Microbial diversity response in thallium polluted riverbank soils of the Lanmuchang

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ABSTRACT

Thallium (Tl) is a toxic element, but little is known about microbial communities' response to TI mobilization and sequestration. Here, we characterize the microbial communities and their feedbacks to Tl-pollution in riverbank soils to understand the distribution of microbial metal tolerance. These soils have been affected by pollution sourced from a Tl-rich mineralized area in Lanmuchang, Guizhou, China. In all studied soil samples, *Proteobacteria, Acidobacteria, and Actinobacteria* were revealed relatively in higher abundance at the phylum level. The results indicated that a number of microbial communities including *Gemmatimonadetes, and Actinobacteria* were correlated with total Tl, suggesting potential roles of these microbes to Tl tolerance. The patterns of phylogenetic beta-diversity in studied samples showed a high diversity of the microbial community in soils with high Tl concentrations. Sequence analysis of microbial community indicated that most of the environmental parameters in soils were associated with the major phylogenetic groups such as *Gemmobacteria, Bryobacteria, Proteobacteria, Actinobacteria, Firmicutes,* and *Rhodobacteria*. Some species of microbes, *Nocardioides* (genus), *Actinomycetales* (Order), *Ralstonia* (phyla) and *Sphingomonas* (genus) might are tolerant of Tl. These results provide direction to the microbial communities in the presence of elevated Tl concentration in Lanmuchang and shed light on bioremediation of Tl polluted locations.

1. Introduction

Thallium (Tl) is a non-essential toxic element for human, animals, plants, and microbes in the earth crust (Peter and Viraraghavan, 2005; George et al., 2018; Liu et al., 2019). Weathering of sulfide-mineralized rocks is the natural source of TI pollution (Wierzbicka et al., 2004; Lindsay et al., 2015), whereas coal combustion and ferrous/non-ferrous smelting are anthropogenic sources (Peter and Viraraghavan, 2005; Jiang et al., 2018). Co-contamination of Tl and arsenic (As) is generally observed in areas surrounded by mining works (Majzlan et al., 2010;

George et al., 2018). Therefore, naturally weathering of thallium-rich sulfide ore, mining and smelting activities have also been found the major sources of Tl contamination (Xiao et al., 2004; Kabata-Pendias and Mukherjee, 2007).

Many studies have demonstrated variations in the microbial diversity, composition, and abundance of environmental elements produced from alkaline or acid mine drainage (AMD) or trace element polluted soils (Azarbad et al., 2015; Bier et al., 2015). Usually, geochemical behaviors of Tl are controlled by various physicochemical factors such as organic matter (OM), Fe–Mn oxides, total organic matter

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(TOC), Eh, and pH (Mitsunobu et al., 2010; Shsharma et al., 2010; Filella, 2011; Liu et al., 2019). Therefore, the migration of the microbial community and speciation of Tl could vary with geographical location (Ettler et al., 2010; Yang et al., 2015). In addition, microorganisms can also facilitate bio-geochemical cycling of Tl by mobilization, reclamation, and precipitation (Hamamura et al., 2013; Kulp et al., 2014). Microbial activities play an important role in processes of oxidation or reduction of sulfides (Schippers et al., 2010; Hamamura et al., 2013), and thus, influence the mobility and speciation of toxic metal(loid)s (Majzlan et al., 2010; Jiang et al., 2018).

Microbial diversity in stream water, sediment, and soils are intensely affected by mine discharge waste (Kuang et al., 2013; Sun et al., 2015). In the places where erosion is caused by mine waste, 56 microbial groups tolerant to corrosive/alkaline, high sulfate, and high metal conditions are frequently discovered, such as, the acid tolerant microbes *Gammaproteobacteria, Nitrospira* and *Euryarchaeota*, and sulfate-reducing *Desulfobacteraceae* (Duester et al., 2005; Bier et al., 2015). The richness and diversity of prokaryotes are extremely large in the soil while the origins of the biological complexity and the factors eventually regulate the growth of microbial community composition (Fierer and Jackson, 2006; Bier et al., 2015). Prokaryotic microbes that are metabolically dynamic in acidic condition limits (pH $<$ 4) were observed to be phylogenetically enhanced, subsidiary inside the phyla *Proteobacteria, Firmicutes, Actinobacteria, Nitrospirae* and *Acidobacteria* (Baker and Banfield, 2003; Swenson, 2011). Therefore, many microbes exist in Tl-rich environments such as planktonic bacteria could oxidize Tl (I) to Tl (III) (Twining et al., 2003; Liu et al., 2019), as well as thallium-tolerant bacterium was isolated from soil of sulfide mineralized area (Majzlan et al., 2010; Bao et al., 2014). However, some of the microbes like *Ralstonia, Arenimonas,* and *Nocardia opaca* were suggesting potential role of these microbes to metal tolerance (Hsieh et al., 2004; Monchy et al., 2006).

Among the techniques to remediate Tl contaminated locations, microbial changes of Tl by encouraging in situ immobilization and precipitation are the most favorable methods. The little data is available about the toxicity of Tl to microbial gatherings in the soil. Mineralization of Tl-rich sulfide, mining, and smelting activities are causing extreme Tl pollution in soils surrounding Qingshui stream in Guizhou province, China. However, it is still unclear whether the microbial communities in Qingshui stream have extensive tolerance for demonstrating new diversities that are evolved and adjusted in Tl contaminated soils. Moreover, the knowledge of the relationship between the microbial community and Tl-rich situations is still inadequate. The response of the microbial community to the environmental factors is unclear, and the most significant geochemical factors to reshape the microbial communities are unknown. For this reason, we investigated Tl-polluted soils located in Lanmuchang. To our knowledge, this is the primary report to examine (1) the impact of Tl pollution on the soil microbial community structure and diversity, (2) relationship between the microbial communities and diverse geochemical constraints; exclusively those directly accompanying to Tl contamination, and (3) the prospective metabolic paths of the soil microbial environments of the studied area in Lanmuchang. This study targets to explore microbial diversity in the soils, which are extremely contaminated by Tl. The conclusions would be substantial for potential implications on biomonitoring of Tl contamination.

2. Materials and methods

2.1. Study area description

The selected research region is situated in Lanmuchang, Guizhou Province, China (Fig. 1), which was basically developed for residential and agricultural purposes. The elevation of the study area ranges between 1000–1600 m (above sea level) with a relative relief ranging between 200–600 m. Pinnacles, despondencies, valleys, caverns,

underground streams, and sinkholes collectively represent karstic geography of the selected region. Qingshui is a local and primary feeding tributary in Lanmuchang, which flows into an innate wetland passing through a Tl mineralized area (Xiao et al., 2003). The water from Qingshui stream is utilized for drinking and farming purposes (Xiao et al., 2003). It is facing mundane anthropogenic effects because of highly populated areas along the whole length of stream, burden of serve mining and smelting exercises near midstream, and metal-related creation near downstream. In our previous studies, sulfide mineralization and nearby topography have been portrayed in detail (Xiao et al., 2003; Sun and Cupples, 2012). In summary, the high quantities of Tl have been brought into nearby water, slit, soils, and crops by practices of mining and exploring Tl-rich minerals and horticultural activities. The contamination of the local environment with Tl-pollution has become a health risk for local inhabitants (Xiao et al., 2012; Sun and Cupples, 2012), who are suffering from severe pain in muscles and joints, intense weakness, hair loss, sleeping disorder, and vision loss (Xiao et al., 2004).

2.2. Sampling and analysis

Soil samples were collected from the bank of Qingshui stream in May 2016 (Fig. 1). The collected samples were solidified by ice collar in the field. For geochemical analysis and DNA extraction, the samples were stored and frozen at 4 °C and −20 °C after transported to the lab, respectively. The soil samples were dried at room temperature, then pestle and mortar machine were used to ground the dried samples, and finally passed through a 100-mesh sieve to separate larger particles. To quantify pH, electrical conductivity (EC), and Eh, mixtures of 10 g dry ground soil sample and 25 ml Milli-Q water were prepared in 100 ml conical flask, shaken for 1 h, and then equilibrated for 30 min. Finally, the above-mentioned parameters were estimated using an aligned HACH HQ30d (HACH, Loveland, USA) pH meter.

A mixture of 10 g dried ground soil sample and 50 ml Milli-Q water was prepared in a 100 ml Erlenmeyer flask to estimate sulfate. After shaking the mixture for 5 min, it was kept still for 4 h to achieve equilibration. The supernatant was centrifuged at 3500 rpm and separated through a 0.45 mm filter membrane. The anions of obtained samples were quantified based on ion chromatography (DIONEXICS-40, Sunnyvale, USA). Likewise, an elemental analyzer (Elementar, Hanau, Germany) was used to estimate the total carbon (TC), total sulfur (TS), total organic carbon (TOC), and total hydrogen (TH) in the samples of soil (Xiao et al., 2016). $K_2Cr_2O_7$–H_2SO_4 oxidation method was adopted to determine soil organic matter (OM). The samples were completely processed with HF (5:1, v/v) and HNO_3, then inductively coupled plasma optical emission spectroscopy (ICP-OES, iCAP6500, Germany) and inductively coupled plasma mass spectrometry (ICP-MS, Agilent, 7700x, USA) were used to estimate cations and trace elements (Xiao et al., 2012; Rasool and Xiao, 2018). The precision testing was performed using confirmed referral materials (SLRS-5) and inside standard (Rh). The investigative accuracy decided given the standard quality control methods using globally ensured reference materials (AMH-1, OU-6, GBPG-1, Montana Soil SRM 2711a), duplicates, and blanks were better than to ± 10%.

2.3. Mineral composition and morphology analysis

Minerals synthesis and morphology were examined by scanning electron microscopy (JSM-6460LV, JEOL, Japan) and vitality dispersive X-beam spectrometry (EDAX-GENESIS, USA) (SEM-EDS). Usually, SEM operates at 15 kV with a 10 mm working separation for EDS examination. However, a quickening voltage of 20 kV was applied to get adequate X-beam tallies (Fig. S1).
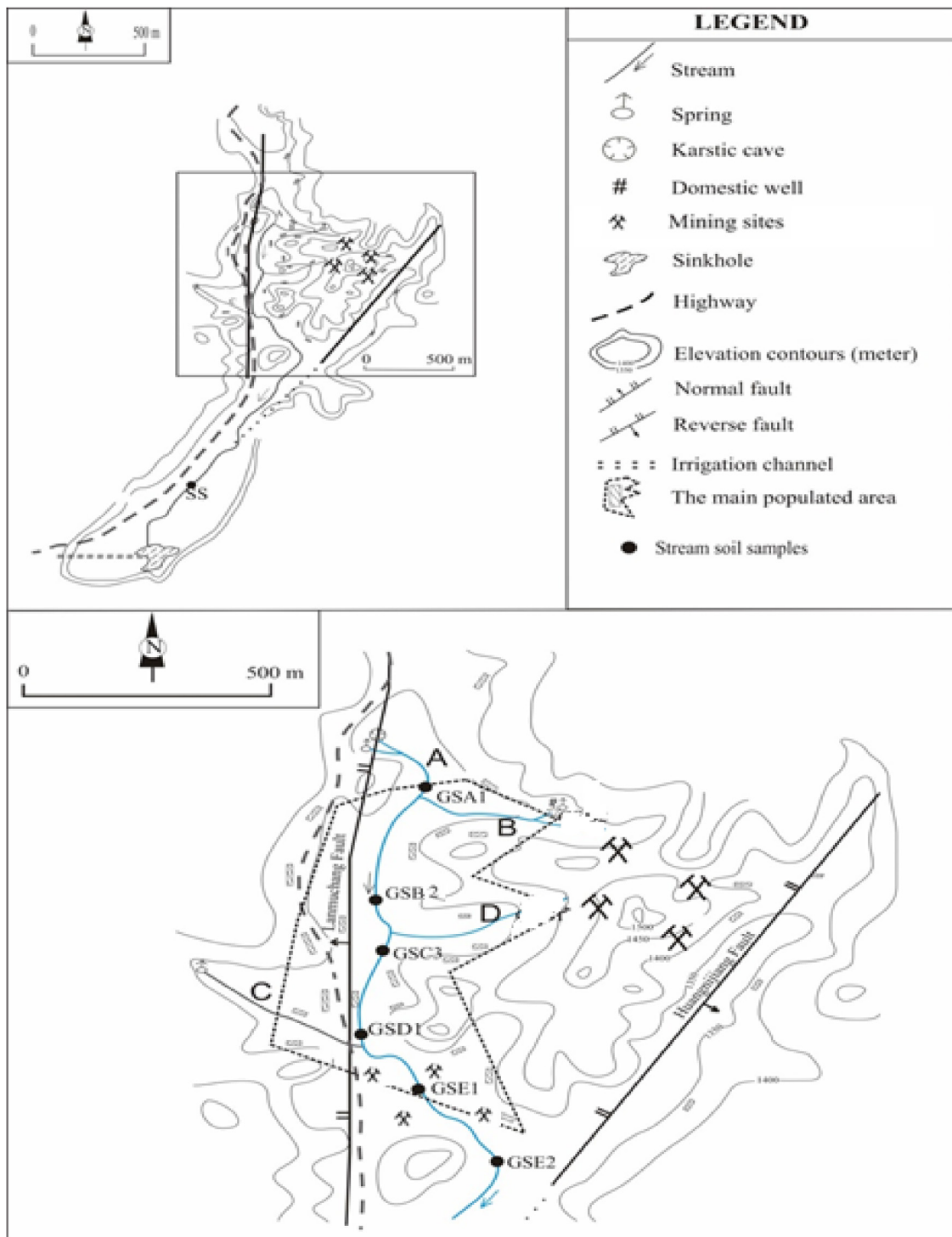
Fig. 1. Map showing the soils sampling sites of Lanmuchang study area, southwest Guizhou.

2.4. High-efficiency sequencing – V3V4 region of 16S rRNA genes

From 250 mg of soil sample, total genome DNA was extracted using FastDNA® turn unit (MP bio, USA) based on the methods indicated by the producer's convention. One percent agarose gels were used to test the purity and concentration of DNA. Sterile water was used to weaken the DNA up to 1 ng/μL. The 16S rRNA characteristics of undeniable region (V3V4) were escalated using the 515f/907r starter set (907r: 5′-CCYCAATTCMTTTRAGTTT-3′, 515f: 5′-GTGYCAGCMGCCGCGG-TAA-3′) (Ren et al., 2014; Xiao et al., 2017). IlluminaHiSeq2500

arranged at Novo quality bioinformatics association in Beijing was used to sequence the library. Matched end peruses were allocated to tests in the light of their extraordinary scanner tag and truncated by disposing of the standardized identification and groundwork grouping. Quantitative Insights into Microbial Ecology (QIIME V1.7.0, http://qiime.org/index.html) was used under specific conditions (Caporaso et al., 2010; Bokulich et al., 2013) to isolate brilliant clean labels. Finally, the powerful labels were acquired by evacuating fabrication arrangements using the reference database (Gold database, http://drive5.com/uchime/uchime_download.html) and UCHIME (Edgar, 2018).

Arrangements with more than or equal to 97% alikeness were allocated to similar operational taxonomic unit (OTUs), utilizing UPARSE programming (http://drive5.com/uparse/). Neat data was analyzed using the Green Gene Database (http://greengenes.lbl.gov/cgi-container/nph-index.cgi) and RDP classifier (V2.2) based on delegate groupings. MUSCLE programming (V3.8.31) was used to examine phylogenetic relationships among various OTUs. Abundance data of OTUs were standardized by a pattern of succession number based on the sample data arrangements. Subsequent investigations of alpha and beta diversity and variety were altogether performed based on standardized information, respectively.

2.5. Data analysis

The grouping was analyzed through QIIME and UPARSE programming (Caporaso et al., 2010). First, the reads were shifted by means of QIIME quality channels based on the standard configuration for Illumina handling. Then OTUs were assigned using UPARSE pipeline based on 97% similarity. A typical sequence was chosen for each OTU and applied to relegate the taxonomic composition using RDP classifier (Wang et al., 2007). The extravagance of species was assessed by calculating the Simpson, Chao 1, and Shannon indices for eleven libraries as represented earlier (Schloss et al., 2009). GraPhlan and Phylo tree were strained by Kruskal-Wallis rank whole to recognize highlights with essentially extraordinary plenitudes of relegated taxa and perform linear discriminant analysis (LDA) to evaluate the impact size of each component with a standardized relative abundance network (Segata et al., 2013). The relation between different geochemical parameters of soils samples was organized by Spearman rank correlation utilizing SPSS software (Version 19), with statistically significant value $p < 0.05$, respectively.

3. Results and discussions

3.1. Environmental factor description

The physicochemical characteristics were analyzed to highlight their interceding role in microbial metabolism and Tl transformation (Table 1). The pH was significantly higher in midstream ranging from 3.53 to 5.97, whereas in upstream ranging from 4.83 to 5.42 and downstream ranging from 3.85 to 4.85 respectively. The EC was significantly higher in upstream soil samples ranging from 131 to 420 μS/cm, then midstream and downstream samples, respectively. The redox potential Eh was found relatively higher throughout the samples (ranging 282 to 244 mV in upstream, 243–278 mV in midstream, and 233–288 mV in downstream soils, respectively), which indicates the higher toxicity in these sites. The results exposed that pH, EC, and Eh were significantly different in soil samples due to the process of mineralization during monsoon season in Lanmuchang (Xiao et al., 2004; Liu et al., 2019). The pH was intensely shaped the inclusive microbial communities established on its concentration discrepancies (Edwards and Santini, 2013; Savio et al., 2015). Likewise, in studies soil samples

EC and Eh were found significant, which can facilitate to reveal a variety of microbial diversity growth (Fierer and Jackson, 2006; Zhang et al., 2012). The sulfate concentration ranges were between 10.65 and 20.17 mg/kg in upstream, 21.23 to 7.24 mg/kg in midstream, and 26.08 to 22.74 in downstream areas, respectively. The higher sulfate concentrations in downstream areas were might be due to acid mine drainage release during the stormy season (Table 2). Although the maximum values of Cl⁻ 8.86 mg/kg in upstream, Ca^{2+} 3931 mg/kg in midstream and Mg^{2+} 3068 mg/kg significantly noted in midstream soil samples (Table 1). Significant differences were noted among different geochemical parameters like total organic carbon (TOC), total sulfur (TS), organic matter (OM), total hydrogen (TH), total nitrogen (TN), and total carbon (TC) in the soils of upstream, midstream and downstream areas (Table 2). Most samples of the soil had higher values of TN, TS, TH, and TC with concentrations of 3.05, 1.69, 10.07, and 47.16 g/kg, respectively. However, the maximum concentrations of TOC and organic matter (OM) were 35.89 g/kg and 61.73 g/kg in downstream samples followed by upstream and then midstream samples, respectively. In our current study, all samples of midstream soil were categorized as high TOC, TC, TN, and OM specifying a suitable condition for bacterial enlargement (Xiao et al., 2016; Rasool and Xiao, 2018; Sun et al., 2019). Therefore, Lanmuchang stream soil has the appropriate pH and maximum quantity of TOC, which may impact the dissemination of microbial activity (Liang et al., 2011; Xiao et al., 2016; Rasool and Xiao, 2018). Insignificant differences were pointed out for average total Fe concentration among the upstream, midstream and downstream soil samples. Total Mn was noted significantly higher in midstream soil samples ranging between 94 and 627 mg/kg followed by upstream (272–404 mg/kg) and downstream soil samples (68–262 mg/kg) (Table 1). All soil samples from the bank of stream showed elevated concentrations of Tl, which were ranged between 5.27 and 10.19 mg/kg in upstream, 11.95–13.17 mg/kg in midstream, and 1.57–27.94 mg/kg in downstream areas, respectively (Table 1). The quantity of Tl in downstream soil samples was more significant in the present study area, which was consistent with the exhaustive disintegration of sulfide-bearing rocks and mining surplus (Xiao et al., 2004; Rasool and Xiao, 2018). At the bank of downstream areas, soil had the highest levels of Tl indicating the direct spillage at the mining and smelting sites. Soil pH, total Fe, and Tl were found to be the most vital variables for influencing microbial communities. Numerous investigations have evaluated the effects of above-mentioned and other comparable variables to be significant as well (Zhao et al., 2013; Zhang et al., 2015; Sun et al., 2017).

Tl was indicated as one of the vital elements in the mineralized formation of soils based on the SEM-EDS analysis. Stimulatingly, the presence of Tl peaks was collected with Fe, Ca, Si and O peaks (Fig. S1). The dispersal of Tl, just as Fe and O peaks in SEM-EDS investigation, described that Tl may exist as oxides and sulfides in the soil, which implies the presence of thallium bearing mineralization, frequently detected in earth crust (Buanuam and Wennrich, 2010). Tl-peaks with higher concentration was observed in the midstream soil, which was taken from the sample (GDS1). The joint occurrence of Tl with total Fe peaks attributed to the potential adsorption of Tl to Fe oxyhydroxides,

Table 1

Physicochemical parameters in bank of stream soil samples of Lanmuchang area southwest Guizhou (mean ± standard deviation of three measurements).

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
GSA1	5.42 ± 0.11	131 ± 1.2	244 ± 1	1.78 ± 0.35	1282 ± 32	775 ± 29	52,674 ± 116	272 ± 12	5.27 ± 1.22
GSB2	4.83 ± 0.09	420 ± 1.1	282 ± 0.9	8.86 ± 1.35	899 ± 26	943 ± 33	49,746 ± 112	404 ± 17	10.19 ± 2.32
Midstream
GSC3	5.97 ± 0.12	354 ± 1.2	243 ± 8	3.05 ± 0.76	3931 ± 45	3068 ± 71	52,250 ± 121	627 ± 19	13.17 ± 1.4
GSD1	3.53 ± 0.10	125 ± 1.1	278 ± 6	1.98 ± 0.45	185 ± 32	235 ± 23	49,730 ± 114	94 ± 15	11.95 ± 1.9
Downstream
GSE1	3.85 ± 0.22	127 ± 1.83	288 ± 11	2.65 ± 0.54	205 ± 1.2	291 ± 11	55,628 ± 123	68 ± 1	27.94 ± 0.8
GSE2	4.85 ± 1.1	85 ± 1.2	233 ± 9	4.04 ± 0.96	97 ± 1	492 ± 13	43,324 ± 124	262 ± 14	1.57 ± 0.7

Table 2
Major elementary structures in Lanmuchang study area (mean $\pm$ standard deviation of three measurements).

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							
GSA1	2.29 ± 0.13	30.37 ± 1.1	10.07 ± 1	0.37 ± 0.05	27.44 ± 0.91	47.19 ± 1.1	10.65 ± 0.82
GSB2	3.05 ± 0.15	36.48 ± 1.3	10.04 ± 0.91	0.43 ± 0.08	28.69 ± 0.93	49.35 ± 1.2	20.17 ± 1.0
Midstream							
GSC3	2.36 ± 0.08	47.16 ± 1.2	9.29 ± 0.91	0.49 ± 0.08	14.73 ± 0.71	25.34 ± 1.1	21.23 ± 0.91
GSD1	2.45 ± 0.12	37.28 ± 0.92	9.46 ± 0.93	0.28 ± 0.05	31.28 ± 0.91	60.82 ± 1.6	7.24 ± 0.9
Downstream							
GSE1	2.38 ± 0.09	43.43 ± 1.1	7.24 ± 0.65	1.69 ± 0.07	35.89 ± 1.1	61.73 ± 1.2	26.08 ± 1.3
GSE2	0.92 ± 0.19	8.85 ± 0.91	9.12 ± 0.82	0.32 ± 0.08	7.01 ± 0.08	12.06 ± 0.93	22.74 ± 1.03

which is established by the recognition of Tl laterally stream soil samples (Fig. S1). Such adsorption is a general phenomenon as stated in previous studies (Savonina et al., 2012), thus, total Fe oxyhydroxides are the main source of Tl in midstream areas.

3.2. Microbial community composition

Analysis of Illumina sequencing resulted in the range from 49,794 to 65,694 reads per sample and agglomerated into 58,223 OTUs with 97% sequence resemblance (Table S1). However, several OTUs were not detected at lower taxonomic levels (for example, genus, family, etc.). These interpretations proposed that OTUs co-occurred in Table S1 may relate to Tl redox reactions or demonstrating vital role in mobilization of this metalloid (Aoki et al., 2014; Jiang et al., 2018). Rarefaction measurement revealed that the observed species increasing sharply before approaching a highland, more data only produce a small number of new species; the bacterial libraries from our samples represented the bacterial communities well (Fig. S2). The alpha diversity was computed following the similar workflow adopted in OUT sequencing. An excellent consistency was observed between replicates (Fig. S2) except in absolutely unfiltered data, which was highlighted by Shannon and Chao1 in analyzed species. The reduction in diversity with TI contamination was confirmed by the scheming of species' diversity (Shannon) and richness (Chao1) indices in the studied stream samples (Fig. S2). However, the OTU did not represent an adjacent matching with any identified cultivated isolates that deliver emphasis to the enigmatic character of *Firmicutes* related OTUs in Tl mineralized linked environments. Thus, exposure to Tl or a combination of these two resulted in a striking decrease in microbial diversity as depicted by rarefaction curves (Fig. S2). In the assessment, the stream soil samples raise diversity indices particularly Shannon and Chao1 comprise more reasonable bacteria. The higher concentrations of Tl can tolerant bacterial activity in downstream soil, designated the impact of mining and smelting to exploit in the Lanmuchang study area.

Using RDP classifier, various taxonomic dimensions from phylum to genus were grouped in each sample (Fig. 2). Based on the phylum analysis, it was identified that outright majority (95%) total 16S rRNA

sequences were made up of 10 bacterial phyla (Fig. 2). Fig. 2 summarized relative abundance for each sample of the phylum level. More than 40 bacterial and archaeal phyla were perceived in samples of stream soil. The most transcendent phylum in soil samples were *Acidobacteria* ($\approx 9.5\%$ of average reads) and *Actinobacteria* ($\approx 14.8\%$ of average reads) over the distinctive area of stream bank while *Proteobacteria* ($\approx 47.8\%$ of average reads) in midstream soil samples, respectively. The other dominant phyla were *Gemmatimonadetes, Chloroflexi, Firmicutes, Verrucomicrobia, Bacteroidetes,* and *Planctomycetes,* respectively (Fig. 2). In sequencing libraries, *Proteobacteria* was accounted viable arrangement and considered as the most conspicuous phylum, whereas *Chloroflexi, Gemmatimonadetes*, and *Actinobacteria* were dynamic successions in the entirety of stream soil samples of Lanmuchang. At the phylum level, Acidobacteria, Gammaproteobacteria, and the Bacteroidetes were most profuse species. Twenty-two percent of the total peruses would not be characterized beyond kingdom bacteria and archaea by RDP classifier. (Fig. 2).

3.3. Association between thallium contaminated environments with bacterial taxa

In GraPhlan five taxonomic classifications were observed (Fig. S3) i.e., class, genus, family, order, and phylum including *Gemmatimonadetes* (phylum), *Rhodobacterales* (order), *Acidobacteria* (phylum), *Phodoferax* (genus), *Proteobacteria* (phylum) respectively. Nevertheless, at the genus level, the top five-group abundance of bacteria in the downstream soil studied samples are Betaproteobacteria, and *Rhodobacter,* and *Firmicutes*, while in upstream soil samples are *Rhodoferax,* and *Hydrogenophaga* and in midstream soil samples are, *Microbacterium*, and *Rihozobiales bacteria*, respectively (Fig. S3). These genera comprise species reveal tolerance to heavy metals or were developed in the enriched metal environment of study area Lanmuchang. We recognized successive bacterial taxonomic assemblages containing thallium and sulfate-reducing bacteria, for instance, *Proteobacteria* (phylum) and *Rhodobacterales* (order) had higher LDA scores in severely Tl polluted study area attributed to mining activities (Segata et al., 2013; Liu et al., 2019). The species *Nocardia opaca* belong to *Acidobacteria* (phylum) *might be* encompass genetic material for thallium mobilization (Jiang et al., 2018; George et al., 2018), *Rhodobacterales* (genera), *Phodoferax* (order) and *Proteobacteria* (phylum) was a thallium-oxidizing bacterium in studied samples. The sulfur-oxidizing bacteria mainly Betaproteobacteria, R*hodobacter,* and *Firmicutes* were abundant in the downstream soil of Lanmuchang study area (Sun et al., 2019).

Despite being directly cultured from the soil, the bacteria from phylogenetic groups (Fig. 3) developed perceptible colonies after prolonged incubation periods usually spanning over many weeks (Cavaletti et al., 2006; Stott et al., 2008). In present work, the phylogenetic groups were identified to contain members from subclass *Rubrobacteridae* of the phylum *Actinobacteria*, in addition to other members of the genus, for example, *Gemmobacter,* and *Gaiella*. *Sphingomonas*, Flavobacterium, Nocardioide, and *Clostridium* were indicated as subdominant groups for soil, comprising 18%, 11.7%, 7.4%, and 5.4%, respectively. These
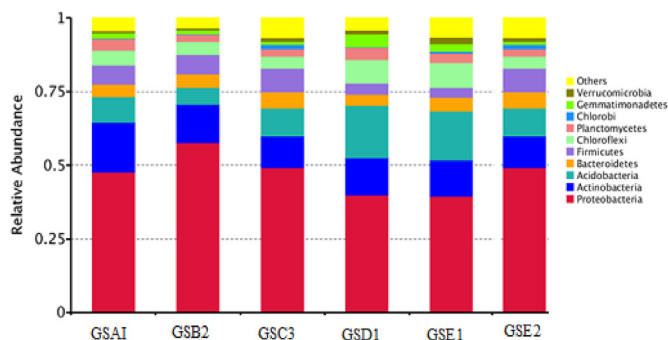

Fig. 2. Taxonomic classification of the bacterial and archaeal reads retrieved from soil samples at the phylum level from 16S rRNA using RDP Classifier.
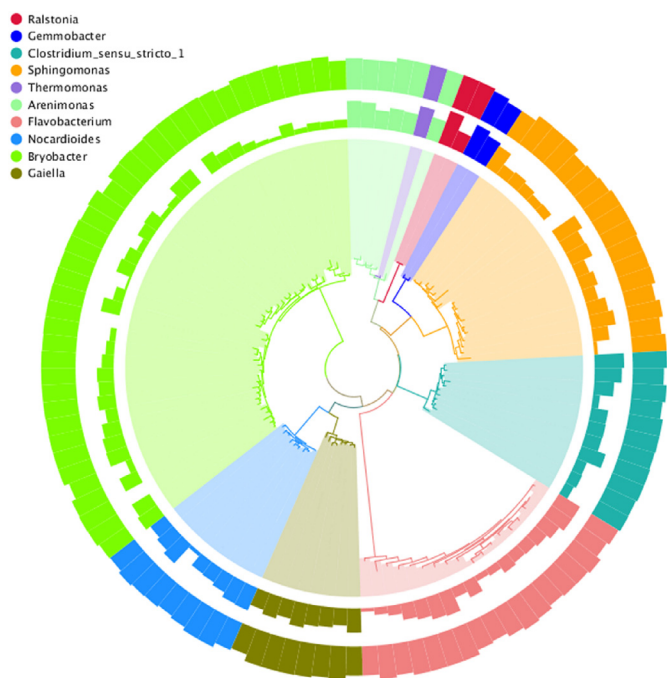
Fig. 3. Microbial communities composition in the soil samples were observed from the Phylo tree at genus and phylum levels. Phylogenetic tree of representative sequences of the bacterial isolates clustered at 97% similarity.

phyla represented approximately 70%–83% of bacteria detected in all soil samples of the study area (Fig. 3). The consequences indicated that most of the main bacteria among diverse samples are tolerance to heavy metals, particularly *Ralstonia, Nocardioides, Flavobacterium, Gemmobacter,* and *Gaiella* (Xiao et al., 2016; Liu et al., 2019).

Eight phyla were most abundant in the heat map (> 0.5%), including *Firmicutes, Acidobacteria, Bacteroidetes, Actinobacteria, Gemmatimonadetes, Cyanobacteria, Proteobacteria* in soil samples from stream banks (Fig. 4). At phylum level the heat map figure elucidate that *Actinobacteria, Nitrospirae, Gemmatimonadetes, Verrucomicrobia,* and *Bacteroidetes* in the downstream soils were more dominant; while *Phyllobacterium, Ralstonia, Rhodobacter, Gemmobacter* were more dominant in midstream soil samples, as well as *Arthrobacter, Acidiferrobacter,* and *Arenimonas* were most abundant in upstream soil samples (Fig. 4). Midstream samples depicted an abundance of *Phyllobacterium, Ralstonia, Rhodobacter, Gemmobacter,* recognized in the sulfide-rich environment of Lanmuchang. Previous research discovered that linkages for *Ralstonia* and *Rhodobacter* are considerably controlled by soil variables (Cao et al., 2015; Jiang et al., 2018). We found that *Actinobacteria, Nitrospirae, Gemmatimonadetes, Ralstonia, Rhodobacter, Gemmobacter, Acidiferrobacter* and *Arenimonas* tent to enrich in bank of steam soils, these bacteria play important role in distribution of metals, such as Tl, As, Mn, Cu, Pb (Akbulut et al., 2014; Moreira et al., 2016). Therefore, in current studied samples, *Rhodobacter and Arenimonas* were found to tolerant Tl in polluted stream soil (Huang et al., 2012). Instantaneously, *Actinobacteria* and *Proteobacteria* were capable to reduce total Fe, and Tl, under various redox circumstances (Saltikov et al., 2005). *Geobacter* and *Euryarchaeota* depicted distinguished capability and could reduce metals in soils (Lovley et al., 2004; Sun et al., 2019). *Thiobacillus* is pervasive in thallium-contaminated environments as they can obtain energy from the oxidation of Tl (Bryan et al., 2009). Desulfomonile tiedjei, a sulfate-reducing bacterium, is also extensively disseminated in Tl rich acid mine drainage (Bruneel et al., 2006; George et al., 2018).
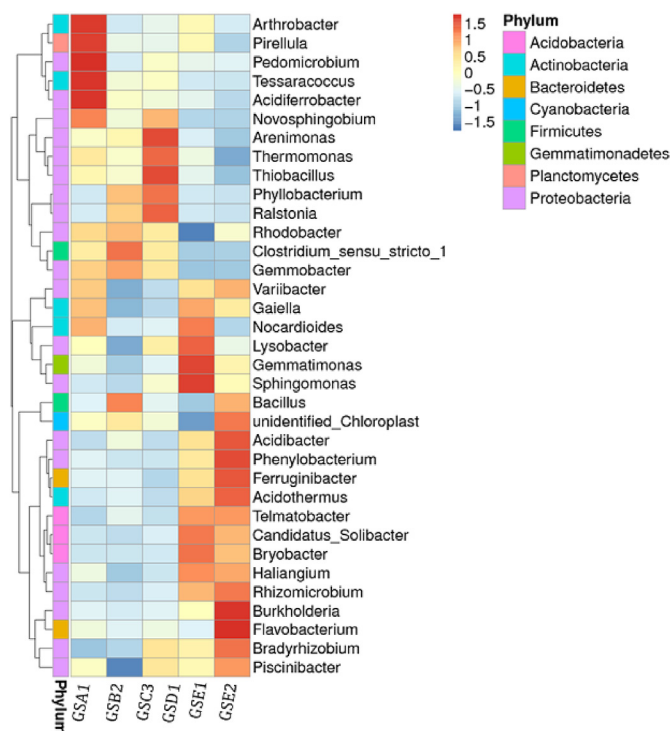

Fig. 4. Heat map analysis of dominant phylotypes with relative abundance greater than one in the soil samples. The value of relative percentage for the microbial genera is indicated by the color intensity, which can be found in the legend. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.4. Relationship between microbial communities and environmental parameters

The correlations between the geochemical parameters and microbial populations were explored using Spearman correlation index at phylum level (Table 3). In all investigated samples, taxonomic groups described that Tl was correlated positively with *Chloroplast* (r = 0.68), whereas negatively with *Actinobacteria* (r = −0.267) shown in (Table 3). A positive correlation between total nitrogen (TN) and *Actinobacteria* (r = 0.504) was observed. Similarly, Eh and *Acidobacteria* (r = 0.527) were correlated positively, whereas Eh had a negative correlation with *Thermoleophilia* (r = −0.614) and *Actinobacteria* (r = −0.517). The TOC had negative correlation with *Tenericutes* (r = −0.689) and positive with *Chloroplast* (r = 0.324), (Table 3). Positive correlations of *Actinobacteria* and *Acidobacteria* with TN and Eh indicate that environments with higher TN and Eh are favorable for these bacteria. Thus, *Chloroplast and Chlamydia* were positively correlated with Tl concentrations, which may suggest that mining, smelting and AMD environments may facilitate the growth of these bacteria. As per our investigations, the species of *Chloroplast* and *Chlamydia* are the aerobic and sulfide-oxidizing, which can be used to eliminate Tl in the mineralized area. Comparable findings were stated by (Finster et al., 2009).

The relative contributions of the geographic location and soil parameters to the impacts of Tl to the bacterial community were identified using Variance partitioning canonical correspondence analysis (VPA). VPA analysis indicated that geochemical factors (68.15%), contaminant factors (13.08%) and a combined factor of them (16.16%) could explain 97.4% of microbial communities' variations (Fig. S4). So, these two variables cannot explain only 2.60% of the community variation (Fig. S4). The outcomes of the variation partitioning exhibit that undergrowth type species enlightened a substantial portion of the microbes' composition buildup even after eliminating the effects of the

Table 3

Spearman rank correlation between selected geochemical data and major abundant with bacterial phyla in soil samples of the study area.

Parameters	Actinobacteria	Acidobacteria	Thermoleophilia	Solibacteres	Gemmatimonadetes	Acidimicrobiia	Chlamydia	Chloroplast	Tenericutes
pH	0.245	−0.393	−0.143	−0.399	−0.168	−0.320	−0.301	−0.132	−0.134
Eh	**−0.517**	**0.527**	0.302	0.495	0.370	0.579	**0.608**	0.218	0.304
EC	0.479	**−0.628**	**−0.609**	**−0.753**	**−0.622**	**−0.719**	−0.318	0.045	−0.663
TN	**0.504**	−0.494	**−0.614**	**−0.612**	**−0.605**	**−0.717**	−0.274	0.148	**−0.714**
TOC	0.219	−0.323	−0.589	−0.523	−0.587	−0.422	0.013	**0.324**	**−0.689**
TH	0.325	−0.347	−0.393	−0.308	−0.217	−0.677	−0.286	−0.104	−0.514
TS	−0.023	−0.446	**−0.841**	**−0.697**	−0.682	−0.600	0.092	0.530	**−0.878**
Cl	0.634	−0.397	−0.147	−0.363	−0.347	−0.482	−0.551	−0.355	−0.264
Sulfate	0.232	**−0.611**	**−0.787**	**−0.826**	**−0.797**	**−0.640**	−0.114	0.246	**−0.844**
Tl	**−0.267**	−0.104	**−0.459**	−0.447	−0.366	−0.153	**0.424**	**0.682**	**−0.506**
Fe	0.166	−0.071	−0.172	−0.014	−0.036	−0.266	−0.156	−0.054	0.009
Mg	0.444	−0.502	−0.201	−0.406	−0.207	−0.548	**−0.633**	−0.314	−0.234
Mn	0.104	−0.401	−0.081	−0.397	−0.029	−0.237	−0.115	−0.167	0.038
TC	0.356	**−0.512**	−0.566	**−0.607**	−0.601	−0.585	−0.310	0.249	−0.617
Ca	0.345	**−0.587**	−0.389	−0.545	−0.294	−0.598	−0.518	−0.040	−0.334

Bold significance illustrate, the correlation is significant at the 0.05 level..

above-mentioned environmental variables. These variables might be concentrations of metals comprised Tl, Ca, and total Fe which has been revealed to affect the species composition of microbial communities in soils of Lanmuchang. Correspondingly, in our studies soil samples (Carlson et al., 2010; Parkes and Duggan, 2012), the percentage of community variation illuminated by measured variables was only 97%. Conversely, the descriptive effect of each variable was not affected by the explained discrepancy of total Fe and Tl accounted for 16.16% of the environmental species owing to sulfide-bearing rocks disintegration in the study area.

4. Conclusions

In current work, our aim was to describe the diversity of microbial communities in thallium polluted soils near the stream banks in Lanmuchang area using high-efficiency sequencing. The geochemical factors including pH, Eh, TOC, TN, and TH are significantly impacted the microbial community. Spearman correlation analysis revealed that most bacteria such as *Chloroplast* and *Chlamydia* are significantly positively correlated with Tl, indicating that shaped the microbial community. But bacteria relating to *Thiobacillus* demonstrated their distribution of metal tolerance in study soil samples. We present GraPhlan, a novel method to generate high-quality circular phylogenies potentially integrated with diverse and high-dimensional metadata. In addition, this study provides the earliest evidence of selective enrichment of various bacterial activities with higher Tl in the soil. The current study provides innovative knowledge of the linkages of microorganisms with Tl and different geochemical parameters. Our current investigations mutually hold the scope to deliver basic knowledge for in-situ bioremediation strategy implications. It is recommended to investigate whether the bacterial diversity of Qingshui stream accumulate Tl or possess physiological variations conferring with TI. Additional experiments on microbial diversity in the future are required to further identify the microbial process in the study area.

Declaration of competing interest

The authors declare that there has no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.109854.

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