

Original Research

Comparative Microbial Communities of Anode Associated Soils in Sediment Microbial Fuel Cells of Rice Field and Drainage Ditch Soils

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Abstract

In this study, the microbial community of the anode-associated soil in sediment microbial fuel cells (MFCs) was investigated. Sediments from the drainage ditch (D) and rice field (RF) were treated with or without autoclaving for single-chamber and mediator-free MFCs. Without sterilization, the voltage output reached a maximum of 124 and 120 mV for the sediments from D and RF on days 30 and 56, respectively. By day 40, the voltage output of the MFCs with sterilization became positive and continued to increase. The microbial communities of the different sediments were clustered together, indicating that the voltage release correlated positively with the presence of soil microbes. Spearman rank correlation analysis showed that the genus *Geobacter* was positively correlated with *Deftuviicoccus*, *Desulfatiglans*, *Sulfurimonas*, *Synthrophobacter*, *Thiobacillus*, and *Thermodesulfovibrio* and negatively correlated with *Holophaga*, *Opiritatus*, *Paludibacter*, and *Pseudomonas*. The genera *Geothrix* showed the dominant changes in the sediments with sterilization. These results indicate that the sediment MFC device could change the microbial composition adapted to the anodic environment.

Keywords: sediment microbial fuel cell, 16S rDNA, microbial community, anode associated soils

Introduction

Microbial fuel cells (MFCs) are a new technology for electrical energy generation that uses microorganisms to oxidize organic matter to produce electrical energy [1, 2]. MFCs can be combined with seabed sediment and wastewater treatment to grow microorganisms

from organic matter supplied by sediments and wastewater to achieve the dual goals of bioremediation and energy production [1, 3, 4]. Suspicious electron transfer pathways in the anode could occur in the exoelectrogenic bacteria, for example, *Shewanella putrefaciens* IR-1, *Geobacter metallireducens*, and *Geobacter sulfurreducens*, supplying electrons to the anode through direct contact or with nanowires. However, when a complex mixture of bacteria is applied to MFC, electron transfer can be due to quorum sensing chemicals, minerals as mediators, or cell-cell

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communication. For example, the oxidation of methane by anaerobic methanotrophic archaea is associated with sulfate reduction by sulfate-reducing bacteria [5, 6].

The content of the microbial community is influenced by environmental conditions and organic wastes. Previous studies have shown that there is a positive relationship between the microbial community and the output of the MFC. The *Geobacteraceae* harvested from marine sediment on the anode could improve the electricity generation [4]. Bacterial community and capacity analysis of sediment MFCs collected from marine sludge, saline and freshwater wetlands showed that *Deltaproteobacteria* were significantly increased on the anode, especially *Geobacteraceae* and *Desulfobulbaceae*, which were positively correlated with power generation efficiency [7]. *Geobacter psychrophilus* and its related species preferentially grow on the anodes of rice paddy-field MFCs and could generate electricity through syntrophic interactions with organisms that excrete electron donors [8]. Furthermore, this symbiotic relationship suggests that *Sporomusa* converts methanol to acetic acid, which is used by *Geobacter* to generate electricity [9]. The anodic biofilm community could also change in response to different nutrients, and the relative abundance of *Geobacter* followed the power density [10]. The surface potential and carbon sources also would affect the microbial communities [11]. Therefore, different sediments with diverse microbial and trophic conditions may affect the performance of MFCs and the adaptability of microorganisms.

This study aims to investigate the microbial communities and electricity outputs affected by different soil sources. We applied sediments from RF and D with and without autoclaving in single-chamber

and mediator-free sediment MFCs for microbial community analyses. The voltages were recorded and 16S rDNA was sequenced on the Illumina MiSeq platform. Microbial phylogeny and correlations were further analyzed using the MetaGenome Analyzer (MEGAN) and the R project.

Materials and Methods

Sediment MFCs Construction and Operation

The soils used in this study was collected from the rice field (RF) and the drainage ditch (D) (24°00'N, 120°34'E) in Dacun Township, Changhua County, Taiwan (Fig. 1) and was divided into two groups with one hour sterilization by autoclave or no sterilization.

The single-chamber, mediator free sediment MFC devices were applied. The soil was placed in a transparent plastic container (23 x 14 x 12 cm) with 5 cm depth, and carbon fiber cloth (11 x 8 cm, skyline carbon co. Taipei, Taiwan) was buried in the sediment soil as an anode (-), and a carbon rod (8 cm in length, 0.5 cm in diameter) was attached to the wall under the buffer solution as the cathode (+). The phosphate buffer solution of pH 7.2 contains 0.137M NaCl, 0.0027M KCl, 0.01M Na₂HPO₄ and 0.0018M KH₂PO₄.

The voltage and current were recorded on a dual-channel data collection multi-meter, Prova 803 (TES Electrical Electronic Corp. Taipei, Taiwan), and the data was downloaded by RS-232 interface connection to the computer. The performance of the MFCs, corresponding to changes in voltage and current, is analyzed by the following sequences of resistances: 22 MΩ, 1 MΩ, 50 KΩ, 10 KΩ, 1 KΩ, 680 Ω, 300 Ω, 150 Ω, 5.1 Ω.

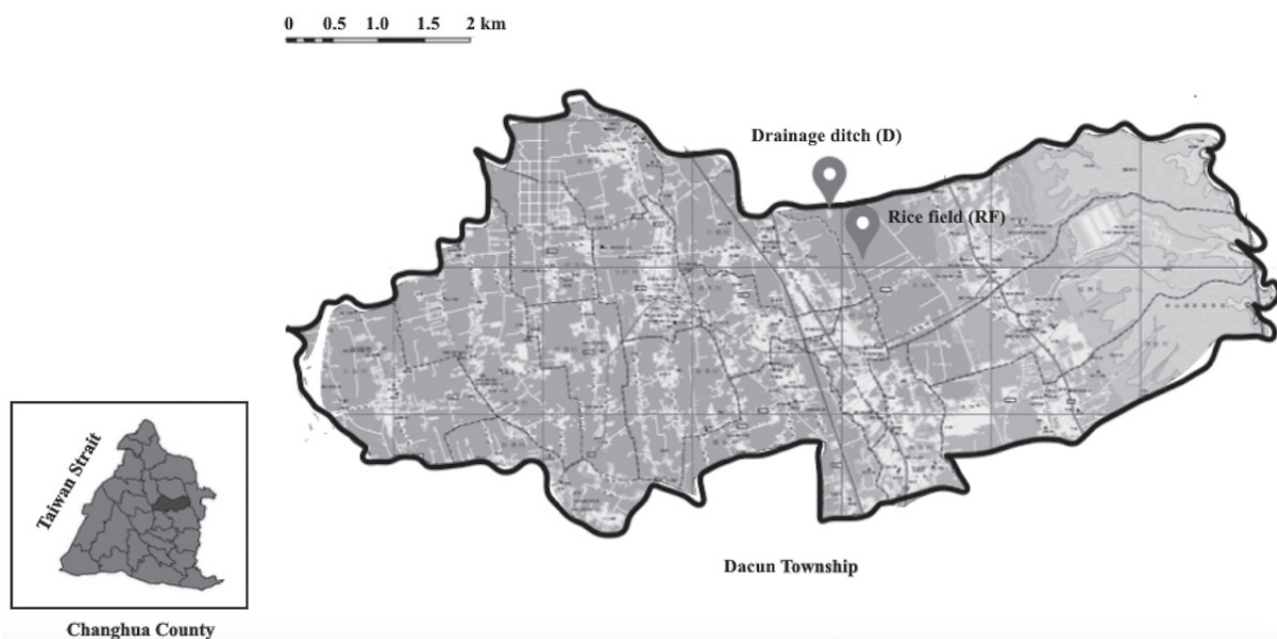


Fig. 1. Location and overview map of the researched area.

DNA Extraction, Polymerization Chain Reaction (PCR) and Sequencing

Bacterial genomic DNA was extracted from 0.5 g of soil by using UltraClean soil DNA isolation kit (MO BIO Laboratory, Carlsbad, CA, USA), according to the manufacturer's instruction. V3 region of 16S rRNA gene was amplified. Sequencing libraries were generated and sequenced on an Illumina MiSeq platform (Illumina, Inc., San Diego, CA). Nucleotide sequences determined in the study have been deposited into the MG-RAST server and the project IDs are mgm4898542.3, mgm4898543.3, mgm4898544.3, and mgm4898545.3 for samples D, DA, RF, and RFA respectively.

Sequence Analyses

Sequence analyses were first compared nucleotides to sequence databases by BLAST (Basic Local Alignment Search Tool) [12], and imported

to MEGAN (MEtaGenome Analyzer) [13] for microbial communities, Shannon index, Simpson index, UPGMA (unweighted pair group method with arithmetic mean) cluster analyses. Heatmap of dominant genera was generated in R version 3.4.1 with the ComplexHeatmap function. Hierarchical clustering was conducted using spearman [14, 15]. Spearman correlation of dominant genera was generated in R version 3.4.1 with the recorr and corplot functions [15].

Results and Discussion

Electricity Generation

The results of daily voltage fluctuations showed that under the condition of 1 K Ω external resistor as load, the output voltage of D sediment MFCs and RF sediment MFCs increased with time without sterilization treatment (Fig. 2). The D sediment MFC reached a maximum voltage of 124 mV on day 30, and

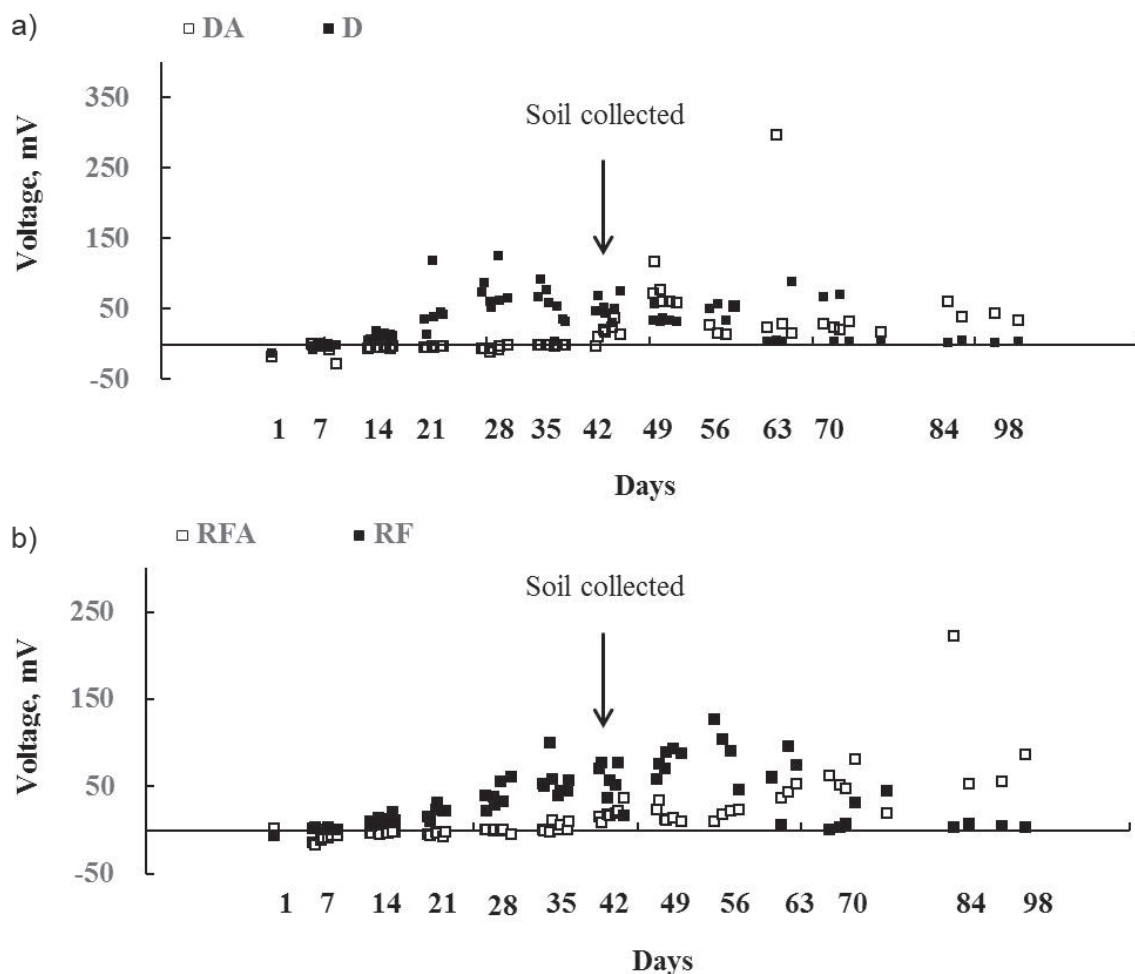


Fig. 2. a) The voltage fluctuation of the drainage ditch sediment NFC as a function of time. b) The voltage fluctuation of the rice field sediment MFC as a function of time. D and RF represent soils from the drainage ditch and rice field sediment. A indicates the soil was treated with sterilization. Solid square represents the soil treated without sterilization. Open square represents the soil treated with sterilization. The soil samples were collected on day 43 for the bacterial DNA preparation, and the overall genome analysis of 16S rDNA sequencing. The voltages of D, DA, RF, and RFA sediment MFC were 49, 37, 76, 22 mV, respectively.

the subsequent voltage output gradually decreased, and the RF-sediment MFC reached a maximum of 120 mV on day 56. Analysis of the polarization curve on day 18 showed that the maximum power densities of the D and RF sediment MFCs without sterilization treatment were 0.1 mWm⁻² and 0.4 mWm⁻², with an external resistance of 50 KΩ and 10 KΩ, respectively (Fig. S1). The voltage output for sterilized sediment MFCs was initially negative. As the number of days increased, the voltage value also increased. After the 40th day, the voltage output reached a positive value and continued to increase. On the 98th day after the end of the experiment, the voltage output was higher than that of the non-sterilized sediment MFCs. The results showed that most of the microorganisms died after sterilization, resulting in a low initial voltage output, then the surviving microorganisms continued to grow and adapt to form anodic microbial communities.

Phylogenetic Analyses of Anode-Associated Soil Microorganisms

Fig. 3 compares the four sediment MFCs by percent composition of the different bacterial species. Without sterilization, the predominant microorganisms in the D MFC were *Proteobacteria* (53.6%), *Bacteroidetes* (16%), *Firmicutes* (7.5%), *Chloroflexi* (5.2%), and *Verrucomicrobia* (3.2%), whereas in the

RF MFC, *Proteobacteria* (47.7%), *Bacteroidetes* (19.2%), *Chloroflexi* (5.6%), *Firmicutes* (4.6%), and *Verrucomicrobia* (4.1%) were predominant. In sterilized sediments (DA and RFA), *Proteobacteria* (47.7%), *Bacteroidetes* (17.5%), *Firmicutes* (13.7%), *Euryarchaeota* (4.7%), and *Verrucomicrobia* (4.3%) were the predominant microorganisms in DA MFC, whereas in the RFA MFC were *Bacteroidetes* (65.4%), *Proteobacteria* (20.1%), *Firmicutes* (6.9%), *Acidobacteria* (1.4%), and *Verrucomicrobia* (1.1%). After sterilization, *Bacteroidetes* showed the most massive increase, and *Chloroflexi* showed the decrease. The change could be due to the growth rate in relation to the current production. Recent research has reported *Proteobacteria* (25-30%), *Chloroflexi* (20%), *Acidobacteria* (15%), and *Actinobacteria* (10%) were the predominant bacteria in rice fields soil [16]. Studies on the metagenomics of anode-associated microbiomes in rice paddy field MFCs suggested that the five predominant bacteria in anode-associated soil were *Proteobacteria* (49.2%), *Actinobacteria* (2.5%), *Bacteroidetes* (2.1%), *Chloroflexi* (1.0%), and *Firmicutes* (0.8%) [8]. Our phylum level classification result was similar to the researches mentioned above. The possible explanations are that the similar natural environment was applied, and those significant predominant microorganisms might be influenced by the MFCs, but still in the larger portions.

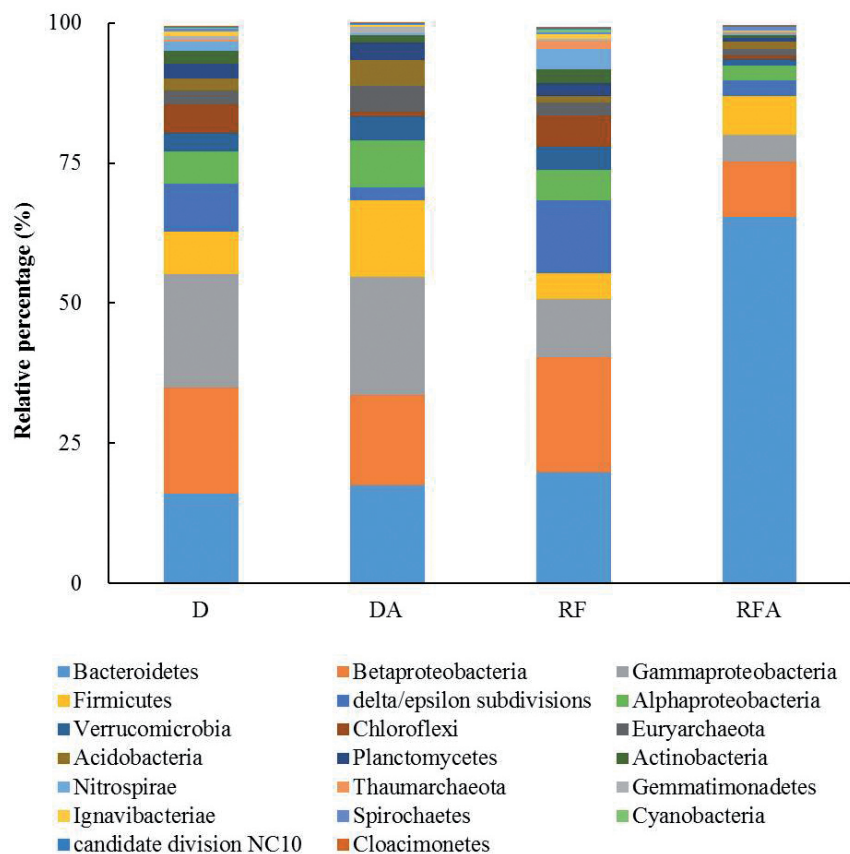


Fig. 3. The relative abundance of phyla of the microbial communities in sediment microbial fuel cells.

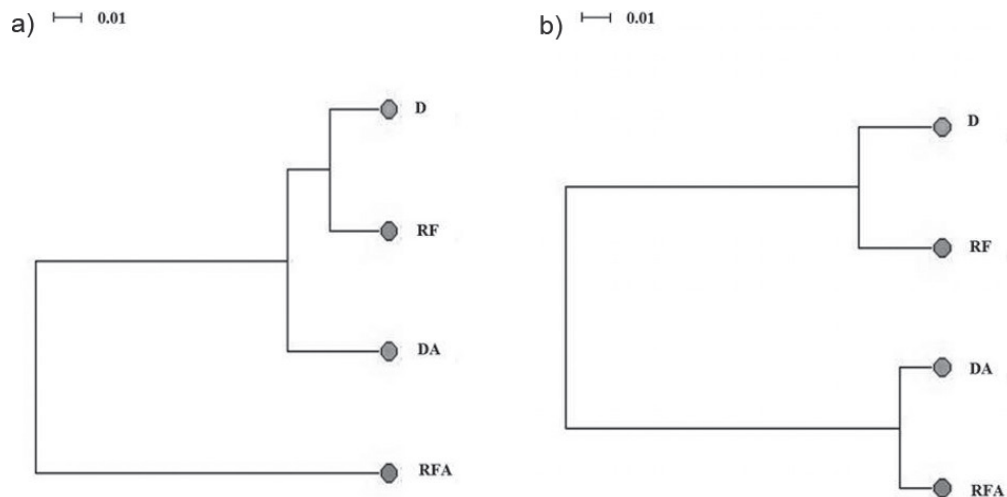


Fig. 4. UPGMA cluster analysis of soil microbial community in sediment microbial fuel cells. a) The tree was constructed with weighted uniform Unifrac metric. b) The tree was constructed with unweighted uniform Unifrac metric.

UPGMA dendrogram of soil microbial community in sediment MFCs showed a clear difference between the microbial communities with and without sterilization (Fig. 4). The energy output circuit may have actually contributed to the acclimation of certain microorganisms [8, 10, 17-19]. Most microorganisms died after sterilization. The microbial communities need time to reform and could evolve in a different direction. Shannon and Simpson diversity index of soil microbial community showed the diversity decreased with sterilization (Table 1), which is probably the cause of the lower energy yield compared to the MFCs without sediment sterilization.

Heatmap Clustering and Correlation Analyses of Anode-Associated Soil Microorganisms

The heat map and clustering analysis used the microbial genera and quantities for graphical representation of clustering (Fig. 5). The result showed that the microbial communities in D and RF sediments were clustered together, indicating that the microbial communities were relative to a higher power output. Thus, the large number of microbes present in the D and RF sediments was the likely candidate for the exoelectrogens when they were relatively fewer in the DA and RFA sediments. We listed the twenty most dominant genera of microbial communities (Table 2), checking with the heatmap result (Fig. 5), revealed that *Geobacter* showed the dominant presence and

contrasting distribution between unsterilized and sterilized sediments. To determine which microbes were responsible for the electricity generation in sterilized sediments, we studied the twenty most diverse species of bacterial communities in sterilized sediments compared to unsterilized sediments (DA/D and RFA/RF, Table 3). *Geothrix* and *Paludibacter* showed the dominant changes in both DA and RFA sediments. Although *Paludibacter* was detected as the dominant species in anodic biofilm, it did not correlate with power output [20, 21]. It is likely that *Paludibacter* did not show the contrast but was abundant in four sediments (Fig. 5, Table 3). *Geothrix fermentans*, a Fe(III)-reducing bacterium, has been reported to be involved in the electron shuttle of electricity generation [22], and the *Geothrix* from rice paddy fields correlated with power [8], and were abundantly detected in anode biofilms [23]. Unfortunately, based on the methods used, we could not confirm that *Geobacter* and *Geothrix* were the major exoelectrogens in the MFCs. However, the change in quantity matched well with the voltage output. We further investigated the possible interaction for *Geobacter* and *Geothrix* with other microorganisms by Spearman rank correlation analysis.

Correlation results ($P < 0.01$) suggested that *Geobacter* positively correlated with *Desulfatiglans*, *Deftuviicoccus*, *Thermodesulfovibrio*, *Thiobacillus*, *Sulfurimonas*, and *Syntrophobacter*, and negatively with *Holophaga*, *Opiritutus*, *Paludibacter*, and *Pseudomonas*; *Geothrix* correlated negatively with *Anaerolinea*,

Table 1. Shannon and Simpson diversity index of soil microbial community in sediment microbial fuel cells.

	D	DA	RF	RFA
Shannon-Weaver index	5.846	4.460	6.229	3.711
Simpson's reciprocal index	26.017	10.082	44.247	5.846

Table 2. The twenty most dominant genera of microbial community.

D		DA		RF		RFA	
Genus	%	Genus	%	Genus	%	Genus	%
<i>Methylococcus</i>	9.93	<i>Paludibacter</i>	13.80	<i>Thiobacillus</i>	18.04	<i>Petrimonas</i>	24.00
<i>Cupriavidus</i>	7.66	<i>Clostridium</i>	12.90	<i>Methylococcus</i>	3.91	<i>Echinicola</i>	19.82
<i>Thiobacillus</i>	5.84	<i>Pusillimonas</i>	8.76	<i>Geobacter</i>	3.89	<i>Paludibacter</i>	10.41
<i>Clostridium</i>	4.09	<i>Pseudomonas</i>	8.44	<i>Prostheco bacter</i>	3.28	<i>Thiobacillus</i>	3.48
<i>Geobacter</i>	3.47	<i>Methanosarcina</i>	4.51	<i>Thermodesulfobivrio</i>	2.63	<i>Persicitalea</i>	2.70
<i>Prostheco bacter</i>	2.91	<i>Prostheco bacter</i>	4.12	<i>Echinicola</i>	2.53	<i>Pseudomonas</i>	2.58
<i>Flexibacter</i>	2.84	<i>Roseomonas</i>	3.33	<i>Desulfatiglans</i>	2.37	<i>Alkaliflexus</i>	2.37
<i>Roseomonas</i>	2.28	<i>Isosphaera</i>	2.91	<i>Chitinophaga</i>	2.32	<i>Tolomonas</i>	2.20
<i>Pseudomonas</i>	1.95	<i>Holophaga</i>	2.46	<i>Roseomonas</i>	1.99	<i>Proteocatella</i>	2.00
<i>Flavobacterium</i>	1.83	<i>Stella</i>	2.17	<i>Alcaligenes</i>	1.84	<i>Nitrosomonas</i>	1.98
<i>Leptolinea</i>	1.69	<i>Geothrix</i>	1.99	<i>Nitrosomonas</i>	1.71	<i>Clostridium</i>	1.89
<i>Candidatus Solibacter</i>	1.64	<i>Thiobacillus</i>	1.91	<i>Pseudomonas</i>	1.67	<i>Roseivirga</i>	1.77
<i>Paludibacter</i>	1.64	<i>Solitalea</i>	1.72	<i>Leptolinea</i>	1.64	<i>Cupriavidus</i>	1.76
<i>Anaeromyxobacter</i>	1.55	<i>Candidatus Solibacter</i>	1.57	<i>Paludibacter</i>	1.43	<i>Barnesiella</i>	1.45
<i>Anaerolinea</i>	1.42	<i>Parasegetibacter</i>	1.53	<i>Deftuviococcus</i>	1.36	<i>Roseomonas</i>	0.99
<i>Parasegetibacter</i>	1.42	<i>Gemmatimonas</i>	1.42	<i>Clostridium</i>	1.33	<i>Geothrix</i>	0.96
<i>Terrimonas</i>	1.33	<i>Kaistia</i>	1.35	<i>Terrimonas</i>	1.30	<i>Olivibacter</i>	0.88
<i>Nitrospira</i>	1.29	<i>Dyadobacter</i>	1.30	<i>Flavobacterium</i>	1.28	<i>Geobacter</i>	0.79
<i>Ruminiclostridium</i>	1.26	<i>Chitinophaga</i>	1.18	<i>Bellilinea</i>	1.27	<i>Prostheco bacter</i>	0.75
<i>Chitinophaga</i>	1.17	<i>Opitutus</i>	1.15	<i>Sulfurimonas</i>	1.25	<i>Sulfurospirillum</i>	0.59

Table 3. The twenty most difference genera of the microbial community in sediment with sterilization relative to the sediment without sterilization.

Genus	DA/D fold	Genus	RFA/RF fold
<i>Isosphaera</i>	24.32	<i>Alkaliflexus</i>	27.26
<i>Holophaga</i>	9.02	<i>Barnesiella</i>	17.04
<i>Geothrix</i>	8.60	<i>Sulfurospirillum</i>	11.53
<i>Paludibacter</i>	8.44	<i>Candidatus Cloacimonas</i>	11.44
<i>Desulfosporosinus</i>	8.24	<i>Campylobacter</i>	10.41
<i>Dehalobacter</i>	7.60	<i>Methanospirillum</i>	9.93
<i>Ethanoligenens</i>	6.36	<i>Echinicola</i>	7.83
<i>Opitutus</i>	5.99	<i>Paludibacter</i>	7.30
<i>Dyadobacter</i>	5.80	<i>Persicitalea</i>	6.69
<i>Methanosarcina</i>	4.67	<i>Proteocatella</i>	6.66
<i>Methanoregula</i>	4.56	<i>Roseivirga</i>	6.59
<i>Pseudomonas</i>	4.33	<i>Parabacteroides</i>	5.37
<i>Sporomusa</i>	4.20	<i>Ruminiclostridium</i>	4.21
<i>Methylarcula</i>	4.19	<i>Geothrix</i>	4.09
<i>Kaistia</i>	4.11	<i>Phenylobacterium</i>	3.76

Table 3. Continued.

<i>Desulfitobacterium</i>	3.91	<i>Sporomusa</i>	3.45
<i>Echinicola</i>	3.72	<i>Magnetospirillum</i>	3.26
<i>Stella</i>	3.57	<i>Spirochaeta</i>	2.94
<i>Solitalea</i>	3.23	<i>Treponema</i>	2.85
<i>Acinetobacter</i>	3.16	<i>Methanoregula</i>	2.65

Flexibacter, *Methylococcus*, and *Thiocystis* (Fig. 6). *Desulfatiglans* [24], *Thermodesulfobivrio*, and *Syntrophobacter* [25] are sulfate-reducing bacteria (SRB), and SRB could cooperate with iron-reducing bacteria, *Geobacter* and *Geothrix* in iron, sulfur, and methane metabolic cycles [26, 27]. *Sulfurimonas* known as the sulfur oxidizing bacteria would anaerobically oxidize sulfur with nitrate as the electron acceptor [28] and could be involved in sulfur, nitrate, iron, and methane metabolic pathways [29]. Metagenomic analysis suggested *Candidatus Defluviococcus*

seviourii, a glycogen accumulating organism, would store polyhydroxyalkanoates and adapt to low nutrient conditions [30]. *Thiobacillus*, *Desulfuromonas* and *Geobacter* were identified as the main members of Fe(III)-reducing bacteria in the sediments of a heavily polluted freshwater lake [31]. *Syntrophobacter* was found to be involved in syntrophic oxidation of propionate in rice field soil, and *Geobacter* would also assimilate propionate [32]. *Opitutus* is an obligately anaerobic bacterium that ferments saccharides to produce propionate and acetate, and reduce nitrate

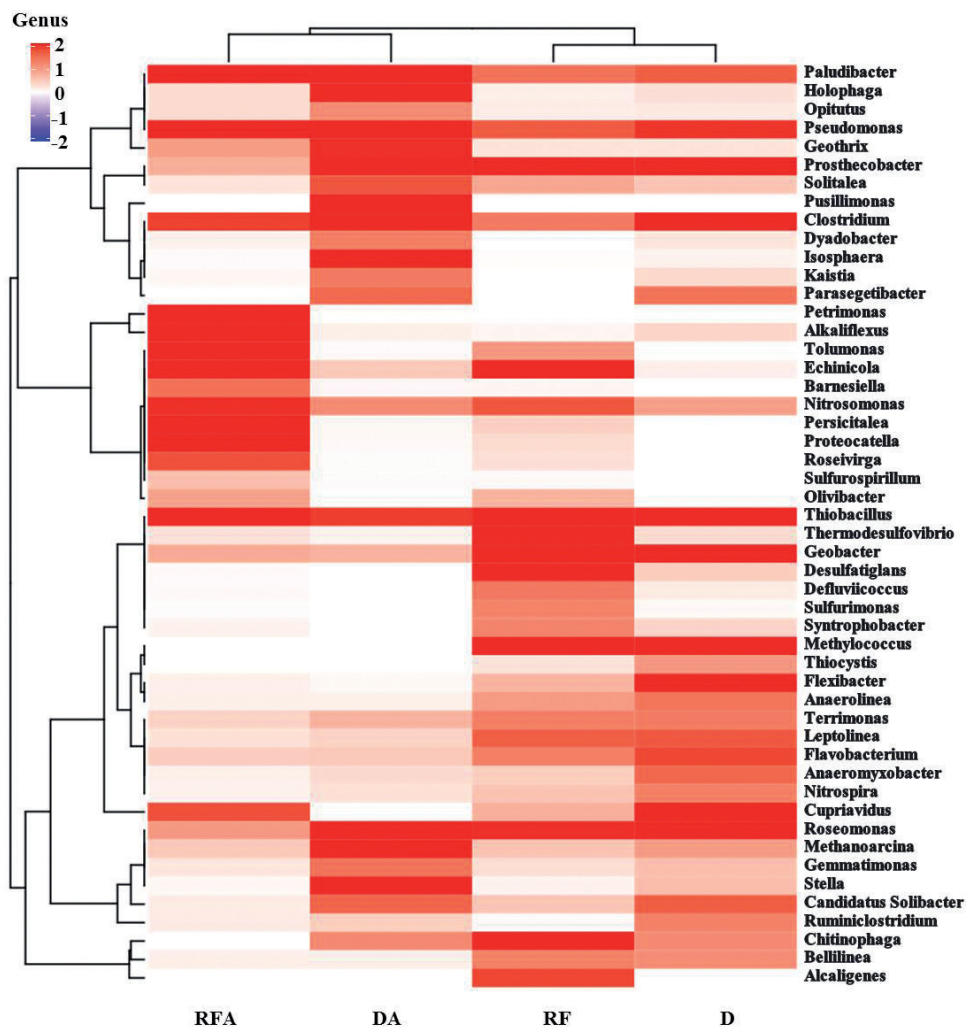


Fig. 5. Heatmap of dominant genera. The abundance distribution of the dominant 50 genera was displayed in the abundance heatmap. The colors of the scale bar represent the 'z' score. The absolute value of 'z' represents the spearman correlation distance between the raw score and the standard deviation mean population. 'Z' is negative when the raw score is below the mean, and vice versa.

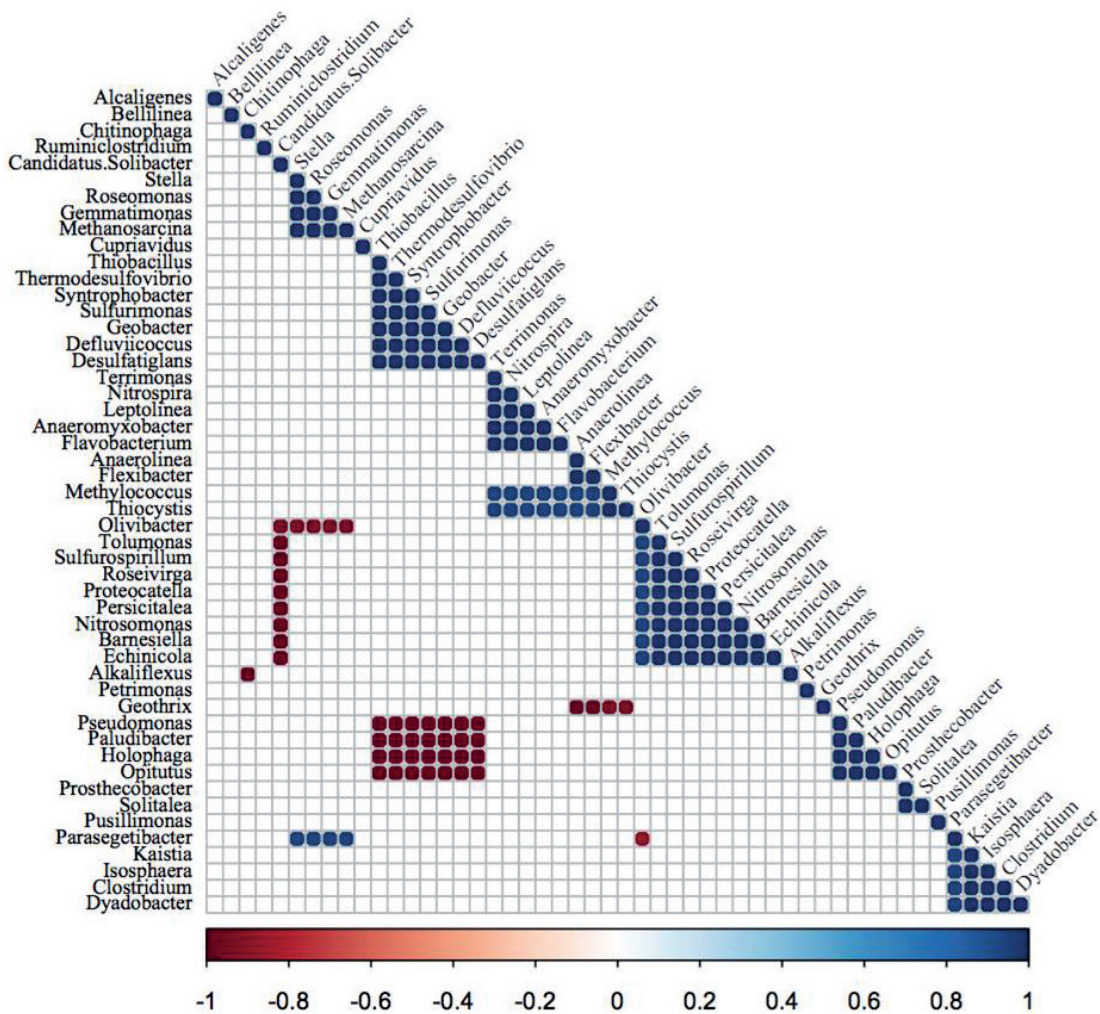


Fig. 6. Spearman rank correlation analysis of the 50 dominant microbial populations in sediment microbial fuel cells. Squares represent the correlation between two microbial communities was significant ($p < 0.01$). Strong correlations are indicated by dark blue squares, whereas dark red squares indicate weak correlations. The scale bar colors denote the nature of the correlation with 1 showing a perfect positive correlation (dark blue) and -1 indicating perfect negative correlation (dark red) between two microbial populations.

to nitrite [33]. *Paludibacter jiangxiensis* is a strictly anaerobic, propionate-producing bacterium isolated from rice paddy field [34]. Study on the rhizosphere selection showed the *Holophaga* 16S rRNA gene number were more abundant in the leek rhizosphere than in bulk soil and the rhizospheres of grass and potato [35]. A cocultures study of *Geobacter* and *Pseudomonas aeruginosa* suggested a transition from syntrophy to competition [36]. *Anaerolinea* and *Syntrophobacter* were found dominantly present in the anode of glucose fed MFC, suggesting syntrophic interaction of these fermentative bacteria with exoelectrogens [8]. *Flexibacter* showed positively correlated with most core bacteria, *Blastocatella*, *Chloronema*, *Desulfobulbus*, *Geobacter*, *Leptolyngbya*, *Rivibacter*, *Spirochaeta*, and *Synechococcus* in response to *indica* and *japonica bar*-transgenic rice paddy soils [37]. With a genome-scale metabolic model for *Methylococcus capsulatus* suggesting the reduced efficiency for the methane monooxygenase to the oxidation of

methane [38]. *Thiocystis* is a purple sulfur bacterium capable of phototrophic growth with sulfide or sulfur as an electron donor under anoxic conditions [39]. Different soil sources in MFCs would yield divergent microbial communities [19, 40]. Soil nutrient and carbon availability has been shown to affect microbial communities, for example, in response to glucose amendment, the microbial communities changed towards to glucose utilization [41]. The microbial community dynamics also could be affected by MFC operation, and the bacteria *Geobacter* and *Thermincola* could be responsible for bioelectricity generation [42]. In a mixed community in MFCs, the microorganisms that would respire at the most negative anodic potential will displace other strains that cannot adapt to the more negative potential [43]. Alternatively, syntrophic interactions have previously been suggested in a study of anode biofilms from glucose-fed and acetate-fed MFC [9]. To further investigate the microbial interaction, metatranscriptomics seems to be the best

method to identify soil microorganisms and protein activity at the time of sampling for interpretation of the regulatory mechanism [44, 45]. Recently, efforts with enhanced electricity generation in paddy plant MFC and paddy-field MFC, the maximum power density could reach 40.3 mWm⁻² and 130 mWm⁻² respectively [46, 47], combined with an ultra-low-power energy harvester specially designed for MFCs which can extract energy even from extremely low-power, inexpensive, and single MFCs for the autonomous environmental sensing [48], suggesting a feasible environmental application for the MFCs.

Conclusion

This study shows that the presence of *Geobacter* and *Geothrix* is relative to power output and the microbial community of the anode-associated soil is in a dynamic syntrophic or competitive relationship. The electrochemical capability modulated by sediment MFCs would cause a change in the microbial community, and an ideal mixed community is likely to form for maximum or sustained power generation. Future studies will study the complex interactions among anode-associated microbes.

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Conflict of Interest

The authors declare no conflict of interest.

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