doi:10.2489/jswc.74.5.439

Effects of heavy metal pollution on soil physicochemical properties and microbial diversity over different reclamation years in a copper tailings dam

T. Jia, R. Wang, and B. Chai

Abstract: The proliferation of copper (Cu) mines has led to a rapid rise of tailings dams comprised of mining metal mineral material, causing serious damage to the ecological environment of mining areas. Soil physicochemical characteristics, enzyme activities, and microbial diversity are important indices for ecosystem functions as well as being important factors in evaluating soil restoration characteristics. This study selected nine Cu tailings subdams in Yuanqu County, Yuncheng City, Shanxi, China, to analyze the effects of heavy metal pollution on soil physicochemical properties and microbial diversity over different years of reclamation. We found that these different years of reclamation exhibited significant differences in physicochemical properties, and as restoration progressed, soil nutrient concentrations (i.e., carbon [C] and nitrogen [N]) significantly increased. Furthermore, we found significant negative correlations between catalase and the soil C and N ratio (C/N). In addition, urease was significantly positively correlated to N, C, and cadmium (Cd) as well as bacterial gene copies but was negatively correlated to zinc (Zn). Sucrase, on the other hand, was negatively correlated to Cd and bacterial gene copies. Over subsequent years of reclamation, we found that environmental factors affected bacteria more than fungi. Soil fungi diversity gradually increased as restoration progressed over consecutive years, while soil bacteria correspondingly exhibited an irregular trend. Results from this study could provide an ecological basis for further studies on soil ecosystem restoration and degradation mechanisms associated with Cu tailings dams.

Key words: copper tailings dam—microbial diversity—soil enzymes—soil physicochemical properties

During metal mineral resource development processes, a large amount of heavy metals are disposed directly into soil along with waste rock, tailings, and other mineral dust in mining districts and their surrounding areas, which subsequently become the main source of environmental pollution (Huang 2015). Such mining waste not only consumes a lot of land and worsens the pervading ecosystem degradation taking place, but it also causes the breakdown of the aggregate structure of soil, reducing soil fertility and soil physical and chemical properties, as well as biological properties, which together cause serious damage to the ecological environment of mining areas (Hao et al. 2017; Xin et al. 2017; Li et al. 2018). The Zhongtiao Mountains copper (Cu) mine, the largest underground Cu mine in China, has an annual output of greater than 4 million t of ore (Liu et al. 2017). This mine is dominated by Cu ore with other available metals, such as iron (Fe), lead (Pb), zinc (Zn), cadmium (Cd), etc. (He et al. 2007). Each year, this Cu mine has increased its mining of metal ore, as well as gradually accelerating the speed of lift of its tailings dam. The extensive accumulation of tailings has led to severe pollution and the degradation of the local ecological environment (Wang et al. 2010b). Accordingly, a resolution to this problem is essential, and the best way to resolve this is to effectuate the reasonable and efficient ecological restoration of the Cu tailings dam. The ecological functional recovery of soil is the key to such restoration as well as the sustainable development of terrestrial ecosystems (Wang et al. 2013; Tong et al. 2017). Thus, along with vegetation, this type of ecological restoration must also focus on soil fertility and the characteristics of dynamic change associated with microbial communities throughout the restoration period.

At present, plant-microbial remediation and characteristics of vegetation succession are mainly used to evaluate restoration effects (Pei et al. 2017a; Zeng et al. 2017). Some studies related to the ecological functional recovery of soil in tailings dams for restoration periods that extend to 45 years have been published (Liu et al. 2017; Ojuederie and Babalola 2017; Tong et al. 2017). Soil enzymes are a type of biological catalyst that can catalyze and decompose polymeric organic matter, which is an important index in evaluating soil quality. Soil enzymes primarily derive from plant root secretion and soil microbial activity as well as animal and plant residue, and they are also involved in processes associated with soil organic matter (SOM) decomposition, synthesis, transformation, and oxidation reduction of inorganic substances. Soil enzyme activities are closely related to soil physical and chemical properties as well as soil types, which are indicative of ecological changes in soil as well as SOM decomposition and element cycling processes (Chen and Yang 2013; Yang et al. 2013b; Liu and Yang 2014; Zhang et al. 2016). Soil enzymes, being one of the most active organic components within the soil ecosystem, are involved in almost all biochemical processes in soil and can consequently reflect the intensity and direction of such biochemical processes. At the same time, soil enzyme activities are closely related to soil physical and chemical properties, and both are critical indicators of ecosystem function (Tong et al. 2017). Soil enzymes can also activate the compounds of various soil elements, and subsequently enhance the availability of soil nutrients and improve overall soil quality. Additionally, soil properties provide a substrate and an environment for enzymatic reactions to occur, which directly affect enzyme activities (Tong et al. 2017). For example, it has been reported that

Tong Jia (corresponding author) is an associate professor, Ruihong Wang is a postgraduate student, and Baofeng Chai is a professor at the Institute of Loess Plateau, Shanxi University, Taiyuan, China.

Copyright © 2019 Soil and Water Conservation Society. All rights reserved.
Journal of Soil and Water Conservation 74(5):439-448 www.swcs.org

different patterns of vegetation restoration processes have a significant impact on both soil physical and chemical characteristics and soil enzymes (i.e., sucrase, catalase, urease, and polyphenol oxidases) in coal mine reparation areas in Shanxi Province (Tong et al. 2017). Furthermore, different artificial forest types have had notable effects on soil urease and sucrase activities in the loess region of China; soil alkali-hydrolyzable nitrogen (N), water content, and available phosphorus (P) were the crucial factors affecting urease activities, while sucrase activities were mainly affected by soil organic carbon (SOC) content (Pei et al. 2017b). However, in addition to the vegetation types, recovery time is also an important factor that influences the soil environment undergoing remediation. Recovery time is also extremely important for soil fertility recovery given that it improves the accumulation of soil nutrients and enzyme activities (Li et al. 2015a).

Soil microorganisms are sensitive to soil quality during restoration processes (Li et al. 2005). Heavy metal accumulation in soil will destroy the natural soil microbial community structure. Consequently, biological community activities not only reduce soil fertility and quality, but also alter the distribution of soil microbial communities (Abraham and Susan 2017; Ding et al. 2017). Soil microorganisms are sensitive to heavy metal stress (Sun et al. 2004); that is to say, the quantity and community structure of microorganisms will change under such conditions. To some extent, soil microorganisms reduce the mobility and biological toxicity of heavy metals through microbial absorption and adsorption, which have certain restorative effects on heavy metal pollution in soil. Soil microorganisms play a significant role in organic matter decomposition, nutrient cycling, and phytoavailability, while they also play a decisive role in soil structure, especially the formation and stabilization of soil aggregates.

Although many studies have shown that anthropogenic activities can cause changes in soil microbial structure and diversity, there have been very few studies to date that have reported on the effects of heavy metal pollution on soil properties and microbial diversity over different reclamation periods in Cu tailings dams in China. In this study, we conducted a survey of heavy metal polluted soil in nine tailings subdams of a Cu mine over different years of restoration. To achieve this objective, we addressed the following questions: (1) How do soil properties and enzyme activities in soil vary with an increase in recovery time, (2) what is the relationship between soil physiochemical properties and enzyme activities for different subdams over various restoration years, and (3) what are the dominant environmental factors that affect soil microbial diversity in a Cu tailings dam? The aim of this study was to provide an ecological basis for the mechanisms of soil ecosystem restoration and degradation in a Cu tailings dam, and to strengthen our understanding of soil property and microbial community biodiversity restoration in an environment subjected to heavy metal pollution.

Materials and Methods

Site Description and Soil Sampling. The Shibahe River tailings of the Northern Copper Mine (35°15′~35°17′ N, 118°38′~111°39′ E) were constructed in 1969 in the southern region of Shanxi Province, China. Each year, waste from mineral processing in the Shibahe River tailings accumulates in the form of ore sand. A new subdam is constructed on the basis of the original subdam every three to five years, and the ore surface of these new subdams is covered with 30 cm of local soil. The elevation from the dam base to the dam crest is 486 m and 509 m, respectively. At this point in time, the Shibahe River tailings of the Northern Copper Mine is composed of 14 subdams, with a stack height of 84 m and a texture ratio of 1:6. It is under the influence of a continental monsoon climate with four distinct seasons, where the annual mean temperature is 14°C, annual precipitation is approximately 780 mm, and frost free days are greater than 200 days (Liu et al. 2018).

In July of 2015, we selected nine subdams under different restoration stages for sampling (figure 1 and table 1). The control soil (referred to as CK) used in this study is from the local (native) soil, free of heavy metal pollution and natural vegetation, taken from the periphery of the subdam. For each subdam, we randomly collected five samples (from a 0 to 10 cm depth) following an S-shaped curve where no vegetation was present. Visible roots and residue were removed prior to homogenizing the soil fraction of each sample. Fresh soil samples were sifted through a 2 mm sieve and divided into two subsamples. One subsample was stored at 4°C to determine physiochemical properties, while the other was stored at –20°C prior to DNA extraction.

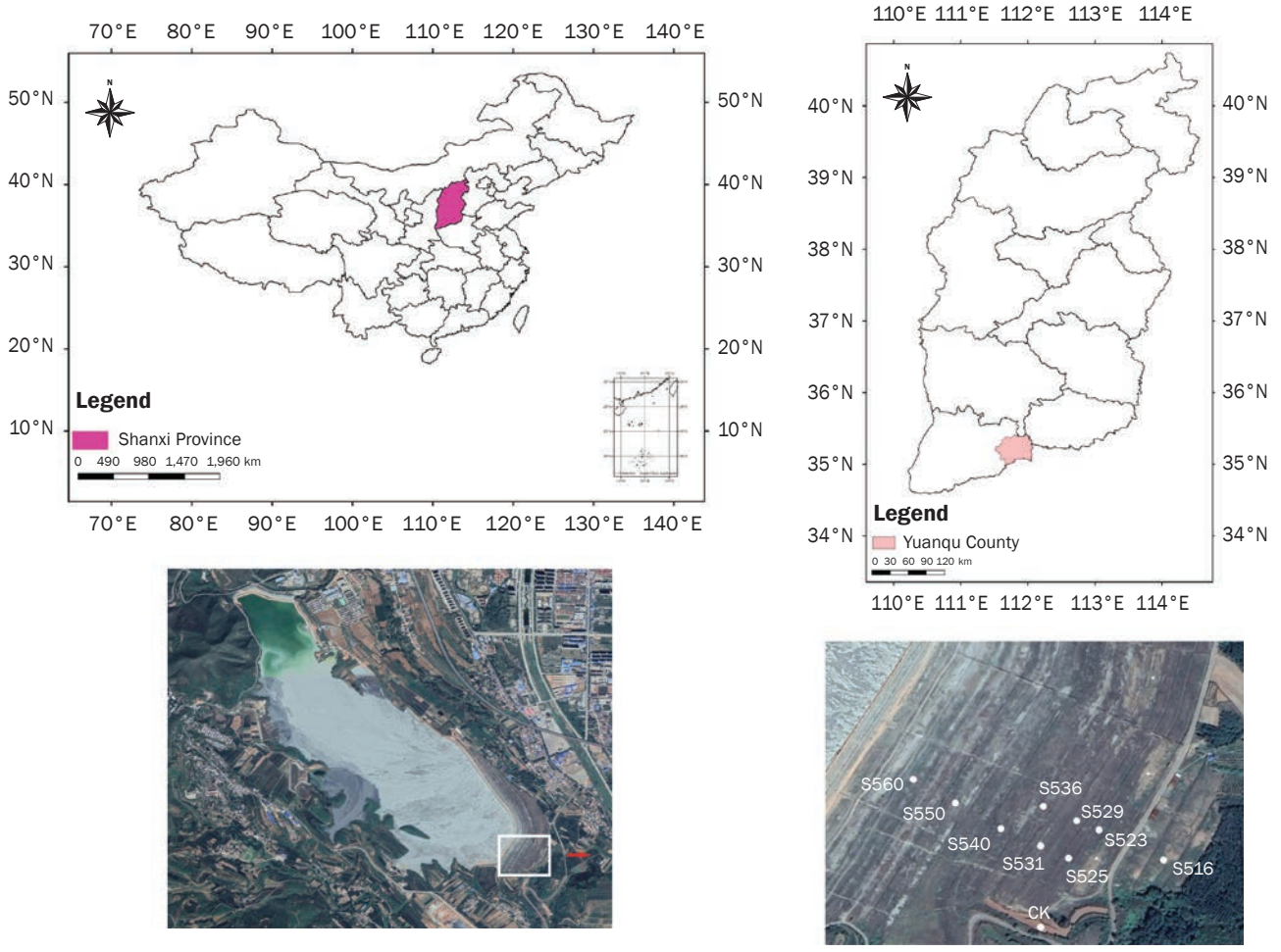
Soil Chemical Properties and Enzyme Activities. Soil pH was measured after shaking in a soil water (1:2.5 mass/volume) suspension for 30 minutes. Soil water content (SWC) was measured gravimetrically. Soil particle size (PS) was measured by using the Mastersizer 3000 laser diffraction particle size analyzer (Malvern Panalytical Ltd., Malvern, United Kingdom). Total soil carbon (C), N, and sulfur (S) content was measured using an elemental analyzer (vario EL/MACRO cube, Elementar, Hanau, Germany). Nitrate nitrogen (NO_3^--N), ammonium nitrogen (NH_4^+-N), and nitrite nitrogen (NO_2^--N) were determined using an automated discrete analyzer (DeChem-Tech, CleverChem 380, Germany). Heavy metal (arsenic [As], Cd, Cu, Pb, and Zn) concentrations of samples were measured using an ICP-AES (iCAP 6000, Thermo Fisher, United Kingdom). Additionally, soil sucrase was measured using 3,5-Dinitrosalicylic acid colorimetry, urease was measured using phenol-sodium hypochlorite colorimetry, catalase was measured using potassium permanganate titration, and phosphatase was measured using the disodium phenyl phosphate colorimetric method (Li et al. 2015a; Qiao et al. 2017).

DNA Extraction, Polymerase Chain Reaction, and Denaturing Gradient Gel Electrophoresis. Total soil DNA was extracted using the E.Z.N.A. Soil DNA Kit (OMEGA, Norcross, Georgia, United States). The quality and quantity of DNA extracts were measured using the Plate reader Infinite 200 PRO (TECAN Group Ltd., Männedorf, Switzerland). DNA purity was assessed by determining A_{260}/A_{280} absorbance ratios, and only DNA extracts with absorbance ratios of about 1.8 to 2.0 were used for bacterial community analysis (Jorquera et al. 2016).

The structure of the bacterial and fungal communities was evaluated using denaturing gradient gel electrophoresis (DGGE) as follows: Partial 16S rRNA genes were amplified by polymerase chain reaction (PCR) with DNA template concentrations of 10 ng μL^{-1}. Fragments of 16S rRNA genes (the V3 region) were amplified by PCR using primers 341F (5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3') and 534R (5'-ATT ACC GCG GCT GCT GG-3'). Hot start PCR was as follows:

Copyright © 2019 Soil and Water Conservation Society. All rights reserved.
Journal of Soil and Water Conservation 74(5):439-448 www.swcs.org

Figure 1
Location of the study area in the Shanxi Province and distribution of the soil samples.



95°C for 4 minutes, followed by 30 cycles at 94°C for 40 seconds, annealing at 63.5°C for 30 seconds, extension at 72°C for 30 seconds, and 72°C for 10 minutes.

Partial 18S rRNA genes were amplified by PCR with DNA template concentrations of 10 ng μL^{-1}. Fragments of 18S rRNA genes (the V4 region) were amplified by PCR using the primers FUNG-GC (5'-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CAT TCC CCG TTA CCC GTT G-3') and NS1 (5'-GTA GTCA TAT GCT TGT CTC-3'). Hot start PCR was as follows: 95°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, annealing at 53°C for 30 seconds, extension at 72°C for 30 seconds, and 72°C for 7 minutes.

Denaturing Gradient Gel Electrophoresis Analysis. The DGGE runs were performed using a DCode system (Bio-Rad Laboratories, Inc., Hercules, California). Eight microliter bacteria PCR products were loaded onto 10% (w/v) polyacrylamide gels over an urea gradient between 45% and 65% (urea and formamide). Eight microliter fungi PCR products were loaded onto 8% (w/v) polyacrylamide gels over an urea gradient between 25% and 35% (urea and formamide). Electrophoresis was run for 12 hours at 65 V. The comb of this system to load samples can only have up to 20 holes. Gels were then stained using the silver staining method (Bassam et al. 1991) and photographed on a gel imaging system (Gel Doc XR, Bio-Rad Laboratories, Inc., Hercules, California). The DGGE image analysis of the band profiles were carried out using Quantity One version 4.6.2 (Bio-Rad Laboratories, Inc.), which detects bands and quantifies the relative concentration of DNA. Based on the results of our analysis, shown in the grayscale value of the band, the ß-diversity of the microbial communities was calculated. The number of distinct DGGE bands was used as an estimate of species richness, and the relative abundance of each band was treated as the proportion of a given species within a sample.

Statistical Analysis. Significant differences between subdam variables were analyzed by one-way analysis of variance (ANOVA) and Duncan test. Pearson correlation coefficient was used to analyze the relationship between soil physicochemical properties and enzyme activities. The number of distinct DGGE bands was imported into SPSS v20.0 (IBM, Chicago, Illinois) to calculate the Shannon–Wiener index, the Margalef's richness index, an evenness index, and the Simpson's Diversity Index (Yang et al. 2015). We used redundancy analysis (RDA) ordination technique and canonical correspondence analysis (CCA) to examine the relationships between environmental variables and microbial communities. The Monte Carlo-based permutation test was used to test the significance level ($p < 0.05$)

Copyright © 2019 Soil and Water Conservation Society. All rights reserved.
Journal of Soil and Water Conservation 74(5):439-448 www.swcs.org

Table 1
The reclamation time of different subdams in copper tailings.

	Subdams number								
Times	**S516**	**S523**	**S525**	**S529**	**S531**	**S536**	**S540**	**S550**	**S560**
Start time (year)	1969	1981	1985	1989	1993	1997	2001	2009	2014
Reclaimed time (a)	47	35	31	27	23	19	15	7	2

between environmental factors and microbial communities. Statistical analyses were performed using Canoco software (version 5.0) and SigmaPlot (version 12.5).

Results and Discussion

Soil Physical and Chemical Properties. Soil physical and chemical properties are important indicators of soil quality. In this study, the soil C, N, and S overall present an ascendant trend as years of restoration progressed, and these contents of S516 subdam were higher than that of other subdams, which indicated that the soil nutrient content was gradually improved along with the increase of restoration years for different subdams (table 2). This result was in accordance with a study by Zhao et al. (2013). The soil was alkaline, and the change of soil pH was not significant in different subdams. Moreover, soil pH decreased as the recovery period increased, which was consistent with results from Yang et al. (2013a). A possible cause for this finding could be that an increase in surface litter and microbial activity within the root system led to an increase in humic acid and organic acid (Shao et al. 2017), effectively improving soil physical and chemical properties (Li et al. 2015b). For soil physical properties, it has been reported that soil porosity and fractal dimensions increased as a result of restoration in a coal mine subsidence area (Huang et al. 2014). The subdams selected in our study, having undergone restoration for a period of 19 years, had a maximum soil particle size, while there were no significant differences in particle size between other subdams with the exception of S536 (table 2), which could be due to the fact that the soil composition of the Cu tailings dam was mainly comprised of ore sand.

Soil urease was in the form of aerobic hydrolase activity associated with soil N (Wang et al. 2010a), and it was significantly positively correlated to soil C content (table 3), which was consistent with results by Li et al. (2015a). One possible reason for this was that urease could convert amide-based organic N to inorganic nitride that plants can directly absorb, and its activity reflects the capacity and level of soil N. Therefore, urease activity in soil improves with an increase in N (Niu et al. 2010). It has been reported that urease activity is significantly positively correlated to pH when soil pH is between 3.1 and 7.1 (Fisher et al. 2017). However, some studies also showed that there was no correlation between pH and urease activity (Xin et al. 2017), which was consistent with our results. It could be that soil pH of a Cu tailings dam was greater than 7.9 (Yuan et al. 2017). Catalase was used as a means of soil oxidation, which was widely available in soil and living organisms. In our study, there was a significant negative correlation between soil catalase and soil C/N (table 3), and the probable cause for this was that vegetation provides C and N to the soil through root exudates and residues during restoration processes, which affected the input of SOM as well as the soil structure and physicochemical properties, thus reducing catalase activity (Cerli et al. 2008; Jin et al. 2009b; Li et al. 2015b). Furthermore, studies have reported on an improvement in soil enzyme activity along with an increase in heavy metal concentrations (Luo et al. 2006). Guo et al. (2012) found that heavy metal pollution in soil was negatively correlated with urease, protease, alkaline phosphatase, and catalase activity in soil. In this study, soil urease was significantly positively correlated with Cd, but negatively correlated with Zn, and sucrase was negatively correlated with Cd (table 3). According to Yang et al. (2001), Cd, Zn, and Pb together caused a negative synergistic inhibition effect on urease.

Relationship between Soil Characteristics and Microbial Community Structure. Soil microorganisms are vital biological indicators for the evaluation of environmental soil quality. They are also important components of soil biological activities and are sensitive to external disturbances (Tong et al. 2017). With the restoration time increase, abundance of soil bacteria and fungi rRNA genes increased in different subdams (figure 2). The DGGE results showed that there was a certain difference in the composition of bacterial and fungal communities in different subdams, and the composition of fungi in the S529 subdam was lower than that of other subdams (figure 3). The soil bacteria and fungi communities in Cu tailings dam were affected by soil chemical properties, heavy metals, and soil enzyme activities. Our results showed that 36.9% of soil bacteria distribution could be explained by soil physical and chemical properties, soil heavy metals, and enzyme activities. Axis 1 of the CCA plot explained nearly 19.1% of the variation; Axis 2 explained a further 17.8% (figure 4a and 4b). For soil fungi, it showed that 41.1% of soil fungi community could be explained by soil environmental factors and enzyme activities. Axis 1 of the CCA plot explained 22.5% of the variation, and Axis 2 explained 18.6% (figure 4c and 4d).

Soil pH is an important regulator of soil microbial communities and enzyme activities at either continental or global scales (Lauber et al. 2009; Sinsabaugh et al. 2010). In our study, the negligible differences we found in pH among the different years of reclamation resulted in no correlations between pH and enzyme activities or microbial abundance (table 3). Similarly, results from Li et al. (2015a) also observed that soil pH was not a significant environmental factor in soil microbial composition. Lauber et al. (2009) reported that soil pH did not correlate to microbial phylogenetic diversity, and they suggested that biodiversity was controlled by the substrate, environmental factors, or biotic competitiveness found in semiarid soils (Fierer and Jackson 2006). In this study, soil bacterial abundance exhibited different correlations along with four enzyme activities, and the ratio of bacteria and fungi was positively correlated with NO_2^--N (table 3). This suggested that bacterial abundance was more affected by environmental factors than soil fungi. Ratios of bacterial to fungal biomass were also correlated to enzyme activities under ecological succession processes because an elevated ratio can be a sign of the amount and composition of litter that enters the soil given that fungi are the dominant decomposers of plant cell wall polymers in litter (Baldrian et al. 2008).

Relationships between Environmental Parameters and Microbial Diversity. The soil microbial diversity and abundance were

Copyright © 2019 Soil and Water Conservation Society. All rights reserved.
Journal of Soil and Water Conservation 74(5):439-448 www.swcs.org

Table 2

Soil chemical properties of the different subdams (topsoil 0 to 10 cm). Values represent mean with standard error in parentheses. Significant differences between sites (Duncan test, $p < 0.05$) are denoted with letters (a > b > c).

	Subdams number									
Properties	**S516**	**S523**	**S525**	**S529**	**S531**	**S536**	**S540**	**S550**	**S560**	**CK**
NH_4^+-N (mg kg^{-1})	8.49ab	4.57b	3.97b	7.66ab	6.03ab	7.09ab	7.33ab	7.04ab	3.41b	12.07a
NO_3^--N (mg kg^{-1})	5.12b	5.16b	5.78b	5.28b	6.65b	5.34b	4.77b	5.35b	3.56b	10.41a
NO_2^--N (mg kg^{-1})	0.39a	0.35a	0.37a	0.34a	0.33a	0.35a	0.44a	0.47a	0.34a	0.45a
Olsen-P (mg kg^{-1})	7.21ab	6.22ab	5.11b	13.43ab	8.68ab	7.97ab	6.03ab	7.59ab	14.15a	6.44ab
N (%)	0.07a	0.05ab	0.05bcd	0.05bc	0.04bcd	0.04bcd	0.03cd	0.03d	0.03d	0.05ab
C (%)	1.71a	1.09bc	1.20b	1.06bc	0.95cd	0.91cde	0.79efg	0.58g	0.67fg	0.64g
C/N	27.41a	21.09a	26.79a	23.19a	24.28a	23.35a	25.27a	22.75a	26.09a	11.50b
S (%)	0.10a	0.07ab	0.06ab	0.06ab	0.05b	0.08ab	0.05b	0.05b	0.05b	0.08ab
SWC%	1.82ab	2.22ab	1.80ab	1.38ab	1.48ab	1.49ab	0.39b	1.03ab	2.16ab	2.90a
pH	7.90ab	7.99ab	7.88b	8.05ab	8.11a	8.02ab	8.03ab	8.12a	8.05ab	8.05ab
PS (μm)	37.34ab	44.98ab	42.60ab	42.00ab	40.66ab	46.66a	39.92ab	42.54ab	38.06ab	24.74b
As (mg kg^{-1})	9.62ab	10.32ab	10.84ab	13.18ab	12.19ab	15.26a	6.33ab	3.32ab	2.13b	12.98ab
Cd (mg kg^{-1})	5.27abc	6.80ab	7.26a	7.47a	7.11a	6.31ab	6.59ab	4.64bcd	3.22cd	2.92d
Cu (mg kg^{-1})	553.53a	366.44b	379.14b	324.41b	326.79b	352.81b	376.43b	340.74b	385.07b	48.80c
Pb (mg kg^{-1})	267.62ab	258.12ab	250.48ab	277.67ab	261.73ab	224.32ab	287.88a	256.91ab	176.36b	226.16ab
Zn (mg kg^{-1})	109.79b	81.01b	81.04b	85.06b	87.30b	69.54b	81.07b	85.52b	67.67b	236.69a

Notes: NH_4^+-N = ammonium nitrogen. NO_3^--N = nitrate nitrogen. NO_2^--N = nitrite nitrogen. N = total nitrogen. C = total carbon. S = total sulfur. C/N = the ratio of carbon and nitrogen. SWC = soil water content. PS = average particle size. AS = arsenic. Cd = cadmium. Cu = copper. Pb = lead. Zn = zinc.

Table 3

The Pearson correlations among soil chemical properties, enzyme activities, and microbial rRNA gene abundances in copper tailings dam.

Properties	NH_4^+–N	NO_3^-–N	NO_2^-–N	Olsen-P	N	C	C/N	S	SWC	pH	PS	As	Cd	Cu	Pb	Zn	Bacteria	Fungi	B/F	Catalase	Urease	Sucrase
NO_3^--N	ns																					
NO_2^--N	(+)**	(+)*																				
Olsen-P	ns	ns	ns																			
N	ns	(+)**	ns	ns																		
C	ns	ns	ns	ns	(+)**																	
C/N	(–)*	(–)**	ns	ns	(–)**	(+)*																
S	ns	ns	ns	ns	(+)*	(+)**	ns															
SWC	ns	ns	ns	ns	ns	ns	ns	ns														
pH	ns	ns	ns	ns	ns	(–)*	ns	ns	ns													
PS	ns	(–)*	ns	ns	(–)*	ns	(+)*	ns	(–)**	ns												
As	ns	ns	ns	ns	ns	ns	ns	(+)*	ns	ns	ns											
Cd	ns	ns	ns	ns	ns	(+)*	(+)*	ns	ns	ns	ns	(+)**										
Cu	ns	(–)**	ns	ns	ns	(+)**	(+)**	ns	ns	ns	ns	ns	ns									
Pb	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	(+)**	ns								
Zn	ns	(+)**	ns	ns	ns	ns	(–)*	ns	ns	ns	(–)**	ns	ns	(–)**	ns							
Bacteria	ns	ns	ns	ns	ns	(+)**	ns	ns	ns	ns	(+)**	ns	(+)**	(+)**	(+)*	(–)*						
Fungi	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	(+)**					
B/F	ns	ns	(+)**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns				
Catalase	ns	ns	ns	ns	ns	ns	(–)**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns			
Urease	ns	ns	ns	ns	(+)*	(+)*	ns	ns	ns	ns	ns	ns	(+)*	ns	ns	(–)*	(+)**	ns	ns	(+)*		
Sucrase	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	(–)*	ns	ns	ns	(–)**	ns	(–)**	(–)**	(–)**	
Phosphatase	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*$p < 0.05$ **$p < 0.01$ ns = no significance

Notes: NH_4^+-N = ammonium nitrogen. NO_3^--N = nitrate nitrogen. NO_2^--N = nitrite nitrogen. N = total nitrogen. C = total carbon. S = total sulfur. C/N = the ratio of carbon and nitrogen. SWC = soil water content. PS = average particle size. AS = arsenic. Cd = cadmium. Cu = copper. Pb = lead. Zn = zinc. B/F = gene abundance ratio of bacteria and fungi. (–) = negative correlation. (+) = positive correlation.

Copyright © 2019 Soil and Water Conservation Society. All rights reserved.
Journal of Soil and Water Conservation 74(5):439-448 www.swcs.org

Figure 2

Abundance of (a) soil bacteria and (b) fungi rRNA genes in different restored subdams. Points show the means of five replicates, and vertical bars show standard errors. The different letters indicate that the means are significantly different among restored subdams ($p < 0.05$) with Duncan test.

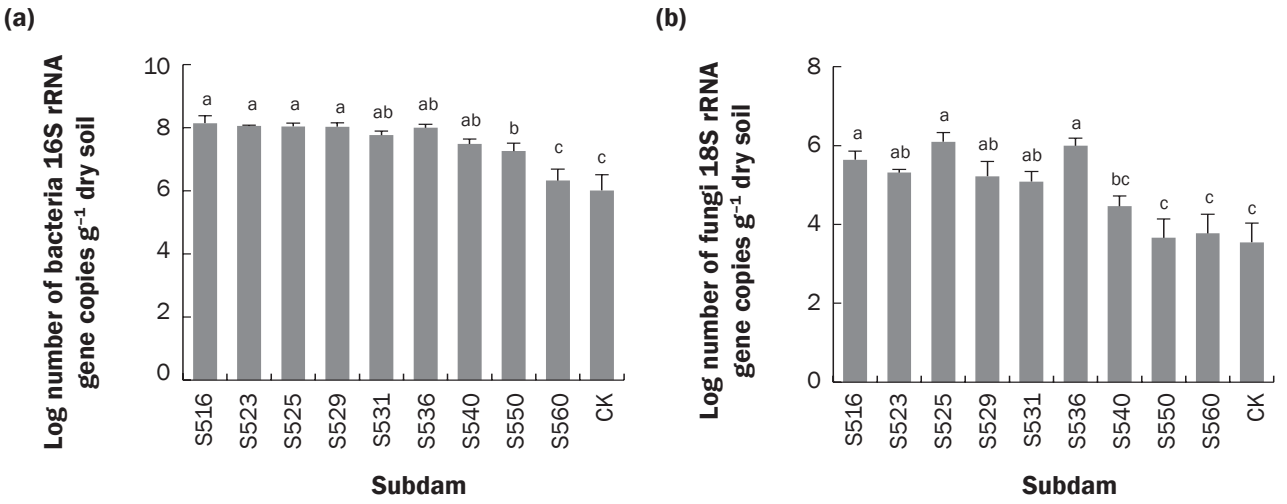
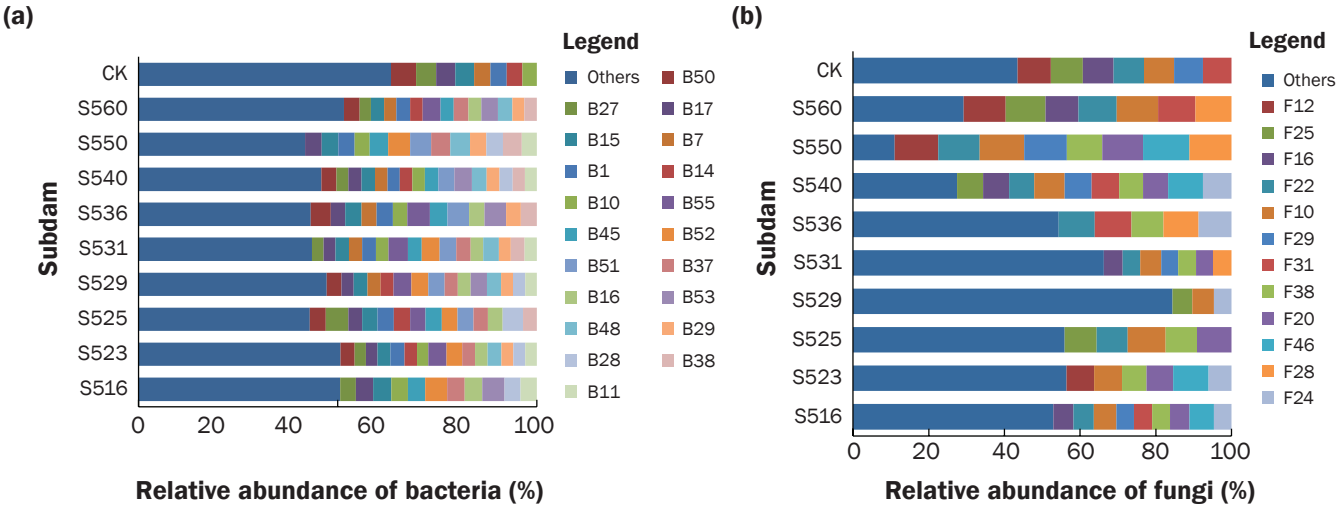


Figure 3

Relative abundance of soil bacteria and fungi from subdams in reclaimed copper (Cu) tailings dam monitored by denaturing gradient gel electrophoresis. (a) Relative abundance of bacteria is greater than 2%. (b) Relative abundance of fungi is greater than 3%. "B" and the following number represent the different bacterial groups; "F" and the following number represent the different fungi groups.



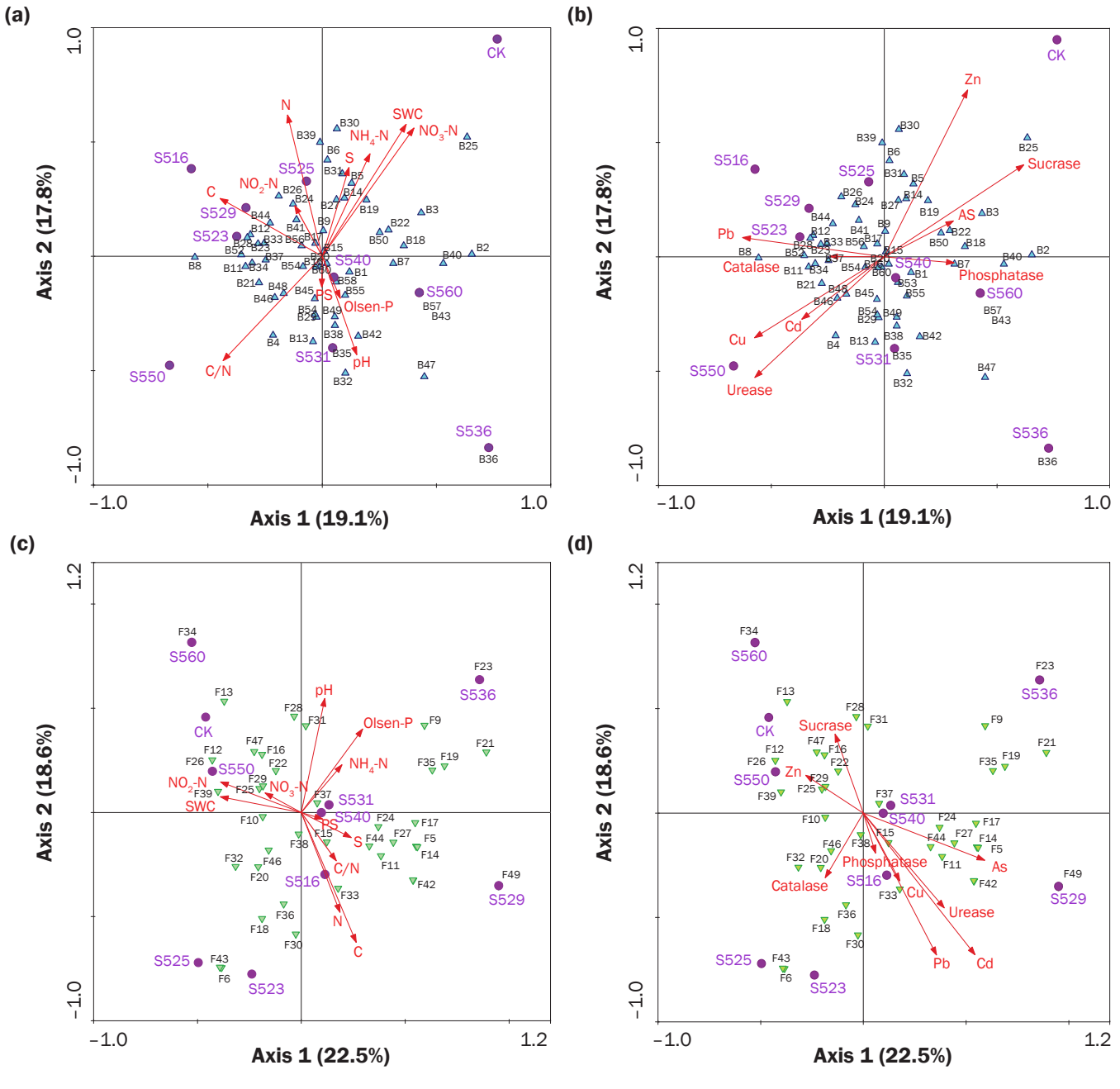
influenced by soil physical and chemical properties. Figure 5 showed that richness index and Margalef of soil bacteria from S516 and S525 were significantly higher than other subdams. We analyzed the soil characteristics on soil microbial diversity with the Redundancy analysis, and the results showed that 99.6% and 97.6% of the variations in soil bacteria and fungi diversity could be explained by soil physical and chemical properties, respectively (figure 6). For soil bacteria, axis 1 of the RDA plot explained nearly 83.9% of the variation, and axis 2 explained a further 15.7% (figure 6a). For soil fungi, axis 1 of the RDA plot explained 97.2% of the variation (figure 6b). Soil bacteria richness and the Margalef's richness index were influenced by soil pH, and soil microbial diversity was generally more affected by soil C content (figure 6a). We also found that fungal diversity was affected by N, PS, and S, and soil bacteria diversity was influenced by C/N and NH_4^+-N (figure 6b). Soil permeability, pH, NH_4^+-N, and NO_3^- were the primary factors that affected microorganism abundance; for example, soil microorganism abundance was higher when soil permeability and pH were higher and NH_4^+-N and NO_3^- were lower (Gu et al. 2018).

It has been reported that light and moderate pollution can improve the richness, diversity, and evenness of the soil microbial community, and severe pollution will activate certain inhibitory effects in them (Guo et al. 2012). Our study showed that Cd and Cu mainly affected soil fungal and bacterial diversity, respectively (table 4). This could be the result of the different sensitivities between bacteria and fungi to

Copyright © 2019 Soil and Water Conservation Society. All rights reserved.
Journal of Soil and Water Conservation 74(5):439-448 www.swcs.org

Figure 4

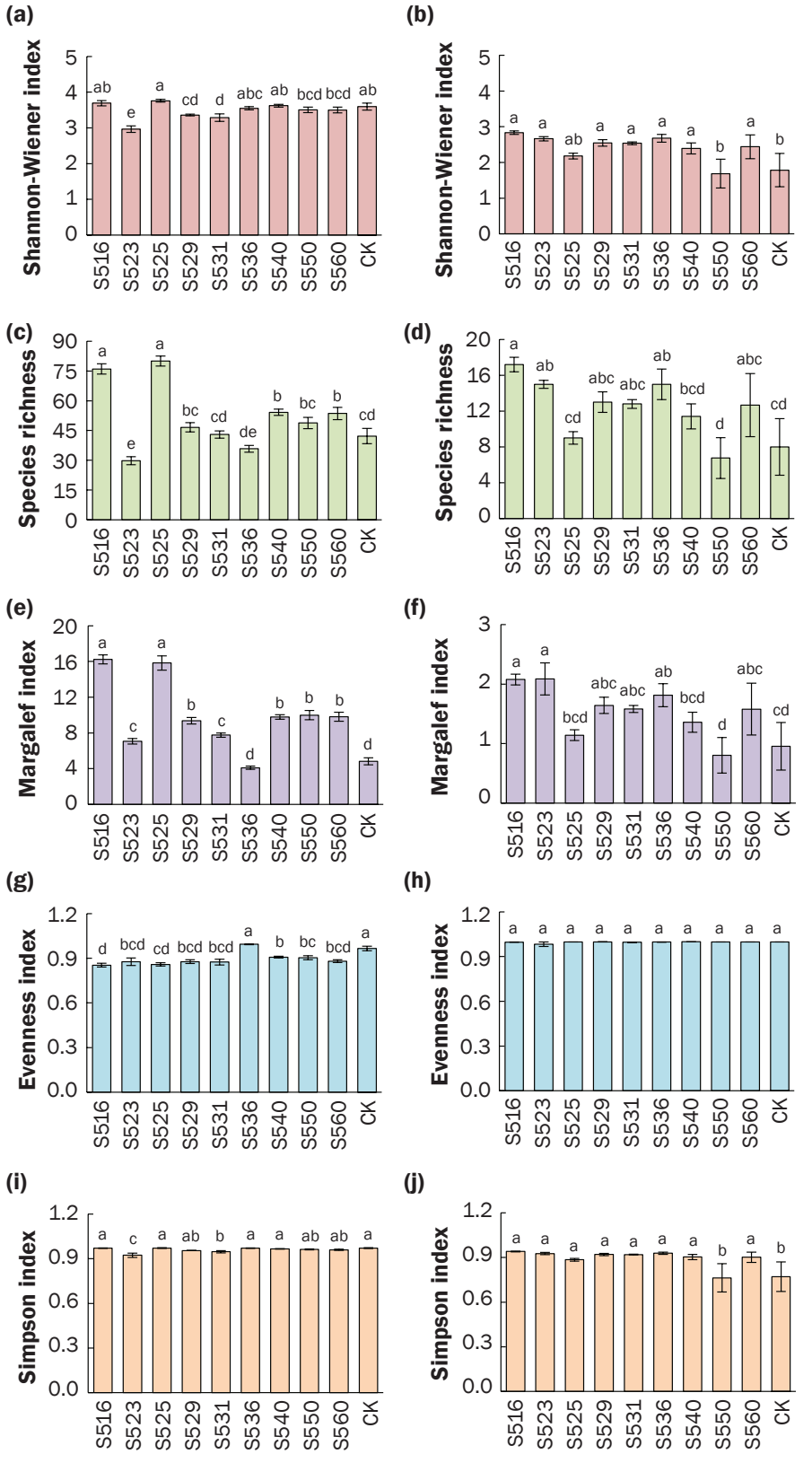
Canonical correspondence analysis (CCA) ordination biplot of environmental factors and enzyme activities for (a and b) soil bacterial and (c and d) fungal communities in copper (Cu) tailings dam. Environmental factors include total nitrogen (N), total carbon (C), total sulfur (S), the ratio of carbon and nitrogen (C/N), soil water content (SWC), soil pH, particle size (PS), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), and Olsen-P.



heavy metal pollution (Jin et al. 2009a). Our results indicated that soil properties have a greater impact on microbial diversity in areas of heavy metal pollution (table 5). We hypothesized that pollution levels would be one of the main reasons for soil microbial diversity in a Cu tailings dam. Richness and Margalef of soil bacteria were positively correlated with soil C content, C/N ratio, and Cu, but negatively correlated with soil pH (table 4 and figure 6a). Conversely, soil bacteria evenness index was negatively correlated with soil C content, C/N ratio, and Cu, but positively correlated with NH_4^+-N (figure 6 and table 4). Research has shown that the degree of heavy metal soil pollution was negatively correlated to the C/N ratio of paddy soil microorganisms, and was positively correlated to the relative abundance of fungus in soil (Wu et al. 2008). On the other hand, severe pollution will decrease richness diversity and evenness of soil microbial communities, while soil microbial community dominance indices will be highest. This indicated that a dominant species within soil microbial communities will prevail under severe pollution (Guo et al. 2012).

Copyright © 2019 Soil and Water Conservation Society. All rights reserved.
Journal of Soil and Water Conservation 74(5):439-448 www.swcs.org

Figure 5
Shannon-Wiener index; species richness; and Margalef, Evenness, and Simpson indices of soil (a, c, e, g, and i) bacteria and (b, d, f, h, and j) fungi communities in different subdams. Different letters indicate significant differences according to Duncan's test ($p < 0.05$).



Summary and Conclusions

This study addressed the effects of heavy metal pollution on soil physicochemical properties and microbial diversity over different years of reclamation in a Cu tailings dam. As restoration progressed, soil nutrient content significantly increased, especially for total soil C and N. Soil enzyme activates also varied along with environmental factors in alkaline soil. Soil bacteria and fungi gene copies gradually increased; the relative abundance of bacteria was more affected by environmental factors than fungi; and soil fungi diversity gradually increased along with an increase in restoration years, while soil bacteria exhibited irregular trends. Results from this study could have important implications for soil ecosystem restoration and could provide an ecological basis for further studies on soil degradation mechanisms in Cu tailings dams.

Acknowledgements

This project was financed through the National Natural Science Foundation of China under Grant No. 31600308, Shanxi Scholarship Council of China under Grant No. 2016-006, Shanxi Province Science Foundation for Youths under Grant No. 201601D021101, and the Higher Education Institution Project of Shanxi Province: Ecological Remediation of Soil Pollution Disciplines Group (Grant No. 20181401).

References

Abraham, M.R., and T.B. Susan. 2017. Water contamination with heavy metals and trace elements from Kilembe copper mine and tailing sites in Western Uganda; implications for domestic water quality. Chemosphere 169:281-287.

Baldrian, P., J. Trogl, J. Frouz, J. Snajdr, V. Valaskova, V. Merhautova, T. Cajthaml, and J. Herinkova. 2008. Enzyme activities and microbial biomass in topsoil layer during spontaneous succession in spoil heaps after brown coal mining. Soil Biology and Biochemistry 40:2107-2115.

Cerli, C., L. Celi, K. Kaiser, G. Guggenberger, M.B. Johansson, A. Cignetti, and E. Zanini. 2008. Changes in humic substances along an age sequence of Norway spruce stands planted on former agricultural land. Organic Geochemistry 39:1269-1280.

Chen, J., and N. Yang. 2013. Changes in SBR and qCO_2 at different re-vegetation stages on sloping-land with purple soils in Hengyang of Hunan Province, south-central China. Journal of Tropical and Subtropical Botany 514-520.

Ding, Q., G. Cheng, Y. Wang, and D. Zhuang. 2017. Effects of natural factors on the spatial distribution of heavy metals

Copyright © 2019 Soil and Water Conservation Society. All rights reserved.
Journal of Soil and Water Conservation 74(5):439-448 www.swcs.org

Figure 6

Redundancy analysis (RDA) biplot of (a) bacteria and (b) fungi diversity, 16S rRNA gene abundance, and environmental factors. Environmental factors include total nitrogen (N), total carbon (C), total sulfur (S), the ratio of carbon and nitrogen (C/N), soil water content (SWC), soil pH, particle size (PS), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), and Olsen-P.

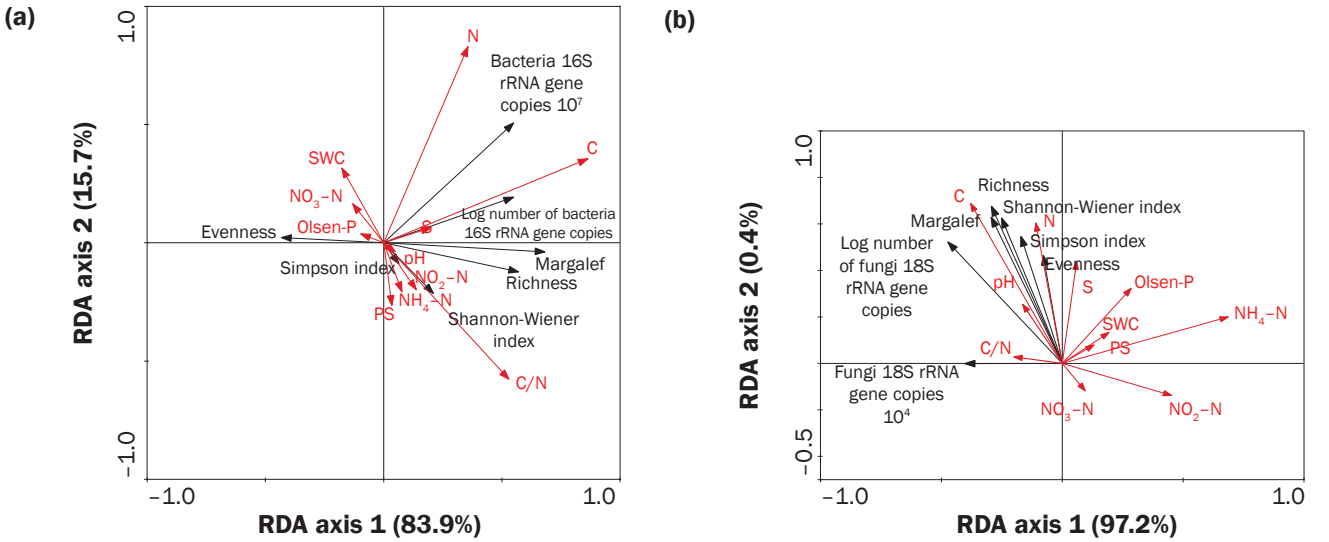


Table 4

The Pearson correlations between soil heavy metals and microbial diversity in copper tailings dam.

	Bacteria					Fungi				
Metal	Shannon-Wiener index	Simpson index	Richness	Margalef	Evenness	Shannon-Wiener index	Simpson index	Richness	Margalef	Evenness
As	0.029	−0.007	−0.067	−0.131	0.124	0.211	0.224	0.174	0.191	−0.072
Cd	−0.153	−0.154	0.005	0.129	−0.175	0.322*	0.328*	0.256	0.250	−0.032
Cu	0.054	−0.038	0.363**	0.492**	−0.376**	0.269	0.270	0.243	0.228	−0.021
Pb	−0.177	−0.134	−0.049	0.052	−0.132	−0.002	−0.027	0.031	0.049	−0.081
Zn	0.137	0.132	−0.030	−0.160	0.199	−0.057	−0.117	0.015	0.047	−0.132

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

Notes: As = arsenic. Cd = cadmium. Cu = copper. Pb = lead. Zn = zinc.

Copyright © 2019 Soil and Water Conservation Society. All rights reserved.
Journal of Soil and Water Conservation 74(5):439-448 www.swcs.org

in soils surrounding mining regions. Science of the Total Environment 578:577-585.

Fierer, N., and R.B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 103:626-631.

Fisher, K.A., S.A. Yarwood, and B.R. James. 2017. Soil urease activity and bacterial ureC gene copy numbers: Effect of pH. Geoderma 285:1-8.

Gu, P., Y. Jiao, W. Yang, H. Wen, S. Bai, and J. Yang. 2018. Effects of different irrigation methods on soil microbial abundance and permeability in farmland. Journal of Irrigation and Drainage 37:21-27.

Guo, X., J. Gu, Z. Chen, H. Gao, Q. Qin, W. Sun, and W. Zhang. 2012. Effects of heavy metals pollution on soil microbial communities metabolism and soil enzyme activities in coal mining area of Tongchuan, Shaanxi Province of northwest China. Chinese Journal of Applied Ecology 23:798-806.

Hao, D., J. Zhou, and J. Han. 2017. Microbial remediation of soil heavy metal and organic pollutants: Bioaugmentation and biostimulation. Biotechnology Bulletin 9-17.

He, Z., X. Xie, S. Xiao, J. Liu, and G. Qiu. 2007. Microbial diversity of mine water at Zhong Tiaoshan copper mine, China. Journal of Basic Microbiology 47:485-495.

Huang, F. 2015. Studies on revegetation and restoration effects of gold and copper wasteland in Zijin Mountain. Journal of Fujian Forestry Science and Technology 74-80:93.

Huang, X., X. Li, N. Liu, and X. Min. 2014. Characteristics of soil particles fractal dimension under different reclamation years in coal mining subsidence. Journal of China Coal Society 39:1140-1146.

Jin, Y., Z. He, and X. Yang. 2009a. Effects of Hg contamination on paddy soil microbial and enzymatic activities. Chinese Journal of Applied Ecology 20:218-222.

Jin, K., S. Sleutel, D. Buchan, S.D. Neve, D.X. Cai, D. Gabriels, and J.Y. Jin. 2009b. Changes of soil enzyme activities under different tillage practices in the Chinese Loess Plateau. Soil and Tillage Research 104:115-120.

Jorquera, M.A., F. Maruyama, A.V. Ogram, O.U. Navarrete, L.M. Lagos, N.G. Inostroza, J.J. Acuna, J.I. Rilling, and M. de La Luz Mora. 2016. Rhizobacterial community structures associated with native plants grown in Chilean extreme environments. Microbial Ecology 72:633-646.

Lauber, C.L., M. Hamady, R. Knight, and N. Fierer. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75:5111.

Table 5
Relationship of microbial structures and diversities to different environmental factors by a Mantel test.

	Soil chemical properties		Heavy metals		Reclaimed years		Enzyme activities	
Structure/ diversity	***rM***	***p***	***rM***	***p***	***rM***	***p***	***rM***	***p***
Bacteria structure	0.410	**0.048**	0.518	**0.012**	0.303	0.058	0.041	0.390
Fungi structure	−0.355	0.975	0.081	0.345	0.133	0.216	−0.102	0.704
Bacteria diversity	0.225	**0.045**	0.135	0.178	−0.036	0.538	0.083	0.259
Fungi diversity	−0.185	0.853	−0.049	0.607	0.237	0.058	−0.148	0.817

Note: Significant *p*-values are in bold print.

Li, C., J. Jing, J. Liu, and B. Chai. 2018. Driving factors of the dynamics of microbial community in a dam of copper mine tailings. Environmental Science 39:1804–1812.

Li, J., F. Liu, and X. Zhou. 2015a. Effects of different reclaimed scenarios on soil microbe and enzyme activities in mining areas. Environmental Science 1836–1841.

Li, X., B. Zhao, X. Li, Y. Li, R. Sun, L. Zhu, J. Xu, L. Wang, X. Li, and F. Zhang. 2005. Effects of different fertilization systems on soil microbe and its relation to soil fertility. Scientia Agricultura Sinica 1591–1599.

Li, J., X. Zhou, J. Yan, H. Li, and J. He. 2015b. Effects of regenerating vegetation on soil enzyme activity and microbial structure in reclaimed soils on a surface coal mine site. Applied Soil Ecology 87:56–62.

Liu, J., C. Li, J. Jing, T. Jia, X. Liu, X. Wang, and B. Chai. 2017. Composition and environmental adaptation of microbial community in Shibahe copper tailing in Zhongtiao Mountain in Shanxi. Environmental Science 38:318–326.

Liu, J., C. Li, J. Jing, P. Zhao, Z. Luo, M. Cao, Z. Ma, T. Jia, and B. Chai. 2018. Ecological patterns and adaptability of bacterial communities in alkaline copper mine drainage. Water Research 133:99–109.

Liu, Z., and N. Yang. 2014. Soil microbial biomass in degenerated and recovered vegetation on sloping-land with purple soils in Hengyang of Hunan Province, China. Ecology and Environment Sciences 1739–1743.

Luo, H., P. Liu, and X. Song. 2006. Effect of compound pollution of Cd, Cu and Ni on soil enzyme activities. Journal of Soil and Water Conservation (in Chinese) 20:94–96.

Niu, S., J. Li, T. Yang, W. Da, J. Yang, Y. Ning, and J. Yao. 2010. The relationships of soil microbial biomass, physicochemical factors and soil enzyme activities in Maqu swamp wetland of Gannan prefecture. Journal of Glaciology and Geocryology V32:1022–1029.

Ojuederie, O.B., and O.O. Babalola. 2017. Microbial and plant-assisted bioremediation of heavy metal polluted environments: A review. International Journal of Environmental Research and Public Health 14:1504–1530.

Pei, D.H., L. Huang, L.I. Hong-Yan, X.Y. Lin, P. Liang, X.U. Jian-Xin, and L. Zhao. 2017a. Demonstration of comprehensive ecological remediation technology of heavy metal pollution in sediment. Modern Agricultural Science & Technology 13:189–190.

Pei, B., L. Zhu, H. Liang, J. Zhang, X. Yang, and Y. Kong. 2017b. Effect of different plantation types on soil properties and enzyme activity in the hilly region of Taihang Mountains. Journal of Arid Land Resources and Environment 31:190–195.

Qiao, S., Y. Zhou, J. Liu, J. Jing, T. Jia, C. Li, X. Yang, and B. Chao. 2017. Characteristics of soil bacterial community structure in coniferous forests of Guandi Mountains, Shanxi Province. Scientia Silvae Sinicae 53:89–99.

Shao, Z., F. Zhang, J. Chen, and B. Zhang. 2017. Soil moisture changes for different land use types in Loess Plateau gully and hilly region of central Gansu. Journal of Arid Land Resources and Environment 129–135.

Sinsabaugh, R., C. Lauber, M. Weintraub, B. Ahmed, S. Allison, C. Crenshaw, A. Contosta, D. Cusack, S. Frey, and M. Gallo. 2010. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11:1252–1264.

Sun, B., H. Sun, and T. Zhang. 2004. Bio-environmental effects and index of remediation of multi-heavy metals polluted red soils. Chinese Journal of Environmental Science 25:104–110.

Tong, J., C. Miaowen, J. Juhui, L. Jinxian, and C. Baofeng. 2017. Endophytic fungi and soil microbial community characteristics over different years of phytoremediation in a copper tailings dam of Shanxi, China. Science of the Total Environment 574:881–888.

Wang, J., L. Guo, Z. Bai, R. Yang, and M. Zhang. 2013. Succession law of reclaimed soil and vegetation on opencast coal mine dump of loess area. Transactions of the Chinese Society of Agricultural Engineering 223–232.

Wang, C., P. Guo, G. Han, X. Feng, P. Zhang, and X. Tian. 2010a. Effect of simulated acid rain on the litter decomposition of *Quercus acutissima* and *Pinus massoniana* in forest soil microcosms and the relationship with soil enzyme activities. Science of the Total Environment 408:2706–2713.

Wang, B., G. Liu, and S. Xue. 2010b. Effects of soil nutrient and soil microbial biomass on soil enzyme activities in abandoned croplands with different restoration age. China Environmental Science 30:1375–1382.

Wu, J., Y. Jiang, Y. Wu, and J. Xu. 2008. Effects of complex heavy metal pollution on biomass and community structure of soil microbes in paddy soil. Acta Pedologica Sinica 45:1102–1109.

Xin, Z., J. Li, X. Zhao, and X. Zhou. 2017. Characteristics of soil organic carbon mineralization and enzyme activities in coal mining area after different reclamation times. Research of Environmental Sciences 1580–1586.

Yang, Z., and S. Liu. 2001. Effect of compound pollution of heavy metals on soil enzymic activities. Acta Scientiae Circumstantiae 21:60–63.

Yang, Y., X. Meng, and P. Zhang. 2013a. Physical and chemical properties of soils in wetlands of Caizi Lake different in restoration history in Anqing, Anhui Province. Journal of Ecology and Rural Environment 29:322–328.

Yang, Y., Y. Song, H.V. Scheller, A. Ghosh, Y. Ban, H. Chen, and M. Tang. 2015. Community structure of arbuscular mycorrhizal fungi associated with *Robinia pseudoacacia* in uncontaminated and heavy metal contaminated soils. Soil Biology and Biochemistry 86:146–158.

Yang, M., N. Yang, R. Guo, F. Zou, L. Zhao, and Z. Lin. 2013b. Numerical properties of soil microbial population in re-vegetation stages on sloping land with purple soils in Hengyang. Ecology and Environment Sciences 2:229–231.

Yuan, Y., Z. Zhao, P. Zhang, L. Chen, T. Hu, S. Niu, and Z. Bai. 2017. Soil organic carbon and nitrogen pools in reclaimed mine soils under forest and cropland ecosystems in the Loess Plateau, China. Ecological Engineering 102:137–144.

Zeng, Q., S. An, and Y. Liu. 2017. Soil bacterial community response to vegetation succession after fencing in the grassland of China. Science of the Total Environment 609:2–10.

Zhang, P., Y. Zhao, L. Huang, Y. Hu, and X. Han. 2016. Effect of revegetation on soil extracellular enzyme activity in the dumping site of an open-pit coal mine in Heidaigou. Acta Ecologica Sinica 36:2715–2723.

Zhao, Z., I. Shahrour, Z. Bai, W. Fan, L. Feng, and H. Li. 2013. Soils development in opencast coal mine spoils reclaimed for 1–13 years in the west-northern loess plateau of China. European Journal of Soil Biology 55:40–46.

Copyright © 2019 Soil and Water Conservation Society. All rights reserved.
Journal of Soil and Water Conservation 74(5):439–448 www.swcs.org