ENVIRONMENTAL BIOTECHNOLOGY

Microbial communities from different subsystems in biological heap leaching system play different roles in iron and sulfur metabolisms

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Abstract The microbial communities are important for minerals decomposition in biological heap leaching system. However, the differentiation and relationship of composition and function of microbial communities between leaching heap (LH) and leaching solution (LS) are still unclear. In this study, 16S rRNA gene sequencing was used to assess the microbial communities from the two subsystems in ZiJinShan copper mine (Fujian province, China). Results of PCoA and dissimilarity test showed that microbial communities in LH samples were significantly different from those in LS samples. The dominant genera of LH was *Acidithiobacillus* (57.2 ∼ 87.9 %), while *Leptospirillum* (48.6 ∼ 73.7 %) was predominant in LS. Environmental parameters (especially pH) were the major factors to influence the composition and structure of microbial community by analysis of Mantel tests. Results of functional test showed that microbial communities in LH utilized sodium thiosulfate more quickly and utilized ferrous sulfate more slowly than those in LS, which further indicated that the most sulfur-oxidizing processes of bioleaching took place in LH and the most iron-oxidizing processes were in LS. Further study found that microbial communities in LH had stronger pyrite leaching ability, and iron extraction efficiency was significantly positively correlated with *Acidithiobacillus* (dominated in LH), which

suggested that higher abundance ratio of sulfur-oxidizing microbes might in favor of minerals decomposition. Finally, a conceptual model was designed through the above results to better exhibit the sulfur and iron metabolism in bioleaching systems.

Introduction

Biohydrometallurgy is gradually becoming a kind of technology because of effectively utilizing low-grade resources to expand copper or other resource reserves in the age of resources lacking. Biological heap leaching is the most widespread technology, for the advantages of low cost, environmental friendliness, mild reaction, higher recovery, and ease of use; however, long production cycle is the main obstacle for its large-scale application (Ehrlich 2001). The first commercial bio-heap leaching plant in China with a capacity of 10,000 t Cu/a in the ZiJinShan Copper Mine (Fujian Province, China) started operation by the end of 2005 (Liu et al. 2010). Nowadays, 20,000 t Cu/year was output from 13,000,000 t ZiJinShan Copper Mine. Bio-heap leaching system is mainly composed of two subsystems, leaching heap (LH) and leaching solution (LS). The LS is formed by collecting leaching liquid from LH, and then the solution of LS could be sprinkled to the LH again, forming a recycle.

The microbial communities were thought as one of the key factors influencing the leaching efficiency (Qiu et al. 2008; Yin et al. 2008). In order to enhance efficiencies of bioleaching, the selection of high-efficient microorganisms or microbial communities is the key point (JOHNSON

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2008). Several studies were focused on the isolation of high-efficient strains and building mixed cultures (Liu et al. 2011; Zhang et al. 2015). However, these measures appeared to be a little blind and unpractical in industry bioleaching, because it seems impossible to build appropriate microbial community before understanding the composition and function in situ microbial communities in bioleaching systems. Therefore, it is important to put insight into the composition, structure, and function of microbial communities in biological heap leaching system and into the relationships between composition and function of microbial communities in bioleaching system. Previous studies have only focused on the composition of microbial community in biological heap leaching systems. Mendez-Garcia et al. reported that the primary biochemical transformation involving iron occurring in solution was ferrous iron oxidation and the related microorganisms were *Acidithiobacillus ferrooxidans* and *Leptospirillum* (Mendez-Garcia et al. 2015). According to Hu et al., the dominant genus of LS was *Acidithiobacillus*, but *Acidiphilium* was predominant in LH in Dexing (Jiangxi, China) biological heap leaching system (Hu et al. 2015; Zhang et al. 2016). However, few studies were involved in functions and its relationship with structure of communities in biological heap leaching system.

There are two opposite views about the relationships between structure and ecosystem function of microbial community. Griffiths et al. supported the view that enhanced species diversity was beneficial to ecosystem functioning (Griffiths et al. 2000). And according to Loranger et al., due to a greater diversity of species, the main limiting nutrient, soil mineral nitrogen, was utilized more completely, and plant productivity increased more rapidly (Loranger et al. 2013). On the contrary, other researchers supported the other view that the characteristics of ecosystem depended more upon the particular functional species than the number of total species (Sophie et al. 2007), that was, composition (species abundance) of microbial community determined ecosystem function. Undoubtedly, in order to enhance the efficiency of mineral's leaching, it was very important to know the relationship between the composition of microbial community and the function, but still few studies were about it.

In this study, 16S rRNA gene high-throughput sequencing and functional experiments were employed to investigate the composition and real functions of microbial communities in bioleaching system. Two important questions should be addressed: (i) What are the differentiations of composition, structure, and function between microbial communities in leaching heap and leaching solution? (ii) What are the relationships between composition and function of microbial communities in biological leaching system? To answer these questions, a total of 12 samples were obtained from leaching heap and leaching solution in ZiJinShan copper mine (Fujian province, China). Our results indicated that mineral

dissolution would be more efficient using the microbial communities composed of higher abundance of sulfur-oxidizing microbes at the initial stage in leaching heap, which is meaningful in bioleaching industry.

Materials and methods

Site description, samples collection, and DNA extraction

The biological heap leaching system of the ZiJinShan copper mine was located at 25° 10′ 41″ ~ 25° 11′ 44″ N and 116° 24′ 00″ ~ 116° 25′ 22″ E in the Fujian province, China. In this study, a total of 12 samples were collected from leaching heap (LH, 1.5 m deep) and leaching solution (LS) with 6 replicates for each subsystem (Fig. S1). Each LH sample was approximately 5 kg and the volume of each LS sample was about 20 L.

The environmental parameters were detected as follows. Inductively coupled plasma (ICP) was used to reveal the elemental composition of each sample. The dissolved ferrous ion and ferric ion were measured using ultraviolet spectrophotometry with microplate reader (ELIASA) by atomic absorption spectrophotometry (AAS), and the pH was measured using a pH meter, but preprocessing was needed for LH samples to detect these parameters. In the preprocessing, 2-g sample was added into 5-mL ddH$_2$O, then it could be measured after 15-min shaking (using an oscillator) and 30-min standing.

LS samples were filtered through qualitative filer paper to remove particles and large cells. Microorganisms were collected with a 0.22-μm filter paper. Each LH sample was washed by sterile dH$_2$O (pH 2.0, volume 10 L), then microorganisms were washed from the ore surface to the solution. Subsequently, they repeated the treatment of LS samples. The collection was used for DNA extraction and later S and Fe oxidation experiments. Total DNA was also extracted separately from each experimental group using a TIANamp genomic DNA purification kit (Tiangen Biotech, Co., Ltd., Beijing, China). 0.8 % (*w/v*) agarose gel was used to visualize the extracted DNA stained with ethidium bromide, while a NanoDrop_ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA) was used to measure the concentration of purified total genomic DNA.

DNA amplification, Illumina sequencing and data processing for DNA samples

These processes were as same as our previous study (Xiao et al. 2015; Yin et al. 2015). The V4 hypervariable region of the 16S rRNA gene was amplified using PCR primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) combined with Illumina adapter sequences, a pad and a linker of two bases,

as well as barcodes on the reverse primers. The amplification mix contained 12.5 µl PCR Mix (Vazyme Biotech, China), 1 µl of a 0.25 mM solution of forward/reverse primer, 1 µl of template DNA, and ddH$_2$O was added to reach a total volume of 25 µl. Genomic DNA (20 ng) was added to the PCR mix. Each sample was amplified under the following conditions: 94 °C for 5 min, 28 cycles of 94 °C for 45 s, 62 °C for 45 s, and 72 °C for 1 min then 10 min at 72 °C. The purified PCR amplicons were analyzed using Agarose Gel DNA Purification Kit (Omega Bio, America). Sample libraries were generated from purified PCR products. The Miseq 500 cycles kit was used for 2 × 250 bp paired-ends sequencing on Miseq machine (Illumina, America). Raw sequences with perfect matches to barcodes were split to sample libraries and were trimmed using Btrim with threshold of QC higher than 20 over five bp window size and the minimum length of 100 bp. The raw data of the high-throughput sequencing has been submitted to the Sequence Read Archive (SRA) of NCBI, and the project ID is SRP067716.

Functional tests of microbial communities in leaching heap and leaching solution

Functions of microbial communities in leaching heap and leaching solution mainly include sulfur and iron oxidation. Sodium thiosulfate and FeSO$_4$•7H$_2$O oxidation rates were measured to evaluate S and Fe and oxidation ability of microbial communities in LH and LS, respectively. In addition, pyrite oxidation rates were measured to evaluate mineral leaching ability. Microorganisms were cultured in 250-mL flasks containing 100 mL of sterile DSMZ or 9 K medium with the same inoculation concentration (1×10^6 cells/mL) and incubated at 30 °C on a rotary shaker (170 r/min). Sodium thiosulfate (5 g/L) was added into medium DSMZ to detect S oxidation, and the initial pH was 4.4. FeSO$_4$•7H$_2$O (44.7 g/L) or pyrite (20 g/L) was added into 9 K to detect Fe oxidation, and the initial pH was 1.5. The iron-free 9 K medium contained 3.0 g/L (NH4)$_2$SO$_4$, 0.5 g/L MgSO$_4$•7H$_2$O, 0.5 g/L K$_2$HPO$_4$, 0.1 g/L KCl, and 0.01 g/L Ca(NO$_3$)$_2$. The DSMZ medium contained 3.0 g/L (NH4)$_2$SO$_4$, 3 g/L KH$_2$PO$_4$, 0.5 g/L MgSO$_4$•7H$_2$O, and 0.25 g/L CaCl$_2$•2H$_2$O. The pH value and concentration of ferrous ions were measured every 24 h.

Data analysis

Data analyses were carried out using the R software (version 3.1.12), including functions from the vegan package (Dixon 2003). Microbial diversity was evaluated by Shannon diversity index Shannon 1948 and Pielous's evenness index (Pielou 1977). Mantel tests were used to evaluate the relationships between the microbial community structure and environmental parameters (Peres-Neto and Jackson 2001; Smith et al.

1997). Principal component analysis (PCA) was used to analyze the dissimilarity of physical and chemical characterize of all samples (Wikström et al. 1999), and principal coordinates analysis was used to analyze the genetic relationship of different microbial community (Li et al. 2015).

To explore relationships among ability of sulfur oxidation, iron oxidation, and pyrite decomposition and species (*Acidithiobacillus*, *Leptospirillum*), regression analysis was carried out using linear regression followed by ANOVA to test for significance.

Results

Physical and chemical characteristics in two subsystems

All samples were characterized with low pH, high content of Fe, S, and heavy metals (e.g., Cu, As, Mn, Zn, Pb). The pH values of LH and LS were about 2.30 and 1.23, respectively. The concentrations of ferric ions and ferrous ions in LS were about 250 ppm (mg/L) and 40 ppm (mg/L), respectively, and those in LH were about 500 ppm (mg/kg) and 20 ppm (mg/kg), respectively. The total amounts of elemental concentrations (except Zn, Cr, Cu, and Co) in LH were higher than those in LS. The total amount of Cu was higher in LS. The data were shown in Table S1. The parameters were analyzed by PCA to determine the dissimilarity in geochemical composition among all samples. The results showed that samples from LH were separated with LS samples (Fig. S2).

Overview of composition, structure, and diversity of microbial communities

In order to explore changes of microbial community composition and structure in LH and LS in detail, 16S rRNA gene amplicon sequencing was used. Rarefaction curves based on the number of OTUs (97 % similarity) and sequencing reads showed that the numbers of OTUs were almost saturated (Fig. S3), and it was indicated that result of sequencing was reliable in representing the real community. Generally, microbial community composition was apparently different between LH and LS samples by dissimilarity test (ADONIS, $R^2 = 0.886$, $p = 0.001$) and PCoA (Fig. 1a). And Table 1 showed both Shannon diversity and Pielou evenness indices were significantly ($p < 0.001$) lower in LH (1.454 ± 0.266, 0.282 ± 0.038) than those in LS (2.971 ± 0.366, 0.498 ± 0.054).

A total of 585 OTUs were detected in all samples; among them, 30 unique OTUs were detected in LH samples and 278 unique OTUs in LS samples. Forty-three OTUs were detected among all 12 samples, which accounted for about 99 % of all reads in LH samples and only about 80 % in LS samples. Among all reads, the number of those belonged to Bacteria
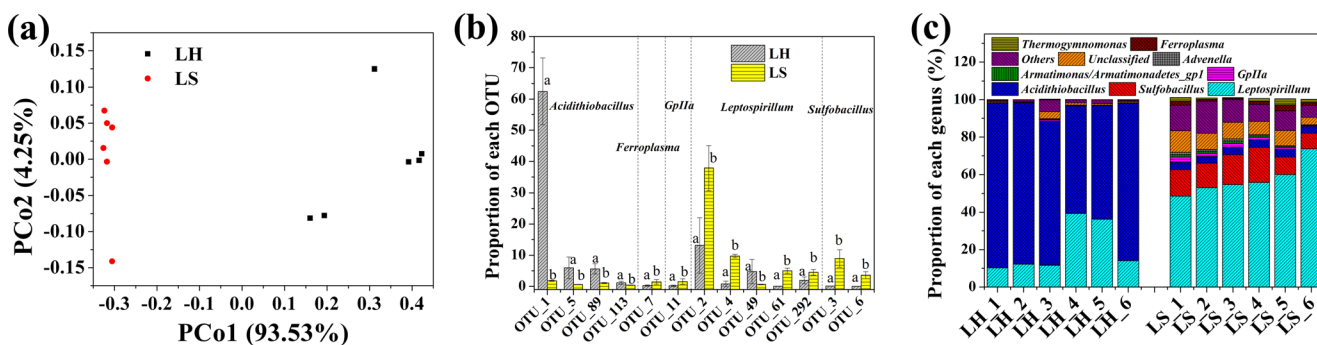
Fig. 1 Summary of microbial communities in leaching heap (*LH*) and leaching solution (*LS*). **a** The ordination plots of all samples for the community structure analyzed by principal coordinates analysis (PCoA). **b** The percentages of dominant OTU of microbial communities in LH and LS, the "*a*" and "*b*" indicated the significant difference between the OTUs of microbial communities in LH and LS. **c** Microbial communities structure in *LH* and *LS* at genus level

was accounted for about 99.7 and 95.7 % in LH and LS samples, respectively, and to archaea was about 0.3 and 4.3 %. LH samples were dominated by OTU_1 (*Acidithiobacillus*, 47.9 % ~ 72.7 %), while OTU_2 (*Leptospirillum*, 30.3 ~ 51.1 %) was the dominant taxonomic group in LS samples (Fig. 1b).

At the phylum level, the microbial communities were mainly composed of *Proteobacteria* (58.4 ~ 88.3 %) and *Nitrospirae* (10.3 ~ 39.3 %) in LH samples (Fig. S4). On the contrary, the proportion of *Nitrospirae* (48.6 ~ 73.7 %) was higher than *Proteobacteria* (8.3 ~ 16.1 %) in LS samples, and another dominant phylum in LS was *Firmicutes* (8.7 ~ 18.9 %), which was lower than 0.5 % in LH samples. The proportions of other phyla, such as *Actinobacteria*, *Cyanobacteria/Chloroplast*, and *Euryarchaeota*, were also much lower in LH samples than those in LS samples. At the genus level, Fig. 1c showed that LH samples were dominated by *Acidithiobacillus* (57.2 ~ 87.9 %) and *Leptospirillum* (10.2 ~ 39.3 %). Different from LH samples, LS samples were dominated by *Leptospirillum* (48.6 ~ 73.7 %), *Sulfobacillus* (8.4 ~ 18.6 %), *Acidithiobacillus* (3.5 ~ 4.1 %), and unclassified species (4 ~ 11.5 %). Particularly, we compared the relative abundance of other predominant genera in LH and LS samples and found that all genera were more abundant in LS.

Linkage of microbial communities and environmental parameters

To explore how environmental condition affected the structure of microbial communities in bioleaching system (LH and LS), the whole environmental parameters (Table S1), e.g., pH,

Fe^{2+}, Fe^{3+}, S, were taken into consideration and Mantel test was performed. The result showed that environment had a significant correlation ($r = 0.795$, $p = 0.001$) with microbial communities. To further determine the most key individual environmental parameters affecting structure of the microbial communities, Mantel test of all 12 samples were performed with 15 individual environmental parameters. The analysis showed that all parameters, except for Ca and Co, were significantly ($p < 0.05$) correlated with the structure of microbial communities (Table 2).

Mantel tests were conducted to explore the relationships between various phyla or genera and environmental parameters or individuality further (Table S2 and Table S3). Significant ($p < 0.05$) or marginally significant ($p < 0.10$) relationships between the whole environmental parameters and various phyla were observed: *Nitospirae* ($r = 0.635$, $p = 0.001$), *Proteobacteria* ($r = 0.836$, $p = 0.002$), *Firmicutes* ($r = 0.523$, $p = 0.005$), OD1 ($r = 0.466$, $p = 0.01$), and *Armatimonadetes* ($r = 0.243$, $p = 0.061$). The environmental parameters had significant effects on several genera, including *Acidithiobacillus* ($r = 0.844$, $p = 0.001$), *Leptospirillum* ($r = 0.635$, $p = 0.001$), *Acidiphilium* ($r = 0.591$, $p = 0.002$), *Sulfobacillus* ($r = 0.523$, $p = 0.002$), *Arthrobacter* ($r = 0.251$, $p = 067$), *Armatimonas/ Armotimonadetes_gp1* ($r = 0.243$, $p = 0.068$), *Advenella* ($r = 0.220$, $p = 0.083$), *Ferruginibacter* ($r = 0.203$, $p = 0.086$), *Rhodobacter* ($r = 0.209$, $p = 0.097$), *Sphingomonas* ($r = 0.165$, $p = 0.099$), and so on. Table S3 showed that the dominant genus, *Acidithiobacillus*, had significant or marginally significant relationships with all environmental parameters in this study, while *Leptospirillum* did except for Ca, Cu, and Co, and *Sulfobacillus* did except for ferrous ions, Ca, Zn, Cr, Cu, and Co. The common

Table 1 The Chao values, Shannon diversity index, and Pielou evenness of microbial communities in leaching heap (LH) and acid mine drainage (LS)

Group	Chao	Shannon diversity index H	Pielou evenness J
LH	2331.2749 (72.75 %)	1.454 ± 0.266^a	0.282 ± 0.038^a
LS	3210.5658 (86.74 %)	2.971 ± 0.366^b	0.498 ± 0.054^b

The "*a*" and "*b*" indicate the significant difference between the samples in LH and LS

Table 2 Summary of statistical analysis of the influences of each environmental parameter on microbial community structures by mantel test

Factors	r value	p value
whole	0.795	0.001
pH	0.923	0.002
Fe2	0.416	0.001
Fe3	0.703	0.002
S	0.685	0.002
P	0.93	0.002
Fe	0.894	0.001
Ca	0.104	0.124
Zn	0.357	0.013
Mn	0.754	0.002
K	0.768	0.004
Cr	0.176	0.002
Pb	0.857	0.006
Cu	0.25	0.013
Co	0.097	0.133
As	0.95	0.001

genus in bioleaching system, *Ferroplasma*, was only significantly correlated with pH, S, P, Fe, K, Pb, and As.

Function of microbial communities in leaching heap and leaching solution

The results of $Na_2S_2O_3$ and $FeSO_4 \cdot 7H_2O$ oxidation experiments showed that pH in LH group decreased faster than that in LS group, while the concentration of ferrous ions in LS group decreased faster than that in LH group (Fig. 2), which indicated that sulfur-oxidizing ability was stronger in LH samples and iron-oxidizing ability was stronger in LS.

In order to further understand the process, functional tests of pyrite leaching were conducted. The results of pyrite bioleaching experiment showed that pH was more quickly decreased and the concentration of ferric ion was more quickly increased in LH samples (Fig. 3), which suggested that microbial communities in LH samples had stronger function of mineral decomposition.
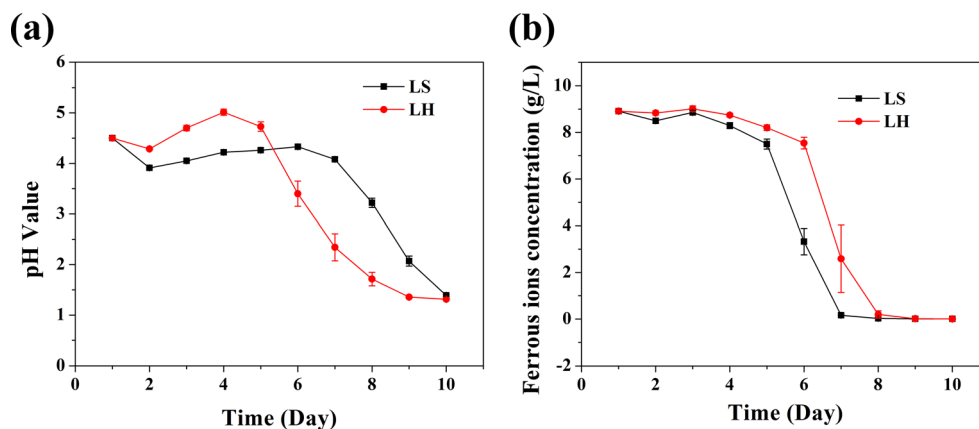
Relationship between species abundance and function of microbial community

Results of linear regression analysis showed that the concentration of H^+ in solution was significantly and positively correlated with *Acidithiobacillus* proportion of inoculums (on day 7 of sodium thiosulfate oxidizing process, Fig. 4), and the concentration of Fe^{2+} with *Leptospirillum* proportion (on day 6 of ferrous sulfate oxidizing process), which indicated that higher proportion of *Acidithiobacillus* enhanced sulfur oxidation ability and higher proportion of *Leptospirillum* enhanced iron oxidation ability. Furthermore, result of linear regression analysis showed that iron extraction efficiency was positively and significantly correlated to *Acidithiobacillus* proportion of inoculum (on day 15 of pyrite leaching process, Fig. 5), which suggested that higher proportion of *Acidithiobacillus* could enhance pyrite leaching ability.

Discussion

The microbial communities are important for minerals' decomposition in biological heap leaching system, which includes two sub-systems, leaching heap, and leaching solution. Due to the different environmental condition in these two subsystems, the composition of microbial communities might be different, finally leading to the difference in the function of microbial communities (Hu et al. 2015).

Differentiation of composition and structure of microbial community in leaching heap and leaching solution

The observed microbial diversity at previously studied bioleaching systems includes organisms belonging primarily to the domains *Bacteria*, *Archaea*, and, to a lesser extent, *Eukarya* (predominantly fungi and algae) (Mendez-Garcia et al. 2015). In this study, our aim was to get insight into the compositions of *Bacteria* and *Archaea* in the systems. Results of 16S rRNA gene sequencing showed that microbial

Fig. 2 Sulfur oxidation (**a**) and iron oxidation (**b**) of microbial communities in leaching heap (*LH*) and leaching solution (*LS*). Variations of pH values and ferrous ions were on behalf of sulfur oxidation and iron oxidation, respectively

Fig. 3 Variations of pH (**a**) and the concentration of ferric ions (**b**) during pyrite bioleaching by microbial communities in leaching heap (*LH*) and leaching solution (*LS*)
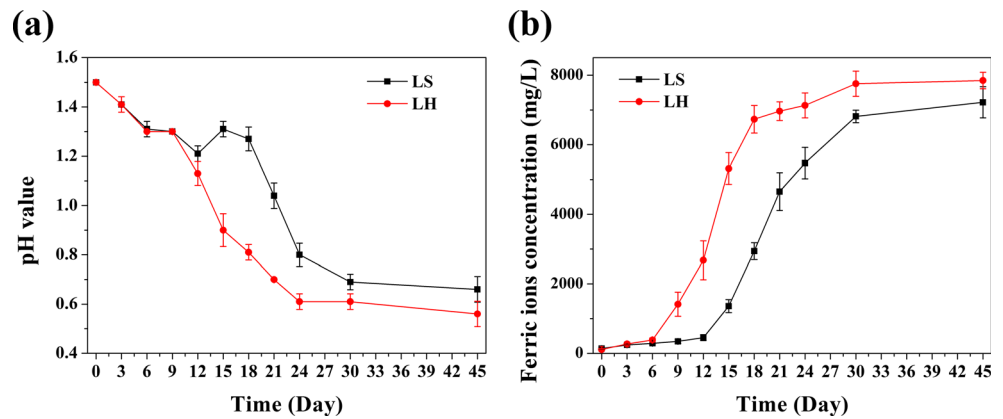
communities in bioleaching systems consisted of *Bacteria* (above 90 %) and *Archaea*. Bacteria in communities were mainly composed of the phyla *Proteobacteria*, *Nitrospirae*, *Firmicutes*, *Actinobacteria*, *Cyanobacteria/Chloroplast*, and *Bacteroidetes*, and *Archaea* were composed of the phyla *Euryarchaeota* and *Crenarchaeota*.

Proteobacteria are widely distributed in acidic ecosystems; among the most common genera, *Acidithiobacillus* are acidophiles (optimal pH value was 2–3) and exhibit mesophilic growth (Williams and Kelly 2013). These bacteria are chemolithotrophic and grow through oxidizing iron and sulfur (including *A. ferrooxidans*, *A. ferridurans*, *A. ferrivorans*) or just sulfur (including *A. caldus*, *A. thiooxidans*, and *A. albertensis*). The results showed that the proportion of *Acidithiobacillus* was higher in LH samples (57.2 ~ 87.9 %) than that in LS samples (3.5 ~ 4.1 %). The reason was mainly the pH value in LH (around 2.3) was more suitable for *Acidithiobacillus* to grow (Williams and Kelly 2013); however, it was too low in LS (around 1.2) and growth of *Acidithiobacillus* might be limited.

Within the phylum *Nitrospirae*, one of the most relevant genera known to inhabit bioleaching systems is *Leptospirillum* (optimal pH value was 1–2), which is chemolithoautotrophs that obtain energy from oxidation of iron (García-Moyano et al. 2007). *Leptospirillum* consisted of three groups, including *L. ferrooxidans* (group I), *L. ferriphillum* (group II), and

L. ferrodiazotrophum (group III) (Goltsman et al. 2009). The results showed that LS samples (48.6 ~ 73.7 %) had higher proportion of *Leptospirillum* than LH samples (10.2 ~ 39.3 %). Firstly, differentiation of the pH value in two subsystems was a reason. The pH in LS was more suitable for *Leptospirillum* to grow. Secondly, take process of minerals dissolution into consideration, iron was dissolved out and mostly became ferrous ions. And ferrous ions dissolved in leaching liquid and flowed into LS, which supplied energy source for *Leptospirillum* to grow. In addition, the higher concentration of ferric ions was another driver to higher proportion of *Leptospirillum* in LS, because some studies revealed that, to a certain extent, the concentration of ferric ions was higher (below 1 g/L or 1000 ppm), *Leptospirillum* grew better (Ouml et al. 2007).

Sulfobacillus (phylum *Firmicutes*) appear to thrive preferentially in lower pH condition (optimal growth at pH around 1.5) (Anderson et al. 2012). These bacteria are mainly mixotrophic, which can grow autotrophically by oxidizing iron or sulfur or heterotrophically on yeast extract. Our results showed that higher proportion of *Sulfobacillus* was in LS (8.4 ~ 18.6 %) than in LH (below 0.2 %). The pH was also one factor, leading to *Sulfobacillus* growing better in LS (pH was around 1.2). In addition, most organics from death bacteria and extracellular metabolites flowed into LS and accumulated, which promoted microorganisms who can utilize organics to grow, e.g., *Sulfobacillus* and the other relatively predominant genera,

Fig. 4 Plot of linear regression analysis showing the relationship between the concentration of H⁺ and proportion of *Acidithiobacillus* (**a**) and the relationship between the concentration of Fe²⁺ and proportion of *Leptospirillum* (**b**)
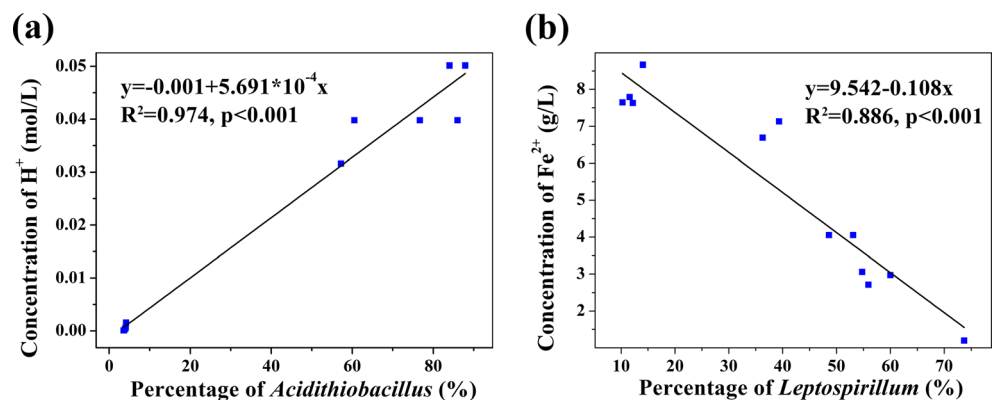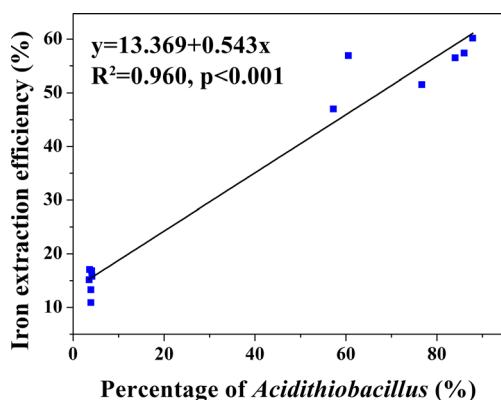
Fig. 5 Plot of linear regression analysis showing the relationship between iron extraction efficiency and proportion of *Acidithiobacillus*

including *GpIIa*, *Armatimonas/Armatimonadetes_gp1*, and *Advenella* (Anderson et al. 2012; Tamaki et al. 2011; Tom et al. 2005; Troesch et al. 1990).

Archaea populating bioleaching systems generally belong to the phylum *Euryarchaeota*, and two of the most abundance genera of this phylum in bioleaching systems are *Ferroplasma* (optimal pH was $1.2 \sim 1.7$) and *Thermogymnomonas* (optimal pH was around 3) (Yang et al. 2014). These two genera were both heterotrophic. Our results showed that the proportion of these two genera were both higher in LS. Lower pH and more organics still might be the reasons.

Through above analyses, we put forward another speculation that minerals were firstly oxidized by sulfur-oxidizing microbes, leading to ferrous dissolved into leaching solution (LS), and then were oxidized by iron-oxidizing microbes, which was consistent with the results by previous studies in our lab (Xiao et al. 2015). Our study revealed that sulfur-oxidizing microbes were the dominant species in the initial stage, while iron-oxidizing microbes were the dominant species in the final stage during bioleaching process.

To sum up, more kinds of common microorganisms in bioleaching systems adapted to habitat LS subsystems, leading to higher microbial diversity of LS samples than LH samples. However, H.R. Watling demonstrated that greater microbial complexity was observed with moderate pH (Watling et al. 2013). It could been seen, although pH was quite an important parameters through this study and demonstration in study by Kuang et al. (Kuang et al. 2013), other parameters (e.g., P, ferric ions, As) were also important to influence the microbial communities in biological heap leaching systems.

Abundance ratio of sulfur- and iron-oxidizing microbes determine functions of microbial community

The composition and structure of microbial communities were different in LH and LS. It was clear that composition of microbial communities was one of the key factors influencing community function, including mineral decomposition in

bioleaching process, ultimately altering leaching efficiencies. As we know, metals almost cannot dissolve out of the minerals until sulfur and iron of mineral were oxidized (Rodríguez et al. 2003a) so that it could be determined that ability of sulfur and iron oxidizing are important functions of microbial communities.

Our study indicated that sulfur-oxidizing ability of microbial communities was stronger in LH samples, and iron-oxidizing ability was stronger in LS samples. The main reasons included two aspects. On the one hand, although the kinetics of mineral dissolution was complicated during bioleaching process, iron and sulfur always were released from mineral together. However, the detached iron was in the form of ferrous ions, which can dissolve into water, while sulfur was always in the form of element sulfur, which cannot dissolve into water and most was attached on the mineral's surface (Rodríguez et al. 2003a, b). Therefore, more ferrous ions flowed into LS and more element sulfur remained in LH, and after a long period of adaptation, it is speculated that microbial community in LH should had stronger sulfur-oxidizing ability and weaker iron-oxidizing ability than those in LS. On the other hand, results of NCBI blast (nr/nt database) showed the taxa of the top 13 OTUs (Fig. 1b and Fig. S5), including OTU_1 belonging to *A. albertensis* strain SO-2 or *A. thiooxidans* SO-1 (100 %), OTU_5, and OTU_113 belonging to *A. caldus* SM_1 (97 %). These three species were all sulfur-oxidizing microbes (Mendez-Garcia et al. 2015; Vardanyan and Vardanyan 2014). To sum up, sulfur- and iron-oxidizing microbes (except microbes oxidizing both sulfur and iron) of microbial communities were about 69 and 21 %, respectively, in LH and were about 3 and 58 %, respectively, in LS. Therefore, sulfur-oxidizing ability of microbial communities was stronger in LH samples, and iron-oxidizing ability was stronger in LS samples. In addition, if microbes attached on the mineral's surface during a long period, they should had stronger minerals bioleaching ability than those separated from mineral. Our study was also proved that microbial communities in LH had stronger pyrite bioleaching ability than that in LS.

Many studies have researched the relationship between species diversity and ecosystem function (Huttenhower et al. 2012; Johnson 1998). Two types were put forward to quantify the form of the relationship (Johnson 1998). Type 1 represented that all species were important for function, while type 2 emphasized the importance of the functional species. In bioleaching system, metals dissolved out after sulfur and iron were utilized by sulfur- and iron-oxidizing microbes; thus, we speculated that functions (including sulfur, iron, and mineral oxidation) should be mainly determined by functional species abundance. That was, the relationship between species diversity and function in bioleaching system should be fit to type 2. On the one hand, there should be no doubt that microbial communities consisting of more sulfur-oxidizing have stronger ability of sulfur oxidation, and consisting of more iron-
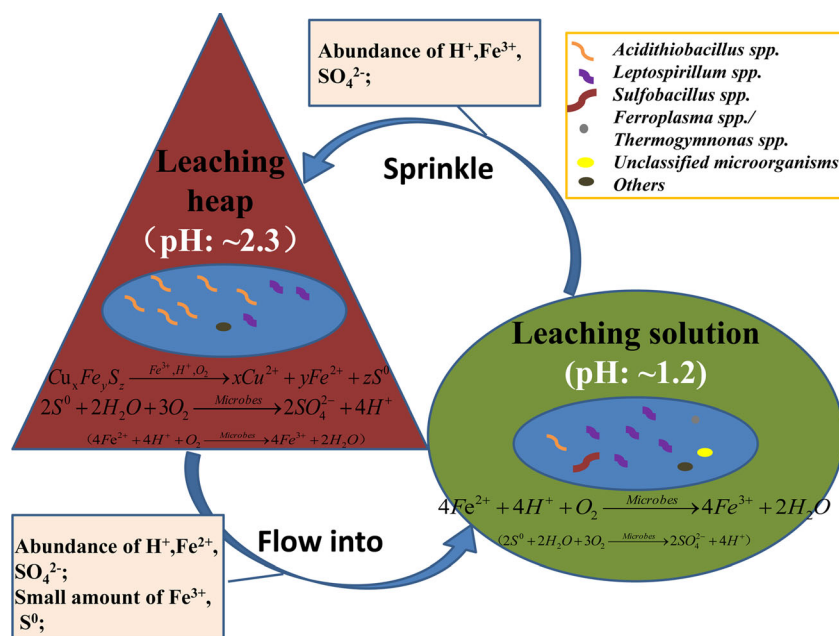
Fig. 6 A conceptual model about microorganisms, sulfur, and iron metabolism in bioleaching systems. Bio-heap leaching system is mainly composed of two subsystems, leaching heap (LH) and leaching solution (LS), and the latter is formed by leaching liquid from the former, then the latter is sprinkled to the former, forming a cycle. *Bigger words* indicate relatively more active reaction; on the contrary, *litter words* and in *brackets* indicate relatively less active reaction. For example, there is stronger sulfur oxidation in LH and stronger iron oxidation in LS. The dominant microbial genera are drawn in blue ellipse, which involved into the sulfur and iron metabolism, the number of the same shape indicates the relative abundance of the represented genus, and the names are labeled at the top right corner

oxidizing microbes have stronger ability of iron oxidation. And it was also proved by linear regression analysis (Fig. 4) showing that sulfur-oxidizing ability was significantly and positively correlated with *Acidithiobacillus* (utilizing sulfur) and iron-oxidizing ability with *Leptospirillum* (utilizing iron). On the other hand, ability of pyrite bioleaching was significantly and positively correlated with *Acidithiobacillus* (utilizing sulfur, Fig. 5). It also suggested that higher abundance of *Acidithiobacillus* (representing sulfur-oxidizing microbes) could enhance mineral bioleaching, which was critically beneficial to improve efficiency of bioleaching industry.

According to the above results and discussion, a conceptual model was designed about differentiations and relationships of environmental condition (pH), microbial community composition, iron, and sulfur oxidizing processes in leaching heap and leaching solution of biological heap leaching system (Fig. 6), which can then be used to deepen our understanding of sulfur and iron metabolism in bioleaching systems. Bio-heap leaching system is mainly composed of two subsystems, leaching heap (LH) and leaching solution (LS), and the latter is formed by leaching liquid from the former, then the latter is sprinkled to the former, forming a cycle. The microbial community in LH, dominated by sulfur-oxidizing microbes, played a key role in mineral decomposition and sulfur oxidation, and the microbial community in LS, dominated by iron-oxidizing microbes, played a key role in iron oxidation. Therefore, it may be that microbial community of higher

sulfur-oxidizing abundance could enhance minerals' decomposition and metals extraction efficiency.

In conclusion, composition and function of microbial communities between LH and LS were significantly different, and species abundance might be the key factor to determine the function of microbial communities.

References

Anderson I, Chertkov O, Chen A, Saunders E, Lapidus A (2012) Complete genome sequence of the moderately thermophilic mineral-sulfide-oxidizing firmicute *Sulfobacillus acidophilus* type strain (NAL (T)). Stand Genomic Sci 6(3):293–303. doi:10.4056/sigs.2736042

Dixon P (2003) VEGAN, a package of R functions for community ecology. J Vegetation Sci 14(6):927–930. doi:10.1111/j.1654-1103.2003.tb02228.x

Ehrlich HL (2001) Past, present and future of biohydrometallurgy. Hydrometallurgy 59:127–134. doi:10.1016/S0304-386X(00)00165-1

García-Moyano A, González-Toril E, Moreno-Paz M, Parro V, Amils R (2007) Microbial ecology of *Leptospirillum* spp. in Río Tinto, a model of interest to biohydrometallurgy. Adv Mater Res 20-21: 409–412. doi:10.4028/www.scientific.net/AMR.20-21.409

Goltsman DSA, Denef VJ, Singer SW, Verberkmoes NC, Mark L (2009) Community genomic and proteomic analyses of chemoautotrophic iron-oxidizing "*Leptospirillum rubarum*" (Group II) and "*Leptospirillum ferrodiazotrophum*" (Group III) bacteria in acid mine drainage biofilms. Appl Environ Microb 75(13):4599–4615. doi:10.1128/AEM.02943-08

Griffiths BS, Ritz K, Bardgett RD, Cook R, Christensen S (2000) Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity–ecosystem function relationship. Oikos 90(2):279–294. doi:10.1007/s00248-002-2043-7

Hu Q, Guo X, Liang Y, Hao X, Ma L, Yin H, Liu X (2015) Comparative metagenomics reveals microbial community differentiation in a biological heap leaching system. Res Microbiol 166(6):525–534. doi:10.1016/j.resmic.2015.06.005

Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH (2012) Structure, function and diversity of the healthy human microbiome. Nature 486(7402):207–214. doi:10.1038/nature11234

Johnson DB (2008) Biodiversity and interactions of acidophiles: key to understanding and optimizing microbial processing of ores and concentrates. T Nonferr Metal Soc 18(6):1367–1373. doi:10.1016/S1003-6326(09)60010-8

Johnson DB (1998) Biodiversity and ecology of acidophilic microorganisms. FEMS Microbiol Ecol 27(4):307–317. doi:10.1016/S0168-6496(98)00079-8

Kuang JL, Huang LN, Chen LX, Hua ZS, Li SJ, Hu M, Li JT, Shu WS (2013) Contemporary environmental variation determines microbial diversity patterns in acid mine drainage. Isme J 7(5):1038–1050. doi:10.1038/ismej.2012.139

Li T, Meng L, Gatesoupe FJ, Zhang Q, Li A, Gong X (2015) Comparative analysis of the intestinal bacterial communities in different species of carp by pyrosequencing. Microb Ecol 69(1):25–36. doi:10.1007/s00248-014-0480-8

Liu X, Chen B, Wen J, Renman R (2010) *Leptospirillum* forms a minor portion of the population in Zijinshan commercial non-aeration copper bioleaching heap identified by 16S rRNA clone libraries and real-time PCR. Hydrometallurgy 104(3):399–403. doi:10.1016/j.hydromet.2010.03.024

Liu Y, Yin H, Liang Y, Shen L, Liu Y (2011) Changes in the composition of an acid mine drainage microbial community upon successive transfers in medium containing low-grade copper sulfide. Bioresource Technol 102(20):9388–9394. doi:10.1016/j.biortech.2011.05.095

Loranger H, Weisser WW, Ebeling A, Eggers T, Luca ED, Loranger J, Roscher C, Meyer ST (2013) Invertebrate herbivory increases along an experimental gradient of grassland plant diversity. Oecologia 174(1):1–11. doi:10.1007/s00442-013-2741-5

Mendez-Garcia C, Pelaez AI, Mesa V, Sanchez J, Golyshina OV, Ferrer M (2015) Microbial diversity and metabolic networks in acid mine drainage habitats. Front Microbiol 6:475. doi:10.3389/fmicb.2015.00475

Ouml BZ, Erkan S, Pauliina N, Kaksonen AH, Puhakka JA (2007) Kinetics of iron oxidation by *Leptospirillum ferriphilum* dominated culture at pH below one. Biotechnol Bioeng 97(5):1121–1127. doi:10.1002/bit.21313

Peres-Neto PR, Jackson DA (2001) How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. Oecologia 129(2):169–178. doi:10.1007/s004420100720

Pielou EC (1977) Mathematical ecology. J Anim Ecol 47

Qiu GZ, Wan MX, Qian L, Huang ZY, Liu K, Liu XD, Shi WY, Yang Y (2008) Archaeal diversity in acid mine drainage from Dabaoshan Mine, China. J Basic Microbiol 48(5):401–409. doi:10.1002/jobm.200800002

Rodríguez Y, Ballester A, Blázquez ML, González F, Muñoz JA (2003b) New information on the pyrite bioleaching mechanism at low and high temperature. Hydrometallurgy 71(1–2):37–46. doi:10.1016/S0304-386X(03)00172-5

Rodríguez Y, Ballester A, Blázquez ML, González F, Muñoz JA (2003a) New information on the chalcopyrite bioleaching mechanism at low and high temperature. Hydrometallurgy 71(1–2):47–56. doi:10.1016/S0304-386X(03)00173-7

Shannon CE (1948) A mathematical theory of communication. Bell System Technical Journal 27(3):379–423. doi:10.1145/584091.584093

Smith TB, Wayne RK, Girman DJ, Bruford MW (1997) A role for ecotones in generating rainforest biodiversity. Science 276(5320):1855–1857. doi:10.1126/science.276.5320.1855

Sophie W, Valérie D, Prosser JI, Franck P, Claire C, Nadine G, Le RX (2007) Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. Environ Microbiol 9(9):2211–2219. doi:10.1111/j.1462-2920.2007.01335.x

Tamaki H, Tanaka Y, Matsuzawa H, Muramatsu M, Meng XY, Hanada S, Mori K, Kamagata Y (2011) *Armatimonas rosea* gen. nov., sp. nov., of a novel bacterial phylum, *Armatimonadetes* phyl. nov., formally called the candidate phylum OP10. Int J Syst Evol Micr 61(Pt6):1442–1447. doi:10.1099/ijs.0.025643-0

Tom C, Elke V, Emly S, Enevold F, Peter L, Peter V (2005) *Advenella incenata* gen. nov., sp nov., a novel member of the *Alcaligenaceae*, isolated from various clinical samples. Int J Syst Evol Micr 55(Part 1):251–256. doi:10.1099/ijs.0.63267-0

Troesch A, Duperray A, Polack B, Marguerie G (1990) Comparative study of the glycosylation of platelet glycoprotein *GPIIb/GPIIa* and the vitronectin receptor. Biochem J 268. doi:10.1042/bj2680129

Vardanyan NS, Vardanyan AK (2014) New sulphur oxidizing bacteria isolated from bioleaching pulp of zinc and copper concentrates. Univ J Microbiol Res 2(2):27–31. doi:10.13189/ujmr.2014.020201

Watling HR, Collinson DM, Shiers DW, Bryan CG, Watkin ELJ (2013) Effects of pH, temperature and solids loading on microbial community structure during batch culture on a polymetallic ore. Miner Eng 48:68–76. doi:10.1016/j.mineng.2012.10.014

Wikström P, Andersson AC, Forsman M (1999) Biomonitoring complex microbial communities using random amplified polymorphic DNA and principal component analysis. FEMS Microbiol Ecol 28(2):131–139. doi:10.1111/j.1574-6941.1999.tb00568.x

Williams KP, Kelly DP (2013) Proposal for a new class within the phylum *Proteobacteria*, *Acidithiobacillia* classis nov., with the type order *Acidithiobacillales*, and emended description of the class *Gammaproteobacteria*. Int J Syst Evol Micr 63(4):2901–2906. doi:10.1099/ijs.0.049270-0

Xiao Y, Xu Y, Dong W, Liang Y, Fan F (2015) The complicated substrates enhance the microbial diversity and zinc leaching efficiency in sphalerite bioleaching system. Appl Microbiol Biotechnol 99(23):10311–10322. doi:10.1007/s00253-015-6881-x

Yang Y, Yang LI, Sun QY (2014) Archaeal and bacterial communities in acid mine drainage from metal-rich abandoned tailing ponds, Tongling, China. Tran Nonferr Metal Soc 24(10):3332–3342. doi:10.1016/S1003-6326(14)63474-9

Yin H, Cao L, Qiu G, Wang D, Kellogg L (2008) Molecular diversity of 16S rRNA and gyrB genes in copper mines. Arch Microbiol 189(2): 101–110. doi:10.1007/s00203-007-0298-6

Yin H, Niu J, Ren Y, Cong J, Zhang X, Fan F, Xiao Y, Zhang X, Deng J, Xie M (2015) An integrated insight into the response of sedimentary microbial communities to heavy metal contamination. Sci Rep 22: 93–102. doi:10.1038/srep14266

Zhang L, Mao F, Li K, Wang Y, Chen X, Zhou H (2015) Enhancement in copper extraction from chalcopyrite by re-inoculation of different acidophilic, moderately thermophilic microorganisms. Hydrometallurgy 156:142–151. doi:10.1016/j.hydromet.2015.06.009

Zhang X, Niu J, Liang Y, Liu X, Yin H (2016) Metagenome-scale analysis yields insights into the structure and function of microbial communities in a copper bioleaching heap. BMC Genet 17:1–12. doi:10.1186/s12863-016-0330-4