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Influence of biochar aged in acidic soil on ecosystem engineers and two tropical agricultural plants

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ABSTRACT

Biochar amendment to soil is predicted globally as a means to enhance soil health. Alongside the beneficial result on soil nutrient availability and retention, biochar is presumed to increase soil macro / microbiota composition and improve plant growth. However, evidence for such an effect remains elusive in many tropical agricultural soils. The influence of biochar aged in soil was assessed on soil microbiota, macrobiota (*Eudrilus eugeniae*), seedling emergence and early plant growth of *Oryza sativa* and *Solanum lycopersicum* in tropical agricultural soil, over a 90 d biochar-soil contact time. Results showed negative impacts of increased loading of biochar on the survival and growth of *E. eugeniae*. LC_{50} and EC_{50} values ranged from 34.8% to 86.8% and 0.9–23.7% dry biochar kg^{-1} soil, over time. The growth of the exposed earthworms was strongly reduced ($R^2 = -0.866$, $p < 0.05$). Biochar significantly increased microbiota abundance relative to the control soil ($p < 0.001$). However, fungal population was reduced by biochar addition. Biochar application threshold of 10% and 5% was observed for (*O. sativa*) and (*S. lycopersicum*), respectively. Furthermore, the addition of biochar to soil resulted in increased aboveground (shoot) biomass ($p < 0.01$). However, the data revealed that biochar did not increase the belowground (root) biomass of the plant species during the 90 d biochar-soil contact time. The shoot-to-root-biomass increase indicates a direct toxic influence of biochar on plant roots. This reveals that nutrient availability is not the only mechanism involved in biota-biochar interactions. Detailed studies on specific biota-plant-responses to biochars between tropical, temperate and boreal environments are needed to resolve the large variations and mechanisms behind these effects.

1. Introduction

Biochar is a by-product produced through the carbonisation of C-based feedstocks (biomass) and is best described as a 'soil conditioner' (EC, 2010). Biochar produced through pyrolysis processing has drawn a lot of international attention as a useful organic material. Irrespective of the different materials proposed as biomass feedstock for biochar (wood, agricultural residues and manures), the sustainability and suitability of the feedstock for such an application depends on chemical, physical, environmental, economic and logistic factors, yet, only few studies have been carried out, especially in the tropics. Biochar has been noted to modify soil biological community composition and abundance (Grossman et al., 2010; Egamberdieva et al., 2016), however, such modifications may also impact on nutrient cycling (Steiner et al., 2008b; Zee et al., 2017), soil structure (Xu et al., 2014); or can change other soil physical characteristics such as texture, pore size and density with influence on soil aeration, water holding capacity and soil workability (Downie et al., 2009) and, thereby affect biota growth (Vaccari et al., 2015; Gale et al., 2016). Thus, careful evaluation of biochar before field scale biochar application is needed.

Biochar is also known for its potential use in contaminated land remediation, however, its long-term influence on soil biota is not widely known. Soil biota (earthworms, enchytraeids, collembolans, microbes) as ecosystem engineers, may have different response to biochar amendments in soil. Studies have shown that adding biochar to soils can have beneficial and/or detrimental impact (Gomez-Eyles et al., 2011; Cornelissen et al., 2013; Domene et al., 2015b; Domene, 2016; Zrim et al., 2017). But, the mechanism is not well understood and the lethal concentrations have not been well established in literature.

Although biochar can be produced from any available biomass material, the effects may vary from soil to soil, region to region, climate to climate, plant to plant, and there are few comparative studies for

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tropical agricultural plants. Adding biochar to soil is recognized to have positive effects on soil quality. Example, it may increase and sustain soil biological, physical and chemical properties (Trupiano et al., 2017), stimulate plant growth (Vaccari et al., 2015; Liu et al., 2016; Zee et al., 2017), result in higher leaf number (Carter et al., 2013; Trupiano et al., 2017), plant height (Abdul and Abdul, 2017; Zee et al., 2017), leaf growth / weight (Liu et al., 2016), and increase plant total biomass (Liu et al., 2016; Trupiano et al., 2017). Also, recent analysis has documented controversial influence on soil–plant interactions and plant biomass response (Vaccari et al., 2015; Buss et al., 2015; Domene et al., 2015b). Yet, there are only few studies on the impact of biochar (over time) on early stages of plant growth (seedling emergence and early plant growth) for plant species grown in tropical agricultural soils. Thus, full analysis of char, climate, and soil conditions for a specific plant is needed before carrying out a large scale biochar application.

Furthermore, several studies have used freshly pyrolysed biochar. But, biochar undergoes aging, and as biochar ages in soil, its structure changes, with surface area increasing, and thus, macro / microbiota will also change (Thies et al., 2015; Domene et al., 2015b; Zrim et al., 2017). Therefore, examining the effects of biochar aged in soil on ecosystem engineers and plant growth becomes essential. Considering the potential role of biochar in carbon sequestration, contaminated land remediation and soil conditioning, the systematic screening of increased loading of biochar in soil (over time) is important to determine the possible effects on soil biota before its use as 'soil conditioner' in tropical agricultural soils. Thus, this current study assessed the impact of rice husk biochar aged in soil on: (i) survival and growth of earthworms (*E. eugeniae*), (ii) soil microbiota abundance and composition and (iii) seedling emergence and early plant growth of *Oryza sativa* and *Solanum lycopersicum* in tropical agricultural soil, over a 90 d biochar-soil-contact-time, using OECD 207, 222 (for earthworms); total microbial count and OECD 208, and USEPA (2012) guidelines (for plants), respectively.

2. Materials and methods

2.1. Biochar production

Feedstock for biochar production was selected on its availability considering the option for agricultural solid waste management in the area. Rice husk biomass was collected from Rice Mill in the area. The collected feedstock was appropriately treated before biochar production by pyrolysis (3–4 h) at temperature > 480 °C. The derived biochar (no brown inside, no white or ash outside, making click sound) was allowed to cool prior experiment.

2.2. Test organisms / plants

Mature earthworms (*Eudrilus eugeniae*) were collected from agricultural soil without history of contamination. The earthworms were conditioned in the laboratory and fed with cow dung for 2 weeks prior exposure.

The seeds of *Oryza sativa* (rice) and *Solanum lycopersicum* (tomato) were purchased from agriculturist in the area. Seeds from each plant species were obtained from plants not previously treated with fungicides.

2.3. Soil preparation

A pristine agricultural soil without history of contamination was used for the study. The soil was collected from the top layer of field (approx. 5–20 cm) and sieved with 2 mm mesh size. The physical and chemical properties of the soil were determined using appropriate laboratory procedures (Data in Brief, Table 1). Soil (1000 g) for each amendment was placed in a bowl and mixed thoroughly with the rice husk biochar to give amendments of 0.5%, 1%, 5%, 10%, 25% and 50% dry biochar kg^{-1} soil, respectively (Anyanwu and Semple, 2016b; Anyanwu et al., 2017). De-ionised water was added to achieve a final moisture content of 70% water holding capacity (WHC) and mixed thoroughly. Soils with no biochar addition were also prepared to serve as control. The amended and un-amended soils were incubated in half closed glass jars and aged in the dark for 0, 30, 60 and 90 d at 24 ± 2 °C. Soil moisture content was regularly measured and lost water was added accordingly with de-ionized water. After each aging time (30 d), earthworm, microbial and plant bioassays were carried out.

Table 1
Summary of acute toxicity of rice husk biochar on survival (LC_{50}) and growth (EC_{50}) of *Eudrilus eugeniae* in tropical agricultural soil after 14 d exposure, over 90 d biochar-soil contact time.

Biochar aging time (d)	LC_{50} (% dry biochar kg^{-1} soil)	EC_{50} (% dry biochar kg^{-1} soil)
Day 0	34.8 (23.8 – 55.4)*	0.9 (0.2 – 1.8)*
Day 30	34.8 (23.8 – 55.4)*	1.230 (0.4 – 2.4)*
Day 60	42.3 (29.5 – 109.4)*	3.780 (1.0 – 10.4)*
Day 90	86.8 (-)	23.7 (9.8 – 172.1)*

Data shows LC_{50} (survival), EC_{50} (weight loss), 95% confidence interval in parenthesis, (–) = 95% confidence interval could not be calculated.
* $p < 0.05$.

2.4. Earthworm bioassay

Acute toxicity of biochar on earthworms in tropical agricultural soil was determined using the guidelines described in OECD 207 and 222 (OECD, 1984, 2016) with little deviation (test duration, temperature and light condition). Mature earthworms (*Eudrilus eugeniae*) weighing 0.5–0.7 g were selected for the study. Before the 14 d exposures, earthworms were left in Petri dishes with moist filter paper and allow to depurate for 24 h, after which the earthworms were weighed (Anyanwu and Semple, 2016b; Anyanwu et al., 2017). Triplicates of 10 earthworms were used for each amendment (0%, 0.5%, 1%, 10%, 25% and 50% dry biochar kg^{-1} soil). Soils (600 g) were weighed into glass trays, de-ionised water was added to moisten the soil to 80% WHC; mixed thoroughly and 10 earthworms were added to each replicate (without food). The samples were covered with perforated foil and incubated in the dark at 24 ± 2 °C. Mortality and growth (weight-change) were the measured parameters to reflect the health condition of the earthworms. Earthworms were weighed before and after exposure (after depurating for 24 h on moist filter paper) to determine changes in growth (weight). Behavioural response and changes on different parts of the earthworms were also recorded (Anyanwu and Semple, 2016b; Anyanwu et al., 2017).

2.5. Microbiota bioassay

Enumeration of the total culturable microbiota was evaluated by colony forming unit count (CFUs g^{-1} soil). This was carried out at the beginning of each ageing time in amended and control soils. Soil samples (1 g) were mixed with 9 ml of Ringer's solution by whirl-mixing for 1 min and allowed to stand for 3 min. Soil solution (0.1 ml) was diluted serially in 0.9 ml Ringers solution; aliquots (0.02 ml) of the dilutions were spread on macConkey agar, nutrient agar and saboraud agar (media for culturing fungi) and incubated. Colonies were counted after 2–5 days of incubation (Anyanwu and Semple, 2016a).

2.6. Plant bioassay

Plant bioassay was carried out according to OECD Guidelines 208 (2006) and USEPA (2012) with little deviations. The bioassay was performed in plant house for 21 days. Soil (50 g) was weighed into plastic pots (3 cm × 3 cm). Controls (soils without biochar addition)