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The shifts of sediment microbial community phylogenetic and functional structures during chromium (VI) reduction

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Abstract The Lanzhou reach of the Yellow River, located at the upstream of Lanzhou, has been contaminated by heavy metals and polycyclic aromatic hydrocarbons over a long-time. We hypothesized that indigenous microbial communities would remediate those contaminants and some unique populations could play an important role in this process. In this study, we investigated the sediment microbial community structure and function from the Lanzhou reach. Sediment samples were collected from two nearby sites (site A and site B) in the Lanzhou reach along the Yellow River. Sediment geochemical property data showed that site A sediment samples contained significantly ($p < 0.05$) higher heavy metals than site B, such as chromium (Cr), manganese (Mn), and copper (Cu). Both site A and B samples were incubated with or without hexavalent chromium (Cr (VI)) for 30 days in the laboratory, and Cr (VI) reduction was only observed in site A sediment samples. After incubation, MiSeq sequencing of 16S rRNA gene amplicons revealed that the phylogenetic composition and structure of microbial communities changed in both samples, and especially Proteobacteria, as the most abundant phylum increased from 45.1 % to 68.2 % in site A, and 50.1 % to 71.3 % in site B, respectively. Some unique OTUs and populations affiliated with *Geobacter*, *Clostridium, Desulfosporosinus* and *Desulfosporosinus* might be involved in Cr (VI) reduction in site A. Furthermore, GeoChip 4.0 (a comprehensive functional gene array) data showed that genes involved in carbon and nitrogen cycling and metal resistance significantly ($p < 0.05$) increased in site A sediment samples. All the results indicated that indigenous sediment microbial communities might be able to remediate contaminants like Cr (VI), and this information provides possible strategies for future bioremediation of the Lanzhou reach.

Keywords Yellow River · Cr (VI) reduction · GeoChip · MiSeq sequencing · Microbial community diversity

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Introduction

The Lanzhou reach is located in one of the biggest industrial bases of the northwest of China, which is in the upper reaches of the Yellow River (Supplementary Fig. S1). Over a hundred industrial companies are placed in this area, causing a lot of environmental contaminations in air, water and soil, especially with heavy metals, and organic contaminants (Chu et al. 2008; Zeng et al. 2007). For example, a recent tap water contamination accident revealed that this area had been contaminated by industrial wastewater since

1950s (Xiaoyan et al. 1989; Yan and Desheng 2007; Zhao et al. 2010). These contaminants might influence the growth of plants (Nagajyoti et al. 2010), threaten human and animal health (Bjerregaard and Hansen 2000), and impact water and soil microbial communities in surrounding areas (Hemme et al. 2010). Microbial communities within this area might have developed unique structure and function to such environments. However, the influence of these contaminants on sediment microbial communities has not yet been investigated.

Chromium is a major contamination in sediment/soil and water environments due to its widely industrial applications in Lanzhou (Liu et al. 2009). Chromium accumulation is harmful for the human body. It could cause diseases, damage to organs (Velma and Tchounwou 2013), and lead to canceration of cell (Zhang et al. 2011). Microorganisms play important roles in the cycling of elements, such as carbon (C), nitrogen (N), phosphorus (P), sulfur (S) and metals, and modify the fate of environmental contaminants (e.g., uranium, chromium) (Cervantes et al. 2001; Faybishenko et al. 2008; Gihring et al. 2011). Some studies have reported microbes could reduce Cr (VI) to less toxic and soluble form (Cr (III)). It is believed that in situ microbial metal reduction is a promising approach for heavy metal remediation (Liang et al. 2012), such as Cr (VI) reduction (Liu et al. 2011; Somenahally et al. 2013).

Recently, high-throughput metagenomic technologies, such as microarrays (He et al. 2012; He et al. 2010; Tu et al. 2014) and sequencing technologies (Loman et al. 2012) have been applied to microbial community analysis. MiSeq of 16S rRNA gene amplicons is suitable to detect the phylogenetic diversity of microbial communities (Caporaso et al. 2012), while high-throughput metagenomic technology may provide functional profiles of microbial communities (He et al. 2012; He et al. 2012). GeoChip 4.0 contains about 84,000 probes and covers 152,000 gene sequences in 410 functional families involved in a variety of key functional processes, such as C, N, P, and S cycling, metal resistance, and antibiotic resistance (Tu et al. 2014). It is preferable to analyze the phylogenetic/taxonomical and functional diversity, composition and structure of microbial communities complementarily using both technologies.

Understanding the functional diversity, composition, structure and potential/activity of microbial communities is essential to develop new strategies for bioremediation of contaminated sites. Earlier research reported several methods for the study of soil microbial community response to environment changes such as in situ bioremediation (Liang et al. 2011) and laboratory incubation (Degens et al. 2001; Nie et al. 2013). In situ bioremediation is better for understanding the changes of microbial community structure in response to complicated environments (Xu et al. 2010), while laboratory incubation could be used to realize the influence of specific factors to microbial communities and their possible mechanisms (Westergaard et al. 2001). For example, Hueso et al. (2012) examined the change of the microbial community structure under drought conditions with laboratory incubation, and found that drought affected the physiology of the microbial community, and soil amended with manure compost mitigated the influence (Hueso et al. 2012). Another study is to observe the effect of cadmium (Cd) and lead (Pb) on soil microbial community structure and activities, and the results revealed that bacteria were more sensitive to heavy metals than actinomycetes and fungi (Khan et al. 2010). Therefore, it is important to combine in situ analysis and laboratory incubation for understanding microbial community composition, structure, and function as well as their linkages with environmental factors.

The aim of this study is to examine the influence of Cr (VI) on the microbial community diversity, composition and structure, and how microorganisms respond to Cr (VI). We hypothesized that indigenous microbial communities would remediate those contaminants and some unique populations could play an important role in this process. Sediment samples were taken from the Lanzhou reach and incubated at the laboratory for 30 days with $K_2Cr_2O_7$, and analyzed by two high throughput metagenomic approaches: GeoChip 4.0 and MiSeq sequencing of 16S rRNA genes. We found that only site A sediment microbial communities had the ability to reduce Cr (VI), and that some unique OTUs/populations as well as functional genes involved in sulfate and metal reduction increased after Cr (VI) reduction in site A. This study provides new insights into our understanding of Cr (VI) reduction mechanisms and possible strategies for bioremediation of contaminated sites in Lanzhou reach of the Yellow River.

Materials and methods

Site description and field sampling

Sediment samples (10–20 cm depth) were collected from two sites (A and B) near the industrial discharging site of the Lanzhou reach with three replicates for each site (Supplementary Fig. S1). Site A is close to the Yellow River at latitude N 36°07′57.3″ and longitude E 103°38′07.3″ with an elevation of 1534 m above sea level, while site B is near the discharge point at latitude N 36°07′53.5″ and longitude E 103°38′02.7″ with an elevation of 1535 m above sea level. There are many petrochemical, metallurgical and mechanical industries in this district, and these industries have seriously polluted air (Chu et al. 2008; Ta et al. 2004) and water (Wang et al. 2010). The distance between A and B is forty meters. The pH and temperature of both sites were

measured on site. All samples were collected in sterile boxes and transported to the laboratory within two hours at room temperature. After transportation to the laboratory, each sample was weighed and divided into three sub-samples: the first set of sub-samples were used for the laboratory incubation with Cr (VI) immediately; the second was kept at −80 °C for DNA extraction and downstream analyses; the third was kept at room temperature for determining sediment physical and chemical properties.

Determination of sediment physical and chemical properties

Water contents of sediment samples were measured by air-drying at room temperature. The concentrations of organic carbon (OC), and available N, P, potassium (K), magnesium (Mg) and calcium (Ca) were measured as previously described (Jackson and Barak 2005; Lu 2000). Cd, Cu, Cr, Pb and Mn concentrations were determined using a Varian AA240 by atomic absorption spectrum (Citak and Tuzen 2010; Olmedo et al. 2010).

Laboratory incubation setup and sampling

For the treatment group, 15 g of sediment (fresh weight) was taken for each sample and mixed with 60 mL sterile water and 2 mL $K_2Cr_2O_7$ (2 mM) in a 100-mL glass flask, and then incubated without shaking at 37 °C, while the corresponding control group was incubated without Cr (VI) (distilled water instead of $K_2Cr_2O_7$ was added) at 37 °C. Also, two other control groups were set up: one was incubated without Cr (VI) at 16 °C, which is similar to the temperature in situ, and the other was the abiotic group, for which samples were autoclaved three times at 121 °C for 20 min. The abiotic control was set to eliminate the effect of Cr (VI) absorbed by sediment samples and to determine whether microbial communities play an important role in decrease of Cr (VI) in the supernatant. Totally, there were eight groups: aA (abiotic control for sediment A), 37A (control for sediment A incubated at 37 °C), 16A (control for sediment A incubated at 16 °C), A (sediment A), aB (abiotic control for sediment B), 37B (control for sediment B incubated at 37 °C), 16B (control for sediment B incubated at 16 °C), and B (sediment B) with three replicates for each group. After incubating for 114 h, another 2 mL $K_2Cr_2O_7$ (2 mM) was added to aA, A, aB and B groups. Sediment samples were collected at day 0 (A0 and B0) (samples kept at −80 °C after transportation to the laboratory) and day 30 (A30 and B30) for DNA extraction, GeoChip analysis and MiSeq sequencing. For samples collected in day 30, the supernatant in 100 mL glass flasks was removed gently using a 10-mL injector first, and then a sterile spoon was used to obtain sediment samples for DNA extraction.

Determination of Cr (VI) reduction

During the incubation, Cr (VI) concentrations in aA, A, aB, and B samples were measured by diphenylcarbazide method (Lovley and Phillips 1994). Briefly, 500 µL supernatant was withdrawn with a syringe and needle and added to 2.5 mL diphenylcarbazide reagent (0.2 g diphenylcarbazide dissolved in 100 mL acetone, and then mixed with 400 mL (1:9) H_2SO_4), and the A_{540} of each sample was measured.

Sediment DNA extraction

The sediment microbial community DNA was extracted from 0.5 g sediment samples using an E.Z.N.A. Soil DNA Kit (Omega Bio-Tek Inc., USA) in accordance with the manufacturer's instructions. DNA quality was estimated using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific Inc., USA) to measure the absorbance ratios of A_{260} /A_{280} and A_{260}/A_{230}. DNA was used for further analysis only when the ratios of A_{260}/A_{280} and A_{260}/A_{230} > 1.8 and 1.7, respectively. Then the purified DNA was dried by CentriVap DNA Concentrator (Labconco, U.S.A) and stored at −20 °C.

MiSeq sequencing of 16S rRNA gene amplicons

The universal primer 515F (5′-GTGCCAGCMGCCGC GGTAA-3′) and 806R (5′-GGACTACHVGGGTWTC-TAAT-3′) with unique barcode was used to amplify the V4 hypervariable region of 16S rRNA genes and sequence using MiSeq sequencer (Caporaso et al. 2012; Caporaso et al. 2011). The PCR mixture (25 µL) contained 1 × PCR buffer, 1.5 mM $MgCl_2$, each deoxynucleoside triphosphate at 0.4 µM, each primer at 1.0 µM and 0.5 U of Ex Taq and 10 ng soil genomic DNA. The PCR amplification program included initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 40 s, 56 °C for 60 s, and 72 °C for 60 s, and a final extension at 72 °C for 10 min. Two PCR reactions were performed for each sample, and they were combined after PCR amplification. PCR products were subjected to electrophoresis using 1.0 % agarose gel. The band with a correct size was excised and purified using SanPrep DNA Gel Extraction Kit (Sangon Biotech, China, Cat# SK8132) and quantified with Nanodrop. All samples were pooled together with an equal molar amount from each sample. The sequencing samples were prepared using TruSeq DNA kit according to the manufacturer's instruction. The purified library was diluted, denatured, re-diluted, mixed with PhiX (equal to 30 % of final DNA amount) as

Table 1 Geochemical data of site A sample (A0), site B sample (B0) and samples incubated with Cr (VI) for 30 days from site A (A30), or from site B (B30)

Sediment geochemical property	A0	B0	A30	B30
pH	6.65 ± 0.013^{a}	6.693 ± 0.01^{a}	6.721 ± 0.021^{a}	6.568 ± 0.017^{a}
Moisture (%)	41.09 ± 1.31^{b}	32.91 ± 1.96^{c}	74.10 ± 2.18^{a}	72.48 ± 2.62^{a}
N (mg/g)	1.19 ± 0.01^{c}	0.86 ± 0.01^{d}	3.51 ± 0.17^{a}	1.44 ± 0.06^{b}
P (mg/g)	1.51 ± 0.02^{a}	1.18 ± 0.01^{b}	nd	nd
OC (mg/g)	17.61 ± 0.21^{b}	14.15 ± 0.12^{c}	28.17 ± 1.67^{a}	13.40 ± 1.21^{c}
K (mg/g)	19.1 ± 0.24^{b}	18.91 ± 0.23^{b}	21.69 ± 0.11^{a}	17.91 ± 0.16^{c}
Fe (mg/g)	46.87 ± 0.34^{a}	44.97 ± 0.72^{a}	34.70 ± 0.07^{b}	30.71 ± 0.15^{c}
Mg (mg/g)	17.05 ± 0.18^{b}	17.05 ± 0.11^{b}	18.04 ± 0.24^{a}	15.44 ± 0.10^{c}
Ca (mg/g)	177.54 ± 0.39^{a}	190.08 ± 9.38^{a}	51.69 ± 0.73^{b}	50.29 ± 0.96^{b}
Mn (mg/Kg)	595.63 ± 12.6^{b}	462.05 ± 3.38^{d}	647.56 ± 7.43^{a}	556.71 ± 6.1^{c}
Pb (mg/Kg)	34.88 ± 7.27^{a}	30.23 ± 1.20^{a}	29.73 ± 2.14^{a}	35.02 ± 3.63^{a}
Cd (mg/Kg)	0.487 ± 0.14^{a}	0.487 ± 0.14^{a}	0.472 ± 0.11^{a}	0.465 ± 0.15^{a}
Cr (mg/Kg)	81.34 ± 2.86^{c}	73.57 ± 0.66^{d}	2968 ± 17.7^{a}	1231.67 ± 22.5^{b}
Cu (mg/Kg)	32.61 ± 1.7^{a}	26.81 ± 0.17^{b}	35.1 ± 2.83^{a}	28.58 ± 3.48^{b}

All the geochemical data were obtained from the sediment samples. The moisture contents in A0 and B0 were determined directly from the field samples, and those in A30 and B30 were determined after the laboratory incubation with Cr (VI). The differences among the four groups were analyzed by ANOVA and Tukey's test with $p < 0.05$ as significance.

N available nitrogen, *P* available phosphorus, *K* available potassium, *OC* organic carbon, *nd* not determined

described in the Illumina library preparation protocols, and then applied to an Illumina MiSeq system for sequencing with the Reagent Kit v2 2×250 bp as described in the manufacture manual.

Sequencing data analysis

The sequence data were processed using QIIME Pipeline–Version 1.7.0 (http://qiime.org/). All sequence reads were trimmed and assigned to each sample based on their barcodes. The sequences with high quality (length > 150 bp, without ambiguous base 'N', and average base quality score > 30) were used for downstream analyses. Sequences were clustered into operational taxonomic units (OTUs) at a 97 % identity threshold. All the samples were randomly-resampled to 12600 reads. Taxonomy was assigned using the Ribosomal Database Project classifier (Wang et al. 2007). Permutational multivariate analysis of variance (ADONIS) was performed to test the dissimilarity between each group using the Bray-Curtis method based on all OTUs detected. Alpha-diversity (phylogenetic distance whole tree, Chao1 estimator of richness, observed species and Shannon's diversity index) was calculated based on MiSeq sequence data. Also, the rarefaction curves were generated from the observed OTUs. Detrended correspondence analysis (DCA) was performed based on the final OTU table. All the sequence data have been submitted to NCBI, the accession numbers are : SAMN05292835 (16A1), SAMN05292836 (16A2), SAMN05292837 (16A3), SAMN05292838 (16B1), SAMN05292839 (16B2), SAMN05292840 (16B3), SAMN05292841 (37A1), SAMN05292842 (37A2), SAMN05292843 (37A3), SAMN05292844 (37B1), SAMN05292845 (37B2), SAMN05292846 (37B3), SAMN05292847 (A1), SAMN05292848 (A2), SAMN05292849 (A3), SAMN05292850 (A301), SAMN05292851 (A302), SAMN05292852 (A303), SAMN05292853 (B1), SAMN05292854 (B2), SAMN05292855 (B3), SAMN05292856 (B301), SAMN05292857 (B302), and SAMN05292858 (B303).

GeoChip analysis

GeoChip 4.0 was used to analyze the functional diversity, composition and structure of sediment microbial communities. The purified sediment DNA (1.0 μg) was labeled with Cy3 and hybridized with GeoChip 4.0 (NimbleGen, Madison, WI) at 42 °C with 40 % formamide for 16 h (Tu et al. 2014). After hybridization, the arrays were scanned (NimbleGen MS200, Madison, WI) at a laser power of 100 %. Signal intensities were background subtracted and only the spots with signal-to-noise ratios (signal intensity-background intensity/background standard deviation) > 2.0 were considered as positive signals and used for further analysis.

The unique and overlapped genes of all samples, and functional gene diversity indices, including Shannon-Weaver index (H'), Simpson's reciprocal index (1/D) and Pielou evenness (*J*) were obtained using the pipeline at the Institute for Environmental Genomics, University of Oklahoma (http://ieg2.ou.edu/NimbleGen). DCA and

clustering analysis were used to examine the overall functional structure of microbial communities among different treatments. Canonical correspondence analysis (CCA) and CCA-based variation partitioning analysis (VPA) were performed to evaluate the relationship between the microbial community structure and environmental variables. DCA, CCA and VPA were performed using R version 2.15.2 (Team 2010). The variance inflation factors (VIFs) were used for selecting environmental variables, and an environmental variable (e.g., K) with the highest VIF was removed, and this CCA repeated until all VIFs < 20. As a result, Pb, Cr, OC and moisture were chosen for VPA.

Results

Sediment chemical properties

Moisture, available nitrogen (N), OC, Mn, Cr, Cu, available potassium (K), Fe, Mg and Ca of sediment samples were significantly different ($p < 0.05$) among the four treatments (A0, B0, A30, and B30) although there were no significant differences for pH, Pb and Cd (Table 1). Also, moisture, and concentrations of N, available phosphorus (P), OC, Mn, Cr and Cu were higher in site A (A0) than in site B (B0) before incubation. After incubation with Cr (VI) for 30 days, some of these geochemical properties significantly ($p < 0.05$) changed for site A samples (A0 vs. A30) with moisture, N, OC, K, Mg, Mn, and Cr increased, and Fe and Ca decreased despite no changes for Cu, while in site B (B0 vs. B30), moisture, and concentrations of N, Mn, and Cr significantly ($p < 0.05$) increased, the concentrations of K, Fe, Mg and Ca significantly ($p < 0.05$) decreased, and OC and Cu remained unchanged (Table 1). Such differences may be due to changed microbial activities, e.g., Cr (VI) reduction, which is in turn expected to modify the sediment microbial community composition, structure and function.

Cr (VI) reduction of sediment samples under laboratory incubation

For each sample, 15 g of sediment was mixed with 60 mL sterile water with 2 mL $K_2Cr_2O_7$ (2 mM) added, and incubated at 37 °C. The concentration of Cr (VI) decreased with time for the site A sediment samples, but this phenomenon was not observed for the site B sediment samples (Fig. 1 and Supplementary Fig. S2). Also, when the same amount of Cr (VI) were re-added to all the samples, a decrease of Cr (VI) concentration was only observed in site A sediment samples. These results indicated that the sediment microbial community from site A could have the capability of Cr (VI) reduction, and that some microorganisms would play an important role in the Cr (VI) reduction process.

Alternations of the taxonomical/phylogenetic composition of microbial populations in response to Cr (VI)

To further examine how microbial populations respond to incubation with Cr (VI), MiSeq sequencing of 16S rRNA gene amplicons was performed and all sequences/OTUs were classified into their possibly affiliated taxa. Alpha-diversity (phylogenetic distance whole tree, Chao1 estimator of richness, observed species and Shannon's diversity index) was calculated based on MiSeq sequencing data (Supplementary Table S1). Rarefaction curves (Supplementary Fig. S3) indicated the sequencing depth was enough for all samples. Microbial community compositions and structures had no significant changes in these two sites after incubated without Cr (VI) at 16 °C (Supplementary Fig. S4 and Supplementary Table S2 and S3). However, the microbial community compositions and structure changed after incubation at 37 °C in both sites, such as phylum Bacteroidetes, and genus *Flavobacterium* (Supplementary Fig. S4, Fig. S5 and Supplementary Table S2). But Adonis results showed no significant difference between A0 and 37A ($p = 0.26$), or between B0 and 37B ($p = 0.622$) (Supplementary Table S3). Alpha-diversity of microbial communities decreased after Cr (VI) incubation (Supplementary Table S1), and DCA of MiSeq sequence data showed that all samples were clustered into three different groups: the first group with A30 samples, the second group with B30 samples, and the third group with all other samples (Fig. 2). Adonis results showed microbial communities significantly changed ($p < 0.05$) after Cr (VI) incubation in

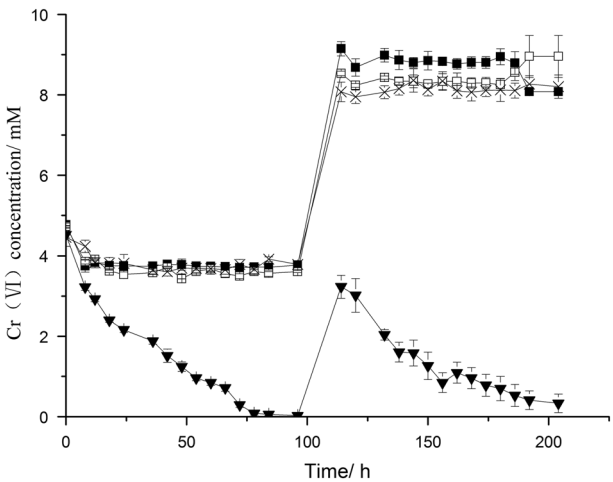

Fig. 1 Concentrations of $K_2Cr_2O_7$ over time in A (▼), B (▢), aA (×) and aB (■) sediments aA and aB are control group as described before. In short, 60 mL sterile water and 2 mL $K_2Cr_2O_7$ solution (2 mM) were added in the four groups. After incubating 114 h, $K_2Cr_2O_7$ were added again. Each group had three replicates. Samples were collected for DNA extraction and metagenomics analysis at the beginning of incubation and after 30-day incubation

both sites (Supplementary Table S3). DCA results and Adonis results revealed that Cr (VI) had greater influence than incubation conditions on microbial communities.

Microbial communities changed after Cr (VI) incubation (Supplementary Fig. S4, Supplementary Table S2 and S3), and the main changes at the phylum level and genus levels are shown (Supplementary Fig. S5). The percentages of Proteobacteria, as the most abundant phylum in those tested samples increased from 45.1 % to 68.2 % in site A, and 50.1 % to 71.3 % in site B after incubated with Cr (VI) for 30 days, while the percentages of Proteobacteria were 38.3 % and 31.9 % for site A (37A) and site B (37B), respectively in the control group incubated at 37 °C. At the genus level, *Flavobacterium* decreased from 28.8 % (A0) and 20.1 % (B0) to 6.2 % (37A) and 3.8 % (37B), respectively after incubation without Cr (VI), and decreased to 1.7 % (A30) and 2.5 % (B30) after Cr (VI) incubation (Supplementary Fig. S5a and Supplementary Table S2), revealing both laboratory incubation and Cr (VI) had negative effect on *Flavobacterium* abundances. *Rhodoferax* occupied similar percentages in site A (2.1 %) and site B (2.7 %) before incubation, and after Cr (VI) incubation, the *Rhodoferax* populations increased to 17.3 % in site A, and 9.1 % in site B, indicating that that *Rhodoferax* populations could tolerate high concentrations of Cr (VI) in both sites. Also, Adonis results showed the overall microbial community composition had significant ($p < 0.05$) difference between A0 and B0 samples, and between A30 and B30 samples. Furthermore, a total of 472 OTUs were increased after Cr (VI) incubation in site A samples, most of which were affiliated with *Geobacter*, *Clostridium, Desulfotomaculum* and *Desulfosporosinus* (Supplementary Table S4) whose members are known to reduce metals (e.g., Cr (VI)) as well as other electron acceptors (e.g., nitrate, sulfate). These OTUs contributed about 9 % of the OTU abundance in A30.

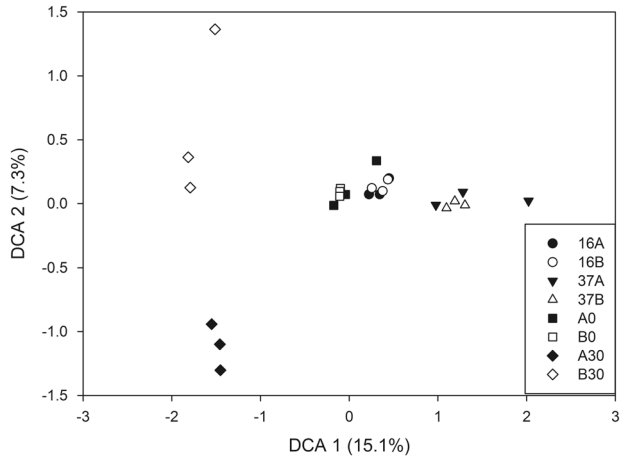

Fig. 2 Detrended correspondence analysis (DCA) of all treatments based on MiSeq sequence data. DCA was performed based on the abundance of OTUs detected. Different labels represent different treatments: 37A (control for sediment A incubated at 37 °C after 30 days), 16A (control for sediment A incubated at 16 °C after 30 days), A0 (sediment A), A30 (sediment A incubated with Cr (VI) at 37 °C after 30 days), 37B (control for sediment B incubated at 37 °C after 30 days), 16B (control for sediment B incubated at 16 °C after 30 days), B0 (sediment B), and B30 (sediment B incubated with Cr (VI) at 37 °C after 30 days)

Shifts of the microbial community functional structure in response to Cr (VI)

To examine if the site and Cr (VI) addition affect the functional structure of microbial communities, GeoChip analysis of key functional genes was performed. No significant changes of functional gene number detected by GeoChip were observed between two sites, or between before and after incubation with Cr (VI) for 30 days although their numbers slightly increased in the site A samples (A30), and decreased in the site B samples (B30) compared to their original samples (A0 and B0) (Table 2).

Table 2 Total number and percentage of functional genes detected by GeoChip, unique and overlapped genes and diversity indices

	A0	B0	B30	A30
Number (%) of genes detected				
A0	*372 (0.85 %)*	**42892 (84.18 %)**	**42526 (84.69 %)**	**41025 (83.95 %)**
B0		*1446 (2.89 %)*	**47163 (90.83 %)**	**44659 (86.58 %)**
B30			*1033 (2.11 %)*	**44135 (86.54 %)**
A30				*788 (1.71 %)*
Total genes detected	43749	50095	48991	46146
Diversity indices				
Shannon Index *H*	10.34918	10.42618	10.38899	10.35553
Simpson Index *D*	21515.62	22332.19	20715.18	20546.68
Pielou evenness *J*	0.9684601	0.9634529	0.9619975	0.9642406

Numbers in italics represent unique genes in each sample; numbers in bold represent genes overlapped between two samples

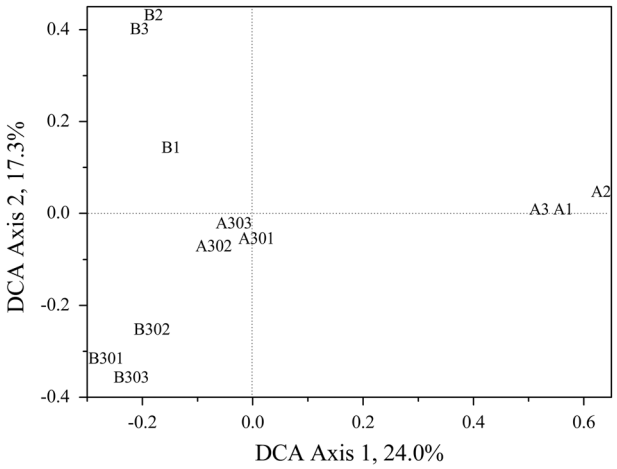

Fig. 3 Detrended correspondence analysis (DCA) of all detected genes by GeoChip 4.0. As described, A0 group genes (A1, A2 and A3) and B0 group genes (B1, B2 and B3) were collected from the initial sediments which hadn't been dealt with Cr (VI), A30 group genes (A301, A302 and A303) and B30 group genes (B301, B302 and B303) were collected from the sediments incubated with Cr (VI) for 30 days

Interestingly, A0 and A30 showed the lowest overlapped genes (83.95 %) while B0 and B30 had the highest overlapped genes (90.83 %), and A0 had the lowest unique genes (372, or 0.85 %) detected while B0 had the highest unique genes (1,446, or 2.89 %) detected (Table 2). However, no significant differences in diversity indices (Shannon, Simpson, and Pielou evenness) were observed (Table 2). DCA of all detected genes showed that all samples were clearly clustered into four groups with site separated by the first axis, and incubation time (0 or 30 days) separated by the first and second axis (Fig. 3). In addition, similar results were seen when those data sets were analyzed by clustering analysis (Supplementary Fig. S6). Therefore, the results indicated that the sediment microbial gene composition was significantly ($p < 0.05$) different between two sites, or between before and after incubation with Cr (VI) for 30 days.

Effects of Cr (VI) on key functional genes

Key functional genes involved in metal resistance/reduction, and C and N cycling were further analyzed, and their significances among different groups were accessed by ANOVA and Tukey's test. First, we examined functional genes involved in metal resistance or reduction, showing that the abundance of seven genes (*arsC*, *cadA*, *czcA*, *chrA*, *copA*, *mer* and *silC*) significantly ($p < 0.05$) increased in the site A samples but only two (*cadA* and *silC*) in the site B samples after 30-day incubation with Cr (VI) (Fig. 4a). The results are consistent with our observations of sediment geochemistry that showed the concentrations of Cr were higher in site A than site B (Table 1), suggesting that *chrA* may play an important role in Cr reduction. Second, the abundance of several genes (*aceB*, *acet*, *amyA*, *xylA*, *pcc* and *pmoA*) involved in C cycling was found to be significantly ($p < 0.05$) increased after incubation with Cr (VI) in the site A samples but not in the site B samples (Fig. 4b). The results are generally consistent with higher concentrations of organic C in site A (Table 1). Third, the abundance of several nitrogen cycling genes (*ureC*, *narG*, *nirK*, *nirS*and *nifH*) significantly ($p < 0.05$) increased in site A samples but only *narG* abundance was increased in site B samples after incubation with Cr (VI) (Fig. 4c). The results appeared to be consistent with higher N concentrations in site A (Table 1). All these genes normalized average signal intensity were supplied in supplementary data (Supplementary Table S5).

CCA analysis

CCA and VPA were performed to evaluate the relationships between the microbial community composition/structure (based on GeoChip data) and sediment geochemical data. From CCA results, we found that Cr and carbon sources were the main factors, which separated all samples into three parts: samples from site A without Cr treatment (A0), samples from site A incubated with Cr (VI) for 30 days (A30), and samples from site B with/without Cr (VI) incubation (B0 and B30). The CCA1 and CCA2 explained about 67 % of the total variation with 35.8 % explained by the first axis and 31.8 % by the second axis (Fig. 5). The results indicate Cr, OC and moisture largely shaped the microbial community functional structure in those four groups studied. The VPA results also confirmed that metal content (Cr and Pb) and carbon sources had an effect on the microbial community functional structure (Fig. 6).

Discussion

This study used GeoChip and MiSeq sequencing technologies to examine the changes of microbial community diversity, composition, structure and function during Cr (VI) incubation in the laboratory. The changes of Cr (VI) concentrations over time indicated they have different ability of Cr (VI) reduction (Fig. 1). GeoChip data showed that nine genes involved in C and N cycling, and metal resistance increased after 30-day incubation with Cr (VI) in site A, and MiSeq sequencing of 16S rRNA gene amplicons also revealed that unique OTUs related to phylum Chloroflexi (e.g., *Anaerolinea*), Firmicutes (e.g., *Clostridium*) and Proteobacteria (e.g., *Geobacter*) in site A samples increased after Cr (VI) incubation (Supplementary Table S2 and S4).

One of our hypotheses is that the microbial community diversity, composition and structure would differ among

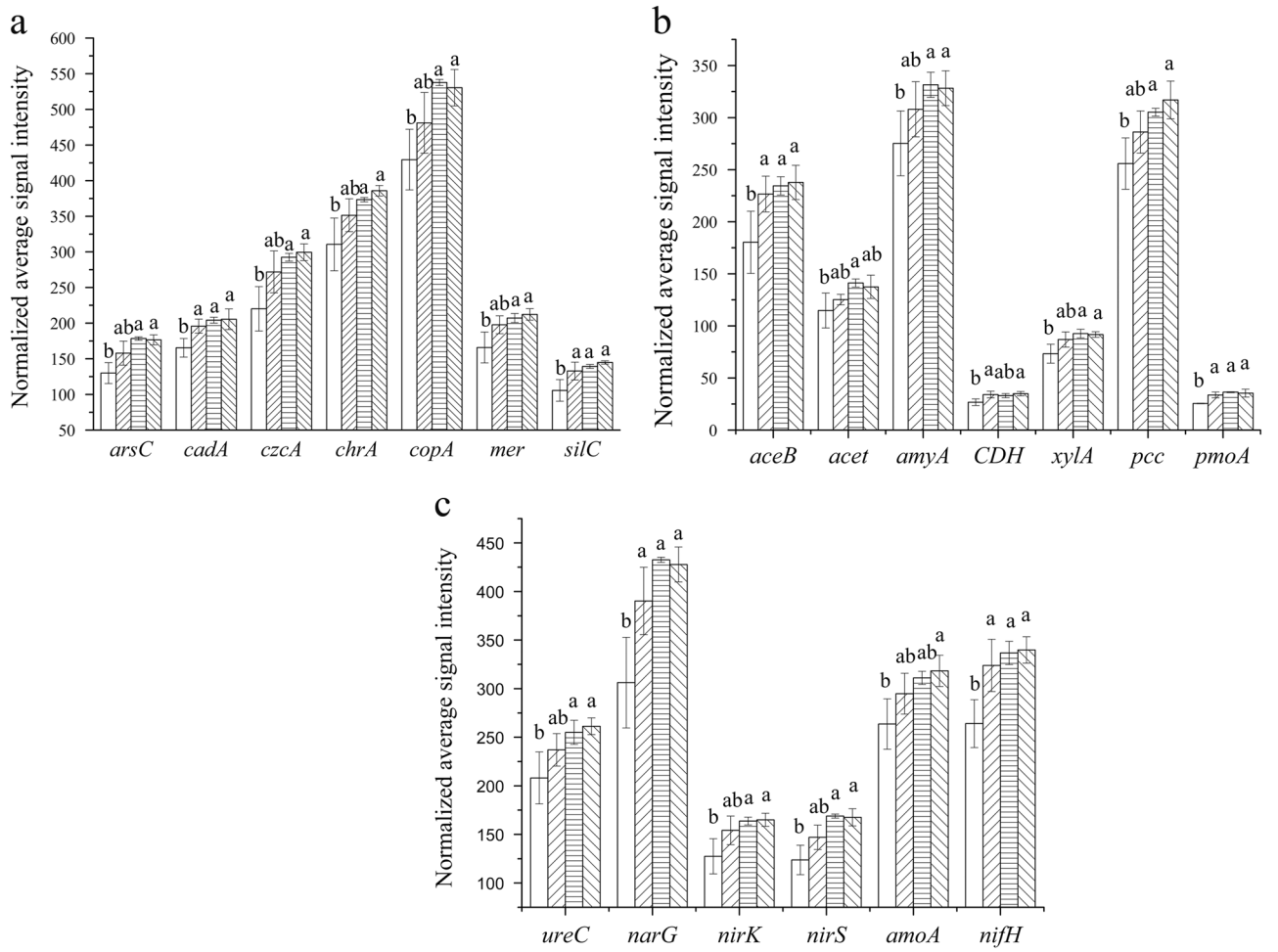

Fig. 4 Significantly changed functional gene families involved in metal resistance/reduction (**a**), carbon cycling (**b**), and N cycling (**c**). Four groups contain site A day 0 (▢), site A day 30 (▨), site B day 0 (▤) and site B day 30 (▧). For each functional gene, the normalized signal intensities were averaged among 3 replicates. All data are presented with mean ± SE and each gene in four groups was compared by Turkey's Test. The metal resistance/reduction gene include *arsC* encoding arsenate reductase, *cadA* encoding a cadmium-efflux , *czcA* and *chrA* transport chromates out of bacteria, *copA* is related to Cu resistance through translocation or/and maintenance of Cu homeostasis and *mer* is related to mercury resistance and reduction in bacteria

different sites, which is largely influenced by available electron donors/acceptors, nutrients and environmental contaminants, and indigenous microbial communities would remediate those contaminants. A previous study at the Oak Ridge FRC site showed that the injection of ethanol as an electron donor stimulated U (VI)-reducing microbial communities and key functional genes, resulting in a shift of microbial community composition and structure (Xu et al. 2010). Another study used a slow-release hydrogen release compound (HRC) to provide electron donors and carbon sources for indigenous microbial growth and reduction of available electron acceptors like Cr (VI) for over 3.5 years (Faybishenko et al. 2008). In this study, although electron donors were not measured, the concentrations of nutrients (e.g., C, N) and electron acceptors (e.g., Cr) were significantly higher in site A compared to site B, and such differences might lead to shifts of microbial community diversity, composition and structure. This hypothesis is supported by CCA, showing that Cr, moisture, OC and Pb largely shaped the microbial community structure. Moisture, available nitrogen, OC, Mn, Cr, and Cu of sediment samples were different between the two sites although the two sites are very close, indicating environmental factors may play a major role in shaping the microbial community structure (Nowakowska and Oliver 2013; Tien et al. 2013) and function (Pepi et al. 2013; Piccirillo et al. 2013). In this case, chromium concentration may be a major reason for reconstructing sediment microbial communities.

Some microorganisms could adapt to high concentrations of environmental contaminants (e.g., Cr (VI)) and perform in situ bioremediation of such contaminants. In this study, we detected such unique OTUs/populations affiliated with *Geobacter*, *Clostridium, Desulfotomaculum* and *Desulfosporosinus* in site A. A previous study suggested that some

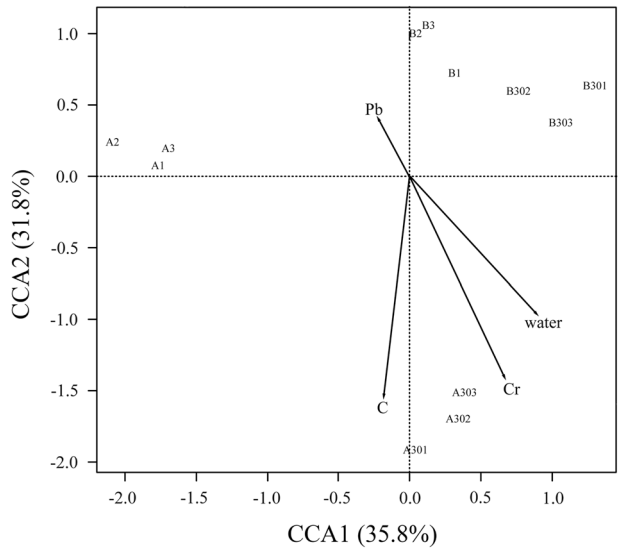

Fig. 5 Canonical correspondence analysis (CCA) of GeoChip 4.0 data. Variables: Pb, Cr, organic carbon (C) and moisture content (water). A1, A2 and A3 are samples from site A; B1, B2 and B3 are samples from site B; A301, A302 and A303 are site A samples incubated with Cr (VI) for 30 days; B301, B302 and B303 are site B samples incubated with Cr (VI) for 30 days

populations of phylum Proteobacteria could take part in biotransformation of Cr (VI) to Cr (III) (Garavaglia et al. 2010), and the genus *Geobacter* is known to be involved in multiple metal reduction processes (Liu et al. 2015; Orellana et al. 2013; Shelobolina et al. 2007), which are consistent with our results, showing that some OTUs related to Proteobacteria, especially the genus *Geobacter*, increased in site A samples after incubation with Cr(VI). Also, the abundance of OTUs related to the genus *Clostridium* increased after Cr (VI) incubation in A30 samples (Supplementary Table S4). *Clostridium* populations (belongs to class Clostridia) are known to be able to reduce U (VI) to U (IV) under anaerobic condition (Francis et al. 1994). Clostridia populations are also involved in reduction of other metals, such as U(VI) and Fe (III) (Francis et al. 1994; Slobodkin et al. 2006). Sulfate reducing bacteria from *Desulfotomaculum* and *Desulfosporosinus* were also enriched in A30 samples after Cr (VI) incubation, and they are known to be involved in sulfate and/or metal (e.g., Cr (VI)) reduction processes (Otwell et al. 2016;Tebo and Obraztsova 1998). In addition, other populations related to the Chloroflexi phylum known to play a role in Fe (III) reduction (Kawaichi et al. 2013), increased after Cr (VI) incubation in site A samples. Although these populations only occupied low percentages (about 9 % of OTU abundance) of the community, they might play important roles in Cr (VI) reduction in site A. For example, a previous study reported low-abundance *Desulfosporosinus* populations (only 0.006 % of the microbial community) could be responsible for sulfate reduction in a peatland system (Pester et al. 2010), and another study indicated low-abundance bacteria drived sulfate reduction-dependent degradation of fermentation products in peat soil microcosms (Hausmann et al. 2016). Therefore, those detected unique OTUs/populations in site A affiliated with *Geobacter*, *Clostridium, Desulfotomaculum* and *Desulfosporosinus* may play important roles in Cr (VI) reduction in the Lanzhou reach of the Yellow River, and further identification and functional characterization of those specific populations (e.g., isolation) are in progress.

Genes involved in metal resistance significantly changed after Cr (VI) incubation, especially in site A samples. In these genes, *czcA* and *chrA* are known to transport chromate out of bacteria (Alvarez et al. 1999; Nies et al. 1990; Nies et al. 1998). An increase of abundance of two genes and a decrease of Cr (VI) concentrations in the supernatant at the same time indicated that microbial Cr (VI) reduction might be performed in site A samples. Also, some genes related to resistance to, or reduction of other metals increased significantly. For example, *copA* gene which is related to Cu resistance through translocation or/and maintenance of Cu homeostasis (Samanovic et al. 2012) increased after the incubation. Our previous study used RNA-Seq to determine the response to Cr (VI) stress in *Staphylococcus aureus* LZ-01, showing that some heavy metal transporters upregulated after Cr (VI) incubation (Zhang et al. 2014). And the similar phenomenon was observed when GeoChip was used to measure the changes of genes under Cr (VI) stress in this study. The mechanism of these results needs further study and investigation. In addition, genes related to carbon and nitrogen metabolism showed significant increased after incubation, for example, *aceB*, which encodes the malate synthase A involved in glyoxylate cycle (Byrne et al. 1988; Griffin et al. 1996) and *pmoA*, which is involved in methane consumption (McDonald and Murrell 2006), and some genes are involved in N fixation (e.g., *nifH*) (Roberts et al. 1978), N mineralization (e.g., *ureC*) (Puskas et al. 2000), and denitrification (e.g., *narG, nirS, nirK*) (Kandeler et al. 2006). This may be due to energy requirements by microorganisms in the process of metal resistance and reduction. A previous study showed that C degradation related genes changed during uranium reduction (Liang et al. 2012). Consistently, our CCA and VPA results showed that C sources and metal were the main factors affecting the microbial communities in this study.

Conclusions

The phylogenetic and functional structure of sediment microbial communities changed during Cr (VI) treatment, and site A sediment samples showed the ability of Cr (VI) reduction. We found key functional genes related to metal

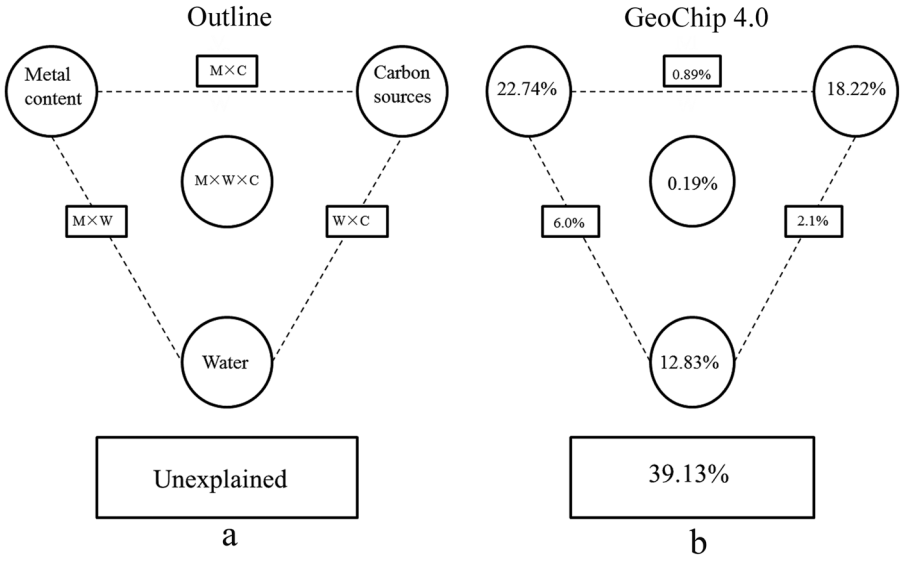

Fig. 6 Variation partitioning analysis (VPA) based on CCA for all functional gene signal intensities. **a** General outline, **b** all functional genes. A CCA-based VIF was used to identify common sets of metal content and carbon sources variables which have influence on the microbial community structure

resistance increased and unique OTUs/populations related to metal and sulfate reduction in site A samples after Cr (VI) incubation. These unique OTUs/populations might play an important role in Cr (VI) reduction in site A. Overall, as the first report about microbial communities in the Lanzhou reach, this study provides a new insight and a potential of bioremediation of contaminated Lanzhou reach of the Yellow River.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict interest.

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