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Response of microbial communities to elevated thallium contamination in river sediments

Atta Rasool[a,b] and Tangfu Xiao[c]

[a]State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, China; [b]University of Chinese Academy of Sciences, Beijing, China; [c]Key Laboratory for Water Quality and Conservation of the Pearl River Delta, Ministry of Education; School of Environmental Science and Engineering, Guangzhou University, Guangzhou, China

ABSTRACT
The study of microbial communities in river sediments contaminated by thallium (Tl) is necessary to achieve the information for *in-situ* microbially mediated bioremediation. However, little is known about the microbial community in Tl-contaminated river sediments. In the present study, we characterized the microbial community and their responses to Tl pollution in river sediments from the Tl-mineralized Lanmuchang area, Southwest Guizhou, China. Illumina sequencing of 16S rRNA amplicons revealed that over 40 phyla belong to the domain bacteria. In all samples, *Proteobacteria*, *Cyanobacteria*, and *Actinobacteria* were the most dominant phyla. Based on the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) tree and PCoA (Principal Coordinates Analysis) analysis, microbial composition of each segment was distinct, indicating *in-situ* geochemical parameters (including Tl, sulfate, TOC, Eh, and pH) had influenced on the microbial communities. Moreover, canonical correspondence analysis (CCA) was employed to further elucidate the impact of geochemical parameters on the distribution of microbial communities in local river sediments. The results indicated that a number of microbial communities including *Cyanobacteria, Spirochaete, Hydrogenophaga,* and *Acinetobacter* were positively correlated with total Tl, suggesting potential roles of these microbes to Tl tolerance or to biogeochemical cycling of Tl. Our results suggested a reliable location for the microbial community's diversity in the presence of high concentrations of Tl and might have a potential association for *in-situ* bioremediation strategies of Tl-contaminated river. Overall, *in situ* microbial community could provide a useful tool for monitoring and assessing geo-environmental stressors in Tl-polluted river sediments.

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thallium pollution; 16S rRNA high-throughput sequencing; microbial community; canonical correspondence analysis

Introduction

Natural weathering process of sulfide minerals and/or mining/smelting activities are major sources of introduction of heavy metals into aqueous environment to cause considerable water pollution (Ayangbenro and Babalola 2017; Chen et al. 2017; Gomez-Gonzalez et al. 2015; Liu et al. 2018; Karbowska et al. 2014; Xiao et al. 2004a,b). Rivers, the main aqueous ecosystem with function for drinking and irrigating water supply in the world, usually present as a primary sink for metals from both natural and anthropogenic inputs into water and sediments; and they may also present as a secondary source of such metals, mainly following water-sediment interaction that fluxes metals from sediments to waters (Ferronato et al. 2013; Grizzetti et al. 2016; Koron et al. 2013; Liu et al., 2016; Taylor and Owens 2009; Vanek et al. 2013). Therefore, the detrimental effect of toxic metals on organisms and water quality and associated remediation in river system has received high environmental concern (Abarikwu et al. 2017; Ferronato et al. 2013; Wang et al. 2012; Wei et al. 2016). Previous studies in literature have more concerns on metal pollutions of Pb, Cd, As, Sb, Hg, and Zn in river system, but less attention was to the toxic metal thallium (Tl).

Thallium, although generally with low concentrations in natural soil and river sediment, is more toxic to mammals than Hg, Cd, Pb, Cu, and Zn, and it is known to have caused many accidental, occupational and therapeutic poisonings since its discovery in 1861 (Mulkey and Oehme 1993; Smith and Carson 1977). Tl usually presents as an associated metal in various metal sulfide minerals, and is quite mobile during the weathering processes (Vink 1993; Xiao et al. 2003, 2004a). Tl generally exists in natural waters as the species Tl(I) and Tl(III), but the Tl(I) species is dominant in the aqueous environment (Vink 1993). The solubility of Tl(I) compounds is quite high, ranging from 40 to 800 g/L (Sager 1994), and suggests that Tl(I) would not precipitate from solution in most aqueous environments. Tl adsorption to geological materials (e.g., Fe–Mn–Al oxyhydroxides) appears to be dominated by cation exchange, as opposed to (co)-precipitation, or absorption into the structure of the solid phase (Kaplan and Mattigod 1998). Cation exchange is a process that does not bond the metals

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CONTACT Tangfu Xiao ✉ tfxiao@gzhu.edu.cn Key Laboratory for Water Quality and Conservation of the Pearl River Delta, Ministry of Education; School of Environmental Science and Engineering, Guangzhou University, Guangzhou 510006, China

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very strongly. All these factors would indicate that Tl is a very mobile metal in most aqueous environments, rather than being locked-up into solid oxides, and can disperse easily during oxidation of Tl-bearing sulfides. Particularly, Tl pollution in river, either from natural weathering of sulfide minerals or anthropogenic activities can pose a significant environmental problem (Turner and Pillsbury 2013; Xiao et al. 2003, 2004b, 2012). For example, Tl pollutions in Beijiang River in South China in 2010 (Xiao et al. 2012; Liu et al. 2018), in Longjiang River in 2012 and Hejiang River in 2013 in the Pearl River Basin in South China (Chen et al. 2017; Dou et al. 2013), and in a mining catchment in Valdicastello Carducci (Tuscany, Italy) in 2016 (Campanella et al. 2017), have detrimentally impacted the water quality for water supply purpose.

Our previous study has identified a river system that shows elevated Tl contents due to naturally occurring Tl enrichment and sulfide mining in the Lanmuchang area, located in southwest Guizhou Province of Southwest China (Xiao et al. 2003). This area was known to have intensive Tl-rich sulfide mineralization, with such minerals of lorandite ($TlAsS_2$), cinnabar (HgS), realgar (As_4S_4) and orpiment (AS_2S_3) (Xiao et al. 2003). The local Qingshui River runs from north to south through the Tl-mineralized area, and is the only surface water system that services for local irrigation. The natural weathering of the Tl-bearing sulfides has introduced elevated Tl into the local river catchment, i.e., 1–33 µg/L in river water and 14–62 mg/kg in alluvial bank soils (Xiao et al. 2003), and 14–53.1 mg/kg in river sediments (Peng et al. 2007). However, little was known the environmental impact of the elevated Tl contents in local sediment on aquatic microorganism.

The adverse effects of various heavy metals on microbial communities have been well documented (Haller et al. 2011; Margesin et al. 2011). Heavy-metal exposure can decrease the microbial diversity, biomass, and the size of possible microbial population (Poll et al. 2003; Sessitsch et al. 2001; Zhang et al. 2007), but certain microbial species can tolerate high metal exposures in river sediments (Kandeler et al. 2000; Margesin et al. 2011; Wang et al. 2007). However, up to date, little knowledge is available for understanding the response of microbial communities to elevated Tl contamination in river sediments.

The study of 16S rRNA sequences exposed that the structure and composition of microbial community in sediments changed significantly across the gradient heavy-metal pollution, and the relative abundances were lower for *Chloroflexi* and *Firmicutes*, though higher for *Proteobacteria* and *Actinobacteria* in mining activity areas (Bier et al. 2015; Monchy et al. 2006). Abundance of several bacterial genera and phylum level including *Vibrio fisheries*, *Nocardia opaca*, *Gemmatimonadetes*, *Sphingomonas*, *Caulobacter*, *Janthinobacterium*, *Geobacter*, *Rhodoferax*, and *Cyanobacteria* may tolerate Tl in Tl-rich minerals (Bao et al. 2014; Hsieh et al. 2004; Monchy et al. 2006). Similarly, microbiological activities within mining area can play an important role in influencing the geochemical behaviors of metal(loid)s (Miao et al. 2015; Schippers et al. 2010).

Therefore, we have characterized for the first time the microbial communities in sediments of the local Qingshui River that represents an excellent site with elevated Tl derived from natural weathering and past sporadic mining of Tl-rich sulfides. Microbial transformations of Tl are promising approaches for remediation of Tl-contaminated environments, by facilitating *in-situ* immobilization and precipitation along with oxidation or reduction of sulfides (DeNicola and Stapleton 2014; Schippers et al. 2010). However, up to date, the knowledge of the relationship between Tl-rich environments and microorganisms is quite limited. It is still undefined that how microbial community response to environmental factors and what geochemical factors mainly influence the microbial community in Tl-polluted river sediments. The present study aimed to explore the correlation between microbial community and different geochemical variables in the Tl-rich river sediments, and to gain new insight into Tl biogeochemical process and microbially mediated Tl transformation in Tl-contaminated river as well. This study provides novel data sets that could be used in an alliance of Tl-contaminated river sediments for a better understanding of microbial processes with geochemical factors. These findings will help to understand how microbes adapt to high Tl concentrations, and possibly provide an insight into Tl remediation potential using high throughput of 16S rRNA gene sequences.

Materials and methods

Study area

The study area (105°30′23″E, 25°31′28″N) is located in Lanmuchang in southwest Guizhou Province, Southwest China, a small town of approximately 1000 residents (Figure 1). It is widely developed for agricultural and residential purposes. The altitude is 1000–1600 m above the sea level with a relative relief of 200–600 m. The karstic topography is characterized by peaks, depressions, valleys, karstic caves, karstic underground rivers, and sinkholes. The local stream (Qingshui River) runs from north to south through the Tl-mineralized zone, into a natural wetland downstream (Xiao et al. 2003). Our previous studies have described the local geology and sulfide mineralization in details (Sun et al. 2012; Xiao et al. 2003). Briefly, the natural weathering of Tl-rich minerals (lorandite, realgar, orpiment, cinnabar, etc.) and past sporadic mining of the sulfides have introduced high amounts of Tl into local soil, water, sediment, and crops, which brought about health risk on local residents (Sun et al. 2012; Xiao et al. 2007, 2012). The local residents suffered from chronic Tl poisoning, with symptoms of weakness, muscle and joint pain, disturbance of vision, and hair loss (Xiao et al. 2004a).

Sampling and analysis

Twelve river sediment samples (upstream, midstream, downstream) were collected in May 2016 (Figure 1). All sediment samples were stored in ice box at site and immediately stored in freezers at −20 °C for microbial analysis and 4 °C for geochemical analysis, respectively. The river sediment samples were thoroughly ground using pestle and mortar before passing through a 200-mesh sieve (FD-1A-80, Beijing, China). To measure pH, oxidation–reduction potential (Eh)

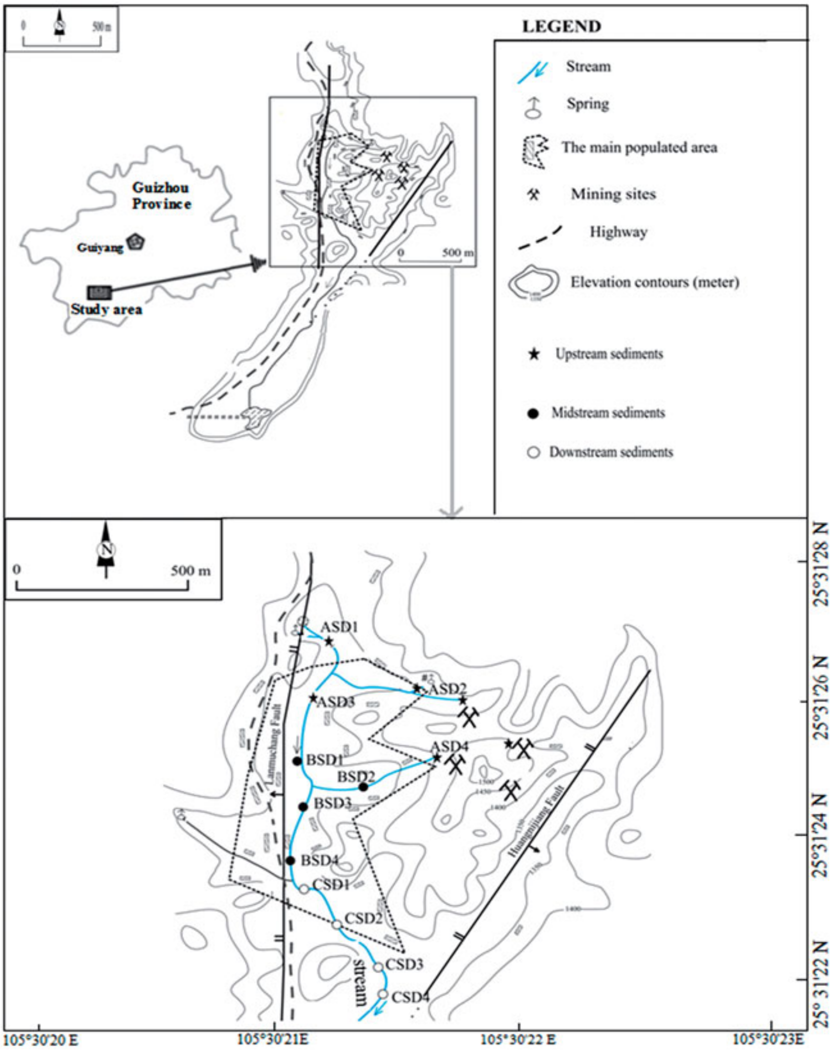

Figure 1. Map showing the sampling sites.

and electrical conductivity (EC), 10 g dry ground samples and 25 ml Milli-Q water were put into a 100-mL Erlenmeyer flask. Afterward, the mixtures were shaken for 1 h and then measured by a calibrated HACH HQ30d pH meter (HACH, Loveland, USA). To measure anions, 10 g of the dry ground samples were mixed with 50-mL Milli-Q water in a 100-mL Erlenmeyer flask. The mixture was shaken for 15 min, then standing to equilibration for 4 h. The supernatant was then centrifuged 3500 rpm for 15 min and filtered through a 0.45-μm filter membrane, and the filtrates were used for anion determination by ion chromatography (DIONEXICS-40, Sunnyvale, CA, USA).

Trace elements and major cations were measured by ICP-MS (Agilent, 7700×, California, USA) and ICP-OES (iCAP6500, Thermo Scientific, Germany) after digesting 50 mg sediment sample in a Teflon tube with HNO_3 and HF (5:1, v/v) (Xiao et al. 2016). Total organic carbon (TOC), total sulfur (TS), total hydrogen (TH), and total nitrogen (TN) were measured by an elemental analyzer (Vario MACRO cube Elementar, Hanau, Germany). The analytical precision, determined based on the standard quality control procedures using internationally certified reference materials (CRM stream sediment GBW07302a, US NIST), internal standards (Rh at 500 μg/L), duplicates, and reagent blanks, was better than ±10%.

Mineral composition and morphology analysis

Mineral compositions were determined using scanning electron microscope (SEM) for the presence and morphology of minerals in sediments, based on a protocol described previously (McBeth et al. 2013). SEM images were taken with a field-emission SEM (JSM-6460LV, JEOL, Tokyo, Japan) with an EDAX energy-dispersive X-ray spectrometer (EDAX-GENESIS, Mahwah, USA) (Figure 2). The SEM was operated at 15 kV with a working distance of 10 mm. For EDAX analysis, to obtain sufficient X-ray counts, an accelerating voltage of 20 kV was used.

DNA extraction and high-throughput of the V3V4 region of 16S rRNA gene sequences

Universal primers (for bacteria and archaea) were used to amplify 16S rRNA genes to normalize bacterial gene abundances to obtained functional gene abundances. 16S rRNA tag-encoded ultra-high-throughput sequencing was carried

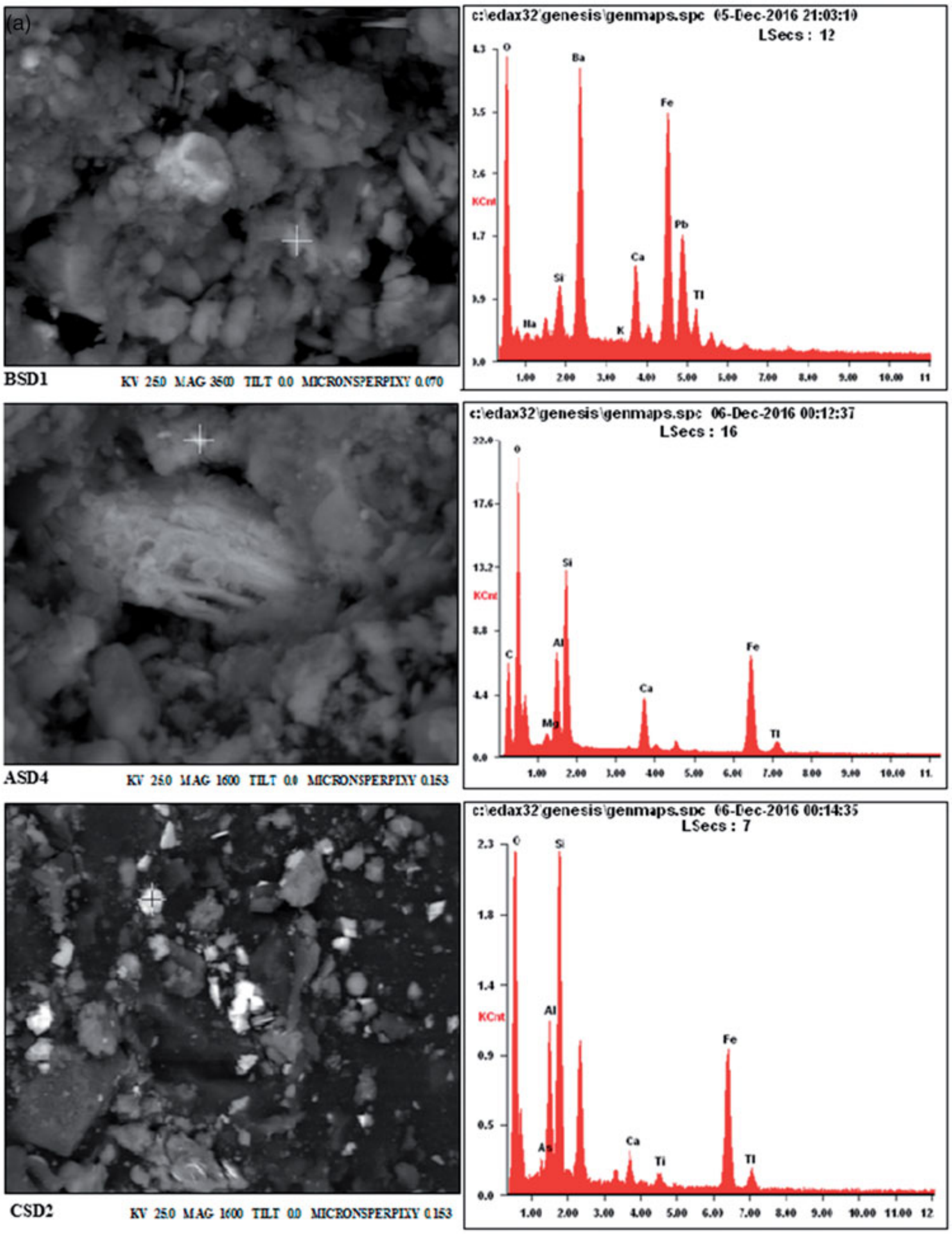

Figure 2. Representative SEM images (left panel) and the corresponding EDX spectra (right panel) of the sediment samples. Sampling sites are labeled on the top left of each panel. The arrows point to the exact area for the EDS analysis.

out on the Illumina MiSeq platform at Novogene (Beijing, China). Total genome DNA was extracted from 250 mg of studied samples using the Fast DNA® spin kit (MP bio, Santa Ana, USA) according to the manufacturer's protocol. DNA concentration and purity were monitored on 1% agarose gels. On the basis of the concentration, DNA was diluted to 1 ng/μL using sterile water. The 16S rRNA genes of distinct regions (V3-V4) were amplified using the 515F/907R primer pairs (515F: 5′-GTGYCAGCMGC CGCGGTAA-3′, 907R: 5′-CCYCAATTCMTTTRAGTTT-3′) (Ren et al. 2014). The library was sequenced on the IlluminaHiSeq2500 platform at Novogene Bioinformatics Company (Beijing, China). The Illumina Next era XT Index kit (Illumina Inc., San Diego, CA, USA) with dual 8-base barcodes were used for multiplexing. Paired-end reads were assigned to the samples in the light of their unique barcode and truncated by getting rid of the barcode and primer sequence. The raw tags were come from merging paired-end reads using FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH). The raw tags were filtered by QIIME (V1.7.0, http://qiime.org/index.html) under specific conditions (Caporaso et al. 2010; Bokulich et al. 2013) to obtain high-quality clean tags. Then the effective tags finally obtained by removing Chimera sequences using UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) to compare with the reference database (Gold database, http://drive5.com/uchime/uchime_download.html). Sequences with ≥97% similarity were assigned to the same OTUs using Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/). Representative sequences were used for annotating taxonomic information by the RDP classifier (Version 2.2) base on the Green Gene Database (http://greengenes.lbl.gov/cgi-bin/nph-

Table 1. Chemical and physical parameters of the Lanmuchang study area.

Sample Id	pH	EC (µS/cm)	Eh (mV)	Cl (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Tl (mg/kg)
Upstream									
ASD1	5.88	345	274	2.87	21556	1356	55570	787	16.3
ASD2	6.49	966	241	2.21	36148	1215	46950	730	36.4
ASD3	6.43	1079	−18	6.97	75746	3751	57788	611	4.3
ASD4	6.07	2420	−85	13.64	49408	2940	51872	522	22.3
Average	6.22 ± 0.29	1202 ± 873	155 ± 181	6.42 ± 5.25	45715 ± 23027	2316 ± 118.9	53045 ± 4740	663 ± 118.9	19.8 ± 13.3
Midstream									
BSD1	3.65	948	356	1.66	709.5	148	24056	102	72.3
BSD2	3.61	951	342	1.68	719.5	149	24356	112	69.3
BSD3	5.8	1443	−13	7.76	22880	1296	61088	775	13.8
BSD4	5.74	2750	−34	22.71	34064	2002	50120	473	16.4
Average	4.7 ± 1.24	1523 ± 850	163 ± 215	8.45 ± 9.93	14593 ± 13664	899 ± 913	39905 ± 18673	366 ± 322.9	42.95 ± 32
Downstream									
CSD1	6.4	273	229	1.98	23492	1959	60716	1002	6.7
CSD2	6.14	2380	−102	11.85	48518	6011	51438	571	14.6
CSD3	6.09	2840	−123	14.73	38196	6255	58070	529	15.2
CSD4	6.89	962	−49	6.69	11376	2049	50852	718	5.8
Average	6.38 ± 0.37	1614 ± 1199	−11 ± 163	8.81 ± 5.64	30396 ± 16316	4069 ± 2386	55269 ± 4888	705 ± 213.9	10.57 ± 5

index.cgi). Phylogenetic relationship of different OTUs was studied using MUSCLE software (Version 3.8.31, http://www.drive5.com/muscle/). Abundance information of OTUs was normalized by the standard of sequence number corresponding to the sample with the least sequences. Subsequent analysis of alpha diversity and beta diversity were all performed basing on normalized data.

Bioinformatics and statistical methods

Statistical analysis was carried out using the SPSS statistical package (version 16.0 for Windows, SPSS Inc., USA), and all the plots were designed by Origin (version 8.5 for Windows, Origin Lab Corp., USA). Linear discriminant analysis (LDA) effect size (LEfSe) (http://huttenhower.sph.harvard.edu/LEfSe) was used to characterize features differentiating the microbial communities under different conditions as described previously (Ling et al. 2014; Segata et al. 2011). LEfSe uses the Kruskal–Wallis rank sum test to detect features with significantly different abundances of assigned taxa and performs LDA to estimate the effect size of each element with a normalized relative abundance matrix (Fierer and Jackson 2006). All tests for significance were two-sided, and $p \leq 0.05$ were considered statistically significant. The complexity of species diversity was performed by alpha diversity indices including Chao1, Shannon, Simpson and ACE base on QIIME (Version 1.7.0) and R software (Version 2.15.3). The Chao 1, Shannon, and the Simpson indexes were determined for 11 libraries to estimate species richness as described previously (Schloss et al. 2009). The differences in microbial communities among different samples were evaluated by UniFrac using QIIME software (Version 1.7.0). Principal Component Analysis (PCoA) was applied to reduce the dimension of original variables using R software (Version 2.15.3). PCoA was displayed by WGCNA package, stat packages and ggplot2 package in R software (Version 2.15.3) on weighed uni fractions. Unweighted Pair-group Method with Arithmetic Means (UPGMA) Clustering was conducted by QIIME software (Version 1.7.0). CCA was done on abundant bacteria genera (relative abundance >1% in at least one sequencing library) and selected physicochemical parameters. The correlations between geochemical parameters were determined by Spearman's rank.

Results

Physico-chemistry and major ion compositions

The physicochemical properties of the river sediments are summarized in Tables 1 and 2. The river sediments presented pH values at 6.22 upstream, 4.7 midstream, and 6.38 downstream, respectively. EC values averaged at 1202 μS/cm upstream, 1523 μS/cm midstream and 1614 μS/cm downstream, respectively. The redox potential (Eh) values varied between 155 to 163 mV in upstream to midstream river sediments, and decreased to −11 mV in the downstream samples (Table 1). The concentrations of sulfate were at 483 mg/kg upstream, 714 mg/kg midstream, and 510 mg/kg downstream, respectively, which may be mainly due to the local acid mine drainage discharge into the midstream in the rainy season.

Other geochemical parameters such as total carbon (TC), total sulfur (TS), total nitrogen (TN), and total hydrogen (TH), total organic carbon (TOC), and organic matter (OM) exhibited different trends across the sediment samples (Table 2). TC averaged at 61.39 g/kg midstream but decreased to 49.49 g/kg downstream. The TH averaged at 8.91 g/kg, 10.18 g/kg, and 11.18 g/kg in upstream, midstream and downstream sediments samples, respectively (Table 2). TOC concentrations averaged at 53.22 g/kg midstream but decreased to 40.46 g/kg downstream. The average contents of organic matter were observed at 79.83 g/kg upstream, 91.15 g/kg midstream, and 69.29 g/kg downstream, respectively (Table 2).

The total concentrations of Fe in local river sediments averaged at 53045 mg/kg upstream, 39905 mg/kg midstream and 55269 mg/kg downstream, respectively, due to the weathering of sulfide minerals. Similarly, Mn averaged at 663 mg/kg upstream, 366 mg/kg midstream, and 705 mg/kg downstream, respectively. Thallium (Tl) concentrations in local river sediments varied from 19.82 mg/kg upstream, 42.95 mg/kg midstream and to 10.57 mg/kg downstream,

Table 2. Major elementary compositions in Lanmuchang thallium mineralized area southwest Guizhou.

Sample Id	Total N (g/kg)	Total C (g/kg)	Total H (g/kg)	Total S (g/kg)	TOC (g/kg)	OM (g/kg)	Sulfate (mg/kg)
Upstream							
ASD1	1.06	24.63	6.97	1.15	20.05	35.26	71
ASD2	2.74	61.21	8.67	1.61	53.04	91.23	189
ASD3	2.79	73.15	8.22	1.23	58.72	100.99	237
ASD4	7.95	73.66	11.77	4.22	53.39	91.83	1433
Average	3.64 ± 2.98	58.16 ± 23	8.91 ± 2.04	2.05 ± 1.46	46.3 ± 17.69	79.83 ± 30	483 ± 637.5
Midstream							
BSD1	3.02	58.1	9.39	2.07	55.54	95.53	504
BSD2	3.12	60.1	9.41	2.17	57.56	97.54	505
BSD3	3.7	48.53	9.71	1.35	38.01	65.38	346
BSD4	7.57	78.35	12.19	3.33	61.75	106.16	1502
Average	4.35 ± 2.17	61.27 ± 12.46	10.18 ± 1.35	2.23 ± 0.82	53.22 ± 10.46	91.15 ± 17.78	714 ± 530
Downstream							
CSD1	2.26	24.82	10.67	1.64	27.12	45.49	30
CSD2	5.22	67.01	11.14	2.35	50.93	87.59	1059
CSD3	9.59	72.96	11.79	2.07	55.78	95.94	643
CSD4	2.78	33.2	11.15	0.82	28	48.16	310
Average	4.96 ± 3.34	49.49 ± 24	11.18 ± 0.46	1.72 ± 0.67	40.46 ± 15	69.29 ± 26	510.5 ± 443

Table 3. OTU number and Chao1, Shannon index in all samples at 97% similarity.

Sample Id	Total tag	Taxon Tag	Unique Tag	OUT	Chao1	Shannon
Upstream						
ASD1	53,982	47,216	6766	4108	4074.78	10.07
ASD2	57,622	51,080	6542	4425	4200.772	10.13
ASD3	57,032	50,740	6292	3682	3849.735	9.489
ASD4	52,404	45,260	7144	4187	3087.148	8.151
Midstream						
BSD1	60,283	54,547	5736	3202	3179.961	8.113
BSD2	53,448	48,449	4999	3205	2636.568	7.446
BSD3	40,353	37,186	3167	2637	3282.597	8.484
BSD4	58,305	54,111	4192	3397	3571.36	9.363
Downstream						
CSD1	50,271	42,668	7603	3917	4055.597	9.637
CSD2	64,176	61,613	2563	2744	3222.893	5.863
CSD3	60,345	53,650	6695	3837	3691.185	8.898
CSD4	49,794	40,769	9025	3769	3719.188	9.502

respectively. The elevated concentrations of Tl were observed in the midstream sediment samples, which were attributed to flooded materials from the mineralized areas (Table 1). The SEM–EDS analysis showed high contents of Tl in local river sediments (Figure 2).

Taxonomic composition of microbial communities

Total Illumina sequencing resulted in average 57,223 sequencing reads after quality filtering from twelve sediment samples, ranging from 40,353 to 65,694 reads per sample (Figure S1 in Supplementary Material), and clustered into 50,713 operational taxonomic units (OTUs). The lightly and moderately contaminated sediment samples BSD3 and ASD2 had the richest diversity with OTU numbers of 2637 to 4425, respectively, followed by the sediment samples from midstream and upstream (Table 3). The Alpha diversity of Chao1 and Shannon index displayed very similar trend as OTU numbers (Table 3). For instance, performed Chao 1 indicated ASD2 and BSD2 maximum and minimum values in upstream and midstream sediment samples, respectively, whereas Shannon analysis revealed highest values in ASD2 and lowest in CSD2, respectively (Table 3).

The classification in each sample was assigned to different taxonomic levels from phylum to genus based on RDP classifier (Figure S2 in Supplementary Material). A total of more than 40 archaeal and bacterial phyla were recognized in all river sediment samples. *Actinobacteria* (13.5% averaging of the reads) and *Acidobacteria* (7.9% averaging of the reads) were the most predominant phyla in twelve river sediments samples across the different location of downstream and upstream, while *Proteobacteria* (51.5% averaging of the reads) was the most predominant phylum in midstream sediment samples, respectively (Table S1 in Supplementary Material). At the genus level, *Ralstonia* (6.4% averaging of the reads) and *Rhodobacter* (5.6% averaging of the reads) were the most predominant genera in upstream, while *Clostridium* (1.5% averaging of the reads) was the most predominant genera in downstream sediment samples, as well as Kaistobacter (1.2% averaging of the reads) was most predominant genera in midstream river sediment samples, respectively (Table S1 in Supplementary Material). Microbial community analysis at lower taxonomic levels, such as genus levels, may provide better relative abundances in sequencing libraries and phylogenetic determination for dominant microbes than higher taxonomic groups such as phylum level (Ho et al. 2016; Sun et al. 2016).

Comparison of bacterial communities between different zones

To further explain the variability of microbial communities within sediment samples, we used Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Principal Coordinates Analysis (PCoA) to cluster the microbial communities as a function of the two factors investigated. Microbial communities differed by site, as shown by the UPGMA tree and PCoA (Figure S3 in Supplementary Material), suggesting that innate geochemical parameters may shape the indigenous microbial communities. The observation indicated that the geogenic process itself is the crucial factor in shaping microbial community, whereas the microbial community in real-time is impacted by anthropogenically disturbed conditions.

The UPGMA tree used to explain the similar and distinct trend of microbial communities in the studied sediment

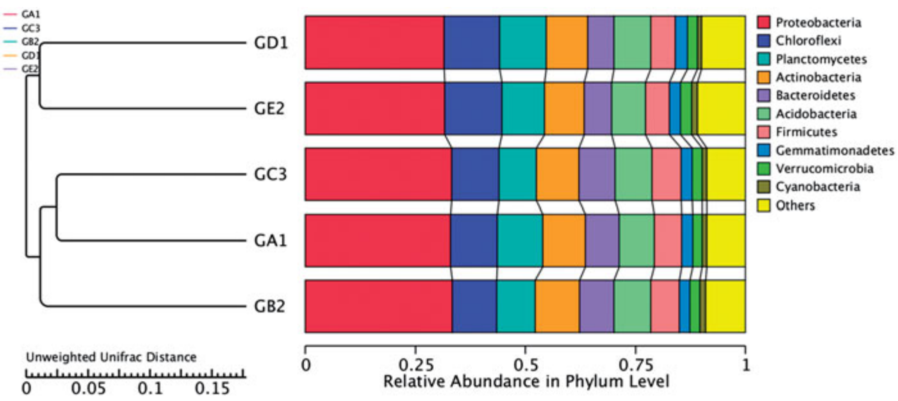

Figure 3. The UPGMA tree showing clusters of microbial communities based on UniFrac with 100% support on all nodes.

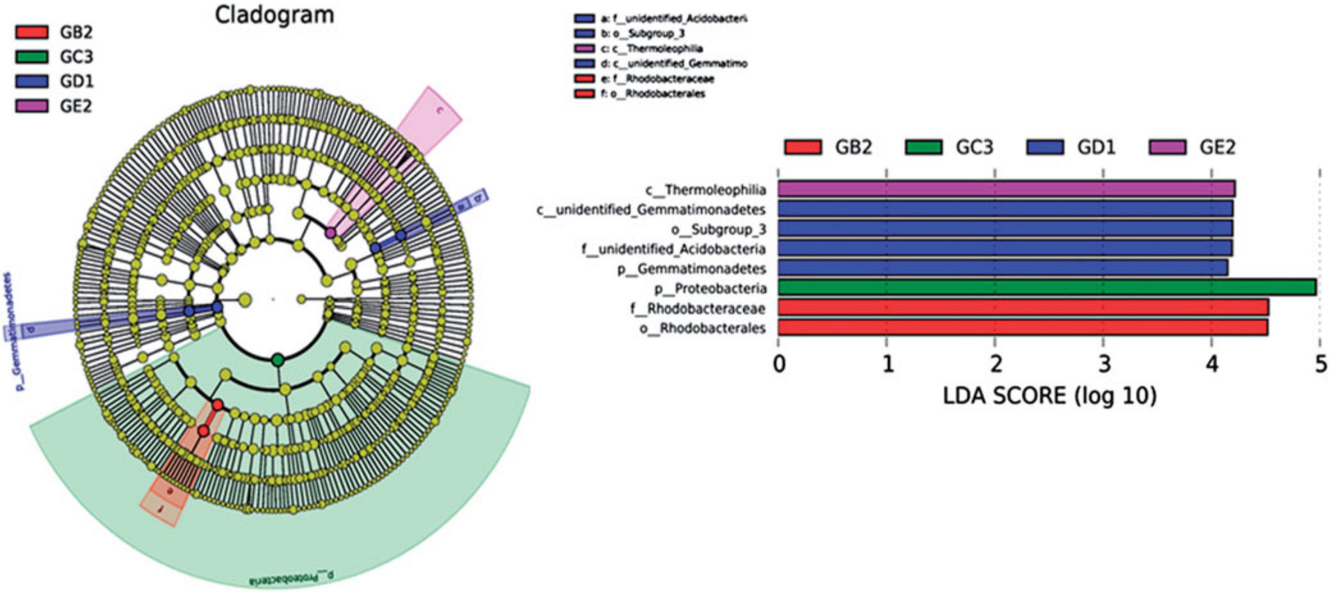

Figure 4. LEfSe identified the abundant taxa more differentially between river sediment samples. Taxa enriched in upstream sediments with a positive LDA score (green) and taxa enriched in midstream sediments have a slightly positive score (red). (a) Only taxa meeting an LDA, threshold significance of 5 are shown. (b) Cladogram obtained from LEfSe analysis 16S sequences. Screening and small circles with different colors in the diagram represent the abundance of those taxa in the respective group. Yellow circles characterize non-significant differences in abundance among upstream and midstream sediments of those particular taxa.

samples groups GAI, GC3, and GB2 (Figure 3 in Supplementary Material). The UPGMA tree showed that microbial communities such as *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Planctomycetes*, and *Chloroflexi* in midstream river sediment samples more closely resembled each other than those engaged from upstream and downstream. Meanwhile, polluted river sediment samples were distantly related to their contaminated counterparts, indicating the influence of microbial activity might be another factor of grouping. The PCoA analysis explained the differences between the sixteen microbial communities (97% OTU sequences similarity) and indicated that the microbial communities from upstream and downstream sediment samples groups (GA1 and GC3) were clustered, while those from midstream sediment samples group (GB2) was distantly related to each other (Figure S3 in Supplementary Material).

LEfSe was conducted from phylum to genus level to evaluate particular bacterial community in local sediments. To identify the unique taxa in a different layer of the profile in the sediment samples, LEfSe is adapted. A Cladograms representative of the structure of the microbial community and their predominant bacteria was shown in Figure 4. Cladograms with LDA taxa showed values higher than five for clarity (Figure 4). At phylum level, *Proteobacteria* was enriched in heavily contaminated samples downstream (green legend) with LDA value higher than five. At the family and order level, *Rhodobacteraceae*, *Rhodobacterales* were enriched with the contaminated samples midstream, while *Gemmatimonadetes* at phylum level and *Acidobacteria* at family level were prevalent in moderately contaminated samples of upstream river sediments.

Clustering heat map of species abundance at the taxonomic level of genus was employed to identify the difference of microbial communities in sediments with the relative abundance of major bacteria (Figure 5). The heat map figure demonstrated that *Thermotogae*, *Actinobacteria*, *Nitrospirae*, and *Synergistetes* were more abundant in the sequences libraries from sediments downstream; *Cyanobacteria*, *Euryarchaeota*, *Chlamydiae*, *Chlorobi*, *Parcubacteria*, and *Deinococcus-Thermus* were more dominant in the midstream sediments, while *Proteobacteria*,

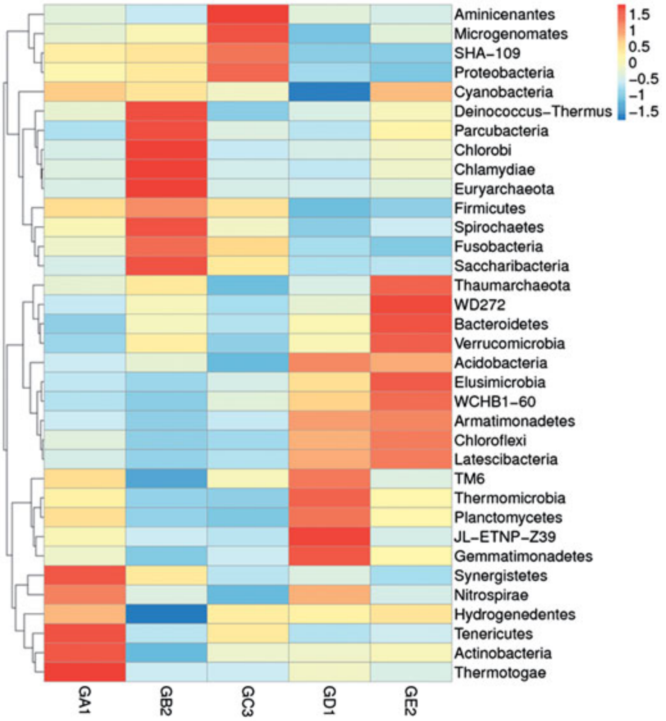

Figure 5. Heat map analysis of dominant phylotypes with relative abundance greater than one of the twelve river sediment samples. The value of relative percentage for the microbial genera is indicated by the color intensity, which can found in the legend.

SHA-109, *Microgenomates*, and *Aminincenantes* were most abundant in the upstream sediments. Detailed information about the relative abundances of dominant groups in each sample was shown in the heat map (Figure 5).

Correlation among geochemical parameters and microbial communities

Spearman's correlation analysis was used to study the correlations among major geochemical factors and microbial communities, mostly bacterial species at phyla and genus level were significantly correlated with EC, TOC, sulfate, and Tl (Tables S2 and S3 in Supplementary Material). In all tested taxonomic groups (phyla level) such as EC were positively correlated with *Proteobacteria* ($r = 0.568$) and negatively correlated with *Acidobacteria* ($r = –0.736$), as well as sulfate was positively correlated with *Proteobacteria* ($r = 0.5294$), and negatively correlated with *Planctomycetes* ($r = –0.7812$), and *Gemmatimonadetes* ($r = –0.863$) in local river sediments (Table S2 in Supplementary Material). At phyla level, Tl was positively correlated with *Cyanobacteria* ($r = 0.6546$), *Spirochaete* ($r = 0.5$) and negatively correlated with *WS3* ($r = –0.6164$), respectively (Table S2 in Supplementary Material). At genus level *Acinetobacter* ($r = 0.531$), *Hydrogenophaga* ($r = 0.618$) were positively correlated with Tl but was negatively correlated with *Solibacter* ($r = –0.556$), *Dechloromonas* ($r = –0.508$) and *Methylibium* ($r = –0.475$) in river sediment samples, respectively (Table S3 in Supplementary Material). Also, *Rhodobacter* ($r = 0.772$) was positively correlated with EC but negatively correlated with *Solibacter* ($r = –0.657$), respectively (Table S3 in Supplementary Material).

Spearman's rank correlations were determined between genera bacterial taxa and various geochemical parameters including Tl contents (Figure 6). *Planctomyces* was positively correlated with Mn in the extractable fractions ($p < 0.01$). *Caldilinea* was positively correlated with Mg, chloride, TN and EC ($p < 0.01$), whereas *Flavihumibacter* was positively correlated with pH ($p < 0.05$). *Phodobacter* was positively correlated with TC ($p < 0.01$), sulfate ($p < 0.01$), chloride ($p < 0.05$), TS ($p < 0.01$), TOC ($p < 0.01$), TN ($p < 0.01$) and EC ($p < 0.01$), while negatively correlated with Eh ($p < 0.05$). Likewise, *Hydrogenophaga* was positively correlated with Tl ($p < 0.01$), TS ($p < 0.05$), as well as *Dechloromonas* negatively correlated with Tl ($p < 0.01$). *Acinetobacter* was positively correlated with Tl ($p < 0.05$) and TS ($p < 0.05$). Other Tl-related microbes were frequently classified to the *Hydrogenophaga*, *Acinetobacter*, *Methylibium*, *Rhodoplanes*, and *Dechloromonas* as well. Some Tl-related microbes were positively correlated with many contaminant parts, suggesting for an adjustment of the microbial community in response to Tl contamination.

Canonical correspondence analysis (CCA) was performed to distinguish probable linkages among geochemical parameters and microbial communities in river sediments (Figure S4 in Supplementary Material). CCA axis 1 explained 42.74% of the phylum-level variability and was positively correlated with pH, EC, TN, TOC, TH, TS, chloride, sulfate, Ca, Fe and Mn, and negatively correlated with Eh and Tl, while CCA axis 2 explained a further 20.97% of the variability and was positively correlated pH, Fe, Mn and Ca, respectively (Figure S4 in Supplementary Material). The most dominate phylum microbes of *TPD-58*, *Verrucomicrobia, Firmicutes, Actinobacteria* were negatively correlated with Tl, TOC and Eh, respectively (Table S4 in Supplementary Material), although *Proteobacteria*, *Acidobacteria*, *Chlorobi*, *Nitrospirae*, *Chlamydiae*, *Cyanobacteria,* and *Synergistetes* were positively correlated with pH, EC, chloride, total Fe, Mg, Mn and Ca, respectively (Table S4 in Supplementary Material). *Proteobacteria* was positively correlated with pH, indicating these bacteria favor the environment with higher pH. Moreover, *TPD-58* and *Verrucomicrobia* portrayed negative correlation with higher Tl concentrations in CCA but positive correlation with prominent *Acinetobacter* and *Hydrogenophaga* in Spearman's rank correlation, which suggests that Tl-contaminated environment facilitates the growth of diverse bacterial communities. Therefore, CCA helps for examining the influence of environmental parameters on the microbial communities.

Discussion

Impact of geochemical parameters on bacterial community

The oxidation–reduction and sorption–desorption processes are controlled by geochemical parameters such as pH, oxygen availability, Fe and S-oxidization, and reduction bacteria (Lindsay et al. 2015). Among these dynamics, the function of bacteria is the most imperative factor. For example, the biological–oxidation rate could be up to several orders of

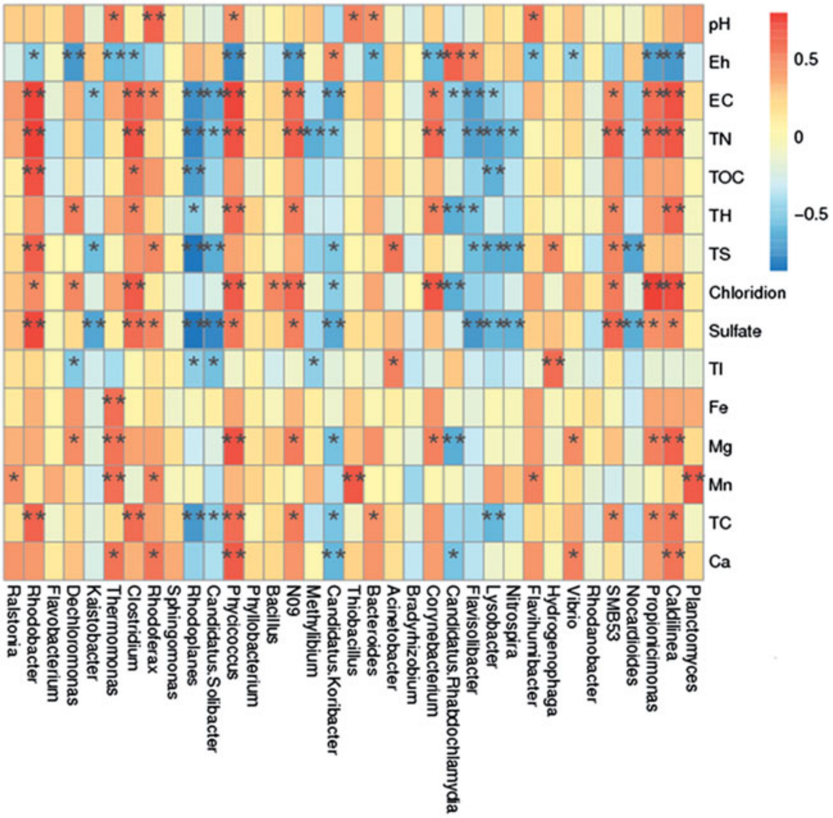

Figure 6. Spearman's correlation coefficients and cluster analysis between the different Tl form and bacterial genera with relative abundances >1% in the least one sample. The correlation coefficient is specified by hue. **Significant correlation ($p < 0.01$). *Significant correlation ($p < 0.05$).

magnitude faster than the abiotic rate at comparable temperatures (Nordstrom 2003). These bacteria were augmented in the present studied samples; though microbial community distribution was determined by mineral constituents, concentration and oxidation state of toxic metals, Eh, TOC, OM, and pH, and intensely influenced the microbial dissemination in river sediments. The environmental conditions, including low pH and higher concentrations of sulfate, total Fe, calcium, and magnesium, represented as geo-environmental stressors for microbial adaptation in the studied river sediment samples. All the observed specimens were categorized with high TOC, TC, EC, OM, and low TS, which designated a favorable condition for bacterial growth. Likewise high levels of Tl in downstream, midstream and upstream sediments delivered an ideal site to study microbial community response to high Tl contents.

The distribution of Tl as well as Fe peaks in SEM–EDS analysis (Figure 2 in Supplementary Material) designated that Tl exists in the form of oxides and sulfides in local river sediments (Buanuam and Wennrich 2010; Oves et al. 2013; Sun et al. 2016). Tl peaks with high intensity was perceived in local sediment samples collected from midstream (BSD1) and downstream (CSD2). The co-occurrence of Tl and Fe peaks indicated potential adsorption of Tl by Fe oxyhydroxides that are chief carrier phases for Tl, and such adsorption is a common phenomenon as explicated formerly (Ashley et al. 2003; Oves et al. 2013). Consequently, low pH and relatively high Fe in BSD1 and CSD2 are reasonable for Tl adsorption. Nonetheless, the co-occurrence of Tl and Fe peaks by SEM–EDS analysis in local sediments samples provide supplementary evidences for co-precipitation hypothesis (Figure 2 in Supplementary Material).

Microbial community compositions in sediments

The Chao1 and Shannon indexes (Table 3) of diversity were also determined to evaluate the biodiversity and phylotype abundance in sediment samples (Saltikov et al. 2005; Savio et al. 2015). The downstream sediment samples obtained more intractable organic substrates from upstream, which leads to increase diversity indices (Shannon and Chao 1). On the other hand, the upstream samples that comprise seasonal fluctuations in the microbial communities include fewer diversity indices because of the influence of acid mine drainage. The widespread discrepancy in these indexes shows that microbial communities in different sediments, and even between samples in the same area, were dissimilar. Some OTUs that may not place within any distinguished taxonomic group were extremely enriched in the upstream samples (Figure S1 in Supplementary Material). For instance, sample CSD2-related OTU was highly abundant approximately 8.3%. Microorganisms belonging to the nominee phylum CSD2 have been mentioned the entire divisions of bacterial phyla that have yet to be cultivated or sequenced (McLean et al. 2013). OTU figured out the microbial community existence in sediment samples due to their higher relative abundances about 97% sequence resemblance (Aoki et al. 2014).

These OTUs in the studied sediment samples were allocated to phylum, class, order, family, and genus to pinpoint the diversity of microbial communities among these samples. *Proteobacteria* has accounted valid sequences and the most prevailing phylum in 12 out of 18 sequencing libraries, whereas *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Gemmatimonadetes* were effective sequences in all river sediment samples. Twenty-two percent of the total reads would not be classified beyond kingdom bacteria and archaea by RDP classifier (Figure S2 in Supplementary Material). This high percentage of unclassified sequences in the Tl-rich sediments suggests the existence of exclusive bacterial and archaeal species. One of the remarkable findings demonstrated that Tl concentrations were prominent in the downstream sediment samples with negative Eh values, which may promise for microbial community adaptation. We proposed that the higher concentration of Tl portrays partial influence on microbial groups because of microbial-mediated Tl cycle. Furthermore, numerous other geochemical parameters specifically sulfate, pH, and iron also represent tremendously impact on the microbial communities (Sun et al. 2016; Xiao et al. 2017). Moreover, the microbial compositions varied considerably in the 12 sequencing libraries at the genus level, while some genera showed relatively high abundances in sequencing libraries derived from the local river sediments. Similar findings at phyla and genus level have been stated previously from some mining-related areas (Fan et al. 2016; Giotta et al. 2006).

Microbial community structure with different Tl pollution gradients

Based on UPGMA tree (Figure 3) and PCoA analyses (Figure S3 in Supplementary Material), microbial communities in the local downstream, midstream, and upstream sediments varied. Overall more than 40 phyla were amended from the river sediments samples. In local river sediments, *Proteobacteria* in upstream, *Acidobacteria* in downstream, and *Chloroflexi* in midstream samples were more dominant, and these species might be tolerant to Tl in Tl-rich environmental media. On the other hand, the present study suggests that *Proteobacteria*, *Gemmatimonadetes*, *Verrucomicrobia*, and *Planctomycetes* are accountable for Tl biotransformation. For example, elimination of Tl and manganese by *Proteobacteria* was reported in mine areas (Li et al. 2014; Silva et al. 2012). In the present study, *Proteobacteria* and *Bacteroidetes* can be used as a monitor for Tl pollution, as well as a pointer for bioremediation of Tl, because microbes can survive at high Tl concentrations, through releasing certain ligands to precipitate Tl and reduce toxicity. The similar features of these bacteria to monitor heavy metals were also found in previous studies (Babu et al. 2014; Bao et al. 2014; Liu et al. 2013; Sun et al. 2012; Xie et al. 2016). Previous studies have displayed that Tl monitor microbes have high capabilities to bioadsorb and bioflocculate Tl in Tl-polluted soils, water and sediments (Plachno et al. 2015; Sun et al. 2012). Although heavy metals exert toxic effects on microorganisms through various mechanisms, certain heavy-metal-tolerant microbes, can live in the metal-polluted environments and are worthwhile for bioremediation of the polluted sites (Kang and So 2016; Pepi et al. 2011). *Proteobacteria* was reported to prefer neutral to some extent acidic pH for optimum growth in sediment samples (Azarbad et al. 2015; Staley et al. 2013). In the present study, majority (97%) of the *Proteobacteria*, *Stramenopiles*, and *Bacteroidetes* 16 rRNA sequencing reads were recognized as metals resistance due to the influence of sulfide-bearing minerals in local sediment sample of upstream GA1. On the contrary, among these factors, metals have major impact on the distribution of bacteria. The dominant bacteria in upstream, midstream and downstream sediments at genus level comprised of *Thiobacillus*, *Kaistobacter*, *Meiothermus*, *Rhodobacter*, *Thiovirga*, *Sulfuricurvum*, and *Ralstonia*, and these genera contain species that could be tolerant to metals were also found in other studied metal-rich environments (Fan et al. 2016; Kang and So 2016; Savio et al. 2015; Wang et al. 2015; Yoon et al. 2010).

In LEfSe (Figure 4), there were four taxonomic groups from family, class, genus, and order to phylum enriched in the midstream, upstream and downstream sediment samples, specifically, *Thermoleophilia* (class), *Gemmatimonadetes* (genus), *Acidobacteria* (family), *Proteobacteria* (phylum), *Rhodobacteraceae* (family) and *Rhodobacterales* (order). The bacterial lineages enriched in the upstream sediment samples were *Proteobacteria* (phylum), while in midstream *Rhodobacteraceae* (family) and *Rhodobacterales* (order) in downstream, respectively. Among these bacterial taxa, only *Proteobacteria* (phylum), *Gemmatimonadetes* (genus), *Acidobacteria* (family), *Rhodobacteraceae* (family), and *Rhodobacterales* (order) showed LDA values greater than 4.5 in the Tl-rich river sediment samples (Figure 4). The LEfSe explored that the microbial communities were affected by high levels of Tl due to local sulfide mineralization. Our results also support that frequent bacterial taxonomic groups holding sulfate and Tl reducing bacteria such as *Syntrophobacterales* (order) and *Proteobacteria* (phylum) had higher LDA scores in the heavily Tl-contaminated sediments, suggesting the tolerance of reducing bacteria to elevated Tl concentrations in contaminated river sediments (Babu et al. 2014; Huang et al. 2012). Furthermore, the species *Proteobacteria* belonging to *Actinomycetales* (genus) contain genetic information for Tl resistance; *Thiobacillus* (genus) of *Rhodobacteraceae* was a sulfur-oxidizing bacterium as previously reported (Hsieh et al. 2004; Monchy et al. 2006). In the present study, the upstream sediments favor to enrich Tl resistance bacterium *Proteobacteria* (phylum), and *Actinomycetales* (family), represent intense impact of acid mine drainage, whereas midstream sediments favor to improve *Rhodobacteraceae* (family), and downstream sediments are characterized as sulfur-oxidizing bacterium *Hydrogenophilaceae* (family) and *Rhodobacterales* (order) because of sulfur and iron compounds that might have influenced the microbial communities primarily by controlling the distribution of Fe and sulfur metabolizing microorganisms (Sun et al. 2015; Vannini et al. 2008). Previous studies reported that these bacteria from mining areas are capable of oxidizing ferrous iron and reducing sulfur, and such that many microorganisms can survive or resist in the Tl-rich environment (Guo et al. 2009; Hsieh et al. 2004; Joe et al. 2007; Moncur et al. 2009).

Heat map groups containing dominant species of river sediment samples like *Actinobacteria*, *Euryarchaeota*, and *Proteobacteria* (Figure 5), enriched along the Tl mineralize area, might play vital role in mobilization of Tl, which may consequently be reduced by Tl reducing bacteria (*Actinomycetales*), and the similar findings were also previously reported (Bao et al. 2006; Huang et al. 2012). The present study pointed out that *Actinobacteria* presented high abundances in local Tl-rich river sediments, which suggested for implication for Tl mobilization in river sediments. Our findings also supported that *Shewanella*, and *Proteobacteria* were capable of reducing Tl under different redox conditions (Fredrickson et al. 2002; Saltikov et al. 2005). *Geobacter* and *Euryarchaeota,* which was also well-known to have ability for reducing metals (Bao et al. 2006; Heidelberg et al. 2002). In the present study, we found that *Thermotogae, Chryseobacterium,* and *Nitrospirae* tend to enrich in downstream, and they were observed resistance to many metals (Moreira et al. 2016), whereas *Cyanobacteria*, *Kaistobacter*, *Rhodobacter*, and *Euryarchaeota* enhance in midstream sediments, and they may struggle in Tl-polluted sediments, and similar findings were reported from previous studies (Mergeay et al. 2003; Mu et al. 2016). Likewise, *Proteobacteria, Rhodobacter, Flavobacterium, and Aminincenantes* enrich in upstream sediments, and they could be tolerant to many metals as previously described (Giotta et al. 2006; Navarro-Noya et al. 2010).

Microbial response to the geochemical parameters

Spearman's rank correlation analysis provided correlations among bacterial taxa and noteworthy environmental factor in the study area (Figure 6; Tables S2 and S3 in Supplementary Material). It is critical that *Acinetobacter* and *Cyanobacteria* were significantly correlated with Tl, and suggested for Tl enrichment in local river sediments. The presence of various Tl-related genes and their positive correlation with contaminant parts indicated the potential for biotransformation and resistance of Tl, constrained by the sediment microbial communities. The common feature of these bacteria is characterized for resistance to toxic heavy metals (Liu et al. 2016; Yoon et al. 2010). We found that *Hydrogenobacter* is another genus positively correlated with Tl ($p < 0.01$). *Cyanobacteria* (phylum) and *Hydrogenobacter* (genus) contain species that are aerobic sulfide-oxidizing species, which can be used to reduce sulfate and Tl derived from weathering of sulfide minerals (Oves et al. 2013; Sun et al. 2012). Sequences assigned to the phylum and genera such as *Cyanobacteria*, *Gemmatimonadetes*, *Actinobacteria*, *Acidobacteria* and *Proteobacteria* revealed high relative abundances in Tl-rich environment (Bao et al. 2014; Kurahashi et al. 2009). In this present study, various *phylotypes* were positively correlated with total Tl, proposing that higher Tl in river sediments could restrain them as much as other microbial taxa. However, bacteria affiliated with *Flavihumibacter* from local Tl-contaminated river sediments were perceived as Tl-oxidizing bacteria, which figured out a relationship of this genus with the Tl-rich environments.

CCA analysis (Figure S4 and Table S4 in Supplementary Material) indicated strong effects on overall microbial communities impacted by geochemical parameters such as TOC, TC, sulfate, total Fe and Eh. The environmental conditions, for example, low pH and high Fe and sulfate concentrations, acted as environmental stressor for microbial adaption. CCA was performed to observe how microbial groups adapt to Tl-polluted environments. Sulfate and total Fe were the main geochemical factors that affected the microbial communities, as designated by CCA (Figure S4 in Supplementary Material). In the Tl-polluted environments, iron and sulfur compounds have impacted the overall microbial groups fundamentally by controlling the conveyance of Fe and S to microorganisms. Total Fe was the key factor that critically shapes the whole microbial communities (Figure S4 in Supplementary Material). Elevated total Fe may encourage the development of Fe-metabolizing bacteria, principally in acidic Fe-rich situations, for example, acid mine drainage (Baker and Banfield 2003; Sun et al. 2015c). Sulfate, which has been observed as crucial geochemical parameters in shaping microbial communities (Sun et al. 2015), represented as one of the most critical factors for organizing the bacterial population in the present study. Sulfate concentrations affected the overall bacterial groups, for example, *Desulfuromonas*, and *Desulfobulbus* were more abundant in upstream and downstream sediments with relatively high sulfate concentrations. In the present study, TOC and TC in local sediments significantly linked to microbial communities. The distribution and composition of TOC and TC could modify the distribution of microorganisms like *Cyanobacteria*, *Anaerolinea*, and *Polaromonas*, which are capable of degrading certain organic matters (Navarro-Noya et al. 2010; Sun et al. 2016).

CCA exhibited a significant relationship between microbial community composition and pH. In downstream sediment samples where pH was moderate, though, expanded amounts of neutrophils were available and consequently changed the overall microbial community. This observation is consistent with the previous investigations on acid mine drainage (Kuang et al. 2013). It was proposed that pH impacts microbial survival directly or potentially through control of geochemical parameters that are definitely related to sediment pH, for example, nutrient accessibility and cationic metal solubility (Miao et al. 2006). The pH shaped the overall microbial communities based on its vector length (Xiao et al. 2016). It is reported that the intracellular pH of most microorganisms is generally <2, and any significant divergence in the environmental pH would impose stress on the microorganisms (Fierer and Jackson 2006). Thus, even though pH did not vary considerably in local river sediments (pH at 3.6–6.89, Table 1), the slight variation of pH might not directly influence the distribution of neutrophilic bacteria, but it may be enough to affect the mobility and bioavailability of Tl in the Tl-rich environment, which could further directly affect the microbial communities (Pokrovsky et al. 2014; Xiao et al. 2016). Nevertheless, more likely the pH gradients in the Lanmuchang Tl-mineralized area might affect the mobility of Tl and other metals. The redox potential (Eh) was also recognized as an essential parameter for structuring the microbial community, and it dramatically changed in upstream to downstream sediments. In summary, microbial community may play an imperative role in weathering of Tl-rich sulfides and biogeochemical cycling of Tl. Further investigations are required to elucidate their specific microbial processes.

Conclusions

In the present study, we characterized the microbial community and their responses to Tl pollution in river sediments from a Tl-mineralized area, based on high-throughput 16S rRNA sequencing genes. The UPGMA and PCoA evaluation exhibited prevailing existence of *Proteobacteria*, *Acidobacteria* in addition to *Chloroflexi* in the Tl-polluted river sediments, which suggest that these species exemplify tolerance to Tl. The LEfSe explored that the microbial communities were affected by elevated Tl due to weathering process of sulfide mineralization. The CCA analysis confirmed that geochemical parameters (pH, Eh, TOC, sulfate, total Fe, and Tl) represented considerable impacts on the overall microbial community. Spearman's correlation analysis indicated that a number of the microbial phylotypes such as *Cyanobacteria,* and *Spirochaete* were positively correlated with Tl in local sediments, suggesting potential roles of these phylotypes in microbial Tl cycling. The heat map analysis proved that *Actinobacteria* presents high abundances in local Tl-rich river sediments, which suggested for implication for Tl mobilization in river sediments. Overall, *in situ* microbial community sequence provided a worthwhile tool for monitoring the sediment quality. The present study enhanced our understanding of microbially mediated Tl interaction and the ability to predict metabolism of uncultured microbes in similar Tl-contaminated areas, which could help to contribute to bioremediation of Tl-related contamination. This research imparted a strong manifestation of widespread bacterial diversity in T1-polluted river sediments and also provided substantial data for further implementing *in situ* bioremediation strategy as well. Further investigations are required to elucidate the specific microbial processes in Tl-polluted river sediments.

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Disclosure statement

The authors declare that there is no conflict of interest.

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