The effects of two different biochars on earthworm survival and microbial carbon levels

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Abstract
Biochar is a material created from the thermoconversion of biomass through pyrolysis for the production of bio-energy. The use of biochar as a soil amendment has been proposed as a means to sequester carbon, thus offsetting the release of CO_2. Management strategies for the use of biochar as a soil amendment are still in development, and the effect of adding biochar to soil on soil organisms, in particular earthworms, is virtually unknown. We studied the effect of two different biochars, pine chip biochar and poultry litter biochar, on earthworm growth and survival in incubated mesosocoms in two different field soils, as well as the effect of the two biochars and earthworms on soil microbial carbon biomass. The poultry litter char adversely affected earthworm survival, but resulted in higher levels of microbial carbon, especially at the higher rates of application. The pine char had a higher survival rate, and did not show any change in the microbial carbon levels.

Key Words
Eisenia fetida, poultry litter biochar, pine chip biochar, earthworm mortality.

Introduction
The organic matter the Terra Preta soils in the Amazon basin are very stable and have an estimated mean residence time of 250-3280 years (Stevenson 1994). The stable, dark component of this soil is biochar, which is created through pyrolysis, which is thermoconversion of biomass with the exclusion of oxygen which produces syngas, bio-oil, and biochar (Antal and Gronli 2003). This process provides a way to dispose of substances like human waste or poultry litter, in a way that could be beneficial to the environment, providing potential carbon sequestration and food for microbial systems. The potential benefits of biochars have generated an interest in creating industrially created chars for soil application outside of their endogenous environment. There are now studies emerging about the effects of artificial biochars on microbial populations. Steinbeiss *et al*. (2009) found that the fertilizer effect of biochar may be derived from an increased recycling of nutrients in biomass due to stimulation of soil microorganisms. Biochar provides a food source for microbes. This food source and the microbial respiration is all dependent on the material the char is made from. Das *et al* (2008) found that adding a poultry litter derived char products in the soil accelerated the microbial respiration rates. Steinbeiss *et al.* (2009) tested two different biochars, a yeast derived char and a glucose derived char, and found that biochar type was the most important factor effecting the microbial community. Work is now emerging on the effects of biochar on microbial populations. Earthworms are biological specie that can impact the microbial cycle, yet there are few studies on biochar that measure the combined effects of microbial biomass carbon and earthworm activity on in the presence of chars.

Methods
We studied the effect of two different biochars, pine chip biochar and poultry litter biochar, on earthworm growth and survival in incubated mesocosms. The containers were controlled cylindrical containers with two different field soils, Cecil and Tifton. Both soils were collected in the field and dried. The pine chip biochar and poultry litter biochar were pyrolyzed with a maximum high temperature of 400°C, a holding time of 0.5 hr, and N_2 as a carrier gas. To establish a baseline of soil properties, a set of control samples were analyzed for mineral N, P, K, pH, total C and N, and microbial biomass. Amendment levels of 0 (control), 22.5, 45, 68, and 90 Mg/ha application were tested, respectively equivalent to 0%, 50%, 100%, 150% and 200% of a 45 Mg/ha application. Four replicates were run with each char at every percentage application rate above 0%; a single set of four replicates at 0% biochar was used as a control for both biochars. Mesocosms were covered in parafilm to maintain moisture and avoid earthworm escape, and incubated at 20°C for 28 days.

To each mesocosm, a total of 10 sub-adult Eisenia *fetida* earthworms were added, with the total earthworm weight per replicate recorded at the beginning and end of the experiment. Rather than total weight, average weights by number of surviving worms were used for comparison. Dead earthworms found at the surface of mesocosms during the incubation were removed. An earthworm was judged to be dead if it did not respond to stimulus with a blunt probe. As dead tissue decomposes rapidly in soil, earthworms not found were assumed to have died during the incubation period. Microbial carbon and nitrogen levels were measured using the fumigation-extraction method by Vance *et al* (1987), using 0.5 M K2SO4 as an extractant. Another sample is fumigated with Chloroform in a vacuum desiccater for three days. Samples were processed on the IL 550 TDC-TN. The levels of Carbon and Nitrogen in the initial non-fumigated soils were subtracted from the fumigated samples. Statistical analysis was performed for microbial biomass using a four way factorial ANOVA using the GLIMMIX procedure for SAS 9.1.

Results and discussion

Earthworm survival rates

Figure 5: Earthworm Survival Rates

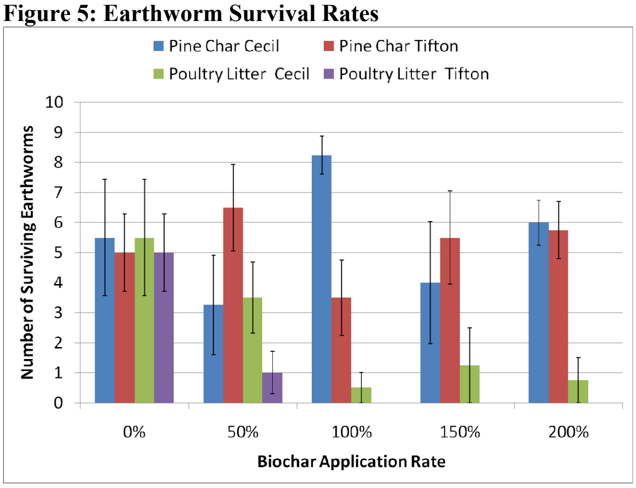

Figure 1. The Pine Chip Biochar survival rates are much higher than the Poultry Litter Biochar rates, and the Cecil soil has higher survival rate. Standard error bars are shown on the graph.

Earthworm survival was dependant on the type of soil and char present. In the Cecil soil, 52% of the total worms survived in the Pine Char, as opposed to only 13% that survived in the Poultry Litter Char. The Tifton Soil had a 47% survival rate for Pine Char, and only a 2% survival for Poultry Litter Char. The number of surviving earthworms is located in Figure 1. The control had a survival rate at 55% and 50% of worms for the Cecil and Tifton respectively. Char type and application rate had a significant interaction (p 0.014). The pine char did not have any significant differences in earthworm survival between the high and low application rates. The Poultry Litter Char had a significant decrease in earthworm survival at all application rates compared to the pine char. At the 50, 100, 150 and 200% application levels, the Poultry litter char are not significantly different from each other, indicating a steep drop in earthworm survival at a low concentration (50%) that persisted in all treatments.

Microbial biomass carbon

There were several significant interactions between the microbial carbon biomass levels and the soil, char type, rate of application and the presence of worms (Table 1). The Cecil soil has a much higher overall microbial biomass than the Tifton soil. There is no significant difference in the microbial biomass in the pine char regardless of the rate of application or the soil. The Cecil soil actually exhibits a net decline in microbial biomass levels with increasing addition of biochar in the Pine Char (Figure 2). There is a significant decline in microbial carbon levels in the Pine Char plots between 0 and 200% and between 50 and 200%. The Tifton soils show no difference in microbial carbon levels for the Pine Char at all treatments.

The presence of poultry litter increased microbial carbon respiration, which agrees with the results of Das *et al* (2008). In the Cecil soil, there was initially a significant decrease in microbial carbon between 0 and 50%, but then there is a steady increase in microbial biomass carbon levels (Figure 2). The Tifton soil poultry litter treatment has a significantly higher carbon level than the pine char at all treatment levels. The microbial carbon increased four times between the 0% treatments to the 200% treatment. This indicates that there is much more carbon available in the Tifton soils as a result of the addition of char, providing a potential nutrient source to plants and microbes. However, most of the earthworms in the poultry litter char died, which could have contributed to the increase in soil carbon levels.

Effect	P Value
Soil (S)	< .0001
Char Type (CT)	< .0001
S x CT	< .0001
Rate (R)	< .0001
S x R	< .0001
CT x R	< .0001
S x CT x R	0.0089
Worms (W)	0.0211
S x W	0.0034
CT x W	0.6045
S x CT x W	0.0222
R x W	0.0046
S x R x W	0.0056
CT x R x W	0.0417
CT x R x S x W	0.0592

When you compare the interaction between soil type, char type, and worms, you find that there is no difference in microbial biomass carbon between the initial, with worm, and without worm treatments for either char. In the Cecil soil pine char treatment, the presence of earthworms was significantly higher than the treatments without earthworms or the initial treatments, which did not differ from each other. This indicates that earthworms were increasing the microbial carbon levels with their activity and respiration. For poultry litter, there is no difference between the presence of worms or not, but the initial measurement was statistically higher in microbial carbon. This could possibly be contributed to a lack of earthworm activity processing and moving the char around the containers.

Figure 6: Microbial Biomass in Two Chars by Rate

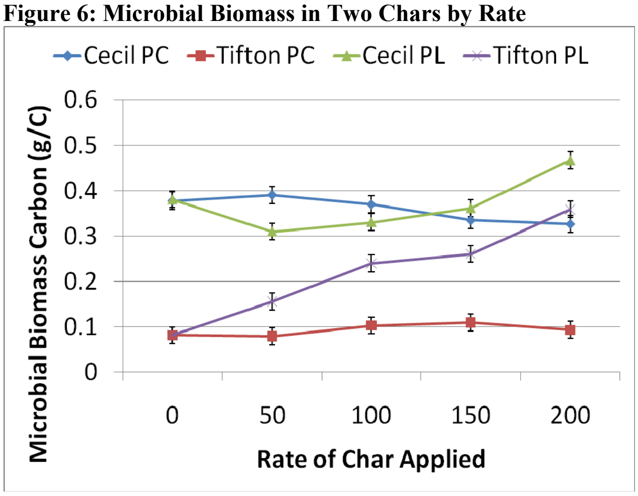

Figure 2. The microbial biomass carbon significantly improved in both Poultry Litter char soils, but remained steady in the Pine Char treatments. Bars are the 95% confidence intervals.

Conclusions

Poultry litter aversively affected the earthworm survival rates in comparison to the Pine Char. The pine char had a stable earthworm survival level that matched the control survival rate. The microbial biomass carbon levels under pine char were also steady in both the Tifton and the Cecil soils, regardless of the rates of char applied or the presence of earthworms. The microbial biomass levels increased in both poultry litter char soils. This may be caused by increased earthworm mortality, or it could provide a better food source for microbes.

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