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Ameliorating acidic soil using bioelectrochemistry systems

Yue Zheng, [a, b, #] Chao Wang, [a, c, #] Zhi-Yong Zheng, [a, b] Jing Che, [c] Yong Xiao, [a] Zhao-hui Yang [b] and Feng Zhao [a, *]

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Soil acidification has been a threat to sustainable agricultural development as a global environmental problem. Bioelectrochemistry system (BES) is an eco-friendly and low-cost technique for ameliorating acidic soil when compared with traditional technologies. Here, operational parameters from soil load and external resistance were investigated through mapping the pH changes of the soil. The result showed that approximately a quarter of soil load in the operating unit was an optimized choice based on the performance at the unit and overall levels. The lower external resistance was more conducive to enhance the electric force for the increase of the soil pH. This study not only proved the feasibility of BES for ameliorating acidic soil, but also comprehensively described the feedback network of soil acidity, Al^{3+} removal and microbial community. After BES-amelioration, the exchangeable Al^{3+} obviously reduced and the microbial community structure shifted. The exploration of operational parameters and feedback network provided theoretical supports for the application of BES-based technologies in reclaiming soil acidity.

Introduction

Soil acidification is one of the serious environmental problems, and over 50% of the potentially arable lands have been acidified in the world [1]. With improper industrialization practices and excessive soil utilization, the problem of soil acidification is worsening. For instance, the topsoil pH of grasslands in China has declined significantly (0.63 units) over two decades [2]. Since acidic soil poses a great threat to the sustainable development of agriculture, it is necessary to develop a feasible and economical amelioration technology to solve the problem.

Among the traditional technologies, various ameliorants, such as calcium carbonate, phosphogypsum and calcium sulphate, are generally applied to control soil acidification [3]. However, the widespread use of these ameliorants results in not only the hardening of the soil but also the imbalance of Ca^{2+}, K^{+} and Mg^{2+} ion in soil, ultimately leading to reduction of soil productivity [4]. By contrast, bioelectrochemical systems (BESs) have been shown to be advantageous due to no emission of pollution and low cost for environmental remediation [5]. Heretofore, BESs have been widely used in sewage treatment [6-9], and extended into the field of soil remediation. Most previous studies focused on the use of BES in the remediation of organic pollutants in soils [10-12].

Microbial fuel cell (MFC), as a representative BES, usually consists of an anode chamber and a cathodic chamber, separated by a membrane. Oxygen reduction reaction on the cathode is common in MFCs due to the inexhaustible oxygen as well as its positive redox potential and harmless reduction product [13]. Since oxygen reduction reaction consumes large amounts of protons, the reaction is directly linked to the pH change of the cathodic chamber. As a result, the phenomenon of cathodic alkalinization could be stated, although the protons coming from anode chamber might slightly relieve this alkalinization process. Rozendal et al [14] found that the pH of the air cathode showed an increase from 7 to over 11 units after 48 hours of operation. The profile implied potential access to improve the pH of the acidic soil with the air cathode of MFCs. Moreover, under the condition that the cathode receives electron, high-valence metal ions such as Cr^{6+} and U^{5+} could be converted into low-valence metal ions, which contributes to reducing metal toxicity [15, 16].

In this study, we constructed an MFC, and mapped the pH changes of the acidic soil loaded in the cathodic chamber under different operational parameters including external resistance and soil load. Red clay soil, a typical acidic soil, was used as a proof of concept to evaluate the applicability of the technology. The

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[a] *Key Laboratory of Urban Pollutant Conversion, Chinese Academy of Sciences Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, 361021, China. E-mail: fzhao@iue.ac.cn; Phone/Fax: +86(0) 592 6190766*

[b] *College of Environmental Science and Engineering, Hunan University, Changsha, 410082, China*

[c] *State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, 210008, China*

[#] *Yue Zheng and Chao Wang contributed equally to this work*

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bioavailability of toxic aluminum (Al) ion in the acidic soil was also investigated before and after the amelioration of BES. To evaluate the biological effect of acidic soil amelioration, the microbial communities of acidic soils were profiled using Miseq sequencing. It is expected to improve our understanding of the feedback mechanism of BES-amelioration through the network analysis of soil acidity, Al^{3+} removal and microbial community.

Materials and methods

Soil sample

The red clay soil used in this study was collected from the ground surface (0-20 cm) of a paddy soil in Yingtan City (28°15′N, 116°55′E), China. The acidic soil used in this study is the red soil from the Quaternary red clay, which is a typical soil type and is widely distributed in the south regions of China, especially Jiangxi, Zhejiang and Hunan provinces. The red clay soil is characterized by the high levels of Al and H^+, and low levels of P, N, K, Ca and Mg. The soil was air-dried and passed through a 1-mm sieve for further use. Some of the soil properties were as follows (per kilogram): pH, 5.08; organic matter, 14.58 g; total nitrogen, 0.84 g; alkali-hydrolyzable nitrogen, 121.22 mg; total phosphorus, 0.86 g; available phosphorus, 6.58 mg; exchangeable Fe 25.43 mg and clay, 38%.

Soil-MFC construction and operation

The MFC consists of two identical perspex frames (5.0 × 5.0 × 5.0 cm^3), which were used as anodic and cathodic chambers separated by a cation exchange membrane (4.0 × 4.0 cm^2, QQ-YLM, Qianqiu Group, China). Both the anode and the cathode used were carbon felts (4.0 × 3.5 cm^2, 8.5 mm thickness, Haoshi Carbon Fiber Co., China). Under closed circuit condition, the electrodes of the two chambers were connected with the titanium wires under external resistance.

During the start-up phase, the soil-MFCs were inoculated with activated sludge (about 1 g dry weight) from a domestic wastewater treatment plant in Jimei, Xiamen, China. The anodic chamber was fed with 125 mL simulation wastewater (pH 7.0) containing the following components (per liter): $NaH_2PO_4{\cdot}2H_2O$, 3.32 g; $Na_2HPO_4{\cdot}12H_2O$, 10.32 g; CH_3COONa, 2.0 g; NH_4Cl, 0.31 g; KCl, 0.13 g; $MgSO_4{\cdot}7H_2O$, 0.2 g; $CaCl_2$, 15 mg; $MnSO_4{\cdot}H_2O$, 20 mg; and trace elements solution, 12.5 mL [17]. For startup, potassium ferricyanide (50 mM, pH 7.0) was used as electron acceptor in the cathodic chamber and the MFC was loaded with an external resistance of 1000 Ω. After obtaining a steady output (650 ± 5 mV), the cathode solution was completely replaced with acidic soil, and a distilled water was simultaneously added to the cathode chamber. Then the soil and the water was thoroughly mixed. After the clarification of the supernatant, a new cathode was horizontally immersed in the water layer and half buried in the soil as shown in Fig. 1. The distance from the water surface to the cathode was maintained at 1 cm.

To evaluate the effects of various factors on the amelioration of the acidic soil, four different external resistances (10, 100, 1000 and 10000 Ω) and five different soil loads (10, 20, 40, 50 and 70 g) were tested. Meanwhile, the controls were carried out in open circuit condition. All experiments were performed at room temperature (28 ± 2 °C) in triplicate.

Electrochemical and chemical analysis

The voltage of MFC was recorded every 5 min using a digital multi-meter (Keithley Instruments Inc., USA). The current density J (mA m^{-2}) was calculated with the equation: $J=U/RA$, where U, R and A are the voltage of MFC (mV), the external resistance (Ω) and the geometrical area of the anode (m^2), respectively.

After amelioration, all the soil and water in the cathode chamber were transferred to a glass flask. The soil was air-dried, and ground to pass a 1-mm sieve for analysis of pH and exchangeable Al^{3+}. The soil pH was determined in a soil-to-water solution (1:2.5 w/v) by a pH meter (UltraBasic Benchtop pH Meter, Denver Instrument, USA). The exchangeable Al^{3+} was extracted with 1 M KCl (1:50 w/v), and after appropriate dilution, the Al concentration in extracting solution was measured by inductively coupled plasma mass spectrometry (7500 Cx, Agilent, USA) [18].

Bacterial community analysis

After 3 days of BES operation, the soil samples were withdrawn from the cathodic chamber and balanced at a room temperature of 28 ± 2 °C and 70% moisture. Each soil sample was evenly mixed, and the genomic DNA was extracted and purified as previously reported [19]. The purified genomic DNA was quantified by a micro-ultraviolet spectrophotometer (Nanodrop Inc., USA) and stored at -20°C for further use. Before sequencing, the purified DNA was PCR amplified with a set of primers (515F/806R) targeting the hypervariable V3 and V4 regions of 16S rRNAs. The specific 6 bp barcode was added to the reverse primer to identify different samples. Each sample was amplified with 20 μL PCR reaction system as previously reported [20]. The PCR products were quantitated by QuantiFluor™-ST, and then mixed for Illumina Miseq sequencing.

The Miseq data of 16S rRNA genes were processed using the Quantitative Insights Into Microbial Ecology (QIIME version 1.8) pipeline. Before the statistical analysis, the paired-end reads of the Miseq data were merged by Fast Length Adjustment of SHort reads (FLASH version 1.2.9). QIIME was used to (a) check the completeness of the barcodes and the primer sequencing and (b) remove reads shorter than 200 bp and with a quality score below 25. Then, the sequences of different samples were exactly assigned using the unique 6 bp barcodes from raw data. Only the sequences with ≥97% identity were divided into OTUs for further analysis, and the most abundant sequence from each OTU was selected as the representative sequence. After being assigned by PyNAST, the representative sequences were used for the taxonomic classification according to the Greengenes database. To minimize the control error, we randomly selected 13056 sequences per sample to measure the α-diversity in each sample and compare the β-diversity between samples by UniFrac metric. To assess the diversity and the structure of bacterial community in each sample, the number of OTUs, Chao1 index, phylogenetic diversity index and Shannon index from each sample were calculated. In light of the UniFrac matrix, we performed principal coordinate analysis in R v. 2.15.3. The heatmap of genus and OTUs distribution generated using MATLAB v. 7.14.0.739.

Results

Influence of operational parameters on pH changes of soil-MFC

With the running of MFCs loaded with soils, the voltage outputs showed a gradual increase (Fig. S1). Although proton might electromigrate from anodic chamber to cathodic chamber under the electric field, our research results demonstrated that there was no significant change in the anodic pH throughout experiment (Fig. S2). Thus, it can be deduced that the proton consumption mainly comes from acidic soils in the cathodic chamber (Fig.1).

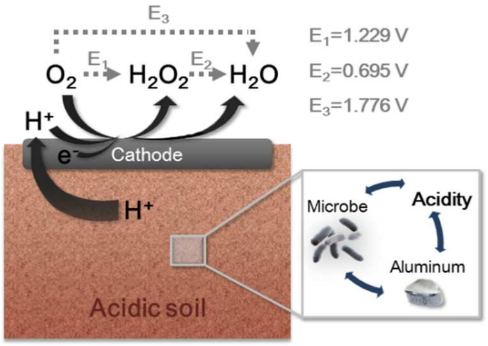

Fig. 1 The schematic diagram of the amelioration of the acidic soil using BESs. There are two electron pathways of oxygen reduction reaction, namely four-electron pathway ($O_2 \rightarrow H_2O$) and two-electron pathway ($O_2 \rightarrow H_2O_2 \rightarrow H_2O$). The E_1, E_2 and E_3 potentials are standard potential vs. standard hydrogen electrode [21].

Influence of running time Six groups of soil-MFCs loaded with 50 g soils were separately operated under different running time (1, 3 and 6 days). As shown in Fig. 2A, the soil pHs in the open-circuit soil-MFCs operated for 1 and 3 days showed no significant change when compared with initial soil. Specifically, there was an obvious tendency for the soil pH to increase with the running time under closed circuit with 1000 Ω external resistance. However, a slight increase of the soil pH was also detected under open circuit after 6 days, which was ascribed to the proton transportation from cathodic chamber to anodic chamber driven by the proton concentration difference. Considering studying the function of BESs, we selected 3 days as the typical operating cycle for the further researches.

Influence of soil load The influence of soil loads was investigated with five rates, namely 10, 20, 40, 50 and 70 g, which accounted for 6.4%, 12.9%, 25.7%, 32.1% and 45.0% of the cathodic volume, respectively (Fig. 2B). The index of $P_{ameliorated}$ was defined as equation 1, which reflected the amelioration of the soil pH at the overall level.

$$P_{ameliorated} = (P_{closed} - P_{open}) * m \qquad \text{(Eq. 1)}$$

where $P_{ameliorated}$ denotes the changed unit after BES-amelioration; P_{closed} and P_{open} denote the soil pHs under closed circuit and open circuit, respectively; and m denotes the mass of soil load.

While a gradual downtrend could be observed in the soil pHs within the range tested, the index $P_{ameliorated}$ increased first and then reached a plateau with the increase of soil loads (Fig. 2B).

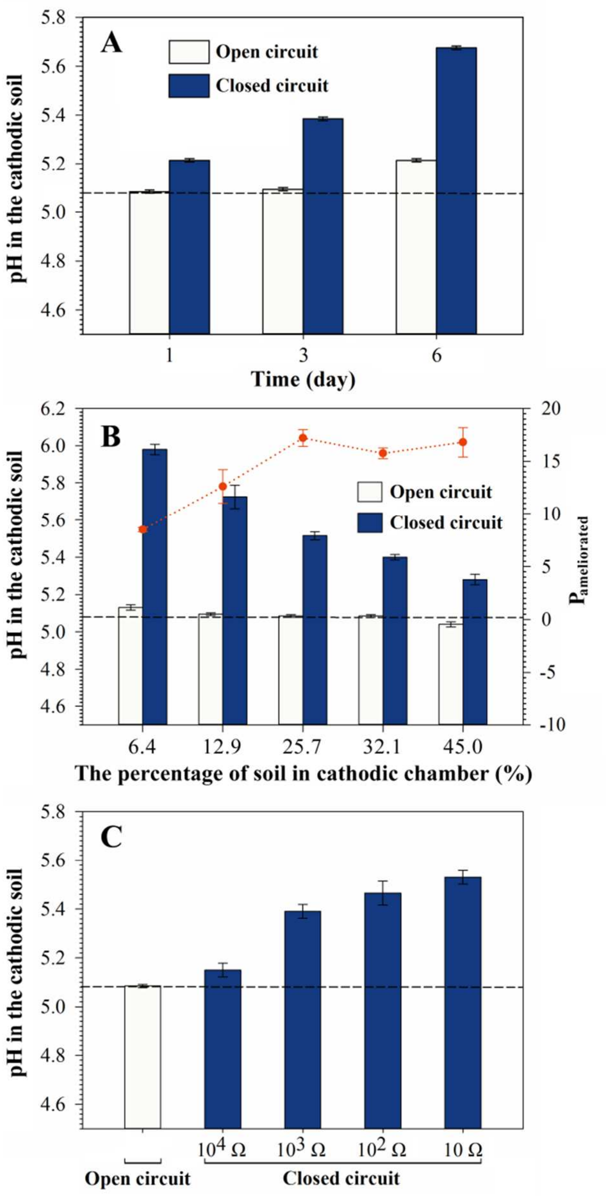

Fig. 2 (A) Soil pHs after amelioration under open circuit and closed circuit as a function of time (1, 3 and 6 days). The MFCs operate with 1000 Ω external resistance and 50 g soil load; (B) Soil pH after amelioration under open circuit and closed circuit with 1000 Ω

external resistance at different soil loads (accounting for 6.4, 12.9, 25.7, 32.1, and 45.0% of cathodic volume). The pH (pillar) uses left Y axis, and the index of $P_{ameliorated}$ (red dot) uses right Y axis; (C) Soil pH after amelioration under open circuit and closed circuit with 50 g soil load in different external resistances (10000, 1000, 100, and 10 Ω). The dotted baseline denotes the pH of raw soil.

Influence of external resistance To analyze the role of electrochemical effects, the performances of soil MFCs were investigated under a series of external resistances ranging from 10 to 10000 Ω. As shown in Fig. 2C, the soil pHs after amelioration correlated in the opposite direction with the external resistance gradient, suggesting that the lower external resistance of MFCs showed the better amelioration for the acidic soil. The current densities of MFCs loaded with 10,000, 1,000 and 100 Ω reached 41.16 ± 7.63, 117.35 ± 8.60 and 123.72 ± 18.98 mA m^{-2}, respectively, except that the current density of 10Ω-MFC fluctuated irregularly. The result suggests that a lower external resistance is more conducive to enhance the electrochemical force for the soil pH increase.

The removal of exchangeable Al^{3+} as the change of soil pH

Al^{3+} toxicity in acidic soil is the main cause of inhibiting growth of plants and microorganisms [22]. The aluminum minerals mainly exist in the forms of layer aluminosilicate minerals, noncrystalline aluminosilicate, and gibbsite. In this study, the soil pH was obviously increased with the decrease of the external resistance as described above. Similarly, with the decrease of the external resistance, the exchangeable Al^{3+} concentration in the ameliorated soil significantly reduced, and the removal rate of exchangeable Al^{3+} showed an exponential correlation with the soil pH (Fig. 3). When the external resistance was 10 Ω, the removal rate of Al^{3+} reached 39% after 3 days of running.

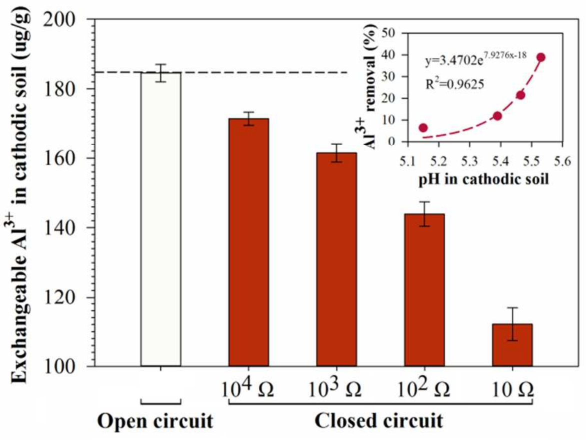

Fig. 3 The exchangeable Al^{3+} removal of acidic soil under open circuit and closed circuit. The correlations between Al^{3+} removal rate and soil pH under closed circuit is shown in insert figure. The dotted baseline denotes the exchangeable Al^{3+} of raw soil.

The shift of microbial community

The microbial communities of acidic soils were investigated before and after BES-amelioration. Six libraries were constructed from the soil samples. A total of 101653 effective sequences with an average length of 271.9 bp were obtained from 16S rRNA gene V3-V4 region amplicons of these samples. Overall sequencing information about six samples was listed in Table S1. To fairly compare the different samples at the same sequencing depth, 13056 sequences were extracted from each sample randomly. Although the rarefaction curves had not yet reached a plateau at 13056 sequences, the Good's coverage was up to 75.55 ± 2.93%, suggesting that the 13056 sequences were enough to reflect the profile of the microbial communities.

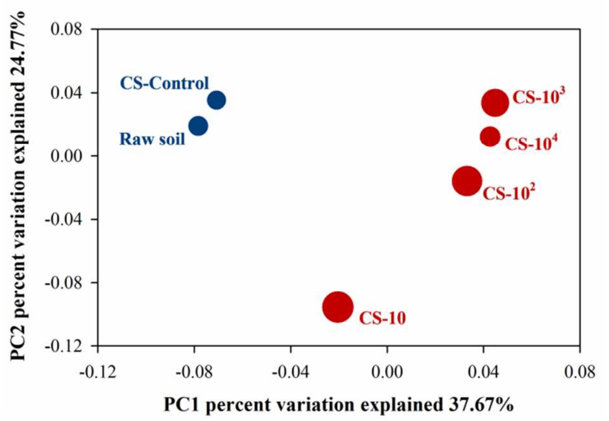

Fig. 4 Weighted principal coordinate analysis of microbial communities from different soil samples using UniFrac matrix. CS-10, CS-10^2, CS-10^3 and CS-10^4 denote the soils of MFCs loaded with 10, 100, 1000 and 10000 Ω external resistances; CS-Control denotes the soil of MFC under open circuit. The dot size reflects the phylogenetic diversity of samples.

As shown in Fig. 4, the size of red dots was bigger than that of the blue ones, indicating that microbial communities of acidic soils after BES-amelioration were more diverse than those without BES-amelioration. The α-diversity indexes, that is observed species, Chao1 index and Shannon index (Fig. S4), were consistent with the phylogenetic diversity. The weighted principal coordinate analysis showed that there was a relatively long distance between the acidic soil samples before (blue) and after (red) BES-amelioration (Fig. 5), suggesting the shifts of microbial communities of the acidic soils when ameliorated by BES. This result indicated that BES technology changed the structure of the microbial community in the acidic soil with respect to α-diversity and β-diversity.

To estimate the effect of BES on phylogenetic distribution at the genus level, 353 classified genera were identified. As illustrated in Fig. 5, the samples after BES-amelioration exhibited similar community composition and weakened the genus of *Sinomonas*, *Methylobacterium* and *Burkholderia*. *Sinomonas* belongs to Actinobacteria with high GC content, which is suitable for extreme environments [23]. *Methylobacterium* is known as methane-oxidizing bacteria [24]. Notably, *Burkholderia* consists of human, animal and plant pathogens, such as *Burkholderia mallei* [25], *Burkholderia pseudomallei* [26] and *Burkholderia cepacia* [27]. The decrease of pathogens contributes to reducing the health and environmental risks. *Geobacter*, as common electrochemically active bacteria [28], were obviously detected in our samples. Those electrochemically active microbes built a biological foundation for the use of BES technologies.

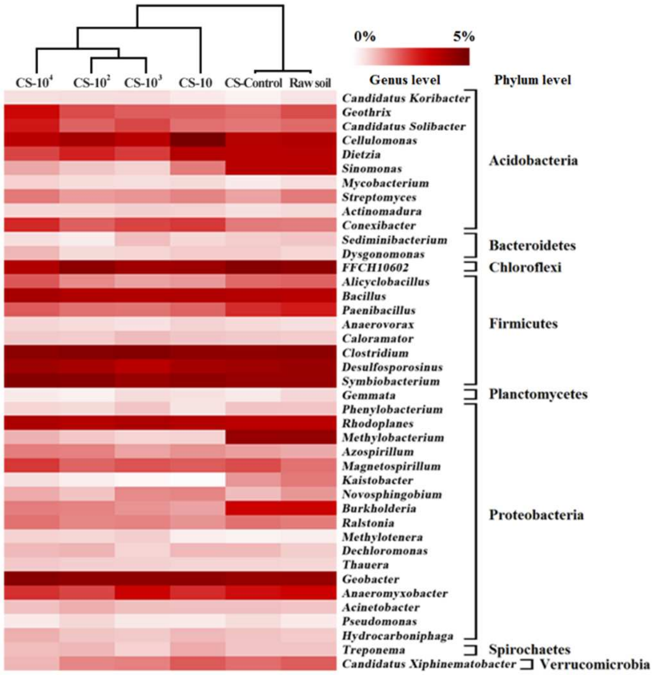

Fig. 5 Taxonomic classification of DNA sequences from the microbial community of soil samples at genus and phylum levels. Relative abundance was defined as the number of sequences affiliated with genus divided by the total sequence number per sample. The genera accounting for less than 0.5% of the total composition in all libraries were ignored in the heatmap graph.

Discussion

The flux of proton into the cathodic chamber during the amelioration of acidic soil could be driven by the interaction of diffusion and electromigration, which accords with Nernst-Planck equation [29]. Under open circuited condition, the diffusion of proton, which is a concentration-driven process, plays a primary role [30]. As shown in Fig. 2, there was no significant increase in the soil pH under 3-day open circuit. At the same time, the exchangeable Al^{3+} removal in the soil was not observed (Fig. 3). Indeed, the pH of the acidic soil showed a very slow increase through concentration-driven diffusion only. For example, the soil pH only had a slight increase initially depending on the diffusion of proton under open circuit until day 6 (Fig.2A). The dilemma was significantly reversed by BES under closed circuit condition. The electrical field promoted the electromigration of protons from soil to cathode, which was influenced by the concentration of various ions, the diffusion coefficient of ionic species and the strength of electrical field [30]. It is also worth mentioning that the consumption of soil protons caused by oxygen reduction reaction should accelerate the flow of protons from soil to cathode surface, thereby increasing soil pH. This process only consumes the soil H^+ by using the oxygen from the air, and does not add other substances into soil, suggesting that BES is an environmental-friendly way for acidic soil amelioration.

External resistance is considered as an important factor affecting BES performance by controlling the electron flow from the anode to the cathode, and further influencing the reduction reaction on the cathode surface [31]. In the present study, a lower external resistance meant a higher current density, except for the irregular fluctuation of the current density of 10Ω-MFC. The gradually enhanced current density strengthened the electrochemical action of BES, resulting in the increase of the electron and proton flow into the cathode from anode and soil, respectively. This further explains why the BES with a lower external resistance shows a better amelioration. The negative correlation between the increased pH and the soil load suggested that the optimization of BES unit numbers and soil load was necessary in practical projects.

Microbial community diversity is an important biological indicator of changes in soil quality, and a higher community diversity in soil generally implies a better soil quality [32]. After BES-amelioration, the advantageous shift of α-diversity, β-diversity and phylogenetic distribution in acidic soil effectively demonstrated the superiority of the biological effect of the BES approach, and the abundant microbial community would facilitate the gradual restoration of the fertility of the acidic soil. In addition, BES-amelioration also affected the function of the microbial community in the acidic soil. For example, *Cyanobacteria* can produce toxins which are dangerous to humans as well as other animals [33]. The disappearance of *Cyanobacteria* in the acidic soil after BES-amelioration may be a good indication of reduction of some hazardous components (Fig. S5).

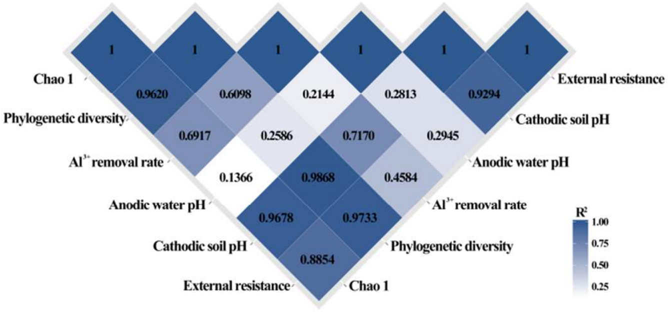

Fig. 6. Correlation Matrix between external resistance, soil pH, anodic water pH, Al^{3+} removal rate, phylogenetic diversity and Chao1 based on Pearson correlation analysis. Values in each cell represent correlation index between each pair of factors; the Chao 1 and phylogenetic diversity are calculated at a sequencing depth of 13056.

Comparing the correlations between each pair of six factors, including external resistance, soil pH, anodic water pH, Al^{3+} removal rate, phylogenetic diversity and Chao1, relatively high association could be observed except for the anodic water pH with others (Fig. 6). There was a strong link between soil pH and external resistance, because soil pH was mainly affected by the levels of electrochemical function through different external resistances. The changed soil pH then led to the shifts of Al toxicity and the microbial community structure, and this result was supported by that the soil pH had high relevance with Al^{3+} removal rate, phylogenetic diversity and Chao 1 (Fig. 6). Definitely, the effects among soil pH, Al toxicity and microbial community are mutual and bidirectional, rather than one completely decided another. The soil pH, Al toxicity and microbial community are regarded as an associated and inseparable system.

Conclusions

In this study, an approach for ameliorating acidic soil was proposed on the basis of the cathodic oxygen reduction in BES. Generally, the acidic soil ameliorated by chemical amendments easily returns to acidity after a long period of time, and the long-term use of chemical amendments often gives rise to soil hardening because of the accumulation of ions such as Ca^{2+} [4]. Environmental-friendly BES technology avoids the supplementation of other exogenous substances, and obtains some electric power in ameliorating acidic soil. The obtained electric power could be further used to supply for environmental sensors [34]. The sensors can be driven by BES to monitor the soil index, which is conducive to establishing the system of performance feedback. This study has verified through investigating the effects of operational factors that the approach of BES is an effective and environmental-friendly way for acidic soil amelioration.

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Notes and references

1 L.V. Kochian, M.A. Pineros, O.A. Hoekenga, *Plant Soil*, 2005, **274**, 175-195.

2 Y. Yang, C. Ji, W. Ma, S. Wang, S. Wang, W. Han, A. Mohammat, D. Robinson, P. Smith, *Glob. Chang. Biol.*, 2012, **18**, 2292-2300.

3 M. Carvalho, B. Van Raij, *Plant Soil*, 1997, **192**, 37-48.

4 T.A. Doerge, E.H. Gardner, *Soil Sci. Soc. Am. J.*, 1985, **49**, 680-685.

5 B.E. Logan, *Appl. Microbiol. Biotechnol.*, 2010, **85**, 1665-1671.

6 Y. Mu, K. Rabaey, R.A. Rozendal, Z. Yuan, J. Keller, *Environ. Sci. Technol.*, 2009, **43**, 5137-5143.

7 Y. Mu, R.A. Rozendal, K. Rabaey, J.r. Keller, *Environ. Sci. Technol.*, 2009, **43**, 8690-8695.

8 Y. Mu, J. Radjenovic, J. Shen, R.A. Rozendal, K. Rabaey, J.r. Keller, *Environ. Sci. Technol.*, 2010, **45**, 782-788.

9 B. Liang, H.Y. Cheng, D.Y. Kong, S.H. Gao, F. Sun, D. Cui, F.Y. Kong, A.J. Zhou, W. Liu, N. Ren, *Environ. Sci. Technol.*, 2013, **47**, 5353-5361.

10 D.Y. Huang, S.G. Zhou, Q. Chen, B. Zhao, Y. Yuan, L. Zhuang, *Chem. Eng. J.*, 2011, **172**, 647-653.

11 X. Wang, Z. Cai, Q. Zhou, Z. Zhang, C. Chen, *Bioeng. Biotechnol.*, 2012, **109**, 426-433.

12 Y.Y. Zhang, X. Wang, X.J. Li, L.J. Cheng, L.L. Wan, Q.X. Zhou, *Environ. Sci. Pollut. Res.*, 2014, DOI: 10.1007/s11356-014-3539-7.

13 K. Rabaey, Bio-electrochemical Systems: From Extracellular Electron Transfer to Biotechnological Application, IWA publishing, 2010.

14 R.A. Rozendal, H.V. Hamelers, R.J. Molenkamp, C.J. Buisman, *Water Res.* 2007, **41**, 1984-1994.

15 G. Wang, L. Huang, Y. Zhang, *Biotechnol. Lett.*, 2008, **30**, 1959-1966.

16 K.B. Gregory, D.R. Lovley, *Environ. Sci. Technol.*, 2005, **39**, 8943-8947.

17 D.R. Lovley, E.J. Phillips, *Appl. Environ. Microbiol.*, 1988, **54**, 1472-1480.

18 C. Wang, X.Q. Zhao, R.F. Chen, H.Y. Chu, R.F. Shen, *Plant soil*, 2013, **367**, 275-284.

19 Z.H. Yang, Y. Xiao, G. Zeng, Z.Y. Xu, Y.S. Liu, *Appl. Microbiol. Biotechnol.*, 2007, **74**, 918-925.

20 Y. Zheng, Y. Xiao, Z.H. Yang, S. Wu, H.-J. Xu, F.Y. Liang, F. Zhao, *Process. Biochem.*, 2014, **49**, 1345–1351.

21 M. Chatenet, M. Aurousseau, R. Durand, F. Andolfatto, *J. Electrochem. Soc.*, 2003, **150**, D47-D55.

22 J.F. Ma, P.R. Ryan, E. Delhaize, *Trends Plant Sci.*, 2001, **6**, 273-278.

23 Y. Zhou, W. Wei, X. Wang, R. Lai, *Int. J. Syst. Evol. Microbiol.*, 2009, **59**, 259-263.

24 T. Patt, G. Cole, R. Hanson, *Int. J. Syst. Bacteriol.*, 1976, **26**, 226-229.

25 G.C. Whitlock, D. Mark Estes, A.G. Torres, *FEMS Microbiol. Lett.*, 2007, **277**, 115-122.

26 T. Nandi, C. Ong, A.P. Singh, J. Boddey, T. Atkins, M. Sarkar-Tyson, A.E. Essex-Lopresti, H.H. Chua, T. Pearson, J.F. Kreisberg, *PLoS Pathog.*, 2010, **6**, e1000845.

27 E. Mahenthiralingam, T.A. Urban, J.B. Goldberg, *Nat. Rev. Microbiol.*, 2005, **3**, 144-156.

28 D.R. Bond, D.R. Lovley, *Appl. Environ. Microbiol.*, 2003, **69**, 1548-1555.

29 T.R. Brumleve, R.P. Buck, *J. Electroanal. Chem. Interfacial Electrochem.*, 1978, **90**, 1-31.

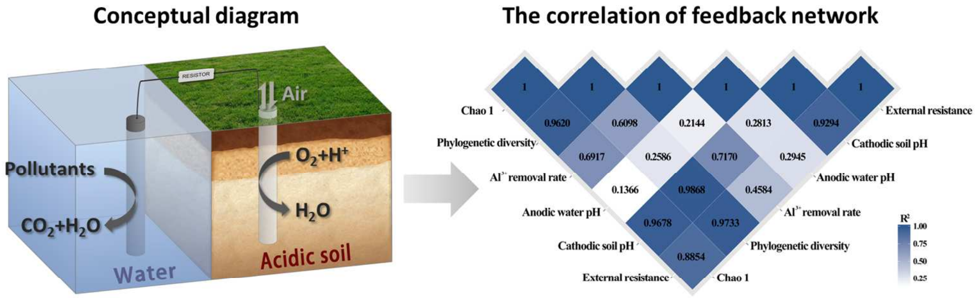
30 F. Harnisch, R. Warmbier, R. Schneider, U. Schröder, *Bioelectrochemistry*, 2009, **75**, 136-141.

31 H.C. Tao, M. Liang, W. Li, L.J. Zhang, J.R. Ni, W.M. Wu, *J. Hazard. Mater.*, 2011, **189**, 186-192.

32 A. Winding, K. Hund-Rinke, M. Rutgers, *Ecotoxicol. Environ. Saf.*, 2005, **62**, 230-248.

33 G.A. Codd, L.F. Morrison, J.S. Metcalf, *Toxicol. Appl. Pharmacol.*, 2005, **203**, 264-272.

34 C. Donovan, A. Dewan, D. Heo, H. Beyenal, *Environ. Sci. Technol.*, 2008, **42**, 8591-8596.



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