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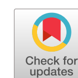
Turning pig manure into biochar can effectively mitigate antibiotic resistance genes as organic fertilizer



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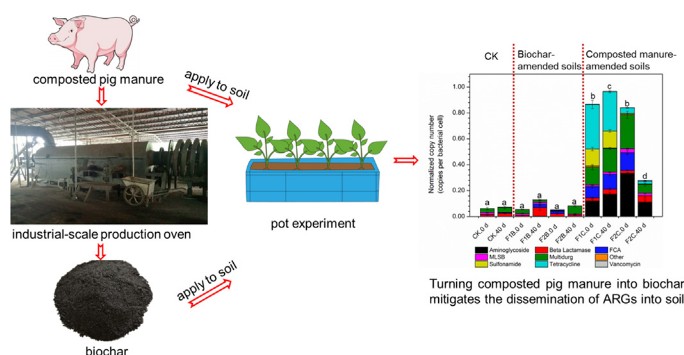
HIGHLIGHTS

- Composted manure and biochar produced from composted manure were applied to soil.
- Applying biochar significantly reduced the abundance of ARGs and MGEs in soil compared with compost.
- No significant difference for ARGs and MGEs was found between biochar-amended and control soils.
- Turning composted manure into biochar is a useful technology to reduce ARGs abundance.

GRAPHICAL ABSTRACT



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ABSTRACT

The composting of fresh manure is an effective way to inactivate pathogens and reduce the levels of antibiotics and some antibiotic resistance genes (ARGs) prior to its application on agricultural land as organic fertilizer. However, some ARGs could still exist and even be enriched after composting. This study investigated whether converting composted pig manure into biochar could reduce the dissemination of ARGs into the soil in comparison with a compost amendment. We performed a pot experiment using pakchoi (*Brassica chinensis*), with two pig manure-based composts and the biochar derived from composted pig manure, as organic fertilizers. The distributions of the antibiotic resistome, mobile genetic elements (MGEs) and bacterial community composition in soils during cultivation were evaluated by high-throughput qPCR and Illumina sequencing. The total ARGs and MGEs abundance in the biochar-treated soils were significantly lower than those in the compost-amended soils during cultivation. The total ARGs abundance in the biochar-amended soils was similar to that in the control soils during cultivation. Thus, the dissemination of ARGs from animal waste to the environment can be effectively mitigated by converting manure into biochar.

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1. Introduction

Antibiotic resistance is one of the most important public health challenges of the 21st century. The number of deaths resulting from antibiotic-resistant bacterial infections is rising and is associated with

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the on-going emergence and dissemination of multidrug resistant "superbugs" (Vikesland et al., 2017). In addition, some Gram-negative bacteria (e.g. *Escherichia coli*, *Salmonella* spp., and *Acinetobacter* spp.) are resistant, in some settings, to almost all currently available antibiotics (Carlet et al., 2012). Thus, there is a growing concern that our globalized society in which common infections and minor injuries will no longer be treatable, as they were before antibiotics became available (Blaser, 2014). To control antibiotic resistance, the World Health Organization has developed a Global Action Plan that calls on all countries to adopt strategies for minimizing the impact of antibiotic resistance (Chereau et al., 2017). In addition, many other regional and national commitments are also being developed and implemented (Vikesland et al., 2017).

Antibiotic-resistant bacteria and ARGs in the environment can enter the food chain and reach clinically relevant niches, and this poses potential risks to human health (Berendonk et al., 2015). Livestock manures are a major source of ARGs in the natural environment (Qiao et al., 2018; Zhu et al., 2017a; Zhu et al., 2017b). Proactive treatments of livestock waste containing ARGs may mitigate their dissemination into the environment (Zhu et al., 2013). However, currently available waste-treatment processes, such as composting, do not efficiently remove ARGs. Response of ARGs varies during composting as a result of the complex microbial ecological processes (Pruden et al., 2013). For instance, after thermophilic composting, some ARGs decrease, while others persist or significantly increase (such as the *sul* ARGs, *tet*X, and *tet*L) (Wang et al., 2015; Xie et al., 2016). The proliferation of ARGs (*tet*X, *sul*1, and *sul*2) was also observed after the aerobic composting of manure (Qian et al., 2016). Technological solutions for the effective removal of ARGs in manure and compost are, therefore, urgently needed.

Turning animal waste into biochar is considered a waste disposal and recycling option (Cao and Harris, 2010). Biochar has been widely applied as a soil amendment to increase fertility (Cao and Harris, 2010). Biochar also serves as green environmental sorbent in the remediation of soils contaminated with various inorganic and organic chemical pollutants, such as heavy metals and pesticides (Ahmad et al., 2014). However, it is not clear if the conversion of composted pig manure into biochar can alleviate the dissemination of ARGs into the soil.

Therefore, in this study, we applied composts and biochar derived from composted pig manure to the soil, and we aimed to (1) determine whether the implementation of biochar derived from composted pig manure reduces the dissemination of antibiotic resistome into soil compared with the compost. (2) characterize the shifts of antibiotic resistome and bacterial community composition in soil after the application of compost or biochar.

2. Materials and methods

2.1. Manure-based compost and biochar preparation

Two large-scale pig farms located in Jiaxing, Zhejiang Province, China were selected. The manure was composted before biochar preparation. Biological waste treatment technologies such as composting and vermicomposting are widely regarded as a clean and sustainable method to manage organic waste like manure (Lim et al., 2016; Wu et al., 2014). For composting, sawdust and straw were added to reduce the water content of pig manure. Then it maintained high temperature for several weeks with proper and regular stirring (one or two times per day). The two pig manure-based composts were collected from Farm 1 and Farm 2. The two farms are typical large-scale pig farms. These farms are located in Tongxiang City, Zhejiang Province, China. The farms have an animal intensity of >10,000 market hogs per year. Without air-drying, the composted pig manure was directly pyrolyzed at 400–450 °C for 30 min in an industrial-scale production oven. The biochar produced from composted pig manure of Farm 1 and Farm 2 was labeled F1B and F2B, respectively. The biochar yield was approximately 30% (ratio of biochar mass to dry compost mass). The compost samples and biochar samples were ground and passed through a 2-mm sieve prior to the pot experiment.

2.2. Pot experiment

Soil was collected from farmland in Zhejiang Province, China. The soil was air dried and passed through a 2-mm sieve before use. Each plastic pot (32 cm in length, 21 cm in width, and 11 cm in height) contained 4500 g air-dried soil. The treatments were designated as follows: (1) control soil (CK); (2) CK plus 4% (dw/dw) compost from Farm 1; (3) CK plus 4% (dw/dw) compost from Farm 2; (4) CK plus 1.2% (dw/dw) F1B; (5) CK plus 1.2% (dw/dw) F2B. The doses of compost and biochar were equivalent in that 4 g of dry compost yielded 1.2 g biochar. Four replicates of each treatment were performed. All of the pots were arranged randomly and incubated in a greenhouse. After the application of compost or biochar, the treated soils were thoroughly watered and pre-incubated overnight. After that, the soil was collected from each pot as the 0 d sample. Then pakchoi (*Brassica chinensis*) seeds were sown into the soil. Deionized water was added every two or three days depending on the weather conditions, to maintain a 70% water-holding capacity. The seedlings were thinned to 12 plants per pot. Pakchoi plants were harvested at 40 d, and the soil was collected on the same day.

2.3. DNA extraction

DNA was extracted from homogenized soil (at 0 d and 40 d), compost, and biochar using a FastDNA Spin Kit (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. DNA was stored at −80 °C before being used.

2.4. High-throughput qPCR (HT-qPCR) of ARGs

HT-qPCR reactions were performed using a Wafergen SmartChip Real-time PCR system as described previously (Wang et al., 2014; Zhu et al., 2013). The SmartChip has 5184 reaction wells with a volume of 100 nL (Wang et al., 2014). Using 296 verified primers, 285 ARGs conferring resistance to almost all major classes of antibiotics were amplified, as were 10 MGEs and 1 16S rRNA gene. PCR cycling conditions, and raw data processing were conducted as previously described (Su et al., 2015; Wang et al., 2014). Relative abundance and normalized abundance of the detected genes were calculated according to previous study (Su et al., 2015; Zhu et al., 2017c). The relative abundance of each ARG/MGE was calculated by dividing the ARG/MGE copy number by 16S rRNA gene copy number (Zhu et al., 2017c). Normalized abundance of each ARG/MGE was calculated by multiplying relative abundance of ARG/MGE by 4 (because the average number of 16S rRNA genes per bacterial cell is estimated to be 4) (Zhu et al., 2017c).

2.5. High-throughput Illumina sequencing

To assess changes in soil bacterial community structure, the 16S primers (515F and 907R) were used to amplify the V4-V5 regions of bacterial 16S rRNA genes (Yusoff et al., 2013). The reaction mixture consisted of 4 μL of 5× FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each 5 μM primer, 0.4 μL of Fastpfu Polymerase, 0.4 μL of BSA, ~10 ng of template DNA, and nuclease-free H_2O to bring the final reaction volume to 20 μL. PCR reactions were then amplified using the following thermocycler conditions: 95 °C for 3 min, followed by 27 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and then 72 °C for 10 min. Amplicons were purified using an AxyPre DNA Gel Extraction Kit. Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq platform (Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China). After sequencing, the paired-end reads were joined, and low-quality reads and ambiguous nucleotides were removed. Raw reads were assembled using FLASH and assigned to individual samples using Quantitative Insights Into

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