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# The dynamic development of bacterial community following long-term weathering of bauxite residue

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## ABSTRACT

Bauxite residue is the industrial waste generated from alumina production and commonly deposited in impoundments. These sites are bare of vegetation due to the extreme high salinity and alkalinity, as well as lack of nutrients. However, long term weathering processes could improve residue properties to support the plant establishment. Here we investigate the development of bacterial communities and the geochemical drivers in bauxite residue, using Illumina high-throughput sequencing technology. Long term weathering reduced the pH in bauxite residue and increased its nutrients content. The bacterial community also significantly developed during long term weathering processes. Taxonomic analysis revealed that natural weathering processes encouraged the populations of Proteobacteria, Chloroflexi, Acidobacteria and Planctomycetes, whereas reducing the populations of Firmicutes and Actinobacteria. Redundancy analysis (RDA) indicated that total organic carbon (TOC) was the dominant factors affecting microbial structure. The results have demonstrated that natural weathering processes improved the soil development on the abandoned bauxite residue disposal areas, which also increased our understanding of the correlation between microbial variation and residue properties during natural weathering processes in Bauxite residue disposal areas.

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## Introduction

Bauxite residue, an industrial waste produced during the production of alumina, represents a large and increasing global flux (Gomes et al., 2016). Currently, the worldwide inventory of bauxite residue had already reached an estimated 4.2 billion tons, increasing by approximately 200 million tons per annum (Xue et al., 2019a). Bauxite residue is rarely

recycled and is frequently stored in bauxite residue disposal areas (BRDAs). The BRDAs commonly presents high alkalinity (pH  $\approx$  10–12), salinity (EC  $\approx$  1.4–28.4 mS/cm), and are oligotrophic with low content of nutrients (C, N, and P) (Jones and Haynes, 2011). Commonly, the BRDAs are bare of vegetation cover, which are more vulnerable to wind and water erosion, causing a seriously environmental concern (Gelencser et al., 2011; Mayes et al., 2011; Ruyters et al., 2011; Renforth et al.,

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2012). Therefore, remediation strategies to reduce the growing mass of stored bauxite residue are urgently required during the management of aluminum industry (Xue et al., 2016).

Direct revegetation often failed due to the harsh residue properties, including high alkalinity, salinity and toxic trace elements, as well as low nutrient (Jones and Haynes, 2011). Currently, various amendments are applied prior to vegetation to enable the residue more hospitable to plants (Jones et al., 2010; Courtney and Kirwan, 2012; Zhu et al., 2017; Kong et al., 2017a; Li et al., 2019; Tian et al., 2019a, 2019b; Xue et al., 2019b, 2019c). However, spontaneous vegetation colonization on abandoned BRDAs indicated natural weathering process may ameliorate bauxite residue to a soil-like medium which can support vegetation cover (Santini and Fey, 2013). The natural weathering processes decreased the alkalinity and salinity in bauxite residue, during which Na was replaced by Ca (Kong et al., 2017b). In addition, the natural weathering process accumulated nutrients and promoted aggregates formation in bauxite residue, eventually lead to successful vegetation establishment (Zhu et al., 2016b). Once colonized in bauxite residue, the plants can further improve their environment by exudation of organic acids and other organic compounds (Gräfe and Klauber, 2011).

Microbes are considered to be sensitive to environmental changes (Griffiths and Philippot, 2013). Numerous studies have demonstrated that extreme conditions (extreme acidic or alkaline, high heavy metal toxicity, low nutrients) can cause adverse effects (e.g., decline of microbial biomass and diversity, and inhibition of microbial activity) on soil microbiota and their ecological functions (Mendez et al., 2008; Brantner and Senko, 2014; Santini et al., 2015; Liu et al., 2018). The natural weathering process can promote microbial community development by regulating soil pH, improving soil structure and accumulating organic substance. The natural weathering process promoted the diversity of nitrogen-fixing microorganism community by increasing residue pH, organic matter and reducing the toxicity of heavy metals (Zhan and Sun, 2011). In the rare earth elements (REEs) contained soil, the increase in TC and TN were identified as two key factors to the development of bacterial community and functions (Chao et al., 2016). In addition, the natural weathering processes influenced the microbial community via plant community by their selective effects (e.g., carbon inputs, antimicrobials exudation, mineral weathering, and soil physicochemical alterations) can regulate the soil microbiota (Hartmann et al., 2009).

For bauxite residues, extreme alkalinity and salinity hindered the development of microbial community. The microbial communities were metabolically inactive in fresh bauxite residue, resulting in fresh bauxite residue was not capable of the normal ecological functions such as organic decomposition, inorganic mineral nutrients mobilization, which are essential to the establishment of vegetation cover. This may be responsible for the failure of vegetation establishment on bauxite residue (Hamdy and Williams, 2001). The addition of organic amendments (biosolid, compost) increased microbial biomass and promoted enzyme activities in bauxite residue (Jones et al., 2010). In addition, the

microbial diversity also developed rapidly in a short rehabilitation period (Banning et al., 2011). The microbial communities shift from haloalkaliphile assemblages to halotolerant assemblages as salinity, sodicity, and alkalinity decrease in bauxite residue during remediation (Santini et al., 2015). The microbial community developed to diverse soil-like microbial communities in a long term restoration (Schmalenberger et al., 2013). However, these studies were conducted under the artificial restoration processes and we still know little on the development of microbial community structure under natural conditions in bauxite residue. Spontaneous vegetation colonization on abandoned BRDAs provided us an opportunity to realize the development of bacterial community and its driving factors under natural weathering process.

In this study, the microbial community structure and its driven factors under natural weathering processes were evaluated by using high-throughput sequencing technology. Thus, the objectives of this study were to (a) investigate the effect of natural weathering processes on the diversity and composition of the bacterial community and (b) evaluate the key environmental factors in shaping bacterial community structure.

## 1. Materials and methods

### 2.1. Site description and sampling

The selected bauxite residue disposal area (BRDA) is located in Central China (35°24'N, 113°25'E) and was in operation for 25 years from 1993 to 2018. The climate is temperate continental monsoon, with an average temperature of 12.8–14.8 °C and average precipitation of 874 mm. Residue samples were collected during October, 2018. Four sampling sites were selected based on the weathering history and vegetation cover: unweathered residue site (UW: weathered for 1 years), young weathered residue site (YW: weathered for 10 years), old weathered residue site (OW: weathered for 25 years) and old weathered residue site covered with grass (OWG: grass appeared on residue weathered for 25 years). These locations were bare of vegetation except for OWG site. For each site, residue samples were collected at the surface layer (0–10) cm in triplicate (Fig. 1). All samples were divided into two parts. One part was dried at room temperature and then sieved (<2 mm) for physicochemical analyses. The other part was stored at –80 °C in the laboratory for microbial analyses.

### 2.2. Determination of residue properties

The residue pH was analyzed by water extraction (M/V, 1:5) using a pH detector and a conductivity meter. Total organic carbon (TOC) was determined by low-temperature external-heat potassium dichromate oxidation colorimetric method. Total nitrogen (TN) was determined by elemental analyzer (VARIO MAX, Elementar, Germany). Available phosphorus (AP) was extracted with 0.5 mol/L NaHCO<sub>3</sub> (pH 8.5) (M/V, 1:100) by shaking at 250 r/min for 30 min and measured using UV spectrophotometry (UV-2450, Shimadzu, Japan) by the Molybdenum blue method.

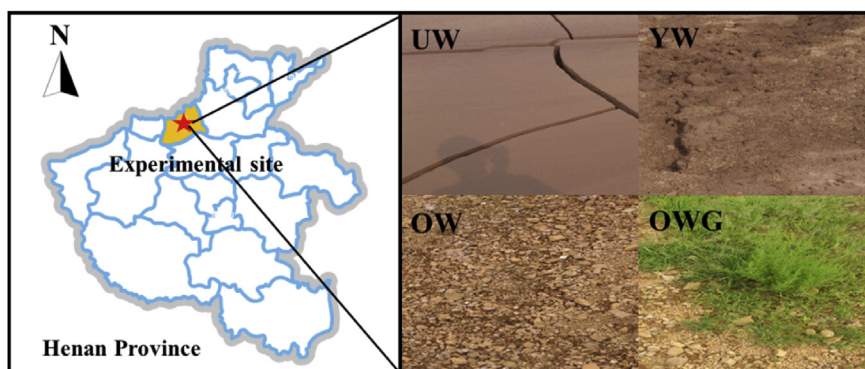


Fig. 1 – The location map and natural weathering condition of bauxite residue disposal areas (BRDAs). UW: unweathered residue; YW: young weathered residue; OW: old weathered residue; OWG: old weathered residue covered with grass.

### 2.3. DNA extraction and PCR amplification

DNA extraction was carried out by using the Ultra Clean Soil DNA extraction kit (Thermo Scientific, USA) according to the manufacturer's instructions. And then the extracted DNA was quantified using a Nanodrop spectrometer (ND-1000, Thermo Scientific, USA). The V4 hypervariable region of the bacterial 16 S rRNA gene was chosen for amplification, using primers 515F and 806R: 5'-GTGCCAGCMGCCGCGGTAA-3' and 5'-GGACTACHVGGGTWTCTAAT-3'. The amplification was conducted under the following conditions: predenaturation for 30 s at 98 °C; 30 cycles of denaturation for 15 s at 98 °C, annealing for 15 s at 58 °C, and extension for 15 s at 72 °C; final extension for 1 min at 72 °C; and holding at 4 °C. The amplicon products were purified using the Agarose Gel DNA purification kit (TIANGEN, China) and then sequenced on Illumina MiSeq 250 platform.

### 2.4. Data analysis and statistical procedures

The raw sequence reads (Access number: PRJNA566082 on NCBI) were first processed on the Qiime pipeline (Caporaso et al., 2010). Low quality sequences with ambiguous bases (quality scores of <20) and short sequences (length <150 bp) were removed. Then, the chimeras were eliminated using UCHIME software (Edgar et al., 2011). The remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% identity threshold in QIIME (Edgar, 2010) for subsequent analysis.

Alpha-diversity and beta-diversity of bacterial communities were calculated on the R statistical platform, using the 'vegan' package. Principal coordinate analysis (PCoA) based on Bray-Curtis distances was conducted to assess the similarities of different microbial communities. The LEfSe (Linear discriminant analysis Effect Size) analysis was performed on the online Galaxy platform (<http://huttenhower.sph.harvard.edu/galaxy/>) for detecting differential biomarkers. Canonical correspondence analysis was employed to reveal the relationship between residue properties and the bacterial community. Linear-regression analysis was conducted to assess the relationships between residue properties and bacterial taxa, using Origin 9.0 software.

## 3. Results

### 3.1. Diversity of bacterial community in bauxite residue

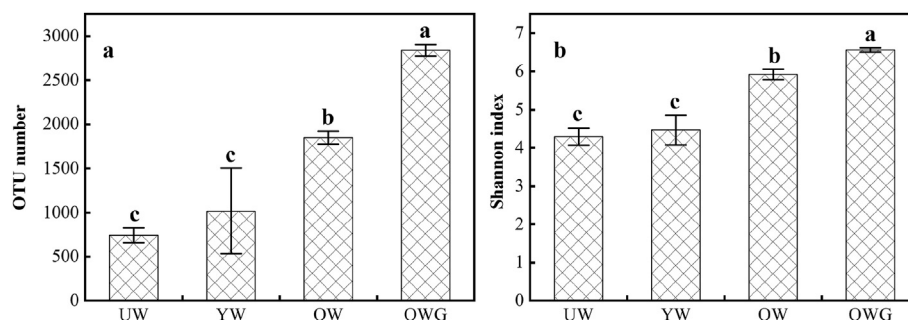
In total, 684,156 effective sequences were obtained from all residue samples and assigned to 3378 OTUs. The rarefaction curves tended to approach the saturation plateau, which well covered the microbial diversity (Appendix A Fig. S1).

The bacterial diversity indexes were significantly higher in old weathered residue than those in unweathered residue. The average OTU numbers and Shannon index increased from  $567 \pm 62$  (UW) to  $1950 \pm 17$  (OWG) and  $4.18 \pm 0.23$  (UW) to  $6.20 \pm 0.06$  (OWG), respectively (Fig. 2). The OTU numbers and Shannon index were significantly increased as the weathering status increased, except for the Shannon index between UW and YW residue ( $p < 0.05$ ).

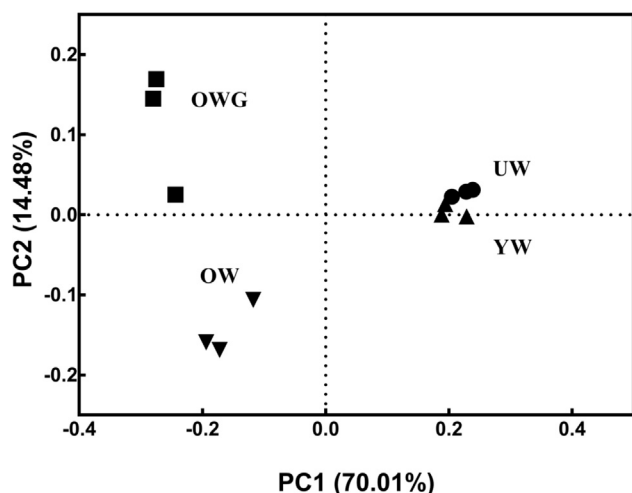
Principal coordinate analysis (PCoA) revealed that the natural weathering processes clearly separated the residue samples with different weathering status (Fig. 3). However, the difference was less pronounced between UW and YW residue compared with that between UW and OW residue. Furthermore, coordinate axes 1 and 2 (PC1 and PC2) can explain 70.01% and 14.48% of the total variation. The high explanation in the first principal-coordinate axis (PC1) indicated that the bacterial structure was significantly different in residue samples with different weathering status.

### 3.2. Composition of bacterial community in bauxite residue

Briefly, a total of 44 phyla were identified at the phylum level in all residue samples (Appendix A Table S1). There were six phyla including Firmicutes, Actinobacteria, Chloroflexi, Proteobacteria, Acidobacteria and Planctomycetes dominated residue samples, accounting for 78.83%–95.45% of all sequences (Fig. 4a; Appendix A Table S2). In UW residue, the most abundant phyla were Firmicutes (45.94%), followed by Actinobacteria (33.51%), Proteobacteria (7.2%) and Chloroflexi (5.04%) (Fig. 4a; Appendix A Table S2). Natural weathering processes significantly changed the composition of bacterial communities in bauxite residue. The abundance of Firmicutes and Actinobacteria significantly decreased, whereas the



**Fig. 2** – Alpha diversity of bacterial community in bauxite residue under natural weathering processes (a) OTU number, (b) Shannon index. Different letters above bars means significantly difference between different samples.



**Fig. 3** – Beta diversity (Principal coordinate analysis) of bacterial community in bauxite residue under natural weathering processes.

abundance of *Chloroflexi* significantly increased as the weathering level increased (Appendix A Table S2). Furthermore, several taxonomic groups including *Acidobacteria* (OW 9.52%) and *Planctomycetes* (OW 9.87%), which are typical bacterial populations in soils, were found to be enriched in OW residue (Fig. 4a; Appendix A Table S2). Furthermore, natural vegetation colonization further increased the abundance of *Acidobacteria* (OWG 18.87%), *Planctomycetes* (OWG 13.67%) and *Proteobacteria* (OWG 19.6%), whereas decreased the abundance of *Chloroflexi* (OWG 19.37%) (Fig. 4a; Appendix A Table S2) (see Fig. 5).

At the class level, a total of 61 classes were obtained across all residue samples, in which 14 classes were abundant at least in one residue sample. These dominated classes consisted of *Bacilli*, *Actinobacteria*, *Acidobacteria*, *Alphaproteobacteria*, *Thermomicrobia*, *KD4-96*, *Planctomycetacia*, *Gemmatimonadetes*, *Gammaproteobacteria*, *Deinococci*, *Phycisphaerae*, *Gitt-GS-136*, *Anaerolineae*, *Betaproteobacteria*, accounted for 82.5–95.4% in each sample (Appendix A Table S3). The relative abundances of *Actinobacteria*, *Bacilli* and *Deinococci* were significantly higher in the unweathered residue (UW) than that in the old weathered (OW and OWG)

residue (Fig. 4b; Appendix A Table S4). However, the relative abundances of *Acidobacteria*, *Alphaproteobacteria*, *KD4-96*, *Planctomycetacia*, *Phycisphaerae*, *Gitt-GS-136*, *Anaerolineae*, and *Betaproteobacteria* were significantly higher in old weathered residue (OW and OWG) than that in unweathered residue (UW) and young weathered residue (YW) (Fig. 4b; Appendix A Table S4). The bacterial composition at the class level showed significantly difference between OW residue and OWG residue. The relative abundances of *Actinobacteria*, *Bacilli*, *KD4-96*, *Thermomicrobia*, *Gitt-GS-136*, and *Gemmatimonadetes* were significantly higher in OW than that in OWG residue, whereas the relative abundances of *Acidobacteria*, *Alphaproteobacteria*, *Planctomycetacia*, *Phycisphaerae*, and *Betaproteobacteria* were significantly lower in OW residue than that in OWG residue (Fig. 4b; Appendix A Table S4).

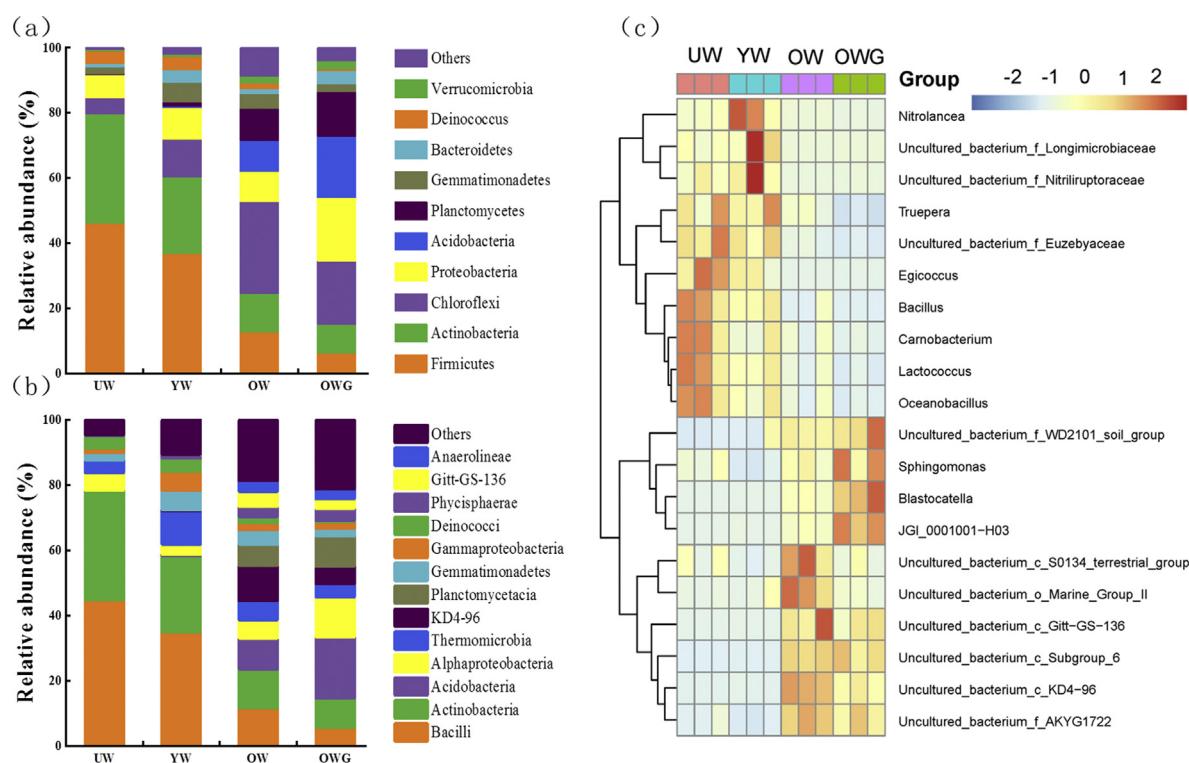
For a further analysis, a hierarchically clustered heatmap was generated to exhibit the relationships of the top 30 genera among the 12 residue samples (Fig. 4c). In UW residue, the most abundant genera were *Lactococcus* (20.2%) followed by *norank\_f\_Euzebyaceae* (16.8%), and *Bacillus* (14.1%) (Fig. 4c). The abundance of these groups was significantly higher than those in OW and OWG residue ( $p < 0.05$ ). Natural weathering processes changed the dominant genera in bauxite residue. In OW and OWG residue, the most abundant genera were *norank\_c\_KD4-96*, followed by *norank\_c\_Acidobacteria*, *Lactococcus*, *norank\_c\_Gitt-GS-136*, *Sphingomonas*, *norank\_f\_Anaerolineaceae*, *norank\_o\_AKYG1722*, *norank\_f\_Blastocatellaceae* and *norank\_f\_Tepidisphaeraceae* (Fig. 4c). The abundance of these groups was significantly higher than those in UW residue sites ( $p < 0.05$ ).

Linear discriminant analysis (LDA) was applied to detect the significantly different species in bauxite residue with different weathering history. According to LDA analysis, 23 bacteria classes presented significant differences among the residue sites with an LDA threshold of 4.0. Specifically, *Bacilli* (class), *Firmicutes* (phyla), *Actinobacteria* (class), *Actinobacteria* (phyla), *Lactobacillales* (order) were enriched in UW residue and *Acidobacteria* (phyla), *Acidobacteria* (class), *Proteobacteria* (phyla), *Planctomycetes* (phyla), *Blastocatellaceae* (family) were enriched in OW residue.

### 3.3. Residue properties

The properties of bauxite residue significantly changed during natural weathering processes (Table 1). The unweathered





**Fig. 4 – Bacterial community composition of bauxite residue under natural weathering processes at the (a) phylum level, (b) class level and (c) genera level.**

residue (UW) presented high alkalinity and salinity, as well as a lack of nutrients. During the natural weathering processes, the values of pH decreased, whereas the contents of TOC, TN, and AP increased. They all showed significantly difference between residue samples ( $p < 0.05$ ). In addition, natural vegetation colonization further significantly decreased the alkalinity and increased nutrients in bauxite residue ( $p < 0.05$ ).

### 3.4. Correlations between bacterial community and residue properties

In this study, five parameters, including pH, TOC, TN and AP were selected to investigate the influence of residue properties on the diversity and composition of bacterial communities in bauxite residue. The Shannon index has been widely used to estimate microbial diversity in ecological restoration. The Shannon index showed positively correlations with the

contents of TOC, TN and AP ( $R^2 = 0.925$ ,  $p < 0.001$  for TOC;  $R^2 = 0.870$ ,  $p < 0.001$  for TN; and  $R^2 = 0.921$ ,  $p < 0.001$  for AP), whilst negatively correlations with the pH ( $R^2 = 0.867$ ,  $p < 0.001$ ) (Appendix A Fig. S2).

Redundancy analysis (RDA) was applied to reveal the driving factors for the development of bacterial community. The first two axes explained 88.85% of the variation of microbial composition, and the correlation of species-environment of both axes was  $>95\%$ , which suggested a remarkable correlation between microbial community composition and residue properties (Fig. 6). In addition, the residue properties also significantly affected the composition of bacterial community. Linear-regression analysis was conducted to reveal the relationships between residue properties and bacterial taxa (Appendix A Figs. S3–S6). The pH showed significant positive correlations with the abundance of Firmicutes and Actinobacteria ( $p < 0.001$ ), while negative

**Table 1 – Selected properties of the residue samples with different weathering history.**

	UW	YW	OW	OWG
pH	11.03 ± 0.11da	10.6 ± 0.07b	10.1 ± 0.09c	9.4 ± 0.10d
TOC (g/kg)	5.71 ± 0.26a	8.00 ± 0.30b	9.24 ± 0.25b	10.81 ± 1.15a
TN (g/kg)	0.039 ± 0.008d	0.150 ± 0.06c	0.729 ± 0.07b	1.532 ± 0.28a
AP (mg/kg)	5.32 ± 0.25d	10.48 ± 0.25c	22.74 ± 5.44b	34.94 ± 5.44a

TOC: total organic carbon; TN: total nitrogen; AP: available phosphorus.

\* Mean ± SD, different letters means significant differences between different samples.

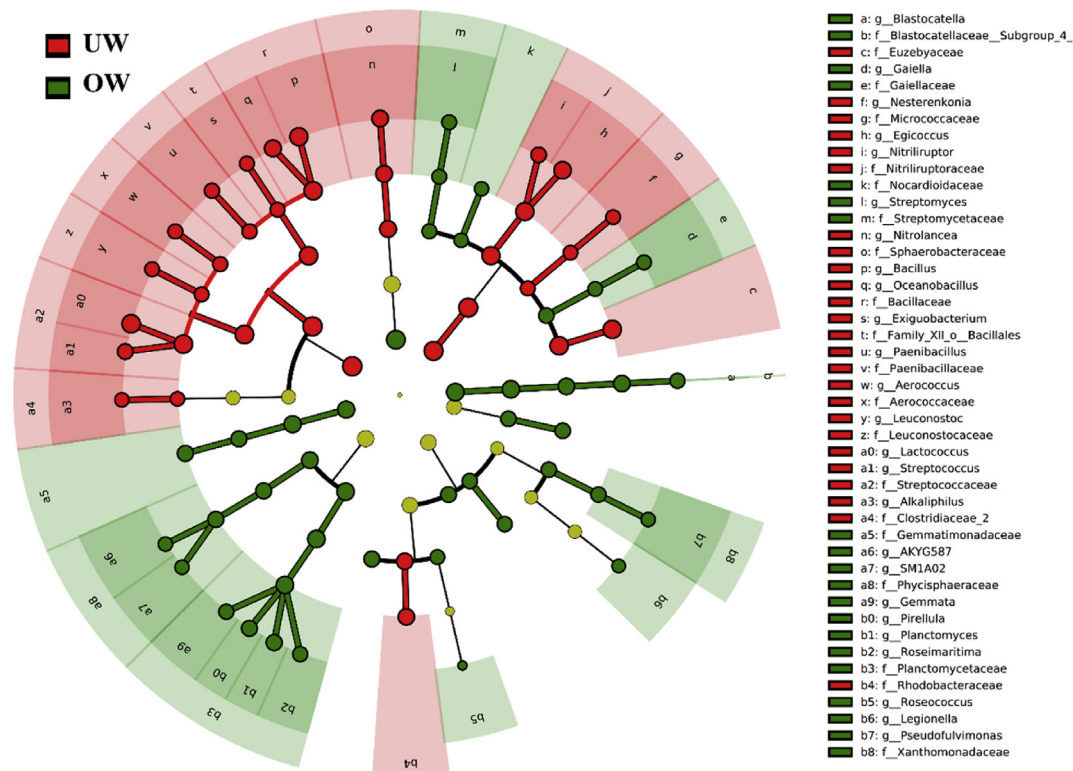


Fig. 5 – LEfSe analysis of bacterial community in bauxite residue under natural weathering processes. Yellow circles represent non-significant differences in abundance between residue samples. Taxa enriched in UW residue with a positive LDA score (red), and taxa enriched in OW residue have a negative score (green).

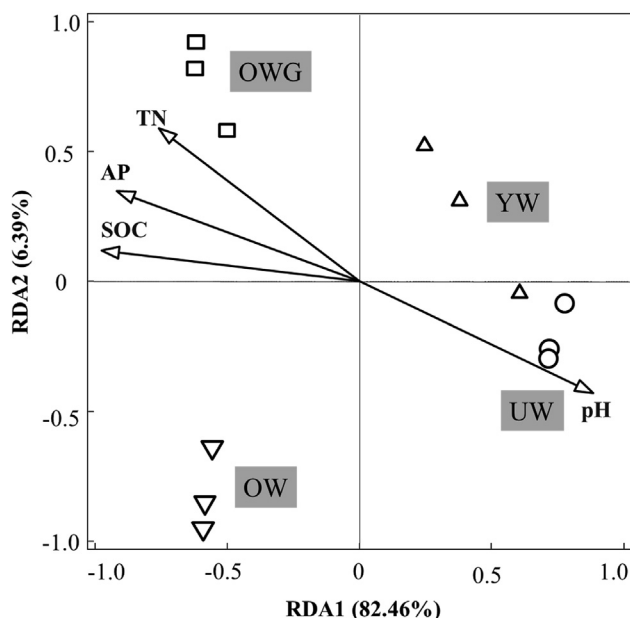


Fig. 6 – The redundancy analysis (RDA) of the bacterial community and residue properties.

correlations with the abundance of Acidobacteria, and Planctomycetes ( $p < 0.001$ ). However, the correlations between pH, EC and Chloroflexi and Proteobacteria are relatively weaker. The nutrient status also significantly influenced the

bacterial communities in bauxite residue. Briefly, the content of TOC, TN and AP showed significant positive with the abundances of Acidobacteria and Planctomycetes ( $p < 0.05$ ), whilst significant negative with the abundances of Firmicutes and Actinobacteria ( $p < 0.05$ ).

## 4. Discussion

### 4.1. Effect of natural process on residue properties

The natural weathering processes caused significant changes to the chemical properties of bauxite residue. The pH value in OW residue was significantly lower than that in UW residue, which may be caused by wind erosion and water leaching. The leaching of free hydroxides, carbonates and aluminates, and dissolution of alkalinity solids including sodalite, hydrogarnet and calcite may be responsible for the alkalinity reduction under natural weathering processes (Kong et al., 2017b, 2018). In addition, some bacterial communities may secrete organic acids, which may benefit to the reduction of alkalinity in bauxite residue (Hamdy and Williams, 2001; Liao et al., 2018; Wu et al., 2019). Furthermore, the natural vegetation colonization may also help to the reduction in alkalinity by secreting organic acids. Furthermore, the natural vegetation colonization at OWG residue may be also responded for the decrease in alkalinity and salinity of bauxite residue (Zhu et al., 2016b). When plants suffer from environmental stresses such as

phosphorus deficiency or aluminum stress (Ryan et al., 2001), they generally release a wide range of compounds, including various organic acids and amino acids to alleviate the stresses in the rhizosphere (Carvalhais et al., 2011).

Contrary to the decrease in alkalinity and salinity, the nutrients content increased in bauxite residue during the natural weathering processes. The natural weathering processes promoted the formation of stable aggregates and the formed stable aggregates may protect the mineralization of organic carbon (Zhu et al., 2016a, 2018). In addition, the shift from haloalkaliphile-dominated assemblages to diverse soil species with diverse functions such as C/N fixation may also be the reasons for the accumulation of nutrients in bauxite residue (Santini et al., 2015). In addition, the plant exudation and plant residue also contribute to the nutrient accumulation in bauxite residue.

#### 4.2. Effect of natural process on bacterial community

Abandoned tailings were generally presented lack of nutrients, which are the major restrictive factors for revegetation and microbial development (Li et al., 2015; Mendez and Maier, 2008). In this study, the microbial diversities in OW residue were higher than those in UW residue (Table 1), indicating that natural weathering processes increased the bacterial diversity in bauxite residue. Similar results were also found in copper mine tailings (Zhan and Sun, 2014), abandoned coal mine site (Harantova et al., 2017) and rare earth elements tailings (Chao et al., 2016). This may be caused by the improvement of residue properties, which significantly shaped the soil microbial communities (Banning et al., 2011). In addition, the vegetation promoted the development of bacterial communities by creating a nutrient enriched environment, such as root exudates, sloughed-off root cells and mucilage (Ryan et al., 2001).

Bacterial communities in unweathered (UW) residue were dominated by Firmicutes and Actinobacteria which was consistent with the previous studies (Krishna et al., 2014). This may due to the strong metabolic capacities of Firmicutes and Actinobacteria in high alkaline and saline conditions (Joshi et al., 2008; Antony et al., 2013). Additionally, *Lactococcus* of the Firmicutes was the most dominant genus in all residue samples, while many strains of *Lactococcus* spp. with the abilities of acid production (Mercade et al., 2000; Prasad et al., 2010; Sahoo and Jayaraman, 2019). Similarly, the genus *Bacillus* of the Firmicutes also contains many strains that are able to weather minerals and are involved in nutrient cycling (Uroz et al., 2011). All of these results suggested that the bacterial taxa adapted to the particular environment and reflected the conditions of the habitat, including those polluted by anthropogenic activities and/or low nutrient availability (Akob et al., 2007).

The composition of bacterial communities significantly changed during natural weathering processes. In old weathered (OW and OWG) residue, the bacterial communities were dominated by Chloroflexi, Acidobacteria, Planctomycetes and Proteobacteria, which is coincided with many previous reports (Krishna et al., 2014; Santini et al., 2015), in which long term restoration resulted in the accumulation of Acidobacteria. Acidobacteria are generally acidophilic, and exist extensively in various ecosystems. The abundance of

Acidobacteria in soils is commonly correlated with soil pH (Jones et al., 2009). Acidobacteria can make up 20% of all bacteria in soils with a pH ranged from 7–8 (Lauber et al., 2009). In this study, the pH in OWG residue was 9.4 and the abundance of the Acidobacteria was in the range of 18% (Table 1), coincide with the discipline that more abundant Acidobacteria exist in lower pH soil.

Proteobacteria is another abundant bacterial phylum in soils that plays an important role in ecological restorations, which positively participate in energy metabolism, such as the oxidation of organic and inorganic compounds and obtaining energy from light, fixation of atmospheric carbon and nitrogen (Ye and Thomas, 2001). In this study, the abundance of Proteobacteria significantly increased during natural weathering processes, which is consistent with previous studies (Schmalenberger et al., 2013; Krishna et al., 2014). For instance, Bondici et al. (2014) found that the relative abundance of Proteobacteria was >50% in uranium mine tailings. Proteobacteria also accounted for 40–80% of all the sequence in restored bauxite residue (Santini et al., 2015).

#### 4.3. Relationship between bacterial community and residue properties

Natural weathering processes improved residue properties and changed bacterial community structure at the disposal area. Redundancy analysis (RDA) analysis showed that the residue properties including pH, TOC, TN and AP were the prime drivers of microbial community.

Previous studies have demonstrated pH is one of the most important environmental factors in regulating bacterial communities (Fierer and Jackson, 2006; Griffiths et al., 2011; Shen et al., 2013). Extreme pH often restricts microbial community diversity by imposing stress on microbial colonization, and regulating the availability of nutrient elements for microbial growth. Under high pH condition, the decrease in pH, such as soil acidification, may enhance the release of mineral nutrients for microbial growth and subsequently influence microbial community composition (Carson et al., 2007). In addition, pH can effectively predict the composition of bacterial community in alkaline sediments (Xiong et al., 2012). The results in our study also showed that residue pH could drive the development of bacterial communities. With the decrease of residue pH, the relative abundance of Acidobacteria increased. This was similar to the distribution patterns of Acidobacteria across the related pH gradient (Jones et al., 2009; Dimitriu and Grayston, 2010; Shen et al., 2013). Furthermore, the relative abundance of Alphaproteobacteria increased at a lower pH, which contrasted with the results in other studies (Shen et al., 2013). It was worth noting that these soils were weakly acidic or nearly neutral (3.5–6.5), whilst the pH in our residue samples ranged from 9.4 to 11.3. The different pH environments may result in different variations in abundance of Alphaproteobacteria.

Besides pH, other environmental factors such as plant types (Wei et al., 2017), salinity gradient (Crump et al., 2004) and water content (Drenovsky et al., 2004) are also of great importance for the geographic distribution of microbial communities. In text of bauxite residue, microbial communities were restricted not only be high pH but also limited nutrients

(Gräfe and Klauber, 2011). The natural weathering processes increased nutrients, including SOC, TN and AP. We hypothesized that affect the bacterial community was also influenced by the nutrient status in bauxite residue. In this study, the relative abundances of six major phyla, including Proteobacteria, Acidobacteria, Firmicutes, Actinobacteria, Chloroflexi and Planctomycetes, had significant correlations with the contents of TOC, TN and AP. Among these groups, the content of TOC, TN and AP significantly decreased the abundance of Actinobacteria and Firmicutes, whilst increased the abundance Acidobacteria, Proteobacteria, Chloroflexi and Planctomycetes. Many previous studies also reported soil nutrients were major factors affecting the bacterial community and bacterial taxa. For example, the bacterial community was pronouncedly driven by soil nutrients (TC, TN, TP and AP) mine site (Zhan and Sun, 2014; Chao et al., 2016). Similar results were also reported in restored bauxite residue. Restored for 17 years, the content of TOC, TN and AP significantly increased, largely promoting the development of bacterial community in bauxite residue (Schmalenberger et al., 2013).

## 5. Conclusions

This study revealed the dynamic development of diversity and structure in microbial communities along with natural weathering processes at bauxite residue disposal areas. Alkalinity decreased, whilst nutrient elements improved during natural weathering processes. Both microbial diversity and composition significantly differed in bauxite residue following long-term natural weathering. The dominant phyla were Firmicutes and Actinobacteria at the unweathered site, whilst Proteobacteria, Chloroflexi, Acidobacteria and Planctomycetes dominated in the old weathered residues. Twenty-three biomarkers were found in the bauxite residue through a linear discriminate analysis (LDA) effect size (LEfSe) analysis. LEfSe analysis revealed that the biomarker changed significantly from Firmicutes (phyla) and Actinobacteria (class) in unweathered residues to Acidobacteria (phyla) and Planctomycetes (phyla) in old weathered (OW) residues. Soil microbial community composition and diversity were regulated by soil nutrients (TOC, TN and AP) and alkalinity (pH), whilst total organic carbon was the primary factor. This study has improved our understanding of development of microbial community in bauxite residue disposal areas and further studies should focus on functional gene prediction to reveal possible mechanisms of metabolic pathways of microorganisms on soil formation in bauxite residue disposal areas.

## Declaration of competing interest

The authors declare that they have no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jes.2019.12.001>.

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