Effects of Cadmium Stress on Microbial Community Diversity in Soil Potted With *Sasa Argenteastriatus*

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Effects of Cadmium Stress on Microbial Community Diversity in Soil Potted With *Sasa Argenteastriatus*

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Abstract. In order to understand the remediation mechanism of cadmium-contaminated soils, pot experiments were conducted to study the effects of cadmium stress on soil microbial biomass and soil microbial diversity in *Sasa argenteastriatus* plantations. The results showed that Cd stress significantly changed the characteristics of soil microbial biomass carbon content, and the level of soil microbial biomass carbon was higher than that of the control when under 0.5mg/kg Cd stress. Heavy metals significantly inhibited soil microbial biomass carbon at higher concentrations of cadmium (>5 mg/kg). Cadmium pollution significantly changed the structure of bacterial communities and promoted the growth and development of some tolerant bacterial species (*hongkongensis* and *ovatus*) at a low level of cadmium stress of 0.5mg/kg. High concentrations of cadmium caused stress that significantly inhibited the growth of *Baclicus thermoamylovorans* and *Baclicus foraminis*. These preliminary results reveal the response of soil microbial community structure to heavy metal pollution, and provide a theoretical reference for early warnings of trends in soil quality changes.

1. Introduction

Soil microorganisms are important participants in the material cycle and energy conversion of soil ecosystems, and constitute the most active part of soil organic components and ecosystems. They participate in almost all processes of material cycle and energy flow in soil ecosystems. Therefore, soil microbes are considered to be the most sensitive biological indicators of soil quality [1-2]. With the rapid development of industrialization and urbanization, as well as the extensive use of chemical fertilizers, pesticides, and herbicides in agricultural production, cadmium pollution has become a more and more serious problem worldwide [3-4]. It has not only caused enormous economic losses [5], but has also significantly affected soil microbial flora the flora of soil microorganisms, and changed the structure of microbial communities and their biological activities [6-8]. This further affects the transformation of effective resources of soil and circulation [9-11]. Therefore, research on the physicochemical properties of, and microbial flora in, soil contaminated with heavy metals is presently receiving much attention from researchers.

Sasa argenteastriatus is a ground cover bamboo of the Gramineae family. It is widely used as ornamental bamboo and for slope protection because of the rapid horizontal growth of its underground

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bamboo whips and developed roots [12]. *Sasa argenteastriatus* is native to Japan, and showed great adaptability after its introduction to China. Increasing levels of Cd pollution seriously affect the growth and mineral metabolism of *Sasa argenteastriatus,* which are closely related to changes in soil microbial community structure under Cd pollution conditions, but there has been a lack of research [13]. Therefore, in order to understand the characteristics of soil microbial community structure under Cd contamination conditions, the microbial biomass and bacterial community structure in soil potted with Sasa argenteastriatus under stress due to different concentrations of Cd were studied using the 16S RNA sequencing technique. It is expected to provide a theoretical basis for a further understanding the response of soil ecological processes, dominated by soil microbial communities, to the actual level of cadmium pollution.

2. Study Methods

2.1. Study Soil

The soil used in this study was collected from the JUZI town of Leshan City, Sichuan province, China. The soil is drab soil (its background value of cadmium is 0.001 mg/kg, pH is 6.8, total nitrogen content is 390.1 mg/kg, total phosphorus content is 481.32 mg/kg). Samples of 0-20 cm surface soil were collected, plant residues and gravel were removed, and then the soil samples were dried naturally and passed through a 2mm screen for reserve.

2.2. Experimental Design

Pot experiments were conducted in the laboratory. Seven different concentration treatments (0 mg kg^{-1}, 0.5 mg·kg^{-1}, 1.0 mg·kg^{-1}, 5.0 mg·kg^{-1}, 10.0 mg·kg^{-1}, 20.0 mg·kg^{-1}, 50.0 mg·kg^{-1}) of single Cd stress were recorded as CK, Cd0.5, Cd1, Cd5, Cd10, Cd20, Cd50, and repeated three times per treatment.

21 plastic basins (20 cm in diameter and 12 cm high) were used in the experiments. The pot was filled with 1 kg of dry soil per basin, and the corresponding concentration of $Cdcl_2 \cdot 2.5H_2O$ solution was applied. The water content was kept constant and the balance maintained for two weeks. Then the same growth of *Sasa argenteastriatus* was transplanted to the plastic basins. Water was irrigated with tap water throughout the growth period, so that the soil moisture content reached 60% of the maximum field capacity. In order to prevent leakage of Cd pollutants, plastic pallets were placed under the basins and Cd leachates were poured back into the basins. Soil microbial biomass carbon and nitrogen (MBC and MBN) were measured 40 days after incubation, and soil microbial diversity was determined using 16SRNA sequencing. The results were analyzed by one-way ANOVA and $P<0.05$ was considered to be significant.

3. Results and Analysis

3.1. Soil Microbial Biomass

With increases of soil Cd pollution, soil MBC and MBN showed a trend of initial increase and then decrease, especially MBC. MBC content was higher at low levels of Cd stress (Cd0.5, Cd1), but lower than the control at high levels of Cd pollution (Cd10, Cd20, Cd50). Microbial biomass reached the lowest level when Cd stress was 50.0 mg·kg^{-1} (Fig. 1). Statistical analysis showed that the correlations between MBC levels and different concentrations of Cd stress were significant ($P=0.028$).

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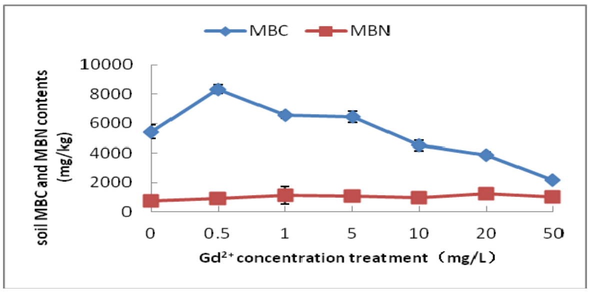

Figure 1. Soil microbial biomass carbon and nitrogen content at different levels of Cd stress

3.2. Effect of Cd Stress on Soil Microbial Community Diversity

Each soil sample was analyzed for 46610 valid reading sequences, and 692 operational taxonomic units (OTUs) were identified at generic the genus level. The OTU values in soils with high Cd concentrations were significantly higher than those in the uncontaminated soil. The data of Chao1 richness estimator, Shannon-Wiener diversity index, and Simpson diversity index all showed that the species of bacteria increased in the Cd-polluted soil (Table. 1).

Table 1. Diversity index of soil bacterial communities under different concentrations of Cd stress

Sample	Valid reads					
		OTU(P)	ACE	Chao1	Shannon	Simpon
CK	45021.3333±3808.5263	2958.3333±41.5251	3757.9576±21.3425	3732.0703±15.1113	9.7401±0.0703	0.9970±0.0004
Gd0.5	43020.6667±3037.9220	2590.0000±344.8869	3266.9022±575.3989	3289.0326±481.8289	9.4329±0.3622	0.9954±0.0018
Gd1	41500.3333±2275.6033	2787.6667±132.8621	3549.6106±153.2662	3594.0926±182.3525	9.6100±0.1345	0.9963±0.0006
Gd5	39420.6667±4133.1671	2998.6667±102.1045	3857.6389±174.4007	3818.5218±150.4979	9.7942±0.0899	0.9969±0.0002
Gd10	37578.0000±7358.1532	2823.0000±168.2914	3645.1475±133.9135	3641.8114±162.8661	9.5235±0.0052	0.9960±0.0002
Gd20	41508.0000±4423.26113	2972.0000±91.0165	3824.7954±117.7755	3848.5028±121.5139	9.5446±0.1528	0.9948±0.0006
Gd50	42937.6670±1275.2887	2912.6667±112.5093	3630.8786±172.5292	3632.5011±163.1302	9.5325±0.1863	0.9957±0.0011
Total			-	-	-	-

-: Data not available.

Intuitively, the microbial diversity indices of Simpson, Chao and Ace at Cd concentration 5 was higher than that at other concentrations. Statistical analysis showed that the microbial diversity index did not reach a significant level under different degrees of Cd stress ($P_{Simpson} = 0.096$, $P_{Shannon} = 0.276$, $P_{Chao} = 0.143$, $P_{Ace} = 0.176$).

3.3. Effect of Cd Stress on Soil Microbial Community Structure

According to the sequencing results, 36 species were classified by phylum, 87 species classified by class, 130 species classified by target, 166 species classified by family, 205 species classified by genus and 34 species classified by species.

Among the seven groups of soil samples, Proteobacteria, Bacteroidetes, Actinobacteria, Chloroflexi and Acidobacteria were the most dominant phyla associated with the BSF, accounting for 85% - 88% of the bacteria identified. Among them, Proteobacteria accounted for almost half of the total number of bacteria. However, the community structure of the flora differed in the soil samples with different levels of Cd stress. For example, at low cadmium stress (Cd0.5, Cd1, Cd5), Acidobacteria, Gemmatimonadetes, TM7, B-42, spirochaetes, GN02, OP11, BRC1 and PRR-12 were present in significantly higher numbers than at high levels of Cd stress (Cd20, Cd50). The spirochaetes, GN02, OP11, BRC1 and PRR-12 contents were also higher than those of the control group. It is suggested that proper Cd stress is beneficial to the growth and reproduction of some bacteria. However, some bacteria showed different trends related to Cd stress. Fig. 2 shows that the content of Kazan-3B-28 decreases with increases in Cd concentration.

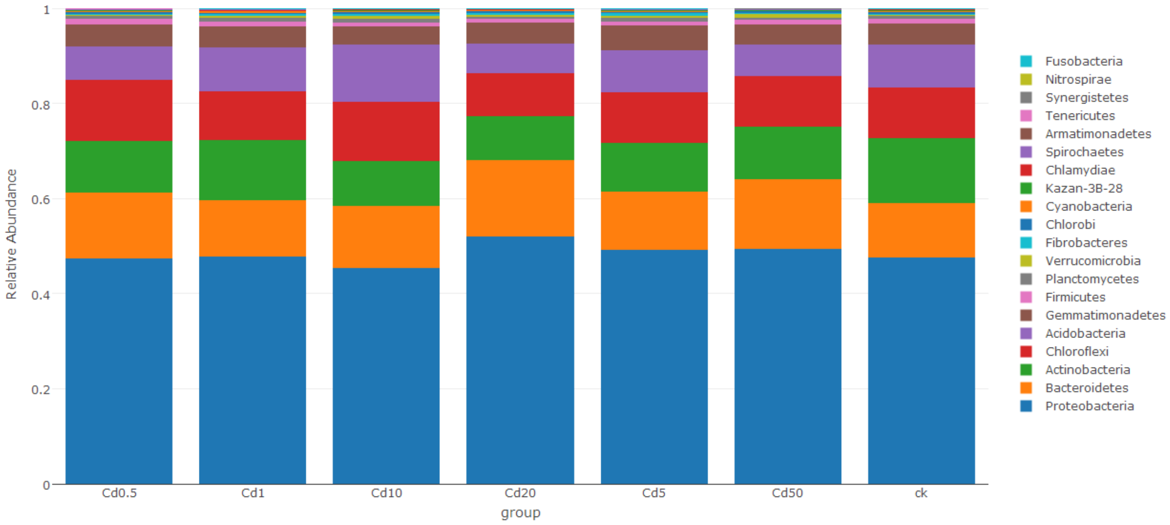

Figure 2. Relative content of main bacterial colonies in soil samples at the phylum level

At the species level, the number of zavarzinii, diminuta and thermoamylovorans was the largest in the seven groups of soils, accounting for 42-59% of the total number of bacteria. The number of diminuta gradually increased with increasing Cd concentrations. When the concentration of Cd was 50 $mg \cdot kg^{-1}$, the number of diminuta was more than 11 times higher than that of the control. However, the number of elegans decreased with increasing Cd pollution. Some other strains showed different trends under different concentrations of Cd stimulation. For example, the number of mucilaginosa, fulvum and wittichii stimulated by moderate cadmium concentrations (Cd10, Cd20) was higher than at other concentrations of cadmium pollution, and significantly higher than CK. The numbers of Thermoamylovorans and foraminis were significantly lower at Cd20 and Cd50, suggesting that high cadmium concentrations inhibited the growth of these two bacterial species. At low levels of cadmium stress, some specific strains were produced, such as hongkongensis and ovatus, whose growth was stimulated by Cd0.5.

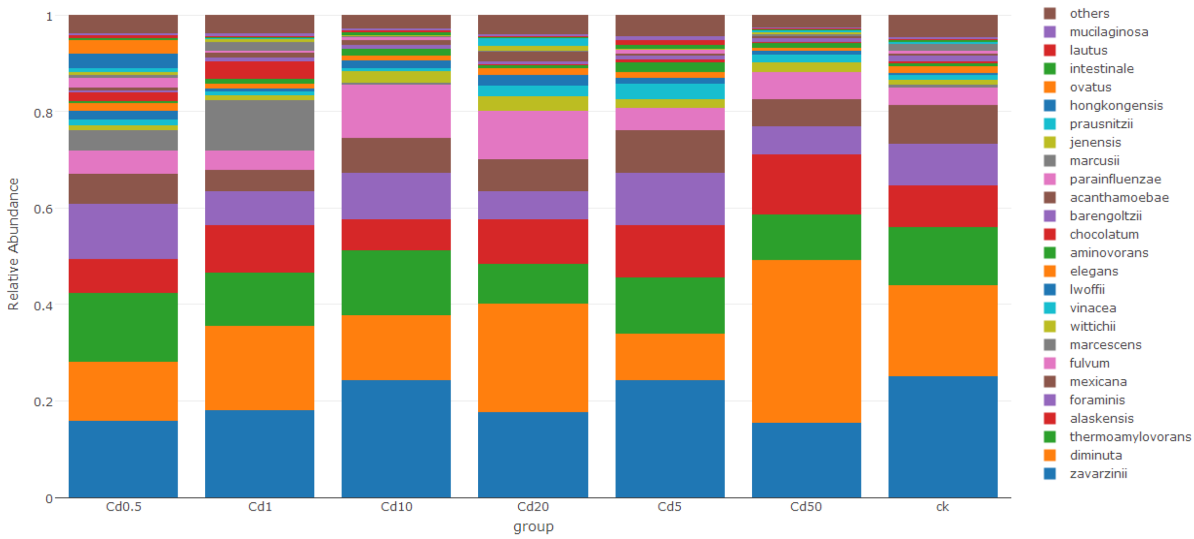

Figure 3. Relative content of the main bacterial colonies in soil samples at the species level

4. Discussion

Microbial biomass is one of the most sensitive indicators for evaluating the effects of heavy metals on soil microorganisms. Over a long period, researchers have carried out research on the effects of heavy metal pollution on soil microorganisms, making some new discoveries. A large number of studies have shown that the soil microbial biomass changes after the soil is polluted with different concentrations of heavy metals, but results are not entirely consistent. Some studies found that long-term heavy metal pollution had a significant inhibitory effect on microbial biomass [14]. Other studies showed that when Cd concentration were low, soil MBC was positively correlated with Cd concentration, while they were negatively correlated with higher Cd concentrations [15-16]. The turning point of Cd concentration was different due to soil properties. The same conclusion is reached in this experiment. The turning point of cadmium concentration is 5mg/kg. In this study, the soil MBC was higher when Cd concentration is less than 5 $mg\cdot kg^{-1}$, while it decreased significantly at higher Cd concentrations (>5 $mg\cdot kg^{-1}$). This may be due to the high tolerance of bacteria that can be cultured to Cd, and the presence of low concentrations of Cd can stimulate plant growth [17], providing more substrates for soil microorganisms. However, when the concentrations of Cd increased further and reached or even exceeded the maximum tolerance level for microorganisms, the normal physiological activities of the microbial population was affected or changed, and ultimately overt toxicity resulted.However, MBN did not show a trend towards significant change with the increase of Cd stress levels in this study. It may be that the ratio of Cd-sensitive groups of bacteria to tolerant groups in soil changed markedly, and the structure of soil microbial community changed, but the total quantity and biomass did not change significantly. Previous study also also fully demonstrated the result [18].

Diversity indices can reflect the complexity of community composition, and the dynamics of these indices can reveal the response characteristics of soil microbial communities to environmental changes [19]. Guo et al. showed that the microbial diversity of soils contaminated with heavy metals decreased significantly and dominant populations appeared. Xie et al. considered that the microbial diversity of soil polluted with medium concentrations of heavy metals was the highest, and the effect of different concentrations of heavy metals on microbial diversity may not reflect a simple linear relationship [20]. In contrast to previous studies, it was found here that the number of different bacterial species bacterial species in soil contaminated by cadmium increased in this experiment, but the soil microbial diversity index showed no significant difference. This phenomenon It may be closely related to the difference of indigenous bacterial communities in soil and their tolerance to Cd, as well as the emergence and disappearance of some dominant groups under Cd stress.

Changes in microbial community structure can predict changes in environmental quality sooner, making them one of the most potential sensitive biological indicators [21]. Many studies show that the microbial community structure, species abundance, and dominant population in heavy metal-contaminated soil change significantly [22-23].

In this study, some specific strains, such as Corynebacterium hongkongensis and ovatus were produced under 0.5 $mg\cdot kg^{-1}$ Cd stress conditions. High levels of Cd stress (20, 50 $mg\cdot kg^{-1}$) severely inhibited the number of Baclicus Thermoamylovorans and Baclicus foraminis. The results showed that low concentrations of Cd promoted the growth and development of some tolerant bacteria and changed the bacterial community structure. The individual populations were more sensitive to cadmium stress, and the population was greatly reduced [23].

Many studies have found that Bacillus spp. has an evident adsorption effect on heavy metal ions [24-25]. However, this study showed that 20 $mg\cdot kg^{-1}$ Cd stress was the cut-off point for the number of Bacillus bacteria. When the concentration of Cd was lower than 20 $mg\cdot kg^{-1}$, Bacillus showed high tolerance and resistance to Cd, and had better repair effects. However, when the concentration of cadmium was higher than 20 mg/kg, the quantity of Bacillus decreased significantly, which significantly affected the repair effect. These results indicate that the Cd stress changed the microbial community structure, affected the physiological growth of microorganisms, and changed their functions.

5. Conclusion

In conclusion, Cd stress significantly changed the characteristics of soil MBC content. High levels of Cd stress (>5 mg/kg) significantly inhibited soil MBC. The bacterial community structure was changed by Cd stress, but it had no significant effect on microbial diversity indices. The growth and development of some tolerant bacteria were promoted at the 0.5 mg·kg^{-1} Cd stress level, and the growth of some Bacillus was inhibited significantly at high Cd stress levels.

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