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Amendment soil with biochar to control antibiotic resistance genes under unconventional water resources irrigation: Proceed with caution☆

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ABSTRACT

The spread of antibiotic resistance genes (ARGs) has become a cause for serious concern because of its potential risk to public health. The use of unconventional water resources (e.g., reclaimed water or piggery wastewater) in agriculture to relieve groundwater shortages may result in an accumulation of ARGs in soil. Biochar addition has been proven to be a beneficial method to alleviate the pollution of ARGs in manure-amended soil. However, the role of biochar on ARGs in soil-plant systems repeatedly irrigated with unconventional water resources is unknown. Under reclaimed water or piggery wastewater irrigation, rhizobox experiments using maize plants in soil amended with biochar were conducted to investigate the variation of typical ARGs (*tet* and *sul* genes) in soil-plant systems during a 60-day cultivation, and ARGs was characterized by high-throughput qPCR with a 48 (assays) × 108 (samples) array. Only piggery wastewater irrigation significantly increased the abundance of ARGs in rhizosphere and bulk soils and root endophytes. Following 30-day cultivation, the abundance of ARGs in soil was significantly lower due to biochar addition. However, by day 60, the abundance of ARGs in soil supplemented with biochar was significantly higher than in the control soils. Antibiotics, bio-available heavy metals, nutrients, bacterial community, and mobile gene elements (MGEs) were detected and analyzed to find factors shaping ARGs dynamics. The behavior of ARGs were associated with antibiotics but not with bio-available heavy metals. The correlation between ARGs and available phosphorus was stronger than that of ARGs with total phosphorus. MGEs had good relationship with ARGs, and MGEs shifts contributed most to ARGs variation in soil and root samples. In summary, this study provides insights into potential options for biochar use in agricultural activities.

1. Introduction

Unconventional water resources (e.g., reclaimed water or piggery wastewater) are now used to supplement groundwater to irrigate agricultural soils during periods of water scarcity. Since these alternative sources typically contain high nitrogen (N) and phosphorus (P) concentrations, their application to agricultural soils may ameliorate nutrient deficiencies and promote crop and grass growth (Cantrell et al., 2009; Chen et al., 2015). However, both reclaimed water and piggery wastewater often harbor several contaminants of emerging concern (CECs), including antibiotics and antibiotic resistance genes (ARGs) (Barker-Reid et al., 2010; Fahrenfeld et al., 2013; Cheng et al., 2016). Irrigation with these unconventional water resources could therefore introduce both antibiotics and ARGs into soil and induce the proliferation of ARGs within soil microbial communities. Several studies have revealed such an effect. Reclaimed water irrigation may have either no influence or a marked effect on the abundance of ARGs (Negreanu et al., 2012; Wang et al., 2014). In contrast, irrigation with piggery wastewater consistently results in significant increases in the abundance of ARGs in receiving soils (Hong et al., 2013; Cheng et al.,

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The fate of ARGs appears to differ between rhizosphere and bulk soil. However, there is no consistent model of the response of ARGs. For instance, Kopmann et al. (2013) observed that the abundance of *sul1* and *sul2* genes, which confer resistance to sulfonamide, in rhizosphere soil were significantly lower than those in bulk soil. However, Wang et al. (2015) and Kang et al. (2016) observed that the abundance of ARGs did not significantly differ between rhizosphere and bulk soils. In addition, the occurrence of ARGs in plant tissues has been attracting increasing attention because humans consume plants directly or indirectly. Organic farming with animal manures has been shown to result in the detection of ARGs in different plant tissues, including the endophytes of both roots and leaves and the phyllosphere (Wang et al., 2015; Zhu et al., 2017). The concentrations of antibiotics and bio-available heavy metals, the abundance of mobile genetic elements (MGEs) and the structure of the bacterial community have all been demonstrated to influence ARG behavior (Cui et al., 2016; Zhu et al., 2017; Duan et al., 2017), however the effect of nutrients in soil and plants remains unknown.

Biochar, a carbon-rich solid formed during pyrolysis of biomass in the absence of air, has potential to reduce concentrations of antibiotics and heavy metals in soil solution by participating in π-π electron donor acceptor surface interactions (Peiris et al., 2017) with both sulfonamides and tetracyclines. The reduced concentrations of antibiotics in soil solution may ameliorate the transfer of ARGs within the soil microbial community. Consequently, many studies have investigated the effect of biochar upon the fate of ARGs in soil and plants. In soil contaminated with organic fertilizer containing a high abundance of ARGs, biochar addition was shown to reduce gene abundance in both soil and leaf and root tissues of lettuce plants grown in the soil (Ye et al., 2016; Duan et al., 2017). The increased use of reclaimed water and piggery wastewater in response to acute drought conditions has also resulted in the accumulation of ARGs (Negreanu et al., 2012; Fahrenfeld et al., 2013; Hong et al., 2013; Wang et al., 2014; Cheng et al., 2016). Few studies have investigated the effect of biochar addition upon the occurrence of ARGs in soil and plants following repeated irrigation of soil with unconventional water resources.

In this study, wheat-straw biochar was added to soil planted with maize to study the effect upon the abundance of ARGs in rhizosphere soil, bulk soil and root endophytes following repeated irrigation with unconventional water resources. We speculated that biochar would reduce the transfer of typical ARGs (*tet* and *sul* genes) in the soil-plant system during a 60-day cultivation. We aimed to investigate the following responses: (1) enrichment of ARGs in rhizosphere soil, bulk soil and root endophytes with different types of water irrigation; (2) the effect of biochar on the bacterial community in rhizosphere soil, bulk soil and root endophytes; and (3) major factors influencing the behavior of ARGs in rhizosphere soil, bulk soil and root endophytes.

2. Materials and methods

2.1. Experimental design

Soil used for the experiments was collected from the surface layer (0–20 cm) of a field in Xinxiang, Henan Province, (35°19′N, 113°53′E at an altitude of 73.2 m) that had previously received only groundwater irrigation. The soil was classified as fluvo-aquic soil (Chinese Soil System) with no history of agricultural management. The collected soil was air-dried and mixed by passing through a 2 mm sieve. Wheat-straw biochar was purchased from Shangqiu Sanli New Energy Co., Ltd, Henan Province, China. The basic properties of the soil and biochar are given in Table S1. Biochar specific surface area and total pore volume were 8.52 $m^2 g^{-1}$ and 0.025 $cm^3 g^{-1}$, respectively.

The experiment was performed in a greenhouse with natural illumination and humidity at a daily average temperature of 25 ± 2 °C. Inorganic fertilizer containing 200 mg kg^{-1} N, 100 mg kg^{-1} P and 200 mg kg^{-1} K were mixed thoroughly with the collected soil before it was divided into two parts: one part was supplied with 1.0% (w/w) biochar, the other was left unamended. The soils were then packed into a rhizobox system of 140 × 120 × 170-mm (length × width × height) (Masud et al., 2014). Each rhizobox consisted of a 20-mm wide central compartment in which plants were sowed (designated rhizosphere soil). On each side of this central compartment, and separated from it by a 48-μm nylon mesh, were two 10-mm wide compartments (designated non-rhizosphere soil), which in turn were separated using the same nylon mesh from a 40-mm wide compartment (designated bulk soil) (Fig. S1). The nylon mesh allowed water and soluble nutrients to move between the compartments but prevented root extension beyond the rhizosphere compartment.

Each rhizobox contained 3 kg soil and each experiment was performed in triplicate. Following packing, soils were thoroughly wetted with distilled water and pre-incubated overnight. Maize seeds (Jundan 20) were sown into the rhizosphere compartment the following day. After thinning, three plants were settled in each rhizobox. The abbreviations of experiments were as follows: (1) S: distilled water irrigation, (2) SR: reclaimed water irrigation, (3) SP: piggery wastewater irrigation, (4) SB: 1.0% (w/w) biochar + distilled water irrigation, (5) SRB: 1.0% (w/w) biochar + reclaimed water irrigation, and (6) SPB: 1.0% (w/w) biochar + piggery wastewater irrigation. Reclaimed water was obtained from the secondary effluent of the Camel Bay sewage treatment plant in Henan Province; piggery wastewater was obtained after anaerobic fermentation at the Xinxiang Shengda Animal Husbandry Co., Ltd, Henan Province, China. The basic properties of the reclaimed water and piggery wastewater are listed in Table S2. Due to its high chemical oxygen demand, the piggery wastewater was diluted five-fold to meet irrigation water quality standards (Department of Rural and Urban Construction and Environmental Protection, 2005) before it was added to the soils. Irrigation with distilled water was included to reveal the behavior of ARGs in soil-plant systems with no pollutant input. All collected water resources were stored at 4 °C in 10-L sealed plastic containers before being used for irrigation. Equal quantity of appropriate water source was added to maintain soil water content every two days.

2.2. Sample collection and chemical analysis

Following a 30-day cultivation, approximately 20 g each of bulk and rhizosphere soil were collected from each rhizobox using a customized soil auger (15 mm diameter and 200 mm in length) which was sterilized with 70% ethanol between sampling each soil compartment. The collected soil samples were air-dried and bio-available heavy metals (Cu, Zn, Pb, and Cd) were extracted using diethylenetriaminepentaacetic acid (DTPA) at a solid-to-liquid ratio of 1:5 (w/v) (Zhang et al., 2016). Their concentrations were determined using graphite furnace atomic absorption spectrometry (ZEEnit 700 P, Analytik Jena AG, Germany) equipped with an automated sampler. On the 60th day, bulk soil and rhizosphere soil were also collected. The harvested plant was washed thoroughly under running tap water followed by distilled water to remove adhering particles and divided into two parts (shoots and roots) after drying using sterilized filter papers. Soil samples were air-dried, and plant samples were oven-dried at 55 °C until a constant weight was achieved and weighted. The concentrations of bio-available heavy metals were also determined as described