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The effects of rice straw biochar on indigenous microbial community and enzymes activity in heavy metal-contaminated sediment

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HIGHLIGHTS

- Invertase and alkaline phosphatase were decreased by high concentration of biochar.
- Intensities of dominant bacteria declined when biochar rate was 50 mg kg^{-1}.
- pH might be related to the decreases in enzymes activity and microbial abundance.
- OM explained the 45% of the variations of microbial community structure by RDA.

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ABSTRACT

Owning to the potential in carbon sequestration and other environmental benefits, biochar has been widely used for in-situ environmental remediation. Understanding the biological effects of biochar is essential. The goal of this study was to explore the response of indigenous microbes under the stress of different concentrations of biochar. The results showed that biochar could significantly change physicochemical properties, enzymes activity and microbial community composition depending on biochar concentration and incubation time. When the concentration of biochar was 50 mg kg^{-1}, the activities of invertase and alkaline phosphatase were obviously inhibited. Meanwhile, bacterial 16S rRNA and fungal 18S rRNA coding gene copies were decreased by 74% and 25%, respectively after 90 days of incubation. Additionally, the bacterial community succession occurred and the relative intensity of dominant species decreased when treated with high concentration of biochar. However, the activity of urease and alkaline phosphatase, as well as bacterial and fungal abundance, were increased when sediment was treated with 10 mg kg^{-1} biochar. Relationships among physicochemical properties, heavy metals and microbes were analyzed by correlation analysis and redundancy analysis (RDA). Correlations between invertase activity and pH value in the experiment were significantly negative. Redundancy analysis showed physicochemical properties and heavy metals explained 92% of the variation in the bacterial DGGE profiles and organic matter content explained the majority (45%) of the variation. This study indicated that indigenous microbes could be affected by biochar either directly or indirectly via changing the physicochemical properties and heavy metals of sediment.

1. Introduction

River sediments are generally considered as the ultimate repositories of past and ongoing discharges of heavy metals (Ghosh et al., 2011). In the south of China, heavy metal pollution in the sediment of Xiangjiang River has been reported in recent years due to the rapid development of metallurgical industry, mining activities and sewage irrigation (Xu et al., 2012). Extensive attention has been paid to heavy metal pollution and a large number of advanced materials have shown their advantages for pollution remediation (Cao et al., 2011; Zhang et al., 2016a; Tang et al., 2012; Tang et al., 2014). As an attractive waste management option, biochar has been used to amend polluted sediment

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recently (Chiang et al., 2012; Ghosh et al., 2011; Lou et al., 2011). Biochar is a not fully carbonized product produced by pyrolysis of biomass (such as crop residues, manure and organic waste) under oxygen-limited condition. Besides, it can promote nutrient availability and increase carbon sequestration as well as soil fertility (Tian et al., 2016). Biochar has been universally applied in soil amendment and its utility in the remediation of heavy metal pollution in soil and sediment is being increasingly considered due to its large surface area, complex porosity and variable surface composition (Oleszczuk et al., 2012; Huang et al., 2017).

Although being successful in pollution remediation, the application of advanced materials always adversely affect the behavior of indigenous microbes (Huang et al., 2016; Zhang et al., 2016b). Das et al. (2012) reported that silver nanoparticles could inhibit natural bacterioplankton production. Fajardo et al. (2012) found that nanoscale zero-valent iron would exert selective pressure on the microbial community. These investigations arouse our interests in the study of the effects on indigenous microbes when heavy metal-contaminated sediment was amended by biochar. Recently, variable effects have been observed on soil microbial community caused by biochar (Jindo et al., 2012; Gul et al., 2015) and the effects mainly depended on soil type, biochar source, biochar concentration and detection method. However, the mechanism of the effects was still unknown and few attention was paid to the effects on sediment microbes.

In surface layer of sediment, bacteria and fungi generally account for the most part of the total sediment microbial biomass (Tong et al., 2012). Indigenous microbes played an important role in nutrient cycling, energy flow and organic matter turnover via ecological processes (Huang et al., 2008). Sediment microbial communities provide important functions in sediment ecosystems and always act as the primary regulators of many sediment processes. Therefore, studying the effects of biochar on sediment microbes is important, which can benefit to the application of biochar. Additionally, being sensitive to environmental changes, enzymes activity are directly related to soil or sediment functionality and widely used to evaluate the microbial activity (Durenkamp et al., 2016). Urease, invertase and alkaline phosphatase are ubiquitous enzymes in soil and sediment, which can be used to study the changes of microbial activity and element cycle related to nitrogen (N), carbon (C) and phosphorus (P). Many researchers indicated that microbial activity and community composition could be affected by soil physicochemical properties (soil organic matter content, moisture, pH, soil type and so on) (Jindo et al., 2012; Abujabhah et al., 2016). Meanwhile, physicochemical properties could be affected by biochar addition (Gul et al., 2015; Sigua et al., 2016). However, the relationship among physicochemical parameters, microbial parameters and biochar concentration has not been evaluated simultaneously (Xian et al., 2015).

In the current study, the responses of indigenous microbes were investigated when heavy metal-contaminated sediment was amended with biochar. The aims of the investigation were to (a) examine the changes of physicochemical properties and microbial enzymes activity upon biochar addition, (b) explore the changes of microbial community composition induced by exogenous biochar using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), (c) discuss the mechanism of the biological effects of biochar by analyzing the relationship among biochar concentration, physicochemical properties, heavy metals and microbial parameters. This study could benefit the application of biochar and contribute to understand the adverse effects of biochar on the function of indigenous microbes.

2. Materials and methods

2.1. Biochar and river sediment

Biochar was produced by rice straw biomass at 600 °C for 3 h using a reported method (Guo and Chen, 2014). Produced biochar was mixed evenly, ground to pass through 0.154-mm sieve and characterized for specific surface area, production yield, pH, and pore width, which were followed by the protocol of Chintala et al. (2014). Selected properties were shown in Table 1.

River sediment was collected from the surface layer of the Xiangjiang River, Changsha, Hunan province by a clam sampler. After removing gravels and plant residues, sediment was put into a sterilized plastic bag and then taken to the laboratory within 1 h (Huang et al., 2015). In order to make sediment and biochar completely incorporated, sediment was slightly air-dried in the dark, then crushed, mixed evenly and sieved through a 0.154-mm sieve. After pretreatment, the sediment was stored at 4 °C in a refrigerator (Lou et al., 2011). Selected sediment properties were determined according to the procedure described by Hu et al. (2014b) and main properties were presented in Table 1.

2.2. Experimental design

The rice straw biochar was used to amend the heavy metal-polluted sediment. Meanwhile, physicochemical properties, heavy metals and microbial parameters were determined. The percentages of rice straw biochar in the sediment were 0% (C_0), 1% (C_1) and 5% (C_2) (w/w). Experiments were designed with three replicates. 300 g (dry weight) of prepared sediment was weighed into 2 L beakers and then 1500 mL of sterile water was added to simulate sediment environment. After incubated for 7 days to acclimatization, biochar was respectively added to sediment and mixed evenly at three different concentration levels. All beakers were covered with apertured plastic wrap and incubated in an incubator at 20 °C. Subsamples were respectively collected from each beaker on day 2, 7, 15, 30, 45, 60 and 90. Then subsamples were vacuumly filtered to obtain the uniform moisture content and stored at −20 °C for subsequent determination.

2.3. Chemical analyses

The pH, organic matter content, total Zn and Cd were measured according to the procedure described by Hu et al. (2014b). Toxicity characteristic leaching procedure (TCLP) was performed to quantify the leachability of heavy metals following the protocol described by

Table 1
The physicochemical properties of experimental sediment and biochar. Data represent the mean ± SE.

	Properties	Value
Sediment	pH	6.65 ± 0.32
	Temperature (°C)	20
	Water content (%)	69.3 ± 0.4
	Organic matter content (%)	6.3 ± 0.3
	Total nitrogen (g kg^{-1})	0.5 ± 0.09
	Total Cu (mg kg^{-1})	69.35 ± 1.6
	Total Zn (mg kg^{-1})	225 ± 15
	Total Pb (mg kg^{-1})	167.1 ± 1.3
	Total Cd (mg kg^{-1})	25.5 ± 0.4
Biochar	pH	10.45 ± 0.5
	Yield (%)	29
	Surface area (m^2 g^{-1})	285.33
	Average pore width (nm)	40
	Pore volume (mL g^{-1})	0.040

Liu and Zhao (2007). Due to the pH value of all sediment samples was higher than 5, the TCLP extractant was 0.1 mol L^{-1} glacial acetic acid. After 18 h shaking, supernatants were filtered through a 0.22-μm pore-size Millipore filter and analyzed for Cd and Zn using atomic absorption spectrometry (AA 240 FS, Varian) (Cao et al., 2011).

2.4. Enzymes activity assays

Three enzymes were analyzed using colorimetric method. Invertase activity was determined using sucrose solution as the substrate as described by Chen et al. (2013). The activity of invertase was assayed based on the product of glucose which was determined colorimetrically at 508 nm using a spectrophotometer. Following a protocol described by Hu et al. (2014a), the activity of urease was assayed by the determination of ammonium released from a solution of urea (10%) and citrate buffer (pH 7) after incubated at 37 °C for 24 h. The activity of urease was expressed as milligrams of ammonium per 100 g of sediment (dry weight). The activity of alkaline phosphatase was measured by the transformation of disodium phenyl phosphate to phenol as described by Jin et al. (2016). The activity of alkaline phosphatase was expressed as milligrams of phenol per gram of sediment (dry weight).

2.5. DNA extraction and quantitative real-time polymerase chain reaction (q-PCR)

DNA of sediment samples was extracted using E.Z.N.A.™ Soil DNA Kit (Omega Bioteke, USA) according to the manufacturer's protocol, then purified with a Clean Up Kit (TianGen, Beijing, China). Purified DNA was stored at −20 °C. The bacterial 16S rRNA gene copies (primer pairs: 338F/518R) and fungal 18S rRNA gene copies (primer pair: Fung/NS1) of all samples were determined in triplicate following the protocol described by Lu et al. (2014). Q-PCR assay was operated in a Cycler iQ5 thermocycler (Bio-Rad, USA). Standard curves were generated using triplicate 10-fold plasmid dilutions of plasmids DNA ranging from 1×10^2 to 1×10^{10} copies per assay.

2.6. PCR-DGGE of bacterial community

For bacterial DGGE analysis, the primer GC-338F/518R was used to amplify bacterial 16S rRNA fragments. PCR amplification was performed in a MyCycler thermal cycle (Bio-Rad, Hercules, CA, USA) and the PCR products from three replicates were mixed to make sure they were representative and then analyzed by DGGE under protocol of Deng et al. (2015). PCR products (40 μL) were loaded onto the 1-mm-thick 8% (w/v) polyacrylamide gels with a denaturing gradient of 35–70%. The gels were run in 1 × TAE buffer at 60 °C, 100 V, for 8 h. After DGGE, the gels were stained with SYBR solution (TianGen, Beijing, China) for 30 min, and visualized with a Gel Doc-2000 Image Analysis System (Bio-Rad, USA).

2.7. Statistical analyses

Statistical analyses were performed using SPSS version 19.0 for Windows (SPSS, Germany). DGGE images for bacteria were digitized by Quantity One software. Besides, Canoco (version 4.5, for Windows) was used to examine the multivariate relationship between bacterial community composition and environmental parameters of sediment. Detrended correspondence analysis (DCA) was used to determine linear or unmoral model. The results showed the length of the first ordination axis was less than 3, which indicated the relationship should be explored with redundancy analysis (RDA). Principal component analysis (PCA) of bacterial DGGE profiles was performed to identify the differences among bacterial community composition based on the relative intensities and positions of bands in DGGE patterns.

Shannon-Wiener diversity index (H) provided a direct indication of the apparent diversity of a bacterial community, which calculated as follows:

$$H = -\sum \left(\frac{Ni}{N}\right) \ln(\mathrm{Ni/N})$$

Where *Ni* was the trace quantity of each band, *i* was the number of bands in each DGGE profile, and *N* was the sum of each sample trace quantities in a given DGGE profile.

3. Results

3.1. Physicochemical properties and heavy metals in river sediment

The physicochemical properties of raw sediment were shown in Table 1. The results showed the sediment was faintly acid and polluted by Cd and Zn. After 90 days of incubation, the pH value and organic matter content were increased by 23% and 100% compared with the control group when treated with 5% biochar. Besides, the application of biochar notably decreased the leachability of heavy metals, especially in treatment with high concentration level of biochar. After 90 days of incubation, the TCLP extractable fraction of Zn was decreased by 21% when treated with 5% biochar. Similarly, a reduction was also found in extractable fraction of Cd by 13.1% compared with that of the control. The results of one-way ANOVA showed strong effect of biochar concentration on physicochemical properties and heavy metals after 7 days of incubation, which were shown in Fig. 1.

3.2. Enzymes activity in river sediment

Invertase, urease and alkaline phosphatase were determined to reflect the dynamic changes of microbial activity caused by the addition of exogenous biochar (Fig. 2). The activity of urease kept stable during the whole experimental period when the concentration of biochar was 1%. But significant increases were found when biochar concentration was up to 5%, and the activity of urease peaked on day 90 with a peak value (204 mg/100 g) which was 1.9-times higher than that of the control group. One-way ANOVA showed the effect of biochar concentration on urease was significant ($p < 0.01$) after 30 days of incubation (Fig. 2). On the contrary, the activity of invertase decreased when treated with biochar along the whole experimental period. The effects of biochar on the activity of invertase were strongly concentration-dependent at all sampling time ($p < 0.01$) by one-way ANOVA (Fig. 2). At the beginning, the activity of alkaline phosphatase temporarily decreased, but increased ultimately when sediment was treated with 1% biochar. However, when the concentration of biochar was up to 5%, the activity of alkaline phosphatase decreased to 0.75-times of the control. One-way ANOVA showed that strong effect of biochar concentration on the activity of alkaline phosphatase after 60 days of incubation ($p < 0.01$) (Fig. 2).

3.3. Indigenous microbial abundance and community structure in river sediment

3.3.1. Bacterial 16S rRNA and fungal 18S rRNA gene copies

The bacterial 16S rRNA gene copies ranged from $2.54 \pm 0.3 \times 10^7$ (C_2, day 45) to $173 \pm 15 \times 10^7$ (C_1, day 90). Meanwhile, the fungal 18S rRNA gene copies ranged from $2.64 \pm 0.3 \times 10^3$ (C_1, day 45) to $23.2 \pm 1.2 \times 10^3$ (C_1, day 90). When sediment was treated with 1% biochar, the bacterial and fungal gene copies were increased by

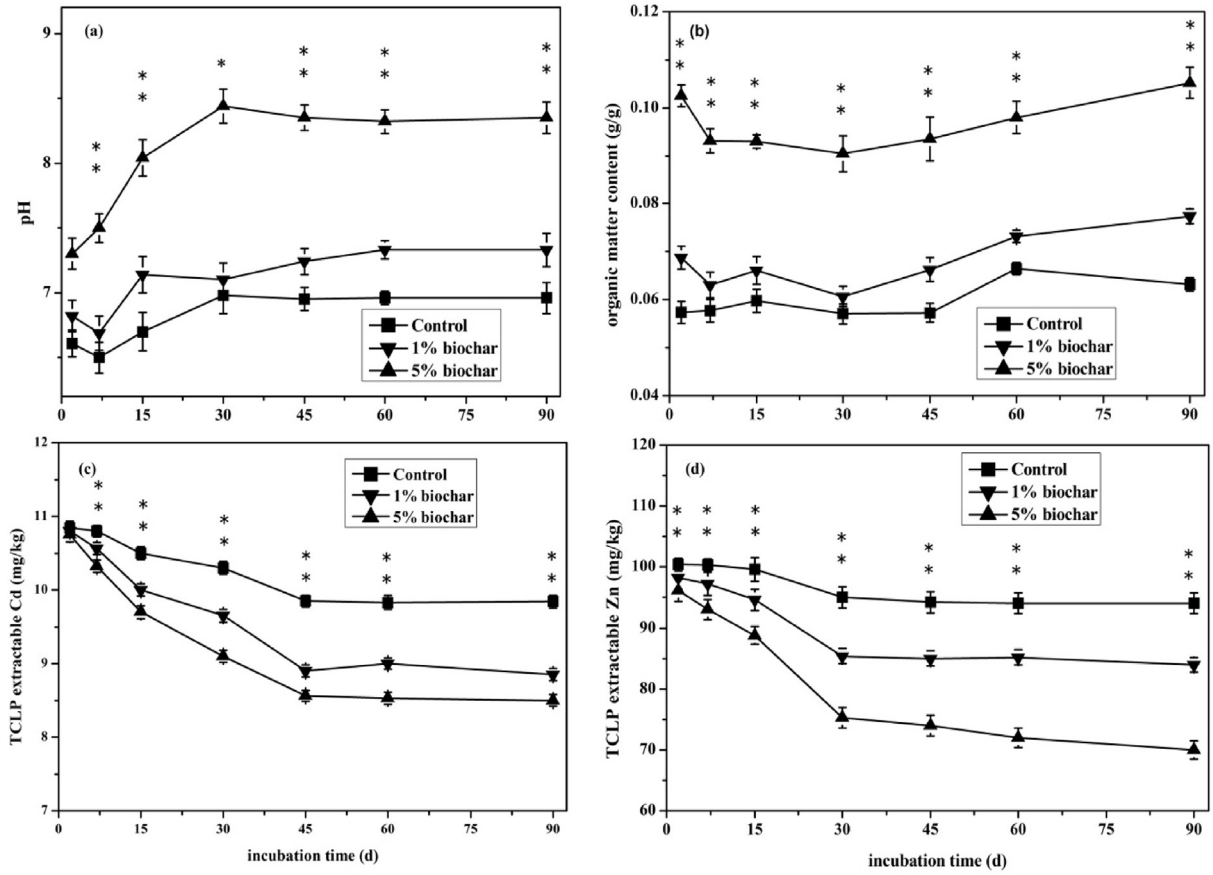

Fig. 1. The effects of different concentrations of biochar on the dynamic changes of pH (a), organic matter content (b), TCLP extractable Cd (c) and Zn (d) during 90 days of incubation. The bars represent the standard deviations of the means ($n = 3$). Differences between biochar concentration level at every sampling time were considered significant at $p < 0.05$ (*) and $p < 0.01$ (**) by one-way ANOVA.

166% and 246% compared with that of control on day 90. The similar increases can be also observed in bacterial gene copies on day 45. On the contrary, significant reductions were found in bacterial and fungal gene copies when the concentration of biochar was increased to 5%. Lg-transformed gene copy numbers of total bacteria and fungi were calculated to clearly reflect the changes caused by biochar addition (Fig. 3). One-way ANOVA showed that the effects of biochar on the abundance of bacterial 16S rRNA coding genes and fungal 18S rRNA coding genes were strongly concentration-dependent on day 90 ($p < 0.01$), however the effects were inconspicuous on day 2 and 45 (Fig. 3).

3.3.2. Bacterial community structure

S1 (C_0, 45d), S2 (C_1, 45d), S3 (C_2, 45d), S4 (C_0, 90d), S5 (C_1, 90d), S6 (C_2, 90d) were used to represent every sample on day 45 and 90. Bacterial DGGE profiles were shown at Fig. S1. Similarity dendrograms were generated by the image analysis of DGGE to clearly show the positions and intensities of these main bands (Fig. 4a). Besides, the relative intensity of each main band was quantified according to the procedure described by Zhang et al. (2011) and the results were exhibited in Table S1. Changes in relative intensity were observed in main bands under different concentration level of biochar treatment, which indicated that the bacterial community succession occurred and some species of bacteria responded differently compared with that of the control. Some of flush obvious stripes (band 2, 6 and 7) appeared in every sample and the relative intensity of them took up more than 38.56% regardless of biochar addition, indicating that the bacterial species represented by the three bands were dominant in bacterial community of every sample. Additionally, the relative intensity of dominant species of bacteria decreased in treatment with high concentration of biochar, which was in accordance with the decreases in bacterial gene copies determined by q-PCR.

The DGGE gel profiles were further visualized by the PCA and the results were shown in Fig. 4b. The cumulative contribution rate of the two principal components reached to 74.2% (51.7% and 22.5% for PC1 and PC2, respectively). Besides, S1, S2 and S3, S4 clustered together respectively and they were well separated from S5 and S6.

3.4. Relationship among sediment enzymes activity, microbial community composition and environmental parameters

Pearson correlation analysis was used to reveal the relationship among environmental parameters, enzymes activity and microbial abundance in sediment (Table 2). The activity of alkaline phosphatase and invertase decreased with the increase of pH value ($p < 0.05$), whereas positive correlation was found between the activity of urease and pH value. There was no significant correlation between organic matter content and enzymes activity. Significant negative correlation was found between the TCLP extractable heavy metals and the activity of urease. However, positive correlation was found between the TCLP extractable heavy metals and the activity of invertase ($p < 0.01$).

According to the results of DCA, RDA was used to assess the correlation between bacterial community and environmental parameters Fig. 4c. The result showed that the first two axes of the

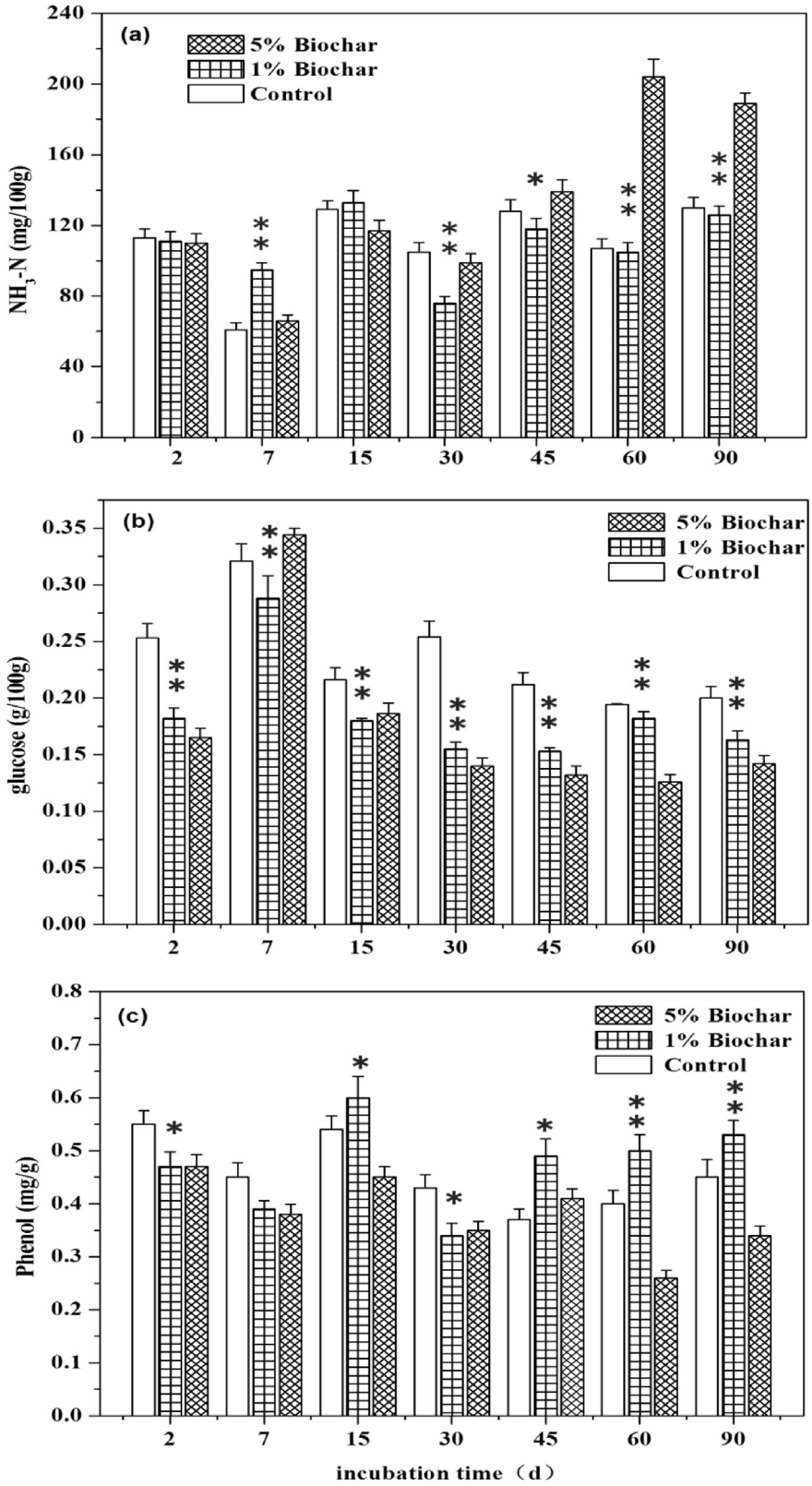

Fig. 2. The effects of different concentrations of biochar on the dynamic changes of urease (a), invertase (b) and alkaline phosphatase (c) during 90 days of incubation. The bars represent the standard deviations of the means ($n = 3$). Differences between biochar concentration level at every sampling time were considered significant at $p < 0.05$ (*) and $p < 0.01$ (**) by one-way ANOVA.

RDA explained 58.9% and 19.6% for bacteria of the variance between environmental variables and species data. Organic matter content and pH were positively correlated with axis 1 for bacterial community structure while the extractable fraction of heavy metals had inverse correlation with dynamic bacterial community structure. According to the RDA profiles, the four environmental

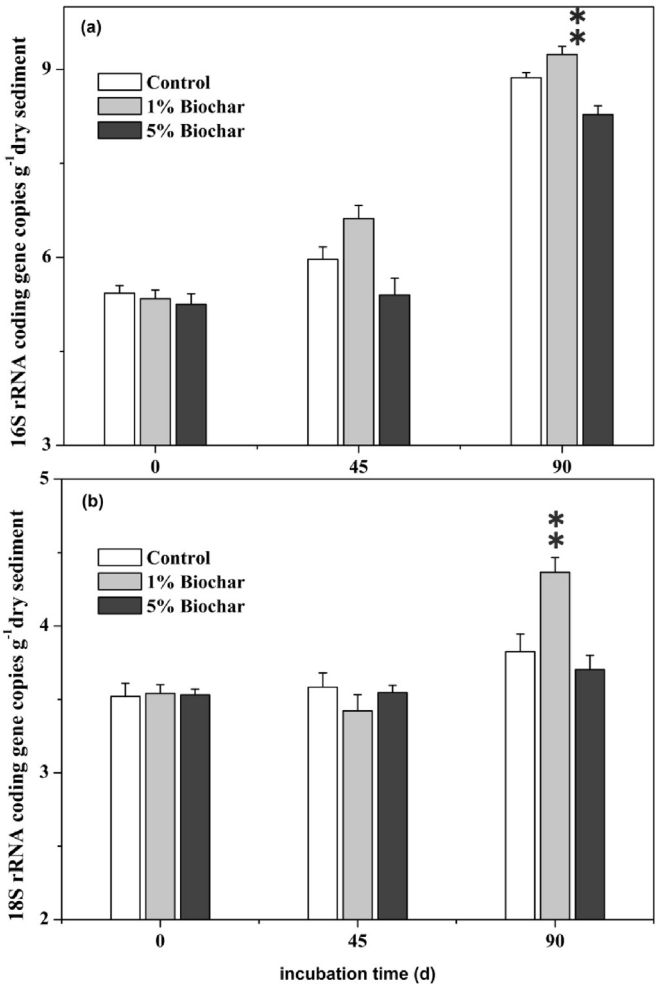

Fig. 3. Lg-transformed gene copies of total bacteria (a) and fungi (b) according to q-PCR analysis in sediment under different concentrations of biochar treatments. The bars represent the standard deviations of the means ($n = 3$). Differences between biochar concentration level at every sampling time were considered significant at $p < 0.05$ (*) and $p < 0.01$ (**) by one-way ANOVA.

parameters explained 92% of the variation in the species data, and organic matter content ($F = 3.266$, $P = 0.002$, 499 permutations) explained 45% of the variation and played a major role in bacterial community structure. The results indicated that significant correlations were existed between microbial community succession and physicochemical parameters.

4. Discussion

4.1. Effects of biochar on microbial enzymes activity in sediment

Enzymes activity which can be used to reflect microbial activity was constantly changing and responded sensitively to the biochar treatments in our study. Various shifts were shown in three enzymes after biochar was added to sediment. The activity of urease increased when treated with 50 mg kg^{-1} biochar and the increase could be explained by two reasons. One is that the biochar could increase the activity of specific enzymes related to N utilization in soil which was supported by the study of Bailey et al. (2011). Besides, Dempster et al. (2012) also indicated that biochar promoted the nitrogen transformation, which might be related to urease activity. The other is that the decreases in extractable fraction of heavy metals contributed to the increase of urease. In our study, there were significant negative correlations between heavy metals and urease activity. Similarly, several studies inferred that correlations were existed between urease activity and heavy metals, and urease activity could be an indicator for assessing the toxicity of heavy metals (Huang et al., 2015). For alkaline phosphatase, increases were observed in treatment with low concentration of biochar. Similar reports mentioned that peanut shell biochar addition at a low concentration level (2.5%) promoted the activity of alkaline phosphomonoesterase (Bhaduri et al., 2016) and the activity of enzymes related to P cycling was increased by biochar addition (Jin et al., 2016).

Nevertheless, when sediment was amended with the high concentration of biochar, decreases were found in invertase and alkaline phosphatase activity, which were consistent with several studies (Elzobair et al., 2016; Bailey et al., 2011). The potential reasons for such decreases might be as follows: (i) enzymes or substrates could be adsorbed by biochar due to the strong adsorption property of biochar, which impeded the catalytic ability of enzymes in sediment. The study of Bailey et al. (2011) showed biochar addition decreased the activities of β-xylosidase, lipase and leucine aminopeptidase and adsorption reaction was one of the reasons. (ii) Biochar addition could be directly detrimental to the microbes and decrease the production of enzymes (Killham, 1985). The study of Masiello et al. (2013) showed that biochar had negative effects on microbial behavior, such as material cell-cell communication and signal delivery in microbic system. (iii) the high pH value caused by high concentration of biochar addition might be another explanation. Increases in pH were observed in our study which was consistent with the previous study of Chintala et al. (2014). Besides, negative correlations were found between the pH value and the activities of invertase and alkaline phosphatase by pearson correlation analysis.

4.2. Effects of biochar on bacterial and fungal abundance

The results of q-PCR accurately reflected the changes of microbial abundance induced by biochar addition. The abundance of bacterial 16S rRNA and fungal 18S rRNA gene copies was mainly affected by biochar concentration on day 90 in this study. Bacterial and fungal abundance was increased compared with the control group when biochar addition at a low concentration level, which was supported by Chen et al. (2013), who has reported that bacterial 16S rRNA gene copies were increased by biochar addition in bacteria dominated microbial community. But when the concentration of biochar was increased to 5%, bacterial and fungal gene copies were obviously decreased by 74% and 25% compared with that of the control. Similarly, the abundance of dominant bacteria declined in treatment with high concentration level of biochar which can be observed from DGGE profile.

The pH value which was significantly affected by biochar concentration might play a key role in microbial abundance (Deng et al., 2015). It was well known that slightly alkaline or neutral conditions favored bacterial and fungal growth comparing with the weak acid conditions (Marstorp et al., 2000; Rousk et al., 2009). After 30 days of incubation, the pH value was stable in our study and it was nearly 7.3 when biochar concentration was 1%. The pH value (7.3) was suitable for microbial growth and could contribute to the increase of bacterial and fungal abundance (Chen et al., 2013). However, when the concentration of biochar was increased to 5%, the pH value of sediment was higher (nearly 8.5) which would inhibit the growth of some bacteria and fungi, and decrease the bacterial and fungal coding gene copies. Similarly, fungal phosphor lipid fatty acid (PLFA) concentration reached its maximum when the pH value was 7.2, but it decreased when pH deviated from the value (Rousk et al., 2009).

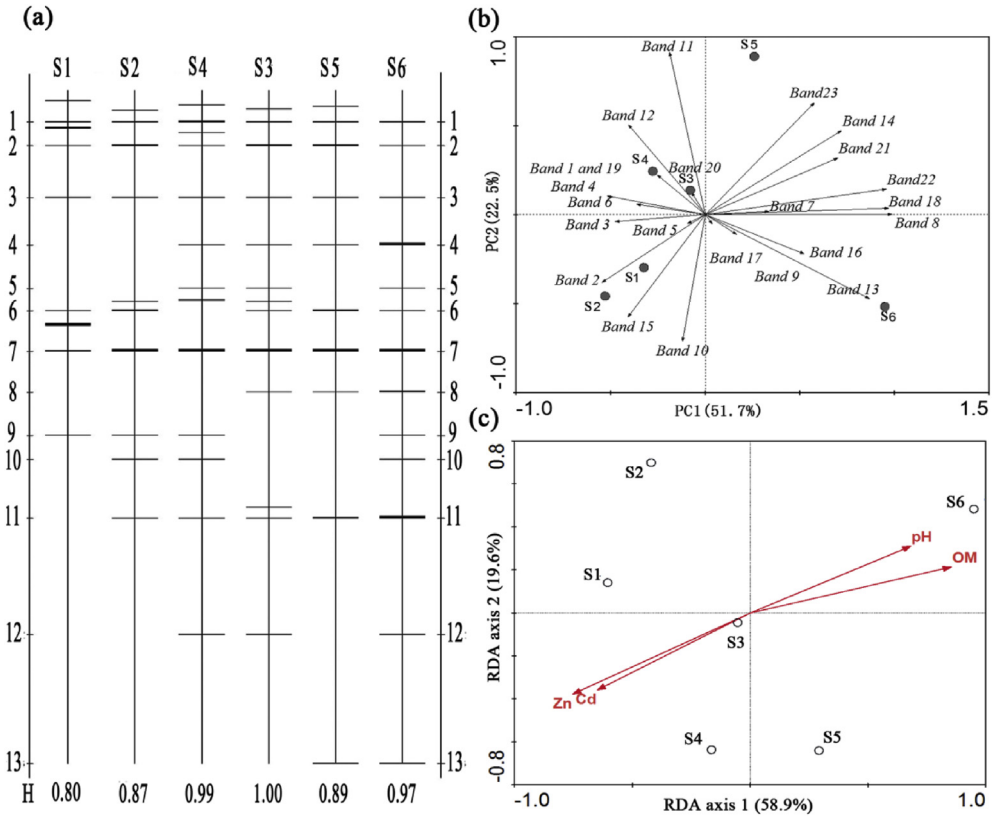

Fig. 4. Similarity dendrograms (a) of banding patterns generated by PCR-DGGE of 16S rRNA gene fragments under different concentrations of biochar treatments. Main bands were numbered at the left and right sides of the figure (a) and the diversity indexes (H) were shown at the bottom. Figure (b) and (c) were the diagrams from PCA and RDA. Samples were represented as circles. S1, S2 and S3 were represented the samples from 0, 1% and 5% biochar treated sediment at day 45; S4, S5 and S6 were represented the samples from 0, 1% and 5% biochar treated sediment on day 90. Bands and environmental variables were represented as sold lines with filled arrows in figure (b) and (c), respectively.

4.3. *Effects of biochar on bacterial community structure*

The diversity indexes (H) can reflect the changes of species diversity which take into account both species richness and species evenness (Deng et al., 2015; Huang et al., 2016). On day 90, the diversity indexes (H) were nearly the same low value in the presence and absence of biochar, which indicated that the species diversity of indigenous microbial community in sediment didn't obviously changed by biochar. However, changes in band position and intensity were found in DGGE profiles which could be quantified to analyze in two ways (Lu et al., 2014). One is the result of PCA of bacterial DGGE profiles which showed that all biochar-amended sediment samples were discrete from their control, especially these samples on day 90. The other one is the obvious changes in relative intensity of specific bands caused by biochar addition which could reflect the succession of bacterial community. The relative intensity of certain bands (4, 8, 11, 12, and 13) were increased with biochar addition which can be interpreted by the enhancement of biochar on these specific bacteria (Zhang et al., 2011). However, biochar addition decreased the relative intensity of other bands (1, 3, and 9) which reflected the adverse effects of biochar addition on these bacterial species. When biochar was at a concentration of 5%, relative intensity of band 2, 6 and 7 decreased and the three bands represented all kinds of dominant species of bacteria. Therefore, the succession of bacterial community occurred and it depended on biochar concentration.

In addition to biochar concentration, changes in physicochemical properties could also explain the variations of bacterial community. Several studies have mentioned that soil physicochemical properties might be related to community structure (Jindo et al.,

Table 2
Correlations among physicochemical properties, heavy metals, enzymes activity and microbial abundance during the whole incubation time.

	pH	Organic matter	Urease	Alkaline phosphatase	Invertase	Cd	Zn	Bacteria abundance	Fungal abundance
pH	1	0.851**	0.520*	−0.504*	−0.595**	−0.769**	−0.904**	0.173	0.052
Organicmatter	–	1	0.421	−0.397	−0.410	−0.485*	−0.668*	0.034	0.038
Urease	–	–	1	−0.201	−0.629**	−0.585**	−0.568**	0.470	0.159
Alkaline phosphatase	–	–	–	1	0.136	0.368	0.546*	−0.051	0.214
Invertase	–	–	–	–	1	0.700**	0.687**	−0.184	−0.100
Cd	–	–	–	–	–	1	0.935**	−0.526	−0.234
Zn	–	–	–	–	–	–	1	−0.374	0.188
Bacteria abundance	–	–	–	–	–	–	–	1	0.797*
Fungal abundance	–	–	–	–	–	–	–	–	1

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

2012; Zhang et al., 2011). Wu et al. (2016) indicated that compost altered microbial community which can be explained by physico-chemical properties. In this study, the changes of bacterial community structure combined with physicochemical properties and heavy metals were analyzed by RDA and the result showed that all four environmental parameters (pH, organic matter, extractable fraction of Cd and Zn) could explain almost 92% of the total sediment microbial community variation, which indicated that biochar affected indigenous microbes either directly or indirectly via changing the physicochemical properties of sediment (Deng et al., 2015). According to the physicochemical parameters matrix which were used to calculate the contribution to microbial community composition caused by biochar addition, organic matter content explained the majority (45%) of these variation. Thus, organic matter content was mainly related to the effects of biochar on microbial community structure. It can be concluded that organic matter content could be affected by biochar and biochar might alter the microbial community structure mainly via changing the organic matter content of sediment. Similarly, Xiong et al. (2015) indicated that organic matter content could directly affect sediment bacterial community structure in Erhai Lake and our previous study also demonstrated the organic matter content was related to microbial community (Huang et al., 2015).

4.4. Environmental implication

Microbial community is an important index of sediment quality. A well functioning microbial community is a prerequisite for resilience to external factors (Bhaduri et al., 2016). Biochar has been widely used for in-situ environmental remediation. However, the application of biochar at a high concentration level may induce adverse impact on sediment microbial community structure and activity, which may ultimately result in changes in soil function. It is important to consider that the effects of biochar on microbial activity and community structure were dependent on several factors, such as soil type, biochar source, addition rate and enzyme type, thus the biological effects of biochar varied from each other in previous studies (Tian et al., 2016; Chen et al., 2013; Hu et al., 2014b). The findings of this study highlighted the adverse effects of biochar on sediment microbial environment when it was widely used and provided an insight to facilitate the application of biochar as a sediment amendment.

5. Conclusions

This study demonstrated that pH and organic matter content increased, whereas extractable fraction of Zn and Cd declined when sediment was treated with biochar. High concentration of biochar addition decreased enzymes activity and microbial abundance, and it also altered microbial community structure. pH, organic matter content, extractable fraction of Zn and Cd explained 92% of the variation in the bacterial DGGE profiles and organic matter content explained the majority of the variation. Up to now, the knowledge about the interaction mechanism of biochar is still lacking and further study is needed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.01.130.

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