Front. Agric. China 2007, 1(3): 289–295
DOI 10.1007/s11703-007-0049-0

RESEARCH ARTICLE

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Response of microbial communities to phytoremediation of nickel contaminated soils

Abstract Through pot experiment, effects of phytoremediation on microbial communities in soils at different nickel treatment levels were studied. Two Ni hyperaccumulating and one Ni tolerant species were planted in paddy soils different in Ni concentration, ranging from 100 to 1 600 mg/kg. After 110 days of incubation, soil microbial activities were analyzed. Results showed that populations of bacteria, fungus, and actinomycetes and biomass of the microorganisms were stimulated when nickel was added at a rate of 100 mg/kg in non-rhizospheric soil. When the rate was over 100 mg/kg in the soil, adverse effects on the soil microbial communities were observed. The plantation of Ni hyperaccumulating species could increase both the population and biomass of soil microorganisms, because, by absorbing nickel from the soil and excreting root exudates, the plants reduced nickel toxicity and improved the living environment of the microbes. However, different plant species had different effects on microorganisms in soil.

Randomly Amplified Polymorphic DNA (RAPD) with five primers was used in this study in 25 soil samples of four types of soils. A total of 947 amplified bands were obtained, including 888 polymorphic bands and 59 non-polymorphic bands. The results indicated that the composition of microbial DNA sequences had changed because of the addition of nickel to the treated soils. Shannon-Weaver index of soil microbial DNA sequences reduced in the nickel contaminated soils with increasing nickel concentration. The changes in Shannon-Weaver index in the four types of soils ranged from 1.65 to 2.32 for *Alyssum corsicum*, 1.37 to 2.27 for *Alyssum murale*, 1.37 to 1.96 for *Brassica juncea*, and 1.19 to 1.85 for non-rhizospheric soil. With the same amount of nickel added to soils, the Shannon-Weaver index in rhizospheric soil with plants was higher than that in non-rhizospheric soil.

Keywords soil, nickel, phytoremediation, microbial community

1 Introduction

Heavy metals are one of the major pollutants in soil, and a number of biological effects on plants, microorganisms, soil enzyme activities caused by heavy metal and bioremediation of heavy metal contamination soils have aroused ever-increasing attention from scientists. Living in long-term heavy metal contaminated soil, microbes need higher basal respiration strength and metabolic quotient, but lower microbial biomass (Insam et al., 1996; Yang et al., 2001). Some reports have found that heavy metals influence the microbial diversity and total community structure in contaminated soils, especially those with a long pollution history. As a result, some population sensitive to heavy metal pollution may decrease or die out, while another population less sensitive or resistant to the heavy mental pollution will increase (Bruins et al., 2000). Phytoextraction, the use of plants to extract metals from soil, which does no damage to soil properties and enables metal recycling, has been reported to be very efficient for cleaning up superficially contaminated soils. There are more than 300 kinds of Ni hyperaccumulating species found in the world at present (Brooks et al., 1998), including *A. corsicum* and *A. murale* (Chaney et al., 1997; Tang and Wilke, 2000).

Much of the previous work on *A. corsicum* and *A. murale* has involved the collection of field samples and measurement of metal concentration in plants, studies of metal tolerance, and the changes of metal forms. However, little or no information is available on the response of microbial communities to phytoremediation of nickel-contaminated soils.

The objectives of this study are to contribute to a better understanding of the response of phytoextraction practices on

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Translated from *Acta Pedologica Sinica*, 2006, 43(6): 919–925 [译自: 土壤学报]

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microbial community in nickel-contaminated soils; specifically, we aim to determine the changes of microorganism population, microbial biomass carbon, basal respiration, and DNA sequence diversity of soil microbial community affected by phytoextraction.

2 Materials and methods

2.1 Materials

2.1.1 Soil preparation

The soils used were collected from the surface layer (0–20 cm) of an agricultural field used for paddy rice at Guangdong Academy of Agricultural Science. The moist soils were air-dried and sieved by using a 3-mm bamboo sieve and stored in closed plastic bags before use. Soils were divided into seven lots added with different Ni concentration of 5 kg per lot. Different masses of $NiSO_4 \cdot 6H_2O$ powdered were mixed with soils carefully. The final Ni concentrations in soils were 0, 100, 200, 300, 400, 800, and 1 600 mg/kg, respectively. Then 1 800 mL distilled and deionized water (DDW) was added to each soil so as to maintain soil moisture. The treated soils were stored in closed plastic buckets with covers for 12 weeks, and then air-dried, loaded in plastic bags. Table 1 shows the Ni concentrations and chemical properties of the collected soils before addition of $NiSO_4 \cdot 6H_2O$.

2.1.2 Plants

Seeds of *Alyssum corsicum* came from Kotodesh, Albania, *Alyssum murale* from Oregon Vineyard, and *Brassica juncea* from South China.

2.1.3 Plant culture

Plastic pots were filled with 500 g (dry weight) treated soils each before seedlings were transplanted. The experiment consisted of seven treatments in total with three duplicates each. The different treated soils without plants were filled to one pot each as controls. Because *B. juncea* planted in high Ni concentration treated soils (400, 800 and 1 600 mg/kg soils) could not grow, the total number of pots was just 61 for three kinds of plants and controls.

The seeds of *A. corsicum* and *A. murale* were planted in plastic basins with 2 kg superior quality peatmoss. With the appearance of the first seven leaves, the seedlings were transplanted into the pots with four plants each. The pots were watered using DDW everyday as required according to the weather condition. A compound fertilizer was added every month in this experiment, with 0.4 g per pot. The growth periods were 110 days for *A. corsicum* and *A. murale*, and 40 days for *B. juncea*.

2.2 Analysis methods

2.2.1 Sample collection

The moist soil samples were collected and analyzed for microorganism population, microorganism carbon, basal respiration and DNA sequence diversity. Soils where *A. corsicum, A. murale*, and *B. juncea* were planted, were defined as rhizosphere soils. Soil samples for DNA extraction were placed in separate polyethylene plastic bags and immediately stored at –20°C. Moisture content of soil samples was analyzed by using the oven dry method. Portions of the soil samples were air-dried prior to Ni concentration measurement.

2.2.2 Soil properties and Ni concentration assay

Some physical and chemical properties of the soil including pH value, total carbon concentration, total nitrogen concentration and cation exchangeble capacity (CEC) were measured with the routine analytical methods (Bao, 2000). The total Ni in the soil samples was determined by the flame atomic absorption spectroscopy (AAS, Hitachi Z-5000) after digestion with a mixture of HNO_3-$HClO_4$-HF. The available Ni concentration in soils were extracted with diethylenetriamine pentaacetic acid solution (DTPA-Ni) and analyzed by AAS.

2.2.3 Soil microbial assay (Department of Microorganism, Institute of Soil Science, Chinese Academy of Sciences, 1985; Lin et al., 1999; Yao et al., 2000; Huang et al., 2004)

The viable count method was used for the estimation of soil-borne bacteria, fungus and actinomycetes. Microbial biomass carbon was determined by the chloroform-fumigation-extraction procedure in which C is extracted by 0.5 mol/L K_2SO_4 before and after fumigation. Basal respiration (CO_2 evolution) was measured by incubating 50 g of fresh soil at 25°C in 500 mL air-tight jars for one day, adjusted to 60% of water holding capacity. Respired CO_2 was trapped in 10 mL of 1 mol/L KOH solution. CO_2 was precipitated with $BaC1_2$ and the excess KOH was titrated with HCl using a phenolphthalein indicator.

The technique of RAPD molecular markers was used for monitoring the DNA sequence diversity of soil microbial community. The total microbial DNA was extracted using

Table 1 Selected soil properties and nickel concentration

pH	Total N/($g \cdot kg^{-1}$)	Total C/($g \cdot kg^{-1}$)	Total Ni/($mg \cdot kg^{-1}$)	Total Zn/($mg \cdot kg^{-1}$)	Total Cu/($mg \cdot kg^{-1}$)	Total Pb/($mg \cdot kg^{-1}$)	CEC(cation exchangeble capacity)/($cmol \cdot kg^{-1}$)
6.37	1.42	18.43	12.50	212.00	41.70	85.80	1.74

sodium dodecyl sulfate method (SDS), and then purified using a Golden Beads DNA purification kit (Sangon Ltd.). Five primers used in the PCR reactions for this study were SBS A02, SBS A03, SBS A11, SBS A19, and SBS A20 from sequences of the DNA primers of SBS A (Sangon Ltd.). The electrophoresis gels were analyzed using Gel analyst software (UVP, INC.) via photograph scanning. For data analysis, all the amplified fragments were scored as present or absent and were assigned a value of 1 or 0, respectively, and then the sequence diversity of soil microbial community was calculated.

3 Results

3.1 Effects of phytoextraction on DTPA-Ni contents in tested soil

The concentrations of total-Ni are listed in Table 2. Results showed that the total-Ni concentration at the same Ni level decreased slightly, but that in the 1 600 mg/kg treated soil was significantly different ($P < 0.05$) among rhizosphere soils and controls.

Table 2 Total Ni concentration in rhizospheric and non-rhizospheric soils

Added Ni level /($mg \cdot kg^{-1}$)	Control /($mg \cdot kg^{-1}$)	*A. corsicum* /($mg \cdot kg^{-1}$)	*A. murale* /($mg \cdot kg^{-1}$)	*B. juncea* /($mg \cdot kg^{-1}$)
0	12.2±0.5A	11.4±0.6AB	9.5±1.0C	10.2±0.9BC
100	110.5±1.7A	112.3±2.4A	111.0±2.0A	110.8±1.0A
200	210.9±1.9A	209.0±0.8A	208.9±3.3A	208.6±3.9A
300	311.3±1.7A	310.8±1.2A	308.0±3.0A	310.4±2.3A
400	411.4±0.6A	407.5±2.5A	407.3±3.0A	
800	810.5±2.2A	805.0±4.1A	807.4±3.6A	
1600	1,600.0±7A	1,550.0±5B	1,544.0±6B	

Note: Figures in the table are Mean±SD. Different letters in one line indicate statistically significant differences by one-way ANOVA and LSD method ($P < 0.05$) between treatments.

The concentrations of the DTPA-Ni are shown in Fig. 1. Ni-resistant plants and hyperaccumulators can take up Ni from the soil, and so the DTPA-Ni concentration in rhizosphere soils was smaller than that in non-rhizosphere soils; the reduction of DTPA-Ni in higher Ni-treated soils was greater than that in low Ni-treated soils.

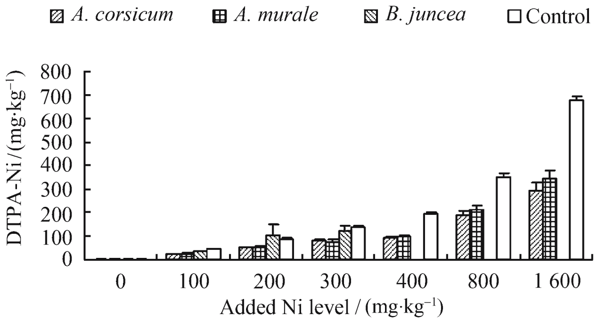

Fig. 1 Changes in DTPA-extractable Ni concentration in rhizospheric soils and control

3.2 Effects of phytoremediation on microbial population

3.2.1 Bacteria population

The total amounts of bacteria in the tested soils are shown in Fig. 2. The highest amount of bacteria in rhizospheric soil was recorded at both control soils where *A. corsicum* (50.30 × 10^6 cfu/g) and *B. juncea* (13.76 × 10^6 cfu/g) were planted and at 800 mg/kg soil where *A. murale* (65.34 × 10^6 cfu/g) was planted, which in control was at 100 mg/kg soil (9.71 × 10^6 cfu/g). The total amounts of bacteria in controls tended to reduce with increasing Ni; however, the changes of total amounts in rhizospheric soils had some difference among the Ni-treated soils due to the different plant species.

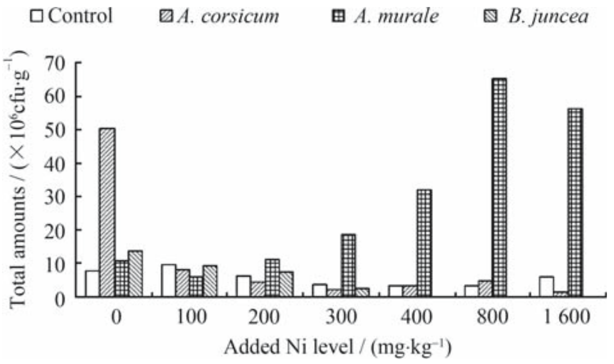

Fig. 2 Populations of bacteria in rhizospheric soils and controls

3.2.2 Fungus population

The total amount of fungi in the tested soils are shown in Fig. 3. The highest amount of fungus in rhizospheric soils was recorded at *A. murale* 100 mg/kg soil (8.30 × 10^6 cfu/g), *B. juncea* 100 mg/kg soil (7.91 × 10^6 cfu/g), *A. corsicum* control soil (8.38 × 10^6 cfu/g), and at 300 mg/kg soil (4.43 × 10^6 cfu/g) in controls.

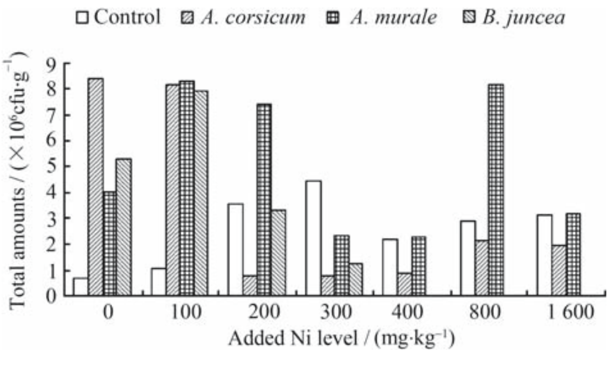

Fig. 3 Populations of fungi in rhizospheric soils and controls

3.2.3 Actinomycetes population

The total amounts of actinomycetes in the tested soils are shown in Fig. 4. The viable amounts of actinomycetes were inhibited by heavy metals. The highest amount of actinomycetes in *A. corsicum* rhizospheric soils was recorded at control soil (9.70×10^6 cfu/g), and then at 100 mg/kg soil (8.92×10^6 cfu/g). The highest amounts of other soils were 4.14×10^6 cfu/g at 200 mg/kg soil for *A. murale*, 3.60×10^6 cfu/g at 100 mg/kg soil for *B. juncea*, and 4.14×10^6 cfu/g in control, respectively.

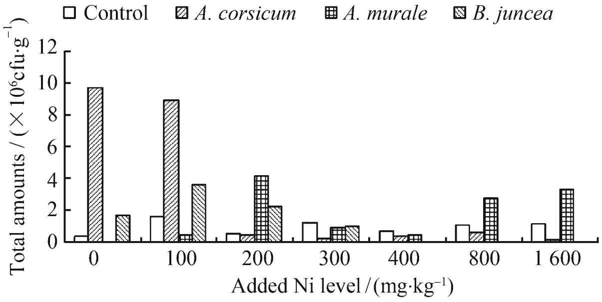

Fig. 4 Populations of actinomycetes in rhizospheric soils and control

3.3 Effect of phytoremediation on soil basal respiration

The basal respirations of the tested soils are shown in Fig. 5. The basal respiration of control soils increased with increasing Ni concentration in soils and then decreased at 200 mg/kg and higher, ranging from 0.01 to 0.09 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$; and in three kinds of rhizospheric soils, it did not change obviously with increasing addition of Ni. Results showed that the basal respiration of the rhizospheric soil, where hyperaccumulators were planted, was obviously higher than that of the control soil.

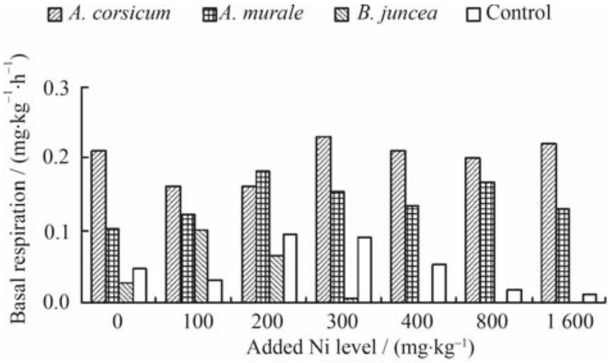

Fig. 5 Basal respiration in rhizospheric soils and controls

3.4 Effect of phytoremediation on soil microbial biomass carbon

Microbial biomass carbon (MBC) of the tested soil samples are shown in Fig. 6. Results showed that microbial biomass in controls ranged from 121.9 to 224.6 mg/kg, the biggest was recorded at 100 mg/kg. Similarly, MBC in *A. corsicum* rhizospheric soils ranged from 162.6 to 513.8 mg/kg, the biggest was at 300 mg/kg. However, as for *A. murale* and *B. juncea* soils, their biggest MBC was at rhizospheric control soil. In other words, the microbial biomass carbon tended to decrease with increasing of the Ni concentration in soils.

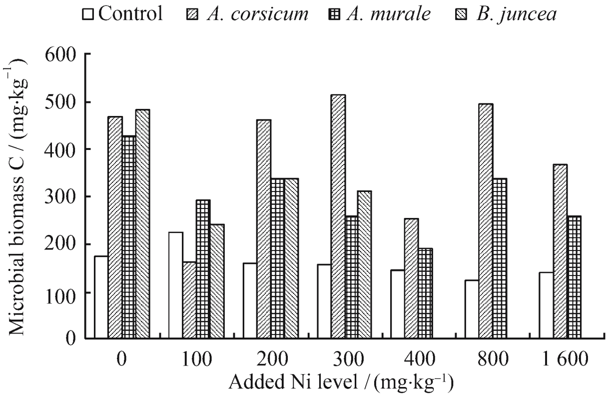

Fig. 6 Microbial biomass carbon in rhizospheric soils and control

3.5 Effect of phytoremediation on the microbial DNA sequence diversity

Results of RAPD-PCR of 25 kinds of soil samples are listed in Table 3. The total bands obtained by amplification with five primers were 947 including 888 polymorphic amplified bands (about 94% of the total) and 59 non-polymorphic bands (about 6% of the total).

Table 3 Summary of the results obtained by amplification with five primers

Primer	Amplified band	Polymorphic amplified band	Non-polymorphic band	Ratio of polymorphic band to total band/%
SBS A2	230	216	14	94
SBS A3	188	149	39	79
SBS A11	201	201	0	100
SBS A19	207	201	6	97
SBS A20	121	121	0	100
Sum	947	888	59	94

The amounts of microbial amplified bands are different when using different primers in the same soil sample, similarly, even if using the same primer in the different soil sample. The statistic results of microbial amplified bands showed that *A. corsicum* rhizospheric soils had the most amplified bands (315 bands) against 284 bands and 216 bands for *A. murale* rhizospheric soils and controls (non-rhizospheric soils), respectively, following the sequence of *A. corsicum* rhizospheric soils > *A. murale* rhizospheric soils > controls with respect to the amount of amplified bands. *B. juncea* rhizospheric soils had 132 bands for four samples. Therefore, the amplified bands of microbial DNA in rhizospheric soils were more abundant than those in controls.

The Shannon-Weaver index (SWI) of microbial DNA sequence diversity calculated in the tested soils are shown in Fig. 7. The changes in Shannon-Weaver index in the four types of soils ranged from 1.65 to 2.32 for *Alyssum corsicum*, 1.37 to 2.27 for *Alyssum murale*, 1.37 to 1.96 for *Brassica juncea*, and 1.19 to 1.85 for control. Results indicated that SWI of soil microbial DNA sequence reduced with increasing nickel concentration, and was generally higher in rhizospheric soils than in control soils.

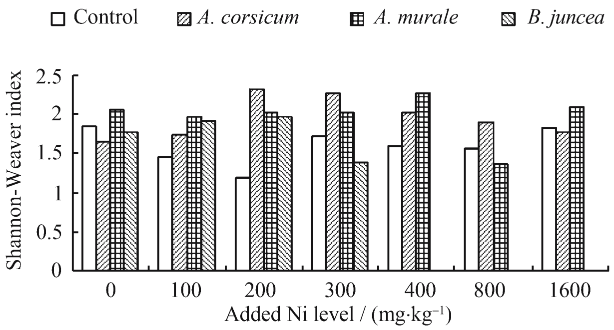

Fig. 7 Shannon-Weaver index of microbial DNA sequence in rhizospheric and non-rhizospheric soils

4 Discussion

In general, the concentration of heavy metals in soils extracted by 0.005 mol/L DTPA can be defined as the available amounts of heavy metals to plants (Zhu et al., 2002). Nickel sulfate added into paddy soils can interact with soil components and cause redistribution of Ni (Cai et al., 2005), but in this experiment, the analyzed data show that the DTPA-Ni in soils were increased with increasing amounts of Ni. The higher of DTPA-Ni in soils, the more available to plants, which would be beneficial to hyperaccumulators (Ernst, 1996; Lombini et al., 1998). In comparison with controls, the amounts of reduction of DTPA-Ni in the treated soils increased with the increase in Ni.

The soil microbial population structure is one of the important indicators to indicate the situation of the soil microbial community and stability of the soil biological system. Generally, heavy metal contamination might lead to two kinds of effects on the microorganisms: on one hand, due to heavy metal toxicities, sensitive populations would be inhibited, the kinds and amounts of microbial populations would be reduced gradually or become extinct; on the other hand, less sensitive or resistant population that had adapted to the contaminated condition would grow, increase and become the dominant population in soil microbial community (Frostegard et al., 1996; Wang, 2002); as a result, heavy metal contamination would cause the changes of the soil microbial population and community. Some results proved that different kinds of microbes had different responses to the heavy metal contamination, in which the sensitive order commonly might be actinomycetes > bacteria > fungi (Sun et al., 2000).

Nickel is one of the essential micronutrient physiologically required by plants, animals and microorganisms, as well as a kind of carcinogenic element (Fu et al., 1996). In this experiment, the highest amount of bacteria and actinomycetes in controls was recorded at the 100 mg/kg treated soil, but that of fungi was at 300 mg/kg treated soil, which indicated that the low dose of nickel has beneficial effects on the growth of soil microorganism, but higher dose may be toxic and inhibitory on the development of microbial populations. The lowest concentration inhibiting the growth of microbes in nickel-contaminated soils was 100 mg Ni/kg for actinomycetes, 100 mg/kg for bacteria and 300 mg/kg for fungi, which meant that both actinomycetes and bacteria were more sensitive than fungi to nickel contamination. The present results are in full agreement with earlier results reported.

The microbial structure in rhizosphere depends on soil type, plant species, plant growth stage, presence of contaminants, temperature and other environmental factors (Kuperman and Carreiro, 1997; Kozdroj and Elsas, 2000). Plants can secrete many kinds of organic compounds into soil, such as organic acids, and various organic acids can bind heavy metals, reduce the toxicity of metal ions, and thus mitigate the negative effects of heavy metals on microorganisms (Sun et al., 2000; Wang, 2002). Hence, these root exudates may cause the changes to the structure of microbial populations in soil. According to the changes of the viable counts in three kinds of the rhizospheric soils, it was found that the functions of plants on the populations of bacteria, fungi, and actinomycetes were different. Phytoextraction could reduce the heavy metal toxicity in the soil because hyperaccumulators or tolerant plants can uptake heavy metals from soils and secrete root exudates to bind heavy metals, improving the living condition of soil microbes, which would be beneficial to the development of the microbial populations. The viable amounts of bacteria, fungi, and actinomycetes in this experiment fluctuated with increasing of nickel concentration in soil, which may be related to the test method. When using a selective media, these results ought to belong to normal data (Hemida et al., 1997; Scragg, 1999). Therefore, phytoextraction made soil microbial species more abundant and microbial populations much higher.

The basal respiration of soil is an important indicator for the assessment of the microbial activities in heavy metal contaminated soils (Gong et al., 1997; Wang et al., 2003). Heavy metal contamination of soil could both reduce the capacity of the use of sole-carbon-source and affect the activity of microorganisms involved in organic matter decomposition and nutrient cycling in contaminated soils (Baath et al., 1998; Sun et al., 2000). In addition to the presence of heavy metals, the basal respiration in long-term heavy metal contaminated soil is closely related to the soil types, vegetations and other environmental factors. Yang et al. (2001) reported that the basal respiration of urban soils was higher than that in rural soils, while the higher heavy metal levels in soils resulted in the elevation of basal respiration. Insam et al. (1996) reported that the results of the studies on the metabolic quotient (qCO_2) response upon heavy metal contamination were contradictory. Our results that the basal respiration in control soils increased at first and then decreased with increasing of nickel concentration in soil indicated that the low dose of Ni might increase the basal respiration, while high dose might decrease it. Phytoextraction was beneficial to relieving the inhibition of heavy metals on the basal respiration.

The changes of soil microbial biomass carbon and the basal respiration may be due to the physiological adaptation or the morphological changes of soil microflora in heavy metal contaminated soils (Wang, 2002; Renella et al., 2004). Microbes may have formed several mechanisms including precipitation, physical exclusion and intracellular sequestration to adapt themselves to the heavy metal stress condition. These physiological activities may increase the consumption of energy and reduce the maintenance energy, the conversion of substrate into new microbial biomass, other metabolic processes, and the result in the microbial biomass may increase slowly or decrease (Bruins et al., 2000; Li et al., 2005). Due to the complex soil microbial composition, the microbial responses are different under stress condition. Some may increase the metabolic activity to get more energy against the stress condition, while others may slow down their activities to reduce the consumption of energy in order to maintain the survival of individuals and the population (this kind of adaptation may be disadvantageous to the development of microbial population). As a result, when the activities of microflora are strong, the basal respiration increases; contrarily, that would be lower.

In addition, the nutrient in the heavy metal contaminated soil can induce the change of the microbial biomass. It is essential that phytoremediation can change the material circulation and the energy flow in the plant-soil system. Soil microorganism can use the plant root exudates including a wide range of organic molecules as nutrients, and thus increase the conversion of nutrients into new microbial and biological activity (Kamnev and Lelie Daniel, 2000), which may explain why the microbial biomass in rhizospheric soils was higher than that in controls.

The diversity of DNA sequence of microbial community in Ni-contaminated soils was highly affected by Ni concentration, soil properties, plant species, and environmental factors. Teng et al. (2004) tested the changes of microbial DNA in mixed heavy metal contaminated farmland soils, indicating that heavy metal accumulation at different levels could markedly cause damage to microbial DNA in farmland soils and change the structural diversity of the microbial community. Chen et al. (2002) used the PCR-DGGE method to investigate the microbial populations of farmland soils, finding the significant microbial community difference among farmland soils where different crops were planted. Griffiths et al. (1999) found that microbial community structure changed consistently with increasing of the substrate, and fungi would dominate over bacteria at high substrate loading rates, suggesting that the quantity of substrate was a governing factor for community composition. In fact, Ni contamination in soils, especially at high Ni-contaminated soils, adversely affects the survival of microorganisms, which could lead to the loss of some sensitive species (populations) or the increase of tolerant species, resulting in the changes of the microbial community (Frostegard et al., 1996; Zhao et al., 2005). In our present results, the ratio of non-polymorphic band was about 6%, which meant that the genetic similarity among treated samples was very low, indicating that heavy metal contamination of soils caused the changes of microbial DNA. The SWI of DNA sequence diversity of the microbial community in controls reduced with increasing Ni concentration in treated soils, and that in rhizospheric soils was generally higher than that in controls, which indicated that phytoextraction may have positive effects on the DNA sequence diversity of the microbial community in Ni-contaminated soils. However, these effects may depend on the plant species, availability of root exudates and other factors prevailing in the habitat of the plant-soil system.

5 Conclusions

When the added Ni in treated soils was lower than 100 mg/kg, the populations of bacteria, fungi, and actinomycetes were stimulated; but with the increasing concentration of Ni in soils, the amount of the microorganisms would tend to decline, which showed that Ni contamination in soils would adversely affect soil microorganisms. By uptaking nickel from soils and excreting root exudates, phytoremediation could increase the amount of the microbial biomass in contaminated soils; however, different plant species might have different effects on microorganisms.

Soil Ni contamination has been proved to affect the microbial biomass carbon, basal respiration and the DNA sequence diversity of the microbial community. Fortunately, phytoremediation can reduce the toxicity of Ni on soil microbes, and improve the habitat conditions of microorganisms, and thus facilitate the development of microbial populations in Ni-contaminated soils.

Acknowledgements This work was partially financed by the National Natural Science Foundation of China (Grant No.20177035), the Provincial Natural Science Foundation of Guangdong (No.051018242) and the New Century Excellent Talents Plan Program of National Education Ministry (No. NCET04-0790). A kind thank is also given to Dr. Li Y M for supplying the seeds of hyperaccumulators.

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