THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Impact of Type and Rate of Application of Biochar on Earth Worm (*Eisenia felida*) and Some Chemical Properties of a Savannah Haplic Acrisol in Ghana

Benjamin A. Osei
Lecturer & Head of Department, Department of Soil Science,
University of Cape Coast, Cape Coast, Ghana

Abstract:
The use of biochar as soil amendment to improve the fertility and productivity of soil and to sequester C is attaining interest in Ghana. However, little is known about possible adverse impacts on soil biota in the Tropics. Two sequential incubation mesocosm experiments respectively were used to determine the toxicity of biochar prepared from corn cob (CCB) and poultry manure (PMB) on Eisenia fetida earthworms and also the interactive effect of biochar and earthworm on earthworm casting activity and selected soil properties.

After 42 days of incubation, significant ($p < 0.05$) and inverse relationship was found between biochar application rates and earthworm survival which negatively correlated in the range of ($r = -0.90$ to -0.95, $p < 0.01$) and ($r = -0.88$ to -0.94, $p < 0.01$) for CCB and PMB respectively. Fifty percent survival of earthworm was observed at respective biochar application rates below 78 t ha^{-1} for CCB and that of PMB was 52 t ha^{-1}. After 56 days of incubation, casting activity correlated negatively with increasing biochar concentrations ($r = -0.66$, $p < 0.01$) with casting activity reducing by 68.4 % to 77.9 % in CCB amended soils and 79.6 - 81.3 % in PMB amended soils. It was also demonstrated that biochar and earthworm synergistically increased pH, mineral nitrogen, available phosphorus, total organic carbon, cation exchange capacity and microbial biomass carbon significantly in the mesocosm.

1. Introduction

Earthworms are highly recognized in the earth ecosystem because of their important contribution to soil fertility improvement (Ampofo, 2007). Their burrowing activities increase soil porosity which enhances soil aeration as well as water infiltration. The casts produced usually are important protective and dispersal vehicles for soil microbes and nutrients respectively. Their mixing activity ensures distribution of nutrients within the soil profile. Further, their burrowing, stimulation, ingestion and mixing activities enhance the decomposition of plant residues and turnover of soil organic matter. It is therefore suggestive that any soil management practices that positively or negatively affect earthworms may indirectly affect soil fertility and productivity. Meanwhile, the use of biochar as soil amendment is continuously receiving global attention due to its potential in improving the fertility of most soils (Jeffery et al., 2011; Spokas et al., 2012; Biederman & Harpole, 2013) and its ability to sequester carbon. Biochar is a carbon (C) - rich product obtained through pyrolysis where agricultural waste or biomass is burnt at low temperatures (< 700 C) and at a relatively low oxygen concentration. During this process heat, flammable gases and liquids are produced together with a solid residue called biochar (Lehmann & Joseph, 2009).

Notwithstanding the benefits of using biochar for improving the quality of soils, few evidences has shown that some biochars may have negative effects on the earthworms (Schmidt et al., 1999; Liesch et al., 2010) resulting in their reduced growth and mortality. The negative impact has been attributed to alterations in soil pH and ammonia concentration (Leisch et al., 2010). Other studies have attributed causes of earthworm mortality to potential physical damage arising from the biochar sticking to the earthworm's body (Schmidt et al., 1999).

However, information on the effect of biochar on earthworm population and activity following the application of biochar is limited in the tropics. In addition, biochar effects may be variable depending on the type of biochar, application rates, earthworm species and the soil environment. It has therefore been suggested that further studies are needed to standardize earthworm studies (Frund et al., 2010). This would help develop appropriate recommendations for the application of biochar. An incubated, mesocosm experiment was setup to determine the impact of biochar prepared from corn cob and poultry manure on survival of tropical earthworm (*Eisenia fetida*) to help identify appropriate rates for biochar application in Ghana. In addition, the interactive effect of biochar and earthworm on selected soil properties of a Coastal Savannah Haplic Acrisol was determined.

2. Materials and Methods

2.1. Sampling of Earthworms

Earthworms were collected from the same sampling sites so that earthworms' variability will be minimized. They were sampled from subsurface of soil litter and within plantain farm after rainfall. The site chosen for earthworm collection was to ensure that the soil contained adequate moisture and with homogenous environment. Preliminary sampling was carried out to identify the earthworm species available at the selected sampling site. The most common earthworms identified were *Eudrilus eugeniae* and *Eisenia fetida.* However,*E. fetida* was used for this study. Live earthworm sampling was done by digging and hand-sorting. A soil core, to a depth of 10 cm, was collected and washed in perforated plastic bowls to collect the worms. Earthworms with well-developed clitellum were sampled and used for the toxicological incubation study at the School of Agriculture Teaching and Research farm, University of Cape Coast.

Worms used for the study did not differ considerably in size and had a relatively homogeneous age structure (measured by weights). In the laboratory, the worms were kept in 5 L plastic pot, half filled with experimental soil, supplemented with dry leaves and moistened with distilled water (Terhivuo & Saura, 1993). The earthworms were kept in the pots for ten days in order to allow them to adapt to experimental conditions.

2.2. Soil

Coastal savannah soil (0-20 cm depth) was sampled from the University of Cape Coast Teaching and Research Farm. It is a typical agricultural soil of Cape Coast with a history of vegetable production. The soil was mixed thoroughly, air dried and sieved through a 4-mm mesh prior to establishment of the experiment. The soil was characterized as light sandy loam with pH (H_2O) averaging 5.01 ± 0.02, total nitrogen; 0.08 ± 0.01%, total phosphorus; of 0.23 ± 0.06%, total carbon; 0.48 ± 0.05% and the soil classified as Haplic Acrisol (ISSS/ISRIC/FAO, 1998).

2.3. Biochar

Biochar was produced from corn cob and poultry manure by slow-pyrolysis process using Lucia biomass pyrolytic stoves 'top-lit updraft'. The feedstocks were sun dried, crushed and loaded into pyrolytic stoves and charred for 45 minutes for corn cob (CCB) and 1 hour, 30 minutes for poultry manure (PMB) respectively. The properties of the chars produced are presented in Table 1.

Property	PMB	CCB
pH	10.14 ± 01	9.57 ± 0.02
Total C (%)	48.01 ± 1.86	89.63 ± 2.25
Total N (%)	1.90 ± 0.07	0.38 ± 0.045
Total P (%)	1.18 ± 0.03	0.21 ± 0.014
Calcium (g kg^{-1})	18.20 ± 011	4.07 ± 0.015
Magnesium (g kg^{-1})	9.55 ± 0.05	3.75 ± 0.384
Potassium (g kg^{-1})	11.95 ± 0.02	8.78 ± 0.005
Sodium (g kg^{-1})	8.59 ± 0.060	7.36 ± 0.010

Table 1: Chemical properties of biochars used for the study

2.4. Experimental Setup

Two sequential experiments were used in the experimentation. The first experiment studied earthworm survival upon exposure to biochar and the second experiment involved an incubation experiment that determined the interactive effect of biochar on selected soil properties.

2.4.1. Experiment 1

To study the toxicity of biochar, a 42-day incubated, mesocosm experiment using biochar prepared from corn cob biochar (CCB) and poultry manure biochar (PMB) were setup in 1.5 L cylindrical containers at the University of Cape Coast Research and Teaching Farm. Biochar at rates of 0 – 156 t ha^{-1} were respectively mixed with 1 kg soil and packed into experimental pots. During potting, moist loose shredded papers were placed in the pots to serve as beddings for the earthworms and ten sub-adult of *E. fetida* earthworms, with average weight range of 0.55 – 0.60 g, were introduced into each pot with five replicates. The pots containing the earthworms were kept in the greenhouse and moisture content of the mesocosm maintained at 60 % of field capacity and earthworms monitored for survival. Data on earthworm survival was taken on days; 3, 7, 14, 28 and 42 upon exposure to the biochar. Surviving earthworms were counted and recorded on each monitoring day. For each sampling day, soil sample from each pot was carefully poured unto a tray and soils dispersed to count earthworms. After the counting, the soils with the earthworms were returned into the experimental pots.

2.4.2. Experiment 2

The second stage of the experiment assessed the interactive effect of biochar and earthworms on selected soil properties. Four earthworms with homogenous weights and size were introduced into mesocosm containing 1 kg soil mixed respectively with 13, 26, 39, 52 and 65 t ha^{-1} each of CCB and PMB as well as a control (no biochar) (Table 2). The amendment rates used were informed by the rates at which 50% earth worms survived in the first experiment. Moisture content of the mesocosm was kept within 60% of field capacity throughout the incubation by mass balance. Pots were kept at the greenhouse, School of Agriculture for 56 days. Entire cast at the top of the soil surface were collected on day 56 and oven dried at 105 ^{0}C till constant weight and results expressed as grams per kilogram oven dry weight. Earthworm cast and remnant soil in the pots were mixed and sampled on day 56 and analyzed for pH, soil mineral nitrogen (MN), available phosphorus (AvP), total organic carbon (TOC), cation exchange capacity (CEC) and soil microbial biomass carbon (SMC).

Treatments	**Biochar rate of application (t ha^{-1})**
Ct (control)	0
CCB1	13
CCB2	26
CCB3	39
CCB4	52
CCB5	65
PMB1	13
PMB2	26
PMB3	39
PMB4	52
PMB5	65

Table 2: Biochar treatments and rates used for the study
CCB = corn cob biochar, PMB = poultry manure biochar

2.5. Laboratory Analyses

Soil pH was determined with Suntex 701 Model pH meter in a soil to water ratio of 1: 2.5 and biochar to water ratio of 1:5 (w/v) respectively. The moisture content of soils was determined using gravimetric method. Soil NH_4^+ and NO_3^- concentrations were determined following the protocol described by Rowell (1994) and soil mineral nitrogen (MN) calculated as the sum of NH_4^+ and NO_3^-. Mineral nitrogen extraction was done with 2 M KCl at a soil to solution ratio of 1: 10 (w/v) (Rowell, 1994). Soil exchangeable bases ($Ca^{2+,}$ $Mg^{2+,}$ K^+) were extracted using 1 N ammonium acetate (CH_3COONH_4)solution at pH 7.0 (Sumner & Miller, 1996). The Ca^{2+} and Mg^{2+} in the extract was determined using the Atomic Absorption Spectrophotometer (AAS) (Buck Scientific 210 VGP) whiles K^+ and Na^+ concentrations were determined using a flame photometer (Jenway PFP 7). Soil available P was determined by using Bray 1 extracting solution at soil to solution ratio of 1: 10 (w/v). Total organic carbon was determined by wet oxidation (Walkley & Black, 1934). Total carbon, total nitrogen and total phosphorus of biochar were determined using ashing method, Kjeldahl digestion method and Ammonium Molybdate-Ascorbic Acid method respectively. Soil microbial biomass carbon concentration were determined by chloroform-fumigation extraction method (Vance et al., 1987). Soil microbial biomass C was estimated from the relationship MBC = 2.22 × EC where EC refers to the difference in soil microbial C in fumigated and unfumigated soils respectively (Wu et al., 1990).

2.6. Statistical Analyses

The data the were obtained were subjected to analysis of variance (ANOVA) by using Genstat 12.1 version (Genstat, 2008) statistical package. Treatment means were compared by the lest significant difference (LSD) method at P=0.05. Pearson's moment correlation was used to determine how the biochar treatments related with mortality rates.

3. Results

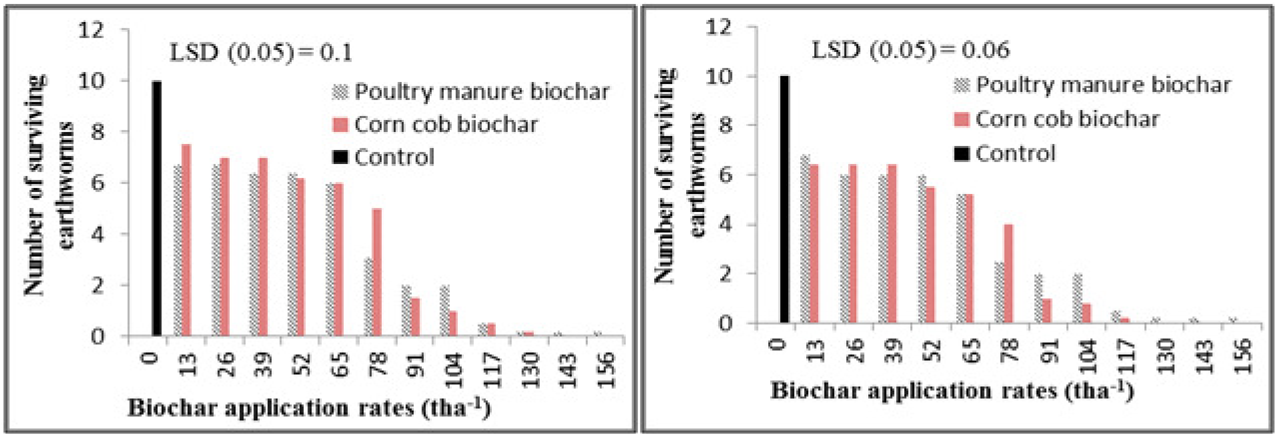
3.1. Earthworm Survival after Exposure to biochar

The number of earthworms that survived on the 3rd, 7th, 14th, 28th and 42nd day of the incubation experiment following the biochar amendment is presented in Figures 1a, b, c, d and e.

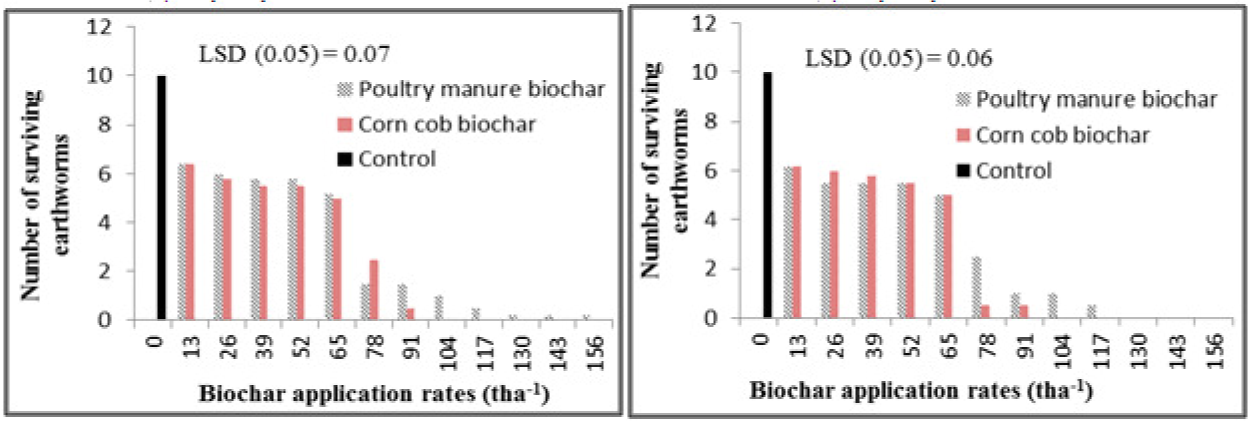
Generally, earthworm survival was dependent on the type of biochar used, application rates and time of exposure. Treatment that received no biochar recorded significantly higher survival (P = 0.05; Number = 10) compared with all other treatments. It was demonstrated that most earthworms' death occurred within the first 3 days of the incubation in all treatments. Most of the dead earthworms were found on the soil surface of the soil. In addition, earthworm survival reduced with increasing biochar application rates. Significant (P=0.05) and inverse relationship was found between biochar application rates and earthworm survival as indicated by Pearson moment correlation analysis. Earthworm survival negatively correlated in the range of (– 0.90 to – 0.95) and (– 88 to – 0.94) at P = 0.05 for CCB and PMB respectively. Evidently, CCB recorded the highest survival rates compared with PMB.

Survival of 50 % of earthworms was observed at CCB application rates of between 13 to 78 t ha^{-1}. Exceeding 78 t ha^{-1} of CCB application, earthworm survival reduced below 50 % and at application rates of 143 and 156 t ha^{-1} all earthworms died by the third day. On day 3, at application rates of 13, 26, 39, 52, 65 t ha^{-1}, mean survival recorded respectively were (8) (7), (7), (6), (6) (5). On subsequent days of the incubation (7, 14, 28 and 42), it was observed that mesocosms containing CCB application rates 13 to 78 t ha^{-1}) lost between 1-2 earthworms. In PMB treatments, 50 % earthworm survival was recorded at rates ranging from 13 and 52 t ha^{-1}.

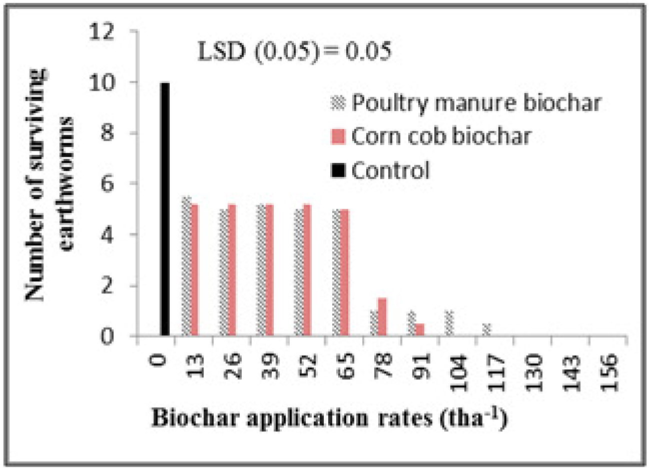
Subsequent days of incubation recorded some number of deaths in PMB amended soils though not pronounced compared with death recorded on the 3rd day. By the 42nd day of the incubation, mortality of earthworm had gone above 50 % for PMB rates above 52 t ha^{-1}.



(a) Day 3 after amendment *(b) Day 7 after amendment*



(c) Day 14 after amendment *(d) Day 28 after amendment*



(e) Day 42 after incubation

Figure 1: The effect of type, rate of application and days after amendments of biochar on survival rate of earthworm

3.2. Interactive Effect of Biochar and Earthworm on Selected Soil Properties

The presence of the earthworms and estimation of weight of earthworm cast produced (Table 3) were used as an indication of earthworm activity. The weight of the cast produced after 56 days of earthworm incubation has been summarized in Table 3. Earthworm cast production varied with types of biochar and application rates. Casting activity correlated negatively with increasing biochar rates (r = -0.66, P= 0.05). The control had significantly (P= 0.05) higher casts (368.3±35.8 g kg^{-1}) produced compared with all the amended soils. Higher casting activity was demonstrated in CCB-amended soils compared with PMB amended soils. Meanwhile casting activity reduced by as much as 68.4% to 77.9% in CCB soils whiles in PMB soils, reduction was by 79.6 - 81.3 %.

Soil pH increased significantly with increasing biochar rates for both CCB and PMB. Soil pH increases ranged between 0.07 – 0.21 units and 0.13 – 0.23 respectively for CCB and PMB amended soils. Soil mineral N increased with increasing rates of both CCB and PMB with highest in soils amended with 65 t ha^{-1} PMB. Mineral N increased by 0.58 – 2.62 units for CCB while in PMB soils, N increased by 2.03 – 8.12 %.

Soil Available P increased significantly in all the amended soil samples compared to the controltreatment. More so, although increasing the rates of CCB increased Available P, it was not significant but PMB demonstrated increased Available P levels with increasing biochar rates. In CCB soils, Available P increased by 0.89 – 1.03 mg kg^{-1} whiles PMB increased by 1.09 – 3.97 mg kg^{-1}. Similarly, TOC significantly increased in all amendments and whiles TOC increased with increasing PMB rates, increasing CCB rates had no effect on TOC. Soil TOC increased by 0.74- 0.93 and 1.36-2.32 % in CCB and PMB respectively.

Microbial biomass C increased significantly in all treatments above the control. In CCB amendments, MBC increased by 0.54 – 1.68 mg kg^{-1} and in PMB application increased MBC by 2.40 to 5.41 mg kg^{-1}. In addition, CEC increased significantly with increasing rates of biochar recording an increase by 0.40 – 0.51 units for CCB and 0.61 – 0.79 for PMB amended soils respectively.

Treatments (t ha^{-1})	Cast (g kg^{-1})	pH	MN (mg kg^{-1})	Av. P (mg kg^{-1})	TOC (%)	CEC (c molkg^{-1})	MBC (mg kg^{-1})
0	376.71 ± 5.80 a	5.04 ± 0.0 j	12.62 ± 0.40 k	4.83 ± 0.09 e	0.47 ± 0.03 g	2.03 ± 0.05 k	14.87 ± 1.20 k
CCB1	119.17 ± 10.75 b	5.40 ± 0.01 i	19.99 ±0.52 j	9.13 ±0.04 d	0.82 ± 0.04 f	3.39 ± 0.03 j	22.89 ± 0.17 j
CCB2	104.77 ± 4.64 b	5.49 ± 0.01 h	25.16 ± 0.41 i	9.47 ± 0.30 d	0.87 ± 0.05 f	3.68 ± 0.02 i	27.54 ± 0.32 i
CCB3	110.43 ± 7.14 b	5.54 ± 0.01 g	34.15 ± 0.82 h	9.51 ± 0.30 d	0.88 ± 0.05 f	3.85 ± 0.02 h	33.22 ± 0.45 h
CCB4	101.43 ± 3.80 b	5.79 ± 0.01 e	42.16 ± 0.56 f	9.81 ± 0.17 d	0.90 ± 0.04 f	4.02 ± 0.03 g	36.26 ± 0.28 g
CCB5	83.43 ± 4.96 c	6.10 ± 0.01 b	45.78 ± 0.67 e	9.21 ± 3.26 d	0.91 ± 0.04 f	4.18 ± 0.02 f	39.81 ± 0.88 f
PMB1	76.96 ± 4.56 cd	5.71 ± 0.01 f	38.33 ± 1.10 g	10.13 ±0.07 d	1.11 ± 0.02 e	5.17 ± 0.03 e	50.63 ± 1.62 e
PMB2	75.63 ± 6.76 cd	5.81 ± 0.01 e	52.17 ± 0.69 d	14.87 ± 0.29 c	1.23 ± 0.02 d	7.03 ± 0.05 d	70.63 ± 1.23 d
PMB3	60.97 ± 6.81 d	5.93 ± 0.01 d	72.27 ± 1.07 c	17.21 ± 0.12 bc	1.34 ± 0.01 c	7.53 ± 0.09 c	82.08 ± 0.61 c
PMB4	63.97 ± 3.58 d	6.00 ± 0.01 c	80.53 ± 0.62 b	18.83 ±0.12 b	1.45 ± 0.02 b	9.13 ± 0.06 b	89.63 ± 0.99 b
PMB5	70.63 ± 4.61 cd	6.21 ± 0.01 a	115.10 ± 1.60 a	24.02 ± 0.66 a	1.56 ± 0.03 a	9.56 ± 0.10 a	95.33 ± 1.13 a

Table 3: Interactive effect of biochar and earthworms on soil properties

Values are expressed as mean ± SD. Different letters following the data in the same column denote significance ($p < 0.05$). MN – mineral nitrogen; AvP – available phosphorus; TOC – total organic carbon; CEC – cation exchange capacity; MBC – microbial biomass carbon

4. Discussion

4.1. Effect of Biochar on Earthworm Survival

Biochar has extensively been documented to improve soil physical and chemical properties. The upsurge in research towards biochar interaction with soil biota is a recent phenomenon (Lehmann et al., 2011). Biochar prepared from corn cob and poultry manure have been used in several research works to interrogate its effect on physicochemical properties but most often have failed to document the impact on soil biota especially on earthworm often referred to as soil engineers (Ampofo, 2007).

Rapid deaths of earthworms were observed by the 3rd day at higher biochar rates and could be associated with the physical damage caused to the earthworm by biochar. Characteristically, it was seen that biochar was stuck to the body of the dead earthworms found on the soil surface. The sticking nature of the biochar prevented the earthworm from penetrating the soil. It has been suggested that biochar is kept wet before or immediately after its application to soil to eliminate the possibility of biochar getting stuck to the body of the earthworm (Li et al., 2011). Although biochar-soil mixture was kept wet, earthworms were seen dead on the surface of the soil in mesocosm having biochar stuck to the bodies'. By evidence from this research, it's recommended that in order to eliminate the propensity of biochar sticking to the body of earthworm, prewetting biochar prior to application could reduce negative impact only at a lower biochar rates observed in this study.

The increasing mortality observed in the current study as a result of increasing biochar application rates is similar to the findings of Liesch et al., (2010) who reported increasing mortality of earthworms with increasing rates of poultry litter biochar. The toxicity of

PMB could be attributed to high N content of the biochar. The consequent mineralization of N might have resulted in the production of high ammonium concentration. When conditions are warm and moist, the ammonium may be converted to ammonia (Cantrell et al., 2007) which may have caused physical injury to the skin or affected gas exchange in the mesocosm causing the death of the earthworm. Then again, biochar has the tendency to absorb moisture, and this property may have created osmotic gradient consequently causing the loss of moisture from the body of the earthworm resulting in the rupture of plasma membrane leading to the loss of cellular contents. This was evident when after the 7th day, the bodies of dead earthworms were seen dried out on the surface of the soils in the mesocosm. Similar conclusions were drawn by Liesch et al., (2010) who associated earthworm's death to osmotic shock. The death of earthworm in this study could also be attributed to elemental composition of the biochar. PMB used in this study contained trace amounts of metals and other micronutrients, including Na, Mg, Cu, Fe (Table 1), and these might have affected earthworm survivorship, growth and activity (Arai et al., 2003). Biochar prepared from PMB contained appreciable levels of potentially toxic elements, such as arsenic, that can be preserved in the production of low-temperature biochars (350 - 400 ^{0}C) (Arai et al., 2003). The estimated concentration of these elements in the present study however were below reported toxic concentrations but the cumulative effect of these metals, the presence of ammonia, high pH and osmotic gradient may have contributed to the mortality of the earthworms. Moreover, biochar has been implicated to have contained polynuclear aromatic hydrocarbons (PAHs) (Naphtahalene and Phenanthrene) which are known toxins to soil biota (Alburquerque et al., 2015) including earthworm. The current study did not estimate the levels of these PAHs but could be a plausible reason for the death of earthworms in this study. Biochar caused 50 % mortality when its rates exceeded 78 t ha^{-1} and 52 t ha^{-1} for CCB and PMB application respectively. Interestingly, some research studies have considered rates above this toxic range to be beneficial for crop production. The long-term application of these rates could destroy some soil biological life, soil quality and productivity. Apart from day 3 where most deaths occurred, there was insignificant reduction in mortality rate with incubation time and it is consistent with the submission of Weyers and Spokas (2011) that biochars negative effects on earthworms may reduce with time.

4.2. Earthworm Activities in biochar Amended Soils

Ingestion of soil-biochar mixture has been inconsistent with some studies even reporting earthworm avoidance of biochar amended soils (Tammeorg et al., 2014). The ingestion and mixing behaviour of earthworm can result in the distribution of biochar within the soil profile. Irrespective, studies on the effect of the interaction of earthworm with soil-biochar mixture is limiting in tropical areas. Our study found increased higher casting activity in the control which could be as a result of low nutrient quality of the soil used. It is known that turnover of the soil by earthworm increases when the quality of soil organic matter is low (Flegel & Schrader, 2000). This difference in cast production can be considered as a compensatory mechanism, where the higher ingestion rate of the control soil compensated for its low nutrient content. On the other hand, PMB-soil mixture was the least to be ingested and this could be attributed to the high nutrient content of the PMB as well as increased microbial population in the treatments. Ingestion of biochar-soil mixture is a positive attribute in that the earthworm could carry the mixture in their gut. In the process, microbes in the gut enhance the mineralization of the biochar or the biochar is carried in the gut and distributed in sublayers within the profile. Similar findings were reported by Topoliantz and Ponge (2005) that *Pontoscolex corethrurus* earthworms evidently grind biochar material and mix it into the soil. Van Zwieten et al. (2010) explained that earthworms' ingested soil-biochar mixture to enable them feed on microbes and microbial metabolites which are more abundant on biochar surfaces. Further, it is possible that earthworm ingested biochar-soil mixture because of biochars' liming effects (Zackrisson et al., 1996) and this could increase the abundance of guts bacteria which could foster elevated production of earthworm's digestive enzymes (Lattaud et al., 1999). The ingested matter as shown in the cast produced (black and brown cast deposition explains why earthworm is important for bioturbation of soil (Garcia & Fragoso, 2002). Biochar and earthworm interaction significantly increased pH, MN, AvP, TOC, CEC and MBC. Soil pH increased significantly ($p < 0.05$) above the control which affirms the assertion that biochar has liming ability and upon incorporation into the soil, increases the pH in most cases. The rise in soil pH could be due to the acid neutralizing effect of the biochar causing adsorption of acidic cations such as (Al^{3+}, Fe^{2+}) onto biochar surfaces, consequently, reducing exchangeable acidity of the soil. Furthermore, the decomposition of more readily decomposable fractions of biochar releases basic cations into solution enhancing the pH of the soil. The released basic cations (Ca^{2+}) ions get hydrolysed. The $Ca(OH)_2$formed reacts with soluble aluminum ions (Al^{3+}) in the soil solution to yield insoluble $Al(OH)_3$.

Mineral N increased significantly ($p < 0.05$) in all treatments above the control. The increase in mineral N could be related to the effect of biochar and the presence of earthworm. In PMB soils mineral N increased probably due to the high N content of the biochar compared with that of CCB. The high N of content of PMB and the low C/N ratio simultaneously offset possible microbial immobilization. Then again, the addition of biochar is documented by previous researchers to enhance microbial proliferation through the creation of a conductive environment for microbes, consequently contributing to higher net N mineralization. The burrowing, casting and mixing activity of earthworm, ensured biochar is mixed with soil particles and microorganisms. Further, the ingestion and mixing of soil-biochar mixture and burrowing activity of the earthworm synergistically might be an ideal reason for influx of mineral N. The presence of biochar in the ingested material might have resulted in elevated production of gut enzymes (protease and urease) by microbes which increased decomposition of biochar. In addition, the mucus secretion associated with cast production has been found to foster microbial activity (Barois, 1986; Coq et al., 2007). Increased mineral N could therefore be an indication of higher mineralization of labile nitrogen and nitrogen held in decomposable recalcitrant portion of biochar through enhanced microbial activity.

Mineralization of native C as a result of priming effect of biochar as well as decomposition of biochar labile C could be the main reasons for the increase in TOC soil. Biochar added to soil could contain an appreciable amount of labile C as well as the degradation of some part of recalcitrant C by microbes (usually about 5 % is degraded) (Brodowski, 2005; Cross & Sohi, 2011). Then again, the addition of biochar might cause positive priming stimulating the mineralization of native soil organic carbon (Kuzyakov et al., 2009). Then again, the presence of earthworm could have aided the decomposition of biochar by increased microbial activity associated with biochar, thus, subsequently releasing carbon into solution. Moreover, earthworm gut is known to provide a conducive environment for the activation of dormant microbes and germination of spores. Some of these gut microbes are responsible for amylase, cellulase, lipase, chitinase and mannose activities in the alimentary canal and through their activity could cause the decomposition of biochar (Munnoli et al., 2010).

Significant increases in MBC were observed in amended soils in the presence of earthworms. This is indicative of increased microbial population as a result of biochar addition and the influence of earthworm activities. Directly, biochar characteristics such as biochar surface characteristics, biochar pores, may offer a suitable habitat and refuge respectively for the proliferation of microbes. Indirectly biochar changes the soil environment such as; through the provision of labile C, micro and macro nutrients which serves as nutrient source for microbes (Glaser et al. 2002). Moreover, earthworm activities such as ingestion, stimulation, burrowing, mixing and casting activities encouraged increase in microbial numbers and activity (ref). Increase in microbial numbers together with earthworm burrowing, ingestion and casting activities accelerated decomposition of labile C of biochar origin as well as native C as a result of priming effect and or microbial decomposition. The increase in organic C provided a readily available carbon energy source for microbes which stimulated microbial activity and consequent assimilation of C.

Available phosphorus increment could be related to the AvP concentrations associated with labile P fraction in biochar material. The presence of readily decomposable organic phosphorus and inorganic fractions contained in biochar ash influences labile P levels and soil microbial community (Lehmann & Joseph, 2009). This is particular with manure based biochar which has been documented to contain higher labile P as in the case of the PMB used in this study. Upon incorporation of biochar, organic P in biochar material is mineralized in addition to the inorganic P, increasing P availability. In addition, Phares et al., (2016) reported an increase in the number of phosphorus solubilisers that correlated with elevated concentrations of AVP. He further explained that these microbes attack biochar and caused mineralization of remnants organic P which may be part of the recalcitrant fraction held in biochar material consequently releasing inorganic P gradually into solution. Phosphorus solubilisers release phosphomonoesterase enzymes that enhance mineralization of phosphorus ((Nannipieri et al. 2011).

Elevation of cation exchange capacity of soils can be attributed to the increase in soil negative charges as a result of surface oxidation and creation of carboxylic and phenolic surface functional groups when biochar was applied to soil (Liang et al., 2006; Cheng et al., 2006). Consequently, chelation of Al and Fe implicated in soil acidity is enhanced. Then again, it could be related to the increase in pH due to the elevation of basic cations (Ca^{2+}, Mg^{2+} and K^{+}) which are products of decomposition of biochar in soil and en route alimentary canal of earthworm.

5. Conclusion

Significant ($p < 0.05$) inverse relationship was found between biochar application rates and earthworm survival. Fifty percent of the earthworm survived was at biochar rates below 78 t ha^{-1} for CCB and 52 t ha^{-1} for PMB. Therefore, high application rate above 52 t ha^{-1} (for CBB) and 78 t ha^{-1} (for PMB) for the soil studied must be considered carefully as it might be harmful to the earthworms in the soil. Then again, the study demonstrated that biochar and earthworm synergistically increased pH, MN, available P, TOC and CECsignificantly in the mesocosm. Based on these findings, *Eisenia fetida* could be integrated with appropriate biochar rate to promote sustainable agriculture and improve the fertility of coastal savannah Haplic Acrisol.

5.1. Conflict of Interests

The author has not declared any conflict of interests.

6. References

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