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The change in biotic and abiotic soil components influenced by paddy soil microbial fuel cells loaded with various resistances

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Abstract: Soil microbial fuel cells (sMFC) are a novel technique that use organic matters in soils as an alternative energy source. External resistance (ER) is a key factor influencing sMFC performance and, furthermore, alters the soil's biological and chemical reactions. However, little information is available on how the microbial community and soil component changes in sMFC with different ER. Therefore, the effects of anodes of sMFC at different ER (2000 Ω, 1000 Ω, 200 Ω, 80 Ω and 50 Ω) were examined by measuring organic matter (OM) removal efficiency, trace elements in porewater and bacterial community structure in contaminated paddy soil. The results indicated that ER has significant effects on sMFC power production, OM removal efficiency and bacterial beta diversity. Moreover ER influences iron, arsenic and nickel concentration as well in soil porewater. In particular, greater current densities were observed at lower ER (2.4mA, 50Ω) compared to a higher ER (0.3mA, 2000Ω). The removal efficiency of OM increased with decreasing ER whereas it decreased with soil distance away from the anode. Furthermore, principal coordinate analysis (PCoA) revealed that ER may shape the bacterial communities that develop in the anode vicinity but have minimal effect on that of the bulk soil. The current study illustrates that lower ER can be used to selectively enhance the relative abundance of electrogenic bacteria and lead to high OM removal.

Keywords: External resistances; Soil microbial fuel cells; Paddy soil; Geobacter; Arsenic; Iron; Organic matter

1. Introduction

Organic carbon in sediments or soils can be used as a new source of energy when employed in microbial fuel cells (MFC). Soil microbial fuel cells (sMFC) are a special type of MFC that produce electricity by using organic chemicals present in soils as energy source [1-4]. In sMFC organic matter (OM) is oxidized at the anode by the soil microorganism, resulting in the production of electrons. The electrons produced during OM oxidation migrate towards the cathode, through an external circuit, where they combine with oxygen and protons to form water. sMFC that employ paddy soil has gained considerable attention recently because of the unique biological and chemical characteristics of paddy soil make them ideal for power production [5]. In addition to power generation, sMFC have also found use in stimulating soil bioremediation. For instance, the anode of the sMFC can serve as an electron sink for anode respiring bacterial (ARB) and accelerate the bioremediation of both polycyclic aromatic hydrocarbons and redox active heavy metals [6,7].

However, sMFC deployment in paddy fields has been shown to alter soil bacterial community structure and abiotic components [8,9]. When an anode of sMFC is embedded into anaerobic soils, it functions as a continuous sink for electrons and this alters soil chemistry [10,11]. This in turn increases soil *Eh*, decreases soil pH and changes the bioavailability of soil nutrients such as phosphate and nitrogen [1,12]. Therefore these changes in soil chemistry may influence the soil bacterial community composition, since soil *Eh*, pH and nutrient bioavailability are the major drivers of soil bacterial community structure [13-15]. Furthermore, the sMFC have also been used to prevent the deterioration of overlying water quality and for the removal or stabilization of toxic heavy metals [7,16,17]. Previous studies have shown that sMFC can be used as a mitigation technology in soils contaminated with chromium, uranium, cadmium, lead or copper [7,17,18].

Anode bacterial community determines the capability of the sMFC to function effectively

as a remediation technology. For example, when non-anode respiring bacteria are abundant in the anode chamber, these microbes may compete with ARB for resources, thereby limiting the performance of the sMFC. However, regulating the anode potential has been shown to effectively tailor the desired ARB consortium and suppress the growth of undesirable microbes, such as methanogens [9,19]. This occurs because the anode potentials determine the accessibility of electron ARB received for growth and development [20]. Thus at certain anode potential, ARB are able to gain higher energy for growth from respiring the anode and out compete methanogens for OM [19].

Moreover, adjusting applied external resistance (ER) in sMFC has also been shown to affect the metabolic rate of ARB on the anode [21]. Previous studies have demonstrated that decreasing ER increases the oxidative degradation of OM and the current output from the sMFC [3]. Furthermore, the current produced by the sMFC can also stimulate microbiological oxidation of soil OM by fermentative bacteria [6,22]. Even though, several studies have examined the influence of the anode potentials on the biofilm community composition in double chamber MFC, to date there is no universal consensus on the effect of the anode potential on the anode community structure. Although the application of sMFC is increasing, studies examining the effect of anode potential on sMFC community have received limited to no attention. Torres, *et al.* [20], observed selective enhancement of *Geobacter sulfurreducens* at lower anode potentials (−0.15V, −0.09V, and 0.02V vs SHE) and an increase in bacterial diversity at higher anode potential (0.37V). On the contrary, Zhu, *et al.* [23] argues that anode community is unaffected by anode potential and that the electrogenic communities acclimatize to different anode potentials. Nonetheless, to date, few studies have been done to examine the influence of the anode at different ER on soil biotic and abiotic constituents collectively.

Thus, the objectives of this study are, therefore, to examine the effects of different ER on soil and anode bacterial community structure, OM removal efficiency and selective soil metal behavior at various resistances away from the anode. The results from this study suggest that lower ER can be used to selectively enhance ARB abundance and increase OM removal.

2. Materials and Methods

2.1. Paddy soils sample

The paddy soil (0–15 cm below the soil–water interface) samples were collected from a rice paddy in Qiyang, Hunan (GPS N26.760 E111.86) and transported directly to the laboratory. These samples were then air-dried and sieved to 2 mm to remove all coarse debris. The soil properties, including texture, pH, OM content, arsenic (As) and Iron (Fe) were measured to be clay, 6, 23.2g/kg, 73.7mg/kg and 53.69 g/kg, respectively.

2.2. Soil Microbial Fuel Cell assembly

Eighteen sMFC were constructed from the paddy soil and operated at different ER 2000 Ω, 1000 Ω, 200 Ω, 80 Ω and 50 Ω in triplicates for 90 days. Of the eighteen sMFC three were controls, the anodes and cathodes were not connected (open circuit). All sMFC were constructed according to a previously reported method by Wang, *et al.* [24] with slight modifications. Briefly, a columnar polyethylene terephthalate container (10 cm diameter × 15 cm depth) with two valve ports (~2 cm above the anode and adjacent to the anode) was used to construct each sMFC. Circular carbon felts with geometric surface area of 50.2 cm^2 were be used as anodes and cathodes. A data logger (USB-7660B, ZTIC, China) was used to record the voltage between the anode and cathode.

Using 700 g (dry weight) of soil sample, ~1 cm depth of soil was placed at the bottom of the sMFC container. Then, the anode was placed on the surface of the soil layer and then buried with additional soil to simulate anaerobic conditions. The cathode was placed above the soil in

aerobic conditions (half submerged in water and the other half in the open air). Deionized water (1L) was added to flood the paddy soil.

2.3. Chemical Analysis

Each constructed sMFC was equipped with a self-made soil pore water sampler. The sampler was made from a 0.45 μm hollow fiber membrane, which was inserted into the soil through the valve port adjacent to the anode and 2 cm above the anode. The soil porewater was sampled and analyzed for As and Fe by inductively coupled plasma emission spectrometry (ICPMS) (NexlonTM 350x, Pekin Elmer, USA) and atomic absorption spectrometry (AAS) (PinAAcle™ 900, PerkinElmer, USA), respectively. Dissolved organic carbon (DOC) concentration was determined with a TOC analyzer (Shimadzu TOC-VCPH, Japan). The loss on ignition (LOI) carbon content was determined gravimetrically. LOI was calculated according to the following equation 1:

$$\text{LOI}\ (\%) = ((D105 - D550)/D105) * 100 \quad (1)$$

where D105 is the dry mass of the sample heated 105 ºC for 12 hours and D550 is the dry mass of the sample after 4 hours of incubation at 550 ºC. Samples were allowed to cool to room temperature in a desiccator before determining D550.

2.4. Bacterial Community Analysis

Total Genomic DNA was extracted from the anode vicinity and bulk soil using the PowerSoil DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA) from all of the treatments including the controls according to the manufacturer's protocol. The quantity of DNA in the extracts was measured at 260 nm using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA). The DNA quality was verified by agarose gel electrophoresis. The DNA samples were stored at -80 °C until sequencing.

The V3-V5 hypervariable regions of the 16S ribosomal RNA (rRNA) gene in bacteria were amplified using primer pair 515f/907R [25,26]. The library preparations and Illumina MiSeq sequencing of the 16S rRNA gene amplicon were outsourced to GENEWIZ, Inc. (Suzhou, China). FLASH was used to merge paired-end reads and QIIME (Quantitative Insights Into Microbial Ecology) (V1.7.0) was employed to optimize the data by removing low-quality sequence and for all taxonomy analysis [27]. The low-quality reads were discarded. Subsequently, the sequences were compared with the reference database (RDP Gold database) using UCHIME algorithm to detect chimeric sequence, and then the chimeric sequences were removed [28,29]. The clean sequences were clustered into operational taxonomic units (OTUs) (97% similarity) by UPARSE. The phylogenetic taxonomy were assigned according to the Ribosomal Database Program (RDP) classifier (Version 2.2) and the Green Genes Database at confidence threshold of 80% [28,30].

2.5. Statistical Analysis

One-way analysis of variance (ANOVA) was performed to test significant differences between treatments using SPSS software (IBM SPSS Statistics 23.0) and OriginPro (Inc., OringinLab, USA). Tukey's honest significant difference (HSD) studentized range tests were applied to differentiate between various treatments and a $p < 0.05$ was considered significant. Alpha diversity indexes were calculated using Faith's phylogenetic diversity, Shannon diversity index and the Chao1 richness estimator using QIIME. Beta diversity was assessed using principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) analysis.

2.6 Nucleotide sequence accession number

Nucleotide sequences were all deposited in the GeneBank database with accession numbers MG814044 - MG815131.

3. Results

3.1. Electricity generation from soil MFCs with different external resistors

In this study each sMFC was loaded with a fixed resistor for the duration of the experiment. Five different ER were tested 50 Ω, 80 Ω, 200 Ω, 1000 Ω and 2000 Ω and the current production from the sMFC for 90 days of operation are illustrated in Figure 1. The current of the all sMFC sharply increased during the initial stage regardless of ER and reached a maximum current at around 10 days. The highest current produced was observed at an ER of 50 Ω (2.4mA), followed by those of 80 Ω (2.1 mA), 200 Ω (1.6 mA) and 1000 Ω (0.8 mA). An ER of 2000 Ω produced the lowest current (0.3mA). However after ca. 30 days all the current outputs irrespective of ER were approximately the same (0.15-0.2 mA). The insert in Figure 1 shows change in the controls voltage with time.

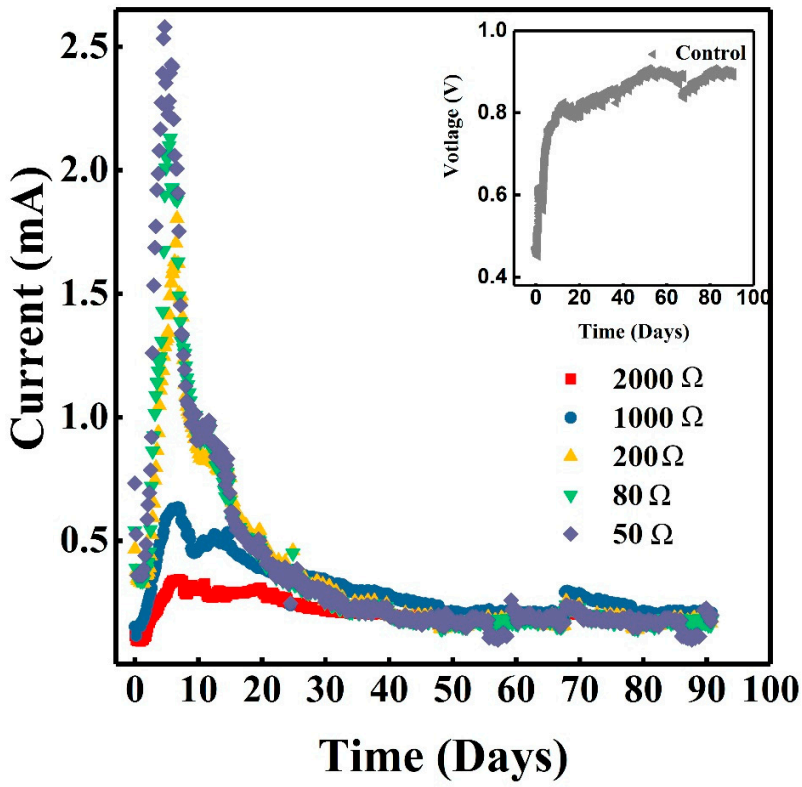

Figure 1. Current production from sMFC fixed with different external resistances during a 90-day operation. The insert represent the change in the control's voltage with time.

3.2. Polarization properties of the sMFC

The power current curve is an important attribute used to determine the performance of MFC. The power current curves in this study were obtained by varying ER from 10KΩ to 10Ω and are compared in Figure 2. The power densities of the sMFC set at 1000 Ω and 2000 Ω ER were similar and those set at lower ER (50 Ω, 80 Ω and 200 Ω) mirrored each other. As shown in Figure 2, the maximum power density observed in the sMFC at different resistance were 22.2, 22.2, 24.1, 36.5 and 40.0 mW/m^2 for 50 Ω, 80 Ω, 200 Ω, 1000 Ω and 2000 Ω respectively. Similarly, the polarization slope method was used to determine the ohmic resistance for each sMFC. The results followed a similar trend to maximum power except for the case of 200 Ω. The 50 Ω ER had the lowest ohmic resistance and 2000 Ω had the highest ohmic resistance. The ohmic resistance increased in the following order 50 (389 Ω) < 80 (390 Ω) < 1000 (476 Ω) < 200 (556 Ω) < 2000 (572 Ω).

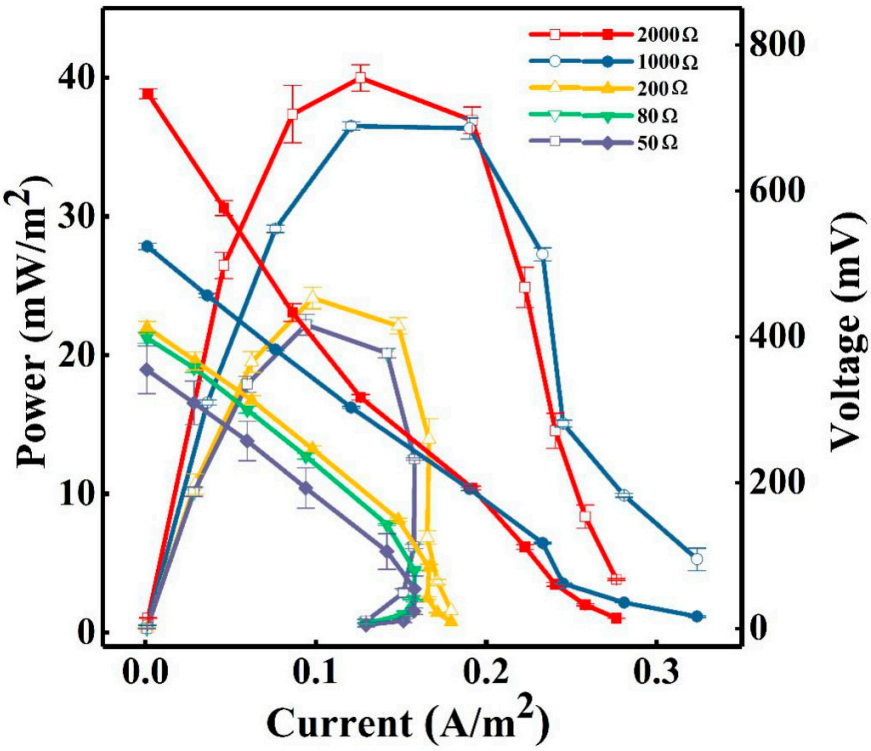

Figure 2. Power density (filled symbols) and polarization curves (open symbols) of sMFCs. The error bars represent standard error of measured concentrations of triplicate samples.

The anode and cathode potentials (versus Ag/AgCl reference electrode) for each sMFC at different ER are shown in Figure S1. In general, the anode potential became more positive with decreasing ER, however there was negligible difference between the potential of the anode at 50 Ω, 80 Ω and 200 Ω. The working potential of the anode for the sMFC with an ER of 2000 Ω was much lower than those with other ER, approximately 345 mv lower than those with ER of 50 Ω, 80 Ω and 200 Ω. Moreover, the highest cathodic potential was observed in the sMFC with 200 Ω ER followed by that of 80 Ω and 1000 Ω. The cathodic potential of sMFC with ER of 50 Ω and 2000 Ω were approximately the same. These findings indicate that the cathode was responsible for the difference in sMFC performance with different ER, especially at ER of 200 Ω, 80 Ω and 50 Ω

[3]. This is due to the sharp decrease in cathode potential with decreasing resistance compared to that of the anode.

3.3. Bacterial community structure

The Illumina MiSeq sequencing of the 16S rRNA gene yielded a total of 2164539 bacterial sequences (average read length 450 bp). The valid reads clustered into 1162 OTUs at a 3% distance. The rarefaction analysis showed a clear saturation trend indicating that the sequencing depth of 30,000 was sufficient (Figure S2). The notion of sufficient depth being met was also supported by the high Good's Coverages (>0.99) (Table S1). The observed alpha diversity indices showed that the bacterial communities in the vicinity of the anode were less diverse than that of the bulk soil and that applying different ER had a negligible influence on bacterial diversity (Table S1).

Beta diversity indices PCoA (Figure 3) and NMDS (Figure S3) analysis of the bacterial community composition, reveal three distinctive clusters. Where the bulk soils bacterial community clustered together regardless of applied ER, while the samples from anode vicinity posed at high ER (1000 Ω and 2000 Ω) clustered with the control and that of the lower resistances (50 Ω, 80 Ω and 200 Ω) formed another cluster. These findings demonstrate that the ER may shape the bacterial communities that develop in the anode vicinity but have minimal effect on that of the bulk soil.

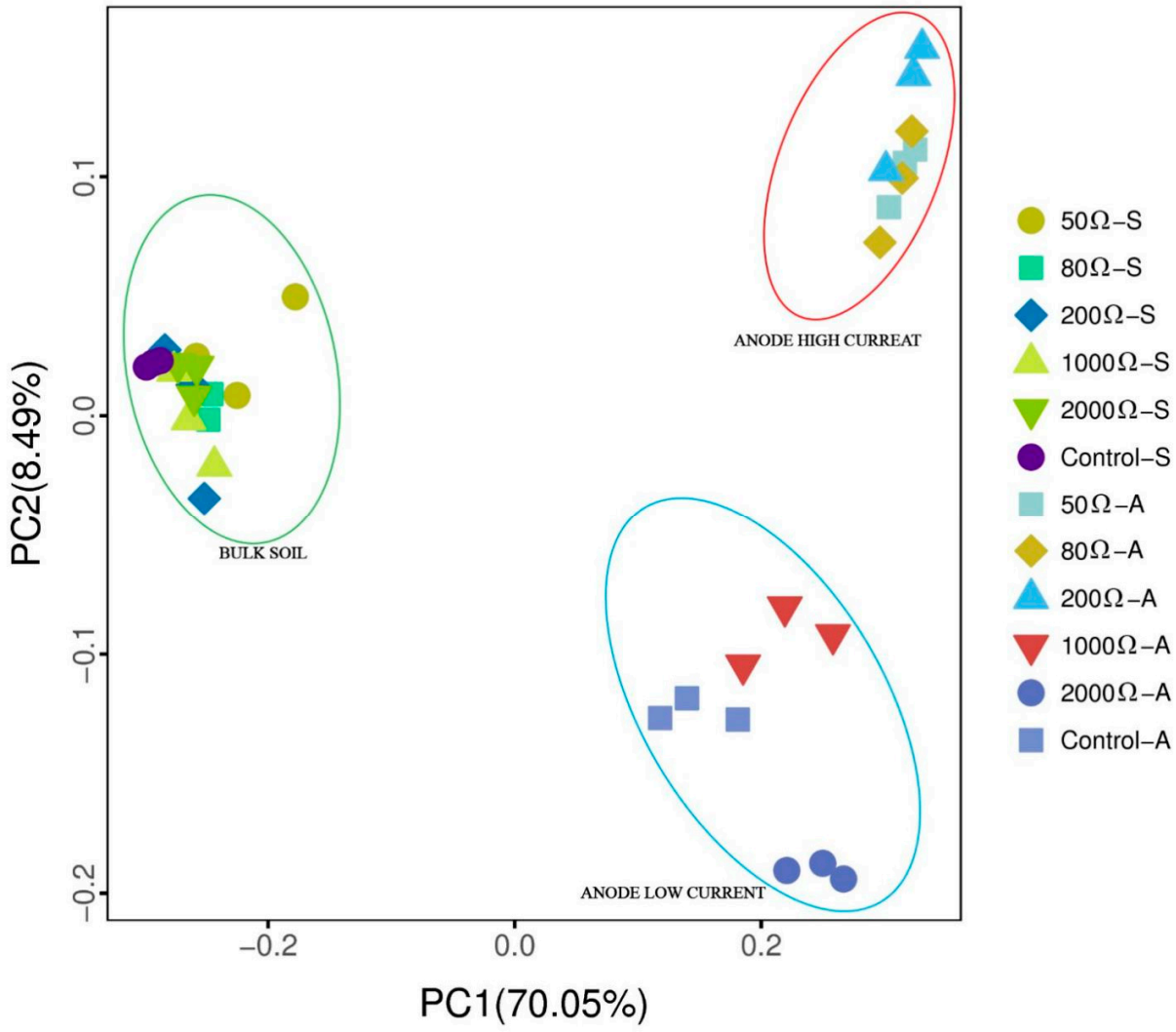

Figure 3. Principal Coordinates Analysis (PCoA) of the sMFC and controls bacterial community composition based on the unweighted UniFrac distance matrix. The x- and y-axes are indicated by the first and second coordinates, respectively, and the values in parentheses show the percentages of the community variation explained.

Phylum and class level analysis reveal that fourteen bacterial phyla and fourteen bacterial classes made up the vast majority of total 16S rRNA gene sequences, accounting for 92% and 78% of total reads, respectively. Taxonomic classification at the phylum showed that the phyla

Proteobacteria, Chloroflexi, Nitrospirae and *Chlorobi* were enhanced with decreasing ER and *Bacteroidetes, Acidobacteria* and *Euryarchaeota* were suppressed (Figure S4). The relative abundance of *Proteobacteria* in the anode biofilms were 29.7% (50 Ω), 26.5% (80 Ω), 27.2% (200 Ω), 23.2% (1000 Ω), 22.6% (2000 Ω) and 21.3% (control) respectively, when *Chloroflexi* accounted for 10.8% (50 Ω), 11.7% (80 Ω), 11.5% (200 Ω), 10.3% (1000 Ω), 8.04% (2000 Ω) and 9.86% (control). The relative abundance of *Nitrospirae* and *Chlorobi* followed a similar trend 11.5 and 2.56 % (50 Ω), 14.1 and 2.36% (80 Ω), 13.9 and 1.64% (200 Ω), 8.01 and 1.06% (1000 Ω), 7.00 and 1.34% (2000 Ω) and 5.2 and 0.86% (control) respectively. Analysis on the class level showed that *Deltaproteobacteria*, *Bacteroidetes vadinHA17*, *Nitrospira* and *Subgroup18* were strongly influenced by ER. Further analysis at the genus level showed that the *Geobacter* and *Lentimicrobium* were most influence genus. *Geobacter* was enhanced with decreasing ER in both the bulk soil and the anode vicinity, while in the bulk soil *Lentimicrobium* was suppressed (Figure 4).

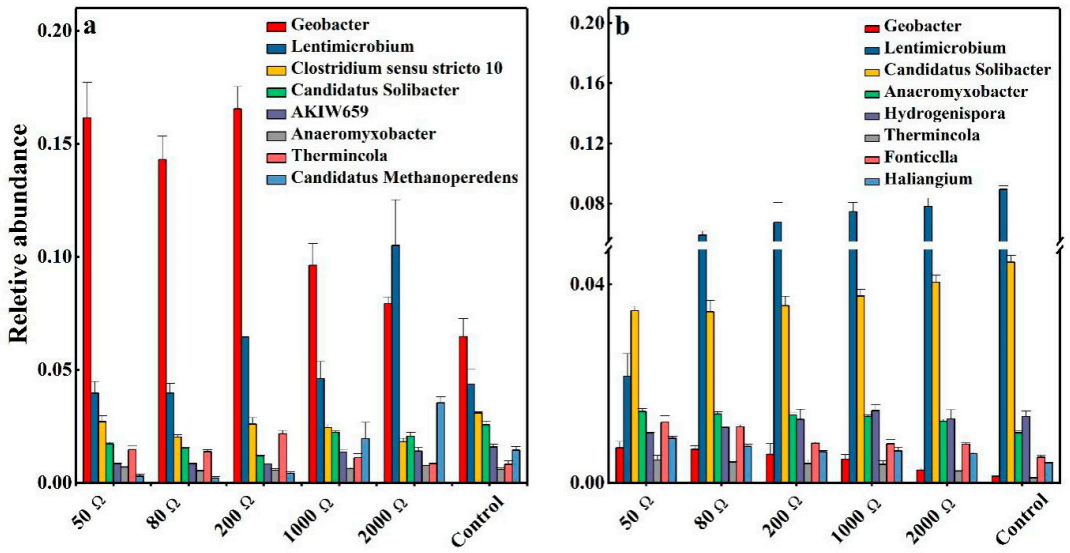

Figure 4. Relative abundance of bacterial community composition at genus level (a) anode vicinity and (b) bulk soil.

3.4. Organic matter removal

The loss on ignition (LOI) value was used as a proxy to estimate the OM content in the soil and its removal efficiency was used to analyze the effect of ER on degradation of organic carbon by the sMFC. Figure 5 illustrates the removal efficiency of LOI from the soil samples at two distances (~1cm and 2cm) away from the anode at different ER. The removal efficiency of LOI increased with decreasing ER and distance away from the anode. The removal efficiency of LOI in the vicinity of the anode were 2.0%, 4.9%, 6.9% and 7.4% higher than the control, for sMFC loaded with 2000 Ω, 1000Ω, 200 Ω, 80 Ω and 50 Ω ER, respectively. A similar trend was observed in the removal efficiency of OM in the bulk soil. A significantly ($p < 0.05$) higher removal efficiency of LOI irrespective of location was observed between the control and all the treatments except for 2000 Ω (anode vicinity). The results obtained here demonstrate that the anode could enhance bacterial oxidation of organics and decreasing ER can further promote this process.

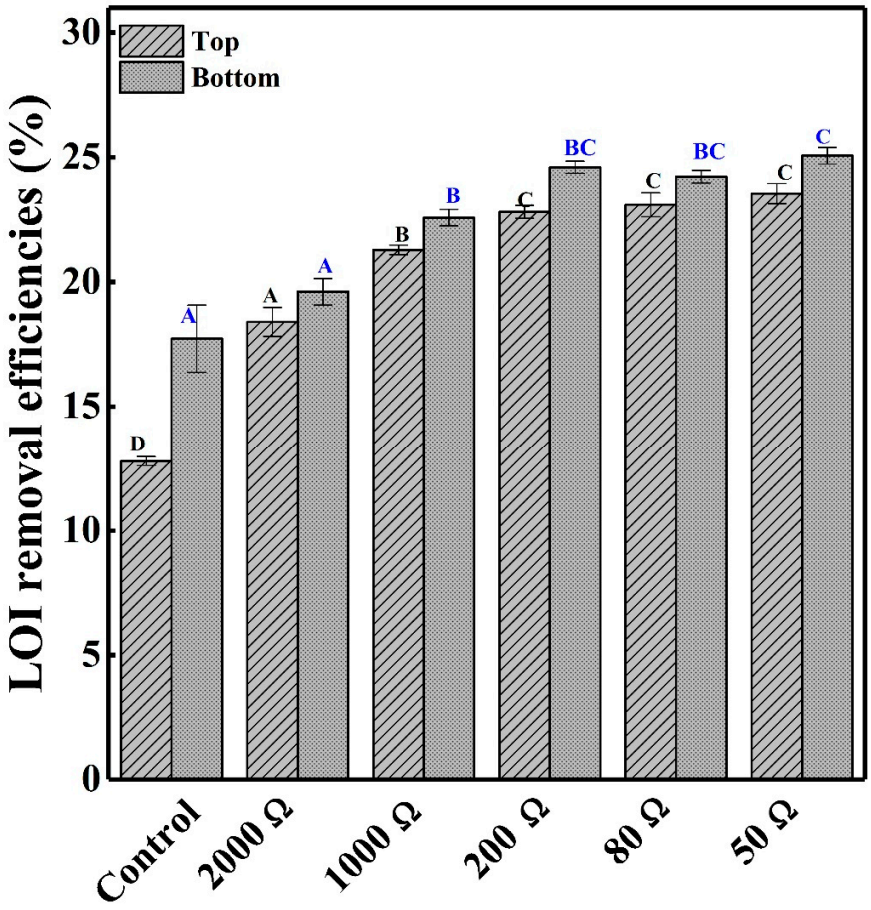

Figure 5. Removal efficiencies of lost on ignition carbon. The error bars represent standard error of measured concentrations of triplicate samples. Different letters represent statistical significance.

3.5. Changes of porewater As, Fe and Ni

The concentrations of As, Fe and Ni in the bulk soil porewater and in the anode vicinity were tested at intervals of 10 days from day 0-60 and then tested at the end of 90 days (Figure 6a-d and Figure S5a-b). As seen in Figure 6a-b and Figure S5a-b no significant (p >0.05) differences was observed in bulk soil porewater for As, Fe and Ni with time irrespective of ER, however the concentration of both Fe and As was slightly elevated in the treatments compared to the controls at the end of 90 days. In the vicinity of the anode the concentration of As, Fe and Ni were lower

in the treatments compared to the control. In general the concentration of these metals decrease with decreasing ER in the anode vicinity.

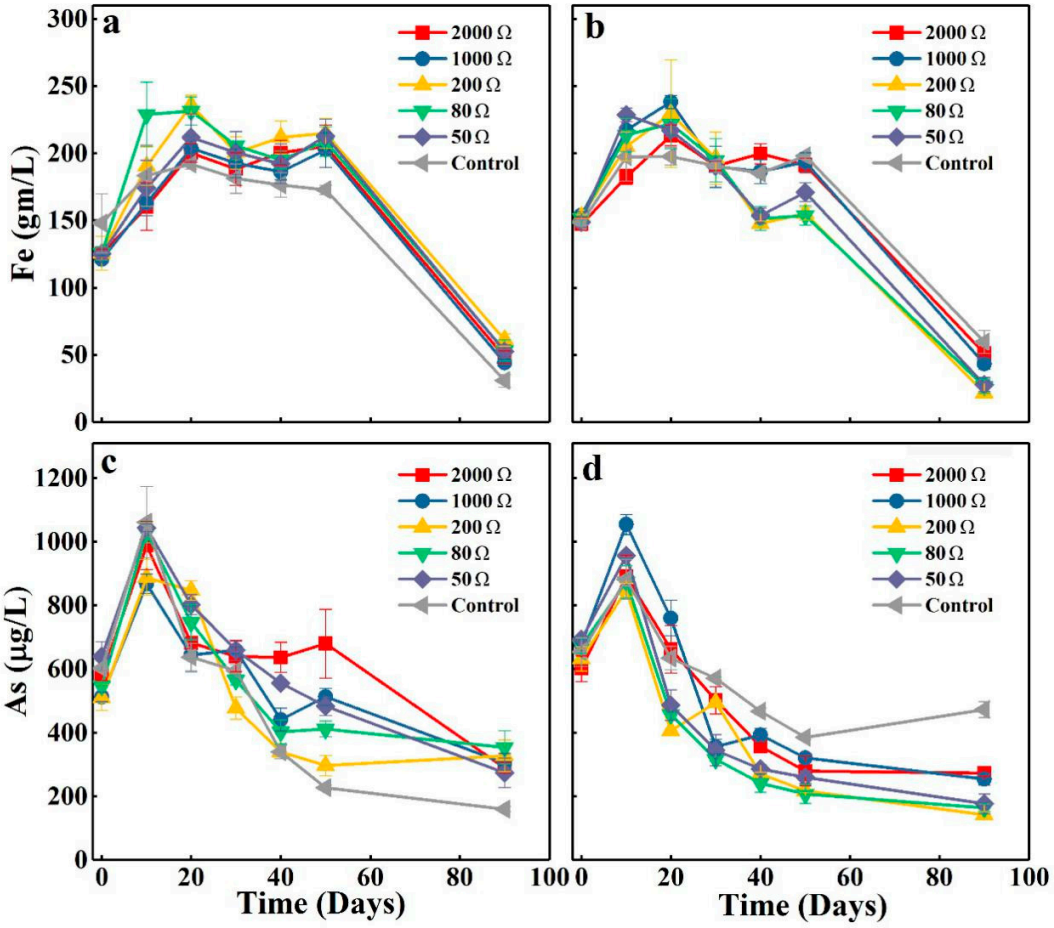

Figure 6. Fe and As variation in soil porewater along with incubation time. Panels a and b represent Fe concentration in the bulk soil and anode vicinity respectively. Panels c and d represent As concentration in the bulk soil and anode vicinity respectively. The error bars represent standard error of measured concentrations of triplicates samples.

4. Discussion

Various studies have shown that the applied ER can greatly affect the current production, because ER plays an important role in the enrichment process of ARB on the anode [21,31]. These

studies revealed that low resistance stimulate ARB growth on the anode and thereby improved current production. As illustrated in Figure 1, the rapid initial increase in current occurred as a result of the high readily oxidized organic content of the soil and the accumulation of the electrochemically active biofilm on the anode (i.e. *Geobacter*). The steady decrease in voltage observed after day 10 can be attributed to reduction in the physiological activity of the ARB on the anode and cathode limitations. The different intensity observed in current peak, occurring at different ER, was probably due to the increase in internal resistance with increasing ER (Figure 2). The results obtained here are in accordance with that of Hong, *et al.* [32] and Holmes, *et al.* [33]. In both studies a maximum current was achieved within 10-20 days which was followed by a gradual decrease in current production with time.

The increase in current with lower ER led to the higher removal efficiency of LOI in treatments fixed at low ER due to stimulated increase of anaerobe metabolic activities (Figure 5). Previous studies have shown that current can accelerate the metabolic reaction rate in anaerobic bacteria by enhancing the release of enzymes and altering the permeability of anaerobes cell membrane [6,22]. Thus, increase in electron transfer rate from anode to cathode due to lower ohmic resistance improved bacterial metabolism of both the anaerobic bacteria in the anode vicinity and the bulk soil. This consequently led to the higher removal of OM by the sMFC with the 50 Ω, 80 Ω, and 200 Ω and 1000 Ω circuit load. The relatively lower current at 2000 Ω circuit load results in minimal OM removal compared to the control. The increase in current and decrease in OM content in the treatment are probably responsible for the slightly lower concentration of As, Fe and Ni in the anode vicinity. Studies have shown that organic carbon is the main electron donor for anaerobic reduction of Fe oxide minerals baring metals and metalloids. A positive correlation has also been observed between Fe and As mobilization and DOC bioavailability [34]. Moreover, the

reduction of Ni in the anode vicinity of the treatment was probably due to electromigration and immobilization of Ni on the anode [35].

The effect of different ER on bacterial community structure enriched in the bulk soil and the anode vicinity of the sMFC after 90 days of operation revealed that differences in community profiles in the vicinity of the anode and minor changes in that of the bulk soils. As shown in the PCoA analysis (Figure 3) and NMDS (Figure S3) the anode community can be divided into two groups. Samples taken from the anode vicinity with lower circuit loads (50 Ω, 80 Ω and 200 Ω i.e. high current) were similar and those from higher ER (1000 and 2000 Ω i.e. low current) were more comparable to the control. The results observed here suggested that distinct bacterial communities developed at different current densities.

The similarity in anode potential in groups with lower or higher ER could be reason for the observed pattern. Numerous studies have shown that bacterial community varied depending on the anode potentials [21,36]. In addition the anode has been shown to accelerate the metabolic rate and development of electroactive bacterial community in its vicinity [21,36]. In a study on the effects of ER on the anode [21], observed higher anode potential and columbic efficiency with lower ER. The authors concluded that lower ER produce higher current and that ER can be used to control anode potential and for the selection of ARB. Thus, it could be assumed that higher ER possibly limited ARB colonization and metabolic activities on the anode and vice versa when ER were reduced.

Moreover taxonomical analysis of the anode community indicated that reducing ER enhances ARB such as *Deltaproteobacteria* and *Nitrospira* (Figure S4). This occurred because when ARB uses anode at higher electrochemical potentials these ARB obtain more energy [36,37]. Thus, at lower ER higher current generation and relative abundance of ARB was observed.

Previous studies have demonstrated that the *Geobacter* sp. current generation properties and growth are strongly affected by the anode potentials [38,39]. Torres, *et al.* [20] investigated the bacterial community at different anode potential and found that lower anode potential had a high selection of the *Geobacter sp.,* since they are able to transfer electrons to the anode efficiently with minimal energy loss. These results are in line with our findings where *Geobacter* relative abundance increased with decreasing ER (Figure 4). The results obtained here demonstrates that ER can significantly influence the composition of anode bacterial communities by selectively enhancing electrogenic bacteria.

5. Conclusion

In this study the effect of different ER on the sMFC performance, OM removal and bacterial community composition was investigated. The results indicated significant influences of ER on current production, OM removal efficiency and bacterial diversity. In particular, greater current densities, OM removal efficiencies and enhancement of *Geobacter* were observed at lower ER (50 Ω, 80 Ω and 200 Ω). The current study illustrates that lower ER can be used to selectively enhance ARB relative abundance and increase OM removal efficiencies while decreasing metal concentration in soil porewater.

Supplementary Materials: The following are available online at www.mdpi.com**.** Table S1. Similarity-based OTUs and species richness and diversity estimates, Figure S1. Anode and cathode polarization curves. The error bars represent standard error of measured concentrations of triplicate samples, Figure S2. Rarefaction curves based on MiSeq sequencing of bacterial communities showing the diversity of OTUs (similarity cut off of 97%). OTUs, Operational Taxonomic Units, Figure S3. Non-metric multidimensional scaling (NMDS) analysis of the sMFC and controls bacterial community composition based on Bray–Curtis dissimilarity matrix for bacterial

communities that consisted of OTUs (97% similarity level), Figure S4. Relative abundance of microbial community composition at phylum ((a) anode vicinity and (b) bulk soil) and class level ((a) anode vicinity and (b) bulk soil), Figure S5. Nickel variation in soil porewater along with incubation time. a and b represent nickel concentration in the bulk soil and anode vicinity respectively. The error bars represent standard error of measured concentrations of triplicates samples.

Author Contributions: Williamson Gustave, Zhao-Feng Yuan and Zheng Chen conceived, designed, and performed the experiments with support from Yu-Xiang Ren and Hu-Cheng Chang. Williamson Gustave and Zhao-Feng Yuan and Zheng Chen worked together to analyze the data. Williamson Gustave, Zhao-Feng Yuan and Zheng Chen wrote the paper with edits from Raju Sekar. All authors read and approved the content.

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Conflicts of Interest: The authors declare no conflict of interest.

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