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The influence of soil properties and geographical distance on the bacterial community compositions of paddy soils enriched on SMFC anodes

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Abstract

Purpose Exoelectrogens are important microorganisms playing crucial roles in the biogeochemistry of elements in paddy soils. But it remains unclear how the soil properties and geographical distances affect the exoelectrogen communities of Chinese paddy soils. So the objectives of this study were to investigate the diversity and composition of these microbial communities which were enriched on the anodes of soil microbial fuel cells (SMFCs) and to elucidate the links between the microbial community compositions and their driving factors.

Materials and methods We used Illumina HiSeq sequencing to determine the bacterial community structures which were enriched on the anodes of SMFCs. Variance partitioning analysis (VPA) was used to obtain the contribution of soil properties and geographical distance to the variations of bacterial communities.

Results and discussion Active bacterial community on anodes of the closed circuit SMFCs differs significantly from the control open circuit SMFCs. Anodes of all the closed circuit SMFCs were characterized by the presence of high numbers of *Nitrospira* and *Anaerolineae*. Taxonomic similarities and phylogenetic similarities of bacterial communities from different paddy soil samples across North and South China were found to be significantly correlated with geographical distances. The relationship between the similarities and the geographic distance exhibited a distance-decay relationship. VPA showed that both geographical distances and soil properties affect the structure of bacterial communities detected on anodes.

Conclusions Our study gives a foundation for understanding the distribution and diversity of exoelectrogens in paddy soils and elucidates the links between the distribution and the diversity of extracellular respiring bacteria and their driving factors. Furthermore, this study also identifies the crucial factors which should be used to evaluate the response of exoelectrogens to environmental perturbations in Chinese paddy soils.

Keywords Exoelectrogen · Geographic distance · SMFCs · Soil property

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1 Introduction

Exoelectrogens play an important role in elements cycling in the earth (Logan and Regan 2006; Hau and Gralnick 2007;

Fredrickson et al. 2008; Shi et al. 2016). The importance of exoelectrogens is ascribed that they can transfer electrons with extracellular electron acceptors such as insoluble minerals (Fe(III) or Mn(IV)) or electrodes, and hence, they can mediate redox reactions (Bond et al. 2002; Lovley et al. 2004; Brutinel and Gralnick 2012). These microorganisms therefore influence biogeochemical cycling of carbon, nitrogen, and sulfur; nutrient availability; greenhouse gas emissions; and transport or fate of contaminants (Ding et al. 2015).

The significance of exoelectrogens in diverse ecosystems is widely accepted and has attracted many studies on their isolation and power production (Girguis et al. 2010). However, further studies are required to understand the distribution and diversity of exoelectrogens in various environments and to identify factors that affect their distribution and diversity. Soil microbial fuel cells (SMFCs) are often used to explore the distribution of exoelectrogens in various environments such as marine sediments and paddy soils (Holmes et al. 2007; Wang et al. 2015). Paddy soil is a typical wetland ecosystem which also plays an important role in biogeochemical cycling of elements, such as carbon, nitrogen, and metals (Ding et al. 2015; Zhang et al. 2015). When paddy soil was flooded, it becomes anaerobic and favors the enrichment of anaerobic microbes including exoelectrogens (Liesack et al. 2000; Li et al. 2011). Therefore, it is easy to study the distribution and diversity of exoelectrogens from paddy soils through enrichment using SMFCs.

It has been recognized that environmental factors influenced the community structure of exoelectrogens (Puig et al. 2010; Wang et al. 2015; Sajana et al. 2016). A study based on five paddy soil samples showed that anode bacterial community compositions were significantly influenced by dissolved organic carbon (DOC), ammonium (NH_4^+), and dissolved ferrous ions (Fe^{2+}) (Wang et al. 2015). Although studies have established the relationship between community structure of exoelectrogens and soil characteristics, it remains unclear how the soil properties and geographical distances together affect the exoelectrogen communities of Chinese paddy soils.

To improve the understanding of the potential exoelectrogens, we investigated bacterial communities at anodes of SMFCs and examined the influence of geographical distances and soil physicochemical properties on potential extracellular respiring bacteria at anodes via Illumina HiSeq sequencing. We hypothesize that both geographical distances and soil properties are crucial to assembly of bacterial community composition on anodes from different paddy soils. Our results supported this hypothesis by demonstrating that the geographical distances and soil properties play key roles in determining not only the bacterial structure but also the bacterial diversity on anodes from different paddy soils over a large range of geographical distance. Our study will provide basic information on the biogeochemical cycling of elements driven by exoelectrogens in paddy soils.

2 Materials and methods

2.1 Soil sample collection and characterization

Soil samples from different paddy ecosystems located at five different geographical locations around China were collected in September of 2014 (Fig. S1, Electronic Supplementary Material). Each sample was a thorough mixture of three soil cores, and it was segregated into two subsamples after homogenization once arrived at the laboratory. One subsample was used for analysis of the soil physicochemical properties, and the other was stored at 4 °C in a sealed container for about 1 week before SMFC experiment. Soil properties which included pH, ammonium, nitrate, C/N, total C and N, DOC, total Fe and Mn, Electrical conductivity (EC), and amorphous iron (aFe) were determined as described in our previous work (Yuan et al. 2016).

2.2 Construction of SMFCs

To study the exoelectrogens in paddy soils two, SMFCs were developed for each sample, namely open circuit SMFCs (control) and closed circuit SMFCs, and were operated for 68 days. For each type of paddy soil sample, three controls and three replicate closed circuit SMFCs were constructed. Therefore, there are 30 SMFCs constructed. Each plastic container was filled with 650 g of paddy soil and subsequently filled with deionized water to make an overlying layer of 2 cm (Fig. S2, Electronic Supplementary Material). In each SMFC, circular carbon felts (Gansu Haoshi Carbon Fiber Co., Ltd., China) with surface area of 36.3 cm^2 were used as anode and cathode (Fig. S2, Electronic Supplementary Material). We used a data logger to record the voltage which was between the anode and the cathode. The anode was buried in the soil sample, while the cathode was placed in the water layer standing above the soil. Anodes and cathodes were connected to a circuit with an external resistance of 1000 Ω using an insulated titanium wire. While the anodes and cathodes of control SMFCs were not connected to any circuit (open circuit SMFCs). The SMFCs were operated at 25 °C before final sampling. Finally, samples from anodes were collected and were stored at −80 °C for further analysis.

2.3 Polarization analyses

Current (I) was calculated according to the equation $I = E/R$, where E is the cell voltage and R is the external resistance. Power (P) was determined using the equation $P = E \times I$. Current density and power densities were calculated based on the anode area. We obtained the polarization curves at the end of the operations of SMFCs as described previously (Ishii et al. 2012). Linear sweep voltammetry analyses were proceeded using a potentiostat (CHI1040B, Chenhua, China) (Ishii et al. 2008b, 2014). The anode and cathode potential were swept from open-circuit voltage to zero with a scan rate of 0.1 mV s^{-1}. Meanwhile, the corresponding current was recorded.

2.4 Nucleic acid extraction and Illumina HiSeq sequencing

At the end of the operation of SMFCs, anode material was collected and the FastDNA Spin Kit (MP, Biomedical) was used to extract DNA. The V3-V4 regions of bacterial 16S ribosomal RNA (rRNA) genes were amplified with the DNA extracted from the samples as template and with 341F and 806R as primers (Kia et al. 2016). PCR amplification of V3-V4 region was conducted in triplicate for each sample using the following conditions in 50-μl reaction mixtures containing ~40 ng of template DNA, 25 μl Dream Taq Green PCR Master Mix (2×), 20.5 μl H_2O, 0.5 μl 1% bovine serum albumin (BSA), and 0.2 μM each primer. The thermal conditions of PCR were initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 30 s; and the final extension step at 72 °C for 7 min. The amplicons were purified, quantified, and pooled as described by our previous work (Yuan et al. 2016). The amplicons were sequenced on an Illumina HiSeq 2500 platform. The obtained sequenced date was processed and analyzed with the method described previously (Carroll et al. 2012). In summary, operational taxonomic units (OTUs) were picked and the diversity was calculated based on OTUs as described in our previous work (Yuan et al. 2016).

2.5 Statistical analysis

We used linear models to analyze the relationship between abundance of *Anaerolineae* and DOC or *I*. The relationship between the geographical distances and the similarity of bacterial community (taxonomic similarity and phylogenetic similarity) was measured by a linear model. Taxonomic bacterial community similarity and phylogenetic bacterial community similarity were determined based on the distance of Bray-Curtis and weighted-Unifrac, respectively (Rodrigues et al. 2013). In order to obtain the contribution of soil properties and geographical distance to the variations of bacterial communities, we used variance partitioning analysis (VPA). Mantel test and partial Mantel test were conducted to analyze the bacterial community correlation with soil properties.

STAMP (2.1.3) (Parks et al. 2014), R (http://www.r-project.org/), and SPSS (SPSS statistics 17.0) were used to carry out the statistical analysis. Moreover, nonmetric multidimensional scaling (NMDS) was performed to cluster the different sites by their bacterial community similarities at anodes of closed circuit SMFCs.

2.6 Nucleotide sequence accession number

The sequences obtained from pyrosequencing analysis of the bacterial community at anodes of SMFCs have deposited with the NCBI Sequence Read Archive (SRA) under accession no. SRP107835.

3 Results and discussion

3.1 SMFC performance and Spearman correlation analysis

All measured basic properties of soil were listed in Table 1. Current densities reached 4.45–22.27 mA m^{-2} (Table 1) after 68 days of incubation with paddy soil. This current was generated from the oxidation of electron donors such as organic matters and humic substances in the paddy soils (Bond et al. 2002). Meanwhile, the maximum stable power density of SMFCs was 0.14–3.65 mW m^{-2}at the 68th day (Table 1). Similar values were obtained in previous studies also but still were lower than the values obtained with amended electric conductor such as biochar and graphene oxide (Kiely et al. 2011; Goto et al. 2015; Wang et al. 2015; Chen et al. 2016).

Spearman correlation analysis displayed that power densities or current densities significantly related to NH_4^+-N, DOC, NO_3^+-N, Fe, Mn, and EC contents and to latitude and longitude (Table 2). SMFCs from different kinds of paddy soils exhibited different power output and paddy soils with higher DOC, and NH_4^+ concentrations showed higher SMFC performance (Wang et al. 2015). A study indicated that SMFCs from agricultural soil had power output about 17 times higher than the SMFCs constructed with forest soil because of higher NH_4^+ concentrations (Dunaj et al. 2012). These results demonstrate that the physicochemical characteristics of paddy soils influence the current densities or power densities directly or indirectly.

It was reported that electric potentials could affect biofilm formation on the electrode and its performance consequently influencing the microbial community compositions (Aelterman et al. 2008). At the end of operation, polarization analyses were carried out and it showed significant enrichment of exoelectrogens on anodes of the SMFCs inoculated with different paddy soils (Figs. S3 and S4, Electronic Supplementary Material).

3.2 Bacterial communities enriched by SMFCs

HiSeq sequencing analysis revealed significant differences of the bacterial community composition at anodes between the control open circuit SMFCs and the closed circuit SMFCs (Fig. 1). NMDS analysis indicated the high heterogeneity of bacterial communities at anodes of closed circuit SMFCs

Table 1 Physicochemical characteristics and SMFC performance of the five paddy soil samples

	mg kg^{-1} dry paddy soil				g kg^{-1} dry paddy soil				µs cm^{-1}	mW m^{-2}	mA m^{-2}
Sample	pH	DOC	NH_4^{-}-N	NO_3^{-}-N	Fe	Amorphous Fe	Mn	C/N	EC	*P*	*I*
HL	6.58 ± 0.58b	210.6 ± 6.2b	20.42 ± 3.38ab	8.81 ± 0.38a	30.68 ± 2.49d	9.65 ± 0.28b	0.93 ± 0.37b	12.48 ± 0.29c	594 ± 82a	0.79 ± 0.08b	10.42 ± 0.56b
DH	7.85 ± 0.34c	130.6 ± 6.0a	16.01 ± 5.06a	12.62 ± 0.97c	32.74 ± 1.88d	12.46 ± 0.47c	0.79 ± 0.21b	11.69 ± 0.06c	2391 ± 412b	0.14 ± 0.02a	4.45 ± 0.35a
TY	5.02 ± 0.05a	278.2 ± 5.0c	19.30 ± 4.12ab	9.93 ± 0.57b	20.33 ± 0.62b	2.61 ± 0.09a	0.21 ± 0.02a	9.01 ± 0.10a	615 ± 86a	0.71 ± 0.32b	9.73 ± 2.33b
YT	5.22 ± 0.07a	484.9 ± 22.1d	22.84 ± 4.94ab	9.76 ± 0.52ab	17.04 ± 0.45a	2.64 ± 0.02a	0.16 ± 0.01a	10.21 ± 0.95b	553 ± 46a	2.36 ± 0.42c	18.00 ± 1.64c
LZ	5.32 ± 0.04a	229.3 ± 5.3b	25.11 ± 2.99b	8.79 ± 0.14a	23.70 ± 0.63c	16.22 ± 0.7d	0.18 ± 0.00a	9.48 ± 0.04ab	419 ± 26a	3.65 ± 1.10d	22.27 ± 3.27d

among the paddy soils (Fig. S7, Electronic Supplementary Material). At class level, the abundance of members of *Nitrospira* increased by 1.27–8.05 times in the closed circuit SMFCs in comparison with the control open circuit SMFCs (Fig. 2). Meanwhile, at the order level, population of *Nitrospirales* also increased significantly in closed circuit SMFCs than the control (Fig. 2). Previous study has shown that most of *Nitrospira* belongs to uncultured nitrite-oxidizing bacteria (Lücker et al. 2010). *Nitrospira* are also among the most diverse and widespread nitrifiers in various environments such as paddy soil and biological wastewater treatments (Lücker et al. 2010). *Nitrospira* and *Planctomycetes* which are anaerobic ammonium-oxidizing bacteria have similar forms of nitrite oxidoreductase which functions in electron transport and respiration (Lücker et al. 2010). Moreover, it is well recognized that the reduction of iron minerals can couple anaerobic ammonium oxidation (Yang et al. 2012). Fe(III) are insoluble minerals in the neutral pH environment, and it could act as a terminal electron acceptor by some microorganisms for anaerobic ammonium oxidation (Ding et al. 2015). Therefore, the extracellular electron transfer driven by exoelectrogen especially *Nitrospira* can be a potential mechanism involved in anaerobic ammonium oxidation coupled to a solid-phase reduction of Fe(III). It is also speculated that *Nitrospira* were the potential exoelectrogens involved in the electron transfer at anode. Herein, *Nitrospira* was enriched on the surface of anode which served as the terminal electron acceptor to replace iron minerals.

Compared to the control, the population of another group of bacteria belonging to the class *Anaerolineae* increased by1.69–2.29 times in the closed circuit SMFCs (Fig. 2). Previous studies have also reported an increase (by 6%) in the population of *Anaerolineae* in SMFCs incubated with pot soil (De Schamphelaire et al. 2010). It has been reported that *Anaerolineae* populations play important multiple roles in microbial fuel cells (MFCs) inoculated with soil, especially paddy soils (Ishii et al. 2008a). In this study, we observed that the abundance of *Anaerolineae* was significantly correlated with DOC concentration (R^2 = 0.471, $P < 0.005$) and I (R^2 = 0.282, $P < 0.050$) (Fig. 3). *Anaerolineae* was known to degrade organic compounds such as carbohydrates and humic acid, which is consistent with our study that *Anaerolineae* enriched on the anodes and might facilitate the growth of other exoelectrogens (Yamada and Sekiguchi 2009). Consequently, *Anaerolineae* were speculated to involve in electron transfer to anodes.

Deltaproteobacteria was also significantly enriched in paddy soils of DH (Fig. 2 and Fig. S5, Electronic Supplementary Material). The abundance of *Geobacter* spp. which is a dominant family within *Deltaproteobacteria* increased significantly in all the SMFCs incubated with soil samples except TY (Fig. S5, Electronic Supplementary Material) (Yi et al. 2013). Previous study has suggested that strong selection of

Table 2 Correlation analysis of physicochemical characteristics and current density or power density

Sample	pH	DOC	NH_4^+-N	NO_3^+-N	Fe	Mn	C/N	EC	Latitude	Longitude	aFe	*I*	*P*
pH	1												
DOC	−0.861**	1											
NH_4^+-N	−0.236	0.296	1										
NO_3^+-N	0.146	−0.104	−0.621*	1									
Fe	0.836**	−0.946**	−0.218	0.032	1								
Mn	0.649**	−0.814**	−0.393	0.142	0.870**	1							
C/N	0.786**	−0.561*	−0.243	−0.021	0.596*	0.552*	1						
EC	0.489	−0.479	−0.329	0.589*	0.461	0.637*	0.418	1					
Latitude	0.502	−0.589*	−0.524*	0.218	0.611*	0.844**	0.687**	0.666**	1				
Longitude	0.502	−0.589*	−0.524*	0.218	0.611*	0.844**	0.687**	0.666**	1.000**	1			
aFe	0.589*	−0.636*	0.218	−0.296	0.614*	0.257	0.164	−0.082	−0.142	−0.142	1		
I	−0.470	0.593*	0.656**	−0.642**	−0.577*	−0.772**	−0.352	−0.817**	−0.786**	−0.786**	0.098	1	
P	−0.470	0.593*	0.656**	−0.642**	−0.577*	−0.772**	−0.352	−0.817**	−0.786**	−0.786**	0.098	1.000**	1

Significance level: $*P < 0.05$; $**P < 0.01$

Geobacter-dominated communities was found at the agricultural SMFC anodes (Dunaj et al. 2012). Moreover, Holmes et al. (2007) have also reported an increase of the populations of several phylogenetic groups including *Geobacter* spp. and *Desulfobulbaceae* in SMFCs (Holmes et al. 2007). Bacterial communities of the anode of SMFCs from agricultural soils were less diverse than that of the anodes of SMFCs from forest soils and were dominated by *Deltaproteobacteria* and *Geobacter* spp. (Dunaj et al. 2012). Another group of bacteria which were abundant on the anodes of closed circuit SMFCs were *Betaproteobacteria*, and their population increased significantly in all paddy soils except DH. Previous study has shown that microbes which belonged to *Betaproteobacteria* could use Fe(III), Mn(IV), and nitrate as terminal electron acceptors for growth on aromatic compounds (Weelink et al. 2009). It has also been suggested that an isolate designated KROX8 that was similar to *Betaproteobacteria* uncultured

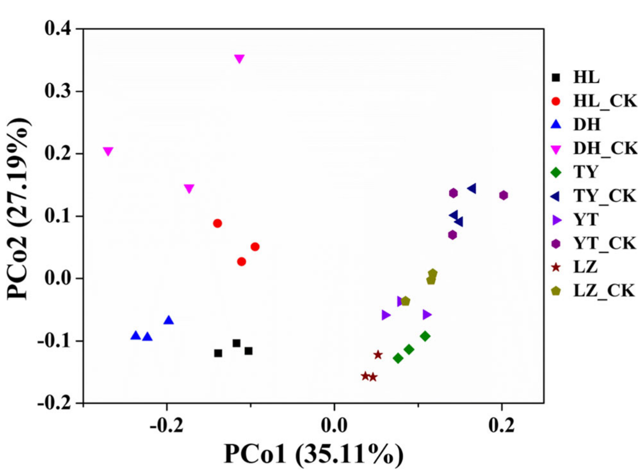

Fig. 1 Weighted-Unifrac-based principal coordinate analysis (PCoA) of bacterial communities at anodes for paddy soils HL, DH, TY, YT, and LZ after 68 days microbial fuel cell (MFC) performance. Each kind of paddy soil had three replicates and three controls without constructing MFCs

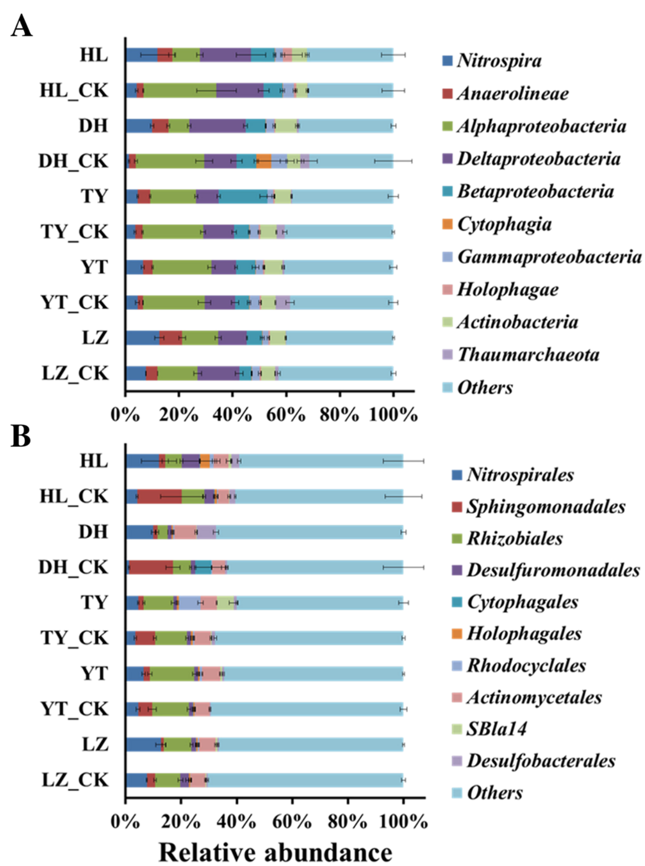

Fig. 2 Relative abundance of bacterial community composition detected at anodes for paddy soils HL, DH, TY, YT, and LZ after 68 days microbial fuel cell (MFC) performance. Each kind of paddy soil had three replicates and three controls without constructing MFCs. **a** At class level. **b** At order level

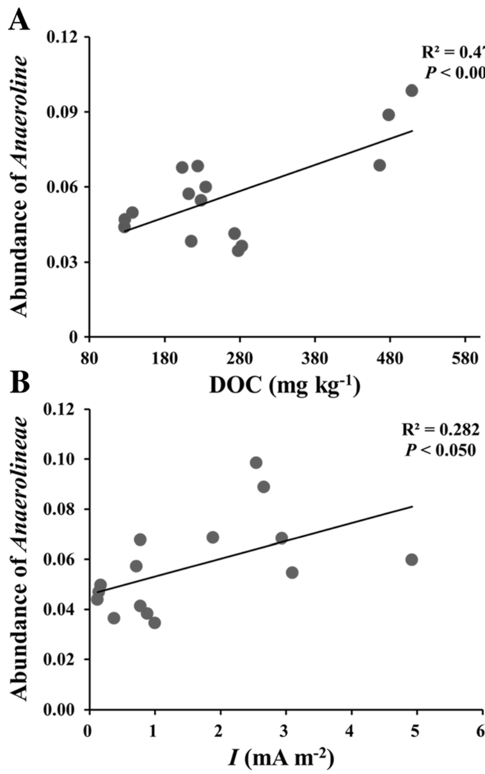

Fig. 3 The relationship between the abundance of *Anaerolineae* detected at anodes of closed of circuit SMFCs for paddy soils HL, DH, TY, YT, and LZ after 68-day MFC performance with DOC (**a**) and *I* (**b**)

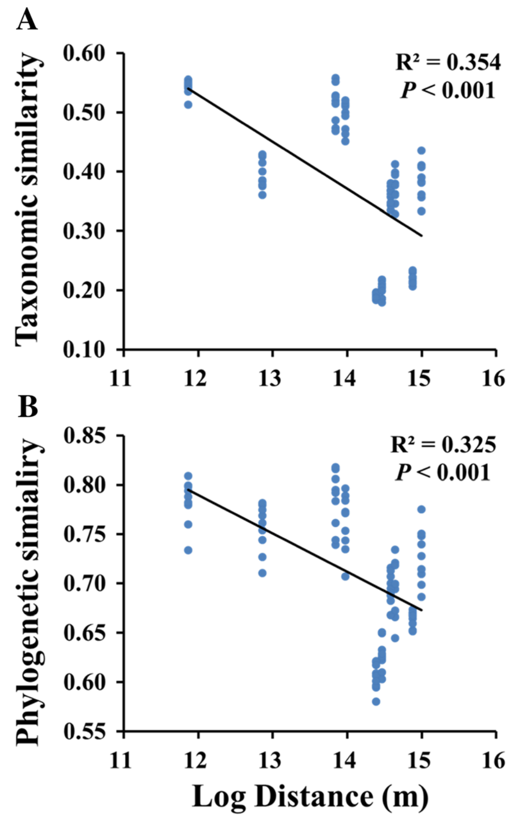

Fig. 4 The relationship between the beta-diversity of bacterial community composition detected at anodes of closed circuit SMFCs for paddy soils HL, DH, TY, YT, and LZ after 68-day MFC performance with geographic distance. **a** The decay of taxonomic similarity (Bray-Curtis) with geographic distance. **b** Decay of phylogenetic similarity (weighted-Unifrac) with geographic distance

clone C11r0 could form an electrochemically active biofilm in MFCs (Huang et al. 2011), which could explain the enrichment of *Betaproteobacteria* at the anodes of SMFCs from paddy soils.

3.3 Relationship between geographical distances, soil properties, and bacterial communities on SMFC anodes

A relationship between geographical distances and bacterial communities on anodes was studied after 68 days operation of SMFCs. The relationships were analyzed by determining the slope between geographical distance and similarity of bacterial communities at anodes at the closed circuit and open circuit SMFCs of five kinds of paddy soils. It was observed that the taxonomic similarity (Bray-Curtis) and phylogenetic similarity (weighted-Unifrac) displayed a relationship with geographical distances from closed circuit SMFCs (Fig. 4) and open circuit SMFCs (Fig. S6, Electronic Supplementary Material). Both taxonomic similarity ($R^2 = 0.354$, $P < 0.001$; $R^2 = 0.361$, $P < 0.001$) and phylogenetic similarity ($R^2 = 0.325$, $P < 0.001$; $R^2 = 0.350$, $P < 0.001$) exhibited a significant, negative distance-decay trend across the distance scale from 142 km up to approximately 3515 km. Therefore, we observed that the 16S rRNA genes of bacterial communities detected on anodes from closed circuit and open circuit SMFCs of five different paddy soils both exhibited a biogeographic pattern which was in line with previous works about the microbial communities' biogeography in soils (Ge et al. 2008). Previous work has shown that the decrease of community similarity with geographic distance is a universal biogeographic pattern which was observed in communities from all domains of life (Martiny et al. 2011). However, the underlying mechanisms of biogeographic pattern are still rare for microorganisms. Previous study has shown that geographic pattern could be driven by environmental heterogeneity across space (Martiny et al. 2011). Mantel and partial Mantel test conducted with Illumina HiSeq sequences found that bacterial community composition at anodes of closed circuit SMFCs correlated significantly with soil properties (Table 3). It has been reported that VPA was used to quantify the influence of soil properties and geographical distances on bacterial community composition detected at anodes for of paddy soils after 68 days SMFC performance. BIOENV procedure was performed to select environmental parameters (pH, aFe, Fe, DOC, EC, NO_3^--N, Mn, C/N, and NH_4^+-N), latitude, and longitude. These variables explained 65.9% of the variation. Additionally, there was 34.1% of the variation unexplained. Thereinto, the soil properties explained 57.1%

Table 3 Mantel and partial Mantel correlations highlight the relationships between soil properties and bacterial community composition at anodes of closed circuit SMFCs

Environmental variables	Mantel score (*r*)	*P* value	Partial Mantel score (*r*)	*P* value
pH	*0.862*	*0.001*	*0.856*	*0.001*
DOC	*0.383*	*0.007*	*0.398*	*0.006*
NH_4^+-N	0.141	0.114	−0.119	0.884
NO_3^--N	*0.574*	*0.003*	*0.570*	*0.002*
Fe	*0.747*	*0.001*	*0.502*	*0.001*
Mn	*0.581*	*0.002*	*0.583*	*0.001*
C/N	*0.571*	*0.001*	0.048	0.271
EC	*0.667*	*0.001*	*0.769*	*0.001*
Latitude	*0.730*	*0.001*	*0.461*	*0.001*
Longitude	*0.658*	*0.001*	−0.207	0.992
aFe	*0.384*	*0.004*	*0.292*	*0.020*

Italicized entries indicate significance values in which $P < 0.05$

and geographical distances explained 8.8% of the variations, and the interaction effect detected was 21.0% (Fig. 5). It was observed that pH, DOC, aFe, Fe, NO_3^--N, EC, and Mn were extremely significant in affecting the variation of bacterial community on anodes of SMFCs, and CCA was used to access the contribution of each significant soil property (Table 4). Geographic distance explained 8.8% of the variation detected, more than any of the other nine of the individual soil properties, though the soil properties contributed more to the variation. Our results demonstrated that geographical distance is more important than individual environmental factors in influencing the community structure of bacteria on anodes from closed circuit of five different paddy soils from across North to South of China which was consistent with previous study (Fierer and Jackson 2006).

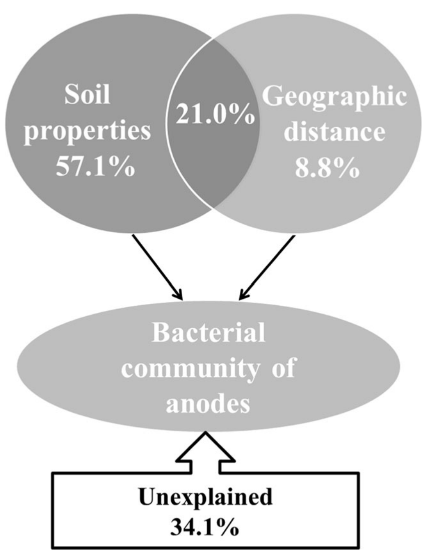

Fig. 5 Variation partition analysis (VPA) of the effects of geographic distance and soil factors on the phylogenetic structure of bacterial communities at anodes for paddy soils HL, DH, TY, YT, and LZ after 68 days MFC performance

Some key environmental factors could explain the variation of microbial diversity (Xia et al. 2016).Our results showed that pH, DOC, aFe, and Fe were the key factors which determined the bacterial community composition. Among these factors, the most important was pH as it seems to influence the microbial community more than any other soil property studied which was consistent with previous study (Scheibe et al. 2015). It has well recognized that soil pH is a dominant factor and regulated bacterial community composition and diversity (Lauber et al. 2009; Chu et al. 2010; Griffiths et al. 2011; Feng et al. 2014; Scheibe et al. 2015; Yuan et al. 2016). It has been reported that the oxidation of DOC could supply electrons for the growth of exoelectrogens and thus affect anode bacterial community composition (Lovley et al. 2004; Yuan et al. 2016). Previous studies showed that DOC was the dominant electron donor in SMFCs (Dunaj et al. 2012). The oxidation of organic carbon by exoelectrogens was found to be coupled with the transfer of electrons to anodes (Doyle and Marsili 2015). Fe(III) minerals especially aFe could be acted as an electron acceptor to accelerate the respiration of exoelectrogens. High level of Fe(III)

Table 4 Explanation and *P* value of soil properties which are significant in affecting the variation of bacterial community at anodes of SMFCs form five types of paddy soils

Soil properties	Explanation (%)	*P*
pH	4.8	0.001
DOC	4.7	0.008
NO_3^--N	4.5	0.002
C/N	4.4	0.013
EC	4.4	0.001
Mn	4.4	0.006
NH_4^--N	4.2	0.038
Fe	4.1	0.001
aFe	4.1	0.001

favored the growth of iron-reducing bacteria which include some exoelectrogens (Ding et al. 2015). Therefore, Fe and aFe are also important factors which regulate the bacterial community composition at anodes.

Our results underscored the importance of geographic distance of paddy soils in a variation of bacterial community compositions detected at anodes of SMFCs from different paddy soils. Our study indicated that geographic distance could affect the bacterial communities at anodes of SMFCs inoculated with different paddy soils across a large scale. Our study will be meaningful in the identification extracellular respiring bacteria which are involved in bioelectrochemical systems. Moreover, our results are important to understand the factors that govern the distribution and community structure of exoelectrogens. Our results have significance for understanding exoelectrogens and the ecology of potential exoelectrogens in terrestrial systems. This study also provides valuable information on how the community of exoelectrogens will respond to environmental disturbances, such as nutrients input and toxic metals.

4 Conclusions

In summary, our results showed that incubation of SMFCs with paddy soil results in the significant enrichment of *Nitrospira* and *Anaerolineae* on the anodes and also results in an increase in the populations of *Deltaproteobacteria* and *Betaproteobacteria*. Bacterial community compositions detected at the anodes of SMFCs from paddy soils were influenced by geographical distances and physicochemical properties of paddy soils. This is the first report demonstrating the influence of geographical distances on the bacterial communities at anodes of SMFCs inoculated with different kinds of paddy soils over a large range of geographic distance. These results will be crucial in the identification of potential exoelectrogens, and for understanding the factors that regulate the distribution and community structure of exoelectrogens in paddy soils. This study also provides information for predicting the response of exoelectrogens to environmental perturbations, such as addition of nutrients or contaminants.

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