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Microbial Diversity and Community Structure in Alpine Stream Soil

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ABSTRACT

Investigations of microbial communities over arsenic-polluted soils in an alpine stream are vital to collect pieces of evidence for their *in situ*-mediated bioremediation. It is the first time that microbial community activities are described in arsenic-polluted soils due to natural and anthropogenic sources along the alpine stream. Greater than 40 phyla from domain bacteria the 16S rRNA Illumina sequencing amplicons are exposed. In overall samples, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) states that the most dominant phyla were *Proteobacteria*, *Cyanobacteria*, and *Actinobacteria*. Our result described that canonical correspondence analysis was used to elaborate more geochemical factors including arsenic, sulfate, total organic carbon, redox potential, and pH influence on the distribution of microbial groups in studies soil samples. Furthermore, Spearman's rank correlation demonstrates a positive correlation between several specific taxonomic groups, refers to the phylotypes' possible role in arsenic biogeochemical cycling. The present research could link the knowledge gap and facilitate our understanding for the bioremediation of arsenic at contaminated sites.

HIGHLIGHTS

- We described the microbial communities in arsenic-contaminated soils.
- CCA revealed that chemical parameters organized microbial communities.
- The effects of geochemical conditions on microbial community composition were examined.

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Introduction

Alpine stream plays its vital role in the river system around the world (Dos Santos et al. 2011) and has a profound influence on the downstream water quantity, quality, and health of the ecosystem (Bishop et al. 2008). Mountains are generally described as extreme and severe climate conditions such as worst weather situations with low temperatures, high solar radiation, and frequent natural degradation such as floods, landslides, and avalanches (Ali et al. 2017). Thus, global change is a significant issue for agricultural, rural livelihoods, and food security for the massive population around the globe (Adnan et al. 2018; Fahad et al. 2013, 2015, 2018; Fahad, Hussain, Saud, Hassan, Ihsan, et al. 2016; Fahad, Hussain, Saud, Hassan, Tanveer, et al. 2016; Fahad and Bano 2012; Khan et al. 2017; Muhammad et al. 2019; Saud et al. 2020; Zahida et al. 2017). Arsenic (As)

contamination in soils, either from the natural conversion of organic matter (OM) into inorganics by microbes (mineralization) or by human activities, has been exposed to be a major environmental concern (Wu et al. 2018). The harmful effects on microbial communities of several heavy metals have been well documented (Arriagada et al. 2009; Margesin et al. 2011). Several studies have also shown that heavy metals can significantly decrease the soil microbial biomass and the size of living microbial population (Hu et al. 2007; Muthusaravanan et al. 2018; Zhang et al. 2010), while some microbial species may be surviving with a high concentration of metals in soil (Margesin et al. 2011; Sousa et al. 2012).

Numerous researchers were discussed the arsenic environmental effects on the microbial communities in arsenic-polluted soils (Bao et al. 2014; Sun et al. 2012). Meanwhile,

at phyla level *Proteobacteria*, *Cyanobacteria*, and *Geobacter* have been identified in arsenic-contaminated soils due to breakdown (weathering) of natural rocks (Bao et al. 2014; Majzlan et al. 2011). Microbiological activities within alpine stream soils were playing a significant role to influence the geochemical reactions of metal(loid)s (Schipper et al. 2010). Therefore, microbial transformation of As is a promising approach to remediate As contamination from the soil by stimulating precipitation and in-situ immobilization (Xiao et al. 2017). Nevertheless, some previous studies have discussed microbes play an important role in the biogeochemical cycling of arsenic contamination (Filella et al. 2002; Kalin et al. 2005). Further investigation using, principal component analysis (PCA) and Unweighted Pair-group Method with Arithmetic Means (UPGMA) method, spearman rank correlation clustering confirmed the difference in microbial communities in present soil samples (Sun et al. 2014).

The new research based on this study analyzes the microbial population in alpine streams soils and correlates the data to the physio-chemical variables, which influence the community structure. Although, the understanding of the association between microorganisms and environmental geochemical factors is still limited. The purpose of the study was to explore (1) the microbial community structure in both anthropogenic and natural environment and to examine the effect of As contamination on microbial networks, (2) to attain new understanding into As biogeochemical cycle and microbially interceded As transformation in this polluted soils condition, and (3) to reveal

the relationship between microbial taxa and geochemical factors. This research aims to contribute new information that can be used to collect geochemical parameters and polluting sections for understanding the microbial processes in polluted areas better. Such outcomes would assist us to know how microbes respond to high levels of As concentrations and potentially provide us useful information with possible microbial bioremediation of As-polluted soils.

Materials and methodology

Description of the study area

Gilgit-Baltistan, a city of Pakistan, is situated in the WA khan corridor in north Afghanistan, Xinjiang China in the north and north-east, with a total area of 72,971 km², and high mountainous area. Gilgit-Baltistan had an estimated population of 1,800,000; its capital city is Gilgit with an estimated population of 216,760. Ten sites of KNP physico-chemical parameters were evaluated (Figure 1). It is located in the border of Pakistan-China and between 34°44' N and, 75°17' E above elevation 4000 m (13,000 ft) adjacent to the natural reserves of Taxkorgan (1,400,000 ha) in China. The climate of the region is characterized by pleasant summers, balmy autumns, and severe winters. The mean temperature varies between 27 °C to below 0 °C. While average temperature in winter (December and January) was −12 °C, while the average temperature in July and August was 14 °C (Ali et al. 2017). Annual precipitation differs from 200 to 900 mm, 90% occurs more locally in the form of snow.

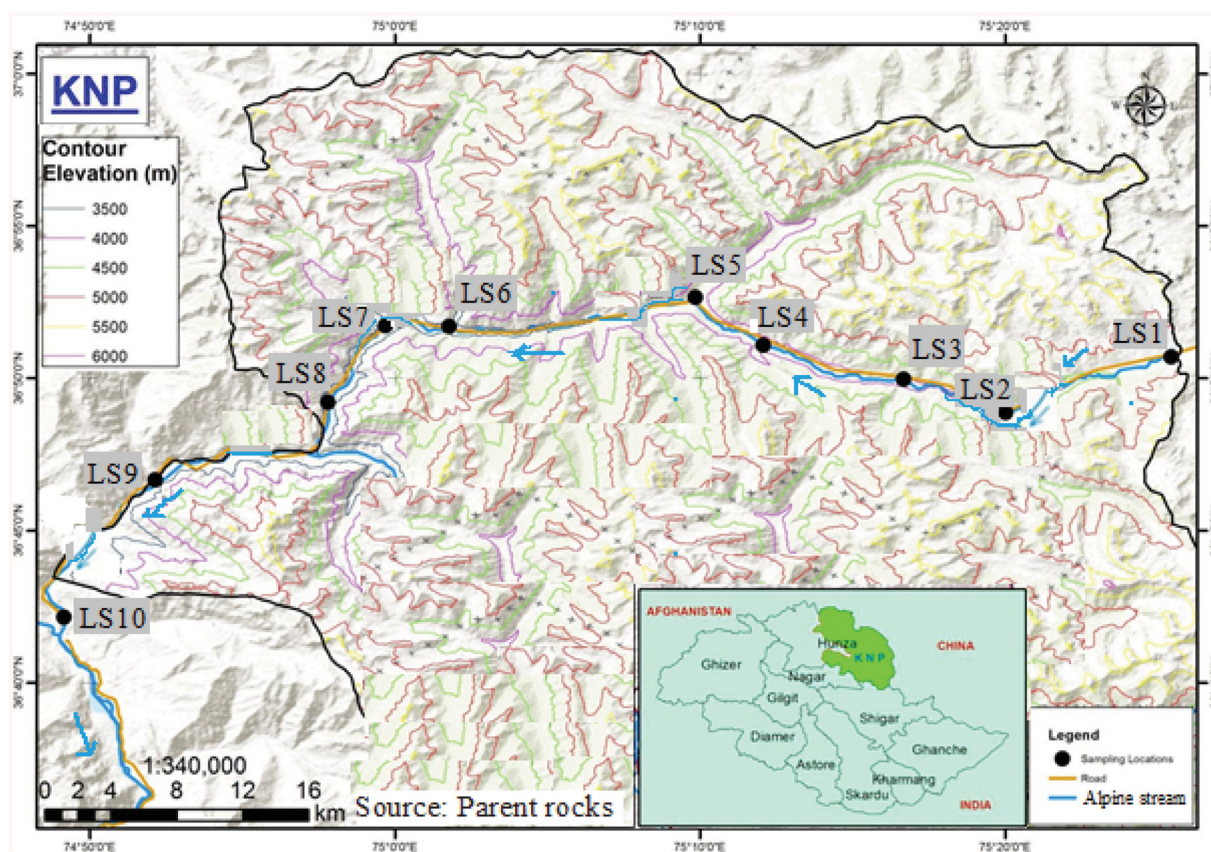


Figure 1. Map showing the soils sampling sites of Alpine stream Gilgit-Baltistan.

The interactions between the westerlies and monsoons affect the amount of precipitation. The area is made up of large, rough, and bare rocks. In these areas, there exist a variety of sedimentary, metamorphic, and igneous rocks. Soil is generally shallow and immature, with small fragments of rocky material, drifted sand, and clay (Ali et al. 2017).

Sample collection and testing

Collection of soil samples was done along the banks of Alpine stream Gilgit Baltistan, in 2017 (Figure 1). All soil samples in the field were cooled by placing them in ice bags and instantaneously stored for geochemical analysis at -4°C and for DNA extraction soil samples stored at -80°C . The samples of soil were dried in air and passed through a 200-mesh sieve after carefully powdered with the help of pestle and mortar. To determine anions, 2 g of dry soil and 10 mL of deionized water were mixed and shaken for 5 min, and then left for 4 h to balance. The supernatant was centrifuged at 3500rpm for 15 min, and filtered by using a filter membrane (0.45 mm). The anion determination was done using filtrates. Elemental analyzer (Elementar-Hanau, Germany) was operated to measure the total organic carbon (TOC). While, the oxidation method $\text{K}_2\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$ measured OM in soil samples (Xiao et al. 2016). Soil samples were completely digested with HF (hydrofluoric acid) and HNO_3 (nitric acid) (1: 5, v/v), then digested samples used to measure arsenic by inductively coupled plasma mass spectrometry (ICP-MS, Agilent, 7700x) and for cations estimation inductively combined with plasma optical emission spectroscopy (ICP-OES, iCAP6500, Germany) was used (Xiao et al. 2012, 2016). The pH and redox potential (TDS) were estimated by a pH meter (AISI pHB9901, Taiwan; solid: deionized water = 1:5) in soil. Based on the international quality control standards, the analytical precision was higher than $\pm 10\%$ since the internationally accredited reference materials (NIST 2711, GBW07310), internal standards (Rh, 500 $\mu\text{g/L}$), duplicates, and reagents blanks were employed.

High-throughput sequencing of the V3V4 region of 16S rRNA genes

Following the standard protocol of fast DNAV R spin kit (MP bio, USA), the total genome DNA was mined from 250 mg of sample. The virtue and concentration of DNA were tested using agarose gels (1%). Then DNA was debilitated up to 1 ng/ μL using sterile water. The 16S rRNA qualities of the evident district (V3V4) were raised using the 515f/907r starter set (907r: 5'-CCYCAATTCMTTTRAGTTT-3', 515f: 5'-GTGYCAGCMGCCGCGGTAA-3') (Ren et al. 2014; Xiao et al. 2017). IlluminaHiSeq2500 scored at the Novo quality bioinformatics relationship in Beijing was utilized to grouping the library. Coordinated end examines allocated to trials in the light of their remarkable scanner tag and abridged by discarding the existing detectible proof and groundwork gathering. Quantitative Insights into Microbial Ecology (QIIMEV1.7) was applied under explicit environment (Caporaso et al. 2010) to isolate excellent clean labels (Edgar 2018). Arrangements with more than or

equivalent to 97% similarity were dispensed to comparable OTUs using UPARSE programming (<http://drive5.com/uparse/>). Ribosomal Database Project (RDP) classifier (Wang et al. 2007) was used to appoint ordered arrangement for each OUT by choosing a typical grouping. The Shannon and Chao 1 archives were resolved for 11 libraries to evaluate the species richness similar to the previous study (Schloss et al. 2009).

Bioinformatics and statistical methods

The statistical package for social sciences (SPSS) version 19 was used for statistical analysis and excel software used for graphical plots. Linear discriminant analysis (LDA) and LEfSe (<http://huttenhower.sph.harvard.edu/LEfSe>) were applied to describe separating structures or features of the microbial networks below various conditions as illustrated already (Ling et al. 2014). Kruskal–Wallis rank sum test is used in LEfSe for detecting the features with reasonably different affluence of allotted taxa. The effective size of each variable is estimated using LDA based on a normalized relative abundance matrix (Rasool and Xiao 2018).

Cluster analysis was followed by principal component analysis (PCA), which was applied to reduce the dimension of the original variables which used the FactoMine R package and plot2 package in R software (Version 2.15.3). A distance matrix of weighted or unweighted unifrac among previously obtained samples was transformed into a new set of orthogonal axes, showing the maximum variation factor is demonstrated by the first principal coordinate, and the second maximum one by the second principal coordinate. Unweighted Pair-group Method with Arithmetic Means (UPGMA) Clustering was carried out using the QIIME. The canonical correspondence analysis (CCA) was performed on rich microorganisms genera (with relative wealth $>1\%$, in any event, each ordering library) and chose physico-chemical variables.

Results and discussions

Physico-chemical parameters and its major ion composition

The concentrations of physicochemical variables are presented in Table 1. The ranged value for pH was 7.6–8.7 in all soil samples, respectively (Table 1). The ranged value of EC was 77–140 $\mu\text{S/cm}$, while the ranged value for TDS was 45–110 mg/kg in soil samples (Table 1). The pH was strongly molded by the microbial communities recognized on its concentration divergences (Savio et al. 2015). Similarly, the EC and TDS are substantial, which can help to expose the various growth of microbial communities in soil samples (Fierer and Jackson 2006; Zhang et al. 2008). The SO_4 concentrations ranging were between 8.45 and 23.11 mg/kg and Ca concentrations ranging were between 11.23 and 46.92 mg/kg, respectively (Table 1). Although the Cl values ranged from 2.02 to 24 mg/kg and NO_3 values ranged from 5.66 to 11.34 mg/kg in soil samples (Table 1).

Table 1. Physicochemical parameters concentration of soil samples from Alpine stream, Pakistan.

Locations	pH	TDS	EC	TOC	OM	Cl	NO3	SO4	Ca	Mg	Na	K	Fe	Mn	As
LS1	8.7	70	105.5	24.22	45.23	3.01	5.66	9.22	15.21	3.6	1.41	20.41	12.22	8.31	8.45
LS2	8	45	78.4	25.33	34.54	2.02	6.43	10.43	11.23	2.36	2.34	31.34	19.34	11.43	7.42
LS3	8	53	77	31.22	57.34	3.03	11.34	12.22	12.11	2.39	2.44	44.44	18.12	16.23	3.31
LS4	7.8	70	96	17.86	61.34	2.04	8.32	8.45	17.45	2.15	1.42	29.42	22.12	23.22	9.34
LS5	7.6	76	109	19.32	77.32	24.02	9.23	11.12	19.87	2.23	3.39	31.39	26.72	25.64	8.32
LS6	7.6	96	137	23.12	63.23	2.12	10.67	13.45	46.92	8.04	2.91	26.91	31.23	30.33	4.33
LS7	7.5	87	127	32.22	81.23	7.14	6.66	18.43	20.22	2.6	1.42	22.42	29.12	14.32	6.34
LS8	7.8	77	97	20.31	44.32	2.13	8.73	23.11	25.63	4.92	0.67	11.67	26.12	28.33	7.23
LS9	8.3	90	102	18.32	84.23	5.15	11.23	9.88	25.22	4.94	0.68	12.68	25.31	18.54	8.23
LS10	7.7	110	140	12	85.21	8.16	10.87	17.44	30.58	3.83	2.47	14.47	29.87	32.33	5.55
Ranges	7.6 – 8.7	45 – 110	77 – 140	12 – 32.22	34.54 – 85.21	2.02 – 24	5.66 – 11.34	8.45 – 23.11	11.23 – 46.92	2.15 – 8.04	0.67 – 3.39	11.67 – 44.44	12.22 – 31.23	8.31 – 32.33	3.31 – 9.34
(Mean ± Std)	(7.84 ± 0.46)	(77.4 ± 19.48)	(106.8 ± 22)	(22.39 ± 6.2)	(63 ± 18)	(5.88 ± 6.75)	(8.9 ± 2.1)	(13.4 ± 4.8)	(22.4 ± 10.56)	(3.7 ± 1.86)	(1.92 ± 0.93)	(24.5 ± 10.3)	(24 ± 6)	(20.86 ± 8.3)	(6.85 ± 1.95)

All cations, anions parameter (mg/kg), TOC and OM (g/kg), TDS (mg/kg), and EC ($\mu\text{S}/\text{cm}$).

There were major variations in the soil samples of alpine streams and various geochemical parameters such as TOC and OM (Table 1). Nevertheless, the optimum concentrations of TOC and OM were 12–32.22 and 34.54–85.21 g/kg in soil samples, respectively. Total Fe and Mn were noted significantly in the ranges from 12.22 to 31.23 and 8.31 to 32.33 in soil samples of alpine stream, respectively (Table 1). All samples were characterized by high TOC, OM, SO_4 , NO_3 , Ca, Fe, and Little amount of Cl, which is a suitable environment for bacterial growth (Abilio et al. 2012). The soil samples of As were ranged between 3.31 and 9.34 mg/kg, respectively (Table 1). The high amount of total As indicated that this toxic metal was enriched to different extents in the soils, such as weathering of rocks, mining wastes, and agricultural runoff (Xiao et al. 2012).

Analysis of illumina-derived data

The average results of Illumina sequencing are 57,223 reads subsequently quality filtering from soil sample studies, ranging from 40,353 to 64,176 reads per sample, and grouped into 50,713 OTUs. Archives (LS3) revealed the maximum OTU numbers with 4425, whereas (LS7) the lowest OTU number 2637 in studies soil samples, respectively (Figure S1, Table S1). Mutually similar trends were displayed by Shannon index, alpha diversity of Chao1, and OTU numbers (Supplementary Table S1). Nevertheless, (LS2) had the highest Chao1, but (LS5) had the lowest Chao1 in studies of soil samples (Supplementary Table S1). Similarly, Shannon had the highest value at (LS2) and the lowest value at (LS10) soil samples (Supplementary Table S1). In studies of soil samples, the diversity indices increased like Shannon and Chao1, which contain good bacteria (Supplementary Table S1). The microbial population efficiency was determined by using the diversity of Chao1 and Shannon indexes in the studies of soil samples from Alpine stream (Savio et al. 2015).

Taxonomic composition of microbial communities

In each sample, based on RDP classification, the classification was allotted to various taxonomic levels from phylum to genus (Figure 2). Figure 2 explains each sample's relative abundance at the phylum level. Overall more than forty bacterial phyla were acknowledged in all studies soil samples. The *Acidobacteria* (7% of total reads), *Actinobacteria* (11% of total reads), and *Proteobacteria* (60% of total reads), were the prevalent phyla in soil samples (Figure 2). Similarly, *Firmicutes*, *Planctomycetes*, *Gemmatimonadetes*, *Bacteroidetes*, and *Verrucomicrobia* were the other dominant phyla (Figure 2). RDP classifier was unable to characterize 22% of the total peruses beyond the archaea and kingdom bacteria (Figure 2).

The presence of unique bacterial species is proposed based on the higher percent of unclassified sequences in As-rich environments (Babu et al. 2014; Savonina et al. 2012). We propose that the bacterial-rich environment might play an important role in the microbially mediated As cycling (Li et al. 2014; Xiao et al. 2017).

Comparison of bacterial communities between different zones

UPGMA tree and PCA were used to satisfactorily explain the microbial networks between various sites of soil samples. The UPGMA group analysis demonstrated that microbial communities were created inside this bioreactor (Figure 3). The PCA (Figure S2) investigation clarified the dissimilarities among the microbial communities (OTU similarity sequences = 97%). It showed the microbial networks from Zone 1 (LS1 and LS7), from zone 2 (LS2), from zone 3 (LS3, LS4, LS5, LS8, and LS10) as well as in Zone 4 (LS6 and LS9) were indirectly identified with one another (Figure 3). In studies soil samples, *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Firmicutes*, and *Bacteroides*, were more dominant based on UPGMA analysis (Figure 3). Furthermore, the growth of *Actinobacteria* associated phylotypes in the bioreactor, specifically in the micro-aerobic groups, was remarkable (Mclean et al. 2013; Xiao et al. 2016). The studies suggested that *Verrucomicrobia*, *Proteobacteria*, and *Gemmatimonadetes* are responsible for the biotransformation of metals in several mine related territories in soil (Li et al. 2014). Similarly, *Proteobacteria* may be tolerant the As from studies of soil samples (Sun et al. 2012; Wu et al. 2012). Also, *Bacteroidetes* might be helpful for the

resistance of As in alpine steam soil (Babu et al. 2014; Lefebvre et al. 2007). PCA (Supplementary Figure S2) identified that the microbial community through natural activity was different than anthropogenic in the bioreactor (Crump et al. 2012; Rasool and Xiao 2018).

Even the microbial networks differed between groups, illustrating the influence on microbial communities of ecological factors (Anjana et al. 2007; Lefebvre et al. 2007). Nonetheless, relative to the previously published research, universal core microbial communities along the river have been recognized, such principal microbial associations were also acknowledged in soil samples of the present study (Savio et al. 2015; Staley et al. 2013).

Relationship between as contaminated environments with bacterial taxa

LEfSe was performed to identify specific bacterial groups augmented from phylum to genus within each type of soil samples (Figure 4). The microbial communities in studied soil samples were divided into three groups LSG1, LSG2, and LSG3 based on LEfSe analysis, respectively. In LEfSe the bacterial lineages enriched in group one were *Proteobacteria*,

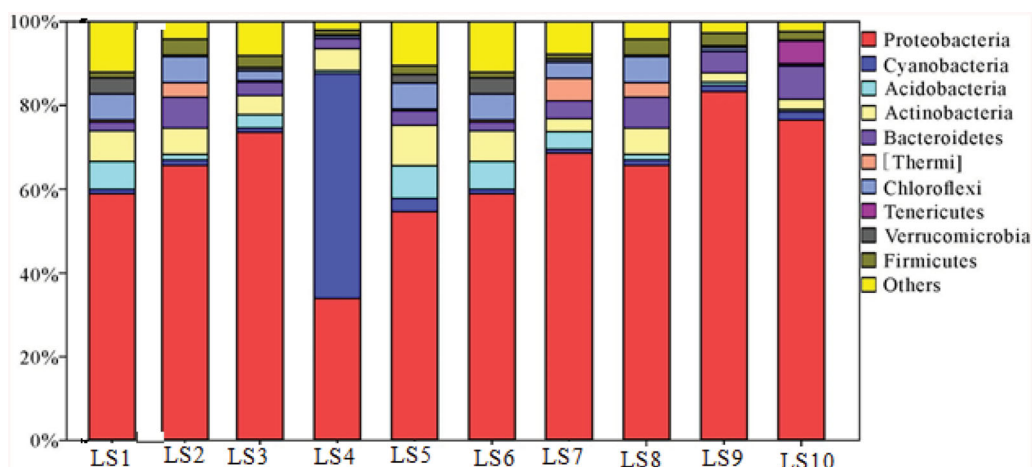


Figure 2. Taxonomic classification of the bacterial and archaeal reads retrieved from soil samples at the phylum level from 16S rRNA using RDP classifier.

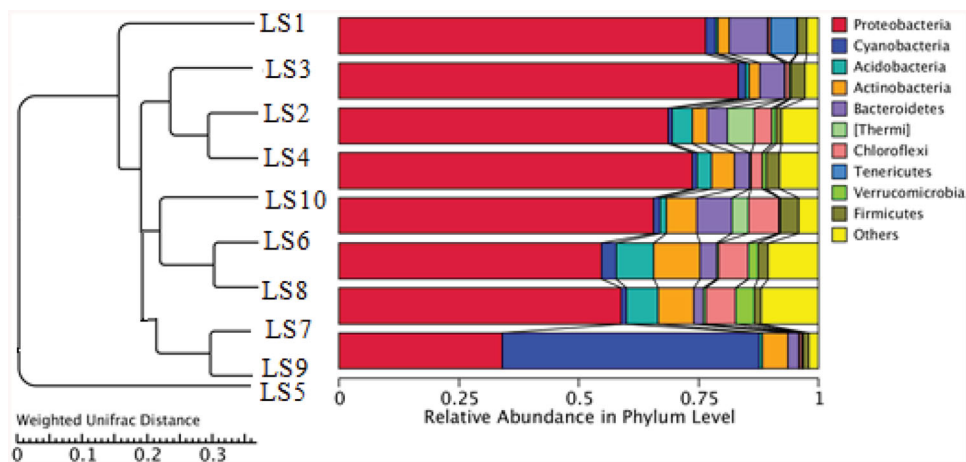


Figure 3. The UPGMA tree showing clusters of microbial communities based on UniFrac with 100% support in all node.

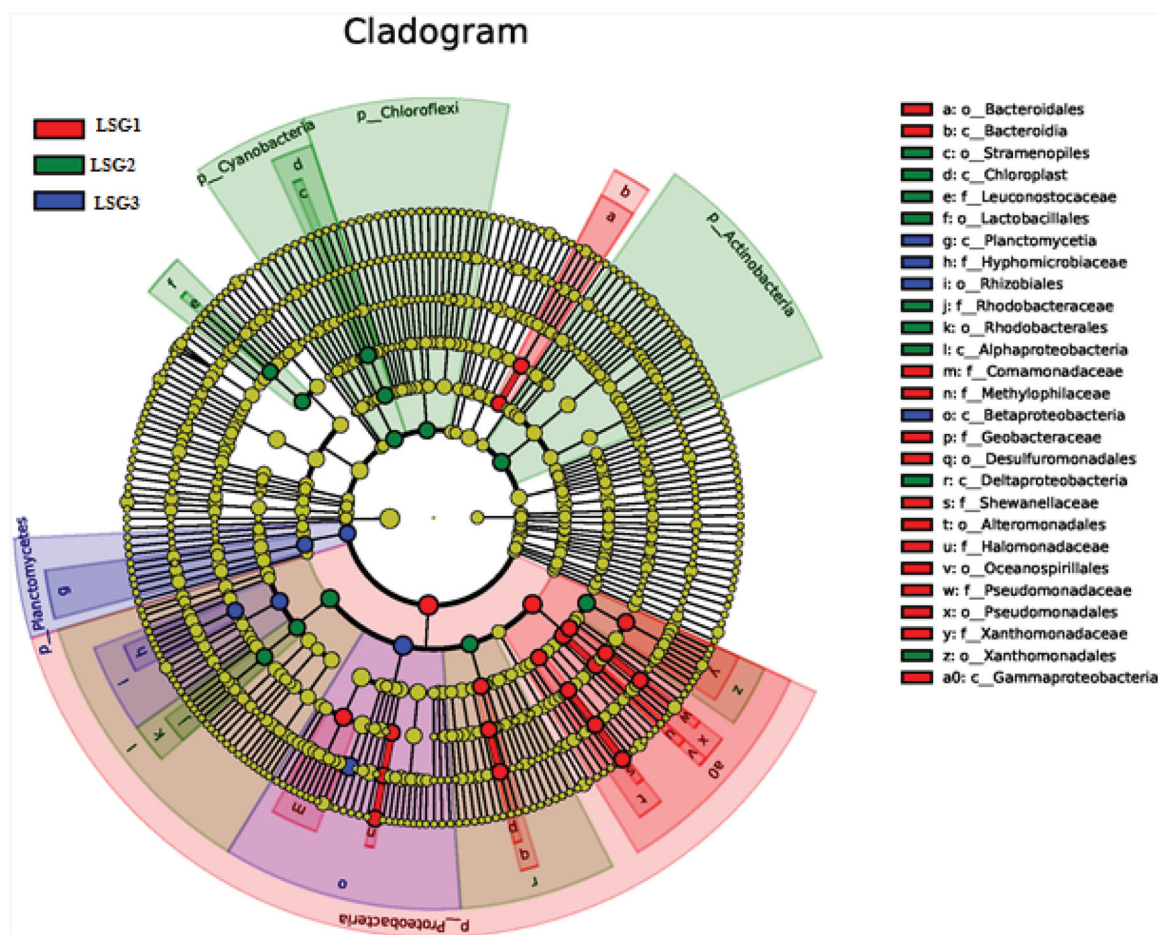


Figure 4. LefSe identified the abundant taxa more differentially in Alpine stream soil samples.

whereas in group two *Rhodobacteraceae*, and group three *Rhodobacterales* in studies soil samples (Cao et al. 2015; Li et al. 2014). Among achieved these bacterial taxa, LDA values were displayed greater than five by only *Proteobacteria*, *Rhodobacteraceae*, and *Rhodobacterales* in the soil samples of the alpine stream (Babu et al. 2014; Johnson and Hallberg 2005). The microbial communities were wrought in parts as confirmed with LefSe, and these parts were based on the As concentration levels. Various bacterial classifications holding sulfate and As diminishing organisms, for example, *Syntrophobacterales* and *Proteobacteria* displayed higher LDA scores in the vigorously As polluted region, proposing the tolerance of reducing microbes to raise As concentration in less contaminated soil (Huang et al. 2012; Sun et al. 2016).

Spearman correlation exposed the correlations between geochemical variables and microbial communities at the phyla level (Table 2). In all the obtained taxonomic groups, such as pH and TDS was positively correlated with *Proteobacteria* (0.547) and *Verrucomicrobia* (0.673), and negatively correlated with *Tenericutes* (−0.7342) and *Proteobacteria* (−0.508), respectively. Similarly, the EC had positive (0.5686) and negative (−0.7358) correlation with *Proteobacteria* and *Acidobacteria*, respectively. OM had a positive correlation with *Tenericutes* (0.6451) and negative with *Acidobacteria* (−0.647). At the same time, TOC was

negatively correlated with *Planctomycetes* (−0.6573) and positively correlated with (0.5022). Sulfate had a positive correlation with *Proteobacteria* (0.5294), *Tenericutes* (0.6213), and negatively correlated with *Planctomycetes* (−0.7812). The As was positively correlated Cyanobacteria (0.5162) and negatively correlated with *Gemmatimonadetes* (−0.5002). Spearman correlations were derived among the bacterial taxa and several geochemical variables, including As at genera level (Supplementary Figure S3). The *Flaviumibacter* was significantly ($p < 0.05$) positively correlated with pH. Meanwhile, *Phodobacter* had a significant ($p < 0.05$) positive correlation with As, SO_4 , Cl, TOC, EC, whereas negative with TDS. Similarly, *Hydrogenophaga* had a positive significant ($p < 0.05$) correlation with As. Spearman correlations analysis detailed information provided in (Supplementary Figure S3).

The *Cyanobacteria* was enriched in alpine stream soil positively with As (Supplementary Table S2). Besides, *Phodobacter* dominantly correlated with As and able to utilize another electron acceptor, including other geochemical parameters (Cao et al. 2015; Li et al. 2014; Wu et al. 2018). However, to our information, *Hydrogenophaga* has been connected with As biotransformation (Babu et al. 2014; Sorokin et al. 2008). Microbial community classification, including *Cyanobacteria*, *Actinobacteria*, *Acidobacteria*, and *Proteobacteria* demonstrated relatively high abundance in

Table 2. Spearman rank correlation between selected geochemical data and major abundant with bacterial phylum.

	Proteobacteria	Actinobacteria	Acidobacteria	Bacteroidetes	Planctomycetes	Gemmatimonadetes	Verrucomicrobia	Cyanobacteria	Spirochaetes	Tenericutes	TPD.58	WS3
pH	0.547	0.2879	-0.4324	0.2404	-0.0257	-0.1909	-0.397	-0.0629	0.2675	0.3524	-0.0734	-0.0247
TDS	-0.508	-0.4716	0.7275	-0.2652	0.3787	0.4716	0.673	0.159	-0.1022	-0.7342	0.4816	0.1517
EC	0.5686	0.3003	-0.7358	-0.1269	-0.5397	-0.69	-0.764	0.1146	0.2117	0.6882	-0.224	-0.5658
OM	0.382	0.2528	-0.647	-0.2817	-0.6571	-0.673	-0.661	0.191	0.0785	0.6451	-0.2741	-0.668
TOC	0.366	0.0154	-0.461	-0.2734	-0.6573	-0.614	-0.283	0.4274	0.3254	0.5022	-0.0331	-0.5327
Cl	0.2156	0.5314	-0.591	0.2383	-0.2693	-0.42	-0.8101	-0.3066	-0.1084	0.5641	-0.569	-0.2472
SO4	0.5294	0.0237	-0.7316	-0.089	-0.7812	-0.863	-0.6388	0.3128	0.3925	0.6213	0.0383	-0.7621
As	0.2239	-0.1991	-0.226	-0.219	-0.4158	-0.5002	-0.2012	0.5162	0.41012	0.1132	0.5595	-0.7123
Fe	0.395	0.26934	-0.2033	0.1475	0.0423	-0.0402	-0.2672	-0.0485	-0.0712	0.2774	0.0033	0.0227
Mn	0.433	0.145	-0.331	0.213	0.0345	-0.023	-0.286	-0.0472	-0.0321	0.154	0.0235	0.0321
Mg	0.517	0.3952	-0.5541	0.1991	-0.1805	-0.3042	-0.665	-0.238	-0.0216	0.5007	-0.4573	-0.1311
NO3	0.5748	0.2156	-0.3725	0.1867	0.2652	-0.0582	-0.4406	-0.1517	0.1384	0.1672	0.1221	0.0771
Ca	0.5789	0.2776	-0.566	0.1744	-0.2261	-0.3828	-0.6119	0.0526	0.1373	0.5704	-0.3206	-0.2013

Significance values mention in bold form.

the As contaminated environment (Park et al. 2015; Sun et al. 2016).

Association between environmental parameters and microbial communities

The CCA was accomplished to separate likely linkages between geochemical parameters and microbial networks in soils (Table 3). The parameters including SO₄, Cl, NO₃, total Fe, Mn, Mg, Ca, OM, TOC, and As were linked strongly to bacterial community compositions (Table 3). The phylum-level variability 42.74% was determined by CCA axis 1 and was positively allied with pH, EC, OM, TOC, Cl, SO₄, Ca, Mn, Fe, Mg, and negatively correlated with TDS, As in soil samples (Table 3). Furthermore, 20.97% of the variability was determined by CCA axis 2 and positively correlated pH, TDS, Fe, Mg, NO₃, Ca, and rest other all parameters negatively correlated in soil samples (Table 3). The bacterial community such as *Synergistetes*, *Betaproteobacteria*, *Chlamydiae*, *Nitrospirae*, and *Cyanobacteria* had a positive correlation with Mn, Fe, Mg, NO₃, Ca and *Firmicutes*, *Acidobacteria*, and *Verrucomicrobia* were negatively correlated with TOC, EC, and As in studied soil samples, respectively.

TOC, TDS, sulfate, and OM have been specified as primary natural environmental variables to shape the microbial communities. It has been found that TOC and OM are significantly associated with microbial communities in the current study area (Li et al. 2014; Sun et al. 2015). The total Fe may faster growth of Fe-metabolizing bacteria; especially in Fe rich environments such as in alpine stream soil (Baker and Banfield 2003; Bao et al. 2014). The composition of TOC and OM might be changed the distributional shapes of heterotrophic microbial communities such as *Cyanobacteria*, and *Anaerolinea* that have been proficient in degrading many OM (Sun et al. 2014). As is considered bio-accessible and mobilize able to encouragement microbial communities (Oves et al. 2013; Savonina et al. 2012; Sharma and Naushad 2020), likewise, other geochemical parameters might be effective with microbial community composition. The pH ascent might be applied as a direct enriching the acidophilus and neutrophils, according to the pH beside soil samples. However, more likely, the pH gradient in mineralized areas might affect the mobility of As and other metals (Babu et al.

Table 3. Canonical correspondence analysis of relative abundance at the phylum level with major physicochemical parameters in the soil samples.

Parameters	CCA1	CCA2	Phylum
pH	0.50055	0.3315	Proteobacteria
TDS	-0.674	0.01734	Actinobacteria
EC	0.59429	-0.3462	Acidobacteria
OM	0.43419	-0.2327	Bacteroidetes
TOC	0.32365	-0.6494	Firmicutes
Cl	0.53764	-0.0296	Chlorobi
SO4	0.44831	-0.3878	Gemmatimonadetes
As	-0.0382	-0.7653	Verrucomicrobia
Fe	0.27962	0.55846	Nitrospirae
Mn	0.284	0.484	Betaproteobacteria
Mg	0.56961	0.00932	Chlamydiae
NO ₃	0.45703	0.23298	Cyanobacteria
Ca	0.51698	0.04387	Synergistetes

2014; Faisal et al. 2020; Nakamaru et al. 2006). In the meantime, TDS is also identified as an important variable for organizing the microbial groups, and its substantially changed in-stream soil (Li et al. 2014; Pokrovsky et al. 2014). In addition to pH, microbial communities were also knowingly associated with TDS. It has been described that TDS and pH are the main parameters that control the As speciation in soil samples (Murciago et al. 2007; Xi et al. 2010). As will impulsive as different As-bearing minerals under numerous TDS and pH (Wilson et al. 2004) with oxidizing conditions (high TDS) favoring higher As solubility (Ashley et al. 2003; Craw et al. 2003).

Conclusions

The geochemical parameters formed diverse microbial communities in alpine stream soil, as indicated by UPGMA and PCA. Furthermore, CCA analysis verified that As contamination and sulfate, TOC, OM, and pH had a considerable influence on the bacterial communities. Several taxonomic groups have been enriched in the substantially contaminated region and mildly contaminated sector. Several microbial phylotypes, which include *Cyanobacteria*, *Phodobacter*, and *Acinetobacter*, were positively correlated with As, suggesting these microbes play an important role in arsenic cycling.

This work provides new knowledge of microbial communities interactions with As and other geochemical variables, enhancing our understandings on As microbial metabolism. Detailed future research is required to isolate the phylotypes, test their capacity in the transformation of AS, and to understand the functionality of genes that are vital for biogeochemical As cycling and resistance. Current data provide useful information for further understanding of the role of the aquatic macrophyte-biofilm system in wetlands in the future.

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Disclosure statement

The authors declare that he has no conflict of interest.

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