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Long term natural restoration creates soil-like microbial communities in bauxite residue: a case for 50-year field study

Hao Wu[1], Tian Tang[1], Feng Zhu[1]*, Xiaomeng Wei[2], William Hartley[3], Shengguo Xue[1]*

1 School of Metallurgy and Environment, Central South University, Changsha 410083, China

2 Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, 410125, China

3 Crop and Environment Sciences Department, Harper Adams University, Newport, Shropshire, TF10 8NB, United Kingdom

Abstract:

Ecological reconstruction on bauxite residue disposal areas is regarded as an effective approach to eliminate potential environmental risks. Establishment of microbial communities and associated functions may improve physical and chemical properties, and may stimulate soil formation in bauxite residue. Spontaneous colonization at a disposal area in Shandong Province, China, over 50 years, indicated that natural weathering can ameliorate residues, and in turn, support the establishment of vegetation communities. Residue samples were collected from unrestored, poorly restored and well restored areas to investigate the development of microbial communities and associated functions. Microbiota significantly developed after long term natural restoration. Microbial biomass, respiration and enzyme activities significantly increased in restored bauxite residue, whereas the metabolic quotient

*Corresponding author. E-mail: sgxue@csu.edu.cn; zhufeng1990@csu.edu.cn;

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significantly decreased. In addition, the long-term natural restoration significantly shaped the microbial structure from alkalophilic and halophilic assemblages (Firmicutes and Actinobacteria) to neutrophilic assemblages (Acidobacteria and Planctomycetes). Both microbial communities and associated functions in well restored residue had high similarity with that in natural soil, indicating that long term restoration created diverse soil-like microbial communities and functions. Redundancy analysis (RDA) revealed that TN, followed by Na^+, ESP, SOC, AP and pH were the major influence factors in the development of microbial communities in bauxite residue. These findings provide us a biogeochemical perspective to reveal soil formation in bauxite residue and suggest that nutrient supplement and regulation of salinity-alkalinity may benefit for the establishment of microbial communities and functions in bauxite residue.

KEYWORDS: Bauxite residue; Natural restoration; Microbial functions; Microbial communities; Soil formation in bauxite residue;

1 | INTRODUCTION

Bauxite residue is an alkaline product which is generated from the extraction of alumina by the Bayer process (Xue et al., 2016). To date, the global inventory of bauxite residues has reached over 4.6 billion tons and increases by 200 million tons per year, thus occupying large areas of the landscape (Xue et al., 2019b). In addition, ecological security issues including wind/water erosion and fugitive dust pollution have become increasingly prominent the surrounding environments (Gelencser et al., 2011; Ruyters et al., 2011). Ecological reconstruction is theoretically, a most promising option to manage such large volumes of bauxite residue. However, it often

fails due to the lack of adequate soil which is beneficial to support vegetation establishment. Currently, converting bauxite residue into a soil-like medium, has been advocated to achieve the ultimate reconstruction of a self-sustaining ecosystem (Santini & Banning, 2016).

The adverse physiochemical properties including extreme high alkalinity, fine grained structure and nutrient deficiency, hinder the soil formation in bauxite residue (Jones & Haynes, 2011). Various chemical and physical amendments have been applied to improve residue properties and encourage soil formation, but reported results have not been satisfactory (Courtney, Jordan, & Harrington, 2009; Zhu et al., 2017; Xue et al., 2019a; Xue et al., 2020). Our previous studies have demonstrated that natural weathering process may ameliorate tailings to the extent that it could support vegetation (Kong et al., 2017; Zhu et al., 2018). Natural weathering promoted the dissolution of alkaline minerals such as calcite, hydrogarnet, and sodalite, and thus decrease the alkalinity and salinity in bauxite residue, consequently benefitting plant growth (Kong et al., 2017; Kong et al., 2018). In addition, natural weathering also induced the formation of stable aggregates, thus improving physical conditions for plant growth (Zhu et al., 2016; Zhu et al., 2018). These changes of physical and chemical properties indicated that long-term natural weathering converted bauxite residue to soil-like medium, which can support vegetation cover. However, the soil formation is not only the development of chemical and physical properties, but also largely associated with the development of microbial communities and biogeochemical functions (Mummey, Stahl, & Buyer, 2002).

Microbial communities are essential promoters in biogeochemical cycling and ecological function construction, and may stimulate soil formation in mine tailings. Microbiota may assisted pH neutralization (You, Zhang, Ye, & Huang, 2019),

accumulate nutrients (Liu, Song, Wang, Li, & Shu, 2012), and form stable aggregates (Kumar, Singh, & Ghosh, 2018). Furthermore, the establishment of stable plant communities is closely correlated to microbial communities (Grandlic, Mendez, Chorover, Machado, & Maier, 2008). For example, high numbers of autotrophic iron- and sulfur-oxidizing bacteria are commonly associated with plant death by inducing soil acidification in mine tailings (Chen et al., 2015). On the contrary, neutrophilic heterotrophic bacteria often assist the establishment of stable plant communities by performing diverse ecological functions associated with nutrient cycling (Chen et al., 2013). Thus, a better understanding of the microbial assemblage and its succession, especially the key geochemical driving factors, is of great importance for the restoration of mine tailings.

Nevertheless, the process of microbial succession in bauxite residue is poorly understood, resulting in restoration failures. The extreme environmental conditions only permit colonization by tolerant microbial communities, which results in relatively low microbial diversity and limited biogeochemical processes (Krishna, Babu, & Reddy, 2014). The input of organic solids, including biosolids and compost, may encourage microbial establishment (Jones, Haynes, & Phillips, 2010, 2011), and thereby enhancing plant growth (Schmalenberger, O'Sullivan, Gahan, Cotter, & Courtney, 2013). To date, no studies have investigated microbial community succession in bauxite residue without the absence of targeted rehabilitation efforts, for example, addition of amendments.

The present study investigated microbial communities and associated geochemical functions in bauxite residue undergoing natural restoration. The objectives of this study were to (1) characterize the development of microbial communities and associated functions, (2) determine the influence factors to

microbial communities and (3) evaluate the differences in microbial communities in restored bauxite residue and natural soil.

2 | MATERIALS AND METHODS

2.1 | Site description and sampling

This study was carried out at a Bauxite Residue Disposal Area (BRDA) in Zibo City, Shandong Province, China. This region has a typical temperate continental monsoon, with an average annual precipitation of 1346 mm and an average annual temperature of 16.0 ℃. Affected by the monsoon, climate change has obvious seasonality, which showed hot and rainy in summer, cold and dry in winter.

After over 50 years of natural restoration, plant communities dominated by perennial herbs and woody plant species including *Artemisia*, *Cynodon*, *Setaria*, *Corispermum* and *Hedysarum* established. According to the development of vegetation cover, we selected three restored residue sites, including unrestored (UR), poorly restored (PR), and well restored (WR) residue site (Fig. 1; Table S1). In addition, one natural soil site (NS) was selected as the reference in order to compare the difference of microbial communities and functions with different developed residue site (Fig. 1; Table S1).

Three quadrats (10 m × 10 m) were randomly selected at each site. The distance between each quadrat was > 30 m, which surpassed the space relatedness for the microbial variables; thus, each quadrat was independent from the others. At each quadrat, five samples were randomly collected, together forming one sample. Gravel and plant residues were removed, and the samples were transported to the laboratory under 4℃ by using icebox. When return to laboratory, the samples were processed and divided into three parts. One part dried and sieved (<2 mm mesh) for

physicochemical properties determination; One part stored at -20℃ for microbial biomass, enzyme assays and One part stored at -80℃ for microbial community. The physicochemical properties of bauxite residue and soil samples are shown in Table S2.

2.2 | Measurement of microbial biomass and activity

Microbial biomass carbon (MBC) was determined by chloroform fumigation-extraction method (Wu, Joergensen, Pommerening, Chaussod, & Brookes, 1990). Briefly, two equal portions of fresh samples (equivalent to about 20 g of oven-dried soil) were fumigated with alcohol-free $CHCl_3$ vapor in darkness for 24hin a vacuum desiccator. Both fumigated and unfumigated samples were extracted with 80 mL of 0.5M K_2SO_4 (residue/extractant ratio 1:4 w/v) by shaking at 250 rpm for 30 min. The resulting suspensions were filtered for the determination of organic carbon. Organic carbon in fumigated and unfumigated extracts was measured using a TOC analyser (TOCVWP; Shimadzu, Kyoto, Japan). Microbial biomass carbon (MBC) was calculated as the difference in organic carbon between fumigated and unfumigated samples with a conversion factor K_{EC} as 0.45.

To determine microbial respiration rate, 50 g of fresh sample and 25 mL of 0.1 $mol \cdot L^{-1}$ NaOH were placed in a hermetically sealed chamber (1 L). The produced CO_2 from microbial respiration can be absorbed by NaOH. The samples were incubated at 25 °C for 24 h in darkness and the absorbed $CO_2$was measured using a TOC analyser (TOCVWP; Shimadzu, Kyoto, Japan).

2.3 | Measurement of enzyme activity

Glucosidase, urease and alkaline phosphatase were selected to reflect the microbial functions involved in the turnover of carbon, nitrogen and phosphorus in bauxite residue. Direct soil zymography was applied as an in-situ technique to study

the enzyme activity in bauxite residue. The glucosidase and phosphatase activities were determined according to the fluorescence enzyme protocol (Deng, Popova, Dick, & Dick, 2013), in which 4-methylumbelliferone (MUF)-substrates can be hydrolyzed by a specific enzyme and then detected by using a microplate fluorometer (SynergyTM H1, Biotek) at 365 nm excitation and 450 nm emission wave lengths. Glucoside and phosphatase activity were detected by using 4-Methylumbelliferyl β-D-glucoside and 4-methylumbelliferyl-phosphate, respectively. Urease activity was measured by using 0.5 $mol \cdot L^{-1}$ urea as the substrate in 0.1 $mol \cdot L^{-1}$ phosphate buffer. The produced NH_4^+–N was measured by using a phenol-sodium hypochlorite colorimetric method (Nannipieri, Ceccanti, Cervelli, & Sequi, 1974).

2.4 | DNA extraction and PCR amplification

Total DNA was extracted from the samples using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc.) after cell enrichment. DNA concentration and quality were determined with a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable region V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′- GGACTACHVGGGTWTCTAAT-3′) by an ABIGeneAmp®9700 PCR thermocycler (ABI, CA, USA). The PCR amplification was performed as follows: pre-denaturation for 3 min at 95℃; 30 cycles of denaturation for 35 sec at 95 ℃, annealing for 35 sec at 58℃, and extension for 45 sec at 72 ℃; final extension for 10 min at 72 ℃; and holding at 4 ℃ using a PCR instrument (XP Cycler, Bioer, Hangzhou,China). The PCR mixtures contain 5 × *TransStart*FastPfu buffer 4 μL, 2.5 mM dNTPs 2 μL, forward primer (5 μM) 0.8 μL, reverse primer (5 μM) 0.8 μL, *TransStart*FastPfu DNA Polymerase 0.4 μL, template DNA 10 ng, and finally ddH_2O up to 20 μL. PCR reactions were performed in triplicate. The PCR product was

extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences,Union City, CA, USA) according to manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA).

2.5 | Illumina MiSeq sequencing

Purified amplicons were pooled in equimolar and paired-end sequenced on anIllumina MiSeq PE300 platform (Illumina, San Diego,USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

2.6 | Processing of sequencing data

Raw sequences were analyzed 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% similarity cutoff by using UPARSE version7.1 (Edgar, 2013). The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 (Wang, Garrity, Tiedje, & Cole, 2007) against the 16S rRNA database (Silva v138) using confidence threshold of 0.7.

2.7 | Statistical analysis

Physicochemical and physiological data were analyzed using SPSS (v 20.0) and R package "vegan". Independent samples t-test and one-way ANOVA were applied to compare the correlations between the physicochemical properties, physiological properties, microbial relative abundances and α-diversity indices. Unconstrained principal coordinate analysis (PCoA) basing on UniFrac distance was applied to analyze the β-diversity of microbial communities. Two nonparametric multivariate statistical tests (ANOSIM and PERMANOVA) were used to determine the

significance of β-diversity. redundancy analysis (RDA) was conducted to reveal correlations between microbial community and environmental factors.

3 | RESULTS

3.1 | Development of microbial biomass and microbial activity

In this study, microbial biomass carbon (MBC) was chosen to reflect the microbial biomass in bauxite residue. The MBC significantly differed among the residue site (Fig. 2A). The microbial biomass carbon (MBC) was significantly lower in UR site compared to that in NS site (Fig. 2A). After 50 years of natural restoration, the microbial biomass carbon (MBC) significantly increased to 137.2 mg/kg (PR) and 317.9 mg/kg (WR), increased by 3.49 times and 9.42 times compared with that in NR (Fig. 2A).

Natural restoration not only increased the microbial biomass, but also significantly promoted microbial activities (Fig. 2B). The microbial respiration rate (PRR) increased to 89.71 CO_2 mg/kg/d (PR) and 111.70 CO_2 mg/kg/d (WR), increased by 0.67 times and 1.07 times compared with that in NR (Fig. 2B).

Notably, both microbial biomass (MBC) and microbial respiration rate (PRR) showed no significantly difference between well restored residue (WR) and natural soil (NS) ($p<0.05$; Fig. 2A-B).

3.2 | Development of enzyme activities

In this study, glucosidase, invertase, urease and alkaline phosphatase were selected to reflect the microbial functions involved in the turnover of carbon, nitrogen and phosphorus in bauxite residue. The activities of glucosidase, invertase, urease and phosphatase significantly differed among the residue site, and increased as the restoration level increased ($P < 0.05$) (Fig.3A-D). Glucosidase in PR and WR

significantly increased by 1.76 times and 3.55 times compared with that in NR (Fig.3A). Invertase was lowest in NR, and increased by 2.12 times and 4.96 times in PR and WR (Fig.3B). Urease was lower in NR and increased by 1.72 times and 5.09 times in PR and WR (Fig.3C). In terms of phosphatase, PR and WR exhibited increased phosphatase by 1.08 times and 2.601 times compared with that in NR (Fig.3D).

Notably, the enzyme activities, except for invertase, showed no significantly difference between WR and NS ($p>0.05$; Fig. 3A-D).

3.3 | Development of microbial communities

3.3.1 | Diversity of microbial communities

In this study, OTU numbers and Shannon index were used to represent the species richness and diversity of bacterial communities. The OTU numbers and Shannon index were significantly lower in UR compared with that in NS ($P < 0.05$; Fig 4A-B). After over 50 years of natural weathering, the richness and diversity of bacterial communities significantly increased ($P < 0.05$; Fig 4A-B). The OTU numbers increased to 1717 ± 368 and 1944 ± 76 in PR and PR residue. The Shannon index in PR and PR significantly increased to 5.80 ± 0.21 and 6.06 ± 0.09 (Table 3). The OTU numbers and Shannon index in PR and PR both were significantly higher than that in LR, whereas no significant difference was observed between PR and PR. Notably, the microbial diversity indices in PR and PR were similar to that in NS ($p>0.05$; Fig. 4A-B).

The analysis of similarities (ANOSIM) ($r = 0.74$, $P < 0.01$) (Fig. 5A) showed that the samples from the restored residue (PR and PR) significantly differed from unrestored residue (UR). The Principal coordinate analysis (PCoA) analysis was applied to evaluate differences in microbial structure across all samples (Fig. 5B).

According to PCoA analysis, the natural restoration clearly separated unrestored residue (UR) and restored residue (PR and PR). In addition, the bacterial communities in restored residue PR showed high similarity with that in natural soil (NS) ($p>0.05$).

3.3.2 | Structure of microbial communities

Based on the taxonomic analysis, six major phyla including Firmictes, Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria and Planctomycetes dominated the microbial communities in residue samples, accounting for 84.4% to 88.1% of all the sequence (Fig. 6; Table S4-S5). However, the microbial communities significantly differed among all the residue samples. In unrestored residue (UR), the most abundant phyla were Firmicutes (33.8%) and Actinobacteria (32.2%), followed by Proteobacteria (11.1%) (Fig. 6; Table S4-S5). After 50 years of restoration, groups including Firmicutes, Actinobacteria, Gemmatimonadetes and Deinococcu were observed to be significantly decreased, whereas Chloroflexi and Proteobacteria were observed to be significantly increased in abundance ($p<0.05$; Fig. 6; Table S4-S5). In addition, several new taxonomic groups such as Acidobacteria and Planctomycetes, which are rarely found in unrestored residue (UR), were found to be increased ($p<0.05$; Fig. 6; Table S4-S5). Although both suffered from long term restoration, significant taxonomic differences were observed in poorly restored residue (PR) and well restored residue (WR). Well restored residue (WR) had higher abundance of Chloroflexi, Acidobacteria and Planctomycetes, whereas the poorly restored (PR) had greater abundance of Firmicutes ($p<0.05$; Fig. 6; Table S4-S5). Notably, compared the microbial community in well restored residue and natural soil, high similarity was found in both dominated groups and abundance ($p>0.05$; Fig. 6; Table S4-S5).

At the genus level, the microbial communities significantly differed during natural restoration, which coincided with the PCoA analysis (Fig. 5). In unrestored

residue (UR), the most abundant genera were *norank_f_Euzebyaceae* (18.9%), *Lactococcus* (14.1%), *Bacillus* (11.0%), *Egicoccus* (5.69%), *Truepera* (4.42%), *Nitrolancea* (4.02%), *Oceanobacillus* (3.19%) (Fig. 7; Table S6-S7). After 50 years of restoration, genus including *norank_f_Euzebyaceae*, *Lactococcus*, *Bacillus*, *Egicoccus*, *Truepera*, *Nitrolancea* and *Oceanobacillus* significantly decreased, in which *Egicoccus*, *Truepera*, *Nitrolancea* and *Oceanobacillus* disappeared in well restored residue (WR) ($p<0.05$; Fig. 7; Table S6-S7). On the contrary, several new genera such as *norank_c_4_KD-96*, *norank_c_Subgroup_6*, *norank_c_Gitt-GS-163*, *norank_f_A4b* and *norank_f_AKYG1722* significantly increased after long term restoration, especially in well restored residue (WR) ($p<0.05$; Fig. 7; Table S6-S7).

3.4 | Relation of Microbial communities and residue properties

To explore whether natural restoration had driven the shift in microbial community structure, as well as what factors contributed to this shift, multivariate analyses were performed on data for microbial communities and residue properties.

The Shannon index showed positively correlations with the contents of TOC, TN and AP (R^2 = 0.8954, P < 0.001 for TOC; R^2 = 0.8771, P < 0.001 for TN; and R^2 = 0.6956, P < 0.001 for AP), whilst negatively correlations with the pH, Na and ESP (R^2 = 0.7776, P < 0.001 for pH; R^2 = 0.8635, P < 0.001 for Na; and R^2 = 0.839, P < 0.001 for ESP) (Fig. 8).

Based on the redundancy analysis (RDA) (Fig. 9), the first two axes explained 76.51% of the variation in microbial composition, and the correlation of species-environment on both axes was > 95%, which suggested a notable correlation between microbial community composition and residue properties. The development of microbial communities was strongly driven by TN (R^2 = 0.9576, P = 0.001), Exchangeable Na (R^2 = 0.9291, P = 0.002), ESP (R^2 = 0.9118, P = 0.001), SOC (R^2 =

0.8642, P = 0.002), AP (R^2 = 0.8544, P = 0.003) and pH (R^2 = 0.8314, P = 0.003).

4 | DISSCUSSION

4.1 Effect of natural restoration on microbial biomass and activity

Soil microbial properties such microbial biomass and microbial respiration are important bioindicators of soil quality (Bastida et al., 2006, Karlen et al., 1994, Puglisi et al., 2006). They were both commonly enhanced during ecosystem succession (Sourkova et al., 2005). In this study, both microbial biomass and microbial respiration significantly increased following long-term natural restoration upon the disposal area ($p < 0.05$; Fig. 2, Table S2), which were also observed on other mining areas (Frouz & Novakova, 2005; Huang et al., 2011; Zhan & Sun, 2014). Normally, microbial biomass increased at earlier stages of natural succession and then decreased gradually due to the limited resources (Baldrian et al., 2008; Fierer, Nemergut, Knight, & Craine, 2010). According to the increased ratio of microbial biomass carbon to total organic carbon (MBC/TOC), the disposal area was supposed to be in the primary stage of ecological succession.

The development of microbial properties was often influenced by plant communities including plant coverage and species richness (Frouz et al., 2008; Singh, Singh, & Singh, 2012), as well as soil properties such as organic matter, total nitrogen and pH (Baldrian et al., 2008; Moreno-de las Heras, 2009). Baldrian et al. (2008) concluded that pH and available nutrients (CNP) were the main influence factors to the development of microbial biomass and respiration. In this study, all the measured residue properties showed significantly correlations with the microbial biomass and respiration in bauxite residue ($p < 0.05$). On one hand, the decreased alkalinity (pH) and salinity (exchangeable Na) may alleviate the microbial metabolic stress, which microorganisms can use limited resources to synthesize more living matter (Padan,

Bibi, Ito, & Krulwich, 2005). On the other hand, the accumulated of TOC, TN and AP regulated basic microbiological activation and element cycling processes, regulating the microbial biomass and microbial activity (Moreno-de las Heras, 2009). In addition, plant community may also protect from wind and water erosion, alleviating the disturbance of temperature and moisture variations, and thus contributed to the development of microbial biomass (Frouz et al., 2008; Singh, Singh, & Singh, 2012).

4.2 Effect of natural restoration on microbial functions

Soil enzyme including glucosidase, invertase, urease and phosphatase played vital roles in the accumulation and circulation of nutrients (e.g. organic carbon, nitrogen and phosphorus) and could be regarded as important indicators to reflect microbial functioning (Raiesi & Salek-Gilani, 2018). In this study, the activities of glucosidase, invertase, urease and phosphatase significantly increased during natural restoration process ($p < 0.05$, Fig. 3), which indicated that natural restoration enhanced enzyme activity in bauxite residues. This coincided with the development of enzyme during successional process (Zhan & Sun, 2014).

Enzyme activities are often restricted by the adverse physicochemical properties such as high salinity, alkalinity (acidity), and nutrient deficiencies in the mine tailings. High alkalinity (pH) and salinity (EC) commonly denature enzymes by destroying protein structures (Rietz & Haynes, 2003). In addition, high alkalinity and salinity may inhibit metabolic activity of bacteria and damage the bacterial cell structure (Hamdy & Williams, 2001). After long term restoration, the reduction of alkalinity (pH) and salinity (EC) alleviated environmental pressure for the development of microbial communities and thus increased enzyme activity in bauxite residue. With the development of plant communities, the content of organic carbon increased, which may provide nutrients for microbial metabolism, thereby increasing enzyme activities

(Wei et al., 2019). The increased organic matter may activate microbial functions and thus accelerate element cycling in mining spoils (Moreno-de las Heras, 2009). Furthermore, enzyme activity was also affected by biotic factors including microbial biomass (Shillam, Hopkins, Badalucco, & Laudicina, 2008). In this study, enzyme activities were positively correlated with microbial biomass carbon, which indicated that increasing microbial biomass may contribute to the increase in enzyme activities (Table S2). Therefore, our results indicated that natural restoration reduced the salinity and alkalinity, accumulated the contents of nutrients, and then improved enzyme activities in bauxite residue.

4.3 Effect of natural restoration on microbial communities

Development of the microbial community was not only affected by environmental resources, but also influenced by environmental pressures (Fierer, Nemergut, Knight, & Craine, 2010). In this study, the Shannon diversity of unrestored residue (UR) was relative low, and was similar to other unrestored alkaline, saline tailings (Santini, Raudsepp, Hamilton, & Nunn, 2018). The low microbial diversity may be caused by the adverse geochemical conditions (e.g., extreme pH, salinity, metal toxicity) and nutrient deficiencies in the mine tailings (e.g., limited nitrogen and phosphorus) (Santini, Raudsepp, Hamilton, & Nunn, 2018). After long term natural weathering, microbial diversity significantly increased, which coincided with other mine tailings (Chao et al., 2016; Harantova et al., 2017).

Sequence analysis indicated that the microbial community was mainly dominated by Firmicutes, Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria and Planctomycetes in all residue samples, but dramatically changed with different restoration status (Fig. 6). In unrestored residue (UR), the most abundant phyla were Firmicutes and Actinobacteria, which were commonly found in other saline-alkali

environments including saline soils, soda lakes and alkaline mine tailings (Antony et al., 2013; Santini, Warren, & Kendra, 2015; Santini, Raudsepp, Hamilton, & Nunn, 2018). Adaptive mechanisms such as generating stress resistant endospores and self-repairing DNA under high alkalinity or salinity, allowed these bacterial to dominate such hostile environments (Johnson et al., 2007). In addition, the abundant genus including *Lactococcus* (Firmicutes) and *Bacillus* (Firmicutes) can produce acid, which alleviate the alkali stress to microbial growth (Meng, Xue, Yu, Gao, & Ma, 2012; Martinez et al., 2013). In addition, *Bacillus* (Firmicutes) also could fix N_2 and solubilize P, thereby providing nutrients for the growth and propagation of organisms colonizing mine wastelands (Uroz et al., 2011).

After 50 years of restoration, the microbial community shifted from alkalophilic and halophilic (Firmicutes and Actinobacteria) in unrestored residue to neutrophilic assemblages (Acidobacteria and Planctomycetes) in long term restored bauxite residue, due to the development of plant community and improvement of residue properties. Schmalenberger found that the microbial community in restored residue dominated by typical soil taxonomic group such as Acidobacteriaceae, Nitrosomonadaceae, and Caulobacteraceae (Schmalenberger, O'Sullivan, Gahan, Cotter, & Courtney, 2013). Wu also found the similar microbial succession under natural weathering processes (Wu et al., 2020).

Proteobacteria played a key role in ecological values and participated in energy metabolism (Bryant & Frigaard, 2006). Chloroflexi could sequestrate organic carbon and nitrogen from atmospheric CO_2 or N_2 (Chen et al., 2008), which may result in the accumulation of C/N nutrients in bauxite residue. Furthermore, the dominated heterotrophic microorganisms including Acidobacteria and Planctomycetes could improve the decomposition of organic matter and the circulation of nutrient (Eichorst

et al., 2018). This successional pattern of microbial communities suggested that the diverse soil-like microbial communities with diverse ecological functions established in long term restored residue.

Development of microbial communities may be explained by the development of plant communities and improvement in residue properties in bauxite residue. With the development of plant community, the input of root exudates, sloughed-off root cells and mucilage significantly increased, providing plenty of nutrients for microbial growth (Haichar et al., 2008). In return, microbial community could secrete acid metabolites or extracellular polysaccharide, further reduced alkalinity and promoted aggregate formation in bauxite residue (Wu et al., 2020). RDA analysis showed TN, followed by Na^+, ESP, SOC, AP and pH were the major factors to influence microbial communities (Fig. 9). These results suggested that the amendment strategy, targeted at the reduction of alkalinity and salinity, as well as accumulation of nutrients, may help to establish microbial communities and their functions in the restoration of bauxite residue.

5 | CONCLUSION

This study investigated the development of microbial communities and functions in bauxite residue after 50 years of natural restoration. Microbial biomass carbon, microbial respiration rate, enzyme activities and microbial diversity, significantly increased after long term natural restoration. Microbial structure changed from alkalophilic and halophilic assemblages (Firmicutes and Actinobacteria) to neutrophilic assemblages (Acidobacteria and Planctomycetes). Both microbial communities and associated functions in well restored residue had high similarity with that in natural soil, indicating that long term restoration created diverse soil-like microbial communities and functions. Redundancy analysis (RDA) revealed that TN,

followed by Na^+, ESP, SOC, AP and pH were the major influence factors in the development of microbial communities in bauxite residue. These findings provide us a biogeochemical perspective to reveal soil formation in bauxite residue and suggest that nutrient supplement and regulation of salinity-alkalinity may benefit for the establishment of microbial communities and functions in bauxite residue. These findings enhance our understanding of soil formation at bauxite residue disposal areas. Further studies are now required that focus on the screening of exchangeable Na-tolerant and pH-tolerant functional microorganisms and their rehabilitation potential in the residues.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Feng Zhu, https://orcid.org/0000-0002-8468-183X

Shengguo Xue, https://orcid.org/0000-0003-2801-0238

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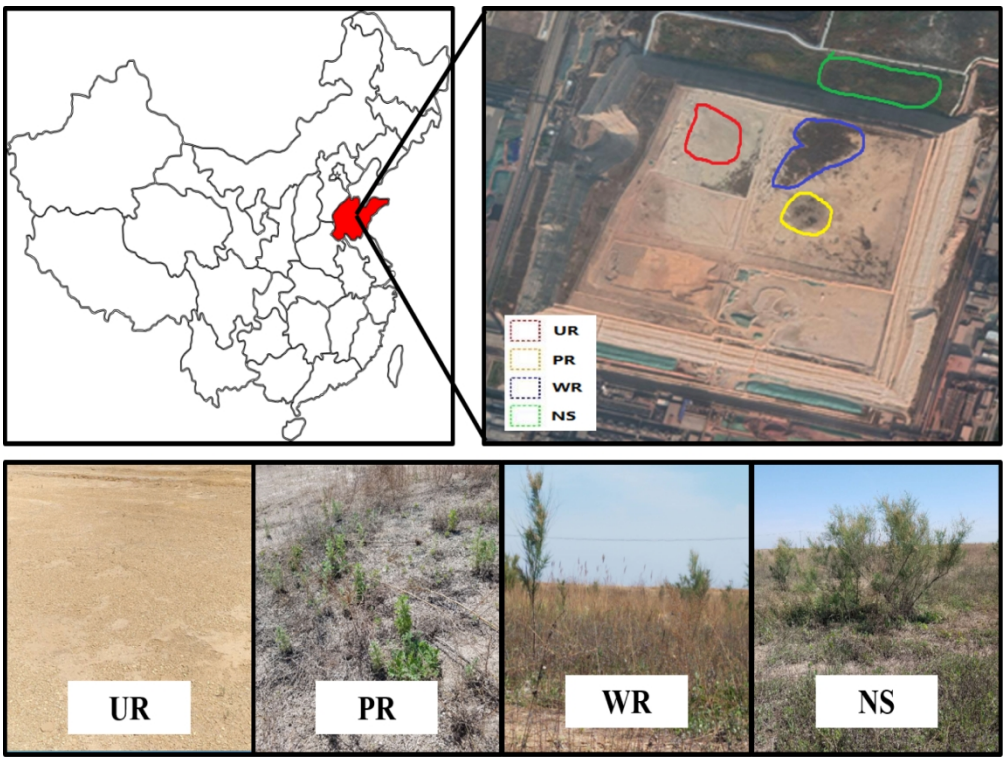

FIGURE 1. The location map and restoration status of bauxite residue and natural soil.

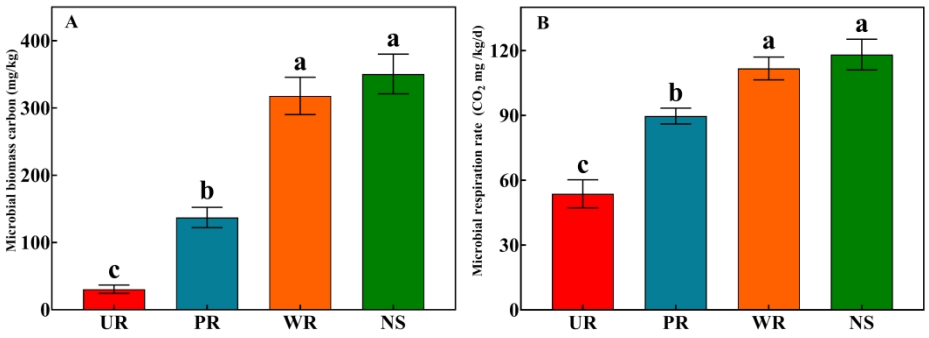

FIGURE 2. Microbial biomass carbon (A) and microbial respiration rate (B) in bauxite residue and natural soil.

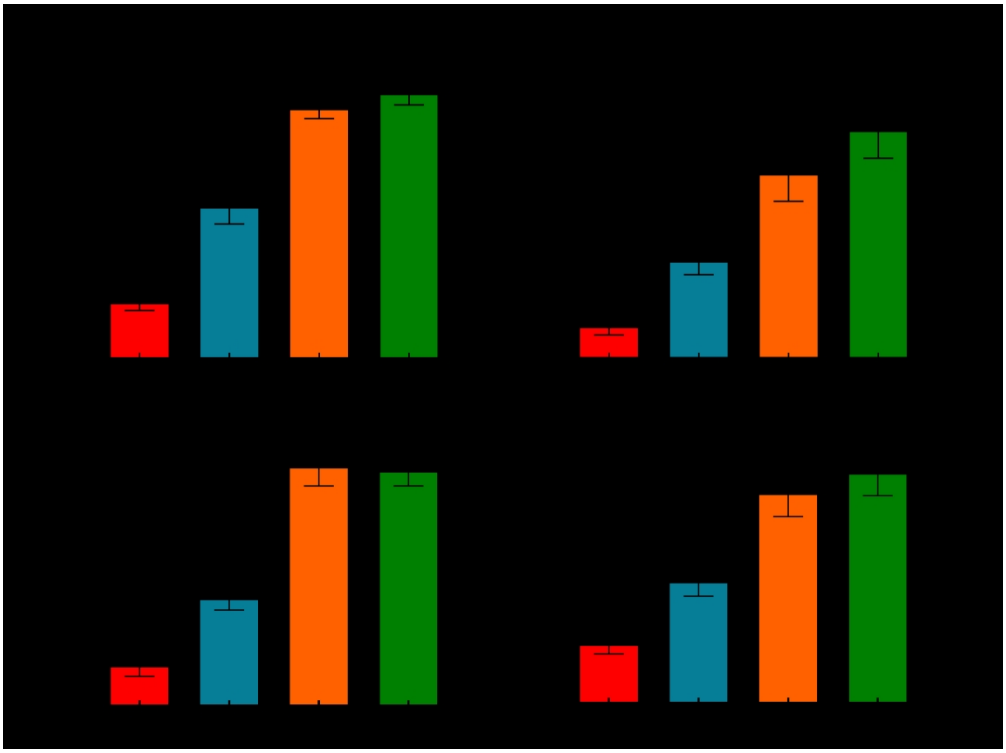

FIGURE 3. Enzyme activity in bauxite residue and natural soil. (A) glucosidase; (B) invertase; (C) urease; (D) phosphatase.

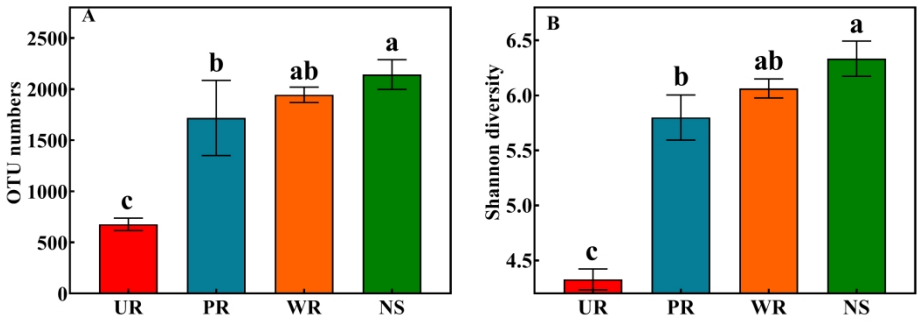

FIGURE 4. α-Diversity of microbial community in bauxite residue and natural soil. (A) OTU richness; (B) Shannon diversity.

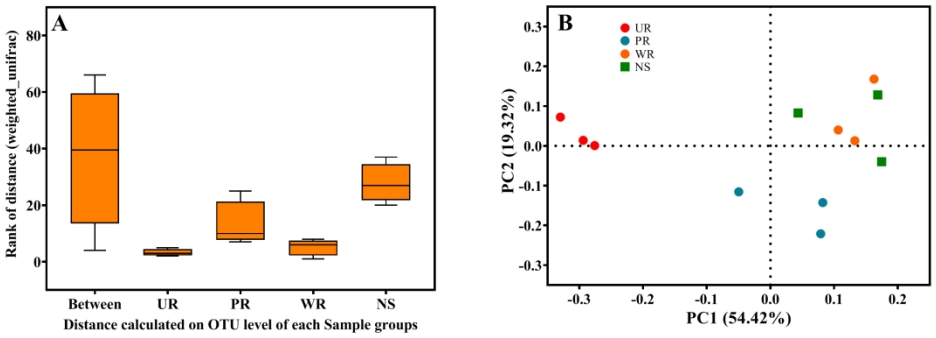

FIGURE 5. β-Diversity of microbial community (PCoA analysis) in bauxite residue and natural soil. (A) weighted_unifrac; (B) unweighted_ unifrac.

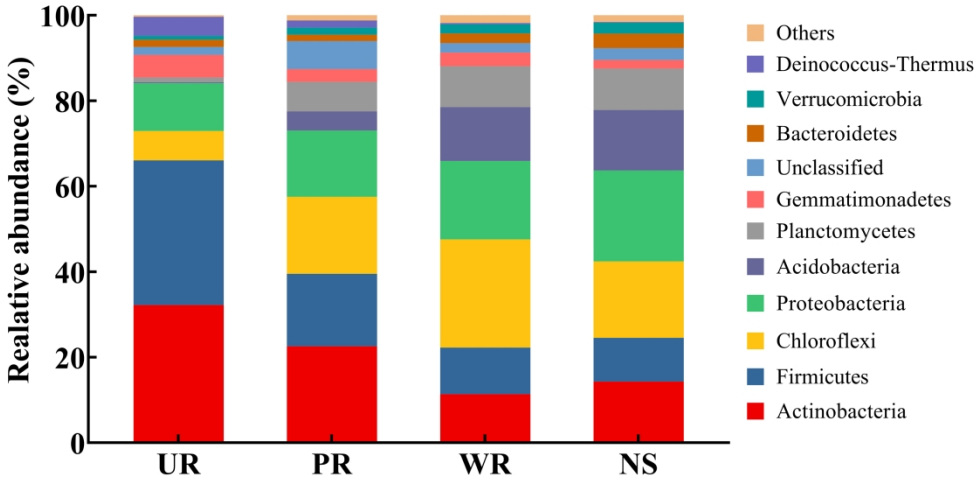

FIGURE 6. Microbial community composition at the phylum level in bauxite residue and natural soil.

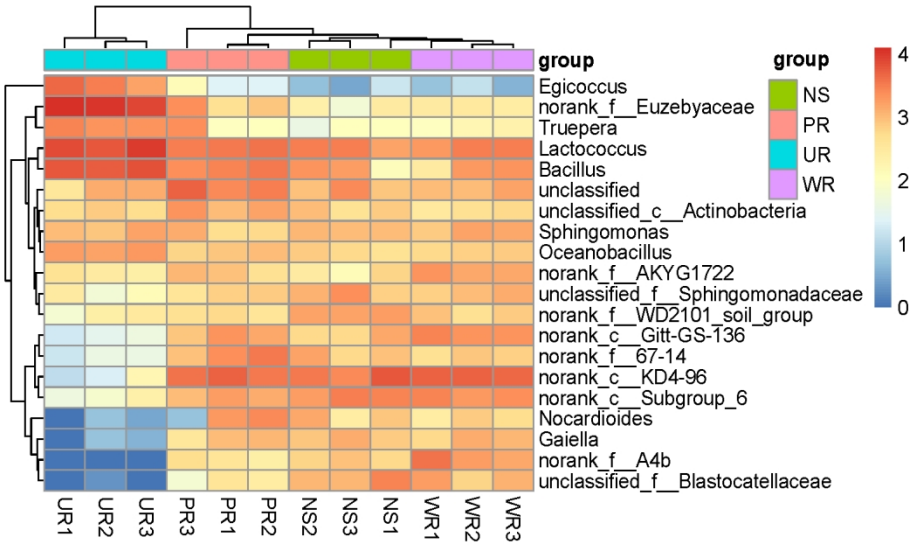

FIGURE 7. Microbial community composition at the genus level in bauxite residue and natural soil.

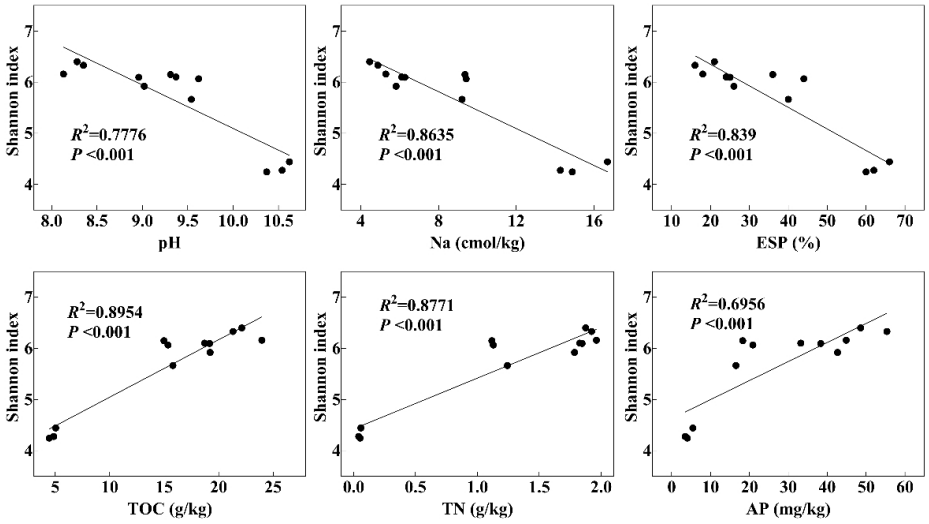

FIGURE 8. Relationships between residue properties and microbial community diversity.

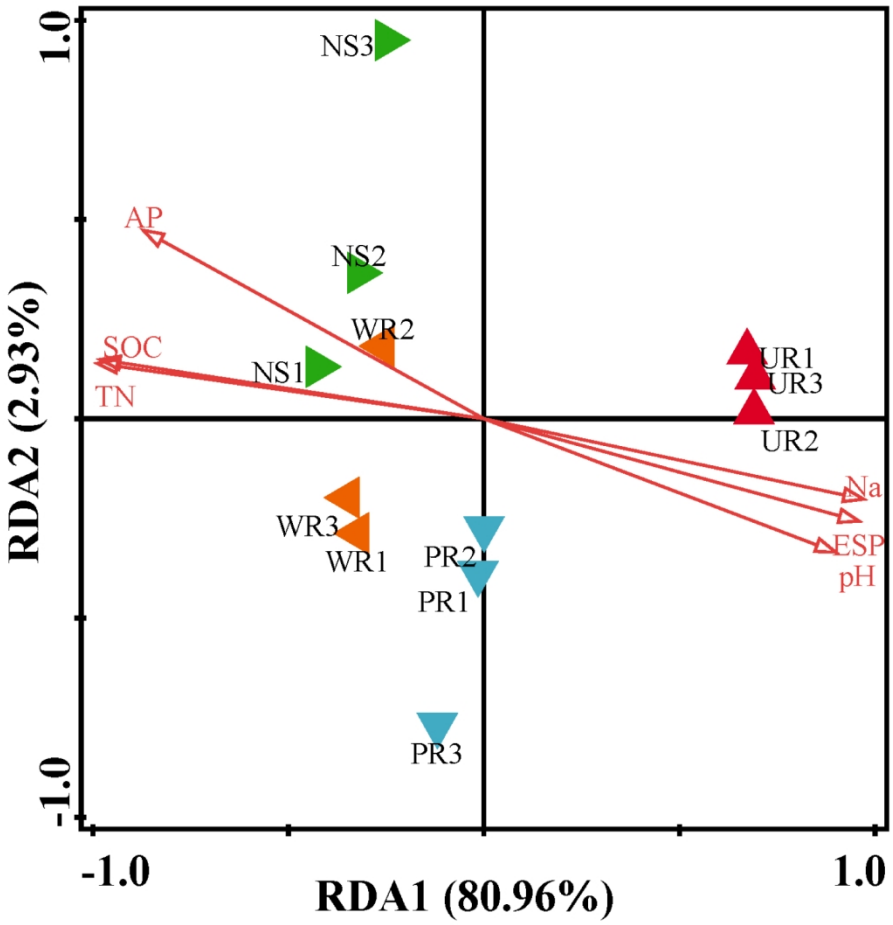

FIGURE 9. Redundancy analysis (RDA) of microbial community vs residue property.