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Bamboo biochar amendment improves the growth and reproduction of *Eisenia fetida* and the quality of green waste vermicompost

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ABSTRACT

Vermicomposting is a promising method for reusing urban green waste. However, high lignin content in the green waste could hinder the development of earthworm and microorganisms and the vermicomposting process, resulting in a low-quality vermicompost product. The objective of this study was to evaluate the effect of bamboo biochar addition (at 0%, 3%, and 6% on a dry w/w basis) on the activity of *Eisenia fetida* and the obtained vermicompost. Biochar addition increased ($P < 0.05$) earthworm biomass, juvenile and cocoon numbers of *Eisenia fetida*, as well as the activities of dehydrogenase, cellulase, urease and alkaline phosphatase. Compared to the control, lignin degradation rate was enhanced up to 13.89% by biochar addition. Biochar addition also improved the vermicompost quality in terms of cation exchange capacity (CEC), dissolved organic carbon (DOC) degradation, humification, nitrogen transformation, toxicity to germinating seeds (*Brassica rapa* L., *Chinensis* group) and heavy metals concentrations. The 6% bamboo biochar addition rate achieved maturity after 60 days of vermicomposting and resulted in the highest quality vermicompost based on parameters such as CEC, DOC, NH_4^+-N/NO_3^--N ratio, germination index and heavy metal concentration. We conclude that 6% biochar addition promoted earthworm growth and the vermicomposting of green waste.

1. Introduction

Urban greening has led to a rapid increase in the quantity of green waste created in large cities in China. In the city of Beijing alone, more than 2.37 million tons of green waste is produced each year (Shi et al., 2013). Green waste has traditionally been incinerated or disposed in landfill, but these practices produce large amounts of greenhouse gases, pollute surface- and groundwater, and occupy land that could otherwise be cultivated (Gong et al., 2016). These practices also fail to capitalize on the value of green waste as a biomass resource that contains substantial amounts of carbohydrates and nutrients.

In contrast to traditional disposal methods, vermicomposting has received increasing attention as an environmentally friendly way to dispose of and utilize organic wastes. Vermicomposting involves the bio-oxidation and stabilization of organic material under aerobic and *meso*philic conditions through the combined action of earthworms and microorganisms (Gong et al., 2017). Vermicomposting can generate a high-quality product that can be used as a soil organic amendment and as a horticultural potting medium.

Although both municipal solid wastes and agricultural wastes have been vermicomposted, there are few reports on the vermicomposting of green wastes. The vermicomposting of green wastes could not only reduce environmental problems caused by landfills and incineration but could also generate a valuable product. Compared to other organic wastes, however, green waste contains a significant amount of lignin, a recalcitrant organic polymer that also reduces microbial access to cellulose and hemi-cellulose. High lignin content could slow the composting process and generate a low-quality compost product (Zhang and Sun, 2014a). The current research determined whether a bamboo biochar amendment can enhance the vermicomposting of green waste.

Biochar is a carbonaceous product resulting from the slow pyrolysis of carbon-rich biomass under low oxygen conditions (Zhang and Sun, 2014b). Biochar is characterized by its high stability, well-developed pore structure, large surface area, high cation exchange capacity (CEC), and abundant surface functional groups, e.g., carboxylic, phenolic, hydroxyl, carbonyl, and quinone groups (Li et al., 2017). Biochar has traditionally been used as a soil amendment in crop production because it increases soil fertility, buffers soil pH, reduces the concentration of exchangeable aluminium, modifies water retention, promotes soil aggregate formation, decreases nitrogen (N) and phosphate leaching, and

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enhances microbial activity (Hijikata et al., 2015)

In recent years, the application of biochar as a compost/vermicompost amendment has gained substantial attention. For example, biochar addition can accelerate the composting process, reduce the bioavailability of heavy metals, increase microbial diversities during the composting of agricultural wastes (Chen et al., 2017), enhance the humification and humic acid synthesis, and improve the quality of the compost product in the composting of pig manure (Wang et al., 2014). Agyarko-Mintah et al. (2017) reported that co-composting poultry litter with biochar reduced total N (51%) and NH_3 (60%) losses, resulting in a higher nutritional value of the compost product. Waqas et al. (2017) also indicated that use of biochar in the composting of food waste improved the process and the physiochemical properties of the final compost by accelerating degradation and mineralization rates. Malińska et al. (2016) found that addition of biochar to the vermicomposting of sewage sludge increased earthworm reproduction and resulted in the faster and more efficient conversion of the sludge into a useful product.

Although many researchers have evaluated the effect of biochar on the composting or vermicomposting of various organic wastes, to our knowledge, no study has evaluated the effect of biochar on the vermicomposting of green waste. Thus, the objective of this study was to evaluate the effect of bamboo biochar on the vermicomposting of green waste with the earthworm *Eisenia fetida*.

2. Materials and methods

2.1. Vermicomposting and sampling

An experiment was conducted at the Beijing Forestry University Forest Science Company Limited Nursery, Beijing, China. Green waste consisting of fallen leaves, grass clippings, and branch cuttings was collected from a municipal green waste treatment plant in Haidian District, Beijing, China. The green waste was shredded into pieces approximately 5 mm in length before the vermicomposting began.

The biochar used in this study was purchased from the Jingyu Charcoal Production Company (Hangzhou, China). The Brunauer–Emmett–Teller (BET) surface area of the biochar was 376.71 $m^2\ g^{-1}$. It was produced by the slow pyrolysis of bamboo biomass at approximately 500 °C for 5 h. The biochar was crushed and passed through a 1-mm sieve. *E. fetida* adults (clitellate) of uniform size (≈ 419 mg fresh weight per individual) were purchased from a commercial earthworm breeding farm in Shunyi District, Beijing, China.

Before commencing the vermicomposting process, the green waste was pre-composted for 14 days to reduce the content of toxic substrates that might be harmful or unpalatable to *E. fetida* in the initial stage, such as ammonia. Then, 2 kg (dry weight basis) of the pre-composted material was mixed with biochar at one of three rates (dry weight basis): 0 (GW), 3 (GW + 3%B), or 6% biochar (GW + 6%B). The mixed materials were loaded into the polyethylene vermicomposting containers (52 cm wide, 68 cm long, and 39 cm high). The bottom of each container had four holes (10 mm diameter) for drainage; these holes were covered with a plastic mesh with a 1 mm opening size to prevent earthworm from escaping. Twenty *E. fetida* adults were added to each container. Each of the three treatments was represented by three replicate containers. The containers were kept in a greenhouse at 24.6–29.3 °C. The moisture content was measured using a digital analyzer MS-70 (A&D Co., Ltd., Tokyo, Japan) and maintained at 65–70% (w/w) by periodic addition of distilled water throughout the vermicomposting process. The chemical properties of the initial raw material (green waste), pre-compost material (after 14 days) and bamboo biochar are given in Table 1.

Earthworm biomass, adult earthworm number, cocoon number, and juvenile earthworm number were determined every 10 days. To accomplish this, all adult earthworms, cocoons, and juveniles were separated from each replicate container by hand sorting; they were counted, washed with distilled water, weighed, and then returned to their original container. Maximum individual biomass gain and maximum individual growth rate were also calculated based on the obtained data of earthworm biomass as following: Maximum individual biomass gain (mg $worm^{-1}$) = Initial individual biomass - Maximum individual biomass; Maximum individual growth rate (mg $worm^{-1}\ day^{-1}$) = (Initial individual biomass - Maximum individual biomass) / Experiment days.

To estimate the changes in the chemical properties of the mixtures, one homogenized sample (30 g of homogenized mixture per sample per replicate) was collected on day 0, 10, 20, 30, 40, 50, and 60. The adults, cocoons, and juveniles were removed from the sample and returned to the respective container before a sample was processed further.

Each sample was divided into two parts. One part was dried at 65 °C, finely pulverized, and stored in sterilized and airtight plastic containers; these dried samples were used to determine chemical properties. The other part of each sample was kept fresh and was used to determine enzyme activities.

Table 1
Chemical properties of the initial raw material (green waste), pre-compost material (after 14 days) and bamboo biochar. Values are means ± SE (n = 3).

Property	Green waste	Pre-compost material	Bamboo biochar
pH	6.95 ± 0.05	8.08 ± 0.02	7.86 ± 0.06
TOC (g kg^{-1})	419.87 ± 5.90	381.35 ± 4.32	566.21 ± 16.31
TKN (g kg^{-1})	11.91 ± 0.19	13.65 ± 0.20	7.80 ± 0.16
C/N ratio	35.29 ± 1.03	27.94 ± 0.10	72.62 ± 2.27
CEC (cmol kg^{-1})	14.78 ± 0.68	21.03 ± 1.23	27.72 ± 0.99
DOC (g kg^{-1})	37.49 ± 0.39	33.08 ± 0.01	5.92 ± 0.36
Humic acid (%)	3.46 ± 0.30	4.53 ± 0.06	ND
Lignin (g kg^{-1})	371.95 ± 9.40	365.66 ± 5.27	263.27 ± 6.56
NH_4^+-N (mg kg^{-1})	74.13 ± 2.86	254.40 ± 11.98	ND
NO_3^--N (mg kg^{-1})	20.88 ± 1.92	109.88 ± 3.95	ND
Cu (mg kg^{-1})	15.72 ± 0.13	16.93 ± 0.35	7.50 ± 0.04
Zn (mg kg^{-1})	90.53 ± 1.45	121.65 ± 0.91	8.36 ± 0.17
Cr (mg kg^{-1})	0.211 ± 0.007	0.257 ± 0.005	0.032 ± 0.002
Pb (mg kg^{-1})	9.74 ± 0.04	12.30 ± 0.09	ND
Cd (mg kg^{-1})	0.207 ± 0.005	0.294 ± 0.003	0.021 ± 0.003
Ni (mg kg^{-1})	24.19 ± 0.22	34.30 ± 0.29	12.37 ± 0.41
BET surface area ($m^2\ g^{-1}$)	–	–	376.71 ± 6.57

ND: not detected. TOC, total organic carbon; TKN, total kjeldahl nitrogen; CEC, cation exchange capacity; DOC, dissolved organic carbon. BET surface area: Brunauer–Emmett–Teller surface area.

2.2. Chemical analysis

The pH was measured in a 1:10 (w: v) aqueous suspension (in distilled water) using a pH meter (Starter 3 C; Ohaus Instrument (Shanghai) Co., Ltd., Shanghai, China). Dissolved organic carbon (DOC) was obtained by aqueous extraction using a 1:10 suspension of sample in de-ionised water (5 g of sample: 50 mL of water), which was shaken for 3 h, centrifuged at 3000 rpm (1400 g) for 10 min, and then passed through a 0.45-μm Millipore membrane filter. The filtered supernatant was analyzed for DOC using an organic carbon analyzer (TOC-Vcp, Shimadzu, Japan). To determine extractable NH_4^+-N and NO_3^--N concentrations, samples were extracted with a 2 M KCl solution (1:5 ratio, w: v) for 30 min. After filtration (0.45 μm Millipore membrane filter), the extracts were analyzed for extractable NH_4^+-N and NO_3^--N concentrations on a continuous flow auto-analyzer (Auto Analyzer 3, Germany). Total organic carbon (TOC) was measured by the wet oxidation method in Yeomans and Bremner (1988). Total Kjeldahl N (TKN) was determined by the Kjeldahl method as described in Barrington et al. (2002) using an automatic Kjeldahl analyzer (KDY-9830; Beijing Tongrunyuan Mechatronics Technology Co., Ltd., Beijing, China). Sodium pyrophosphate was used to extract soluble HA (Brittain et al., 2012).