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Relationships between Soil Organic Matter, Nutrients, Bacterial Community Structure, And the Performance of Microbial Fuel Cells

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Supporting Information

ABSTRACT: Microbial fuel cells (MFCs) offer the potential for generating electricity, mitigating greenhouse gas emissions, and bioremediating pollutants through utilization of a plentiful renewable resource: soil organic carbon. We analyzed bacterial community structure, MFC performance, and soil characteristics in different microhabitats within MFCs constructed from agricultural or forest soils in order to determine how soil type and bacterial dynamics influence MFC performance. Our results indicated that MFCs constructed from agricultural soil had power output about 17 times that of forest soil-based MFCs and respiration rates about 10 times higher than forest soil MFCs. Agricultural soil MFCs had lower C:N ratios, polyphenol content, and acetate concentrations than forest soil MFCs. Bacterial community profile data indicate that the bacterial communities at the anode of the high power MFCs were less diverse than in low power MFCs and were dominated by *Deltaproteobacteria*, *Geobacter*, and to a lesser extent, *Clostridia*, while low-power MFC anode communities were dominated by *Clostridia*. These results suggest that the presence of organic carbon substrate (acetate) was not the major limiting factor in selecting for highly electrogenic bacterial communities, while the quality of available organic matter may have played a significant role in supporting high performing bacterial communities.

INTRODUCTION

Microbes from organic matter rich soils and sediments have shown value as an alternative energy source in microbial fuel cells (MFCs). MFCs have been generated at low cost, sustained for long periods of time, and have minimal impact on the environment.[1,2] MFCs rely on bacterial oxidation of organic carbon coupled to transfer of electrons to an anode in order to produce an electric current.[3] Many electrogenic microbes are also known to reduce Fe^{3+} and Mn^{4+}, humic acids, and sulfate in organic-rich sediments and soils.[3] Substrates used for MFC electrogenic production have included wastewater, activated sludge, liquid medium enhanced with acetate and fumarate, and organic-rich soils and sediments.[1,3−5] Limitations have occurred in pure culture MFC systems due to thick biofilm production on the anode, diminishing the ability for proton diffusion and nutrient availability.[4,6] These results support the feasibility of utilizing soil or sediment based MFCs, where the potential of a heterogeneous bacterial community colonizing the anode could be a potential benefit in preventing inhibitory metabolic waste accumulation. Soil or sediment-based MFCs are also extremely inexpensive to construct and maintain, in contrast with pure-culture systems.

Despite the advantages of MFC construction from natural sediments, the availability of labile organic substrates to fuel microbial generation of electricity may limit MFC performance. Also, MFC studies have focused on bacterial communities at the anode, but have neglected the bulk sediment and cathodes communities.[7,8] These components, along with the nutrient content and soil characteristics related to electrogenic activity,

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need to be further investigated to better understand the factors that govern MFC performance. In the current study, we analyzed bacterial community structure, MFC performance, and soil characteristics in different microhabitats (bulk soil, anode, and cathode) within MFCs constructed from agricultural or forest soils in order to determine how soil type and bacterial dynamics influence MFC performance. Our results indicated strong selection of electrogenic bacteria within the anodes of high-performing agricultural soil MFCs.

MATERIALS AND METHODS

Soil Sample Collection and Study Site. Soil samples were collected from forested and agricultural plots at Harvard Forest (Petersham MA) on November 15, 2009. Forest soil was taken from Barre Woods, which is a transitional hardwood forest stand,[9] and agricultural soils were obtained from a garden plot that was located within a few meters of the Barre Woods stand. MFCs were set up using a 1:1 homogenized mixture of organic and mineral soil layers from each site.

Percent water-soluble polyphenol content within the separated organic layer was analyzed utilizing the Folins-Denis method.[10] Organic layer samples were analyzed to determine the carbon and nitrogen percent content and carbon to nitrogen molar ratios via a CHN analysis following drying and weighing of samples (Perkin-Elmer 2400 CHN analyzer).

Microbial Fuel Cells Construction and Treatment. The MFCs were developed by Trophos Energy, Inc. (Somerville MA) (currently available through KeegoTech LLC, Somerville MA). MFCs consisted of the following: a one-liter clear hard PET container with top valve ports allowing for CO_2 sampling, a valve port adjacent to the anode for pore water sampling, and a controller circuit and data logger (Supporting Information Figure S1). The electrodes were constructed of circular carbon cloth for the anode and carbon felt for the cathode, and the total geometric area (GA) of the anode electrode was 81.07 cm^2. The cathode had a diameter of about 8.89 cm, thickness of 1.27 cm, with eight thin (about 1.91 cm wide) carbon cloth strips woven into the top of the cathode.

Each constructed MFC contained a 1:1 mixture of the collected organic and mineral soil layers that were homogenized and saturated with deionized water. The saturated soils were left for 24 h and were allowed to drain before placement within each MFC. The headspace within each MFC was measured after draining excess water from the saturated soil. About 1 cm of soil was placed at the base of the MFC container before installing the anode, and additional soil was deposited on top of the anode until the container was $\sim^2/_3$ full. The carbon felt cathode was placed above the soil, allowing for oxygen interaction with the cathode.[4,5] MFC treatment controls were constructed using the same soils, treatments, and engineered systems but with no electrodes. For each type of soil ("agricultural" or "Barre Woods" soil), one control (i.e., no electrodes) and two replicate closed circuit MFCs were constructed and analyzed. All of the assembled MFCs were then incubated at 30 °C in the dark until final destructive sampling and analysis (total of 78 days).

Power Output Measurements. Electrical power output was measured on a continual basis. Once a day, with the recorded voltage measurements, the MFC control circuit scanned between a range of resistances and set load resistance to generate the highest power output possible. Recorded voltage and current data from the circuits were stored via the data logger connected to each individual MFC until disassembly; however, some of the data loggers powered down before deconstruction and final sampling. This was overcome by utilizing a multimeter to measure the voltage and power output data from each MFC immediately before disassembly and subsequent final sampling.

Analysis of Bulk MFC Soil Parameters. MFC pore water was sampled from the anode port and the concentrations of phosphate (PO_4^{3-}), ammonium (NH_4^+), and nitrate (NO_3^-) were measured. PO_4^{3-} was measured using the method prescribed by Murphy and Riley,[11] NH_4^+ was analyzed with a modified version of a phenol-hypochlorite method,[12] and NO_3^- concentration was determined with a Lachat instrument.[13] Two gas samples collected over a two-hour period were obtained from each MFC through the top port valves on days 0, 4, and 10 to measure the rates of CO_2 production. CO_2 gas samples were collected via syringe and analyzed within four hours using gas chromatography on a Shimadzu GC-14A analyzer equipped with a 1 mL gas sample loop, a Supelco (Sigam-Aldrich) Porapak Q 100/120 column with He as a carrier gas, and maintained at 80 °C. A thermal conductivity detector was used to measure CO_2. For samples taken after day 10, the rate of CO_2 respiration was determined by using a Li-Cor 6400 portable photosynthesis system.

Interstitial pore water was collected using the sampling port near the anode with a sterile syringe and collection vial for analysis of anions using ion chromatography (IC). Samples were held on dry ice or at −20 °C until transfer to −80 °C freezer, where they were stored until analysis. A Dionex DX-600 IC and Chromeleon software (Dionex, Sunnydale, CA)[14] were used to analyze nitrite (NO_2^-), NO_3^-, PO_4^-, sulfate (SO_4^-), and acetate.

Bacterial Community Sampling. To determine links between bacterial community structure, bulk soil parameters, and MFC performance, soil and electrode samples were collected throughout each MFC for 16S rRNA gene terminal restriction length polymorphism (T-RFLP) analysis and clone library construction and sequencing. Bacterial community profiling samples were collected in triplicate from each of the following microhabitats of each MFC: cloth anode, bulk soil immediately adjacent to the anode, bulk soil half way between the cathode and anode (includes mid section of treatment controls), and felt cathode (top soil layer for control). Each bulk soil sample was homogenized and placed in a cryovial for analysis. The carbon cloth anodes were divided and cut into three small equivalent (2.5 ×2.75 cm) rectangular sections for analyses of anode communities and biomass.[15]

Genomic DNA Extraction. Genomic DNA was extracted with the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH), with three freeze (liquid N_2) and thaw (65 °C) cycles.[8,16] DNA was further purified using a QIAquick PCR Purification Kit (Qiagen, Germantown MD), and eluted with 30 μL of preheated (60−70 °C) EB buffer. Purified genomic DNA was analyzed by agarose (0.7% in 0.5 × TBE buffer) gel electrophoresis,[17] and quantified using a PicoGreen (Invitrogen, Carlsbad CA) assay on a SpectraMax M2e plate reader (Molecular Devices, Sunnyvale CA). The PicoGreen assay utilized was validated against humic acid interference via a humic acid standard addition determination with extracted DNA from a similar MFC. The concentration of extracted DNA from the each of the anodes was also utilized as proxy for biomass determination.[15]

Terminal Restriction Fragment Length Polymorphism (T-RFLP). Terminal restriction fragment length polymorphism

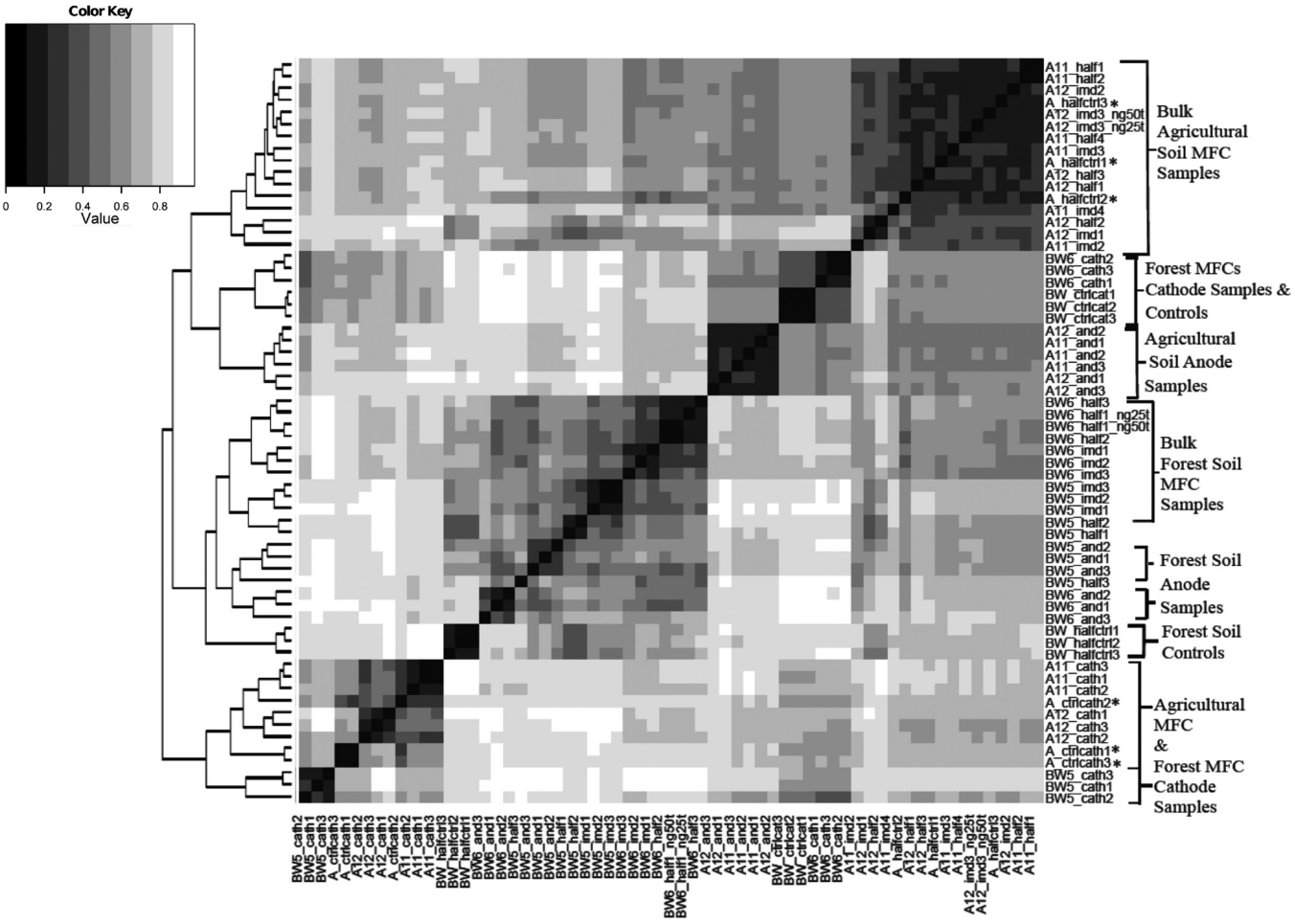

Figure 1. Hierarchical clustering analysis and heatmap of Bray−Curtis distances among MFC Samples. Sample name abbreviations denote the following. A: agricultural soil MFCs; BW: Barre Woods forest soil MFCs; 5, 6, 11, 12: unique identifiers for distinct MFCs; 25 ngT and 50 ngT suffix indicates PCR template amount in nanograms for protocol validation purposes; *imd:* soil immediately adjacent to anode; *half:* bulk soil half way between cathode and anode; *and:* anodes, *cath: cathodes,* and *halfctrl:* midsection bulk soil from control MFC treatments. Agricultural control samples are marked with an asterisk.

(T-RFLP) analysis is an established method for generating community structure profiles and determining the dynamics of complex bacterial communities.[7,16,18] T-RFLP analysis was completed utilizing primers that target the 16S rRNA gene of most *Bacteria*, 27F (5′-AGAGTTTGATCMTGGCTCAG-3′),[19] 5′ end-labeled with 6-carboxyfluorescein *(6-FAM)*, and 1492R (5′-GGTTACCTTGTTACGACTT-3′).[5,20−23] Fifty ng of genomic DNA was used as template in each 50 μL PCR reaction, except in cases for which environmental contaminants inhibited PCR. In these latter cases, 25 ng template was used and comparisons of the T-RFLP profile obtained from 25 ng or 50 ng of the same template DNA revealed no substantial differences in the resulting community profiles (see Figure 1, sample notations: A12imd3_25 ngt, A12imd3_50 ngt, BW6half1_25 ngt, and BW6half1_50 ngt). Subsequently, for a small number (12 replicates) of bulk soil samples, PCR products from 25 ng and 50 ng template reactions were combined for further analyses. T-RFLP PCR was completed for each sample type using the following conditions with a final volume of 50 μL: 1X II buffer, 1.5 mM $MgCl_2$, 200 μM each dNTPs, 400 ng μL^{-1} BSA, 0.2 μM 27F (*6-FAM*) primer, 0.2 μM 1492R primer, 2.5 U *Taq* polymerase, and template DNA (50 ng or 25 ng).[16] The temperature profile for thermocyling included an initial denaturation step at 94 °C for 2 min, 30 cycles of 94 °C for 1 min (denaturing), 46 °C for 1 min (annealing), and 72 °C for 1 min (extension), followed by a final extension step for 10 min at 72 °C.[19,20]

PCR products were analyzed by agarose gel electrophoresis,[17] 4−8 replicates were combined, and combined products were purified using a QiaQuick PCR purification kit. The concentrations of the combined PCR product were determined using a NanoDrop^{tm} 2000.

After purification and quantification, 500 ng of the combined PCR product were digested with *Hha*I (Promega-Madion, WI) restriction enzyme.[8,22,24,25] The generated restriction fragments (RFs) were analyzed via capillary electrophoresis (CE) utilizing the ROX1000 size standard and a 60 s injection time (Roy J. Carver Biotechnology Center at the University of Illinois). The resulting peaks were initially analyzed via the PeakScanner Software version 1.0 (Applied BioSystems, Carlsbad CA). These peaks were then binned into "operational taxonomical units" (OTUs), based on fragment lengths and relative fluorescence intensity using R (The R Foundation for Statistical Computing) programs Interactive Binner and Automatic Binner.[26] Interactive Binner settings that were used included molecular size limits of 50−1500 bps, a relative peak fluorescence (RFI) cutoff of 0.09%, window size of 3, and a shift size of 0.3.[27,28] In order to provide higher resolution of

the smaller fragments, an additional OTU binning step was completed with both Automatic and Interactive Binner for fragments between 50 - 200 bps, settings include: size cutoff of 50 bps, RFI cut off of 0.01%, window size of 1, and a shift size of 0.1.[28] These binning output data were combined and normalized and all peaks with an RFI < 1% of the total peak fluorescence intensity a given sample were removed. Comparative analysis of the resulting bacterial community profiles was conducted by calculating intersample Bray−Curtis distances using R program, Ecodist, and a hierarchical clustering algorithm with average linkage clustering were used to construct a dendrogram depicting relationships among the samples' T-RFLP profiles in R.

Clone Library Construction and Sequence Analysis. T-RFLP results were used to guide sample selection for clone library construction, by combining PCR products from the same anode type (i.e., either agricultural soil MFCs or forest soil MFCs) in order to capture as many of the dominant community members as possible. The 16S rRNA gene clone libraries were constructed, inserts were sequenced, and sequences were analyzed to infer the phylogenetic identity of dominant community members and identify those members represented by T-RFLP peaks via in silico digestion of resulting sequences.

16S rRNA gene amplicons were generated from the selected anode samples with the same PCR conditions as described above (except the 27F primer was unlabeled). PCR products were purified (QiaQuick PCR purification kit), 3′A-overhangs were added post PCR purification and the 16S rRNA gene PCR products were cloned into the pCR 4-TOPO vector (Invitrogen, Carlsbad CA).

About 400 clones were screened for the correct insert via sequencing with a single primer (Beckman Coulter Genomics Inc., Danvers MA). From those, 290 (158 from the agricultural MFC anode and 132 from the Barre Woods MFC anode) were found to have inserts of the correct size and were sequenced in both directions, at a sequencing depth of between 2× and 4×. After trimming primer sequences, putative chimeras (26 sequences) detected using the programs Bellerophon and Mallard were removed from further analyses.[8,31,32] Sequences were then aligned to the Ribosomal Database Project (RDP),[30] allowing for secondary structure consideration in sequence analysis, and alignments were inspected and edited manually in ARB.[29] Maximum likelihood phylogenetic trees were generated with the RAxML algorithm (version 7.2.8-Alpha) and edited in Dendroscope (version 2.7.4).[33−35] The 16S rRNA gene sequences obtained and analyzed in this study were deposited in GenBank under accession numbers JN540099 to JN540281.

RESULTS AND DISCUSSION

Our results show that soil organic carbon, mineralization rates, and bacterial community structure are key factors in determining the performance of soil-based MFCs, with agricultural soil MFC performance about 17-fold higher than forest soil MFCs (average peak powers of agricultural and forest soil MFCs, respectively) (Table 1). Both T-RFLP profiles and clone library analysis revealed substantial differences between the high performing agricultural and low performing forest soil (Figures 1−4). Bray−Curtis distances and hierarchical clustering analysis of T-RFLP profiles showed that bacterial communities colonizing high-performing agricultural soil anodes were highly similar to each other, yet distinct from communities inhabiting other microhabitats within the MFCs,

Table 1. Summary of Soil Sample Characteristics and Microbial Community Profile Data[a]

sample ID	acetate (μM)[d]	phosphate (μM)[d]	nitrite (μM)[d]	nitrite (μM)[d]	water-soluble polyphenols in initial soil samples (% tannin)[c]	organic C(%)[c]	organic C(%)[c]	C:N ratio[c]	net nitrogen mineralization rate ($(NH_4^+)\mu M$/day)	CO_2 respiration rate ($(CO_2)\mu$mol/day/cm^3)	peak power output (mW/m^2)	final power output (mW/m^2)	anode (DNA): proxy for biomass (ng/cm^2)[d]	number of T-RFLP peaks[d]
Ag Control MFC	123	1	ND	0	0.027	4.3	0.30	16.9	47.4	−0.037				24
Ag MFC Rep 1	69	12	ND	0	0.021	4.5	0.30	17.3	38.3	0.29	40.98	11.82	32.2	17
Ag MFC Rep 2	b	b	b	b	0.033	4.1	0.29	16.4	38.3	0.29	44	10.83	32.7	17
BW Control MFC	232	40	ND	0	0.267	19.6	0.80	28.1	−4.01	−0.0047				28
BW MFC Rep 1	814	63	6.2	0	0.231	18.8	0.80	27.3	−10.6	0.033	0.66	0.23	11.6	28
BW MFC Rep 2	302	97	1.9	0	0.302	20.4	0.82	28.9	−10.6	0.033	4.21	1.08	13.3	31

[a]Interstitial porewater was analyzed to determine concentrations of acetate and inorganic nutrients, while organic matter was analyzed for C:N ratio and polyphenol content. Lastly, biological activity was measured by determining net nitrogen mineralization rates, CO_2 respiration rates, and MFC power output, and the microbial biomass colonizing MFC anodes was determined using total genomic DNA as a biomass proxy. Abbreviations refer to: Ag, agricultural soil; BW, Barre Woods soil; and Rep, replicate number. ND, No peak for ion [b]Unable to complete IC analysis due to low pore water availability. [c]Analyzed at the beginning of study. [d]Analyzed at the end of study.

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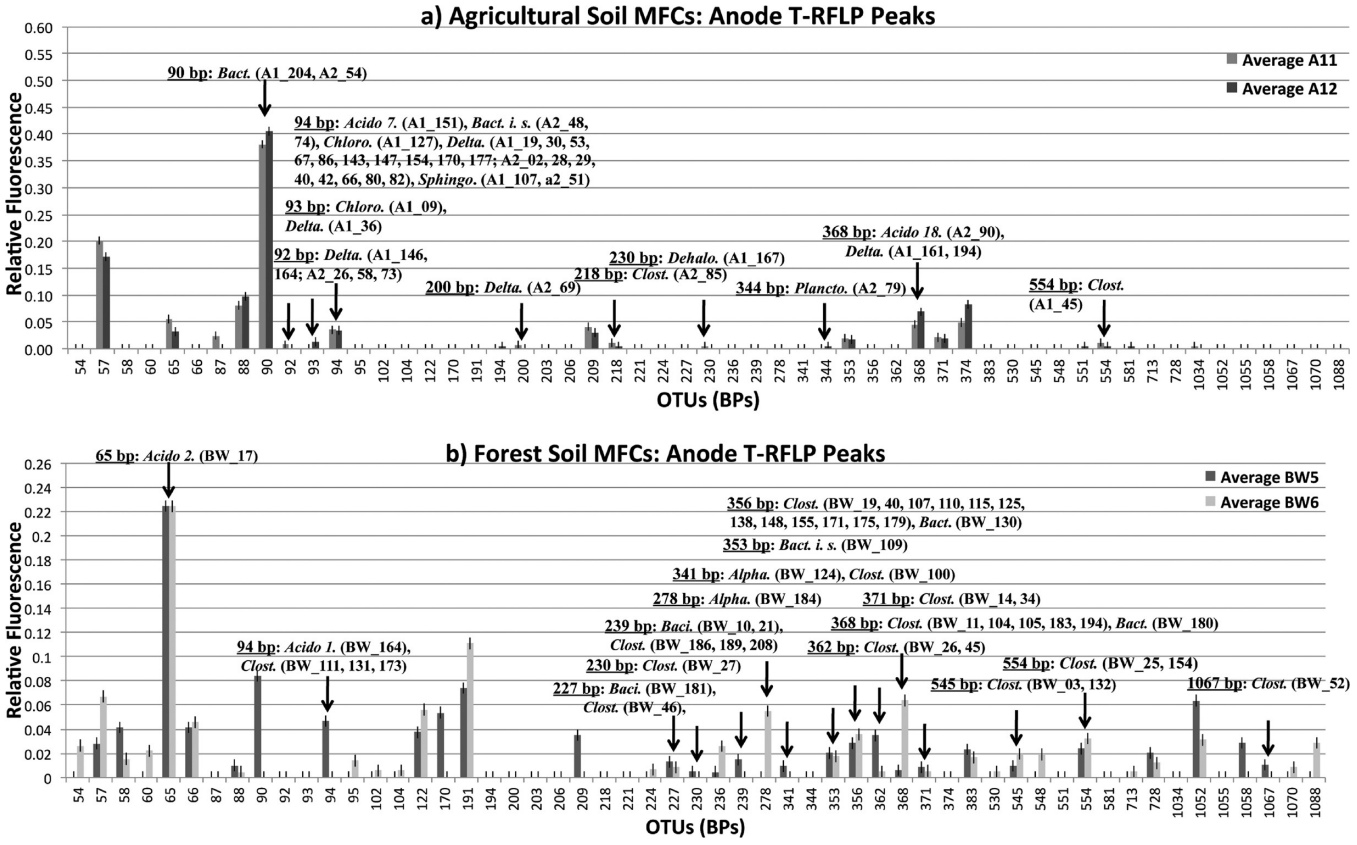

Figure 2. Graphical representation of microbial community structure T-RFLP Profiles. Arrows indicate peaks that have been matched to clone sequences (Classification IDs: *Acidobacteria Gp 1 = Acido 1., Acidobacteria Gp 2 = Acido 2., Acidobacteria Gp 7 = Acido 7., Acidobacteria Gp 18 = Acido 18., Alphaproteobacteria = Alpha., Bacilli = Baci., Bacteroidia = Bact., Bacteroidetes incertae sedis = Bact. i. s., Clostridia = Clost., Chlorobia = Chloro., Dehalococcoidetes = Dehalo., Deltaproteobacteria = Delta., Planctomycetacia = Plancto., Sphingobacteria = Sphingo.* Also noted is the matching clone ID numbers, where *A* = agricultural anode library (1 and 2), and BW = forest soil anode library. Additional abbreviations: agricultural soil MFC replicates (11 = MFC ID, 12 = MFC ID), BW = forest soil MFC replicates- soil from Barre Woods plot in Harvard Forest (05 = MFC ID, 06 = MFC ID. Each replicate MFC was analyzed in triplicate and average peak intensities were plotted against fragment length (OTU).

even the soil immediately adjacent to the anode material (Figure 1). Clone library analysis indicated that agricultural soil anode communities were dominated by *Geobacter* spp., with one closely related cluster of previously uncultivated *Geobacter* strains particularly prevalent (Figure 3). Together, these results suggest that strong selection of an active, rapidly growing, *Geobacter*-dominated community was found within the agricultural soil MFC anodes, but not within the forest soil anodes.

MFC Performance and Soil Characteristics Measurements. The average peak power output levels for the agricultural MFCs (42.49 mW m^{-2} (anode GA)) were 17 times higher than the forest soil-based MFCs (2.44 mW m^{-2} (anode GA)) (Table 1). The peak power output of the high performing agricultural MFCs were fairly similar to or higher than other MFC studies that were constructed using organic-rich soils and sediments, for example, 26 mW m^{-2} in rice paddy MFCs[36] and 30 mW m^{-2} in other sediment MFCs, as cited by Logan and Regan.[2] These results also compare well to substrate-fed anaerobic sludge MFC systems that were found to have power outputs of 48.4 mW m^{-2} (acetate-fed) and 40.3 mW m^{-2} (glucose-fed).[37]

Bulk soil characteristics differed substantially between agricultural and forest soils (Table 1). Both water-soluble polyphenol content and C:N ratios were much higher in forest soils than in agricultural soils, suggesting that forest soil organic matter was more recalcitrant and had lower nitrogen availability than its agricultural soil counterpart (Table 1). The higher rates of both nitrogen mineralization and respiration observed in agricultural than forest soil samples also support this finding. Lastly, a higher level of bacterial activity in agricultural vs forest soil MFCs was also clear from MFC performance (~17-fold higher in agricultural soil MFCs) and the genomic DNA content in anodes (almost 3-fold higher in agricultural soil MFC anodes), which was used as a proxy for biomass.[15]

Acetate levels were about eight times higher in forest than agricultural soil MFCs (Table 1). This result was somewhat surprising, as acetate is thought to be an effective substrate for electrogenic bacteria, such as *Geobacter* spp. within MFCs.[3,6,38] In contrast, in the current study, high levels of acetate did not correlate with high MFC performance in the forest soil MFCs. The dominance of *Clostridia* spp. and other acetogens in the forest soil MFC anodes may be indicative of acetogenesis as a terminal electron accepting process in these systems, despite the fact that both an electron donor (e.g., acetate) and an electron sink (the anode) were present. Taken together, these results indicate that acetate availability did not limit MFC performance in forest soil MFCs (Table 1), but rather that electrogenic microorganisms were either limited by some other nutrient, substrate, or condition, or were restricted by an inhibitor. For example, the higher water-soluble polyphenol content and C:N ratios found in forest soils are indicative of

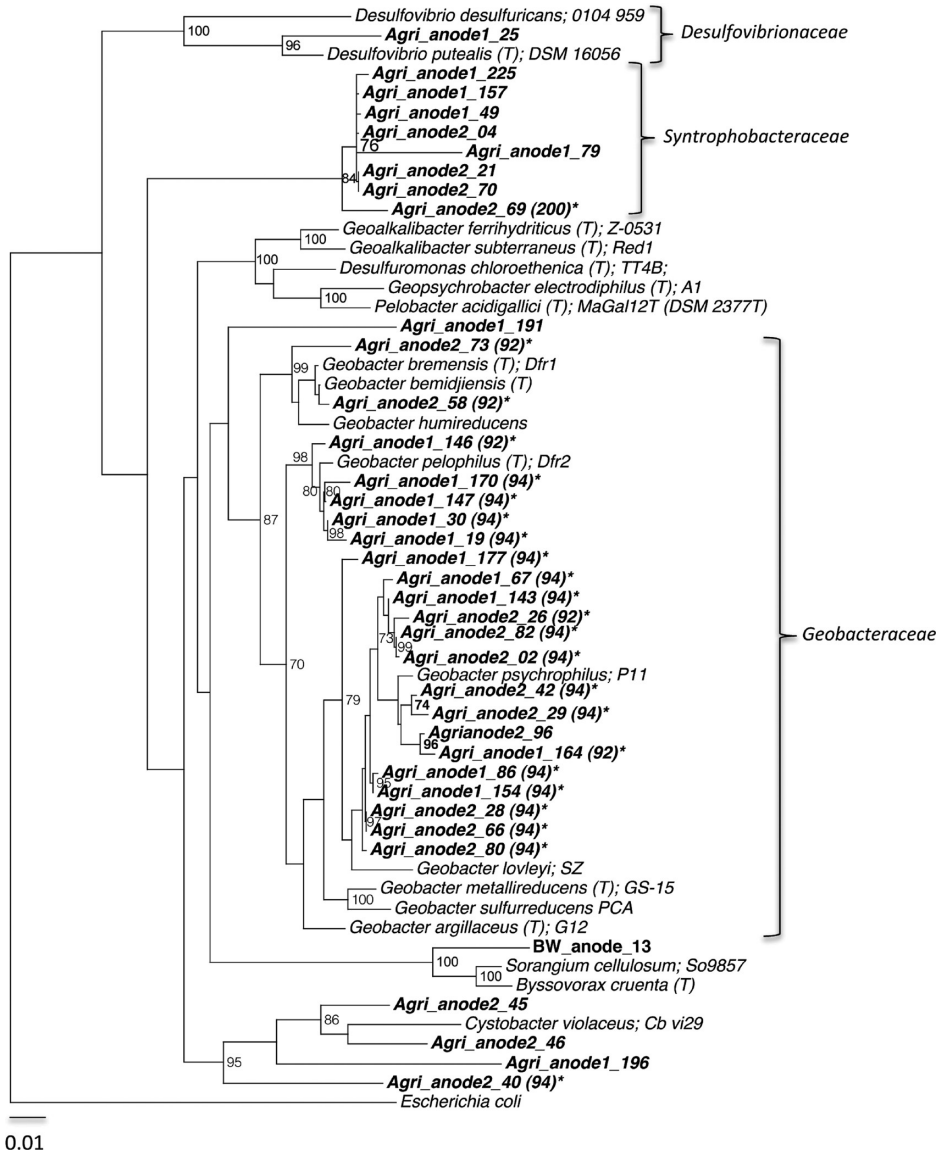

Figure 3. Maximum likelihood phylogenetic tree of all MFC anode clones within the class Deltaproteobacteria. Bootstrap values greater than 70% (for 100 iterations) are shown at the nodes of the tree. Agricultural soil anode clones are shown in bold italics and forest soil anode clones are shown in bold. Asterisks denote clones whose in silico peaks matched with T-RFLP peaks (numbers in parentheses indicate length of the RF). The scale bar reflects sequence distance values.

humics and other polyphenol compounds that may have sequestered macronutrients,[39] thereby reducing their microbial availability. Alternatively, polyphenol compounds may act as inhibitors of microbial activity.[40] Previous studies have also found this phenomenon of acetate accumulation occurring in saturated forest soils;[41] however, the mechanisms that control this shift between acetate accumulation and consumption is poorly understood and requires further investigation.[14]

T-RFLP Community Profiles. Both the total number of T-RFLP peaks (or "OTUs") and the evenness of their distribution were lower in the anode communities of high-performing agricultural soil MFCs than in forest soil MFCs, although communities from the bulk soil samples from both MFC types had similar numbers of OTUs (Table 1 and Figure 2a and b). In addition, community genomic DNA, a proxy for biomass,[15] was found to be over 2-fold higher in anodes of agricultural vs forest soil MFCs (Table 1). Together, these results indicate strong colonization and specific selection of a relatively simple electrogenic bacterial community in agricultural soil anodes (13−14 OTUs), even though the bulk agricultural soil harbored communities with higher diversity (i.e., 24 OTUs), while forest soil anodes supported lower bacterial growth and higher community complexity (22−23 OTUs) (Table 1 and Figure 2a and b). Strong selection of a distinct, presumably electrogenic, bacterial community in agricultural soil anodes was also evident from comparison of T-RFLP profiles from agricultural anodes and the soil immediately adjacent to the anodes, which were clearly distinct from each other (Figure 1). In fact, communities from the soil immediately adjacent to anodes clustered together with communities from the bulk soil (taken several centimeters away from the anode) and from the control agricultural MFCs (with no electrical circuit), but not with anode communities (Figure 1). Thus, it appears that direct physical contact with the anode material is necessary for selection and support of electrogenic communities in these systems. Interestingly, these distinct communities over microspatial scales (e.g., on the

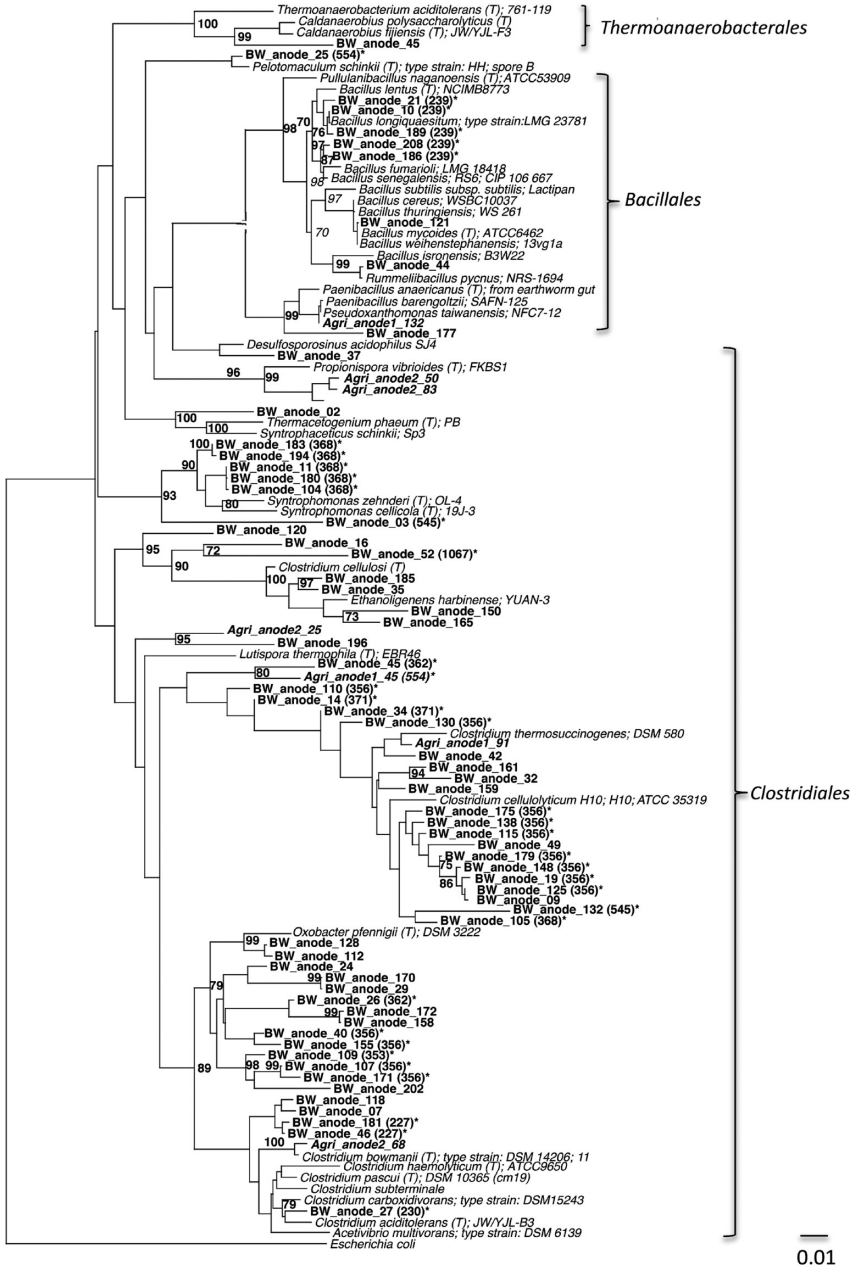

Figure 4. Maximum likelihood phylogenetic tree of all MFC anode clones within the phylum firmicutes. Bootstrap values greater than 70% (for 100 iterations) are shown at the nodes of the tree. Agricultural soil anode clones are shown in bold italics and forest soil anode clones are shown in bold. Asterisks denote clones whose in silico peaks matched with T-RFLP peaks (numbers in parentheses indicate length of the RF). The scale bar reflects sequence distance values.

anode vs immediately adjacent to it) were not clearly evident in the forest soil MFCs (Figure 1), where Bray−Curtis distances between different anode communities were much higher (0.5−0.7) than for agricultural soil MFCs and the anode communities did not clearly cluster away from all other community types (Figure 1).

16S rRNA Clone Library and Phylogenetic Analysis. The major constituents of the agricultural anode clone library were as follows: 41.4% *Deltaproteobacteria* (53.4% of which were *Geobacter* species) (phylogenetic tree, Figure 3), 11.4% *Clostridia*, 5.7% *Bacteroidetes incertae sedis*, 4.3% *Bacteroidia*, 4.3% *Alphaproteobacteria*, 3.6% *Actinobacteria*, 2.9% *Chlorobia*, 2.9% *Planctomycetacia*, 2.9% *Anaeorlinea*, 2.9% *Spirochaetes*. Other studies have also found these phylogenetic groups to be dominant members of MFCs constructed from complex, organic-rich, natural materials.[8,37,42] Mixed microbial MFC systems are thought to have higher performance than pure-culture MFCs, probably because they benefit from microbial consortium interactions and in situ electrogenic substrate production.[37] Padmanabhan et al.[43] showed that *Bacteroidetes* tend to be the initial soil bacterial community members that metabolize labile organic matter; therefore, the presence of this group could have improved the availability of certain metabolic and electrogenic substrates, which, in turn, may have increased bacterial activity within the high-performing agricultural soil MFCs. *Clostridium* spp. may have played several roles in the agricultural soil MFCs, including generating fermentation products that can be consumed by electrogens[3] and direct

involvement in electrogenic activity.[44] Among the relatively few agricultural anode clones that fell within the *Firmicutes* phylum, most of those were not phylogenetically distant from forest soil MFC anode *Firmicutes* sequences (Figure 4). Interestingly, two exceptions were sequences *Agri_anode2_50* and *Agri_anode2_83* that did not cluster with forest anode sequences, but did cluster with a known Fe (III)-reducer *Firmicutes* species clone isolated from rice paddy soil.[45]

The major constituents of the forest soil MFC anode clone library were *Clostridia* (73.6%) (phylogenetic tree, Figure 4), *Bacilli* (8.3%), *Acidobacteria GP1* (3.3%), *Actinobacteria* (2.5%), and *Alphaproteobacteria* (2.5%). The absence of *Geobacter* members in open circuit/noncurrent producing MFCs was also noted in a rice paddy plant MFC.[8] In addition, *Clostridia* found in the forest soil MFC anodes did not cluster with other Fe (III)-reducers or sequences from other MFCs (including agricultural MFC anodes analyzed here) (Figure 4).

In Silico Digests and T-RFLP Peak Comparisons. In silico digests of 42 of the dereplicated agricultural anode 16S rRNA gene sequences and 45 of the dereplicated forest anode clone sequences were matched to T-RFLP peaks, with *Bacteroidetes*, *Geobacter*, and *Clostridium* members representing major T-RFLP peaks in agricultural anode communities, whereas *Clostridia* dominated forest anode T-RFLP profiles (Figure 2a and b, 3, and 4). These results provide further evidence that *Clostridia* members dominanted the forest soil, but not the agricultural soil, MFC anodes.

T-RFLP analysis indicated that cathode communities from high-performing MFCs were not clearly distinct from other oxic microhabitats in the MFC systems analyzed. For example, agricultural MFC cathode communities were found to cluster together and with their respective controls, for which samples were taken from the oxic layer of soil, but where no cathode was present (Figure 1). The agricultural cathodes also clustered loosely with one of the BW cathodes, but not with both (Figure 1). The most likely explanation is that the cathode community represents an oxic microhabitat community that did not select strongly for a community that is specific to functioning MFCs.

In conclusion, we found that MFCs constructed from the agricultural soil selected for and sustained an active, highly electrogenic bacterial anode community (dominated by *Geobacter* spp.), whereas MFCs from nearby forest soils did not. These results underscore the importance of soil type in MFC bacterial communities and performance and point to a need for further research to determine how different soil types and characteristics influence potential MFC performance. Interestingly, acetate, a widely used substrate for electrogenic bacterial activity, accumulated in the low-performing forest soil MFCs but not in the high-performing MFCs. This phenomenon, in which acetate accumulates rather than fueling terminal electron accepting processes, has been observed in other biogeochemical studies involving forest and other saturated soils, but has yet to be explained or fully understood.

■ ASSOCIATED CONTENT

Supporting Information

Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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