# Effects of modifiers on soil enzyme activity and microbial community diversity in cadmium-contaminated farmland soil

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# Abstract

Cadmium (Cd) contamination, with great toxicity, seriously threatens soil environment. In this study, the effects of biochar and biofertilizer used as modifiers on the biochemical properties, enzyme activity, and microbial diversity in Cd-contaminated soils (1, 2, and 4 $mg \cdot kg^{-1}$) were investigated. The results showed that the soil C/N ratio and electrical conductance could be increased by biochar and biofertilizer ($P < 0.05$). The soil pH in the biochar and biofertilizer treatments increased by 0.99 and 0.95, respectively, and the soil available Cd concentration decreased by 60.24% and 74.34% ($P < 0.05$), respectively, compared with those in the control group. The increase of soil alkaline phosphatase activity was observed in the biochar treatment ($P < 0.05$), and the increases of the activities of soil invertase, alkaline phosphatase, catalase, and urease were observed in the biofertilizer treatment ($P < 0.05$). The relative abundances of *Acidobacteria*, *Gemmatimonadetes*, and *Proteobacteria* were increased and the soil microbial community structure was improved in the biochar and biofertilizer treatments. Redundancy analysis (RDA) and structural equation model (SEM) showed that soil available Cd concentration had negative effects on soil pH, C/N ratio, urease and alkaline phosphatase activity, and relative abundances of *Acidobacteria* and *Proteobacteria*. The increase of soil urease activity was the main reason for the increase of soil microbial diversity. In summary, the applications of biochar and biofertilizer could decrease soil available Cd concentration, increase soil microbial community diversity, and reduce the damage of Cd stress.


# Introduction

Cd contamination caused by sewage irrigation and application of fertilizers and pesticides is very common in the farmlands in China (Qian et al. 2005; He et al. 2013; Ye et al. 2015). Great Cd accumulation negatively impacts soil biochemical properties (Huang et al. 2016; Meier et al. 2017; Nahar et al. 2020) and microbial activity (Zeng et al. 2019c). However, soil enzymes play important role in various biochemical processes. When the soil is contaminated by Cd, soil urease, phosphatase, and catalase activities are obviously decreased (Kandeler et al. 1996). For example, Wang et al. (2007) have shown that the phosphatase activity in Cd-contaminated soil (10 $mg \cdot kg^{-1}$) could be obviously decreased, while no difference could be found in the urease activity. Moreover, soil microbes, an essential part of the ecosystem, are also greatly impacted by Cd contamination (Harris 2009). Fritze (2000) has shown that the number of *Actinomycetes* and fungi could be decreased in Cd-treated soil. Heavy metals mainly accumulate in organic materials on soil surface. They always inhibit the activities of microbes through damaging the cell membranes and DNA structure and influencing cell functions (Choi et al. 2009; Kamal et al. 2010; Ji et al. 2012), and cause toxicity to microbes. Therefore, for Cd-contaminated soils, it is indispensable to find an eco-friendly remediation method to improve the degraded soil ecosystem.

Biotic and abiotic remediation are important methods for the remediation of heavy metal-contaminated soils (Guo et al. 2006). Abiotic remediation includes electro kinetic remediation, soil replacement, soil isolation, chemical leaching, organic matter fixation, etc. (Guo et al. 2006). Biochar is an environmentally friendly adsorbent that could be used for abiotic remediation, with the characteristics of low cost and high efficiency (Mu et al. 2018). It could reduce soil available Cd concentration, and increase soil pH

(Bandara et al. 2017; Mu et al. 2018), organic carbon concentration (Liu et al. 2017), enzyme activity (Sun et al. 2014), and biochemical properties. Bioremediation uses microorganisms or plants to detoxify heavy metals or remove from soils. Biofertilizer, an atoxic multifunctional fertilizer, could be used in the inoculation with functional bacteria to enhance soil fertility and quality, and reduce heavy metal toxicity (Vassilev et al. 2015). Previous study has reported that the Cd-removal rate after inoculating with *Bacillus* in soil reached more than 80%, and the adsorption capacity was 62.0–159.5 mg Cd (Zouboulis et al. 2004). Moreover, the application of modifiers is certain to impact soil microbes and enzyme activity. Chen et al. (2019) have shown that the application of biochar (40 t $ha^{-1}$) could increase phosphatase and catalase activities, and change the microbial biomass by changing soil carbon and nitrogen. In the remediation of Pb- and As-contaminated soils using biochar, the relative abundance of *Actinomycetes* could be increased obviously, while the relative abundances of *Acidbacteria* and *Chloroflexi* were decreased (Ahmad et al. 2014b); however, the urease activity could be increased obviously after application of biofertilizer (Shang et al. 2017). Although biotic and abiotic remediation have been gradually adopted for the remediation of heavy metal-contaminated soils, the micro-mechanisms in the remediation with biochar and biofertilizer needs further studies.

The planting area of cotton in China is as high as 3339.2 kha in 2019, accounting for 9.98% of the total area of cotton fields in the world. The planting area of cotton in Xinjiang Province is up to 2540.5 kha, accounting for 70% of the total area in China. In recent years, due to the unreasonable agricultural activities and the rapid development of industry, twenty percent of the farmland in Xinjiang Province has been contaminated by heavy metals (Wang et al. 2016; Jing et al. 2018). The potential ecological risk index (RI) of soil Cd in Karamay (Wang et al. 2016) and Yanqi (Mamut et al. 2018), Xijiang Province are as high as 185.05 and 11.34, respectively. Therefore, this study selected biochar and biofertilizer as modifiers to explore whether the modifiers could increase the diversity of soil bacterial community in Cd-contaminated cotton field, and to clarify the micro-mechanism of modifiers in the remediation of Cd-contaminated soil. We hypothesized that: 1) the biochemical properties and microbial diversity might have difference in the soils with different concentrations of Cd; and 2) the two modifiers might change soil enzyme activity and microbial structure, and the effects of biochar and biofertilizer on soil microbial community might be different.

# Materials And Methods

### Experimental site

This study was conducted at the Experimental Station of Agricultural College of Shihezi University, Xinjiang Province, China (44°18'42.37"N, 86°03'20.72"E), where there has a temperate arid continental climate. The average annual temperature is 7.5 - 8.2 ℃. During the experiment, the lowest temperature was 12.1℃, and the highest temperature was 27.3℃. The annual sunshine duration is 2318 - 2732 h, the frost-free period is 147 - 191 d, the annual rainfall is 180 - 270 mm, and the annual evaporation is 1000 - 1500 mm (Mamut et al. 2018). The soil texture is sandy loam.

## Preparation of experimental materials

Soils were collected from the cotton field with twenty-five years of continuous cropping in the study area. After removing residues, soils were air-dried and sieved through a 5 mm sieve to determine soil biochemical properties (Table 1). Solid $CdCl_2 \cdot 5H_2O$ was mixed with the soil to prepare soil samples with different Cd concentrations. Solution (1.2 $g \cdot L^{-1}$of $Cd^{2+}$) of 10 mL, 20 mL, and 40 mL were mixed with 12 kg soil to prepare the soil samples with 0.25 (H0), 1 (H1), 2 (H2), and 4 (H3) $mg \cdot kg^{-1}$ exogenous $Cd^{2+}$. These levels were equivalent to three, six, and eleven times of the average soil Cd concentration globally (Adriano 2001; Rawlins et al. 2012). Finally, soil samples were stored for 60 d for subsequent tests (Bashir et al. 2019).

Biochar (B) was prepared using cotton stalk according to Lehmann et al. (2011). Biochar was air-dried and sieved through a 0.2 mm sieve, and then the biochemical properties, including pH, organic matter, total nitrogen, total phosphorus, and total potassium, were measured (Sun et al. 2007). Dried biochar of 0.5 g was accurately weighed and digested with a mixture of nitric acid and muriatic acid (v:v=1:3) (Guaranteed reagent). The Cd concentration of biochar was determined using the Hitachi Z2000 graphite atomic absorption spectrophotometer (PinAAcle900T, PerkinElmer, USA) (Table 1). The biofertilizer containing dominant functional bacteria of *Bacillus* (J) was purchased from Shandong lvlong Biotechnology Co., Ltd, China, and the biochemical properties were measured according to the *Standards of Microbial Inoculants in Agriculture* (SMIA, National Standard of China, GB20287-2006). Biofertilizer was sieved through a 0.2 mm sieve. The Colony-Forming Units (CFU) was greater than or equal to 20 $billion \cdot g^{-1}$, and the miscellaneous bacteria rate was less than 0.4%. The moisture was less than 10%, and pH was 7.8. Total Cd concentration was 0.0001 $mg \cdot L^{-1}$, total nitrogen concentration was 900 $mg \cdot L^{-1}$, and total organic carbon concentration was 3791 $mg \cdot L^{-1}$.

## Experimental design

The experiment employed a randomized block design with two factors. Four levels of soil Cd concentration were set, which were 0.25 (H0), 1 (H1), 2 (H2), and 4 (H3) $mg \cdot kg^{-1}$, and two modifiers were applied (T means no modifier). There were twelve treatments in total, and each treatment had five replicates (Table 2). Cd-contaminated soil (12 kg) was mixed with 3% (w/w, 46.8 $t \cdot ha^{-1}$) biochar and 1.5% (w/w, 0.45 $t \cdot ha^{-1}$) biofertilizer separately, and transferred into ceramic pots with a height of 40 cm and a diameter of 25 cm. After that, they were stored in a greenhouse (25 °C) for one week. Soils were irrigated with deionized water to keep the water holding capacity at 60%. Rhizosphere soil samples were collected after 120 days of cultivation. Part of the soil samples was air-dried for the analysis of soil pH, enzymes, total Cd concentration, and available Cd concentration; the other was sieved through a 2 mm sieve and stored at -80 °C for microbial diversity analysis.

## Determination of soil indices

## Soil biochemical indices

Soil pH was measured with a pH meter (Thermo Orion 920A, Thermo Orion, USA) (soil: water = 1: 5). Soil organic carbon was measured with the wet oxidation method (Mulder et al. 2001). Soil total nitrogen concentration was measured with a semi-micro-Kjeldahl procedure (Bandara et al. 2017). Soil available Cd concentration was measured with the diethylenetriaminepentaacetic acid (DTPA) extraction method using a graphite furnace atomic absorption spectrophotometer (Z2000, Hitachi, Tokyo, Japan) (Bandara et al. 2017). Soil urease activity was measured with indophenol-blue colorimetry; invertase activity was measured using 3,5-dinitrosalicylic acid colorimetry; and alkaline phosphatase activity of disodium phenyl phosphate was measured using colorimetric method (Bandara et al. 2017).

To determine the water holding capacity, damp soil of 50 g was accurately weighed and transferred into the tube with mesh base (3.5 cm in diameter and 5 cm in length). Then, the tube was placed in a container with water and allowed to be wetted by capillary action. When the soil surface became glossy, soil cores were removed from the water and allowed to drain until they stopped dripping. The soil in the cores was then gently removed and weighed. The water holding capacity of the soil was determined as the weight of water held in the soil cores compared with the oven-dry weight (105 °C) of the sample (Jamal and Mark 2020).

### Analyses of the structure and diversity of soil microbial community

DNA was extracted from soil samples using the E.Z.N.A.® Soil DNA Kit (OMEGA, USA). Soil samples stored at -80 °C were weighed to extract the total DNAs according to the instructions of the kit. After that, the DNAs were stored at -80 ℃. PCR amplification was conducted using 0.8 μL of bacterial synthetic primers (Forward Primer: ACTCCTACGGGAGGCAGCAG; and Reverse Primer: GGACTACHVGGGTWTCTAAT). 16S rRNA gene V3-V4 was targeted using the primer set. The product was cycled 30 times at 95 °C. The PCR products were detected using 2% agarose gel electrophoresis, and then AxyPrep DNA Gel Extraction Kit and Quantus ™ Fluorometer were used to purify and quantify the products. Illumina MiSeq System (Milq PE300 platform of Illumina company, USA) was used for sequencing by Shanghai Meiji Technologies Corporation, China.

### Data process and analysis

Sequences were clustered at a 97% similarity level using Quantitative Insights Into Microbial Ecology (QIIME) package (version 1.9.1), and OUTs were obtained, with 0.005% as threshold. To compare the species richness of soil bacteria after applying biochar and biofertilizer, the total community richness was calculated using different statistical methods, including Chao1, Simpson, and Coverage indices. The structural equation model (SEM) analysis was performed using AMOS 20.0 software (AMOS, IBM, USA) with a maximum-likelihood method (Chen et al. 2019).

## Results

### Soil biochemical properties

The applications of biochar and biofertilizer had different effects on soil biochemical properties (Table 3). After the application of Cd, the soil C/N ratios in the H1T, H2T, and H3T treatments decreased by 3.91%, 7.31%, and 14.55%, respectively, while the soil EC increased by 90.38%, 61.54%, and 28.85%, respectively ($P < 0.05$), compared with those in the control group (H0T treatment).

The soil pH, C/N ratio, and EC could be increased after the applications of biochar and biofertilizer (Table 3). The soil C/N ratio and EC in the biochar and biofertilizer treatments were higher than those in the control group. For example, soil pH, C/N ratio, and EC in the H2B treatment increased by 10.78%, 56.11%, and 45.24%, respectively, and those in the H2J treatment increased by 6.02%, 76.87%, and 41.96%, respectively, compared with those in the H2T treatment ($P < 0.05$).

Regression analysis showed that the application of Cd had no effect on soil pH, C/N ratio, and EC ($P > 0.05$), and the application of modifiers greatly impacted soil C/N ratio ($P < 0.05$). The modifiers and Cd greatly impacted soil C/N ratio ($P < 0.05$), but no differences were found in soil pH and EC ($P > 0.05$).

### Effects of biochar and biofertilizer on soil available Cd

The soil available Cd concentration in the H1T, H2T, and H3T treatments increased after the application of exogenous Cd ($P < 0.05$) (Fig. 1). The highest soil available Cd concentration was 1.1320 $mg \cdot kg^{-1}$ which was found in the H3T treatment. The soil available Cd concentration decreased in the biochar (H1B, H2B, and H3B) and biofertilizer (H1J, H2J, and H3J) treatments ($P < 0.05$). Soil available Cd concentration in the H0B and H0J treatments decreased by 88.26% and 95.96%, respectively ($P < 0.05$), compared with that in the H0T treatment. Soil available Cd concentration in the H1B and H1J treatments decreased by 52.32% and 68.54%, respectively ($P < 0.05$), compared with that in the H1T treatment. Soil available Cd concentration in the H2B and H2J treatments decreased by 36.30% and 65.17%, respectively ($P < 0.05$), compared with that in the H2T treatment. Soil available Cd concentration in the H3B and H3J treatments decreased by 60.24% and 74.34%, respectively ($P < 0.05$), compared with that in the H3T treatment.

### Effects of modifiers and Cd on soil enzyme activities

Soil enzyme activity decreased after the application of exogenous Cd (Fig. 2). Soil invertase activity in the H1T, H2T, and H3T treatments decreased by 18.36%, 37.25%, and 45.07, respectively ($P < 0.05$), compared with that in the H0T treatment. Soil alkaline phosphatase activity in the H2T and H3T treatments decreased by 7.21% and 35.53%, respectively ($P < 0.05$), and soil urease activity decreased by 18.54% and 27.33%, respectively ($P < 0.05$), compared with those in the H0T treatment. The activities of soil invertase, alkaline phosphatase, catalase, and urease in the H3T treatment were the lowest, which decreased by 45.07%, 35.53%, 68.01%, and 27.33%, respectively ($P < 0.05$), compared with those in the H0T treatment.

Soil invertase enzyme activity increased after the applications of biochar and biofertilizer (Fig. 2A). Soil invertase activity in the H1B and H1J treatments increased by 17.51% and 61.29%, respectively, compared with that in the H1T treatment ($P < 0.05$). The activity of alkaline phosphatase also increased

after the applications of biochar and biofertilizer, and difference was found between biochar and biofertilizer treatments ($P < 0.05$). For example, soil alkaline phosphatase activity in the H3B and H3J treatments increased by 16.16% and 43.74%, respectively ($P < 0.05$), compared with that in the H3T treatment (Fig. 2B). Soil catalase activity in the H1B and H1J treatments increased by 23.08% and 53.85%, respectively ($P < 0.05$), compared with that in the H1T treatment (Fig. 2C). Soil urease activity in the H2B and H2J treatments increased by 13.27% and 28.94%, respectively ($P < 0.05$), compared with that in the H2T treatment (Fig. 2D).

### Effects of the applications of biochar and biofertilizer on soil microbial community diversity

Coverage indices showed that the sequencing coverage indices of each sample was more than 97.97%, which could reflect the reliability of this sequencing result (Table 4). The Simpson index increased after the applications of biochar and biofertilizer ($P < 0.05$). The Simpson's diversity index in the H2B and H2J treatments increased by 66.67% and 50.88%, respectively ($P < 0.05$), compared with that in the H2T treatment. The Chao1 index in the biochar and biofertilizer treatments increased. The Chao1 index in the H0B and H0J treatments increased by 20.21% and 17.66%, respectively ($P < 0.05$), compared with that in the H0T treatment; similar trends were also found in the H3B and H3J treatments.

Regression analysis showed that Cd and modifiers greatly impacted Chao1 index and Coverage index ($P < 0.05$), but there was no difference in the Simpson's diversity index ($P > 0.05$). Moreover, the applications of Cd and modifiers had a combined effect on soil microbial diversity ($P < 0.05$).

### Effect of the applications of biochar and biofertilizer on the relative abundance of soil bacteria

The applications of modifiers and Cd could obviously impact the relative abundance of bacteria (Fig. 3). In this study, *Acidobacteria*, *Proteobacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Bacteroidetes*, and *Actinobacteria* were the dominant phyla, accounting for 91.27% - 95.52% of bacteria in soil samples. The relative abundance of *Acidobacteria* in the H1T and H3T treatments decreased by 10.77% and 14.92%, respectively, and the relative abundance of *Chloroflexi* decreased by 36.94% and 39.64%, respectively, compared with those in the H0T treatment. However, the relative abundances of *Acidobacteria* and *Gemmatimonadetes* in the H0B treatment increased by 12.63% and 2.09%, respectively, compared with those in the H0T treatment. Similar trends were found in the relative abundances of *Acidobacteria* and *Gemmatimonadetes* in the H3B treatment. The relative abundances of *Acidobacteria* and *Proteobacteria* in the H0B treatment increased by 16.88% and 11.58%, respectively, and the relative abundances of *Acidobacteria* and *Gemmatimonadetes* in the H2J treatment increased by 10.48% and 0.39%, respectively, compared with those in the H2T treatment. The relative abundances of *Acidobacteria* and *Gemmatimonadetes* in the H3J treatment also increased by 20.83% and 6.12%, respectively, compared with that in the H3T treatment.

### Relationship between soil microbial diversity and biochemical properties

Redundancy analysis (RDA) revealed the relationship between soil microbial community diversity and soil biochemical properties (Fig. 4). The first principal component of RDA accounted for 46.52% of the total variation, and the second principal component accounted for 26.49% of the total variation. So all variables could be well explained. The results showed that soil biochemical properties (pH, C/N ratio, and soil enzyme activity) and the relative abundances of *Acidobacteria* and *Proteobacteria* were closely in the first quadrant, indicating that the relative abundances of *Acidobacteria* and *Proteobacteria* were greatly impacted by soil biochemical properties. In the third quadrant, the longest arrow for soil available Cd concentration indicated that soil available Cd concentration had the greatest impact on soil microbial diversity. Soil available Cd had a large angle with soil biochemical properties (pH, C/N ratio, and soil enzyme activity) and the relative abundances of *Acidobacteria* and *Proteobacteria*, indicating that soil available Cd negatively impacted soil biochemical properties and the relative abundances of *Acidobacteria* and *Proteobacteria*. H3B and H2B treatments were also closely located in the third quadrant, indicating that the bacterial community structure in the H3B and H2B treatments were similar.

To determine the main factors responsible for the change of microbial community structure and available Cd concentration in Cd-contaminated soil, the direct and indirect effects of soil biochemical properties (soil enzyme and pH) and microbial diversity on soil available Cd were determined using structural equation model (SEM) (Fig. 5). The results showed that soil urease and alkaline phosphatase activities had negative correlations with soil available Cd ($\beta$ = -0.752 and $\beta$ = -0.757, $P < 0.001$), indicating that soil available Cd could suppress soil urease and alkaline phosphatase activities. Soil available Cd had negative correlation with microbial diversity ($\beta$ = -0.743, $P < 0.001$), indicating that exogenous Cd could decrease soil microbial diversity (Fig. 3). However, urease activity had positive correlation with soil microbial diversity ($\beta$ = -0.829 and $\beta$ = -0.757, $P < 0.001$), indicating that soil urease activity could increase soil microbial diversity.

# Discussion

The effects of biochar and biofertilizer on soil biochemical properties was evaluated in this study, and the relationships between Cd and soil biochemical properties were also measured. Previous studies have shown that the bioavailability of Cd in the soil may increase when soil pH decreases; while the soil adsorption of Cd may increase when soil pH increases (Ardestani et al. 2016; Yin et al. 2020). In this study, soil pH and EC increased after the application of biochar, which is consistent with the results of Qureshi et al. (2004) and Bandara et al. (2017). The increase of soil pH may be due to the conversion of basic cations (such as Ca, Mg, K, and Na) in biochar into oxides, hydroxyl oxides, and carbonates (ash), which adhere to biochar during pyrolysis (Ahmad et al. 2012a; Ahmad et al. 2014b). The dissolution of the alkaline substances and the application of microbial fertilizer could also increase soil pH. In this study, soil pH increased by 0.95 unit after the application of biofertilizer, which is consistent with the results of Blaya et al. (2015). It may be due to the high pH of the biofertilizer. Besides, biochar could accelerate the dissolution of most salts in the soil (Bandara et al. 2017), resulting in the increase of soil EC (Yang et al. 2016). The increase of soil EC after the application of biofertilizer may be due to the change in total dissolved solids (TDS) concentration and the interaction of biofertilizer with inorganic and

organic ions in the soil (Iftikhar et al. 2019). Moreover, El-Kherbawy et al. (1989) have showed that the concentration of available heavy metals in the soil with pH greater than 7.2 was lower than that in the acid soil, indicating that a high soil pH could positively impact Cd fixation, and soil pH could increase after the application of biochar (Chen et al. 2019). In this study, the soil available Cd concentration decreased after the application of biochar ($P < 0.05$). Cd ions precipitate with the alkaline ions in the soil, which reduces the soil available Cd (Mu et al. 2018). The oxygen-containing functional groups of biochar (carboxyl, carbonyl, and ester) (Table 1) induce Cd fixation, and absorb Cd on the surface through cation exchange (Li et al. 2017). *Bacillus* subtilis in biofertilizer is a gram-positive, rod-shaped, and aerobic bacterium in the soil. Due to the different cell wall structures, *Bacillus* subtilis is more likely to bind with metals than gram-negative bacterium. Teichoic acid associated with the cell wall is unique to gram-positive cells, and its phosphate group is a key component of metal uptake (Matyar et al. 2008; Çolak et al. 2011). In this study, the soil available Cd concentration decreased by 74.34% ($P < 0.05$) after the application of biofertilizer with *Bacillus* as the main component (Fig. 1). This is consistent with the results of Li et al. (2019).

Soil enzyme activity is an important biological indicator to evaluate soil quality, especially to the evaluation of soils contaminated by heavy metals (Wang et al. 2020). The urease, alkaline phosphatase, and catalase are the most sensitive to heavy metals (Papa et al. 2010; Hu et al. 2014). Microorganisms secrete large amounts of urease. The decomposition of urease and the formation of bicarbonate, ammonium, and hydroxyl ions could increase the pH. All the urease-producing isolates could increase the pH of medium, which may greatly impact the bioavailability of soil heavy metals (Jalilvand et al. 2020). The reason for the decrease of soil enzyme activity after the application of exogenous Cd is that the molecular reaction between heavy metals and enzyme-substrate complexes or protein active groups denatures enzyme protein and reduces enzyme activity (Nannipieri 1995; Cui et al. 2019). Yang et al. (2016) have shown that soil urease, alkaline phosphatase, and catalase activities could be increased after the application of biochar ($P < 0.05$). In this study, soil urease, phosphatase, catalase, and sucrase activities increased by 16.55%, 15.51%, 31.33%, and 24.50%, respectively ($P < 0.05$), after the application of biochar. It may be due to that the application of biochar improves the soil biochemical properties, creating a good soil micro-environment for soil microbes' growth and metabolism. Thereby, soil enzyme activities are increased (Jaiswal et al. 2018; Palansooriya et al. 2019). Biofertilizer (*Bacillus*) also increased soil enzyme activity in this study, which is similar to the results of Zhang et al. (2015). It may be due to that the application of biofertilizer promotes the growth of some soil microbes, and the reproduction of microbes reduces the activity of Cd and increases the activity of soil enzymes (Sun et al. 2007). Urease, relating to soil pH, plays an important role in the conversion of soil nitrogen. Urease and sucrase are related to the cycling of nutrients needed by microbes (Makoi 2008). Catalase could be used to indicate soil redox capacity. Phosphatase is an enzyme involved in organophosphorus mineralization (Palansooriya et al. 2019). The activities of these enzymes could be used for the evaluation of Cd contamination. In this study, soil alkaline phosphatase and urease activities had a negative correlation with soil available Cd concentration ($P < 0.01$) (Fig. 5), which is similar to the results of Ahmad et al. (2012a) and Hu et al. (2014).

Heavy metal stress not only negatively impacts soil biochemical properties, but also causes changes in size, composition, and activity of soil microbial communities (Deng et al. 2015). In this study, the application of Cd reduced the relative abundance of *Chloroflexi* (Fig. 3). It may be due to the difference in the absorption of heavy metals by soil microbes (Brookes 1995). Besides, the soil carbon and nitrogen cycle is the main factor affecting soil microbial community (Peralta et al. 2013). The range of soil C/N ratio of 3.5 - 19.5 is the most beneficial for the growth and composition of soil microbes (Ghani et al. 2013). It is reported that biochar and biofertilizer are rich in nutrients. When biochar and biofertilizer are applied to the soil, the carbon and nitrogen required by soil microbes greatly enrich (Liu et al.2017; Palviainen et al. 2018). In this study, the C/N ratio of soil was 16.41 - 18.66 after the applications of biochar and biofertilizer (Table 3), indicating that it was a favorable condition for microbial community. Besides, the application of biochar increased soil microbial diversity (Table 4) and the relative abundance of *Acidobacteria*. Compared with the treatments without modifiers, the application of biochar obviously impacted the microbial diversity in Cd-contaminated soil. It may be due to the increase of soil C/N ratio caused by the high nutrient concentration of biochar (Hass et al. 2012; Meier et al. 2017a). Moreover, the variations in soil microbial community structure may also be due to the reduction of soil available Cd concentration (Fig. 5). Jambhulkar et al. (2009) have found that the number of soil bacteria increases from 18 to $9.8\times10^7$ CFU·$g^{-1}$ after the application of biofertilizer, which is similar to the results of our study. In this study, the Cd ions in the soil were adsorbed and fixed after the applications of biochar and biofertilizer, thereby the Cd toxicity could be reduced and the microbial diversity could also be changed. The dominant phylum (*Proteobacteria* and *Cyanobacteria*) in the soil is related to specific soil enzyme. These microbes absorb heavy metal ions in the contaminated soil. Thus, the activity of soil enzymes could be increased, and the soil quality could be improved (Zeng et al. 2019c).

Redundancy analysis and SEM showed that soil microbial diversity was closely related to soil enzyme activity, while soil available Cd had negative correlation with microbial diversity and soil enzyme ($P < 0.001$), indicating that the application of modifiers could reduce soil available Cd concentration and Cd toxicity to the ecosystem, which is consistent with the results of Hu et al. (2014).

# Conclusion

Exogenous Cd contamination could decrease soil quality through decreasing soil C/N ratio, enzyme activity, and microbial activity. The application of modifiers (biochar and biofertilizer) could positively impact soil biochemical properties (pH, EC, C/N ratio, and urease and alkaline phosphatase activities), leading to the increase of soil microbial diversity and the relative abundances of *Acidobacteria* and *Gemmatimonadetes*. Thus, soil available Cd could be greatly fixed and soil available Cd concentration could be greatly decreased.

# Declarations

**Author contribution** Yongqi Zhu analyzed the data and wrote the manuscript; Tian Tian, Jingang Wang, Jianghui Song and Xiaoyan Shi did the experiment and collected the data; Xin Lv revised the manuscript;

Haijiang Wang designed the experiment and revised the manuscript.

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**Ethics approval** This study does not involve human participants.

**Consent to participate** This study does not involve human participants nor human data or human tissue.

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**Conflict of interest** The authors declare no conflict of interest and no financial and nonfinancial competing interests.

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# Tables

**Table 1** Biochemical properties of biochar and soil.

| Property | Biochar | Soil |
|---|---|---|
| pH | 9.50 | 7.76 |
| Total nitrogen ($g \cdot kg^{-1}$) | 0.89 | 0.46 |
| Total P ($g \cdot kg^{-1}$) | 2.54 | 0.82 |
| Organic matter ($g \cdot kg^{-1}$) | 625 | 14.73 |
| Total K ($g \cdot kg^{-1}$) | 8.62 | 246.83 |
| Total Cd ($mg \cdot kg^{-1}$) | 0.002 | 0.25 |
| Available Cd | - | 0.121 |
| Total salinity ($g \cdot kg^{-1}$) | - | 3.36 |
| Carboxyl ($mmol \cdot g^{-1}$) | 0.20 | - |
| Lactone ($mmol \cdot g^{-1}$) | 0.25 | - |
| Phenolic hydroxyl ($mmol \cdot g^{-1}$) | 0.21 | - |

**Table 2** Amount of Cd, biochar, and biofertilizer in each treatment

| Treatments | Cd ($mg \cdot kg^{-1}$) | Biochar (%) | Biofertilizer (%) |
|---|---|---|---|
| H0T | 0.25 | 0 | 0 |
| H0B | 0.25 | 3% | 0 |
| H0J | 0.25 | 0 | 1.5% |
| H1T | 1 | 0 | 0 |
| H1B | 1 | 3% | 0 |
| H1J | 1 | 0 | 1.5% |
| H2T | 2 | 0 | 0 |
| H2B | 2 | 3% | 0 |
| H2J | 2 | 0 | 1.5% |
| H3T | 4 | 0 | 0 |
| H3B | 4 | 3% | 0 |
| H3J | 4 | 0 | 1.5% |

T, no modifiers; B, 3% biochar was applied; J, 1.5 % biofertilizer was applied; H0, no Cd; H1, 1 $mg \cdot kg^{-1}$ of Cd was applied; H2, 2 $mg \cdot kg^{-1}$ of Cd was applied; H3, 4 $mg \cdot kg^{-1}$ of Cd was applied.

**Table 3** Effect of the applications of biochar and biofertilizer on soil biochemical properties.

| Cd ($mg \cdot kg^{-1}$) | Modifiers（%） | pH | C/N ratio | EC ($ms \cdot cm^{-1}$) |
|---|---|---|---|---|
| H0 | T | 7.44±0.21 b | 10.54±0.39 d | 2.08±0.02 h |
| | B | 8.49±0.24 a | 16.93±0.78 bc | 2.88±0.02 ef |
| | J | 8.42±0.24 a | 18.51±0.53 a | 2.41±0.02 g |
| H1 | T | 7.23±0.20 b | 9.60±0.38 d | 3.96±0.03 b |
| | B | 8.58±0.25 a | 16.41±0.62 c | 4.91±0.04 a |
| | J | 8.57±0.24 a | 17.62±0.48 abc | 4.96±0.04 a |
| H2 | T | 7.97±0.23 ab | 10.55±0.36 d | 3.36±0.03 c |
| | B | 8.63±0.24 a | 16.47±0.54 c | 4.88±0.04 a |
| | J | 8.45±0.24 a | 18.66±0.48 a | 4.77±0.04 a |
| H3 | T | 7.56±0.21 b | 9.57±0.33 d | 2.68±0.02 gf |
| | B | 8.46±0.21 a | 16.92±0.51 bc | 3.30±0.03 cd |
| | J | 8.57±0.24 a | 17.98±0.40 ab | 3.03±0.05 de |
| Regression Analysis | | | | |
| H | | ns | ns | ns |
| BJ | | ns | * | ns |
| BJ*H | | ns | * | ns |

T, no modifiers; B, 3% biochar was applied; J, 1.5 % biofertilizer was applied; H0, no Cd; H1, 1 $mg \cdot kg^{-1}$ of Cd was applied; H2, 2 $mg \cdot kg^{-1}$ of Cd was applied; H3, 4 $mg \cdot kg^{-1}$ of Cd was applied. Different lowercase letters in the same column indicate significant differences ($P < 0.05$). **, $P < 0.01$; *, $0.01 < P < 0.05$; ns, $P \geq 0.05$.

**Table 4** Changes in microbial diversity after the applications of biochar and biofertilizer

| Cd ($mg \cdot kg^{-1}$) | Modifiers（%） | Diversity index of soil microbial community | | |
|---|---|---|---|---|
| | | Simpson | Chao1 | Coverage |
| H0 | T | 0.0070±0.0002 f | 1801±51.99 e | 0.9853±0.028 a |
| | B | 0.0117±0.0003 c | 2165±62.48 bcd | 0.9819±0.028 a |
| | J | 0.0095±0.0003 d | 2119±61.17 cd | 0.9820±0.028 a |
| H1 | T | 0.0059±0.0002 g | 1964±56.69 de | 0.9835±0.028 a |
| | B | 0.0070±0.0002 f | 2030±58.61 d | 0.9841±0.028 a |
| | J | 0.0128±0.0004 b | 2397±69.22 a | 0.9809±0.028 a |
| H2 | T | 0.0057±0.0002 g | 2083±60.13 cd | 0.9824±0.028 a |
| | B | 0.0095±0.0005 d | 2453±70.81 a | 0.9800±0.028 a |
| | J | 0.0086±0.0004 de | 2277±65.74 abc | 0.9799±0.028 a |
| H3 | T | 0.0078±0.0004 ef | 2333±67.36 ab | 0.9797±0.028 a |
| | B | 0.0090±0.0003 d | 2251±64.99 abc | 0.9811±0.028 a |
| | J | 0.0315±0.0009 a | 2251±64.98 abc | 0.9806±0.028 a |
| Regression Analysis | | | | |
| H | | ns | * | ** |
| BJ | | ns | * | ** |
| BJ*H | | ns | * | ** |

T, no application of modifiers; B, 3% biochar was applied; J, 1.5% biofertilizer was applied; H0, no application of Cd; H1, 1 $mg \cdot kg^{-1}$ of Cd was applied; H2, 2 $mg \cdot kg^{-1}$ of Cd was applied; H3, 4 $mg \cdot kg^{-1}$ of Cd was applied. Different lowercase letters in the same column indicate significant differences ($P < 0.05$) in Cd content. **, $P < 0.01$; *, $0.01 < P < 0.05$; ns, $P \geq 0.05$.

# Figures

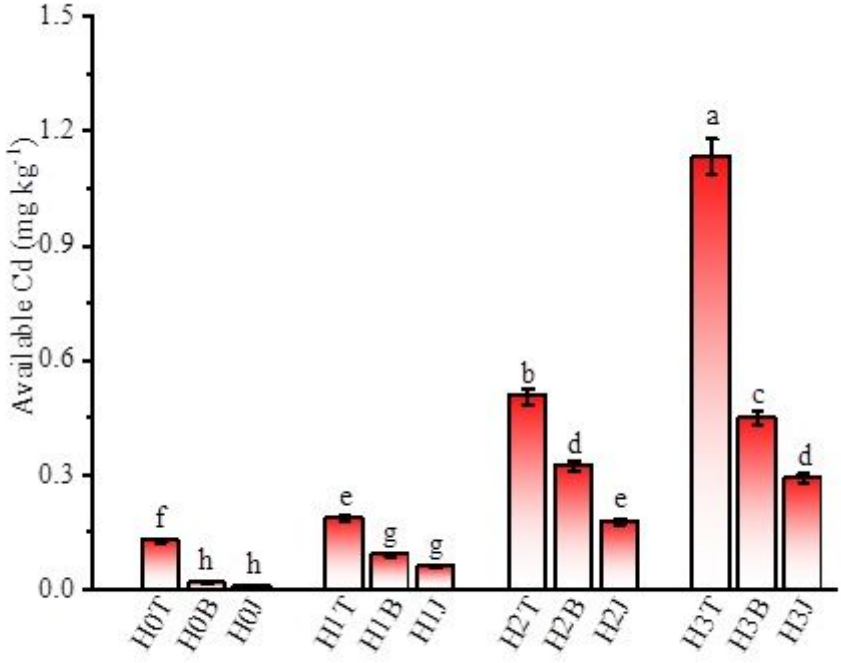



**Figure 1**

Effect of the applications of biochar and biofertilizer on soil available Cd. Values show the mean of five replicates ± SE. Means followed by same small letters are not significant different at P < 0.05 by using the Duncan test

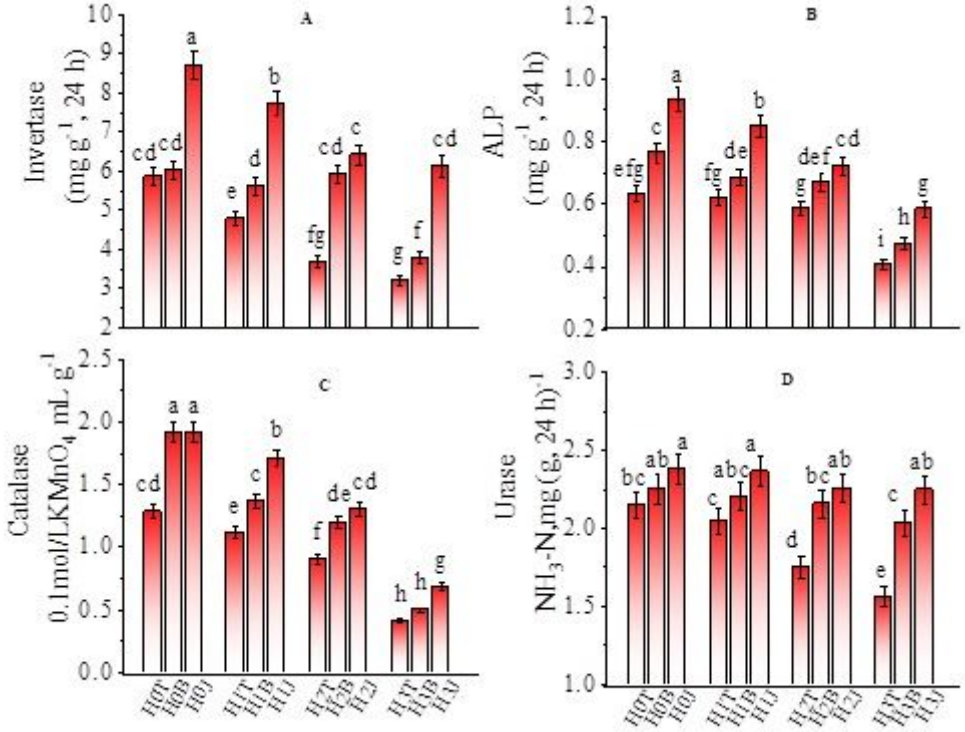
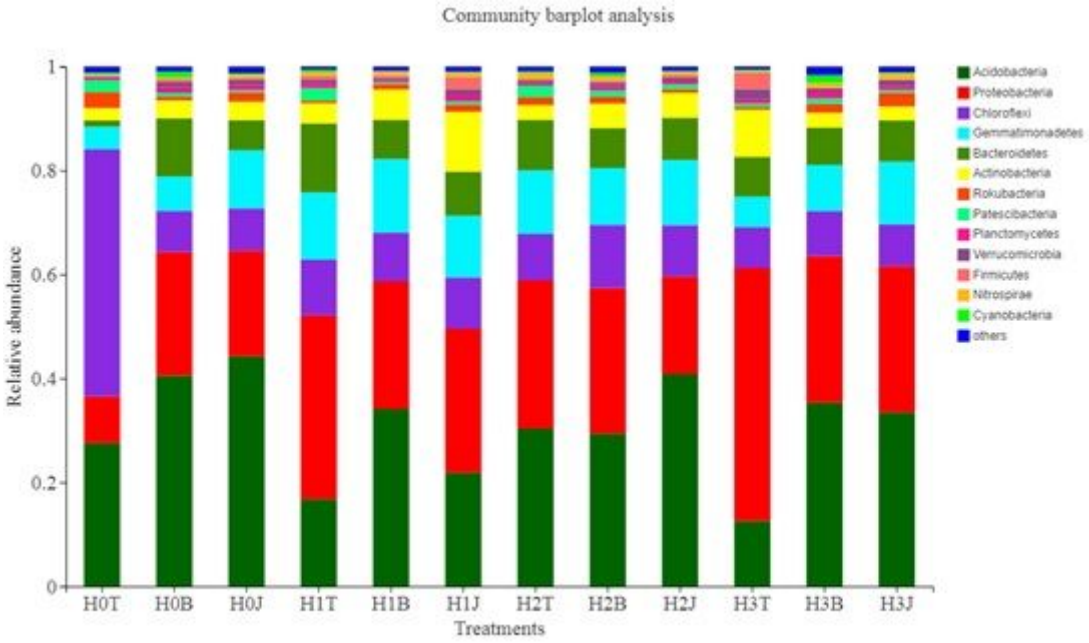



**Figure 2**

Effect of the applications of biochar and biofertilizer on soil invertase (A), urease (B), alkaline phosphatase (C), and catalase (D) activities. Values show the mean of five replicates ± SE. Means followed by same small letters are not significant different at $P < 0.05$ by using the Duncan test




## Figure 3

Relative abundances of soil microbes after the applications of biochar and biofertilizer

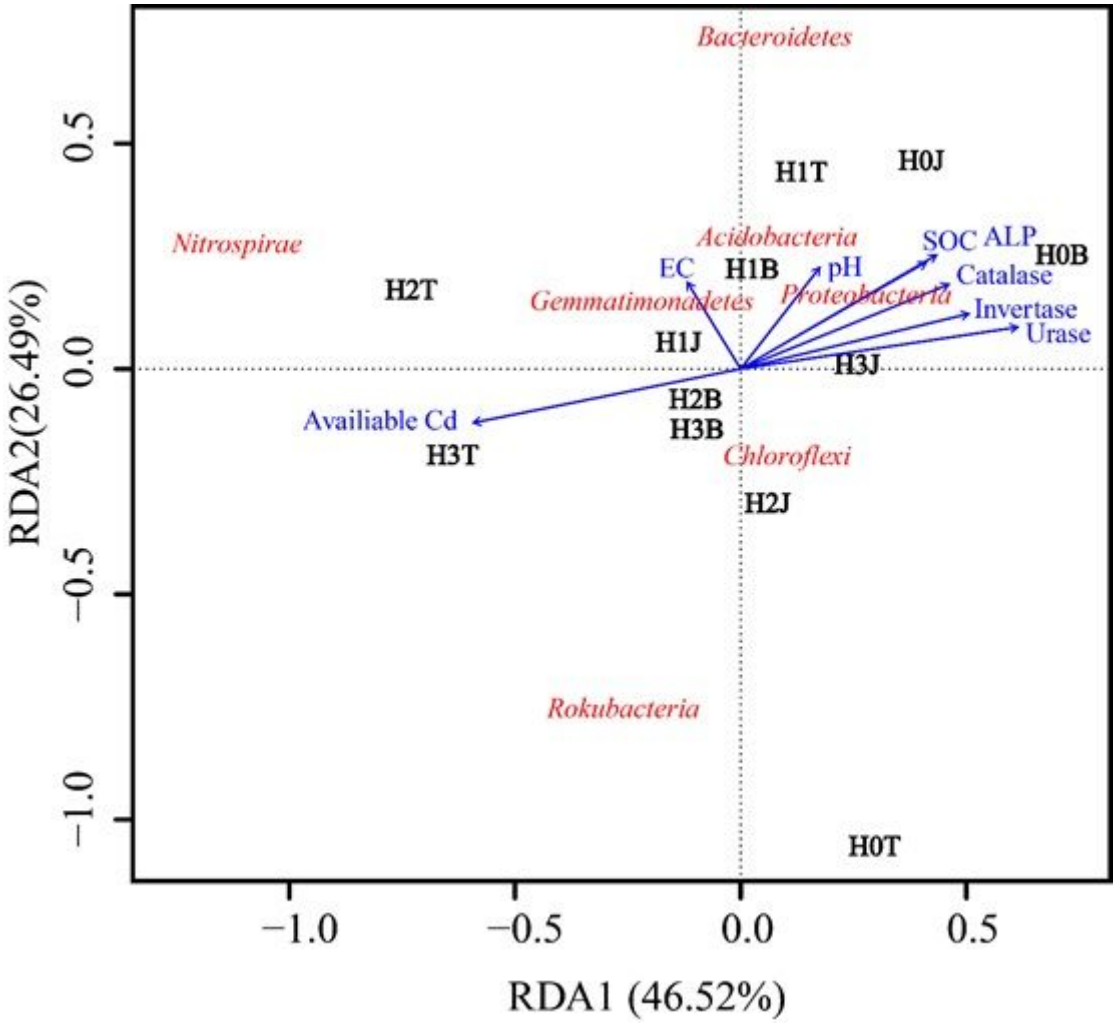



**Figure 4**

RDA analysis between soil biochemical properties and soil microbial structure at phylum level. Abbreviations: soil available Cd, available Cd; pH, soil pH; Urase, soil urase activity; Sucrase, soil sucrase activity; Catalase, soil catalase activity; ALP, soil alkaline phosphatase activity.

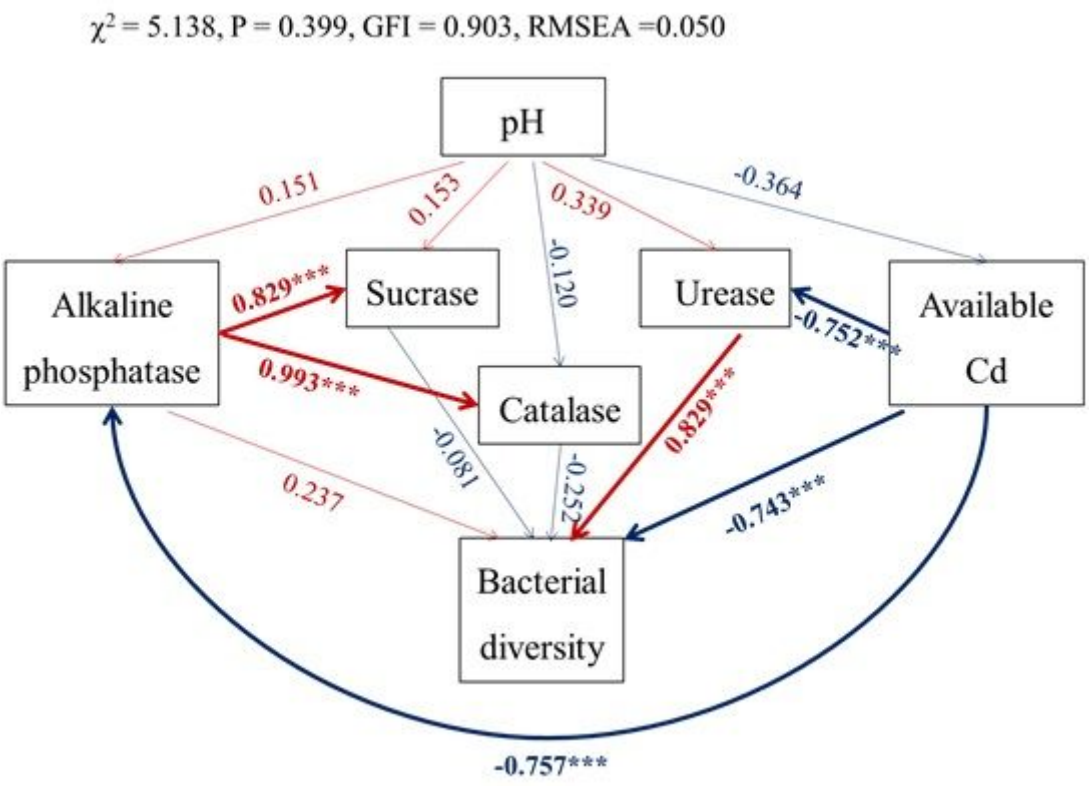



**Figure 5**

Structural equation modeling (SEM) showing the relationship between soil properties. Blue lines indicate negative relationships, while red lines indicate positive relationships. The microbial diversities are represented by the Chao1 and Simpson indexes based on the rarified same sequencing depth. The width of arrows indicates the relevance of significant standardized path coefficients (P < 0.05). ***P < 0.001, **P < 0.01.

## Supplementary Files

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