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Responses of ammonia-oxidizing microorganisms to biochar and compost amendments of heavy metals-polluted soil

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ABSTRACT

Heavy metal pollution affects soil ecological function. Biochar and compost can effectively remediate heavy metals and increase soil nutrients. The effects and mechanisms of biochar and compost amendments on soil nitrogen cycle function in heavy-metal contaminated soils are not fully understood. This study examined how biochar, compost, and their integrated use affected ammonia-oxidizing microorganisms in heavy metal polluted soil. Quantitative PCR was used to determine the abundance of ammonia-oxidizing archaea (AOA) and bacteria (AOB). Ammonia monooxygenase (AMO) activity was evaluated by the enzyme-linked immunosorbent assay. Results showed that compost rather than biochar improved nitrogen conversion in soil. Biochar, compost, or their integrated application significantly reduced the effective Zn and Cd speciation. Adding compost obviously increased As and Cu effective speciation, bacterial 16S rRNA abundance, and AMO activity. AOB, stimulated by compost addition, was significantly more abundant than AOA throughout remediation. Correlation analysis showed that AOB abundance positively correlated with NO_3^--N ($r = 0.830$, $P < 0.01$), and that AMO activity had significant correlation with EC ($r = -0.908$, $P < 0.01$) and water-soluble carbon ($r = -0.868$, $P < 0.01$). Those seem to be the most vital factors affecting AOB community and their function in heavy metal-polluted soil remediated by biochar and compost.

Introduction

Contamination with heavy metals is of great environmental concern and has serious negative effects on environmental quality and human health (Bai et al., 2020; Tóth et al., 2016; Yuan et al., 2021). Human activities, including modern industrial production, have intensified the global problem of heavy metals pollution (Lu et al., 2015; Yuan et al., 2021). Natural behaviors and mining activities directly and indirectly pollute

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agricultural soil with heavy metals. Heavy metals in farmland mainly come from sewage irrigation, mining and smelting, fertilizer application and sludge reuse (Jin et al., 2016). The increasing pollution issue cause that the government and the public radically cannot afford the risk of losing more valuable farmland. Therefore, it is essential to take effective measures to remediate soils contaminated by heavy metals and recover their function.

A number of remediation techniques such as physical, chemical, and biological remediation have been used to address to heavy metal pollution (Al-Shannag et al., 2015; Huang et al., 2020; Li et al., 2019a; Raklami et al., 2021; Tang et al., 2020). Some are costly and potentially hazardous for soil ecology (Hua et al., 2017). Selecting low-cost, environmentally-friendly and new materials is imperative research task. As an effective remediation material, biochar has demonstrated remediation effectiveness by reducing the hazard posed by heavy metal bioavailability (Wang et al., 2018) to agricultural production (Godlewska et al., 2017; Mao et al., 2019). It facilitates land revegetation (Shen et al., 2016) and increases crop yields (O'Connor et al., 2018). Biochar changes soil properties, such as increasing cation exchange capacity, combining nutrients, and promoting nitrogen fixation (Jin et al., 2016). Compost, the product of controlled biodegradation of organic waste, increases soil structure porosity, stimulates microbial activity, thus increases soil water storage and erosion resistance (Sanchez-Monedero et al., 2018). Compost microbial activity affects organic matter degradation, humification, nutrient cycling, and greenhouse gas emissions (Xiao et al., 2017). Compost has the capacity to passivate heavy metals to some extent by changing soil environments and structure (Zhang et al., 2018a).

Applying biochar, and compost, changes the physical and chemical parameters of heavy metal contaminated soil (Huang et al., 2020; Li et al., 2019a; Tang et al., 2020), resulting in changes in microbial community function (Huang et al., 2017; Liu et al., 2020; Yang et al., 2019). Of all the nutrients, nitrogen is one of the most vital for crops (Ge et al., 2018; Van Der Heijden et al., 2016). Ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) are ubiquitous and play a primary role in nitrogen cycling (Li et al., 2019b). Ammonia monooxygenase (AMO) significantly influences ammoxidation which is the rate-determining step of ammonium (NH_4^+-N) to hydroxylamine (NH_2OH) reaction process (Urakawa et al., 2019). Enzyme activity is of utmost importance for accessing microbial community function and soil quality. Heavy metals significantly influence AMO enzyme synthesis (Kuypers et al., 2018; Tang et al., 2019). The literature reports that microbial communities related with nitrogen transformation were affected by heavy metals (Cui et al., 2017). Microbial properties including diversity and abundance of microbial communities in soil, are often used as indicators of heavy metal pollutions, because of their high sensitivity to metal-induced stress (Bai et al., 2020; Song et al., 2018; Tang et al., 2019). Biochar and compost significantly increase NH_4^+-N concentration and levels of microorganism population (DeLuca et al., 2015; Li et al., 2019a). Little is well-understood about ammonia-oxidizing microbial communities and functional enzyme activity or their key factors which shape their behaviors in heavy metal contaminated soil.

Thus, we hypothesize that the addition of biochar and compost can reduce the effective state of heavy metals and increase the microbial ammonia-oxidizing activities in soil. This study investigated the actions of ammonia-oxidizing communities, AMO enzyme activity, and their key shaping factors both in order to determine biochar and compost impacts on the remediation of heavy metal contaminated soil. Bacterial 16S rRNA, AOA and AOB *amoA* gene abundances were obtained by quantitative PCR. AMO activity was determined by enzyme-linked immunosorbent assay (ELISA). Pearson correlation analysis was used to examine the relationships between the *amoA*, 16S rRNA gene abundance, AMO enzyme activity and environmental factors. It is hoped that this study will deepen the understanding of the microbiological mechanism of nitrogen transformation and the community dynamics of AOA and AOB in heavy metals polluted soils under different biochar and compost remediation strategies.

1. Materials and methods

1.1. Experimental preparation

Soil samples were collected from Changde City, Hunan, China. They were taken from the topsoil (0–20 cm). Due to mining activities, the soil of this area was contaminated by heavy metals (e.g., Cu, As, Zn, and Cr). Soil samples were air-dried to a constant weight at ambient temperature. Biochar was obtained from rice husk in a hypoxia environment (500 °C, 3 hr) using the tubular carbonization furnace (Li et al., 2019a; Zeng et al., 2018). Compost samples from agricultural waste including rice straw, vegetable leaves, fresh soil, and bran were mixed at a volume ratio of 11:3:8:2 to homogeneity as had been done in previous studies (Ren et al., 2018; Wu et al., 2020; Zeng et al., 2011). The characteristics of biochar, compost, and soil are given in Table 1.

1.2. Experimental treatments and sample collection

Prior research suggested that a 5% biochar and compost mix was the optimal ratio for farmland soil remediation (Li et al., 2019a; Liu et al., 2020; Ren et al., 2018; Tang et al., 2020). Four treatments were conducted: Treatment A, the control without any addition; Treatment B, 5% of biochar added; Treatment C, 5% of compost added; and Treatment D, with 5% biochar and 5% compost added. Moisture content was maintained at 70%. All treatments were cultured in artificial climate chambers at 25 °C (RGX-280F, Lichen, Shanghai, China). Treated samples of 150 g were collected on day 0, 15, and 30. Before used for physico-chemical property determination and microbiological analysis, the samples were stored at 4 °C and −20 °C, respectively.

1.3. Physico-chemical parameters determination

Moisture content, electrical conductivity (EC), pH, NH_4^+-N, nitrate (NO_3^--N), and water-soluble organic carbon (WSC) were measured using methods described in previous studies (Li et al., 2019a; Liu et al., 2020; Tang et al., 2020; Wu et al., 2020). $CaCl_2$-extractable heavy metals were extracted with a

Table 1 – The characteristics of the soil, biochar, and compost.

Parameters	Soil	Biochar	Compost
pH	5.98±0.01	9.10±0.02	8.83±0.02
EC (μS/cm)	0.21±0.01	0.16±0.02	7.99±0.01
WSC (mg/kg)	2.76±0.11	8.72±0.77	71.24±2.02
NO_3^--N (mg/kg)	48.06±0.13	6.98±0.07	82.53±4.27
NH_4^+-N (mg/kg)	38.32±0.21	42.20±1.39	354.56±9.71
Specific surface area	0.69±0.05	60.18±3.12	–
Ash content (%)	–	49.52±1.23	–
Moisture (%)	17.95	7.12	23.13
Total pore volume (cm^3/g)	–	0.05±0.002	–
Organic matter content (%)	62.33±3.01	816.20±15.23	274.41±10.27
As (mg/kg)	55.07±0.48	4.25±0.16	6.04±0.79
Cd (mg/kg)	0.46±0.03	0.15±0.01	1.88±0.09
Cu (mg/kg)	50.15±1.33	301.62±0.89	29.93±3.16
Zn (mg/kg)	100.55±0.58	483.47±13.75	150.31±11.45

EC: Electrical conductivity; WSC: Water-soluble carbon; As/Cd/Cu/Zn content: Total heavy metals. Values are given the mean ± SE ($n = 3$).

0.01 mol/L $CaCl_2$ solution, centrifuged, and filtered with a 0.45 μm organic filter. 1 mL of 1 mol/L HNO_3 solution was then added to prevent heavy metal precipitation and reduce microbial activity. Finally, extract concentration was determined by ICP-MS (PerkinElmer, NexION 300 ×, USA) (Liang et al., 2017).

1.4. AMO activity measurement

An AMO ELISA Kit was used to determine AMO activity. The Stop Solution color changed from blue to yellow. Color intensity was determined at 450 nm with a Spectrophotometer (SpectraMax iD5). AMO ELISA Kits have a series of calibration standards to determine AMO concentration in samples. Calibration and sample standards were determined simultaneously. A standard Optical Density (O.D.) curve versus an AMO concentration was produced by the operator. AMO activity data was determined by comparing the sample O.D. value to the standard curve (Taylor et al., 2017).

1.5. DNA extraction and quantitative PCR

Soil DNA was extracted and purified using a Power Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, California, USA). DNA extract was collected and stored at −20 °C before use. Primers 338F and 543R (Muyzer et al., 1993), amoA-1f/amoA-2r (Rotthauwe et al., 1997), and CrenamoA-23f/CrenamoA-616r (Tourna et al., 2008) were used for 16S rRNA, AOB and AOA *amoA* gene, respectively. Quantitative PCR was performed by a Cycler IQ5 Thermocycler (Bio-Rad, USA) in a 10 μL system containing 0.5 μL DNA extract, 0.2 μL each primer (10 μmol/L), 5 μL 2 × SYBR real-time PCR premixture (Bioteke, Beijing), and 4.1 μL sterile water. PCR amplification condition was as follows: 95 °C for 10 min; 40 cycles at 55 °C for 30 sec (16S rRNA and AOB), or 58 °C for 60 sec (AOA); 72 °C for 40 sec; and 83 °C for 20 sec (Li et al., 2019a; Zhang et al., 2012). Readings were collected at 83 °C. Standard curves and quantitative PCR inhibitory effects were obtained using the methods described by Li et al. (2019a).

1.6. Statistical analysis

SPSS 25 (Chicago, IL) was used to analyze data, and one-way analysis of variance (ANOVA) with less than 95% confidence level was applied. All the data are presented as mean ± SE ($n = 3$). Mean values for the treatments were conducted to compare through a Tukey test. Correlation coefficients between parameters were evaluated using a Pairwise Pearson's correlation matrix by partial Mantel tests.

2. Results and discussion

2.1. Physico-chemical parameters

The pH values for all treatments were stable and ranged from 5.8 to 6.6 (Table 2). On day 30, pH in Treatments B, C and D were increased by 9.40%, 10.9% and 17.43%, respec-

Table 2 – Results of pH, EC and WSC for different treatments.

Days	Treatments	pH	EC (μS/cm)	WSC (mg/kg)
0 day	A	^{a}5.98±0.03	^{c}0.21±0.01	^{d}27.62±4.21
	B	^{b}4.32±0.14	^{c}0.22±0.01	^{c}35.02±1.26
	C	^{b}4.38±0.09	^{a}0.57±0.01	^{a}204.46±3.40
	D	^{a}6.16±0.08	^{b}0.46±0.02	^{b}183.34±0.78
15 day	A	^{a}5.75±0.04	^{c}0.20±0.01	^{c}44.71±2.89
	B	^{b}6.35±0.03	^{c}0.16±0.01	^{b}37.21±12.26
	C	^{b}6.32±0.01	^{b}0.44±0.03	^{a}97.48±0.09
	D	^{b}6.48±0.02	^{a}0.57±0.01	^{a}106.60±2.53
30 day	A	^{a}5.68±0.06	^{b}0.22±0.01	^{b}32.00±0.258
	B	^{b}6.27±0.06	^{b}0.16±0.01	^{b}34.84±2.48
	C	^{bc}6.30±0.02	^{a}0.64±0.01	^{a}101.78±18.73
	D	^{c}6.67±0.03	^{a}0.58±0.03	^{a}109.60±6.52

A: soil (control); B: soil + biochar (5%); C: soil + compost (5%); D: soil+ biochar (5%) + compost (5%). EC: Electrical conductivity; WSC: Water-soluble carbon. Values are given the mean ± SE ($n = 3$). Different letter means the statistical difference on each sampling occasion ($P < 0.05$).

tively, compared to the control (Treatment A). Biochar has alkaline feature due to negatively charged surface hydroxy, carboxylic, and phenolic groups (Chintala et al., 2014). Carbonates, bicarbonates, and silicates in biochar can bind with the H^+. This reduces H^+ content and increases pH in both soil and water (Gul et al., 2015). Previous research indicated that soil pH would be increased by adding sufficient biochar (Lehmann and Joseph, 2015). The presence of biochar could gradually release alkali and alkaline-earth metals into the soil (Zheng et al., 2015). Soil pH increase resulting from compost being added might be due to the presence of vast lignin, cellulose and ammonia.

Adding compost increased EC, with the most significant increase on day 15 ($P < 0.05$) (Table 2). EC of Treatments C and D increased by 17.54% and 23.91% on day 30, respectively compared with Treatment A. Compost-treated soil samples showed the greatest EC amounts which could be interpreted as continuous compost mineralization (Glaser et al., 2015). Microbial decomposition of organic matter produced a large amount of salts and humic acids which significantly increased soil EC. Anionic and cationic nutrient release also contributed to the increase soil EC. Addition of biochar reduced soil EC somewhat which is consistent with the results of other study (Wang et al., 2013). EC values decreased in Treatment B due to biochar increase of mineral salt precipitation (Li et al., 2019a; Tang et al., 2020). Biochar application did not significantly affect WSC (Table 2). WSC content increased, probably due to soil microbial community secretions or organic compound decomposition. WSC had a positive correlation to the compost, likely due to material that was easily biodegradable and decomposed by soil microorganisms. This result is consistent with previous research (Zhang et al., 2018b).

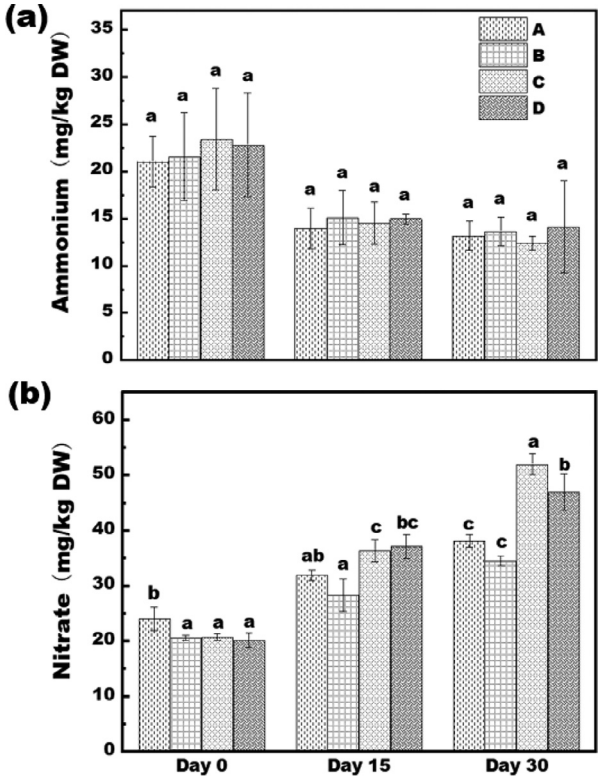

Fig. 1 – Transformation of NH_4^+-N (a) and NO_3^--N (b) for different treatments. A: soil (control); B: soil + biochar (5%); C: soil + compost (5%); D: soil+ biochar (5%) + compost (5%). Different letters above bars indicate significant differences ($P < 0.05$) at each sampling occasion.

2.2. *Nitrogen conversion*

The transformation of NH_4^+-N and NO_3^--N is presented in Fig. 1. NH_4^+-N decreased gradually with time. No significant differences were obtained for the differing treatments. NO_3^--N amounts increased from day 0 to 30. This indicates that ammonia-oxidizing microbial communities were active during incubation. Adding compost rather than biochar stimulated nitrogen conversion. NO_3^--N contents in Treatments C and D were significantly higher than that of Treatments A and B ($P < 0.05$) on days 15 and 30.

Unsurprisingly, NH_4^+-N and NO_3^--N values increased after compost was added. The slight NH_4^+-N increase, resulting from adding compost, explains the ammonification and mineralization of organic nitrogen (Zhang et al., 2019). Compost contains nutrients including N, P, K, and S as well as a number of essential trace elements. Previous study suggested that adding compost could significantly increase NH_4^+-N conversion rate (Zhang et al., 2018b). NO_3^--N increase indicates increased nitrification likely from AOB and/or AOA activity (Li et al., 2019a). NH_4^+-N produced and accumulated, and was attributed to the ammonization of size-fractionated dissolved organic nitrogen in the compost. Adding compost increased soil fertility and was conducive to microbial growth. This might make the soil structure more stable (Fischer and Glaser, 2012). Enzymatic activity is a good indicator of microbial activity (Tang et al., 2019). Urease associated with nitrogen-cycling catalyzes urea hydrolysis to produce carbonic acid and ammonia. Previous study suggests that using compost and biochar would increase urease activity commensurately with the increased amendments (Liang et al., 2020).

2.3. *$CaCl_2$-extractable heavy metal*

$CaCl_2$-extractable heavy metal refers to the form that can be easily used by organisms. Biochar and compost addition significantly reduced the Cd and Zn effective speciation, especially in Treatment D (Table 3). Amending with biochar and compost, the reduction of Cd effective speciation in Treatments B, C, and D were found 67.16%, 68.60%, and 86.62%, and the reduction of Zn effective speciation were 60.21%, 76.34%, and 86.02% compared with control treatment on day 30. However, the effective speciation of As and Cu were obviously increased by adding compost.

Effective heavy metals speciation is a key indicator for assessing of heavy metal toxicity (Sundaray et al., 2011). It is well known that the heavy metal bioavailability depends on pH levels. By adding biochar and compost, pH increase could be a critical explanation for heavy metal passivation. Previous studies indicated adding biochar increased heavy metal immobilization rates (Chen et al., 2019) and decreased their bioavailability (Lu et al., 2017). This was accomplished via changing soil properties and providing reaction sites for heavy

Table 3 – Heavy metal effective speciation changes of Cd, Zn, As, and Cu for different treatments.

Days	Treatments	Cd (μg/kg)	Zn (μg/kg)	As (μg/kg)	Cu (μg/kg)
0 day	A	^{a}41.53±3.09	^{a}0.45±0.03	^{a}31.60±3.88	^{a}d37.08±9.79
	B	^{b}23.46±4.70	^{b}0.27±0.15	^{b}46.27±3.66	^{b}42.06±21.43
	C	^{c}16.79±0.96	^{c}0.22±0.05	^{c}113.48±6.12	^{c}139.36±14.86
	D	^{d}8.64±1.14	^{d}0.16±0.06	^{d}140.45±22.17	^{d}166.84±53.51
15 day	A	^{a}51.54±3.38	^{a}0.76±0.09	^{a}33.64±3.83	^{a}55.01±15.70
	B	^{b}17.92±1.63	^{b}0.32±0.06	^{b}46.88±2.68	^{b}30.90±8.47
	C	^{b}17.95±1.39	^{c}0.24±0.04	^{c}103.78±5.87	^{c}135.27±5.99
	D	^{c}10.39±3.24	^{d}0.17±0.05	^{d}117.34±7.54	^{c}133.14±7.54
30 day	A	^{a}56.27±1.43	^{a}0.93±0.04	^{a}30.41±6.59	^{a}59.30±11.84
	B	^{b}18.48±0.57	^{b}0.37±0.09	^{b}55.76±4.64	^{b}43.88±15.53
	C	^{b}17.67±1.47	^{c}0.22±0.04	^{c}108.89±8.18	^{c}139.23±23.17
	D	^{c}7.53±0.90	^{d}0.13±0.01	^{d}144.55±5.91	^{c}136.38±18.21

A: soil (control); B: soil + biochar (5%); C: soil + compost (5%); D: soil+ biochar (5%) + compost (5%). Values are given the mean ± SE ($n = 3$). Different letter means the statistical difference on each sampling occasion ($P < 0.05$).

metal redox reaction. Biochar surfaces have hydroxyl, carboxyl, and carbonyl groups, which are involved in electrostatics, ion exchanges, and cation-π interactions with heavy metals in the soil (Cao et al., 2009). Compost also has the potential for adsorbing heavy metals (Zhang, 2011). Compost can immobilize heavy metals primarily using its microorganisms, humus substance, and inorganic components. Humic substances in compost include organic functional groups, such as carbonyl, carboxyl and phenols, which combine with metal ions through complexation (He et al., 2014). Some microorganisms inoculated by compost addition could immobilize heavy metals using biomineralization and biosorption (Chen et al., 2019). Biochar surfaces have special functional groups which, via adsorption, provide active sites for heavy metal redox reactions. Rich humus induced by adding compost greatly mobilized effective Zn and Cd speciation. As and Cu increased due to the higher background concentration of these heavy metals in biochar and compost (Table 1). Biochar and compost addition deliver humic matters to soil which changed the microenvironment and activated As and Cu. Careful selection of remediation materials is needed to avoid inducing other environmental risks (heavy metals, antibiotics, and pesticides) (Chen et al., 2015).

2.4. *AMO enzyme activity*

The activities of AMO enzyme are presented in Fig. 2. AMO activities in Treatments B, and D increased on day 15 and 30. The compost additions in Treatments C and D significantly reduced AMO enzyme activity. Treatment C (compost only) had the lowest AMO activity. These results indicate that the biochar amendment promoted of NH_4^+-N microbial conversion and increased AMO enzyme activity.

AMO enzyme is encoded by the *amoA*, *amoB*, and *amoC* genes (Feike et al., 2012). It has an enormous influence on NH_4^+-N to NH_2OH, the rate limiting step of nitrification (Kuypers et al., 2018; Urakawa et al., 2019). Enzyme activity can sensitively reflect the direction and extent of soil biochemical reactions. It is also diagnostic of soil nutrition health conditions as a potential biological indicator (Tang et al., 2019). AMO inhibition is the result of multiple factors. Some NO_3^--N production does not require AMO participation (Huang et al., 2019). Without the intermediate (NH_2OH), NH_4^+-N would be transformed into NO or N_2O by *Pseudomonas putida* Y-9, a bacterium isolated from long-term submerged rice rhizosphere soil (Huang et al., 2019). Additionally, comammox, a complete nitrification microorganism which produces ammonia monooxygenase had the same effect on the nitrogen cycle along with AOB and NOB (Van Kessel et al., 2015).

The differing responses of AOB, AOA, and AMO are important indicators of potential physico-chemical/ biological condition changes in heavy metal polluted soil. Most studies have shown heavy metals in the soil depress enzyme activity (Yang et al., 2016). Heavy metals were likely to influence enzyme activity by affecting microbial activity and changing their environment (Fan et al., 2016; Lemire et al., 2013). Previous study indicated that AMO activity was significantly increased by biochar addition (Liang et al., 2020). The biochar also changed soil CEC, organic matter levels, soil aggregation, and reduced bulk density, thus increasing enzyme activity and microbial biomass (Liang et al., 2020).

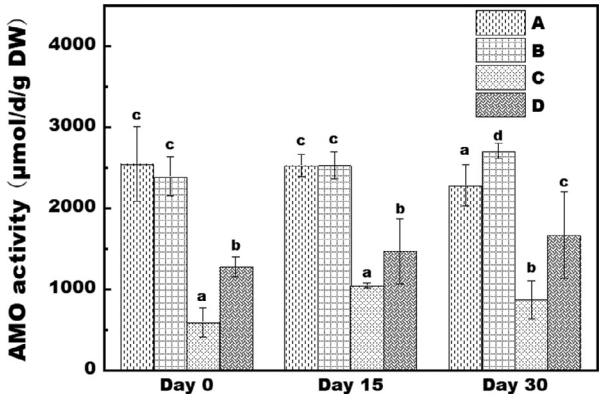

Fig. 2 – Changes in AMO activity for different treatments. A: soil (control); B: soil + biochar (5%); C: soil + compost (5%); D: soil+ biochar (5%) + compost (5%). AMO: ammonium monooxygenase activity. Different letters above bars indicate significant differences ($P < 0.05$) at each sampling occasion.

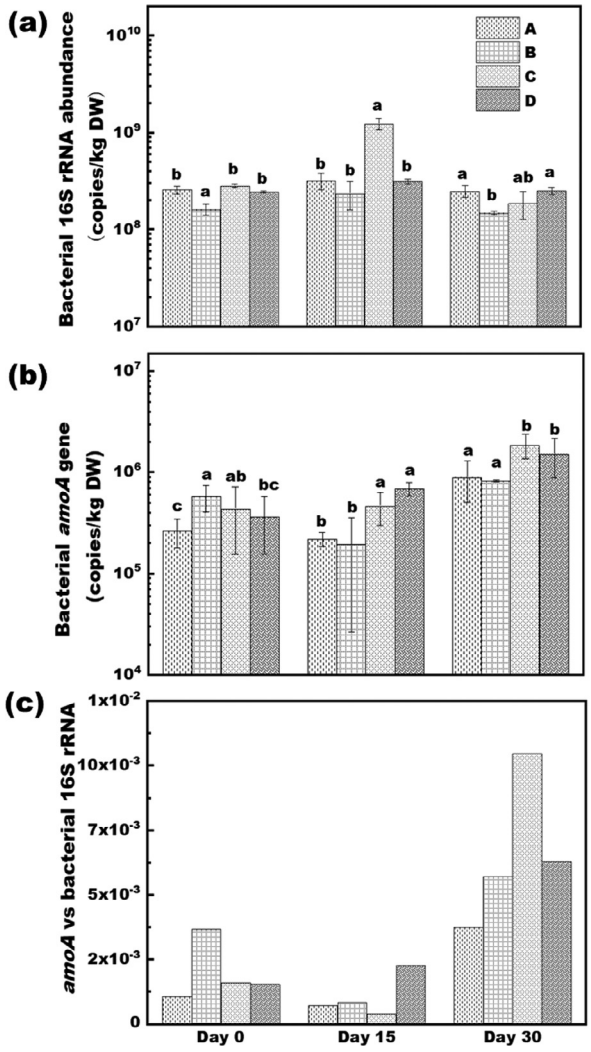

Fig. 3 – Bacterial 16S rRNA gene abundance (a), AOB *amoA* gene abundance (b), and AOB *amoA* gene to 16S rRNA ratio (c) for different treatments. A: soil (control); B: soil + biochar (5%); C: soil + compost (5%); D: soil+ biochar (5%) + compost (5%). Different letters above bars indicate significant differences ($P < 0.05$) at each sampling occasion.

2.5. *Bacterial 16S rRNA and AOA/AOB* amoA *gene abundance*

Adding biochar to the soil markedly decreased the bacterial community abundance (Fig. 3a). Adding compost increased bacterial 16S rRNA gene abundance, with the maximum abundance appeared on day 15. AOA was not detected in all samples, while AOB was abundant. AOB gene abundances in Treatments C and D were significantly higher than that of Treatments A and B (Fig. 3b). This suggests that adding compost stimulated AOB activity. AOB *amoA* gene to bacterial 16S rRNA gene ratios were used to indicate AOB percentages in the bacterial community (Fig. 3c). Biochar, compost, and their combination increased the proportion of AOB, but only compost addition had the better stimulation effect (day 30).

Similar results were reported that biochar changed bacterial community richness (Harter et al., 2014). Biochar influences soil microbial community structure including α-diversity and relative abundance of taxa (Xu et al., 2014). The toxicity of heavy metals in soil is often associated with soil pH. In acidic soil, AOA is a major contributor for ammonia oxidation and plays more vital roles than AOB (Wang et al., 2015; Zhang et al., 2012). While in the agricultural soil, nitrogen-rich grassland ecosystem and mangrove sediment, AOB has obvious influence (Ding et al., 2014). High Cu concentrations significantly inhibits soil potential nitrification activity (Li et al., 2009). Nitrification was significantly reduced at the stress of Hg, and gradually recovered with Hg concentration decreases (Zhou et al., 2015).

Previous research indicates that biochar increases WSC in soil pore water, which can play a vital part in the redox processes (Yuan et al., 2017). Biochar increased dehydrogenase activity (DHA) which is an indispensable oxidoreductase excreted by active microorganisms. Yang et al. (2019) suggests that biochar facilitated microbial proliferation via providing necessary organic matter which related to water stability of soil aggregated sand. Biochar's relatively large surface area and porous structure are suitable microbial habitat. Humus in compost also provides nutrients for microbial growth (Fischer and Glaser, 2012). Biochar's useful effects correlate with its concentration. Previous study suggests that applying low content biochar will stimulate enzyme and microbial activity by providing small amounts of nutrients and improving soil physicochemical properties (Liang et al., 2020). Higher biochar levels might have negative effects. For a more detailed explanation of this phenomenon see: Liang et al. (2020).

Prior study indicated that AOA was more active in low NH_4^+-N concentration environments such as paddy soil rhizosphere (Chen et al., 2008). The ammonia-oxidizing community activities are better described by the AOB *amoA* gene than its AOA counterparts (Zhang et al., 2017). It could be explained by AOB being a major contributor to the aerobic ammonium oxidation process in nitrogen-rich grassland ecosystems and mangrove sediments (Di et al., 2009). Much of the *amoA*-based research, viewed as a whole, argues that diversity and abundance of AOA and AOB depends on several factors. Adding biochar and compost elevates soil nutrients which promotes the microbial community growth and activity. Soil physico-chemical parameters, especially pH, are key factors for microbial communities and activities. Previous research showed that bacterial diversity positively correlates with how much biochar has been applied (Xiao et al., 2017). Microbial communities increased when bamboo stick biochar was added to sandy loam soil (Luo et al., 2017). Some studies have indicated that biochar rates and soil types affected the structure and abundance of nitrogen-cycling bacteria in the soil (Abujabhah et al., 2018). Organic matter mineralization and compost stabilization could be activated, over time, by microorganisms. Carbon and nitrogen could be used by microorganisms for cell growth and energy production.

2.6. *Microbial community and activity key factors*

Pearson correlation analysis indicated a positive correlation between pH and heavy metal effective speciation of Cd ($r = -0.917$, $P < 0.01$) and Zn ($r = -0.878$, $P < 0.01$), respec-

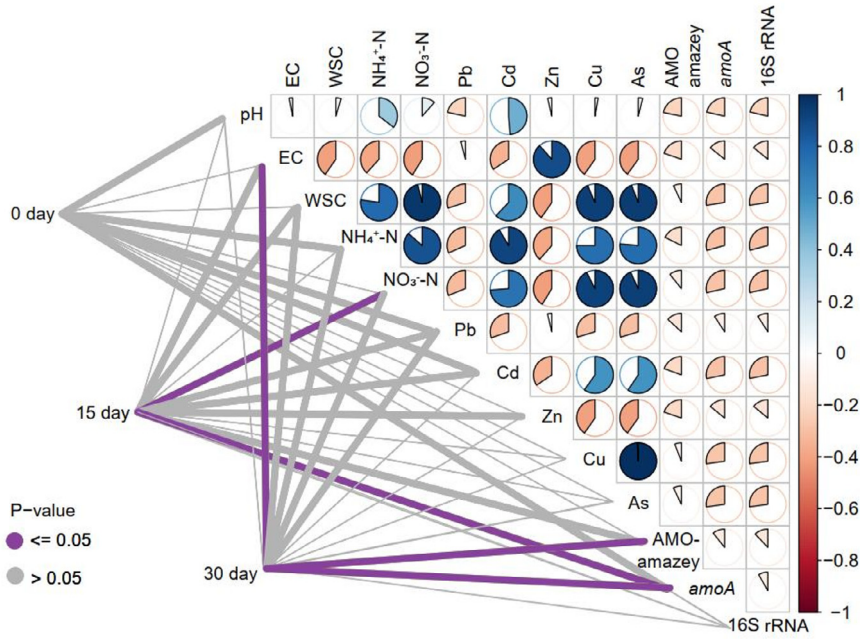

Fig. 4 – The correlations between bacterial 16S rRNA gene, AOB *amoA* gene, AOB *amoA*/16S rRNA ratio, and physico-chemical parameters for different treatments. Pairwise correlation matrix of factors was shown with pie graphs and different treatments were related to each factor by partial Mantel tests. Edge width means the Mantel's statistic, and edge color means the statistical significance. EC: Electrical conductivity; WSC: Water-soluble carbon; AMO: ammonium monooxygenase activity.

tively (Fig. 4). AMO activity significantly correlated with EC ($r = -0.908$, $P < 0.01$) and WSC ($r = -0.868$, $P < 0.01$). AOB *amoA* gene abundance has significant correlation with NO_3^--N ($r = 0.830$, $P < 0.01$). Moreover, the AOB *amoA* to16S rRNA ratio significantly related to NO_3^--N ($r = 0.728$, $P < 0.01$) and WSC ($r = 0.622$, $P < 0.01$).

WSC correlates most strongly with bacterial, and archaeal community in the soil (Dang et al., 2016). Recent research suggests that AOB possesses the most energy efficient pathway for carbon fixation discovered so far (Könneke et al., 2014). Genomic analysis suggests that AOA and AOB have different ammonia oxidation pathways (Walker et al., 2010). This study also found a significant positive relationship between AOB *amoA* gene abundance and NO_3^--N. High NO_3^--N concentration can speed AOB community growth. WSC is an AMO substrate and a key agent in the rate-limiting step of the NH_4^+ to NH_2OH reaction process. WSC had been reported to significantly influence AMO. This might be caused by that more nutrient being provided for microbial ammonia oxidation in soils (Wang et al., 2015). A previous study indicated that the AMO substrate of in the first procedure of ammonia oxidation reaction could be NH_3 rather than NH_4^+ (Di et al., 2010). There may be an indivisible relationship between pH, NH_3, NO_3^--N and NH_4^+-N, which plays a vital role in the construction of AOA and AOB community structures.

3. Conclusions

This study suggests that heavy metal toxicity levels were significantly changed by adding biochar and compost to soil. Adding compost rather than biochar improved nitrogen conversion. AOB abundance and the AOB to total bacterial community ratio were increased by adding biochar and compost. AOB abundance positively correlated with NO_3^--N. AMO activity had a significant correlation with EC and WSC. Those seem to be the most vital factors to explain AOB community variation and their function in heavy metal-polluted soil remediated by biochar and compost.

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