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Original article

Changes in soil microbial community structure and enzyme activity with amendment of biochar-manure compost and pyroligneous solution in a saline soil from Central China

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ABSTRACT

Salt stress has been increasingly constraining crop productivity in arid and semiarid lands of the world. In a previous study, salt stress was alleviated and maize productivity improved remarkably with soil amendment with biochar poultry-manure compost (BPC) in conjunction with pyroligneous solution (PS) in a saline soil from Central China Plain. In 2010, before maize sowing, BPC was incorporated into topsoil at 12 t ha^{-1} following surface spray of diluted PS solution at 0.15 t ha^{-1} one week in advance (BPC-PS2). Such an experiment was repeated in adjacent fields in 2011 (BPC-PS1). Both bulk and rhizosphere samples of these experiment plots were collected at the vegetative growth stage of maize in 2012. Microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}), and soil enzyme activity were measured. Based on 16S rRNA and 18S rRNA gene, bacterial and fungal community structure and abundance were respectively characterized using denaturing gradient gel electrophoresis (DGGE) and quantitative real-time PCR (qPCR). With the amendment, C_{mic} and N_{mic}, and bacterial gene abundance were significantly and greatly increased in both bulk and rhizosphere samples, being greater under BPC-PS2 than under BPC-PS1. On contrast, smaller increase in fungal gene abundance was observed, along with a significant reduction in fungal diversity under BPC-PS2. In addition, two single bands belonging respectively to *Alphaproteobacteria* and *Deltaproteobacteria* emerged in the amended soil. Meanwhile, activities of urease, invertase and phosphatase in both bulk soils and rhizosphere soils were increased by 19–44% with the amendment except of urease in rhizosphere soils. Therefore, with the great enhancement of microbial growth and enzyme activities, combined use of biochar and poultry manure with pyroligneous solution could be a practical option to alleviate salt stresses on plant and soil microbial community in order to improve crop production in saline soils.

1. Introduction

Increasing salinity has been a critical problem in arid and semi-arid area of the world [1,2], which would challenge global food production in the mid-21st century with climate change [3]. Soil salinity constrains crop growth and development [3] with stresses of high osmotic potential on water and nutrient uptake by plants [4], restricts microbial growth and biochemical functioning [5]. Increased salinity could lead to reductions in soil microbial biomass, metabolical efficiency as well as deterioration of soil bio-physical properties [6,7].

Biochar, produced via pyrolysis of biomass under limited oxygen, recalcitrant carbon-rich material with more or less nano-sized pore structure [8]. Generally, biochar soil amendment (BSA) helps improving soil structure, soil water retention and soil tilth [9,10]. Moreover, BSA is generally effective for promoting microbial

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growth [11], improving nutrient availability, thus enhancing crop productivity [12]. BSA has been widely recommended for promoting carbon sequestration and mitigating N_2O emission from croplands [13,14]. However, they have been increasing studies showing biochar's role in enhancing resilience of biological systems, including enhanced microbial and plant health [15], enhanced systemic resistance to pathogens and diseases [16] as well as promoted seed germination and plant development [17,18]. Therefore, BSA could be potentially used for restore soil fertility and microbial activity in salt-stressed agricultural soils [19].

Pyroligneous solution (PS) is a mixture of volatilized substances captured during pyrolysis of plant residues [20]. In slightly acid reaction, PS is generally a mixture of reacting organic compounds with relatively small molecular weights (mainly organic acids and phenolic compounds) [21]. And PS has been shown beneficial for crop performance and the tolerance to pest and disease infection [22]. BSA supplemented with PS reduced salinity and soil pH, and increased crop yield in a salt-stressed soil [10]. Under this combined amendment, improvement was observed of plant growth and leaf biological activity of maize on the soil [23]. However, how this combined amendment affect microbial growth, activity and community composition in the treated salt-stressed soil has not yet been examined.

In this study, we hypothesize that biotic/abiotic stresses to soil microbial community will be alleviated and thus microbial growth and activity improved in the salt stressed soil treated with soil amendment with biochar composted with poultry manure and supplemented with PS field experiments for years. This is now tested by characterizing the changes with the treatment in microbial biomass carbon and nitrogen by chemical assay, in gene abundance and in community structure by molecular biology assay and in enzyme activities by biochemical assay in this study. We aim to address improving soil biological quality and functioning and the recovery of salt-stressed soils for better crop productivity with biochar products.

2. Materials and methods

2.1. Experimental site

The field experimental site was the same as reported in previous work [10,23], and is situated in Kangzhuang Village (34°32′N, 115°30′E), Liangyuan District, Shangqiu Municipality, Henan, China. Located in the center of North China Great Plain, the local area has a semi-humid temperate monsoon climate. For the period of 2008–2012, the average mean annual temperature was 13.9 °C and total potential evaporation was 1735 mm. Annual precipitation was 770 mm and 785 mm in 2011 and in 2012, respectively. In addition, the area occupied a total sunshine time of 2510 h and 230 frost-free days annually. The soil was classified as Aqui-Entisol [24] formed on paleo-sediments of Yellow River. The soil is saline with a slightly alkaline reaction with a high cation exchange capacity while it is low in organic carbon content and moderately compacted (Table 1). Rotation of summer maize with wheat in winter has been practiced as conventional cropping system since the 1980s in the region.

2.2. Biochar and pyroligneous solution

Biochar used for the field experiment was produced through pyrolysis of wheat straw at 450 °C in a vertical kiln at Sanli New Energy Company in Shangqiu, China. Approximately 350 kg of biochar and 250 L of PS are produced per ton of wheat straw dry matter [20]. PS was obtained via condensation with cycling water of volatile organic compounds released in pyrolysis and stored in a closed underground tank. And PS was a mixture containing mainly ethylene, phenolic and ketones groups. The detail chemical composition of PS [21] and basic properties of the biochar [25] has already been reported. Here, Table 1 also contained some information. Prior to use for composting, biochar was ground to pass a 2-mm sieve and homogenized thoroughly. The PS was diluted 5-fold in distilled water before use.

2.3. Biochar poultry manure compost (BPC)

For production of biochar manure compost, poultry manure was collected from a local poultry farm and placed in open-air storage for a week under ambient conditions to air dry. The poultry manure (PM) was then mixed with biochar (BC) at a ratio of 1:3 (PM: BC, v/v) for composting for 6 weeks. The produced biochar poultry manure compost (BPC) was thoroughly mixed prior to its use as an amendment. The compost was a dark neutral loose organic material (pH 7.5 in water) containing 419.7 g kg^{-1} of organic carbon, 25.0 g kg^{-1} of total N, and 0.82 g kg^{-1} of alkaline-releasable N, 12.2 mg kg^{-1} of Olsen-P and 0.83 mg kg^{-1} of NH_4NO_3 exchangeable K (Table 1).

2.4. Experiment design

A field experiment using a combined amendment of BPC-PS was conducted on an abandoned salt-affected cropland in 2010. BPC was broadcast at 12 t ha^{-1} one week after spraying of the diluted PS at 0.15 t ha^{-1} on soil surface, after which maize was sown one week later. The broadcast BPC was thoroughly mixed with the topsoil by ploughing to a depth of 20 cm and then discing to homogeneity. A control with ploughing and discing but without this amendment was set up for comparison. A similar treatment was performed in an adjacent un-reclaimed soil in 2011 before wheat sowing (BPC-PS1) and was compared to the BPC-PS2 field amended in 2010. No more BPC but the same dosage of PS was applied to BPC-PS2 plots in 2011.

Maize cultivar Zheng Dan 958 was directly sown after the wheat harvest each year. A based application of phosphorus and potassium fertilizer was applied at the time of sowing. One third of total nitrogen fertilizer was applied as basal and the remaining was applied twice as a top dressing. The conventional practice in the region is to return the crop straw by ploughing it into the soil.

All of the treatments were performed in triplicate and the plots were arranged in a complete randomized block design. Each individual treatment plot had an area of 0.15 ha and was separated by surrounding border rows to avoid possible surface water fluxes across plots. The crop growth management was consistent across the plots. No irrigation was performed during maize production as the cropland was rain fed during this crop production period.

2.5. Soil sampling

Bulk and rhizosphere soils were collected at the vegetative growth stage of maize plants. Composite bulk topsoil (0–10 cm) was collected from six random locations in each plot using an *S*-shaped pattern. Cores were taken using an Eijkelkamp soil core sampler. At the same time, six random maize plants were excavated from each plot and a composite rhizosphere soil was obtained following the procedure used by Butler and co-authors [26]. Samples were sealed in plastic bags, stored on ice and shipped to laboratory within 24 h. Samples were stored at −20 °C for microbial community structure analysis and at 4 °C for soil enzyme activity and microbial C and N analyse, respectively. For DNA extraction soil samples were processed within one week of storage.