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Bacterial community composition at anodes of microbial fuel cells for paddy soils: the effects of soil properties

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Abstract
Purpose Anode electrogenic bacteria (AEB) widely exist in paddy soils and play an important role in element biogeochemical cycling. However, little information is available on the role of soil characteristics in shaping AEB community. Therefore, the objective of this study was to evaluate the role of soil properties in driving the evolution of anode bacterial communities.
Materials and methods Microbial fuel cells (MFCs) were constructed for five paddy soils with different chemical properties. The bacterial communities at anodes of closed (MFC running) and open (control) circuit MFCs were characterized using 16S rRNA gene-based Illumina sequencing.
Results and discussion Paddy soils with higher dissolved organic carbon (DOC) and ammonium (NH_4^+) concentrations in porewater showed higher MFC performance. Without MFC running, the dominant bacterial community composition was similar among the used five soils with *Clostridia* as the dominant bacteria at class level. Compared to control treatments, MFC running significantly decreased bacterial diversity and altered the bacterial community composition at anodes. However, the shift of bacterial communities varied with different types of soils. *Betaproteobacteria* was enriched by 4–30 times after MFC running for low MFC performance soils, while *Deltaproteobacteria* enriched (4–20 times) for high MFC performance soils. Redundancy analysis (RDA) indicated that DOC, NH_4^+, and dissolved ferrous (Fe^{2+}) significantly shift anode bacterial communities for the five soils with MFC running.
Conclusions We found that high-performing MFCs constructed from paddy soils with high DOC and NH_4^+ concentrations in porewater selected for an active, highly electrogenic bacterial community (dominated by *Deltaproteobacteria*) at anodes, while the dominant bacterial community for the low-performing MFCs from soils with low DOC and NH_4^+ was *Betaproteobacteria*. These findings imply that soil properties shape the AEB composition, therefore influencing MFC performance. This study provides new insights into the microbial-mediated carbon and nitrogen cycling in paddy soils.

Keywords Anode electrogenic bacteria (AEB) · Illumina sequencing · Microbial fuel cells · Paddy soil

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1 Introduction

Paddy field is a typical agricultural ecosystem and plays an important role in food supply and ecosystem sustainability (Kögel-Knabner et al. 2010). When it is flooded, the redox potential below overlying water immediately decreases and

becomes negative (Chen et al. 2012). The reductive dissolution of iron (Fe) minerals in paddy soil influences the bioavailability of nutrients or toxic elements to the plants, e.g., phosphate and arsenic that are easily adsorbed onto iron oxides and would be released when ferric oxides are reduced to ferrous ions (Islam et al. 2004; Scala et al. 2006).

The process that microorganism respire solid Fe minerals is one of the most important forms of extracellular respiration (Lovley 1997). These microorganisms could oxidize the substrates and transfer electron to various extracellular electron acceptors, such as iron (Fe) oxides, manganese (Mn) oxides, electron shuttles like humic substance, and even other microorganisms to yield energy for their growth (Lovley 2013; Scott et al. 1998; Thamdrup 2000). The finding of the microorganism with the extracellular respiration functionality provides a new perspective for understanding the evolution of microbial respiration and element biogeochemical cycling in paddy soils. However, in natural environment, when the solid minerals (e.g., Fe and Mn oxides) were used to enrich these microorganisms able to extracellular respiration, these oxides would be gradually reduced and consumed, limiting the long-time enrichment of target microorganisms (Lovley et al. 2004; Wang et al. 2009).

The microbes able to extracellular respiration can also grow at the electrode of microbial fuel cells (MFCs). In MFCs, these microbes are defined as anode electrogenic bacteria (AEB), which could transfer electrons supplied by oxidation of organic carbon to the anode in order to produce electricity (Logan and Regan 2006). Many electrogenic microbes are also known to reduce Fe and Mn oxides and humic acids in organic-rich sediments and soils (Lovley 2006). The anodes of sediment MFCs, unlike the solid minerals, are chemically inert and can serve as a stable electron acceptor to facilitate AEB enrichment (Wrighton and Coates 2009). Moreover, MFCs have been widely applied in various environmental media (e.g., marsh, lake, marine sediment, anaerobic soil, wastewater, and forested and agriculture soil) to study the diversity and functions of the bacterial communities at the anodes (Dunaj et al. 2012; Kaku et al. 2008; Kan et al. 2010; Li et al. 2014; Miceli et al. 2012). To date, more than 50 microbial species have been reported to have extracellular respiring function in MFCs, most of which belong to *Proteobacteria*, *Firmicutes*, *Acidobacteria*, and *Bacteroidetes* (Stams and Plugge 2009; Summers et al. 2010; Yates et al. 2012). Thus, the enrichment of anode bacterial communities by MFCs becomes an excellent tool to study the bacteria able to extracellular respiration in paddy soils.

The substrate is a key factor influencing the anode bacterial communities. It has been reported that anode bacterial communities respond to the type and available amount of organic carbon (C). For example, anode bacteria in an acetate-fed MFC was found to be dominated by *Deltaproteobacteria*, while *Betaproteobacteria* was mostly enriched in butyrate- and glucose-fed MFCs (Chae et al. 2009; Lee et al. 2003; Logan and Regan 2006). Dunaj et al. (2012) investigated the electrogenic bacterial communities from agricultural and forest soils and showed that the amount of available organic matter rather than the presence of organic C substrate (acetate) played the significant role in selecting highly electrogenic bacterial community. Ammonium, widely applied to agricultural soils, can also be the substrate for the growth of bacteria and shape the anode bacterial communities (Clément et al. 2005; Ding et al. 2014). Ammonium was involved in electricity generation either directly as the anodic fuel or indirectly as substrates for nitrifiers to produce organic compounds for heterotrophs in MFCs (He et al. 2009). Most of these previous studies were performed by adding exogenetic C and N into incubation solution or slurry and provided preliminary information on influences of organic carbon and ammonium on anode bacterial communities. However, little information is available on the *in situ* effect of paddy soil characteristics (e.g., organic carbon and ammonium) on anode bacterial communities. The variation of anode bacterial communities among paddy soils with different chemical properties is a research shortfall needed to be addressed.

Therefore, the objective of this study was to evaluate the role of soil properties in driving the evolution of anode bacterial communities. We collected five paddy soils from Southern China with various chemical properties and constructed paddy soil MFCs. The specific objectives were (1) to determine the bacterial community composition at MFC anodes and compare it to control treatment without MFC running, (2) to investigate the variation of the bacterial communities at MFC anodes among the five paddy soils, and (3) to study the influence of soil characteristics on anode bacterial community composition.

2 Materials and methods

2.1 Soil sampling and characterization

Five paddy soils from Leizhou (LZ) of Guangzhou Province, Jiaxing (JX) of Zhejiang Province, Yingtan (YT) of Jiangxi Province, Changde alluvium (CA) and Changde red clay (CR) of Hunan Province, China were collected. Paddy soils were classified to different soil types according to a scientific book named Soils of China (Institute of Soil Science, Chinese of Academy of Science 1978). Upon arrival at the laboratory, each soil sample was divided into two subsamples after homogenization. One subsample was air dried and passed through a 2-mm nylon sieve for the physical and chemical property analysis. The remainder was stored moist at 4 °C for MFC experiment. Soil total carbon (TC) and nitrogen (TN) contents were measured using an element analyzer (Vario

MAX CNS, Elementar, Germany). Soil pH was measured in soil to deionized water suspension of 1:2.5 (*w*/*v*) using a Dual Channel pH/Conductivity Meter (XL60, Fisher Scientific, USA). Details on sampling site and soil basic properties are listed in Table S1 (Electronic Supplementary Material).

2.2 Soil microbial fuel cell construction and treatment

A columnar polyethylene terephthalate container (10 cm diameter × 15 cm depth) with a valve port (~1 cm above the bottom) was used to construct each soil MFC (Fig. 1). Circular carbon felts (Gansu Haoshi Carbon Fiber Co., Ltd, China) with geometric surface area of 50.2 cm^2 were used as anodes and cathodes. A data logger (USB-7660B, ZTIC, China) was used to record the voltage between the anode and cathode.

Each MFC contained 400 g (dry weight) of paddy soil. Initially, ~1 cm depth of soil was placed at the bottom of the MFC container. The anode was placed on the surface of soil layer, and additional soil was deposited on top of the anode. Then, the cathode was placed above the soil. Deionized water (400 mL) was added to flood the paddy soil. An external resistance of 500 Ω was used to connect the anode and cathode. For each MFC, a soil porewater sampler (3S-10, Institute of Soil Science, Chinese Academy of Sciences, Nanjing) was horizontally inserted into the soil through the valve port adjacent to the anode. Assembled MFCs were incubated in dark for total 58 days at 25 °C before final destructive sampling. For MFC control treatments, anode and cathode were installed but not connected via an external resistance.

Thirty MFCs were constructed from the five paddy soils. Each soil had three control replicates (open circuit MFCs) and three MFC replicates (closed circuit MFCs). The voltage data was recorded using the data logger connected to the MFCs once a day. At day 58, the pH and electrical conductivity (EC) of overlying water were measured using the Dual Channel pH/Conductivity Meter (XL60, Fisher Scientific, USA). Porewater samples were collected from the anode port using soil solution samplers with sterile syringes and analyzed for ammonium (NH_4^+) using ion chromatography (ICS-3000, Dionex, USA) (Mou et al. 1993) and dissolved organic carbon (DOC) using total organic carbon (TOC) analyzer (Shimadzu TOC-VCPH, Japan). Ferrous ions (Fe^{2+}) in the porewater samples were fixed with 0.5 M HCl and measured using colorimetry with ferrozine solution (Stookey 1970). Owing to the unique characteristics of paddy soil and the abundance of iron, dissimilatory Fe(III) reduction is prevalent and thought to be central to many other biogeochemical processes in flooded paddy soils (Yi et al. 2013). Therefore, we analyzed Fe ions for these paddy soils.

At the end of the experiment, the anode potential was measured using an Ag/AgCl reference electrode located about 5 mm below the anode electrode. Anode potentials were converted into standard hydrogen electrode (SHE) values. Then, carbon felt anodes were collected by destructive sampling and slightly washed with sterile water to remove soils at the anodes. The anodes were cut into small pieces (1.5 × 1.5 cm) and stored in sterilized centrifuge tubes at −80 °C prior to DNA extraction. Following anode collection, soil samples around anodes were retrieved, freeze-dried, and measured for TC and TN using an element analyzer.

2.3 Genomic DNA extraction, PCR, and sequence analysis

Genomic DNA from the collected anode carbon felts for control and MFC treatments was extracted with FastDNA SPIN Kit (MP Biomedicals, Solon, OH, USA). The extracted DNA was dissolved in 80 μL DES, quantified by a UV–Vis Spectrophotometer (ND-1000, NanoDrop, USA) and stored at −20 °C. The V3 region of the 16S ribosomal RNA (rRNA) gene was targeted using the primers 338 F (5′-ACT CCT ACG GGA GGC AGC AG-3′) and 533R (5′-TTA CCG CGG CTG CTG GCA C-3′) (Huse et al. 2008). Each pair of primers used to amplify a certain sample were barcoded with a unique error-correcting eight-base barcode on both forward and reverse primers (Hamady et al. 2008).

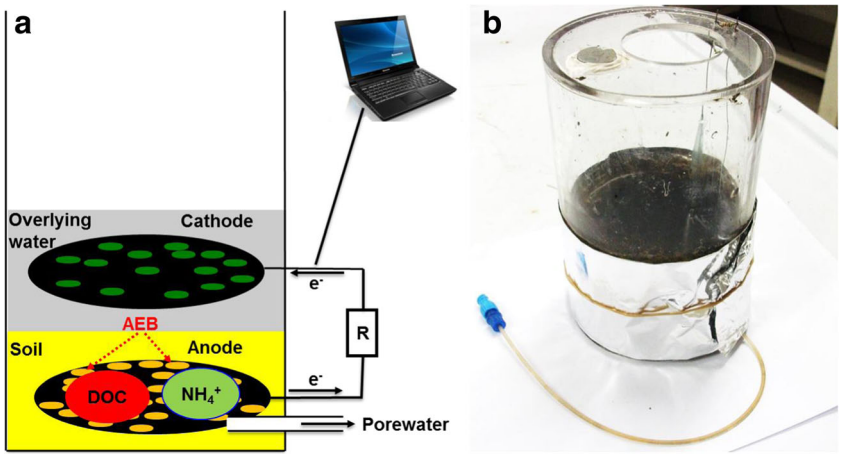

Fig. 1 **a** Schematic and **b** picture of the paddy soil microbial fuel cells (MFCs). The anode and cathode were a piece of circular carbon felt

PCR amplification of V3 region was conducted in triplicate for each sample using the following conditions with a final volume of 50 μL: 25 μL SYBR® Premix Ex TaqTM II (2×), 1 μL BSA (20 mg mL^{-1}), 1 μL 338 F (10 μ M), 1 μL 533R (10 μM), 18 μL ddH_2O, and 4 μL template DNA (100–200 ng). PCR as the control without template DNA was also completed by the above conditions. The thermal profile includes an initial denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 30 s (denaturing), 56 °C for 30 s (annealing), 72 °C for 30 s (extension), followed by a final extension step for 5 min at 72 °C. PCR products were visualized by agarose gel electrophoresis, combined and purified using a universal DNA Purification Kit (Qiagen, Germany). The DNA concentrations of the combined PCR products were quantified using a UV–Vis Spectrophotometer (ND-1000, NanoDrop, USA). Equal amounts of PCR products from different samples were mixed, purified, and quantified. PCR combination products were sent to BGI, Shenzhen, China, for Illumina sequencing.

2.4 Data processing and statistical analysis

The power density (P_d, W m^{-2}) and current density (I_d, A m^{-2}) of MFCs were calculated as the following:

$$P_d = \frac{U^2}{R \times A} \quad (1)$$

$$I_d = \frac{U}{R \times A} \quad (2)$$

where U represents voltage between cathode and anode (V); R represents external resistance (Ω); A is anode area (m^2).

Sequence data were processed using the Quantitative Insights into Microbial Ecology (QIIME) toolkit version 1.7.0 following a procedure similar to Caporaso et al. (2010). After removing any low quality or ambiguous reads, qualified sequences were clustered into operational taxonomic units (OTUs) at 97 % similarity level by default. The most abundant sequence from each OTU was selected as the representative sequence for that OTU and was assigned to taxonomy using an RDP classifier (version 2.2) (Wang et al. 2007). To assess the internal (within-sample) complexity of individual microbial populations, the observed species were calculated. Rarefaction curves were generated to compare the level of bacterial community diversity between samples. Principle component analysis (PCA) was carried out using the unweighted UniFrac metric (Lozupone et al. 2006), which calculates the distance between any pair of communities based on the fraction of unique branch length of their sequences in the tree (Lozupone and Knight 2005).

To find out which factors are important in shaping bacterial communities, redundancy analysis (RDA) was conducted using R software (2.14.0, http://www.r-project.org/) with the community ecology package vegan (2.0-10) (Oksanen 2013). Envfit function with 999 permutations was used to remove environmental variables which insignificantly contributed to the total anode bacterial community variance. Adonis was used to test the bacterial community composition differences among various treatments. Pearson correlation between the influencing factors and the dominant genera for five paddy soils was conducted. One-way analysis of variance (ANOVA) was conducted to test the differences in soil (pH, TC, TN, and EC) and porewater (dissolved NH_4^+, DOC, and Fe^{2+}) properties among different paddy soils. All statistical analyses were conducted using Statistical Analysis Systems (SAS, Version 9.1.3 for Windows, Cary, NC, USA). All data were processed by Microsoft Excel, and diagrams drawn using SigmaPlot 10.0.

3 Results

3.1 Soil characteristics

The porewater properties varied among the tested paddy soils at the end of the experiment (Table 1). For both the control and MFC treatments, porewater NH_4^+ and DOC concentrations in LZ, JX, and YT were significantly lower than those in CA and CR at day 58. Porewater Fe^{2+} concentration in LZ was significantly ($p<0.05$) lower than the others. Compared to the control, MFC running decreased porewater NH_4^+ and DOC concentrations in soils YT, CA, and CR and Fe^{2+} concentrations in LZ, JX, and YT.

For MFC treatments, overlying water for soil LZ was acidic (pH=6.40), while pH values for other soils being ~8 at day 58 (Table S2, Electronic Supplementary Material). Overlying water EC for soil CA was significantly higher than that for other soils. Soil TC and TN for CA and CR were significantly higher than the other soils at day 58.

3.2 MFC performance

The output voltage of the soil MFCs increased gradually until 40 days and then reached a steady value for the next 18 days. At day 58, the average power densities for CA and CR were 2.3–10 times those for LZ, JX, and YT and the average current densities for CA and CR were 1.5–3 times those for other soils (Table 1 and Fig. S1, Electronic Supplementary Material). The anode potentials for soils LZ, JX, and YT (~−135 mV vs SHE) were significantly higher than those for soils CA and CR (~−240 mV vs SHE) at the end of the experiment (Table S2, Electronic Supplementary Material).

Table 1 Porewater characteristics and MFC performance parameters in the control and soil MFCs for the used five paddy soils at the end (day 58) of the experiment

Paddy soil	Treatment	NH_4^+ (mg L^{-1})	DOC (mg L^{-1})	Fe^{2+} (mg L^{-1})	P_d (mW m^{-2})	I_d (mA m^{-2})
LZ	Control	20.3±1.67^{d}	17.1±2.65^{f}	2.78±0.07^{d}	–	–
	MFC	19.1±1.32^{d}	10.2±2.34^{f}	1.87±0.11^{e}	1.02±0.2^{d}	21
JX	Control	19.5±1.31^{d}	98.9±6.78^{c}	43.2±2.65^{b}	–	–
	MFC	19.3±0.89^{d}	78.6±8.93^{d}	32.4±4.24^{c}	1.95±0.3^{c}	28
YT	Control	17.9±3.22^{de}	72.4±8.76^{d}	48.2±2.32a^{b}	–	–
	MFC	14.5±2.70^{e}	48.3±13.3^{e}	31.7±4.16^{c}	4.69±1.3^{b}	43
CA	Control	52.2±3.24^{a}	189±4.51^{a}	52.0±5.63^{a}	–	–
	MFC	28.4±0.42^{c}	169±8.68^{b}	47.3±3.82^{ab}	10.4±1.2^{a}	65
CR	Control	48.1±2.78^{b}	167±9.73^{b}	30.0±4.27^{c}	–	–
	MFC	28.4±1.34^{c}	97.8±13.1^{c}	29.6±5.31^{c}	10.6±0.9^{a}	65

Data were given as mean±standard deviation (n=3) in MFC soil

Different upper letters indicated significant (p<0.05) differences in soil characteristics among paddy soils MFC

NH_4^+ ammonium, *DOC* dissolved organic carbon, Fe^{2+} ferrous ion, P_d power density, I_d current density, *JX* Jiaxing soil, *LZ* Leizhou soil, *YT* Yingtan soil, *CA* Changde alluvium soil, *CR* Changde red soil

3.3 Bacterial community composition at MFC anodes

A total of 930,709 high-quality sequences were generated from 30 samples. All the sequences were only from bacteria. Rarefaction curves showed that MFC running significantly decreased bacterial community diversity, with the bacterial community diversity at MFC anodes for YT and JX being higher than other soils at a sampling depth of 20,150 sequences (Fig. 2)

After 58-day MFC running, the dominant bacterial classes at MFC anodes were *Betaproteobacteria*, *Bacilli* and *Betaproteobacteria*, *Clostridia*, *Bacilli* and *Deltaproteobacteria*, and *Clostridia* and *Deltaproteobacteria* for soils LZ, JX, YT, CA, and CR, respectively (Fig. 3). Among these classes, the dominant bacterial genera were *Bacillus*, *Clostridiun*, and *Geobacter* (Fig. S2, Electronic Supplementary Material). The bacterial community compositions at anodes were significantly different among the five soils with MFC running according to Adonis analysis (p<0.05) (Fig. S3, Electronic Supplementary Material). The first axis could explain 50.2 % of the total soil microbial community variation (Fig. S3, Electronic Supplementary Material).

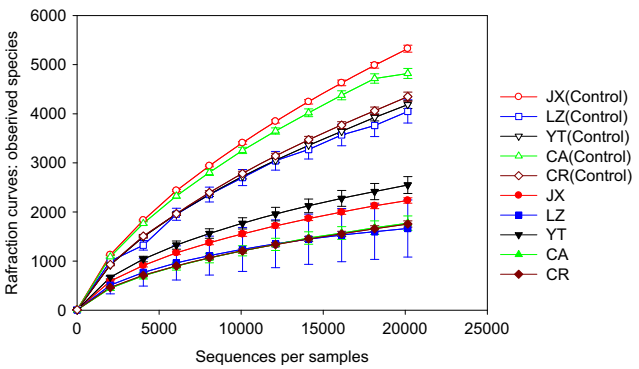

Fig. 2 Bacterial community diversity plots in the used five types of paddy soils

3.4 Bacterial communities enriched by MFCs

Compared to control treatments, MFC running significantly altered bacterial community composition at anodes in each soil. At class level, MFC running significantly enriched *Betaproteobacteria* for LZ, JX, and YT soils by a factor of 4–30 times (Fig. 3), which showed low P_d (Table 1). In the soils with high P_d (CA and CR), *Deltaproteobacteria* was enriched 4–20 times at anodes. In addition, *Bacilli* was also significantly enriched at anodes for all soils except for LZ. In contrast, *Gammaproteobacteria* was only enriched in the soil LZ. For all soils, MFC running did not lead to an enrichment of *Clostridia*. Although not enriched, *Clostridia* shared a great portion of microbial communities at anodes for YT, CA, and CR (Fig. 3).

The relative abundance of order *Burkholderiales* which dominates *Betaproteobacteria* was significantly enriched by MFC running in soils LZ, JX, and YT, while genus *Bacillus* which dominates *Bacilli* was enriched in all soils except for LZ (Fig. 4). *Geobacter* which are the dominant genus of *Deltaproteobacteria* was significantly enriched with MFC running in soils CA and CR. However, in all soils with MFC running, the relative abundance of *Clostridium* (belongs to *Clostridia*) was not increased.

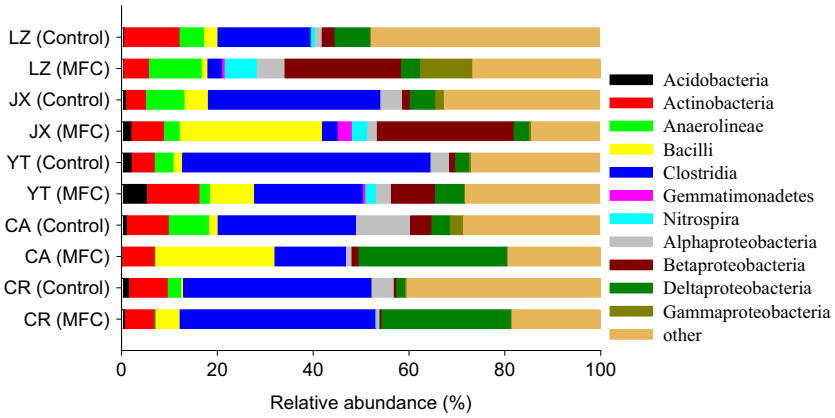

Fig. 3 Relative abundance of bacterial community composition at class level detected at anodes for paddy soils LZ, JX, YT, CA, and CR after 58-day MFC performance. Each type of paddy soil had three MFC replications and three control samples without constructing MFC. Other includes low abundance (less than <1 %) bacteria and the taxonomically unassigned sequences at class level

3.5 Relationship between soil characteristics and bacterial communities

RDA showed that porewater NH_4^+, DOC, and Fe^{2+} were major factors that attributed to the variation of microbial community composition among all possible influence factors (porewater DOC, NH_4^+, Fe^{2+}, soil TC and TN, overlying water pH and EC, and E_{ah}) (Fig. 5). In total, these factors could explain 55.1 % of the soil microbial community composition variation. The correlations between relative abundance of the four main bacterial taxons (*Burkholderiales*, *Bacillus*, *Clostridium*, and *Geobacter*) and porewater NH_4^+, DOC, and Fe^{2+} were developed, showing that *Burkholderiales* was significantly negatively correlated with DOC and NH_4^+ (Table 2). The relative abundance of *Clostridium* and *Geobacter* were significantly positively correlated with NH_4^+ and DOC, and that of *Bacillus* was only positively correlated with DOC. Significant correlations with Fe^{2+} were not found for all the four bacterial taxons.

4 Discussion

4.1 Effect of soil properties on MFC performance

In the current study, we examined electricity generation of MFCs constructed using the five paddy soils. The average power density for soils CA and CR were significantly higher than the other three soils (Table 1). The power output of soils CA and CR (~10 mW m^{-2}) were higher than the value previously reported to forest soil-based MFC (2.44 mW m^{-2}) (Dunaj et al. 2012), while similar to other

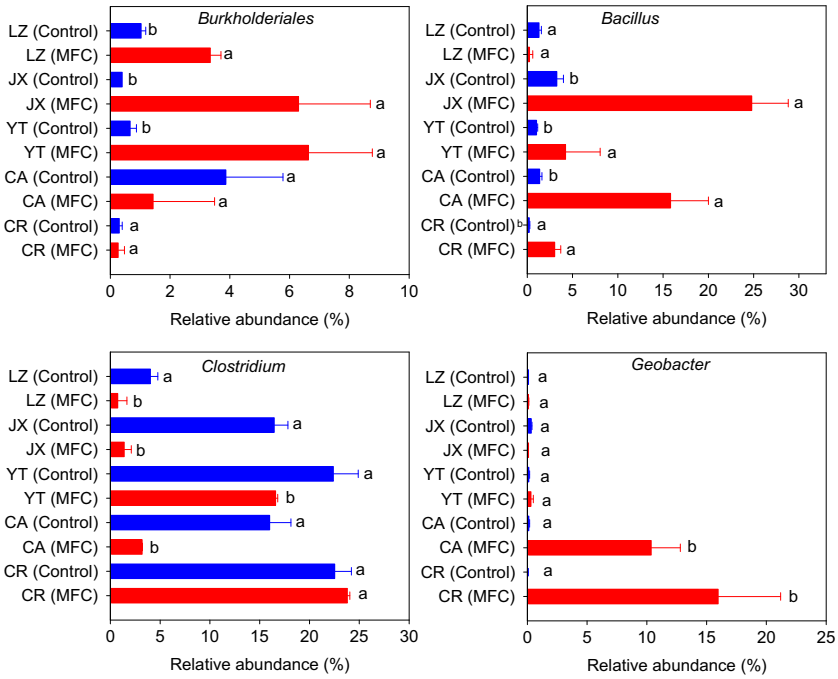

Fig. 4 Relative abundances of order *Burkholderiales* and three genera (*Bacillus*, *Clostridium*, and *Geobacter*) detected at anodes for paddy soils. *Different letters* indicated significant ($p<0.05$) differences in control and MFC treatments for each type soil

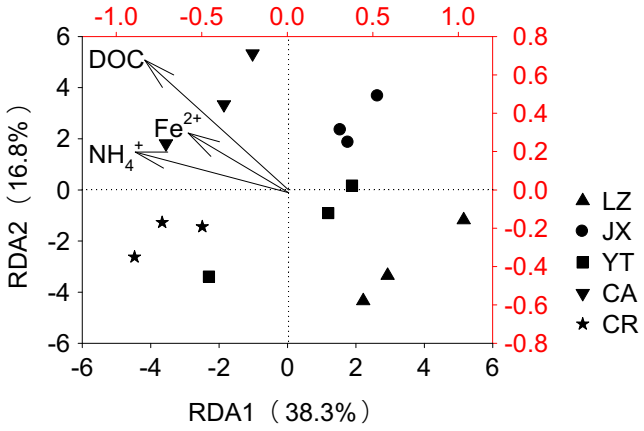

Fig. 5 Redundancy analysis (RDA) of bacterial community composition detected at anodes for paddy soils LZ, JX, YT, CA, and CR after 58-day MFC performance. *Left and bottom coordinate axis* showed species variables values; *right and top coordinate axis* showed the arrows of environmental variables values

MFCs study that was constructed using organic-rich soils, for example, 9.82 mW m^{-2} in paddy-field MFCs (Takanezawa et al. 2010).

Previous studies revealed that MFC electricity could be affected by many factors, such as the oxidation of organic matter, addition of ammonium, the ionic strength, and EC of the electrolyte (Domínguez-Garay et al. 2013; He et al. 2009; Ryckelynck et al. 2005; Tender et al. 2002). In the current study, the MFC constructed from paddy soil LZ, in which porewater DOC was the lowest among soils (10.2 vs 48.3–169 mg L^{-1}) (Table 1), produced lowest power, while soils CA and CR having higher DOC showed higher power density. This suggests that amount of available organic carbon is one of the key factors in determining MFC performance, because organic carbon is the main electron donor for AEB growth (Esteve-Núñez et al. 2008; Lovley 2006). Another possible reason for the high MFC performance of soil CA may be its high EC of overlying water (Table S2, Electronic Supplementary Material). The high EC has an advantage of low ohmic internal resistance due to its high concentration of salts, leading to high power production (Dumas et al. 2008). However, overlying water EC of LZ was significantly higher than JX, YT, and CR, being in contrast with the results that MFC power density was lowest for LZ. Therefore, it can be inferred that overlying water EC might not be a dominant influencing factor in MFC performance compared to porewater DOC, whose variation was in line with the differences in power density among soils. Several studies have shown that the current increased with the increasing of the anode potential in a lower (more negative) potential range (Zhu et al. 2012), while our results showed that the output power was significantly increased (from ~2.0 to ~10 mW m^{-2}) with the decreasing of the anode potential (from~−130 to~−240 mV), possibly implying that the anode potential might not be the dominant influencing factor for the MFC performance in our study.

Furthermore, the lower power density for soils LZ, JX, and YT than CA and CR was consistent with the finding of significantly lower NH_4^+ in porewater for LZ, JX, and YT (14.5–19.3 vs 28.4 mg L^{-1}), implying that amount of available NH_4^+ is another factor controlling MFC performance. Previous studies showed that ammonium was involved in electricity generation either directly as the anodic fuel or indirectly as substrates for nitrifiers to produce organic compounds for heterotrophs in MFCs (He et al. 2009). Compared to control treatments without MFC running, the decreased porewater NH_4^+ and DOC concentrations for all five MFCs especially in CA and CR confirmed that NH_4^+ and DOC might be consumed to produce electricity. These results indicated that NH_4^+ and DOC together influenced MFC performance.

Table 2 Pearson correlation coefficient (*r*) between soil characteristics and the relative abundance of major anode bacteria detected at anodes at the end of 58-day MFC performance for the used five paddy soils

Bacteria	Ammonia	Ferrous	DOC
Burkholderiales	−0.83**	−0.49	−0.51*
Bacillus	0.03	0.30	0.53*
Clostridium	0.81**	0.31	0.71**
Geobacter	0.78**	0.27	0.55*

* and ** indicate significant correlations at $p<0.01$ and <0.05

4.2 Enrichment of electrogenic bacteria at MFC anodes

MFCs rely on the oxidation of organic carbon by electrogenic bacteria to supply electrons, which could be transferred to an anode to produce electricity (Lovley 2006). Therefore, MFCs could facilitate the growth of AEB and made the AEB to be the dominant bacteria communities, decreasing bacterial diversity and changing the bacterial community composition. Compared to control treatments, MFC running significantly decreased bacterial community diversity (Fig. 2) and changed bacterial community composition (Fig. 3). Without MFC running, the dominant bacteria were *Clostridia* among the five soils (Fig. 3). However, after MFC running, some bacteria were enriched at anodes of MFCs, and the bacterial community compositions at MFC anodes were significantly different among the five soils. The enriched bacteria at anodes were *Betaproteobacteria* and *Gammaproteobacteria*, *Bacilli* and *Betaproteobacteria*, *Bacilli* and *Betaproteobacteria*, *Deltaproteobacteria* and *Bacilli*, and *Bacilli* and *Deltaproteobacteria* for LZ, JX, YT, CA, and CR, respectively (Fig. 3). As MFCs running facilitate the growth of AEB at anodes, these enriched classes (i.e., *Betaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, and *Bacilli*) might be AEB. MFC running did not lead to an increase in

the relative portion of *Clostridia* in all soils. However, it was still the dominant bacterial community at MFC anodes for three (YT, CA, and CR) of the five soils (Fig. 3) which showed high power output. This indicates that some bacteria of *Clostridia* (e.g., *Clostridium*) may also have an important role in power output of MFC, although they are not enriched with MFC running. Previous studies have shown that *Betaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Bacilli*, and *Clostridia* are extracellular electrogenic bacteria (Chae et al. 2009; Dunaj et al. 2012; Kim et al. 2006; Phung et al. 2004).

4.3 Variation of anode bacterial communities among low and high MFC performing soils

Clostridia, *Deltaproteobacteria*, *Bacilli*, and *Betaproteobacteria* were found to be the dominant classes on the anodes of paddy soil MFCs (Fig. 3), being similar to previous soil MFC studies that focused on bacterial communities at MFC anodes (Da Rosa 2010; De Schamphelaire et al. 2010; Dunaj et al. 2012). However, the bacterial community composition varied between high- and low-performing MFCs. The bacterial communities at the anodes of the low-power MFCs constructed from soils LZ, JX, and YT were dominated by *Betaproteobacteria*, *Bacilli*, and/or *Clostridia*, while for high-power MFCs (CA and CR), anode communities dominated by *Deltaproteobacteria*, *Clostridia*, and/or *Bacilli* (Fig. 3). The most significant differences were that high relative abundance of *Deltaproteobacteria* (~30 %) was at anodes of high-power MFCs (CA and CR), while large percentage of *Betaproteobacteria* (10–30 %) was detected only at anodes of low-power MFCs (LZ, JX, and YT). The different bacterial community composition (e.g., *Betaproteobacteria* for LZ, JX, and YT; *Deltaproteobacteria* for CA and CR) is the reason for the different MFC performance at microbial levels. These results suggest that *Deltaproteobacteria* has higher electrogenic ability than *Betaproteobacteria*. *Betaproteobacteria* was assumed to be involved in organic carbon oxidation using nitrate and oxygen as electron acceptors besides the electrode, which may cause electron loss in MFCs, therefore lowering its eletrogenic ability (Chae et al. 2009; Logan et al. 2005). Previous study also found that high-performing MFCs constructed from agricultural soil sustained an active, highly electrogenic bacteria community dominated by *Deltaproteobacteria* at anodes, while low-power MFCs from forest soil selected for *Clostridia* (Dunaj et al. 2012).

4.4 Effect of soil properties on bacterial community composition at anodes

As many previous studies have shown, soil properties are important factors in shaping bacterial community composition at MFC anodes (Dunaj et al. 2012; Schaetzle et al. 2008). To explain why bacteria communities responded differently to MFC running among the five paddy soils, the relationships between soil properties and bacterial community composition were developed. Among all possible properties (porewater DOC, NH_4^+ and Fe^{2+}, soil TC and TC, overlying water pH and EC, and anode potential) that might influence bacterial communities, porewater DOC, NH_4^+, and Fe^{2+} were the key factors in determining the bacterial community composition at the anodes (Fig. 5). The oxidation of DOC, Fe^{2+}, and NH_4^+ in soil MFCs could supply electrons for anode bacterial growth and influenced bacterial community composition.

In this study, strong selection of the active, rapidly growing, highly eletrogenic bacteria (*Deltaproteobacteria* dominated by *Geobacter*) were found at MFC anodes for soils CA and CR but not at anodes for other three soils. One main reason behind this is the significantly higher porewater DOC concentrations for CA and CR (~170 and 98 mg L^{-1}) than LZ, JX, and YT (10–80 mg L^{-1}). During MFC running, DOC was the major electron donor (Weber et al. 2006) and the organic carbon was oxidized by AEB coupled with transfer of electrons to anodes. The involvement of *Geobacter* in organic matter oxidation has been well characterized. Compared to control treatments, MFC running significantly decreased porewater DOC concentrations for all soils (Table 1), suggesting that part of lost DOC was used as electron donors and consumed by AEB. Another reason for the loss of DOC may be attributed to the release of methane for soils with MFC running. Previously, studies have reported that *Methanosaetaceae* as the main methanogenic microorganism could involve in organic carbon oxidation in acetate-fed MFC (Jung and Regan 2011). The role of methanogenic microorganism in DOC oxidation in MFCs needs further study.

As suggested by Dunaj et al. (2012), the quantity of available DOC might be a key factor in determining the selection for bacterial communities at anodes. Low DOC may not provide enough substrates for the growth of *Deltaproteobacteria*. Therefore, large portion of *Geobacter* (the main genera of *Deltaproteobacteria*) was only found at anodes for soils CA and CR (Fig. S2, Electronic Supplementary Material), and it was positively related with DOC (Table 2). In contrast, *Betaproteobacteria* may not be so sensitive to DOC level as *Deltaproteobacteria*, and thus, it can grow and be enriched at MFC anodes at low DOC conditions. Previous studies showed that the dominance of *Betaproteobacteria* at anodes of wastewater and riverwater-fed MFCs might be attributed to the introducing of oxygen in the anode area through aeration or diffusion from the cathode, creating a favorable environment for the growth of the bacteria (Kim et al. 2004; Phung et al. 2004). In the current study, oxygen can be diffused to anode areas through soil water interface, and oxygen level near anodes might be relatively higher for soils (LZ, JX, and YT) with lower DOC content, because degradation of DOC at early flooding period consumed oxygen. The low DOC induced relatively high oxygen

near anodes may be the reason of the enrichment of *Betaproteobacteria* (dominated by *Burkholderiales*) at MFC anodes for low DOC soils LZ, JX, and YT, explaining the negative relationship between porewater DOC and *Burkholderiales* (Table 2).

Similar to *Deltaproteobacteria*, DOC can promote the growth of *Bacilli* (dominated by *Bacillus*) and *Clostridia* (dominated by *Clostridium*) at MFC anodes as suggested by their significantly positive correlations with DOC (Table 2). Studies have shown that *Bacillus* and *Clostridium* could also oxidize the organic substrate and transfer electrons to extracellular Fe oxides while growing under anaerobic conditions (Hobbie et al. 2012; Raiswell and Canfield 2012; Shanh et al. 2014). *Bacilli* and *Clostridia* have been commonly found at anodes of soil MFCs (Chae et al. 2009; Dunaj et al. 2012). High amount of available DOC provides enough substrate for their growth to transfer electrons. The finding that *Bacilli* was not enriched at MFC anodes for LZ (Fig. 3) was possibly attributed to the lowest available DOC level of LZ (10 mg L^{-1}) (Table 1), which was too low to support the growth of *Bacilli*. However, an interesting finding was that *Gammaproteobacteria* was only enriched at MFC anodes for LZ. Logan et al. (2005) reported a significant abundance of *Gammaproteobacteria* in a cysteine-enriched MFC inoculated with sediments. The unique enrichment of *Gammaproteobacteria* at MFC anode for LZ may be related to its different style of DOC from other soils, possibly soil LZ had dissolved organic carbon similar to cysteine. Further study is needed to study the effects of different types of organic carbon on AEB community composition.

In addition to DOC, porewater NH_4^+ was found to be another factor influencing anode bacterial communities (Fig. 5). There were significantly positive correlations between NH_4^+ and *Clostridium* and *Geobacter*, while *Burkholderiales* was negatively related to NH_4^+ with correlation coefficient r=~0.8 (Table 2). Compared to DOC (r=0.5–0.7), *Burkholderiales*, *Clostridium*, and *Geobacter* were more related with NH_4^+, suggesting the strong influence of NH_4^+ on anode bacterial communities. Several mechanisms have been proposed for the interaction between NH_4^+ and anode bacterial communities. Ammonium might have a role in Fe(III) reduction as an electron donor, implying the possibility of anaerobic NH_4^+ oxidation by anode bacteria (Clément et al. 2005; Ding et al. 2014). However, direct pure-culture evidence is still required to support the observation of anaerobic NH_4^+ oxidation. Another possibility is that ammonium might be oxidized by ammonia oxidizing bacteria, and the electron from ammonium oxidation might be transferred to anode bacteria by interspecies electron transfer, therefore indirectly influencing anode bacterial communities (Shrestha et al. 2013). Other processes except NH_4^+ oxidation might also be responsible for the anaerobic AEB growth. For example, ammonium oxidizing bacteria could grow close to the soil water interface, decreasing oxygen diffusion into the soil (Jetten et al. 1998), and then, the anaerobic condition helps anaerobic bacteria growth at anodes. The mechanisms between NH_4^+ and the AEB still need further studies.

5 Conclusions

We found that high-performing MFCs constructed from paddy soils with high DOC and NH_4^+ concentrations in porewater selected for an active, highly electrogenic bacterial community (dominated by *Deltaproteobacteria*) at anodes, while the dominant bacterial community for the low-performing MFCs from soils with low DOC and NH_4^+ was *Betaproteobacteria*. These results highlight the importance of soil properties in shaping bacterial communities at MFC anode and point to a need of future studies to study how both quantity and style of DOC influence MFC bacterial communities, especially AEB composition. Interestingly, NH_4^+ was found to be an important factor influencing anode bacterial community. The mechanism between NH_4^+ and AEB has yet to be fully understood and require further studies.

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