water

MDPI

Supplementary Materials:Article

Activated Carbon Mixed with Marine Sediment is Suitable as Bioanode Material for *Spartina anglica* Sediment/Plant Microbial Fuel Cell: Plant Growth, Electricity Generation and Spatial Microbial Community Diversity

Emilius Sudirjo, Cees J.N Buisman and David P.B.T.B Strik

Figure

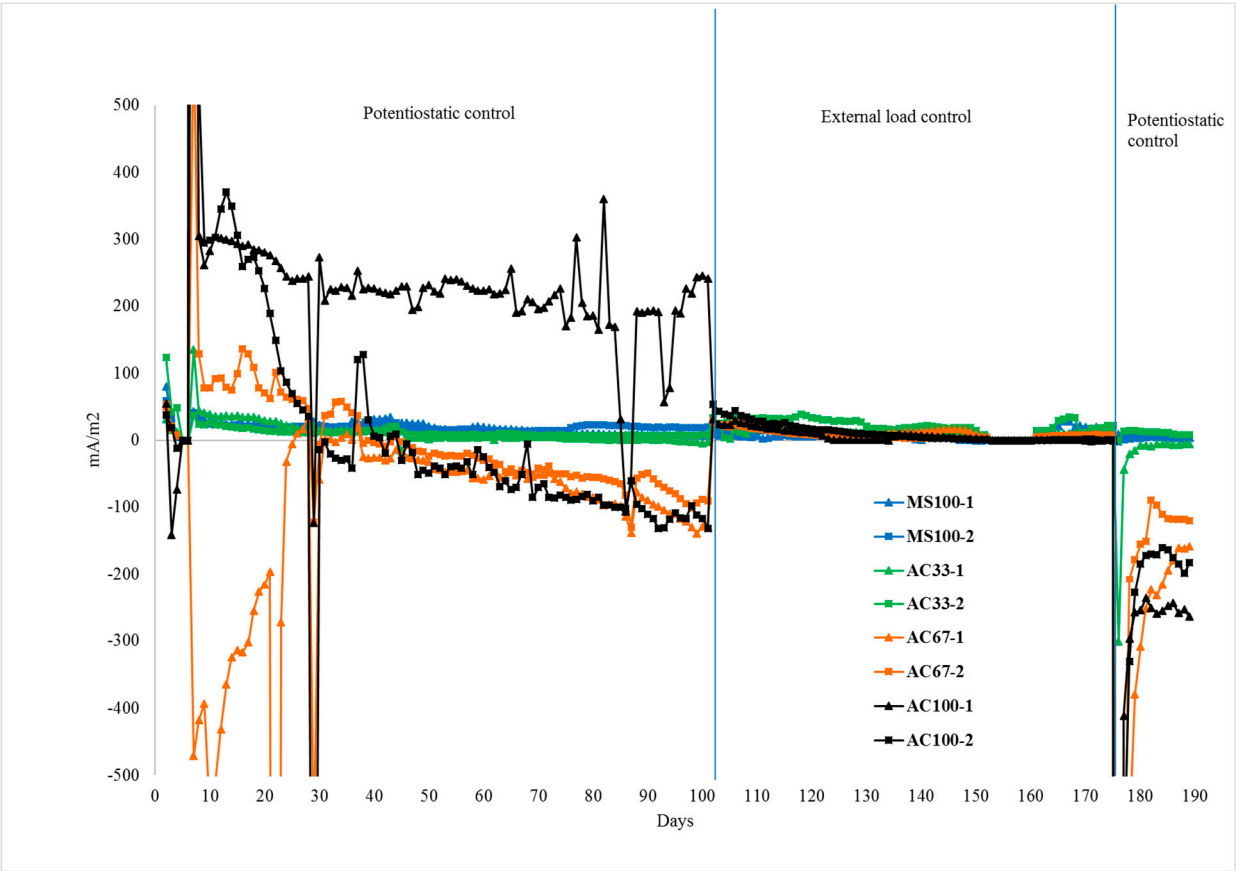
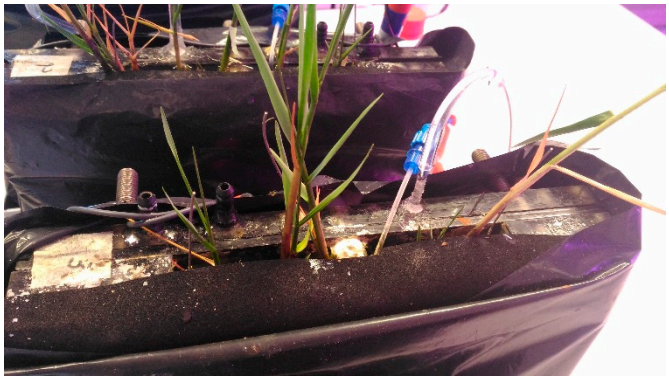
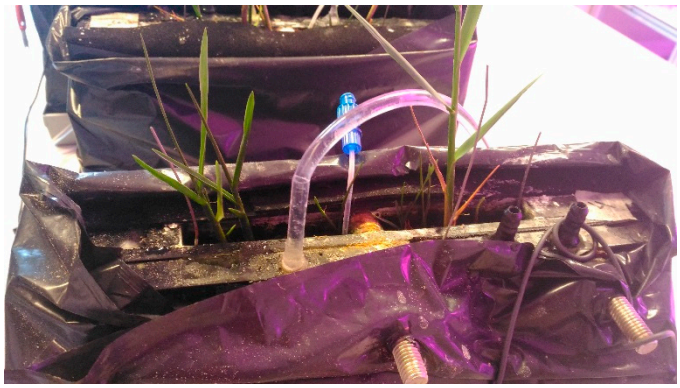


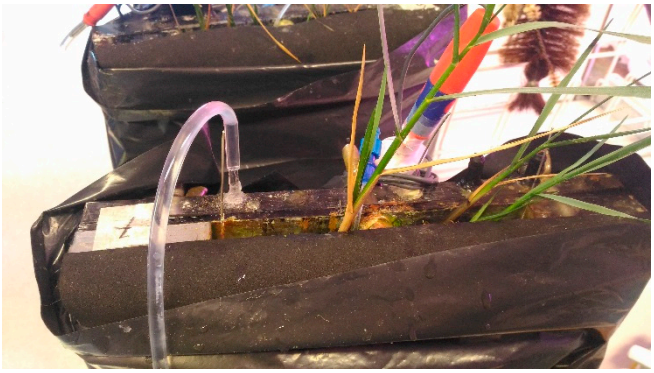
Figure S1. Plant-MFC performance on two different control modes.



MS100 (Plant-MFC 3)

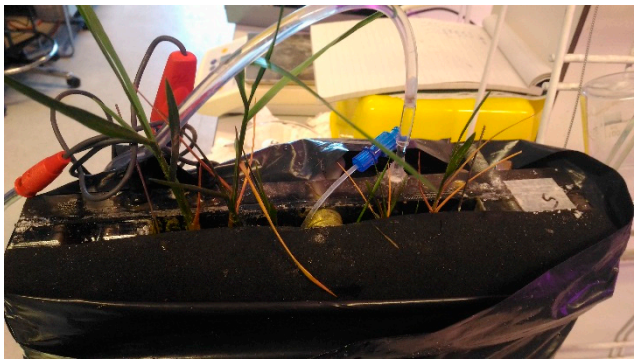


MS100 (Plant-MFC 4)

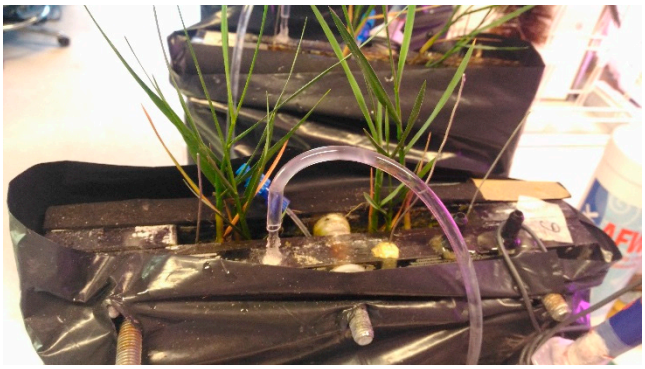


AC33 (Plant-MFC 7)

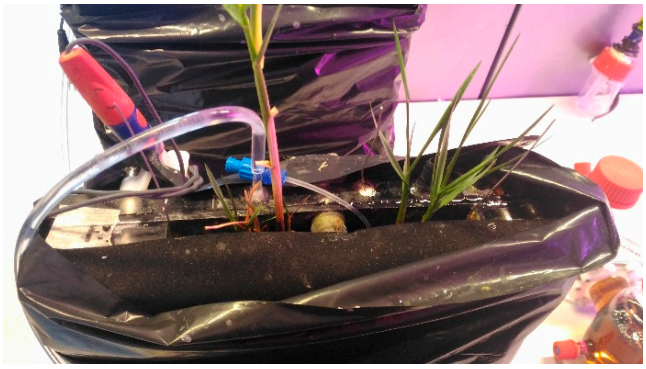
AC33 (Plant-MFC 8)



AC67 (Plant-MFC 5)



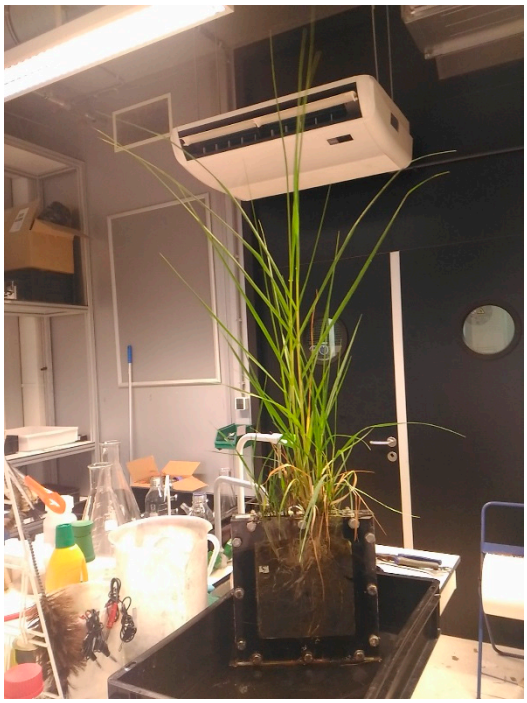
AC67 (Plant-MFC 6)



AC100 (Plant-MFC1)

AC100 (Plant-MFC2)

Figure S2. Plant condition at the beginning of the experiment (during transplantation).



MS100 (Plant-MFC 3)

MS100 (Plant-MFC 4)

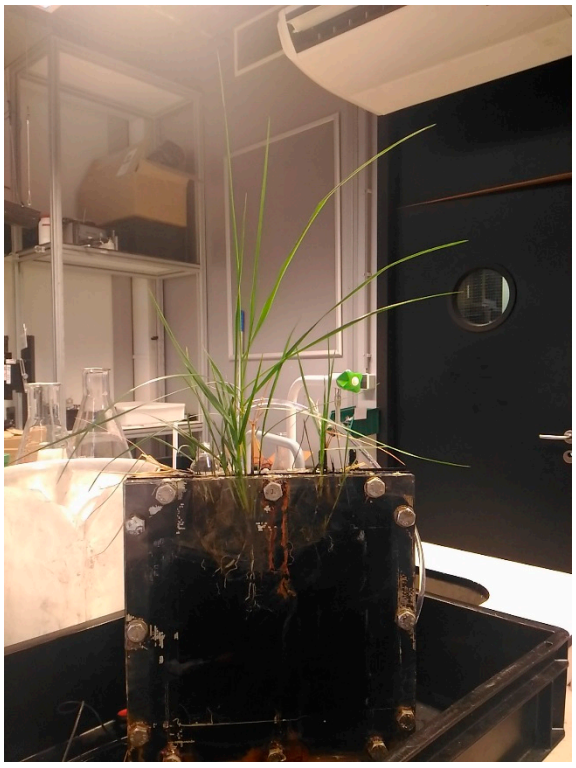
AC33 (Plant-MFC 7)

AC33 (Plant-MFC 8)

AC67 (Plant-MFC 5)

AC67 (Plant-MFC 6)

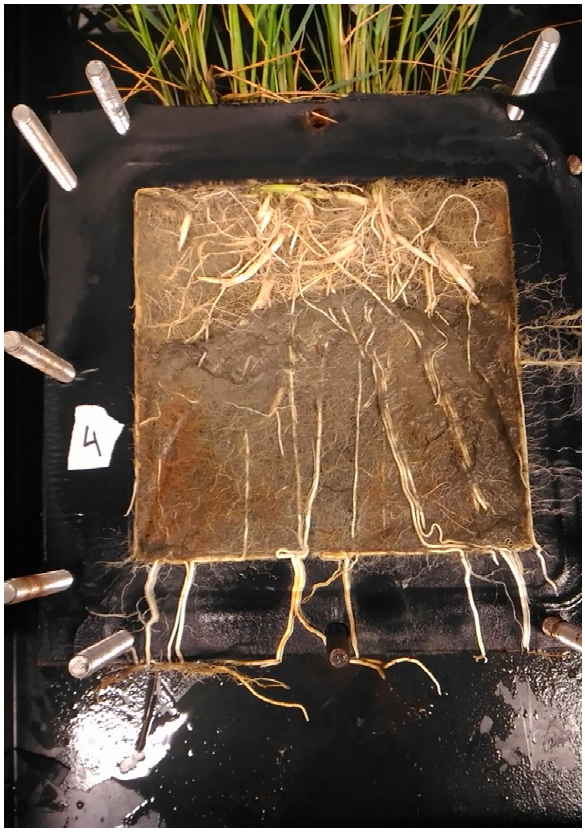
AC100 (Plant-MFC1)



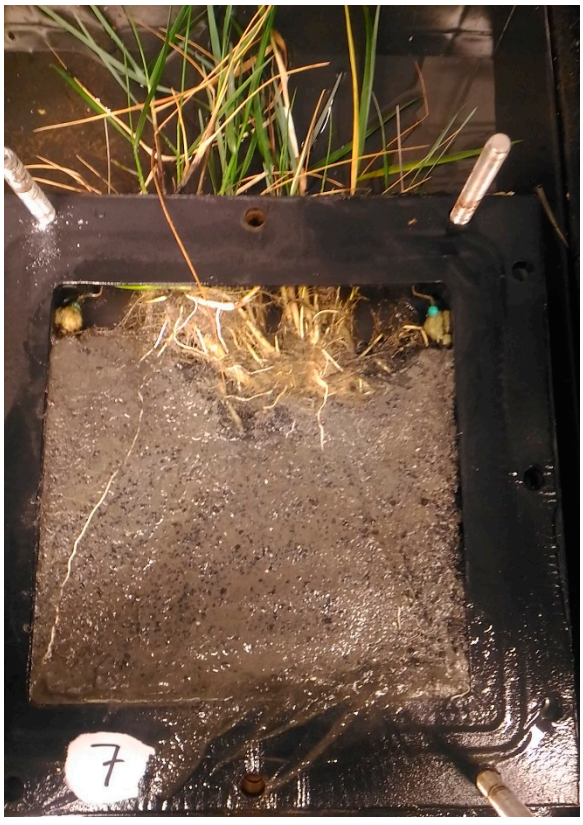
AC100 (Plant-MFC2)

Figure S3. Plant condition at the end of the experiment. Showing roots penetration; stem and leaf condition.

MS100 (Plant-MFC 3)

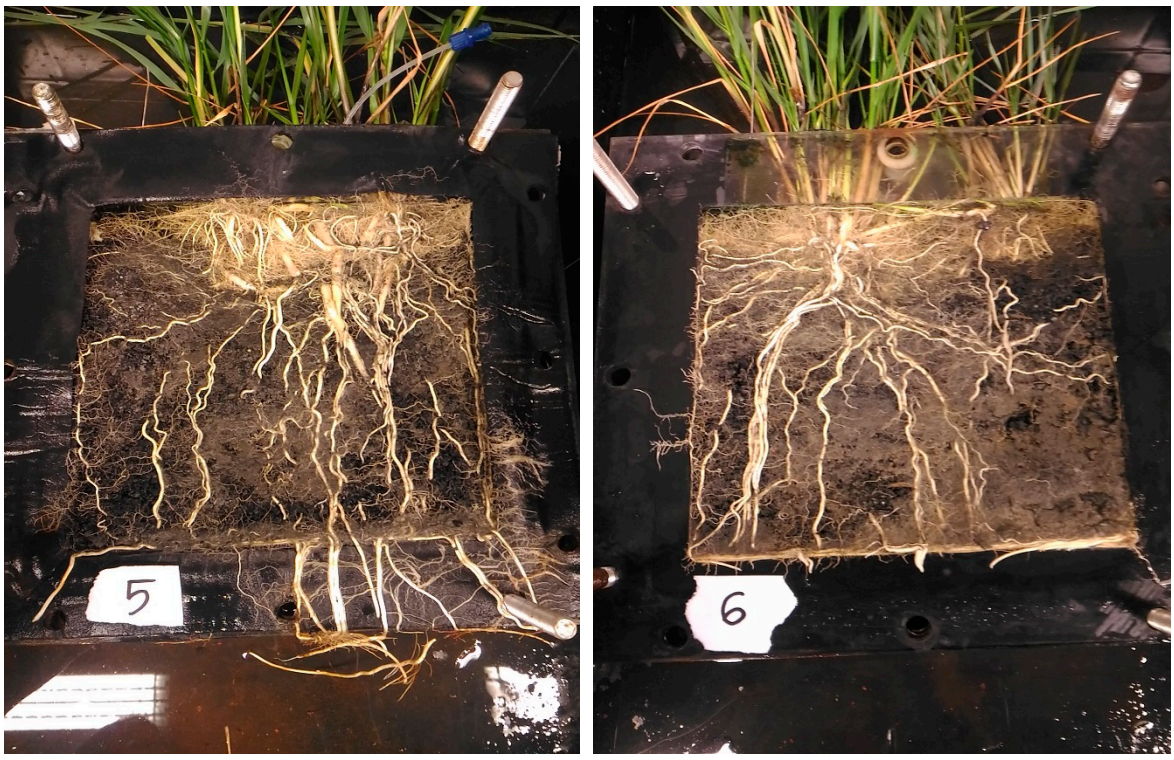


MS100 (Plant-MFC 4)



AC33 (Plant-MFC 7)

AC33 (Plant-MFC 8)



AC67 (Plant-MFC 5) AC67 (Plant-MFC 6)

AC100 (Plant-MFC1) AC100 (Plant-MFC2)

Figure S4. Root condition at the end of the experiment.

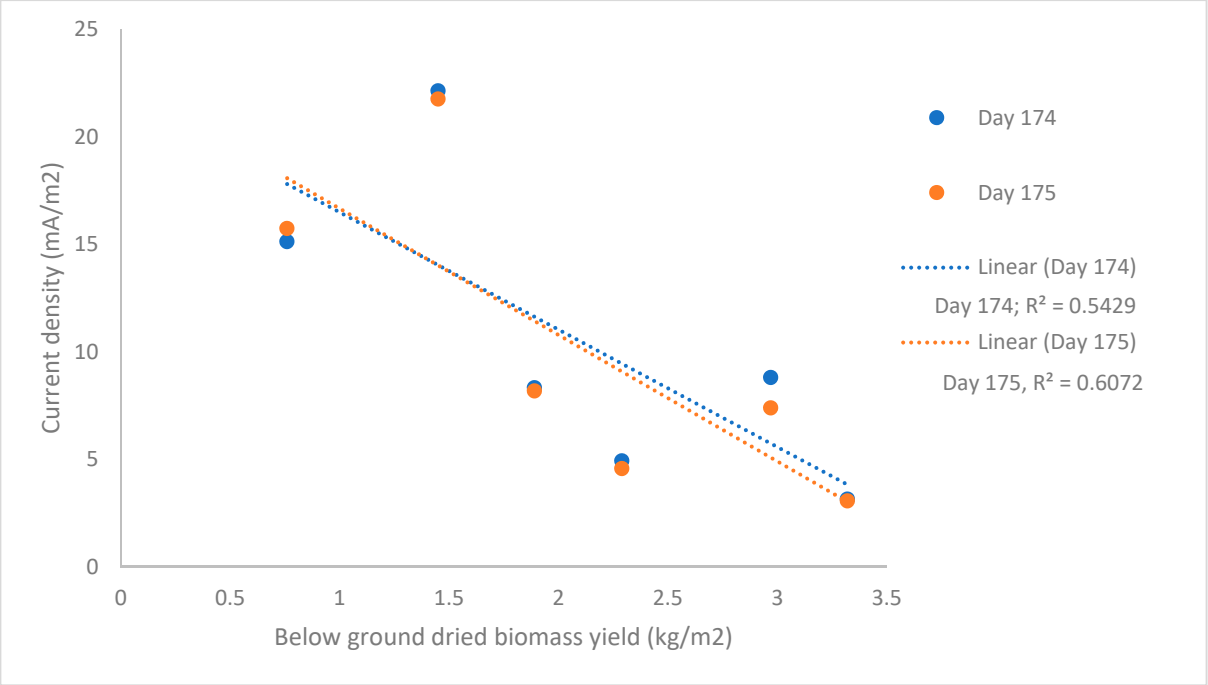
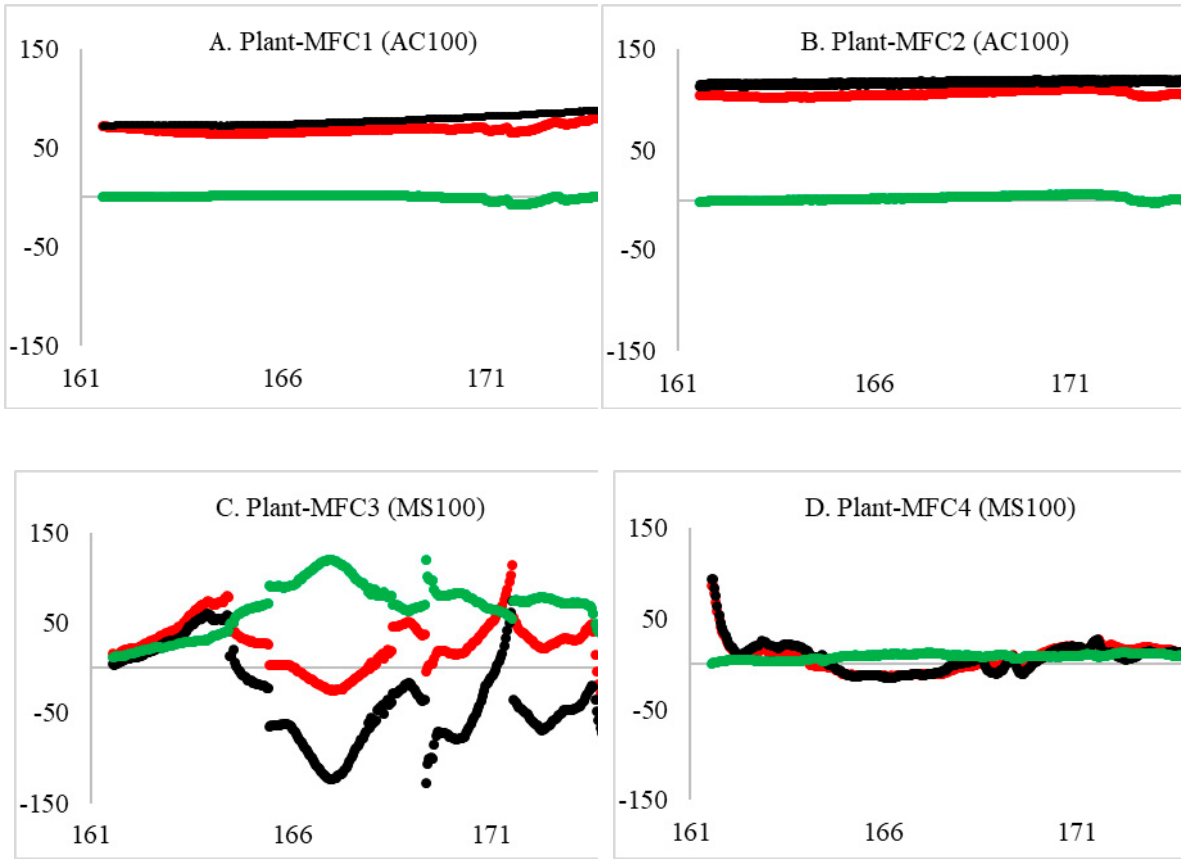


Figure S5. Correlation between root density and current density.

Below ground dried biomass was calculated from harvested roots in the end of the experiment (day 190)



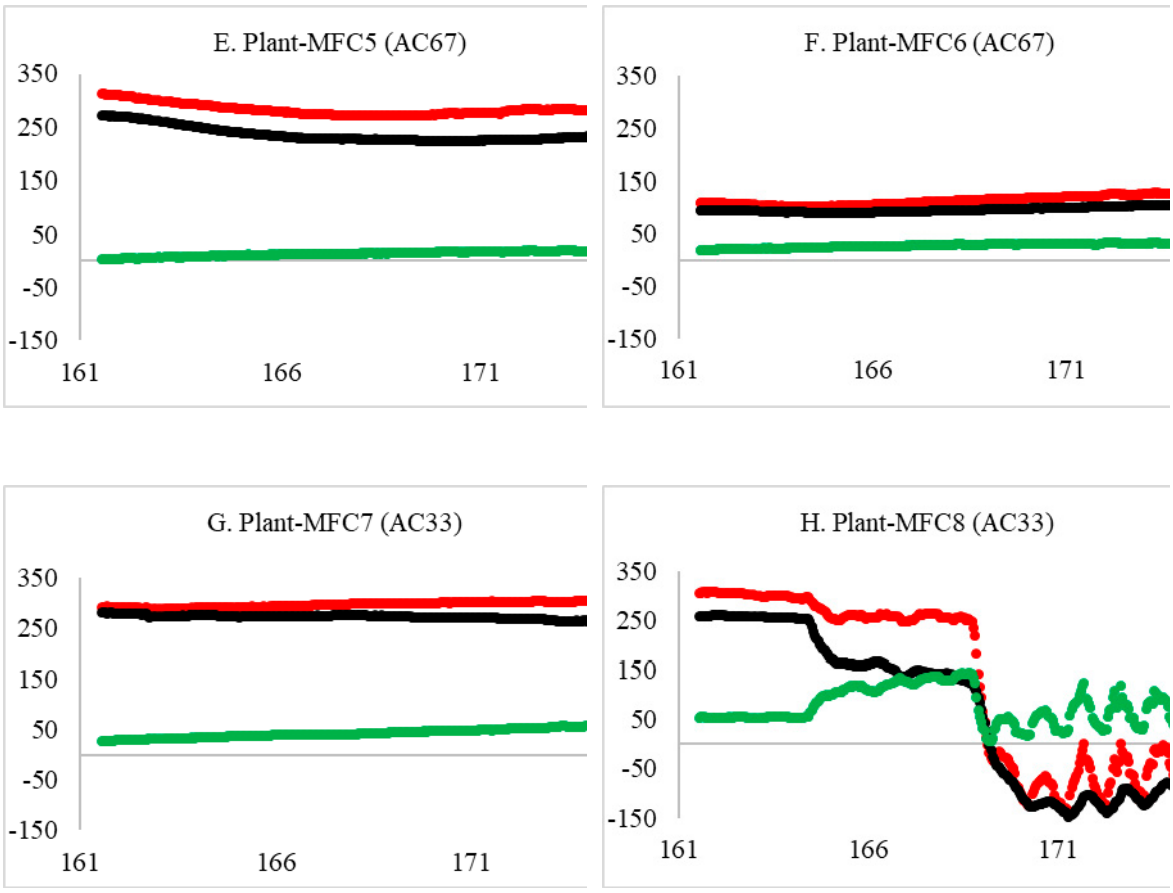
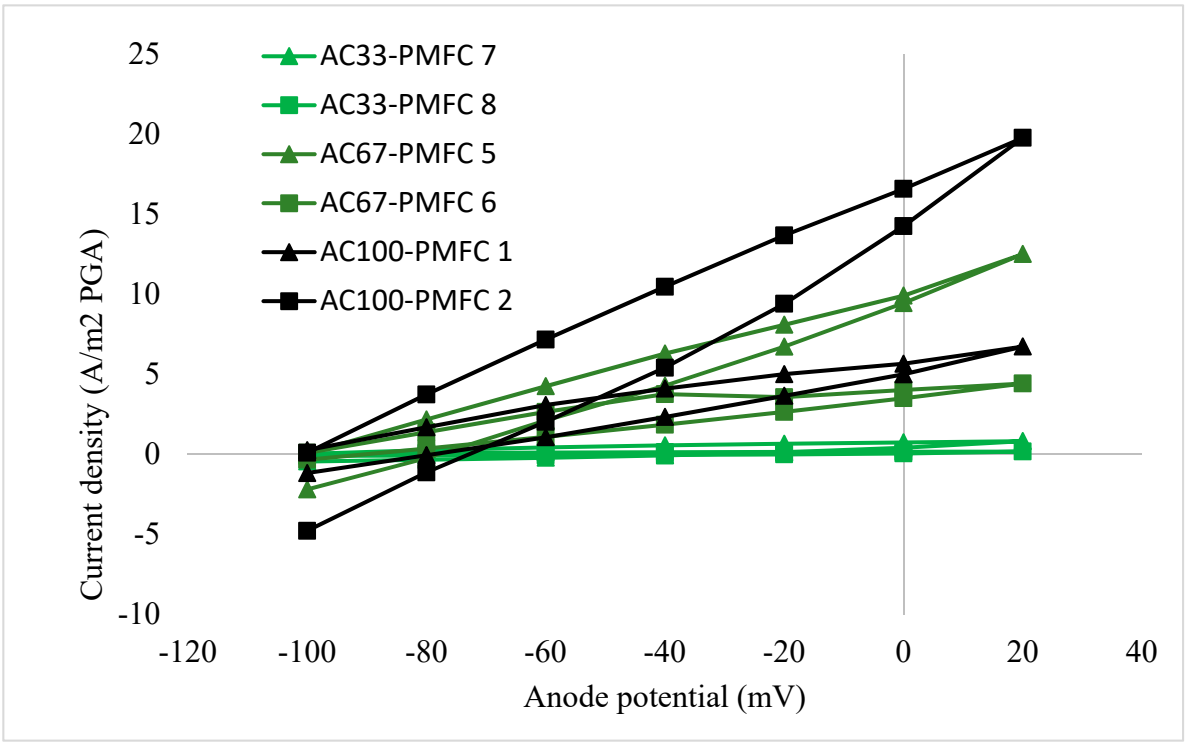
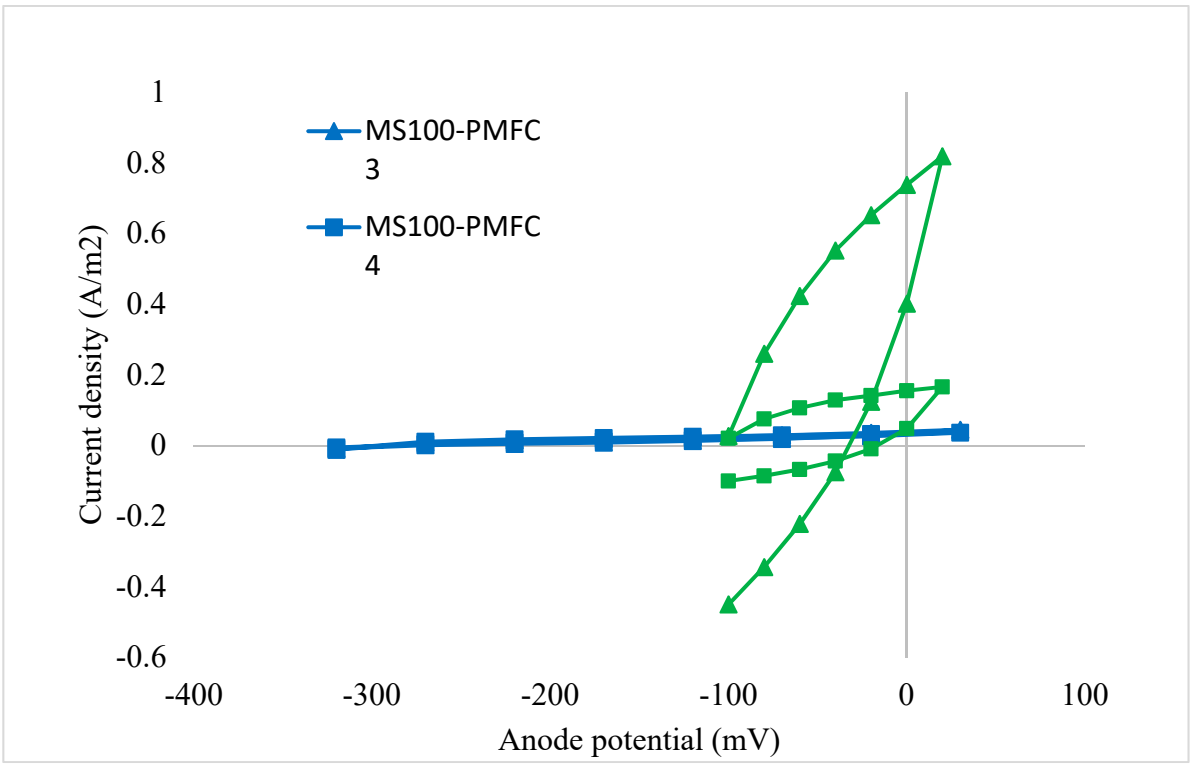


Figure S6. Anode potential (black), Cathode Potential (red) and Cell potential(Green) of plant-MFCs between day 161 and 175. The vertical axes are the potential (mV) and the horizontal axes are the day.



(**a**)



(**b**)

Figure S7. Polarization curve on day 28. (**a**)All Plant-MFC, excluding MS100 Plant-MFC. (**b**)Zoom in for MS100 and AC 33 Plant-MFCs.

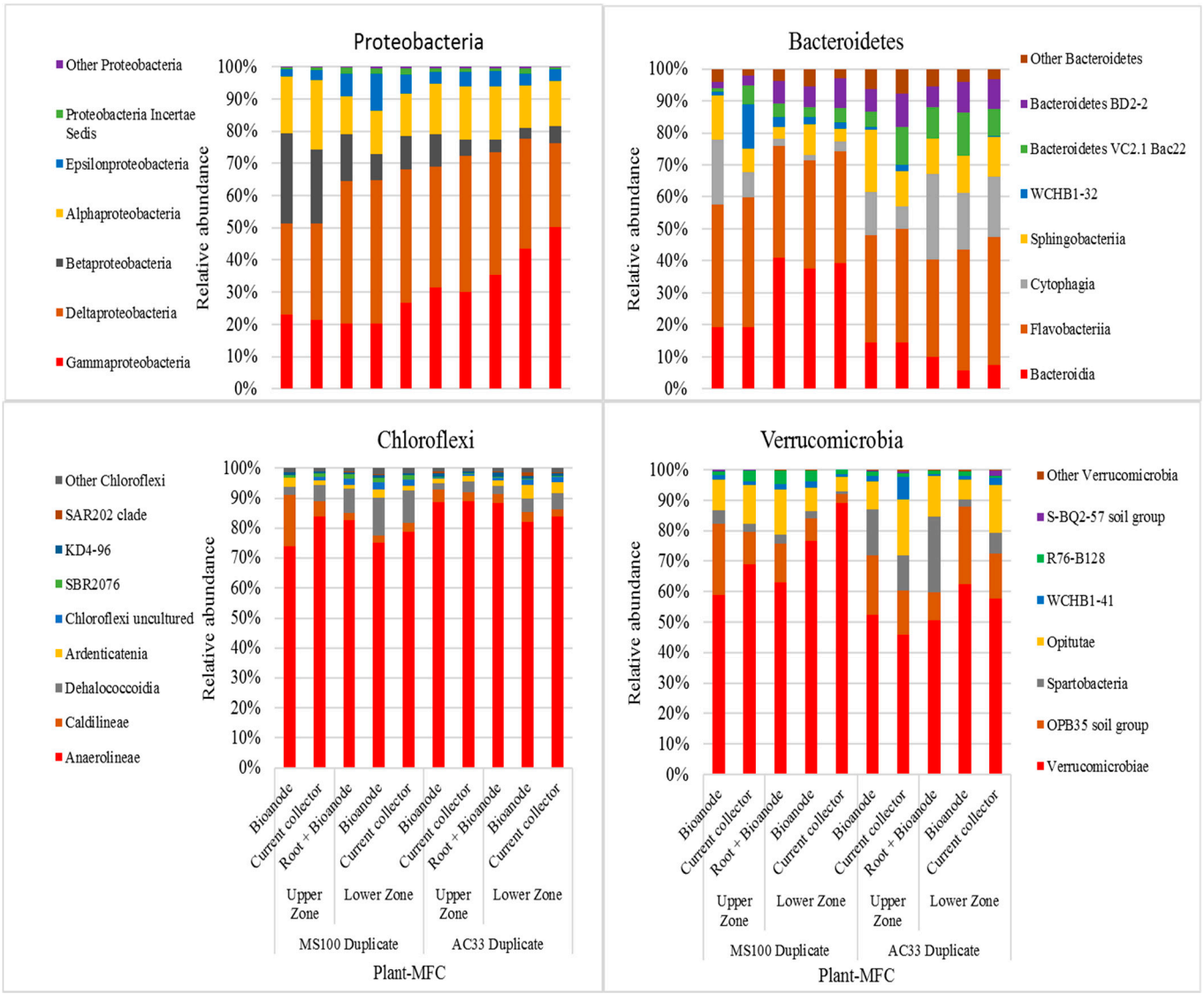


Figure S8. Relative abundance of classes within a phylum from 4 most abundance phyla.

Table

Table S1. Initial planted plants compositions.

Systems	Wet mass of the stems (gr)	Number of planted stems
Plant-MFC 1 (AC100)	37.8	7
Plant-MFC 2 (AC100)	35.7	8
Plant-MFC 3 (MS100)	35.1	6
Plant-MFC 4 (MS100)	33.4	7
Plant-MFC 5 (AC67)	31.7	7
Plant-MFC 6 (AC67)	37.2	6
Plant-MFC 7 (AC33)	36.8	7
Plant-MFC 8 (AC33)	32.4	12

Table S2. Most abundant bacteria at order level with at least 5% relative abundance. Heat map abundance colors: red > 5%; orange 2 to 5%; white <2%.

CLASS **ORDER**	**Average relative abundant (± standard error)**									
	MS100 Duplicate					**AC33 Duplicate**				
	Upper Zone		**Lower Zone**			**Upper Zone**		**Lower Zone**		
	Bioanode (Sediment)	**Current collector**	**Root+ Bioanode**	**Bioanode**	**Current collector**	**Bioanode (Sediment)**	**Current collector**	**Root+ Bioanode**	**Bioanode**	**Current collector**
Deltaproteobacteria **Desulfobacterales**	6.95 ±2.66	8.85 ±0.75	10.76 ±2.29	16.26 ±0.63	15.1 ±1.1	13.15 ±0.58	14.4 ±1.16	15.06 ±1.47	16.85 ±8.25	10.86 ±6.29
Gammaproteobacteria **Chromatiales**	1.8 ±0.46	2.41 ±0.08	4.59 ±1.25	6.04 ±1.17	7.77 ±2.54	6.5 ±0.15	6.98 ±1.78	7.29 ±0.89	5.26 ±0.12	8.46 ±2.61
Flavobacteriia **Flavobacteriales**	8.15 ±4.79	7.59 ±3.43	6.95 ±0.34	2.52 ±0.47	2.95 ±0.29	2.42 ±0.53	1.78 ±0.12	1.87 ±0.2	1.35 ±0.22	2.56 ±1.02
Betaproteobacteria **Hydrogenophilales**	9.47 ±0.15	4.99 ±0.19	3.87 ±0.17	1.12 ±0.96	1.52 ±1.38	1.87 ±0.72	1.15 ±0.52	0.66 ±0.42	0.26 ±0.04	0.66 ±0.6
Verrucomicrobiae **Verrucomicrobiales**	3.35 ±0.38	3.98 ±3.1	1.27 ±0.77	3 ±0.56	7.42 ±0.83	1.72 ±0.7	0.31 ±0.02	0.99 ±0.66	0.77 ±0.63	0.82 ±0.24
Epsilonproteobacteria **Campylobacterales**	1.03 ±0.19	1.47 ±0.18	2.9 ±0.67	6.13 ±1.64	3.06 ±1.32	2.24 ±0.19	2.98 ±0.04	2.96 ±0.72	2.25 ±0.75	2.22 ±0.65
Deltaproteobacteria **Desulfuromonadales**	2.85 ±1.09	1.44 ±0.24	1.6 ±0.38	2.58 ±1.32	1.49 ±0.41	7.52 ±5.46	10.29 ±4.38	5.91 ±0.75	0.4 ±0	0.58 ±0.1
Gammaproteobacteria **Gammaproteobacteria Incertae Sedis**	0.99 ±0.15	1.27 ±0.34	1.14 ±0.4	1.64 ±1	1.39 ±0.06	3.07 ±1.17	5.24 ±1.5	6.45 ±0.31	6.76 ±1.59	6.51 ±1.47
Anaerolineae **Anaerolineales**	2.76 ±0.08	4.18 ±0.22	3.76 ±0.56	2.07 ±0.97	3.72 ±0.21	4.34 ±0.72	5.56 ±0.29	4.95 ±0.3	3.51 ±1.59	3.29 ±0.04

Table S3. Most abundant bacteria at family level with at least 5% relative abundance. Heat map abundance colors: red > 5%; orange 2 to 5%; white <2%.

Order **Family**	**Average relative abundant (± standard error)**									
	MS100 Duplicate					**AC33 Duplicate**				
	Upper Zone		**Lower Zone**			**Upper Zone**		**Lower Zone**		
	Bioanode (Sediment)	**Current collector**	**Root + Bioanode**	**Bioanode**	**Current collector**	**Bioanode (Sediment)**	**Current collector**	**Root + Bioanode**	**Bioanode**	**Current collector**
Desulfobacterales; **Desulfobacteraceae**	4.56 ±1.67	6.22 ±0.16	13.41 ±2.24	13.79 ±0.94	12.24 ±0.26	7.64 ±0.78	10.11 ±1.01	9.11 ±0.14	8.25 ±1.39	6.31 ±2.65
Chromatiales; **Ectothiorhodospiraceae**	1.37 ±0.23	1.7 ±0.1	4.84 ±1.48	5.05 ±1.08	6.39 ±2.15	6.11 ±0.35	6.2 ±1.65	6.67 ±0.85	4.25 ±0.09	7.79 ±3.06
Anaerolineales; **Anaerolineaceae**	2.76 ±0.08	4.18 ±0.22	3.69 ±0.56	2.07 ±0.97	3.72 ±0.21	4.34 ±0.72	5.56 ±0.29	4.95 ±0.3	3.51 ±1.59	3.29 ±0.04
Hydrogenophilales; **Hydrogenophilaceae**	9.47 ±0.15	4.99 ±0.19	2.38 ±0.17	1.12 ±0.96	1.52 ±1.38	1.87 ±0.72	1.15 ±0.52	0.66 ±0.42	0.26 ±0.04	0.66 ±0.6
Flavobacteriales; **Flavobacteriaceae**	7.66 ±4.64	7.3 ±3.36	2.9 ±0.34	2.41 ±0.5	2.69 ±0.26	1.55 ±0.4	1.25 ±0.03	1.02 ±0.28	1.27 ±0.23	1.59 ±0.29
Campylobacterales; **Helicobacteraceae**	1 ±0.19	1.43 ±0.2	3.33 ±0.6	5.86 ±1.68	2.93 ±1.2	2.16 ±0.15	2.87 ±0.04	2.95 ±0.72	2.24 ±0.75	2.19 ±0.68
Verrucomicrobiales; **Verrucomicrobiaceae**	3.27 ±0.39	3.96 ±3.1	2.37 ±0.74	2.93 ±0.57	7.34 ±0.82	1.69 ±0.7	0.27 ±0.01	0.94 ±0.65	0.72 ±0.65	0.77 ±0.24
Desulfobacterales; **Desulfobulbaceae**	2.39 ±0.99	2.63 ±0.58	2.31 ±0.06	2.46 ±0.31	2.83 ±0.83	5.5 ±0.2	4.28 ±0.14	5.94 ±1.33	8.59 ±6.86	4.54 ±3.64
Gammaproteobacteria Incertae Sedis; **Unknown Family**	0.99 ±0.15	1.27 ±0.34	1.08 ±0.4	1.64 ±1	1.39 ±0.06	3.07 ±1.17	5.24 ±1.5	6.45 ±0.31	6.76 ±1.59	6.51 ±1.47
Desulfuromonadales; **Desulfuromonadaceae**	0.62 ±0.1	0.4 ±0.18	0.37 ±0.06	0.52 ±0.03	0.55 ±0.18	6.57 ±4.92	9.78 ±4.4	5.5 ±0.84	0.07 ±0.01	0.31 ±0.21

Table S4. Most abundant bacteria at genera level with at least 3% relative abundance. Heat map abundance colors: red > 3%; orange 1 to 3%; white <1%.

	Average relative abundant (± standard error)									
	MS100 Duplicate					**AC33 Duplicate**				
	Upper Zone		**Lower Zone**			**Upper Zone**		**Lower Zone**		
Order Family **Genus**	**Bioanode (Sediment)**	**Current collector**	**Root + Bioanode**	**Bioanode**	**Current collector**	**Bioanode (Sediment)**	**Current collector**	**Root + Bioanode**	**Bioanode**	**Current collector**
Burkholderiales; Alcaligenaceae; **Castellaniella**	1.47 ±0.8	2.17 ±0.25	5.62 ±0.04	6.48 ±0.74	4.95 ±0.05	3.28 ±0.51	2.88 ±0.18	3.75 ±0.19	2.99 ±0.53	3.97 ±0.77
Hydrogenophilales; Hydrogenophilaceae; **uncultured**	2.25 ±0.17	3.25 ±0.25	2.98 ±0.53	1.55 ±0.74	2.88 ±0.27	3.63 ±0.52	4.17 ±0.2	4.11 ±0.25	2.89 ±1.23	2.95 ±0.05
Propionibacteriales; Propionibacteriaceae; **Brooklawnia**	0.77 ±0	0.66 ±0.1	3.67 ±1.7	2.56 ±1.02	2.12 ±0.11	5.06 ±0.74	3.62 ±1.61	4.55 ±0.16	1.97 ±0.34	2.53 ±3.67
Xanthomonadales; Xanthomonadaceae; **Thermomonas**	3.23 ±0.49	2.82 ±0.05	2.91 ±0.23	8.64 ±5.78	2.74 ±0.25	1.92 ±0.06	2.66 ±0.39	2.37 ±0.56	2.16 ±0.24	2.41 ±0.06
SAR324 clade; uncultured δ proteobacterium; **uncultured delta proteobacterium**	0.12 ±0.02	0.15 ±0.08	1.31 ±0.85	3.1 ±2.64	0.72 ±0.66	0.71 ±0.28	0.08 ±0.04	0.03 ±0	0.05 ±0.02	0.04 ±0.11
uncultured bacterium (ML635J-21); uncultured bacterium; **uncultured bacterium**	3.02 ±2.49	0.47 ±0.36	0.12 ±0.08	0.05 ±0.05	0.06 ±0.04	0.11 ±0.01	0.42 ±0.1	0.82 ±0.46	0.41 ±0.19	0.1 ±0.21
Phycisphaerales; Phycisphaeraceae; **Z195MB87**	0.42 ±0.1	1.44 ±0.3	0.66 ±0.11	0.74 ±0.36	1.15 ±0.47	2.92 ±0.59	1.56 ±0.44	3.8 ±1.75	6.81 ±6.03	3.3 ±2.42
Rickettsiales; Rickettsiaceae; **uncultured**	0.37 ±0.12	0.59 ±0.07	0.29 ±0.19	0.45 ±0.3	0.65 ±0.15	1.98 ±0.82	2.68 ±1.21	4.04 ±0.13	4.14 ±0.13	3.61 ±1.64
Cytophagales; Cyclobacteriaceae; **Indibacter**	0.39 ±0.08	0.28 ±0.18	0.24 ±0.05	0.33 ±0.07	0.44 ±0.16	6.36 ±4.95	2.64 ±4.32	5.34 ±0.85	0.02 ±0.01	0.15 ±0.06
SubsectionICyanobacteria; FamilyI; **Synechococcus**	0.75 ±0.09	0.78 ±0.24	0.37 ±0.15	0.3 ±0.18	0.53 ±0.32	1.42 ±0.68	1.16 ±0.33	1.16 ±0.4	3.18 ±1.42	1.95 ±0.47

Table S5. Acetate concentration, pH and ionic conductivity from anolyte and catholyte of Plant-MFCs.

Days	AC100-PMFC 1	AC100-PMFC 2	MS100-PMFC 3	MS100-PMFC 4	AC67-PMFC 5	AC67-PMFC 6	AC33-PMFC 7	AC33-PMFC 8
Acetate concentration in the anolyte (mg/l)								
1	195	1752	31	13	41	7	89	7
22	6	9	17	5	5	6	8	7
31	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0
77	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
105	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
190	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Anolyte pH								
1	8.94	9.38	9.14	9.17	9.15	8.77	8.98	9.12
22	6.72	6.76	7.72	7.95	7.56	7.06	7.29	7.95
31	6.22	6.72	7.22	7.73	7.15	6.77	7.4	7.51
37	6.45	7.23	7.38	7.73	7.18	6.99	7.27	7.46
45	7.19	6.99	7.32	7.37	7.08	7.02	7.04	7.46
77	7.2	7.23	6.79	6.69	6.6	6.85	6.77	6.92
105	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
190	7.28	7.41	6.71	6.24	7.07	6.63	6.75	7.05
Anolyte ionic conductivity (S/m)								
1	0.783	0.77	0.966	1.084	0.676	0.941	0.905	0.764
22	0.829	0.767	0.927	0.932	0.841	0.938	0.932	0.824
31	0.929	0.758	0.964	0.953	0.933	0.879	0.9	0.851
37	0.862	0.751	0.984	0.931	0.926	0.899	0.903	0.892
45	0.903	0.769	1.017	0.941	0.98	0.904	0.987	0.926
77	1.044	0.722	1.248	0.945	1.004	0.954	0.978	0.997
105	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
190	0.441	0.447	0.379	0.298	0.342	0.591	0.404	0.341
Catholyte pH								

1	9.19	8.55	8.78	8.81	8.98	9.22	9.02	9.09
22	8.16	8.36	6.12	5.4	7.98	8.2	5.68	5.71
31	7.72	8.26	6.54	6.83	7.65	8.28	6.21	6.6
37	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
45	7.99	8.25	5.37	7.55	7.7	8.41	6.63	7.14
77	7.59	7.79	7.05	7.47	7.69	8.36	7.1	7.55
105	6.5	6.22	6.23	6.68	7.03	7.72	6.46	6.85
190	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Catholyte ionic conductivity (S/m)								
1	0.764	0.388	0.558	0.616	0.284	0.532	0.55	0.476
22	0.703	0.413	0.558	0.589	0.745	0.574	0.649	0.499
31	0.744	0.433	0.591	0.519	0.638	0.569	0.665	0.541
37	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
45	0.803	0.45	0.845	0.576	0.686	0.659	0.75	0.57
77	0.745	0.376	0.722	0.508	0.678	0.569	0.689	0.663
105	0.887	0.571	0.926	0.655	0.774	0.689	0.86	0.718
190	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a

n.a = not analysed.

Table S6. Dried biomass yield after 190 days.

Reactors	Above ground biomass Yield (Kg/m² PGA)	Below ground biomass yield (Kg/m² PGA)
MS100 (Plant-MFC 3)	2.74	2.97
MS100 (Plant-MFC4)	2.29	3.32
AC33 (Plant-MFC7)	0.45	0.76
AC33 (Plant-MFC8)	0.82	1.45
AC67 (Plant-MFC 5)	1.71	2.29
AC67 (Plant-MFC 6)	1.47	1.89
AC 100 (Plant-MFC 1)	0.63	0.79
AC100 (Plant-MFC 2)	0.47	0.87

Method

Method S1. DNA extraction protocol modification.

A. DNA extraction: Power soil® DNA Isolation Kit

Original procedure Instruction manual version 07272016 PowerSoil® DNA Isolation Kit can be downloaded from https://mobio.com/media/wysiwyg/pdfs/protocols/12888.pdf

The procedure is the same mentioned in the manual on pages 10-11.

Some modifications have been done and they are described by steps.

3–After this step put samples in heat block at 55 degrees Celsius for 15 min.

6–Tubes were centrifuged per 1 minutes instead of 30 seconds

14–Solution C4 was added twice (600 uL first time and 500 ul second time) and vortexed twice as well.

15–Spin filter was loaded with 650 uL instead of 675 uL to leave space on the tube for centrifugation.

20–30 uL of solution C6 were added instead of 100 uL. This was done because we didn't know what could be the DNA concentration and we didn't want to dilute the DNA.

23–Put in heatblock for 15 min 55degrees Celsius just before spinning C6 down.

B. DNA quantification – Qubit

No modifications to the protocol were made. Qubit® dsDNA HS Assay Kits protocol can be downloaded from https://assets.thermofisher.com/TFS-Assets/LSG/manuals/Qubit_dsDNA_HS_Assay_UG.pdf

C. DNA concentration - Speed Vac

Original protocol on Concentrator 5301 Eppendorf can be downloaded from https://sydney.edu.au/medicine/bosch/facilities/molecular-biology/5301_900_017_11_0906_en.pdf

Evaporation with heating was used (page 21 – section 3)

The temperature used was 45C and the time was between 5-10 minutes.