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ARTICLE

Effects of uranium on soil microbial biomass carbon, enzymes, plant biomass and microbial diversity in yellow soils

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Abstract – We conducted an experiment to investigate the effects of uranium (U) on soil microbial biomass carbon (MBC), enzymes, plant biomass and microbial diversity in yellow soils under three concentrations: 0 mg kg^{-1} (T1, control), 30 mg kg^{-1} (T2) and 60 mg kg^{-1} (T3). Under each treatment, elevated U did not reduce soil MBC or plant biomass, but inhibited the activity of the soil enzymes urease (UR), dehydrogenase (DH) and phosphatase (PHO). The microbial diversity was different, with eight dominant phyla in T1 and six in T2 and T3. Furthermore, *Proteobacteria* and *material X* were both detected in each treatment site (T1, T2 and T3). *Pseudomonas sp.* was the dominant strain, followed by *Acidiphilium sp.* This initial study provided valuable data for further research toward a better understanding of U contamination in yellow soils in China.

Keywords: uranium / soil enzymes / MBC / microbial diversity / yellow soils

1 Introduction

Yellow soil, which is widespread in the humid subtropical areas of the world, is the most important soil resource for agriculture in the southwest region of China (Liu *et al.*, 2014). However, yellow soils are threatened by radionuclides such as ^{238}U and ^{234}U with the rapid development of the nuclear industry in recent years. Soil microbial diversity is an important component of the soil ecosystem. It can represent the stability of the soil microbial community structure and predict changes in soil environmental quality comparatively early (Zhalnina *et al.*, 2015). Soil enzymes can also participate in the whole process of the formation of soil fertility (Densilin and Srinivasan, 2014). There is strong evidence that soil microbial diversity and enzyme activities are sensitive to radionuclide contamination (Yan and Luo, 2015). The reduced soil microbial diversity and soil enzyme activities, in turn, affect the fertility of the radionuclide-contaminated soils.

However, the effect of U on soil MBC, enzymes, plant biomass and microbial diversity is less known. Thus, we conducted this experiment using yellow soil by simulating U pollution levels around uranium milling tailings. The specific objectives of our experiment were to examine (1) the effects of different concentrations of U on soil MBC, soil enzyme activity (UR, DH and PHO) and plant biomass, and (2) the effects of different concentrations of U on soil microbial diversity.

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2 Materials and methods

2.1 Soil and plants for the experiment

Soils for the experiment were collected from farmland situated around uranium mill tailings in northwest China. For each sample, 0.050 g of the sediment samples were taken and digested with HF and HNO_3. The organic matter (OM) content of soil samples was determined based on the Walkley-Black method. Available nitrogen, available phosphorus, exchangeable potassium, exchangeable calcium and exchangeable magnesium were determined based on the Kjeldahl method. Uranium was determined by inductively coupled plasma mass spectrometry.

2.2 Experimental design

The experiment was a factorial design with three U levels in yellow soils cultivated with orchardgrass. In addition to the background values (0.35 mg kg^{-1}) in the soil samples, three levels of U (0, 30 and 60 mg kg^{-1}) were designed to simulate three levels of U pollution sites around the uranium milling plant, respectively. U (^{238}U + ^{234}U) was applied as uranyl nitrate ($UO_2(NO_3)_2{\cdot}6H_2O$). After 4 weeks of U addition, 12 soil samples were collected from each U treatment to determine the actual total U concentrations using inductively coupled plasma mass spectrometry (ELEMENT 2/XR, Thermo Scientific, USA). The detection limit was 0.0003 μg L^{-1} for

U. The accuracy of inductively coupled plasma mass spectrometry analysis is estimated to be better than ±3% (relative) for the elements determined. The total U concentrations of the 0, 30 and 60 mg kg^{-1} U treatments were 5.7 ± 0.02 mg kg^{-1}, 35.4 ± 0.31 mg kg^{-1} and 66.3 ± 0.35 mg kg^{-1}, respectively.

Thirty-six mesocosms (50 cm × 50 cm × 20 cm) were used in this experiment. There were 12 mesocosms for 0 mg kg^{-1} U treatments (control), 12 mesocosms for 30 mg kg^{-1} U treatments and 12 mesocosms for 60 mg kg^{-1} U treatments. Each mesocosm was filled with 7 kg of yellow soil. The mesocosms were arranged in a greenhouse in a complete randomized block design. The total plant density was 15 for each mesocosm. Plants were maintained under natural light and the average air temperature was 15–30 °C during the experiment from March to October 2014. Plants were watered daily to keep soil moisture at 70–90% of the water-holding capacity and no U leached from soils.

2.3 Soil and plant sampling

Eight months after seedling, the plant and soil samples were collected. Root systems were separated from shoots. The shoot biomass was dried at 85 °C for 48 h and weighed. For each mesocosm, three soil samples were taken to a depth of 10 cm and were then combined (about 90 g). All samples were immediately transported to the laboratory and stored at 4 °C. Each combined soil sample was used for determination of MBC, soil enzymes and soil microbial diversity.

2.4 Measurements

MBC was determined mainly following the fumigation-extraction method (Vance *et al.*, 1987). Fresh soil samples for analyzing enzyme activity were sieved (<2.0 mm) before the measurement. Soil enzyme activities of UR, DH and PHO were determined as described by Gianfreda *et al.* (2005).

For each soil sample, gDNA was extracted using a sludge genomic DNA rapid extraction kit (Baisaike Company, Nanjing, China). PCR amplification of bacterial 16S rDNA from gDNA was performed using the universal primers GC-338F (with 40 GC clamps) and 518R. The PCR reactions were carried out in 50-μL volumes, containing 2 ng gDNA, 1 μL GC-338F (20 mM) and 1 μL 518R (20 mM), 5 μL 10 × PCR buffer, 3.2 μL dNTP (2.5 mM) and 0.4 μL rTaq (5 U/μL), made up to 50 μL with ddH_2O. The cycling conditions involved an initial 5-min denaturing step at 95 °C, followed by 30 cycles of 1 min at 95 °C, 50 s at 60 °C and 1 min at 70 °C, and a final extension step of 10 min at 60 °C. After verification of PCR products in agarose gel, 10 μL PCR products were analyzed by DGGE (Yan and Luo, 2015).

Finally, the bands were checked using the BLAST search program at the National Center for Biotechnology Information. Sequences showing the highest homology with the sequences in GenBank were selected. Furthermore, the phylogenetic characteristics of the soil samples were determined for a mixture of the soil; briefly, the sequences of the microorganisms were transformed into the FASTA form, and were then checked using the Clustal-X software. Next, Neighbor-Joining was used to calculate the evolutionary distance, followed by phylogenetic tree construction with MEGA V4.1, in combination with the bootstrap test (Yan and Luo, 2015).

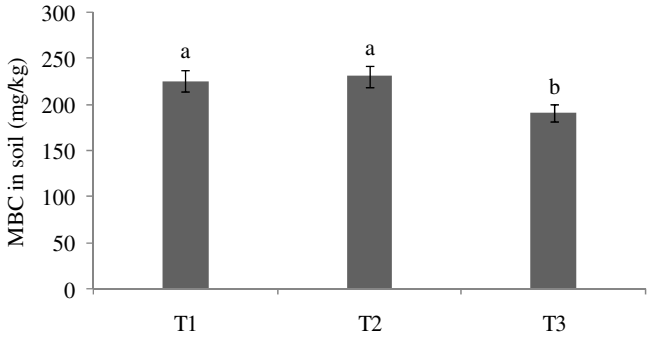

Figure 1. MBC of yellow soils under different U treatments. Different letters indicate significant differences ($P < 0.05$). [a] There was a significant difference compared with T1 and T2. [b] There was a significant difference compared with T3.

2.5 Data analysis

Multivariate analysis and analysis of variance (ANOVA) were used to demonstrate differences among the samples. The test data acquired in the present study were subjected to statistical analysis including the independent sample T-test by SPSS 19.0.

3 Results

3.1 The physicochemical properties of the soil

The main results are as follows: the soil had 21.35 g kg^{-1} organic matter, 133.0 mg kg^{-1} available nitrogen, 12.9 mg kg^{-1} available phosphorus, 81.9 mg kg^{-1} exchangeable potassium, 209.7 mg kg^{-1} exchangeable calcium and 39.7 mg kg^{-1} exchangeable magnesium. The uranium concentration in the soil samples was 5.3 mg kg^{-1}. The plant species orchardgrass (*Dactylis glomerata L.*) was selected for the experiment.

3.2 Soil microbial biomass carbon (MBC)

Compared with T1 (control), the MBC was increased by 2.3% (not statistically significant) after the treatments with 30 mg kg^{-1} U (T2). After the treatments with 60 mg kg^{-1} U (T3), there was a statistically significant decrease in the MBC of 15.5% (Figure 1).

3.3 Plant biomass

Compared with T1 (control), the total shoot biomass was increased by 0.5% (not statistically significant) after the treatments with 30 mg kg^{-1} U (T2). After the treatments with 60 mg kg^{-1} U (T3), there was a statistically significant decrease in the total shoot biomass of 27.9% (Figure 2).

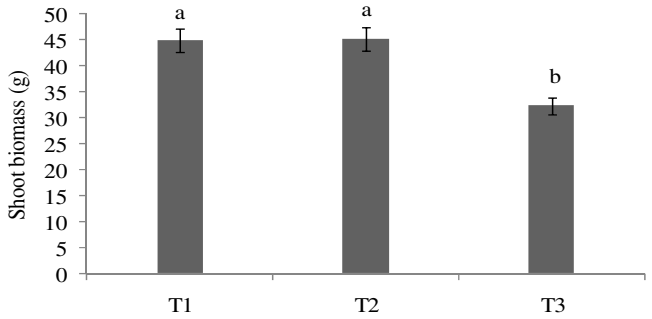

Figure 2. Total shoot biomass under different U treatments. Different letters indicate significant differences ($P < 0.05$). [a] There was a significant difference compared with T1 and T2. [b] There was a significant difference compared with T3.

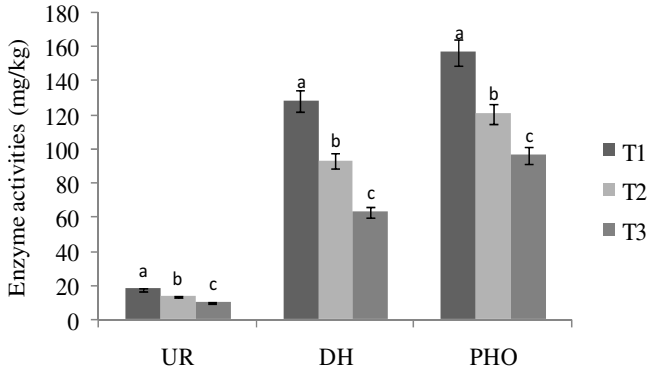

Figure 3. Enzyme activities of yellow soils under different U treatments. Different letters indicate significant differences ($P < 0.05$).

3.4 Soil enzyme activities

Significant effects of U on soil enzyme activities were observed in the present study (Figure 3). Additionally, the activities of soil enzymes (UR, DH and PHO) decreased with the increase in the U concentration (Figure 3). There was a statistically significant decrease in the concentrations of all the soil enzyme activities (UR, DH and PHO) in T2 and T3 compared with T1 (Figure 3).

3.5 Soil microbial diversity

Soil microbial diversity was analyzed by denaturing gradient gel electrophoresis (DGGE). A total of 17 bands (T1), 23 bands (T2) and 24 bands (T3) were sequenced to detect the representative microorganisms *via* BLAST in GenBank (Figure 4). For T1, *Proteobacteria*, *Chloroflexi, Actinobacteria, Acidobacteria, Bacteroidetes, Firmicutes, Nitrospirae* and *material X* (the exact bacteria name could not be identified, as there is no detailed information of a similar sequence related to the "unknown environmental samples" in the NCBI databases) were identified (Table 1).

For T2, *Proteobacteria*, *material X, Firmicutes, Actinobacteria, Bacteroidetes* and *Actinobacteria* were identified (Table 2).

For T3, *Proteobacteria*, *material X*, *Actinobacteria, Firmicutes, Acidobacteria* and *Nitrospirae* were identified (Table 3).

4 Discussion

In our experiment the elevated concentration of U (T2), which was estimated at about 29.7 mg kg^{-1}, did not significantly reduce the soil MBC. These results may be due to the increasing growth and plant biomass of orchardgrass under elevated U treatments (T2) in the short term; higher plant production could enhance MBC transportation in soil, and then promote soil microbial development (Chen *et al.*, 2004). However, a large increase in the concentration of U (T3), which was estimated at about 60.6 mg kg^{-1}, did significantly reduce the soil MBC. The results show that excessive amounts of U can restrain soil microbial development.

Soil enzyme activities are generally related to soil characteristics, and soil microbial quantity and diversity, and are also related to external contamination such as radionuclides (Peyrot *et al.*, 2014). In fact, U inhibits soil enzyme activities by interaction with their SH group and/or imidazole ligand, which is competitive inhibition (Gao *et al.*, 2010). It is concluded that the soil enzyme activity may be a sensitive tool for assessing toxic effects on soil biochemical parameters. In the present study, we found that soil enzyme activities significantly decreased in T2 and T3 compared with T1. The results of our experiment indicated that elevated U, which was estimated at about 29.7 mg kg^{-1} in T2 and 60.6 mg kg^{-1} in T3, impacted the activities of the soil enzymes UR, DH and PHO similarly. That is, as the concentration of U rises, soil enzyme activities decline.

Uranium contamination is widespread in subsurface soils in mining and milling sites across China. However, less is known about the adverse effects of U on soil microorganisms than on plant growth. This is not surprising, since the effects of radionuclides added to soils are difficult to assess, and few experiments exist. In this study, the genetic fingerprinting technique of DGGE, which enables the separation of the double-stranded DNA fragments with lengths up to 500 bp, was used to analyze the diversity of microorganisms in different sample sites. DGGE can screen the soil bacterial communities by detecting community changes. Many bacteria and fungi have been detected in radionuclide-contaminated soils around the world (Akob *et al.*, 2008; Chapon *et al.*, 2014; Feng *et al.*, 2015). The microbial structures in T1, T2 and T3 were simple, with limited strains of microbial populations. Also, we can note that the microbial population in T3 was significantly lower – only 75% – compared with T1.

The results showed that *Proteobacteria* was the dominant phylum in yellow soils. Other studies have similar results. Akob *et al.* (2008) found that α-, β-, δ- and γ-*Proteobacteria* can be found in U-contaminated subsurface sediments. Following *in situ* biostimulation of microbial communities within contaminated soils, sequences related to previously cultured metal-reducing *Proteobacteria* increased from 5% to nearly 40% of the clone libraries (North *et al.*, 2004). Luo *et al.* (2007) found that microbial communities

Table 1. Soil microbial diversity analysis of T1 soil.

Samples	The most similar strains	Accession number	Similarity	Diversity
Band 1 (T1)	*Pseudomonas sp.* SC18	HF566312	99%	*Proteobacteria*
Band 2 (T1)	uncultured *Chloroflexi* bacterium	AY921926	99%	*Chloroflexi*
	uncultured *Anaerolineae* bacterium	AM935798	99%	*Chloroflexi*
Band 3 (T1)	*Pseudomonas sp.* AB72	KC019209	100%	*Proteobacteria*
Band 4 (T1)	*Pseudomonas* putida	KC129032	100%	*Proteobacteria*
	Pseudomonas teessidea	KC108719	100%	*Proteobacteria*
Band 5 (T1)	*Arthrobacter sp.* WS20	JN899573	99%	*Actinobacteria*
Band 6 (T1)	*Prevotella sp.* oral taxon 314	GU409788	100%	*Bacteroidetes*
Band 7 (T1)	*Granulibacter bethesdensis*	EF408914	100%	*Proteobacteria*
Band 8 (T1)	uncultured *Alicyclobacillus sp.*	JX504950	92%	*Firmicutes*
Band 9 (T1)	uncultured bacterium	DQ906087	99%	environmental samples
Band 10 (T1)	uncultured soil bacterium	GU375449	99%	environmental samples
Band 11 (T1)	*Acidisoma sibirica*	NR_042706	99%	*Proteobacteria*
Band 12 (T1)	uncultured bacterium	JX967680	100%	environmental samples
Band 13 (T1)	uncultured *Rhizobiales* bacterium	GU983325	100%	*Proteobacteria*
Band 14 (T1)	*Sphingomicrobium sp.* CC-AMZ-30M	JX235672	99%	*Proteobacteria*
	Novosphingobium sp. FNE08-86	JN399173	99%	*Proteobacteria*
Band 15 (T1)	uncultured *Acidobacteriaceae* bacterium	EF072275	100%	*Acidobacteria*
Band 16 (T1)	*Microbacterium sp.* MA21	JX255398	100%	*Actinobacteria*
	Agrococcus sp. MDT1-82	JX949729	100%	*Actinobacteria*
Band 17 (T1)	*Leptospirillum ferriphilum*	JX966411	100%	*Nitrospirae*

Table 2. Soil microbial diversity analysis of T2 soil.

Samples	The most similar strains	Accession number	Similarity	Diversity
Band 1 (T2)	*Achromobacter* sp. enrichment culture clone	HQ694767	100%	*Proteobacteria*
Band 2 (T2)	*Rhodanobacter* sp. BJC16-A24	JX483760	99%	*Proteobacteria*
Band 3 (T2)	*Serratia sp.* DCM0915	KC007128	100%	*Proteobacteria*
Band 4 (T2)	uncultured *proteobacterium*	GQ141784	100%	*Proteobacteria*
Band 5 (T2)	*Staphylococcus epidermidis*	KC107217	100%	*Firmicutes*
Band 6 (T3)	uncultured bacterium	JQ815799	97%	environmental samples
Band 7 (T2)	uncultured actinobacterium	EU849325	99%	*Actinobacteria*
Band 8 (T2)	*Arthrobacter sp.* WS20	JN899573	99%	*Actinobacteria*
Band 9 (T2)	*Prevotella sp.* oral taxon 314	GU409788	100%	*Bacteroidetes*
Band 10 (T2)	uncultured bacterium	DQ906087	99%	environmental samples
Band 11 (T2)	uncultured *Alicyclobacillus sp.*	JX504950	92%	*Firmicutes*
Band 12 (T2)	uncultured bacterium	DQ906087	99%	environmental samples
Band 13 (T2)	*Rhodanobacter* sp. BJC16-A24	JX483760	99%	*Proteobacteria*
Band 14 (T2)	uncultured *Firmicutes* bacterium	EU299155	99%	*Firmicutes*
Band 15 (T2)	*Acidiphilium sp.* MS8	AB561883	99%	Proteobacteria
Band 16 (T2)	uncultured bacterium	AB729745	100%	environmental samples
Band 17 (T2)	uncultured bacterium	JQ387473	100%	environmental samples
Band 18 (T2)	*Sphingomicrobium sp.* CC-AMZ-30M	JX235672	99%	*Proteobacteria*
	Novosphingobium sp. FNE08-86	JN399173	99%	*Proteobacteria*
	Altererythrobacter sp. OB38-2	JN942147	99%	*Proteobacteria*
Band 19 (T2)	uncultured bacterium	JQ380053	100%	environmental samples
Band 20 (T2)	*Agrobacterium sp.* S1-A4	KC139688	100%	*Proteobacteria*
Band 21 (T2)	*Rhizobium sp.* TDB-198	AB730559	100%	*Proteobacteria*
Band 22 (T2)	*Microbacterium sp.* MA21	JX255398	100%	*Actinobacteria*
	Agrococcus sp. MDT1-82	JX949729	100%	*Actinobacteria*
Band 23 (T2)	*Alicyclobacillus sp.* HJ	JN092119	100%	*Firmicutes*

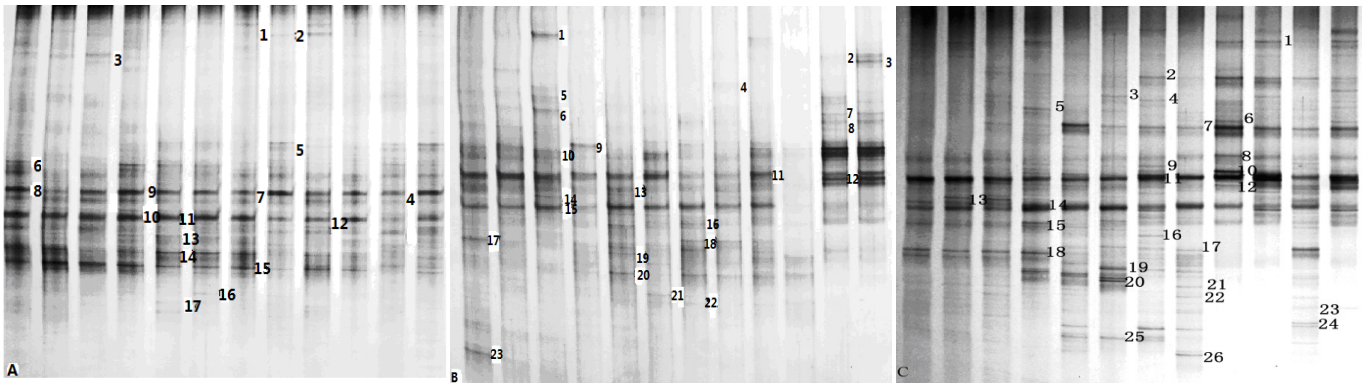

Figure 4. The denaturing gradient gel electrophoresis (DGGE) profiles of amplified 16S rDNA fragments from soil samples used for sequencing. A, B and C represent soil samples collected from T1, T2 and T3.

Table 3. Soil microbial diversity analysis of T3 soil.

Samples	The most similar strains	Accession number	Similarity	Diversity
Band 1 (T3)	*Pseudomonas congelans*	HF566284	100%	*Proteobacteria*
Band 2 (T3)	*Rhodanobacter sp.* BJC16-A24	JX483760	99%	*Proteobacteria*
Band 3 (T3)	uncultured *proteobacterium*	GQ141784	100%	*Proteobacteria*
Band 4 (T3)	*Staphylococcus epidermidis*	KC107217	100%	*Firmicutes*
Band 5 (T3)	uncultured bacterium	JQ815799	97%	environmental samples
Band 6 (T3)	uncultured soil bacterium	GU599014	99%	environmental samples
Band 7 (T3)	*Arthrobacter sp.*	JN899573	99%	*Actinobacteria*
Band 8 (T3)	*Granulibacter bethesdensis* CGDNIH1	NR_043197	100%	*Proteobacteria*
	Rhodopila globiformis	M59066	100%	*Proteobacteria*
Band 9 (T3)	*Finegoldia sp.* S5-A7	JX262677	100%	*Firmicutes*
Band 10 (T3)	uncultured bacterium	DQ906087	99%	environmental samples
Band 11 (T3)	uncultured *Alicyclobacillus sp.*	JX504950	92%	*Firmicutes*
Band 12 (T3)	uncultured bacterium	DQ906087	99%	environmental samples
Band 13 (T3)	uncultured bacterium	JQ379109	100%	environmental samples
Band 14 (T3)	*Acidiphilium sp.* MS8	AB561883	99%	*Proteobacteria*
Band 15 (T3)	uncultured bacterium	AB729745	100%	environmental samples
Band 16 (T3)	uncultured *Rhizobiales* bacterium	FJ037044	100%	*Proteobacteria*
Band 17 (T3)	*Sphingomicrobium sp.* CC-AMZ-30M	JX235672	99%	*Proteobacteria*
	Novosphingobium sp. FNE08-86	JN399173	99%	*Proteobacteria*
Band 18 (T3)	uncultured bacterium	JQ376454	99%	environmental samples
Band 19 (T3)	uncultured *Acidobacteria* bacterium	HQ598915	100%	*Acidobacteria*
Band 20 (T3)	*Acidiphilium sp.* WJ52	AY495956	99%	*Proteobacteria*
Band 21 (T3)	*Agrobacterium sp.* S1-A4	KC139688	100%	*Proteobacteria*
	Rhizobium sp. TDB-198	AB730559	100%	*Proteobacteria*
Band 22 (T3)	*Microbacterium sp.* MA21	JX255398	100%	*Actinobacteria*
	Agrococcus sp. MDT1-82	JX949729	100%	*Actinobacteria*
Band 23 (T3)	*Leptospirillum ferriphilum*	JX966411	100%	*Nitrospirae*
Band 24 (T3)	uncultured *Acidobacteria* bacterium	GU205315	98%	*Acidobacteria*
Band 25 (T3)	uncultured bacterium	DQ660866	99%	environmental samples
Band 26 (T3)	*Alicyclobacillus sp.* HJ	JN092119	100%	*Firmicutes*

in U-contaminated soils (up to 2.8 g U kg^{-1}) were dominated by species from the *Geothrix* genus and *Proteobacteria* phylum in all microcosms. Gillow *et al.* (2000) detected that *Proteobacteria* was the most dominant phylum in U-contaminated soils. Mondani *et al.* (2011) found using DGGE that *Proteobacteria* and *Acidobacteria* were the primary phyla in uranium-contaminated soils (0.26%–25.5% U in mass). Moreover, *Pseudomonas sp.* was the dominant strain, followed by *Acidiphilium sp.* These results indicated that microorganisms can survive in soil environmentally polluted with U. U pollution does not always inhibit microorganism growth. Phylogenetic relationship analysis of representative microorganisms showed two main clusters, and most microorganisms showed high similarity with *Proteobacteria* (Figure 5). However, *Bacteroidetes* in T1 and T2 showed low similarity with most microorganisms and it was not found in T3. The reasons for this are still not clear and need to be further investigated. The current analysis concerning the influence

of U on soil microbial diversity should provide a better understanding of U contamination and promote the treatment of U-contaminated soils.

5 Conclusions

In conclusion, the U concentrations used in this study did not significantly affect the MBC or plant biomass. However, U in yellow soils showed a profound influence on soil enzyme activities and microbial diversity. This initial study provided valuable data for further research toward a better understanding of the radioactive contamination in yellow soils in southeast China.

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