

Microbial diversity and community structure in an antimony-rich tailings dump

Enzong Xiao^{1,2} · Valdis Krumins³ · Yiran Dong⁴ · Tangfu Xiao^{1,5} · Zengping Ning¹ · Qingxiang Xiao^{1,2} · Weimin Sun^{1,6,7}

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Abstract To assess the impact of antimony (Sb) on microbial community structure, 12 samples were taken from an Sb tailings pile in Guizhou Province, Southwest China. All 12 samples exhibited elevated Sb concentrations, but the mobile and bioaccessible fractions were small in comparison to total Sb concentrations. Besides the geochemical analyses, microbial communities inhabiting the tailing samples were characterized to investigate the interplay between the microorganisms and environmental factors in mine tailings. In all samples, *Proteobacteria* and *Actinobacteria* were the most dominant phyla. At the genus level, *Thiobacillus*, *Limnobacter*, *Nocardioides*, *Lysobacter*, *Phormidium*, and *Kaistobacter* demonstrated relatively high abundances. The two most abundant genera, *Thiobacillus* and *Limnobacter*, are characterized as sulfur-oxidizing bacteria and thiosulfate-oxidizing bacteria, respectively, while the genus *Lysobacter* contains arsenic (As)-resistant bacteria. Canonical correspondence analysis (CCA) indicates that TOC and the sulfate to sulfide ratio strongly shaped the microbial communities, suggesting the

influence of the environmental factors in the indigenous microbial communities.

Keyword Illumina sequencing · Antimony · Sulfur-oxidizing bacteria · Canonical correspondence analysis

Introduction

Mine tailings or mill tailings, which can account for more than 80–99 % of the raw ore by weight (Edraki et al. 2014), are the remained materials after extracting economically minerals from ore (Diaby et al. 2007). These materials contain high concentrations of sulfide and metal(-loid)s such as arsenic (As), copper (Cu), and cadmium (Cd) (Dold and Fontboté 2001; Johnson and Bradshaw 1977). In addition, they contain limited organic matter and nutrients (Johnson and Bradshaw 1977; Krzaklewski and Pietrzykowski 2002). Mine tailings can be major sources of contamination, and microbiological activities

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✉ Tangfu Xiao
xiaotangfu@vip.gyig.ac.cn

✉ Weimin Sun
swm@envsci.rutgers.edu

¹ State Key Laboratory of Environmental Geochemistry, Chinese Academy of Sciences, Guiyang 500081, China

² University of Chinese Academy of Sciences, Beijing 100049, China

³ Department of Environmental Sciences, Rutgers University, New Brunswick, NJ 08901, USA

⁴ Department of Geology, University of Illinois-Urbana Champaign, Urbana, IL 61801, USA

⁵ Innovation Center and Key Laboratory of Waters Safety & Protection in the Pearl River Delta, Ministry of Education, Guangzhou University, 99 Lincheng Road West, Guiyang, Guizhou Province 550081, People's Republic of China

⁶ Department of Microbiology and Biochemistry, Rutgers University, 14 College Farm Road, New Brunswick, NJ 08901, USA

⁷ Guangdong Institute of Eco-environment and Soil Sciences, Guangzhou 510650, China

within mine tailings play an important role by influencing the morphology and chemistry of metal(loid)s (Schippers et al. 2010). For instance, various sulfur-oxidizing bacteria (SOB) and Fe-oxidizing bacteria (FeOB) can harness the energy via redox reactions occurring in mine tailings (Garcia et al. 2001; Li et al. 2014). These microbially mediated reactions are important determinants of the acidity generation, metals mobilization, and release of key nutrients (such as phosphate and metal cations) required not only for their own metabolism but for plant growth and eutrophication potential (Uroz et al. 2009; Welch et al. 1999). A number of bacteria from diverse genera such as *Azospirillum*, *Sphingomonas*, *Shewanella*, and *Staphylococcus* are implicated in such processes (for detailed information, see the review of (Uroz et al. 2009)).

Antimony (Sb) is a naturally occurring metalloid that commonly co-occurs with As (Filella et al. 2002). Sb and its compounds are considered priority pollutants by the US Environmental Protection Agency (USEPA 1979) and the European Union (Communities of the Europe 1976). Sb is mined mostly from hydrothermal ores where stibnite (Sb_2S_3) is the dominant mineral form, along with other Sb-bearing minerals such as berthierite (FeSb_2S_4) and gudmundite (FeSbS) (Fowler and Goering 1991). Sb mine tailings remain after extraction of Sb from the primary ores. Extraction agents such as Na_2CO_3 are sometimes added during smelting. These could neutralize the acidity caused by weathering of S-bearing Sb minerals (Anderson 2012). The biogeochemical changes due to physical and chemical processes that occur in Sb tailings after extraction may change Sb speciation. This affects bioavailability and mobility of Sb species, which may affect toxicity to aquatic and terrestrial organisms. Much of the transformation processes these minerals undergo are catalyzed by microbes; therefore, characterization of microbial assemblages in mine tailings can inform the study and prediction of biogeochemical changes of Sb mine tailings.

We investigated the microbial communities in an Sb mine tailings pile, located in Dushan County, Guizhou Province, Southwest China. These Sb tailings originated from the local Banpo Sb Mine and are characterized as neutral pH. Inefficient metals extraction due to outdated metallurgy technology results in elevated concentrations of Sb (as high as 5181 mg/kg) and As (as high as 206 mg/kg) in the Sb tailings. These metals thus pose potential threats to the surrounding ecological environment. We selected this Sb tailing to study (1) the variation of Sb extractable fractions along with other metal(loid)s within the mine tailings, (2) microbial community and diversity in the tailings, and (3) the correlation between microbial communities with Sb extractable fractions and other geochemical parameters.

Materials and methods

Site information, sample description, and sampling procedure

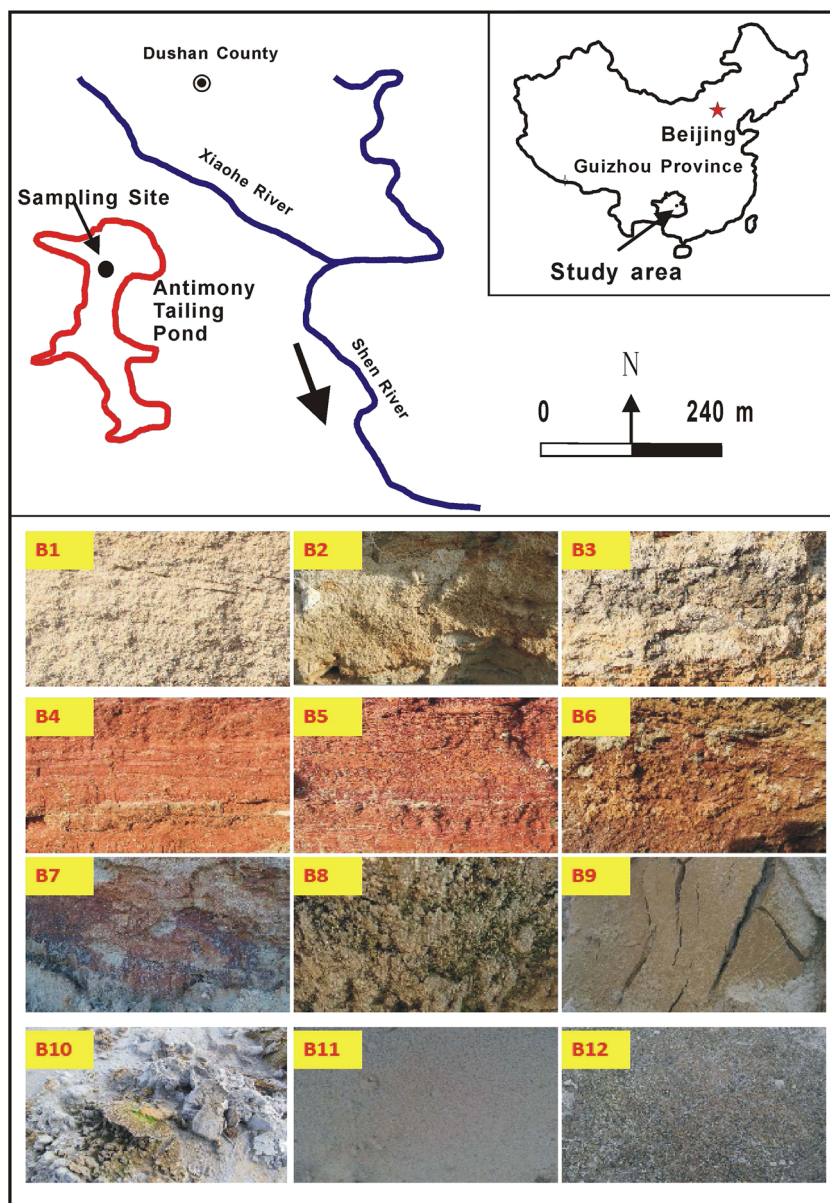
The Xiaohé Sb tailings pile is located in Dushan County, Guizhou Province, Southwest China ($25^\circ 47' 48.4'' \text{ N}$, $107^\circ 32' 52.2'' \text{ E}$) (Fig. 1). The tailing wastes were deposited there starting in June 2006 from the Banpo Sb Mine ($25^\circ 49' 8.2'' \text{ N}$, $107^\circ 37' 29.1'' \text{ E}$). Samples were collected in October 2014 following the procedure of Chen et al. (Chen et al. 2013). Briefly, tailings with different depositional faces were selected for physicochemical and microbial community analysis (Fig. 1). Twelve tailings samples (B1–B12), about 0.5 kg each, were collected using a soil corer from the top 0–5 cm of the tailings piles. Each sample was mixed and homogenized before transferring to a 500-ml sterile serum bottle and then transported to the laboratory (within 5 h) in cool boxes. Thereafter, samples were stored in freezers at -20° C (samples used for molecular analysis) and 4° C (for physicochemical characterization), respectively.

Chemical analysis

The samples were freeze-dried for 48 h and thoroughly ground using mortar and pestle before passing through a 200-mesh sieve for the subsequent analysis. To measure pH, 10-g dry ground tailing samples were placed into a 100-ml Erlenmeyer flask and mixed with 25-ml Milli-Q water, shaken for 5 min, and then left to equilibrate for 20 min. A calibrated HACH HQ30d pH meter (HACH, Loveland, USA) was used to measure pH, oxidation-reduction potential (Eh) and electrical conductivity (Ec). For nitrate and sulfate measurement, 10-g dry samples were placed into a 100-ml Erlenmeyer flask, mixed with 50-ml distilled water and shaken for 5 min, and followed by 4 h of equilibration. The supernatant was filtered through a $0.45\text{-}\mu\text{m}$ membrane after centrifuging at 3500 rpm for 10 min. The sulfate (SO_4^{2-}), chloride (Cl^-), and fluoride (F^-) concentrations were determined by ion chromatography (Dionex ICS-1500, Sunnyvale, CA, USA).

For major elements analysis, 1-g ground sample was combusted at 900° C for 2 h and the difference in sample weight before and after combustion was reported as loss on ignition. Total sulfur (TS), soluble sulfur, total organic carbon (TOC), and total carbon (TC) in tailings were measured using an elemental analyzer (vario MACRO cube, Elementar, Hanau, Germany) (Schumacher 2002). Trace elements were determined by ICP-MS (Agilent, 7700 \times , California, USA) after digestion using USEPA method 3050B (Kimbrough and Wakakuwa 2002). For trace elements analysis, certified reference materials (SLRS-5 (Fornieles et al. 2011; Rueda-Holgado et al. 2012)) and internal standards (Rh at 500 $\mu\text{g/L}$ (Ning et al. 2015)) were used for accuracy testing. Standard

Fig. 1 Sample location map including tailings pile and tailings pond. Field photographs of the 12 tailings samples and sample numbers are labeled in each subfigure. *Blue line* represents river and *red line* represents boundary of antimony tailing pond (color figure online)



reference material GBW07310 (Chinese National Standard) was used for analytical quality control. The measured total Sb concentration in GBW07310 was 6.5 ± 0.8 mg/kg, which is comparable to the certified value of 6.3 ± 0.9 mg/kg. All chemical analyses were determined in triplicate.

Mineral composition and morphology analysis

Scanning electron microscopy (JSM-6460LV, JEOL, Tokyo, Japan) and energy dispersive X-ray spectrometry (EDAX-GENESIS, Mahwah, USA) (SEM-EDS) were employed to determine the presence and morphology of minerals, based on the methods of McBeth et al. (2013). The SEM was operated at 15 kV with a working distance of 10 mm. For EDS

analysis, an accelerating voltage of 20 kV was used to obtain sufficient X-ray counts.

Sequential extraction analysis of metals (metalloids) in tailing samples

A three-stage sequential extraction was adapted from the procedure for As by Wenzel et al. (2001) and Gault et al. (2003) to target a range of metal(loid) phases in the tailings. Briefly, 1 g of tailing sample was placed in a 50-ml centrifugation tube. The “easily exchangeable fraction” (M_{exc}) was extracted by adding 10 ml of 0.05 M $(\text{NH}_4)_2\text{SO}_4$ solution and shaking for 4 h. The samples were centrifuged and supernatant was removed for total metal(loid) concentration analysis by ICP-MS. Next, the “specifically-sorbed surface-bound fraction”

(M_{spp}) was extracted by adding 10 ml of 0.05 M $\text{NH}_4\text{H}_2\text{PO}_4$ to the solids remaining after the previous step, and shaking for 16 h. Finally, a fraction containing “amorphous hydrous oxides of iron (and aluminum)” (M_{amr}) was targeted by extraction with 10 ml of 0.2-M ammonium oxalate buffer (pH 3.0) for 4 h in the dark. Acid-washed glassware was used and all reagents were analytical grade (Kemoiou, Tianjing, China). Extractions were performed at room temperature ($\sim 20^\circ\text{C}$), and all shaking was done at 200 rpm. After each extraction stage, samples were centrifuged at 3000 rpm for 15 min, and the supernatant was collected and analyzed by ICP-MS (Agilent, 7700 \times , California, USA).

High-throughput sequencing of the V4–V5 regions of 16S rRNA genes

Total genomic DNA was extracted using the FastDNA[®] spin kit (MP bio, Santa Ana, USA) following the manufacturer’s protocol. All DNA extracts were stored at -80°C until further analysis. DNA concentration and purity was monitored on 1 % agarose gels. The V4–V5 regions of 16S rRNA gene were amplified using the 515f/907r primer set (515f: 5'-GTGYCAGCMGCCGCGGTAA-3', 907r: 5'-CCYCAATTCMTTTRAGTTT-3'). 16S rRNA tag-encoded ultra-high-throughput sequencing was carried out on the Illumina MiSeq platform at Novogene (Beijing, China). Sequences were analyzed with the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (Caporaso et al. 2010). Default settings for Illumina processing in QIIME were used ($r=3$, $p=0.75$ total read length; $q=3$; $n=0$), and then UPARSE (Edgar 2013) was used to cluster operational taxonomic units (OTUs) at 97 % similarity. Taxonomy was assigned to the OTUs using the RDP classifier (Wang et al. 2007). Chao1 and Shannon indices were used to estimate species richness for the six libraries (Schloss et al. 2009). The reads were deposited into the NCBI short reads archive database under accession number of SRP067961.

Statistical analyses

Circos software (<http://circos.ca/>) was used to graph the bacterial community of each sample at phylum and genus level in different weathering stages. The similarity of microbial communities indifferent tailing samples was determined using weighted UniFrac after the samples had been rarefied to the size of the smallest sequencing library. Principal coordinate analysis (PCoA) was then conducted on the weighted UniFrac (Kuczynski et al. 2012). Canonical correspondence analysis (CCA) performed by CANOCO 4.5 (Microcomputer Power, Ithaca, NY) was used to measure the major physicochemical parameters that had the most substantial influence on microbial community structure. CCA was performed to discern possible linkages between the microbial

communities (limited to those genera with relative abundance $>1\%$ in at least one sequencing library) and selected physicochemical parameters. A symbol’s position in relation to a vector head indicates the correlation between the community and the environmental factors. The length of a vector reflects the relative importance of those environmental factors in discriminating the overall microbial community within one library (Zhang et al. 2008). Manual forward selection with Monte Carlo permutation tests was then performed to determine the significance of the environmental variables with 999 permutations (Lepš and Šmilauer 2003). The correlations between geochemical parameters were determined by Spearman’s rank correlation using SPSS (v19) package. Unless stated, p values <0.05 were considered statistically significant.

Results

Distribution of antimony and arsenic in the weathering tailings

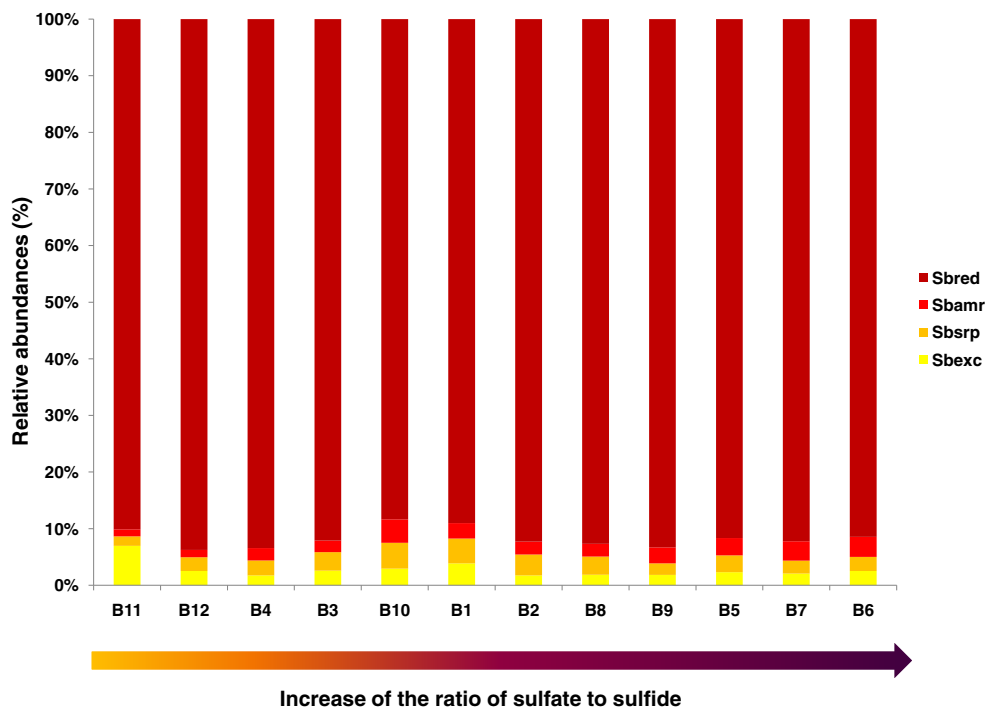
A total of 12 samples (named B1–B12) were taken from the mine tailings. Different Sb extractable fractions, including total Sb concentrations (Sb_{tot}) and various extractable fractions, are summarized in Table S1. The Sb_{tot} varied from 1534 in sample B11 to 4963 mg/kg in B9. The Sb concentrations in the bioaccessible fractions including easily exchangeable Sb (Sb_{exc}) and specifically sorbed (Sb_{spp}) (Savonina et al. 2012) ranged from 25.6 (B11) to 148 mg/kg (B10) (Table S1), accounting for 3.87 % (B9) to 8.64 % (B11) of Sb_{tot} in the tailings samples (Fig. 2). Meanwhile, the less mobilizable and bioaccessible Sb concentrations associated with amorphous crystalline hydroxides of Fe and Al (Sb_{amr}) ranged from 18.9 (B11) to 163 mg/kg (B7) (Table S1). SEM-EDS images also revealed the presence of Sb-bearing minerals in all tailing samples. Representative sample images are presented in Fig. 3.

Because As and Sb commonly co-occur (Fawcett and Jamieson 2011; Ritchie et al. 2013), As fractions were also measured (Table S1). As concentrations were generally lower than Sb in the current study. Total arsenic (As_{tot}) ranged from 63.1 (B1) to 207 mg/kg (B9), while As concentrations in the two bioaccessible fractions including easily exchangeable As (As_{exc}) and specifically sorbed (As_{spp}) ranged from 1.75 (B1) to 5.42 mg/kg (B9).

Other physicochemical parameters

Due to the occurrence of Sb in sulfur-bearing minerals (such as pyrite, arsenopyrite, and jamesonite) (Ritchie et al. 2013), various forms of sulfur including total sulfur, sulfate, and sulfide (Table 1) and total Fe (Table 2) were measured as well. Total sulfur concentration averaged 7.10 mg/g but varied from

Fig. 2 Ratio between bioaccessible antimony fractions (Sb_{exc} and Sb_{srp}) and non-bioaccessible antimony fractions (Sb_{amr} and Sb_{red}). Sb_{red} refers the difference of total sediment Sb and the sum of three extractable Sb fractions. The bioaccessible Sb fractions are represented by yellow shades and the non-bioaccessible Sb fractions are represented by red shades (color figure online)



1.70 mg/g (B11) to 16.10 mg/g (B6). Sulfate and sulfide accounted for over 95 % percent of total sulfur, but their relative amounts varied in the tailings. Sulfate ranged from 0.30 (B11) to 14.80 mg/g (B6), while sulfide varied from 0.80 (B6) to 1.50 mg/g (B2). The ratio of sulfate to sulfide (sulfate/sulfide, which we interpret as an extent of oxidation of the tailings) varied from 0.23 (B11) to 18.5 (B6). Total Fe averaged 8713 mg/kg, varying from 1893 (B11) to 15473 mg/kg (B6). Other physicochemical parameters such as pH, Eh, and EC were also tested in this study (Table 2). All samples exhibited pH greater than 6.5, suggesting minimal acidification during the weathering process. Eh varied little among samples, ranging from 122.9 to 165.9 mV, suggesting oxidized environments prevailed in the tailings. In contrast, EC varied significantly, increasing from 82 (B11) to 5365 μ S/cm (B5). The concentrations of total nitrogen (TN), TC, TOC, and total hydrogen (TH) were also determined and are shown in Table 1. All 12 samples were characterized as relative low TOC (<5.46 mg/g, B10), TC (<13.78 mg/g, B10), and TN (<0.73 mg/g, B8), indicating unfavorable conditions for bacterial growth.

High-throughput sequencing analysis

In total, 1,515,469 sequences were derived from 12 sequencing libraries after passing the highly stringent quality control. Although the current study site may be less than optimal for bacterial growth (low nutrients, low TOC), there still were 5098 OTUs detected by Illumina sequencing among the 12 sequencing libraries.

Libraries B5 and B10 demonstrated the highest OTU numbers with 2306 and 2016, respectively, while B1 and B9 had the lowest OTU number (1453 and 1531, respectively). The alpha diversity indices including Chao1 and Shannon were also determined and exhibited very similar trend as OTU numbers. For instance, B5 and B10 had the highest Chao1 and Shannon, but B1 and B9 had the lowest Chao1 and Shannon (Table S2).

Proteobacteria accounted for 60.5 % of total valid sequences (24.3 to 83.8 % per sample) and were the most dominant phylum in 11 out of 12 sequencing libraries (Fig. 4). *Actinobacteria* accounted for 10.3 % of all effective sequences, while *Chloroflexi*, *Cyanobacteria*, *Acidobacteria*, and *Gemmatimonadetes* each accounted for more than 4 %. Thirty-three percent of total reads could not be classified beyond kingdom (bacteria and archaea) by the RDP classifier. This high proportion of unclassified sequences suggested that a large number of microorganisms in the Sb-rich environments belonged to unrecognized or novel bacterial and archaeal species. Two archaeal phyla, *Euryarchaeota* and *Crenarchaeota*, were also detected in the current study but only accounted for 0.4 % of total valid reads. At the class level, *Betaproteobacteria* (30 %), *Alphaproteobacteria* (16.2 %), and *Gammaproteobacteria* (12.2 %) were the most abundant classes accounting for more than 10 % of total effective reads (Table S3). All other classes accounted for less than 10 % of total reads. At the family level, *Hydrogenophilaceae*, *Comamonadaceae*, and *Xanthomonadaceae* were the top three families, accounting for more than 5 % of all effective reads (Table S4).

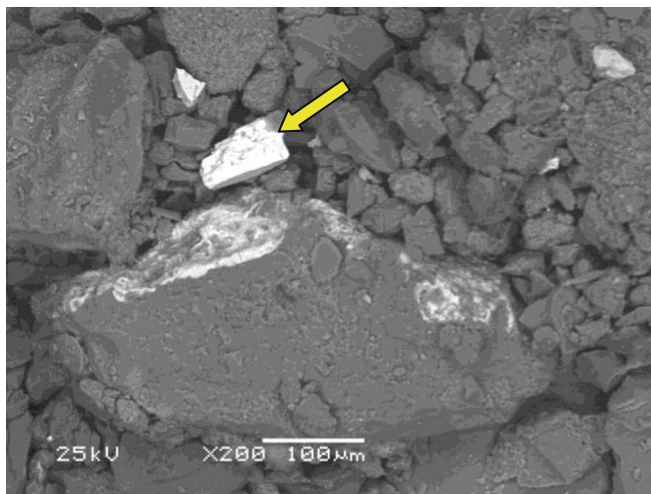
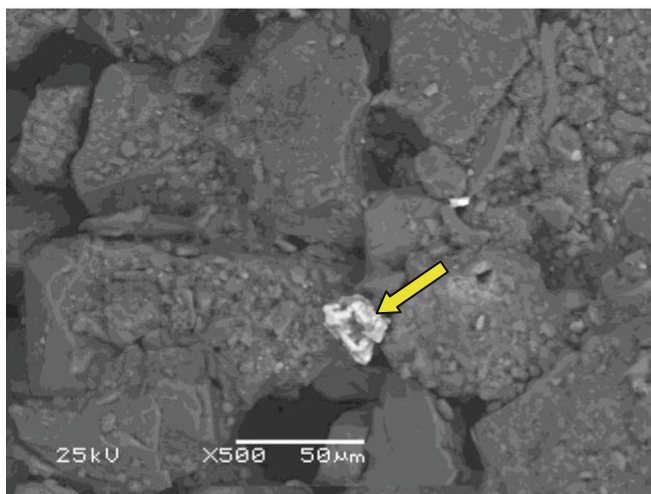
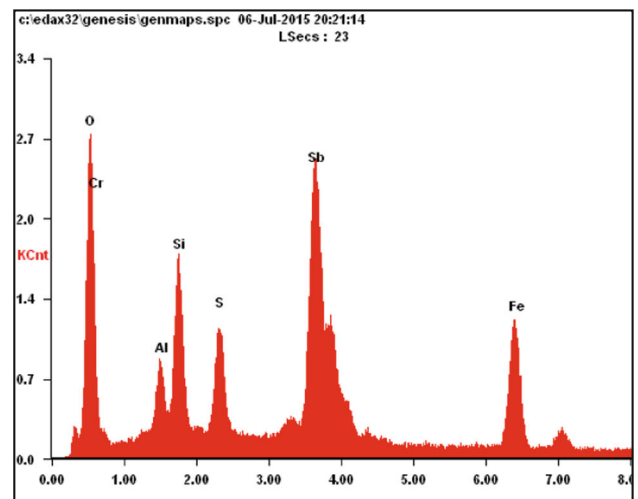
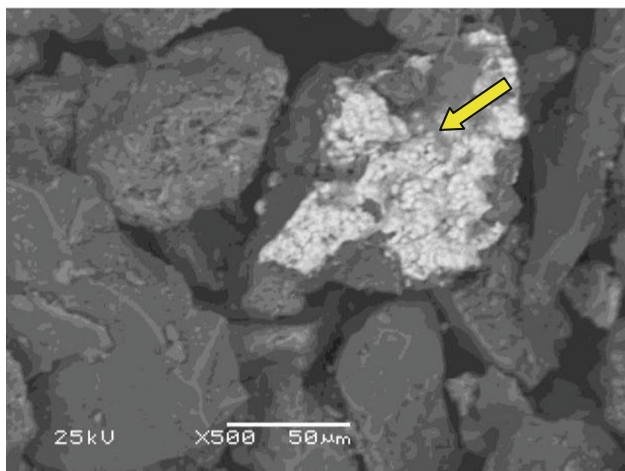
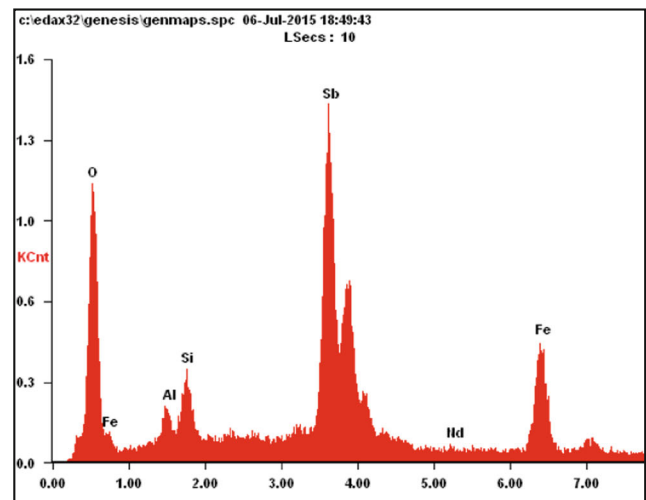
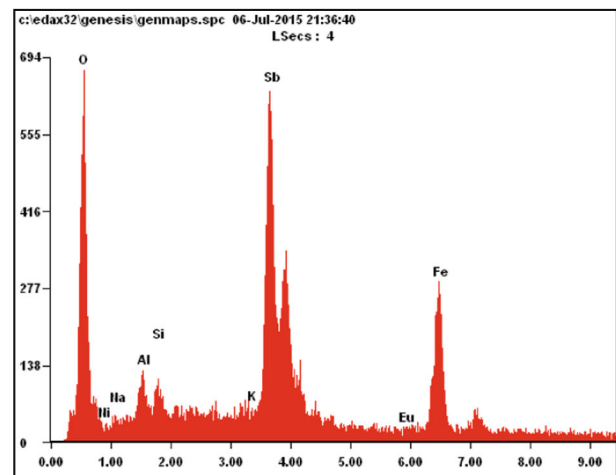
**B12****B3****B7**

Fig. 3 Representative SEM images (*left panel*) and the corresponding EDX spectra (*right panel*) of the sediment samples. Sampling sites were labeled on the *top* of each figure

Table 1 Major elementary compositions of Sb tailings (mean \pm standard deviation of three measurements in mg/g)

Sample	Total N	Total C	Organic C	Total H	Total S	Sulfate S	Sulfide S	Sulfate/sulfide
B1	0.25 \pm 0.11	3.53 \pm 0.40	0.58 \pm 0.07	1.56 \pm 0.14	5.10 \pm 0.10	3.50 \pm 0.10	1.10 \pm 0.10	3.18
B2	0.46 \pm 0.14	5.96 \pm 1.16	2.55 \pm 0.67	2.58 \pm 0.44	8.50 \pm 0.10	6.70 \pm 0.10	1.50 \pm 0.10	4.47
B3	0.37 \pm 0.10	3.11 \pm 0.35	0.64	1.75 \pm 0.58	5.10	3.30	1.30	2.54
B4	0.36 \pm 0.03	3.73 \pm 0.34	0.92 \pm 0.14	0.12 \pm 0.43	1.90 \pm 0.10	0.80	0.80	1.00
B5	0.29 \pm 0.02	2.73 \pm 0.18	0.75 \pm 0.28	2.37 \pm 0.43	11.00 \pm 0.60	9.70 \pm 0.20	0.90 \pm 0.10	10.78
B6	0.33 \pm 0.11	3.51 \pm 0.54	0.72 \pm 0.40	3.23 \pm 0.14	16.10 \pm 0.60	14.80 \pm 0.10	0.80 \pm 0.10	18.50
B7	0.34 \pm 0.04	3.52 \pm 0.52	0.92 \pm 0.01	3.25 \pm 0.45	14.10 \pm 0.30	12.70 \pm 0.20	1.00 \pm 0.10	12.70
B8	0.73 \pm 0.34	12.29 \pm 1.97	3.82 \pm 1.23	3.27 \pm 0.27	7.00	5.40 \pm 0.30	1.10 \pm 0.10	4.91
B9	0.45 \pm 0.16	2.85 \pm 0.36	1.27 \pm 0.28	2.79 \pm 0.23	7.50 \pm 0.10	6.20 \pm 0.10	1.10 \pm 0.10	5.64
B10	0.69 \pm 0.07	13.78 \pm 3.08	5.46 \pm 0.79	3.19 \pm 1.53	6.00 \pm 0.20	4.20 \pm 0.10	1.20	3.50
B11	0.20 \pm 0.01	1.48 \pm 0.15	0.65 \pm 0.01	0.92 \pm 0.20	1.70	0.30 \pm 0.10	1.30	0.23
B12	0.38 \pm 0.11	3.65 \pm 0.55	2.42 \pm 0.67	1.45 \pm 0.16	1.80	0.40	1.40 \pm 0.10	0.29

A total of 256 genera were identified from 12 sequencing libraries. Only six genera demonstrated relative abundance greater than 1 % of total sequences. They are *Thiobacillus*, *Limnobacter*, *Nocardioides*, *Lysobacter*, *Phormidium*, and *Kaistobacter* (Fig. 5, Fig. S1, and Table S5). Among them, *Thiobacillus* and *Limnobacter* were the most abundant genera. *Thiobacillus* was most dominant in B1 and B2, with relative abundances greater than 28 %. *Limnobacter* demonstrated relative abundances greater than 10 % in B3, B4, B5, B6, and B7. These two genera accounted for 14.2 % of total valid reads. Other genera, such as *Sphingomonas*, *Hydrogenophaga*, *Iamia*, and *Methylothera*, only demonstrated relatively higher abundances in one or a few sequencing libraries each.

Spearman correlation revealed the correlations between microbial population (at phylum and genus level) and selected geochemical profiles (Table S6 and S7). Among all tested taxonomic groups, sulfate was

positively correlated with *Limnobacter* (genus) and *Rhodobacter* (genus) but was negatively correlated with *Actinobacteria* (phylum) and *Gemmatimonadetes* (phylum). Sb_{tot} and Sb_{amr} were negatively correlated with *Kaistobacter* (genus) and *Iamia* (genus), while Sb_{srp} was negatively correlated with *Kaistobacter* (genus), *Acidobacteria* (phylum), and *Actinobacteria* (phylum). TOC was negatively correlated with *Limnobacter* (genus) and *Lysobacter* (genus), and pH was negatively correlated with *Thiobacillus* (genus).

Effect of environmental factors on microbial communities

Overall, the microbial community shared some similarities according to their sampling locations. For example, B1–B7, which were collected in close physical proximity to one another were clustered together, while B8–B9 and B11–B12 were clustered together. B10 is a notable

Table 2 Chemical and physical parameters of the tailing samples (mean \pm standard deviation of three measurements)

Sample	pH	Eh mv	EC μ S/cm	F ⁻ mg/kg	Cl ⁻ mg/kg	SO ₄ ²⁻ mg/kg	Total Fe mg/kg	Cr mg/kg	Mo mg/kg	Cd mg/kg
B1	6.81 \pm 0.04	163 \pm 3	2400 \pm 28	0.39 \pm 0.21	8.04 \pm 0.35	1420 \pm 225	6704 \pm 1406	58.3 \pm 16.1	7.22 \pm 0.51	0.65 \pm 0.53
B2	6.82 \pm 0.01	165 \pm 1	2220 \pm 28	1.23 \pm 0.33	2.09 \pm 1.06	1150 \pm 155	11893 \pm 1096	124.0 \pm 27.8	8.62 \pm 5.91	1.17 \pm 0.70
B3	6.80 \pm 0.02	165 \pm 3	2520 \pm 28	1.83 \pm 1.40	8.15 \pm 6.09	1777 \pm 21	5289 \pm 805	84.1 \pm 26.8	6.00 \pm 0.36	0.50 \pm 0.39
B4	6.94 \pm 0.02	153 \pm 1	1020 \pm 4	0.83 \pm 0.15	2.63 \pm 0.28	502 \pm 11	5841 \pm 895	54.9 \pm 17.5	7.71 \pm 1.01	0.44 \pm 0.50
B5	7.62 \pm 0.04	163 \pm 5	5370 \pm 21	8.25 \pm 0.04	16.9 \pm 0.20	2673 \pm 22	6559 \pm 813	53.8 \pm 7.2	8.64 \pm 0.33	0.33 \pm 0.14
B6	7.64 \pm 0.03	152 \pm 1	3860 \pm 14	6.43 \pm 0.10	25.5 \pm 0.73	2188 \pm 44	14058 \pm 820	70.7 \pm 11.0	13.13 \pm 0.85	0.29 \pm 0.26
B7	7.56 \pm 0.03	156 \pm 6	2020 \pm 14	5.17 \pm 0.27	14.3 \pm 0.22	1949 \pm 17	15473 \pm 658	70.3 \pm 7.9	15.17 \pm 0.28	0.41 \pm 0.20
B8	7.64 \pm 0.05	149 \pm 2	2020 \pm 4	1.12 \pm 0.15	1.39 \pm 0.23	1340 \pm 14	9775 \pm 632	94.5 \pm 47.6	6.65 \pm 0.47	1.13 \pm 0.61
B9	7.50	156 \pm 2	2220 \pm 14	2.42 \pm 0.18	0.90 \pm 0.13	1472 \pm 15	11317 \pm 745	80.6 \pm 11.3	9.16 \pm 0.84	0.66 \pm 0.16
B10	7.54 \pm 0.05	154 \pm 2	4790 \pm 127	0.90 \pm 0.03	24.1 \pm 1.10	1513 \pm 1	8325 \pm 341	64.4 \pm 44.5	9.56 \pm 1.37	0.36 \pm 0.15
B11	7.73 \pm 0.04	123 \pm 3	82.0 \pm 2	8.69 \pm 0.17	1.32 \pm 0.15	122 \pm 4	3766 \pm 495	60.5 \pm 11.4	6.08 \pm 1.31	0.37 \pm 0.19
B12	7.70 \pm 0.03	149 \pm 4	1130 \pm 9	0.64 \pm 0.01	3.77 \pm 0.02	306 \pm 2	5562 \pm 619	85.4 \pm 34.6	11.61 \pm 3.51	0.97 \pm 0.52

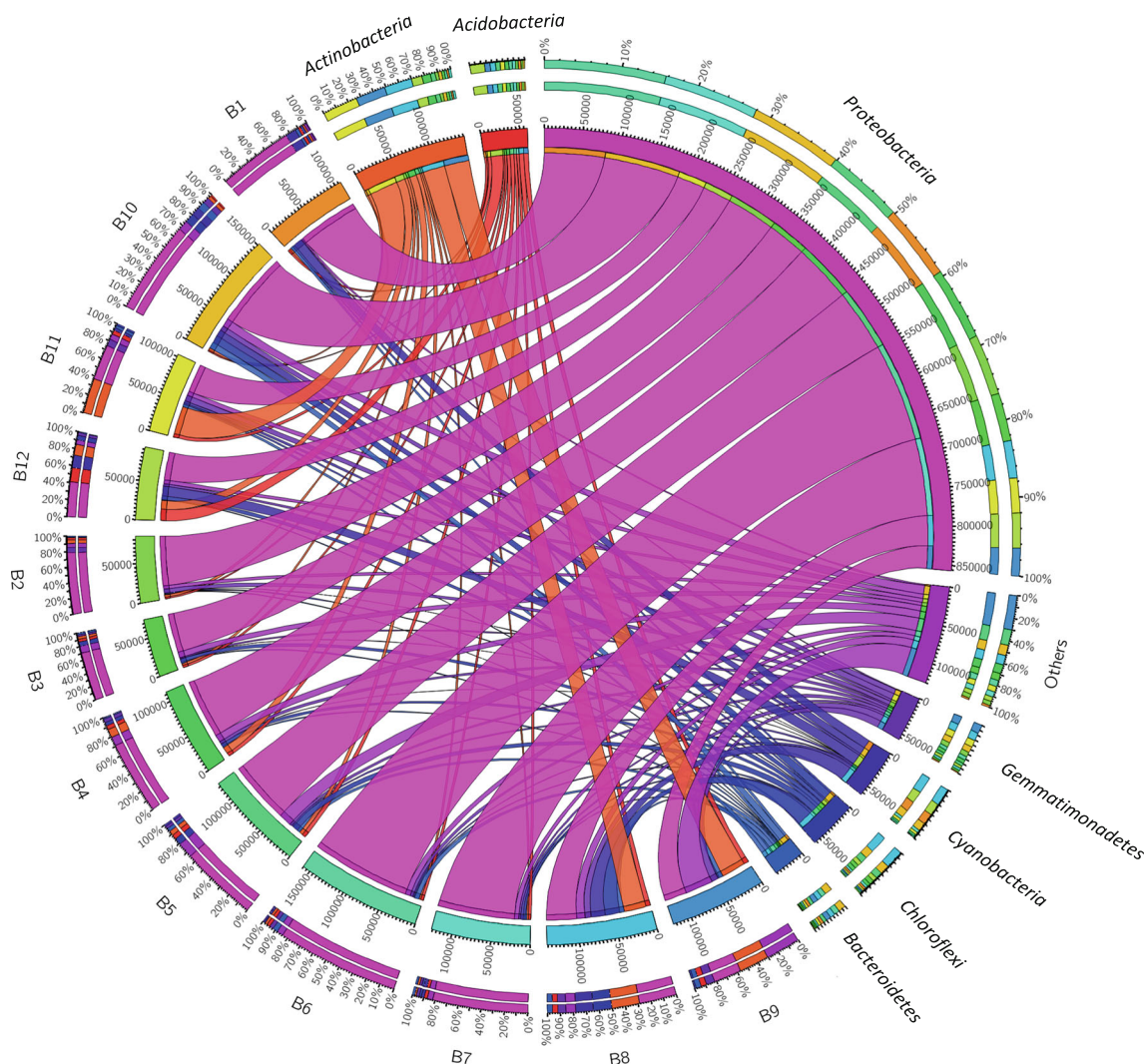


Fig. 4 Circos graph showing the taxonomic classification of bacterial reads at phylum level was produced by the Circos software (<http://circos.ca/>)

exception that was distantly correlated with other samples (Fig. 6). CCA was used to correlate the effect of environmental factors on microbial communities. The first CCA axis explained 30 % of the total variance and was positively correlated with pH, TOC, and sulfate/sulfide, and negatively correlated with Sb_{tot} and the three Sb extraction fractions. The effect of the four Sb fractions on the microbial communities was weaker than those of TOC, pH, and sulfate/sulfide, as indicated by the lengths of the vectors (Fig. 7), with Sb_{srp} demonstrating the least influence and Sb_{tot} exhibiting the strongest effect on microbial communities. The strongest determinant for the microbial communities was TOC (Fig. 7). *Thiobacillus* and *Limnobacter*, the two most dominant genera, were negatively correlated with TOC. CCA axis 2, which explained 23.7 % of the total variance, was positively correlated with all three Sb extractable fractions and negatively correlated with the sulfate/sulfide ratio, pH, and TOC.

Discussion

It is surprising to observe such a diversity of microorganisms inhabiting mine tailings, which contain limited TOC and nutrients such as TN but elevated Sb and As concentrations. At the phylum level, *Proteobacteria* accounted for the highest abundances (>60 %) of reads and was dominant in 11 out of 12 samples. Members of *Proteobacteria* are metabolically versatile and have been reported to be predominant in a wide diversity of environments including acid mine drainage, petroleum-contaminated sites (Sun et al. 2015b, c, d), and in black shale weathering (Li et al. 2014). *Actinobacteria* were the second most dominant phylum, accounting for more than 10 % of total reads. *Actinobacteria* are ubiquitously distributed in different habitats including terrestrial and aquatic environments (Glöckner et al. 2000; Kaplan and Kitts 2004; Margesin et al. 2003) and are of importance for their metabolic versatility and resilience to harsh environments (Arenskötter et al. 2004; Hamamura et al. 2006; Larkin et al. 2005). Other

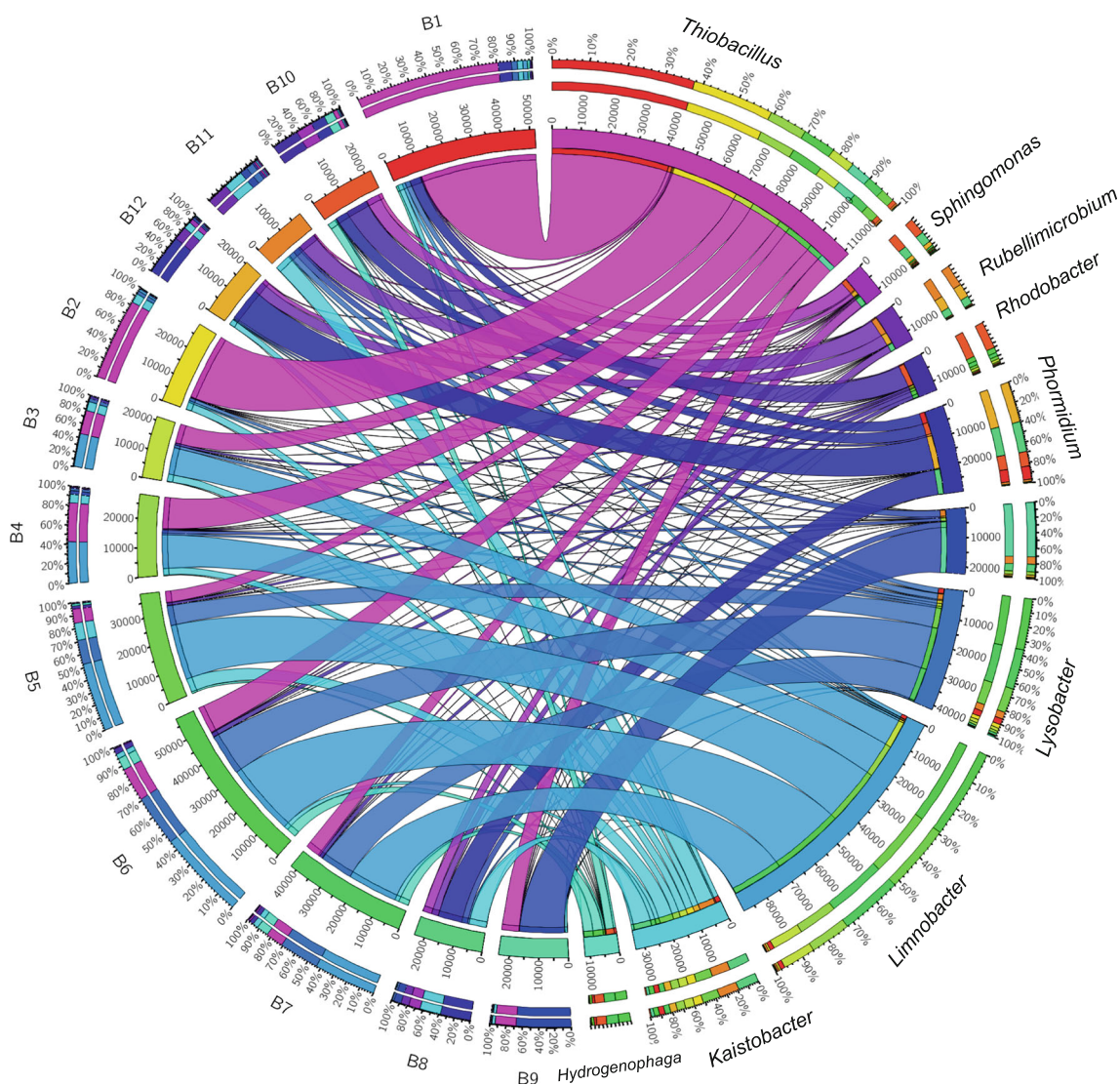


Fig. 5 Circos graph showing the taxonomic classification of bacterial reads at genus level was produced by the Circos software (<http://circos.ca/>). Only genera accounting for more than 10,000 reads in all 12 libraries are included in this graph

phyla observed in relatively high abundance included *Chloroflexi*, *Cyanobacteria*, and *Acidobacteria*, which have been also reported in mining-related areas (Kishimoto et al. 1991; Kuang et al. 2013).

The elevated concentrations of sulfate and sulfide provided electron donors or acceptors for S-metabolizing bacteria. *Thiobacillus* and *Limnobacter*, which have been identified as sulfur-oxidizing bacteria (SOB), were the two most abundant genera identified in the 12 samples, suggesting possible dynamic biogeochemical sulfur cycling. *Thiobacillus ferrooxidans*, often found in acid mine drainage (Schrenk et al. 1998), can derive energy for growth from oxidation of Fe(II) and sulfur compounds with CO₂ as the carbon source (Jensen and Webb 1995). *Thiobacillus denitrificans* is capable of autotrophic denitrification (Claus and Kutzner 1985), while *Thiobacillus thioparus* strain TK-m can grow autotrophically on carbon disulfide (CS₂) or carbonyl sulfide (COS) (Smith

and Kelly 1988). The capability of autotrophic and lithotrophic growth by some *Thiobacillus* species could explain their dominance in the mine tailings characterized as low TOC. Moreover, some members of *Thiobacillus* contain As resistance genes, which have also been reported to confer resistance to Sb (Butcher et al. 2000; Kondratyeva et al. 1995). Taking together the autotrophy, sulfur and iron oxidation, and As and Sb resistance, it is not surprising that *Thiobacillus* was the most abundant genus in the Sb tailings. It is also not unexpected to see the enrichment of *Limnobacter* in the tailings, as this genus contains species including *Limnobacter thiooxidans* and *Limnobacter litoralis* able to oxidize thiosulfate (Lu et al. 2011; Spring et al. 2001). Unlike *Thiobacillus*, the two *Limnobacter* species are heterotrophic. Unfortunately, thiosulfate was not measured in the current study. According to previous studies and the elevated sulfide concentrations in the current study site, we can still

Fig. 6 Principal coordinates analysis (*PCoA*) plot showing clusters of microbial communities based on weighted UniFrac with 100 % support at all nodes. The percentages are the percentage of variation explained by the components

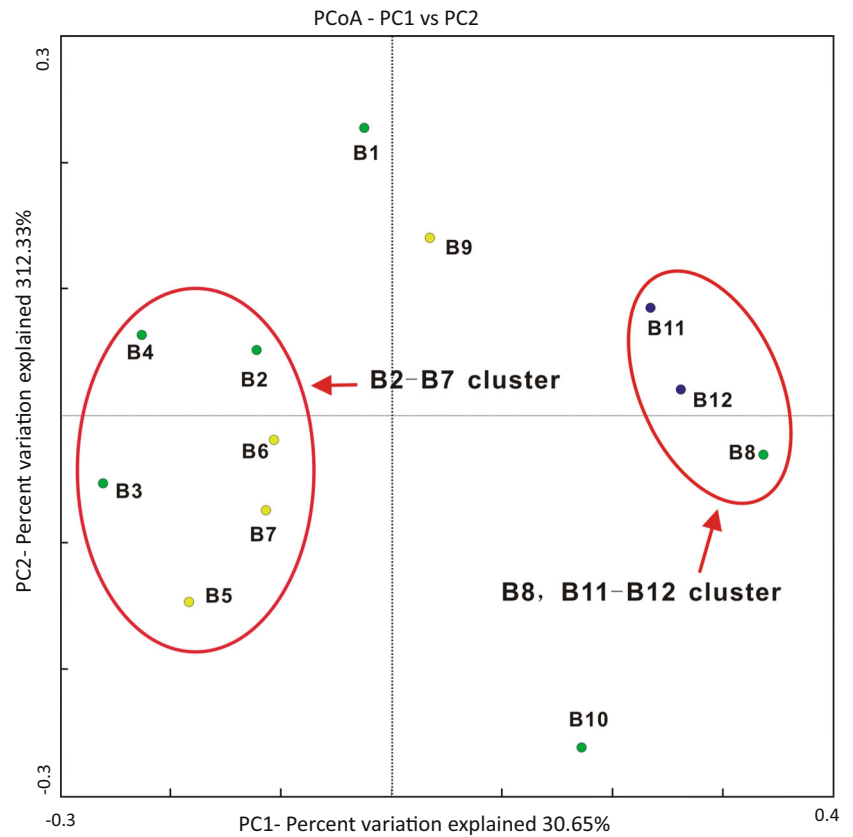
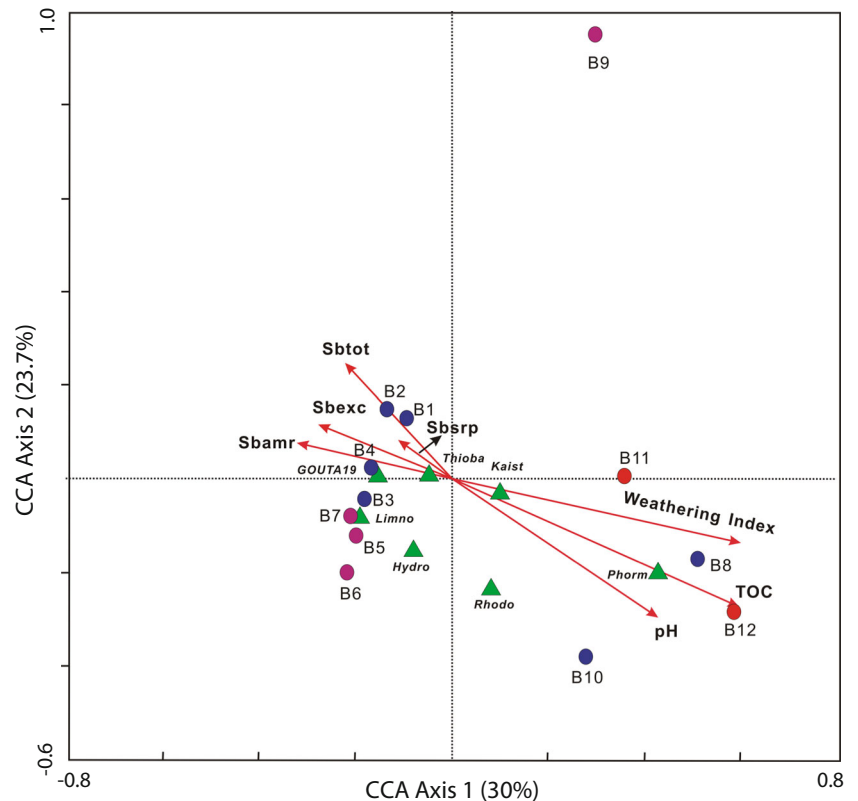


Fig. 7 Canonical correspondence analysis (*CCA*) of relative abundances at the genus level and major physicochemical parameters in the tailings. *Arrows* indicate the direction and magnitude of geochemical parameters associated with bacterial community structures. Microbial communities were represented by *circles* shaded to indicate sulfate/sulfide ratios (weathering index). *Sb_{tot}* total antimony in sediment, *Sb_{exc}* easily exchangeable forms of Sb, *Sb_{srp}* specifically sorbed forms of antimony, *Sb_{amr}* amorphous hydrous oxides of Fe and Al, *TOC* total organic carbon, *Thioba* *Thiobacillus*, *Limno* *Limnobacter*, *Hydro* *Hydrogenophaga*, *Kaist* *Kaistobacter*, *Rhodo* *Rhodobacter*, *Phorm* *Phormidium*



propose that *Limnobacter* spp. may be responsible for the sulfur or thiosulfate oxidation. Other SOB including *Sulfuritalea*, *Sulfuricurvum*, and *Sulfurimonas* were also detected, however, at much lower abundances than *Thiobacillus* and *Limnobacter*.

In accordance with the elevated sulfate concentrations, we observed a number of sulfate-reducing bacteria (SRB) in the weathered tailings. Because it is unlikely that SRBs would proliferate in aerobic environments, we hypothesize that SRBs survive in oxygen-depleted micro-environments in deeper portions of the sample cores. Members of the family *Thermodesulfovibrionaceae*, which have been identified as thermophilic sulfate-reducing bacteria (Sekiguchi et al. 2008), were detected in all samples with relatively high abundances (up to 1.57 % of reads in some samples). These bacteria have also been detected in other mining-related environments (Medeiros et al. 2015; Sun et al. 2015a). Other SRB, such as *Desulfosporosinus*, *Geobacter*, *Desulfocapsa*, *Desulfobulbus*, and *Desulfurispora*, were also detected in the tailings. Detection of both SOB and SRB suggests dynamic sulfur cycling in the weathered Sb tailings.

Other enriched genera have been frequently reported in mining environments. *Kaistobacter* was detected in uranium mining and milling site (Radeva et al. 2013) and heavy metal contaminated soil (Navarro-Noya et al. 2010). *Arenimonas* contains species isolated from a Fe mine, and *Nocardioides* was observed in waste mine tailings (Hur et al. 2011). Unfortunately, the literature contains sparse information about these genera. Further investigations may be required to reveal their role in the mine tailings. The genus *Lysobacter* contains a species, *Lysobacter arseniciresistens*, which was isolated from Fe-mined soil and is arsenite (As(III))-resistant (Luo et al. 2012). In addition to *Lysobacter*, *Hydrogenophaga* contains As-oxidizing bacteria: *Hydrogenophaga* sp. str. NT-14 is able to oxidize As(III) and contains arsenite oxidase genes such as *aroA* and *aroB* (Vanden Hoven and Santini 2004). Given the chemical similarity between As and Sb (Wilson et al. 2010), we cannot exclude these bacteria's roles in Sb resistance and even Sb cycling.

We previously characterized the microbial community of sediments of a nearby Sb-contaminated river. Sequences assigned to the genera *Flavobacterium*, *Sulfuricurvum*, *Halomonas*, *Shewanella*, *Lactobacillus*, *Acinetobacter*, and *Geobacter* demonstrated high relative abundances in that Sb-rich environment (Sun et al. 2016). The remarkable difference of microbial community composition in two adjacent Sb-rich environments (river sediment versus mine tailings) indicates that historical factors (sediments or tailings) and environmental conditions may be an important filter in governing the distribution of microbial assemblage.

The CCA analysis elucidated the interaction between the microbial community and geochemical parameters (Fig. 7). It is not surprising to see that Sb_{tot} and all extractable Sb

fractions except Sb_{snp} were important in shaping the microbial communities due to the elevated concentrations of Sb and various Sb fractions. pH also strongly shaped the overall microbial communities based on its vector length. It is reported that the intracellular pH of most microorganisms is usually within 1 pH unit of neutral, and any considerable divergence in the environmental pH should impose stress on the microorganisms (Fierer and Jackson 2006). So, even though pH did not differ substantially among the 12 samples (ranging from 6.78 to 7.70), the slight variation in pH may not have been sufficient to directly influence the distribution of acidophilic and neutrophilic bacteria, but it may be enough to affect the mobility and bioavailability of metal(loid)s in the tailings, which could indirectly affect the communities. It has been reported that adsorption of Sb species is strongly related to pH (Cai et al. 2015; Leuz et al. 2006; Ritchie et al. 2013; Tighe et al. 2005). Thus, the pH effect on the microbial community structure may be indirect, mediated by the bioavailability of Sb species or other metal(loid)s. TOC also demonstrated strong effect on the microbial community. Autotrophic *Thiobacillus* are negatively correlated with TOC, suggesting that the availability of organic carbon may impact the distribution of autotrophic and heterotrophic bacteria. This effect may be more apparent at a low C environment. The ratio between sulfate and sulfide also exhibited a strong effect on the microbial community structure. Given that sulfide is oxidized to sulfate as weathering proceeds (Chen et al. 2013; Schippers 2004), this ratio sometimes was used as an index of weathering. This index varied substantially across 12 samples, indicating the occurrence of a possible weathering process in the tailing dump. A direct effect of the remarkable alteration of this index is the change the allocation of SOB and SRB, resulting in remarkable shift of microbial communities between samples. The effect of weathering process, which can generate acidity and release metal(loid)s to the surrounding environments, on microbial community composition has been reported previously and may shape the indigenous microbial communities in the current study as well (Chen et al. 2013; Garcia et al. 2001; Li et al. 2014).

In summary, the physicochemical characteristics and microbial communities of a total of 12Sb mine tailings samples were analyzed. All these tailings exhibited elevated Sb concentrations. In all samples, the bioaccessible and mobile Sb fractions in the tailings only accounted for a small proportion of the total Sb, suggesting a limited threat to the surrounding environment. An in-depth analysis of the microbial communities by high throughput sequencing revealed the dominance of *Thiobacillus* spp., *Limnobacter* spp., and *Thermodesulfovibrionaceae*-related bacteria in the tailings. The enrichment of these sulfur-oxidizing bacteria and sulfate-reducing bacteria indicates dynamic sulfur cycling in the tailings. In addition, the enrichment of a wide diversity of bacteria suggests the metabolic versatility of the microbial

communities inhabiting the tailing piles. These bacteria may play an important role in tailings weathering and biogeochemical cycling of Sb. Additional experiments are required to further elucidate these microbial processes in tailings piles.

Compliance with ethical standards

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Conflict of interest The authors declare that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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