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Response of soil micro-ecology to different levels of cadmium in alkaline soil

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ABSTRACT

Although the effect of heavy metal on soil microbial diversity was widely studied, the interaction among micro-ecological environment in heavy metal contaminated soil was less known. In this study, we systematically investigated the influence of cadmium (Cd) on soil micro-ecological environment (pH, nutrient content, soil enzyme activities, microbial biomass, bacterial and fungal diversities). Results showed that pH values slightly decreased with the Cd level increase, whereas the nutrient content including of Olsen-P (OSP), Alkeline-N (ALN), Olsen-K (OSK) and organic matter (OM) did not show significant difference in different treatments. In contrast to physicochemical properties, the biochemical qualities were easily influenced by Cd pollutant, resulting in soil microbial numbers and enzyme activities significantly decreased. High-throughput sequencing showed that microbial community composition was significantly affected by heavy metal. For bacteria, *Actinobacteria* abundance significantly decreased in Cd treated soil, corresponding to *Proteobacteria* and *Firmicutes* increased. For fungi, the most dominant phyla member (*Ascomycota*) was significantly decreased whereas *Zygomycota* significantly increased with Cd addition. These results further revealed the integral interrelation of micro-ecology environmental players under the stress of different Cd levels.

1. Introduction

Soil ecological environment has been seriously threatened by heavy metal, which has possibly caused the alteration of soil main properties. In the previous reports, the main nutrient elements (Nitrogen, Phosphorus, Potassium and Organic matter) varied among different heavy metal contaminated soil, which might have a relation with the heavy metal pollution (Khan et al., 2017; Chaffei et al., 2004). More importantly, soil is one of the largest microbial libraries on earth and contains large of microbial diversity with an estimated 10^7–10^9 distinct bacterial species and 1.5 million fungi taxa worldwide (Narendrula-Kotha and Nkongolo, 2017). The diversity and abundance of microbes are closely related with regional distribution, environmental conditions and human activities. In recent years, many studies found that the microbial structure and composition were directly and indirectly influenced by heavy metal. Among the varieties of heavy metal pollutants, Cadmium (Cd), a non-essential trace element, could cause toxic reactions even in low concentrations (Khan et al., 2015). The enrichment of Cd in soil caused by natural and anthropogenic activities has been considered to be a great concern, which has threated the ecology and food safety (Pan et al., 2016). Therefore, the Cd was chosen to comprehensively understand the effect of Cd on microbial diversity, which would be also benefit for the remediation of Cd contaminated soil.

With the studies between soil and contaminant going further, many researches have focused on the connection between indigenous microorganism and contaminant (Bouskill et al., 2010; Sheeba et al., 2017). Nowadays, high-throughput sequencing techniques have been widely applied to study the microbial diversity and abundance (S. Li et al., 2017; Luo et al., 2017; Wang et al., 2015). Although many works have been done on the understanding of the interaction between heavy metals and microbes, there was no consensus on the effect of heavy metals on microbial diversity and abundance. For example, Gołębiewski et al., (2014) reported the bacterial diversity was decreased in Zn contaminated soil. However, some reports showed that the bacterial diversity in forest soil was not influenced by heavy metal (Chodak et al., 2013). Because the energy and nutrient cycling remained unclear, few studies revealed the interactions of microorganisms among heavy metal and other compounds, especially in alkaline soil. In addition, studies of fungal diversity on heavy metal contaminated soil are very lacking. More importantly, not only the microbial diversity should be studied, but the whole micro-ecology under Cd stress should be evaluated.

In this study, we systematically investigated the influence of Cd at different levels on micro-ecology in alkaline soil. The physicochemical and biochemical properties including of pH, Olsen-P (OSP), Alkeline-N (ALN), Olsen-K (OSK), organic matter (OM), urease, invertase,

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dehydrogenase, bacterial and fungi diversities were determined to discuss the interaction of main environmental factors under Cd stress.

2. Material and methods

2.1. Soil preparation

Soil samples were collected from the campus of Sichuan University, Chengdu, China. The soil samples were air dried, sieved through 2 mm mesh, and then carefully mixed with cadmium chloride solution. The Cd levels added into soil were NCS (0 mg/kg), CS1 (5 mg/kg), CS2 (10 mg/kg), CS3 (15 mg/kg) and CS4 (20 mg/kg), which were conducted with three replicates. After the soil adequately mixed with Cd, the soil was sieved again to pass 2 mm mesh and then placed into pots (2 kg soil/pot). The soil was wetted with deionized water to approximately 65% soil field water capacity and then covered with plastic foil. Finally, the soil contaminated Cd was incubated for 60 days.

2.2. Soil properties analysis

After 60 days incubation, soil pH was measured by the pH meter (METTLER-S220) with a soil/water ratio of 5 g/25 mL. OSP, ALN and OSK of soil were measured as the method ascribed by Liu et al. (2017). Soil organic matter was determined according to the method ascribed by Walz et al. (2017). In addition, the available Cd was measured by the TCLP method (Xu et al., 2017). BCR sequential extraction method was used to analysis the Cd formation according to Wu et al. (2016). The sequential extraction method was conducted as follows: (1) 1 g of the sieved soil was shaken at 25 ℃, 150 rpm for 16 h with 40 mL 0.11 M CH_3COOH and then centrifuged for 5 min at 8000 rpm to acquire supernatant; (2) The above mentioned residue was shaken at 25 ℃, 250 rpm for 16 h with a 40 mL mixture of 0.5 M $NH_2OH{\cdot}HCl$ and 0.05 M HNO_3. Then the mixture was centrifuged for 5 min at 8000 rpm and the supernatant was collected for assay. (3) The above mentioned residue was mixed with 10 mL 30% H_2O_2 (pH = 2.5) and placed in a bath at 85 ℃ for about 1 h until the volume of liquid was less than 3 mL, then the residue was extracted with 10 mL 30% H_2O_2. Finally, 50 mL 1.0 M CH_3COONH_4 (pH = 2) was added and the mixture was centrifuged for assay when the volume of above liquid was less than 1 mL; (4) the above residual soil was digested by using a microwave digestion method that 0.2 g above residual soil was mixed with 6 mL HNO_3, 5 mL $HClO_4$ and 4 mL HF, then the mixture was respectively heated at meddle-low, meddle and meddle-high temperature for 5 min. The method partitioned metal into HOAc extractable, reducible, oxidizable and residual fractions. Finally, the Cd concentration was measured by atomic absorption spectroscopy (AAS; VARIAN, SpecterAA-220Fs).

2.3. Soil microbial biomass and enzyme analysis

Soil microbial biomass was determined according the method described by Cheema et al. (2009). Aqueous extracts of 3 g soil samples were serially diluted and spread on nutrient agar for bacteria and streptomycin-rose bengal agar for fungi through the plate spread method. The total number of microbes was counted after 3–5 days cultivation at 28 ℃ in the dark.

The soil enzyme activities were determined according to the method ascribed by Wu et al. (2016). Dehydrogenase activity was assayed at 37 ℃ for 24 h by incubation of the mixture containing 1 g soil, 4 mL Tris-HCl buffer (pH = 7.6) and 2 mL 0.5% TTC. After that, 10 mL methanol added into the mixture. The above mixture was centrifuged at 4500 rpm for 5 min and the supernatant was determined spectrophotometrically at 492 nm. Dehydrogenase activity was evaluated by triphenylformazan (TPF) and expressed as μg TPF/(g soil·24 h). Urease activity was assayed at 37 ℃ for 24 h with the mixture of 1 g soil, 200 μL methylbenzene, 2 mL 10% urea solution and 4 mL of citrate buffer (pH 6.7). The urease activity was evaluated by the NH_4^+ color complex that was measured at 578 nm and the urease activity was expressed as μg NH_4^+/(g soil·24 h). Invertase activity was assayed at 37 ℃ for 24 h with the mixture of 1 g soil, 3 mL 8% sucrose solution, 1 mL phosphate buffer (pH = 5.5) and the supernatant was measured at 508 nm. The invertase activity was expressed as μg glucose/(g soil·24 h).

2.4. Microbial diversity analysis

To analysis the diversities of active bacteria and fungi in soil, the microbial RNA was extracted from 0.5 g fresh soil with OMG soil RNA Kit (Vazyme Biotech Co.,Ltd) according to the manufacturer's instructions. After that, the cDNA gene was obtained by RNA inverse transcription with HiScript Q RT SuperMix for qPCR (Vazyme Biotech Co.,Ltd). For amplification of bacterial 16S rRNA, PCR was performed using primer pair 530F (5′ GTG CCA GCM GCN GCG G) and 1100R (5′ GGG TTN CGN TCG TTR). For amplification of fungal internal transcribed spacer (ITS), PCR was performed using primer pair ITSF (5′ TCC GTA GGT GAA CCT GCG G) and ITSR (5′ TCC TCC GCT TAT TGA TAT GC). PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5x FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol.

Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego,USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Raw fastq files were quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (i) The reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window. (ii) Sequences whose overlap being longer than 10 bp were merged according to their overlap with mismatch no more than 2 bp. (iii) Sequences of each sample were separated according to barcodes (exactly matching) and Primers (allowing 2 nucleotide mismatching), and reads containing ambiguous bases were removed.

Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) with a novel ‘greedy’ algorithm that performs chimera filtering and OTU clustering simultaneously. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using confidence threshold of 70% (X. Li et al., 2017).

2.5. Statistical analysis

In this study, mean and standard deviation values of three replicates were calculated. Statistical significance was performed using SPSS 18.0 package, and means values were considered to be different when $P < 0.05$ using least significant difference (LSD). All statistics were performed using Origin 8.0 (USA).

3. Results and discussion

3.1. Soil main properties analysis

The main properties of soil before treatment with Cd were showed in Table S1. The soil was sandy loam texture and alkaline (pH 8.89). The organic matter was low with a value of 12.53 g/kg, whereas the total N, P and K were 0.65, 1.39, 23.15 g/kg, respectively. After treatment with Cd, the main characteristics of soil were weakly influenced and the results were showed in Table 1. As illustrated in Table 1, the pH values of contaminated soil weakly decreased with Cd increase. The OM, OSK, ALN were 12.15–12.45, 39.55–45.30, 42.00–44.50 mg/kg, respectively, which were not obviously influenced in different Cd