biblio.ugent.be

The UGent Institutional Repository is the electronic archiving and dissemination platform for all UGent research publications. Ghent University has implemented a mandate stipulating that all academic publications of UGent researchers should be deposited and archived in this repository. Except for items where current copyright restrictions apply, these papers are available in Open Access.

This item is the archived peer-reviewed author-version of:

Title: Greenhouse gas emissions from rice microcosms amended with a plant microbial fuel cell.

Authors: Jan B. A. Arends, Jonas Speeckaert, Evelyne Blondeel, Jo De Vrieze, Pascal Boeckx, Willy Verstraete, Korneel Rabaey, Nico Boon.

In: Applied Microbiology and Biotechnology 98 (7) 3205-3217

Link: http://link.springer.com/article/10.1007%2Fs00253-013-5328-5

To refer to or to cite this work, please use the citation to the published version:

Arends, J. B. A., Speeckaert, J., Blondeel, E., Vrieze, J., Boeckx, P., Verstraete, W., Rabaey, K., Boon, N. 2014. Greenhouse gas emissions from rice microcosms amended with a plant microbial fuel cell. Applied Microbiology and Biotechnology, 98(7), 3205-3217. DOI: 10.1007/s00253-013-5328-5

Greenhouse gas emissions from rice microcosms amended with a plant microbial fuel cell.

Jan B. A. Arends[1], Jonas Speeckaert[1], Evelyne Blondeel[1#], Jo De Vrieze[1], Pascal Boeckx[2], Willy Verstraete[1], Korneel Rabaey[1], Nico Boon[1]*

[1]Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, B-9000 Gent, Belgium.
[2]Isotope Bioscience Laboratory (ISOFYS), Ghent University, Coupure Links 653, B-9000 Gent, Belgium.
Currently at: EnBiChem, HOWEST, Graaf Karel de Goedelaan 5, B-8500 Kortrijk, Belgium

Corresponding author. Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, B-9000 Ghent, Belgium;
phone: +32 (0)9 264 59 76;
fax: +32 (0)9 264 62 48;
E-mail: Nico.Boon@ugent.be;
Webpage: www.labmet.Ugent.be.

Key words:

Bioelectrochemical system, methane, microbial ecology, hydrogenotrophic methanogens, electrode material, nitrous oxide, rhizosphere, *Oryza sativa.*

Abstract

Methane (CH_4) release from wetlands is an important source of greenhouse gas emissions. Gas exchange occurs mainly through the aerenchyma of plants and production of greenhouse gases is heavily dependent on rhizosphere biogeochemical conditions (i.e. substrate availability and redox potential). It is hypothesized that by introducing a biocatalyzed anode electrode in the rhizosphere of wetland plants, a competition for carbon and electrons can be invoked between electrical current generating bacteria and methanogenic archaea. The anode electrode is part of a bioelectrochemical system (BES) capable of harvesting electrical current from microbial metabolism. In this work, the anode of a BES was introduced in the rhizosphere of rice plants (*Oryza sativa*) and the impact on methane emissions was monitored.

Microbial current generation was able to outcompete methanogenic processes when the bulk matrix contained low concentrations of organic carbon, provided that the electrical circuit with the effective electro-active microorganisms was in place. When interrupting the electrical circuit or supplying an excess of organic carbon, methanogenic metabolism was able to outcompete current generating metabolism. The qPCR results showed hydrogenotrophic methanogens were the most abundant methanogenic group present, while mixotrophic or acetoclastic methanogens were hardly detected in the bulk rhizosphere or on the electrodes. Competition for electron donor and acceptor were likely the main drivers to lower methane emissions. Overall, electrical current generation with BESs is an interesting option to control CH_4 emissions from wetlands but needs to be applied in combination with other mitigation strategies to be successful and feasible in practice.

1. Introduction

Methane (CH_4) emissions to the atmosphere arise from various (a)biotic sources. CH_4 has a global warming potential (GWP) of 25 times the GWP of CO_2. Therefore these emissions need to be carefully managed (Forster et al. 2007). The main anthropogenic sources of methane emissions are ruminant farming (15-32%) and rice agriculture (9-19%). Taking also natural emissions into account, wetlands and rice agriculture combined amount to 32-47% of total methane emissions (Denman et al. 2007).

Anaerobic decomposition of organic matter resulting in the formation of methane is the consequence of a series of biochemical transformations. Bacteria ensure the formation of monomers from complex organic polymers. The monomers are subsequently transformed into various organic acids. In the third step acetic acid, CO_2 and H_2 are formed which are finally transformed into methane by the hydrogenotrophic or the acetoclastic methanogens (Appels et al. 2008; Conrad 2002). CH_4 formation occurs under specific biogeochemical conditions such as redox potential (Eh), pH and ammonia concentration (Appels et al. 2008; Conrad 2002; Johnson-Beebout et al. 2009; Majumdar 2003). These parameters give, next to competition for substrate, various handles for control of CH_4 production. The main pathway of gas exchange with the rhizosphere under waterlogged conditions is by transport through the aerenchyma of plants (90%). Other mechanisms include diffusion and ebullition (Aulakh et al. 2000; Bazhin 2010). Therefore a promising route for mitigation of CH_4 emissions is to prevent the formation of CH_4 in the rhizosphere. Next to introduction of alternative electron acceptors, other mitigation strategies include fertilizer management, water management, biochar addition to the soil and choosing crop varieties with little aerenchyma or little rhizodeposition (Aulakh et al. 2001; Aulakh et al. 2000; Conrad 2002; Majumdar 2003; Singh et al. 1999; Zhang et al. 2012).

The last decades microbial extra cellular electron transfer has gained attention on the promise of generating electrical energy directly from various sources of organic matter (Rabaey and Verstraete 2005). During anaerobic respiration of organic carbon in a microbial fuel cell (MFC), microorganisms are able to use an electrode (the anode) as an electron acceptor. When such a biocatalyzed anode (bioanode) is connected with a (bio)cathode, electrical current can be directly harvested from the microbial decomposition of organic matter in a bioelectrochemical system (BES). The typical substrate for the anodic process is acetate although it has been shown that H_2 can also be used (Logan et al. 2006). Waterlogged soils and sediments containing organic matter have been exploited for direct electrical current generation in sediment-MFCs (Donovan et al. 2011; Reimers et al. 2001; Tender et al. 2008). A drawback of sediment-MFCs is the low flux of organic matter towards the anode, limiting high current production. To overcome this issue, plants have been introduced into

the anode of a sediment-MFC creating a Plant-MFC which enables sustained current generation from organic matter due to rhizodeposition processes (Strik et al. 2008). An example is the use of rice plants, as they are able to withstand prolonged inundation of their rhizosphere (De Schamphelaire et al. 2008; Kaku et al. 2008).

Methane emissions from rice paddies can be estimated to range from 0 up to 60 mgCH$_4$ m^{-2} h^{-1} (Gogoi et al. 2005; Singh et al. 1999; Xu et al. 2007). This corresponds to the equivalent of an electrical current of 0 to 804 mA m^{-2} (See supplementary information for calculations). Plant-MFCs have been reported to produce a current up to 120 mA m^{-2} (De Schamphelaire et al. 2008). These values are well within the same order of magnitude, therefore it has been hypothesized that current generating metabolism can mitigate CH$_4$ emissions from rice paddy soils and in more general terms, waterlogged wetlands (Cabezas da Rosa 2010; De Schamphelaire et al. 2008; Hong et al. 2009; Ishii et al. 2008; Kaku et al. 2008). Introducing an electrode (anode) in the rhizosphere of waterlogged plants possibly enables the removal of electrons from the commonly present methanogenic metabolism (Arends and Verstraete 2012; Cabezas da Rosa 2010; De Schamphelaire et al. 2008; Kaku et al. 2008). This is analogous to CH$_4$ emission mitigation strategies such as the addition of ferric iron or sulphate to stimulate iron reducing or sulphate reducing microorganisms in their competition for reducing equivalents with methanogens (Conrad 2002; Liesack et al. 2000).

Anodic oxidation of organic matter leads to a decrease in pH during current generation (Rozendal et al. 2006; eq. S2). Methanogenic metabolism can be inhibited by a decrease in pH (Appels et al. 2008). Acidification due to current generating metabolism can possibly form a second route to inhibition of methanogenic activity, next to competition for substrate.

Cabezas de Rosa (2010) has observed a 47% decrease in pore water CH$_4$ concentrations in a rice paddy soil sediment-MFC without plants. Kaku et al. (2008) were not able to show lower CH$_4$ emissions in closed circuit operation of a Plant-MFC during a field trial in a rice paddy. In this work the results of a microcosm study are presented in which rice plants were grown in vermiculite with a BES in the rhizosphere. The aim of this work was to investigate 1) the influence of the external resistance 2) the mechanism and 3) the archaeal pathways involved in possible methane emission mitigation in Plant-Sediment MFC rhizosphere/anode environments.

2. Materials and Methods

2.1 Microcosm setup

All experiments were carried out in microcosms consisting of Perspex tubes (14 cm inner diameter, 35 cm high). The bottom 15 cm contained 12 sample ports at various heights while the top 10 cm contained 4 sample ports (Figure 1, S2). The containers were filled with graphite granules (type 00514, Mersen, Belgium) and vermiculite (Nestaan, Belgium) in a volume ratio of 2:1, to a height of 18 cm. This ratio was chosen in order to have a good electrical conductivity in the rhizosphere (Arends et al. 2012) and to ensure that only added organic carbon (COD) or rhizodeposition were the sources organic carbon. On top of this mixture, a layer of 3 cm vermiculite was added which prevented electrical contact between the anode and the cathode. Vermiculite and not rice paddy soil was chosen to create uniform rhizosphere conditions and to be able to measure and/or control the amount of organic carbon in the rhizosphere/anode as much as possible. The sides of containers were covered with black tape to create a dark root environment. At 5 and 15 cm height, a piece of graphite felt (10*10*0.3 cm; Alfa Aesar, Germany) interwoven with a carbon rod (5 mm diameter; Morgan, Belgium) was placed as the anodic current collector. The cathode was made of two sections of 5*10*0.3 cm graphite felt interwoven with a carbon rod. The two sections were placed so that the plants were able to grow in the middle of the Perspex tube. A watertight connection between the wire and the carbon rod was established by means of conductive carbon cement (CCC, Leit-C; Fluka, Germany) and vulcanizing tape. An Ag/AgCl reference electrode (BASi, United Kingdom) was placed at 10 cm height in the rhizosphere/anode compartment and also in the cathode compartment. To be able to measure soluble pore liquid components, a rhizon sampler was located at 6.7 and 16.7 cm height (10 cm porous, Rhizosphere Research Products, The Netherlands). In order to collect gas emissions, the top of the Perspex container contained a ledge for a water lock. This ledge was able to support a tube of 12.3 cm inner diameter and 1 m height (Figure S2). The top of this tube contained four connectors for sampling or other uses. One connection was occupied by the electrical connections for a fan to mix the contents of the headspace; another was used for temperature measurements during headspace gas sampling. All sampling i.e. pore liquid, trace gas and polarization curves was performed at the same time for a microcosm during the whole experimental period to minimize circadian (day/night) influences on the results. For the exploratory study, additional square containers were used with the same configuration except for the surface area (18*18 cm). In experimental setups meant for open circuit conditions, only 1 anode and 1 rhizon sampler were placed in the container. An overview of all microcosms and experimental conditions can be found in table S1.
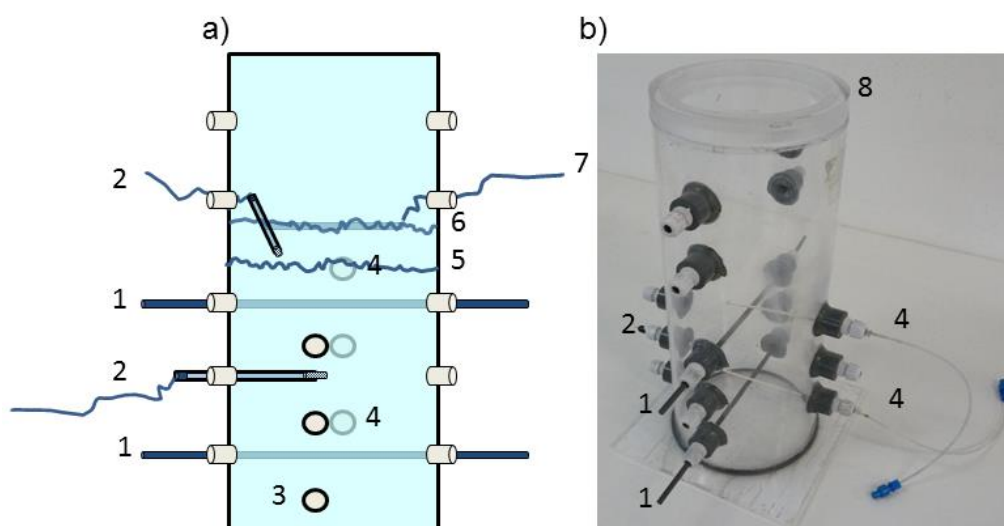
Figure 1: a) Schematic overview of microcosm, not to scale. b) photograph of empty microcosm. 1) anode current collector, 2) Ag/AgCl reference electrode, 3) port for liquid addition, 4) positions for Rhizon samplers, 5) vermiculite level, 6) water level, 7) cathode electrode and wire, 8) rim to hold gas cover.

2.2 Rice plants & rhizosphere organic carbon

Rice seeds (*Oryza sativa*, ssp. *indica*, cultivar C101 PKT) were germinated in ¼ Hoagland nutrient solution at 28°C in the dark (De Schamphelaire et al. 2008). The seedlings were subsequently transplanted into the Perspex containers, which was considered the start of the experiment. Two seedlings were planted per microcosm. The microcosms were placed in a light (12/12 light/dark using Osram 400W plantastar and Osram 400W powerstar daylight HQI-BT Lamps) and temperature (30 ± 2 °C) controlled growth room. Development of the plants was scored according to Counce et al. (Counce et al. (2000). To maintain a constant water level above the vermiculite, plants were regularly watered with tap water and once per week with ½ Hoagland nutrient solution. Upon good visible plant development, nitrate was removed from the Hoagland nutrient solution (Helder et al. 2012). Dried and ground barley straw was added to the rhizosphere as additional organic carbon in the exploratory study (Table S1). Soluble organic carbon mimicking exudation was added to the rhizosphere in the main study as an equal weight mixture of acetate, starch, glucose, malate and succinate. The amount of organic carbon added was calculated to attain approximately 30 mg CH_4 m^{-2} h^{-1} emission resulting in an organic carbon addition rate of 2.9 gCOD m^{-2} d^{-1} (~ 401 mA m^{-2}; starting from day 39; See supplementary information S1.).

2.3 Electrochemical analysis

Cell potential over an external resistor (500 Ω, unless stated otherwise), anode and cathode potentials (E_{an} respectively E_{cath}) versus a reference electrode were measured continuously at 5 min intervals (HP 34970A, Agilent). The potential of the Ag/AgCl reference electrodes were regularly

monitored relative to a calomel electrode (+244 mV vs. Standard Hydrogen Electrode (SHE); QIS, the Netherlands) for correct conversion of the electrode potentials compared to the SHE. Electrode potentials are consequently reported versus the standard hydrogen electrode. Polarization and power curves were recorded on a weekly basis with a Bistat potentiostat (Biologic, France) at a scanrate of 1 mV s^{-1} following a 20 min stabilization period in open circuit. Electrochemical calculations were performed according to Rabaey et al. (2005) and are based on hourly averages. Current and power density are reported normalized to the plant growth area (0.015 m^2; Table S1).

2.4 Chemical analysis

Liquid and headspace gas samples were taken twice a week. Anions (PO_4^{3-}, NO_3^-, NO_2^-, SO_4^{2-}) in the pore liquid were analysed using a metrosep A Supp 5-150 column after a metrosep A 4/5 guard column in a 761 Compact IC with a conductivity detector (Metrohm, Switzerland). Pore liquid soluble chemical oxygen demand (COD) was determined using commercial kits according to the manufacturer's instructions (Machery-Nagel, Germany). Volatile fatty acids (VFA) were determined, after etheric extraction of 2 mL pore liquid, on an EC-1000 Econo-Cap column in a gas chromatograph (2014, Shimadzu) with a flame ionization detector (FID). Pore liquid and overlying water pH and conductivity (EC) were determined using a handheld probe (SP10B and SK20, Consort, Belgium). Headspace air temperature was monitored during gas sampling.

2.5 Trace gas analysis

Twice a week the headspace of the microcosms was closed to determine gas emissions from the rhizosphere-plant continuum. Every 30 min. duplicate 15 mL gas samples were taken from the headspace over a period of 2 hours. A duplicate 15 mL background air sample was taken to account for gas concentrations already present in the rice cultivation room. All gas samples were stored in a 12 mL vacutainer, allowing at least 3 subsamples for analysis. Vacutainers were stored in the dark at ambient temperature (20 ± 2 °C) until analysis. CH_4 and CO_2 were analysed using a gas chromatograph (Trace GC Ultra, Thermo Fisher Scientific, Germany). Methane was determined using FID while CO_2 was determined using a thermal conductivity detector (TCD). Nitrous oxide was measured using a gas chromatograph (14B, Shimadzu, Japan) with a 63Ni electron capture detector (ECD).

2.6 PCR-DGGE and qPCR

At the end of the experimental period, samples were taken for microbial community analysis. Sample positions were: anode current collector, anode felt (top & bottom) and anode granules with and without rice roots (Figure S2). Anode granules without rice roots are considered as bulk vermiculite. Total DNA was extracted using the method as described by Boon et al. (Boon et al. 2000). The 16S rRNA-gene was amplified using PCR (Table 1). The bacterial community structure was visualized by means of denaturing gradient gel electrophoresis (DGGE) of the obtained PCR amplicons using an 8% polyacrylamide gel and a denaturing gradient of 40-60% on an INGENY phorU2X2 system for 16 h at 120 V (Goes, The Netherlands). The gel was stained with Sybr green in 1x TAE buffer. The resulting community structures were analysed using BioNumerics software v5.1 (Applied Maths, Belgium). DGGE profile similarities were based on band based clustering using the Jaccard coefficient to minimize the influence of background subtraction on the clustering. Theoretical ecological parameters, community organization (Co) and richness (Rr), were used to numerically describe the bacterial communities. In brief, Co describes the species abundance distribution based on the gini coefficient of the microbial community. Rr describes the species richness of the microbial community and can be interpreted as the carrying capacity of a certain environment (Read et al. 2011). The archaeal community structure was analysed by means of qPCR (Table 1 & S2) using the GoTaq qPCR MasterMix (Promega, Belgium) on a StepOnePlus qPCR machine and accompanying software (Applied Biosystems, United Kingdom).

Table 1: (q)PCR primers and conditions used for elucidating the microbial communities. D: denaturation, A: Annealing, E: Elongation phases of the PCR cycle. For the specific methanogenic groups, taxonomic level and major metabolism are indicated.

Reaction	Target	Primers	Program	Reference
PCR-DGGE	Bacterial 16S rRNA gene	338F-GC 518R	D: 94°C, 300s 30 cycles D: 95°C, 60s A:53°C, 60s E: 72°C, 120s E: 72°C, 600s	(Boon et al., 2000; Ovreas et al., 1997)
qPCR	Total Bacteria 16S rRNA gene	338F 518R	D: 94°C, 10min 40 cycles D: 94°C, 15s A&E: 60°C, 60s	
	Total Archaea 16S rRNA gene	ARC787F ARC1059R	D: 94°C, 10min 40 cycles D: 94°C, 10s A&E: 60°C, 60s	(Yu et al., 2005)
	Methanosaetaceae Family (acetoclastic)	Mst 702F Mst 862R	See total archaea	

	Methanosarcianceae Family (mixotroph)	Msc 380F Msc 828R	See total archaea	
	Methanobacteriales Order (H_2/CO_2)	MBT 857F MBT 1196R	See total archaea	
	Methanomicrobiales Order (H_2/CO_2)	MMB 282F MMB 832R	D: 94°C, 10min 40 cycles D: 94°C, 10s A&E: 63°C, 60s	

3. Results

A first set of exploratory experiments was used to determine the key operational parameters (electrode position, organic matter content and initial microbial community) relevant for CH_4 emissions. A second, more detailed study focused on the effect of placing a sediment MFC in the rhizosphere of rice plants on greenhouse gas emissions.

3.1 Exploratory study

From the exploratory study no clear difference could be distinguished in methane emissions between closed and open circuit conditions as well as between planted and sediment conditions. Methane emissions averaged across the open (n=7) and closed circuit (n=3) configurations (table S1) were 157 ± 76 vs. 169 ± 68 mg CH_4 m^{-2} h^{-1} respectively. These values are high compared to natural field conditions (0-60 mg CH_4 m^{-2} h^{-1} (Gogoi et al. 2005; Singh et al. 1999; Xu et al. 2007)). The microcosm that was not supplemented with straw was the configuration that came close to natural CH_4 fluxes in the order of 35 mgCH_4 m^{-2} h^{-1}. Interestingly, the exploratory study revealed that interrupting the current flow from a well performing Plant-MFC resulted in a doubling of CH_4 emission flux (Figure S3). This doubling was about 20 times higher than the loss in electrons due to current (See supplementary information for calculations). This suggests the influence of changing electrochemical or thermodynamic conditions, possibly related to local acidification close to the anode electrode, next to direct competition for electrons between the current generating and methanogenic microbial populations. Based on the results of the exploratory work, it was chosen to work in the detailed study with no added organic carbon at the start of the experiment and also to not add methanogenic sludge.

The next sections provide the results of the detailed study.

3.2 Plant growth characteristics

4 Microcosms were set up for the detailed study. One microcosm (M4) lagged behind in development rate in the first growth phase, probably due to leakage of copper from the current

collector wire at the cathode i.e. at the top of the microcosm. This was amended by replacing the wire and removing the affected vermiculite, after which the rate of development of the plants in M4 as well as electrochemical and gas emissions were similar to comparable microcosms. Final above ground dry biomass were M1: 9.5 ± 1.2 gDW plant^{-1} M2: 10.9 ± 0.1 gDW plant^{-1} M3: 11.5 ± 1.7gDW plant^{-1} M4: 4.3 ± 2.2 gDW plant^{-1}.

3.3 Current, power and electrochemical performance of the Plant-MFCs

Cell potentials of all four plant-MFCs increased during the first 21 days after planting to an average maximum value of 0.23 ± 0.039 V resulting in a current density of 27.9 ± 4.2 mA m^{-2} (figure 2b). After day 21, the cell potentials decreased. Addition of organic carbon (starting at day 39) resulted in an increase in current density for M3 and M4 (days 45-55) to 45.5 ± 11.8 mA m^{-2} for M3 and 25.9 ± 13.7 mA m^{-2} for M4 (Figure 2b). M1 and M2 did not receive any organic carbon (Table S1). Consequently, no increase in current nor in greenhouse gas emissions were observed. M2 is not plotted in figure 2 as data were similar to M1. The high standard deviations are due to a circadian rhythm with daily maxima up to 69.6 mA m^{-2} and 62.4 mA m^{-2} for M3 and M4 respectively and nightly minima as low as 22.9 mA m^{-2} and 9.7 mA m^{-2} for M3 and M4 (Figure 3). After the open circuit period (day 63-72), the current density did not recover up to these values. Attempts to increase current density by lowering the external resistance did not result in an increased current flow (Figure 2b). The main cause for the low current density was a deterioration of the cathode performance as evidenced by the cathode potential (E_{cath}). While the cathode potential reached daily maxima of 0.84 V and 0.62 V for M3 and M4 and nightly minima of 0.07 V and 0.17 V during days 45-55 (Figure 3), daily cathode maxima went only up to 0.40 V and 0.37 V for M3 and M4 upon re-closing the opened electrical circuit with a 500 Ω resistor. Decreasing the external resistance to 100 Ω (d 79) resulted in an average (including day & night) E_{cath} over the remainder of the experimental period of -0.11 ± 0.13 V for M3 and -0.11 ± 0.07 V for M4. The anode potential of M3 and M4 remained low after day 39, -0.20 ± 0.16 V for M3 and -0.19 ± 0.09 V for M4 (Figure 1c and 3). Variations in anode potential are mainly caused by polarization curves (invasive technique) or sampling. Overall average power production during days 45-55 resulted in 22.2 ± 9.5 mW m^{-2} for M3 and 6.6 ± 6.6 mW m^{-2} for M4. Here again high standard deviations can be seen, due to the circadian rhythm influencing cathode performance. From the polarization and power curves the maximum attainable current and power can be estimated. The maximum power output was attained at day 61, 72 mW m^{-2} for M3 and 56 mW m^{-2} for M4. The maximum short circuit current generated during the polarization measurements was 0.58 A m^{-2} for M3 at day 53 and 0.20 A m^{-2} for M4 at day 61. For M1 and M2, the maximum values amounted to 9 mW m^{-2} and 1 mW m^{-2} respectively, obtained at day 19

a) Chemical oxygen demand in pore liquid

b) Current density
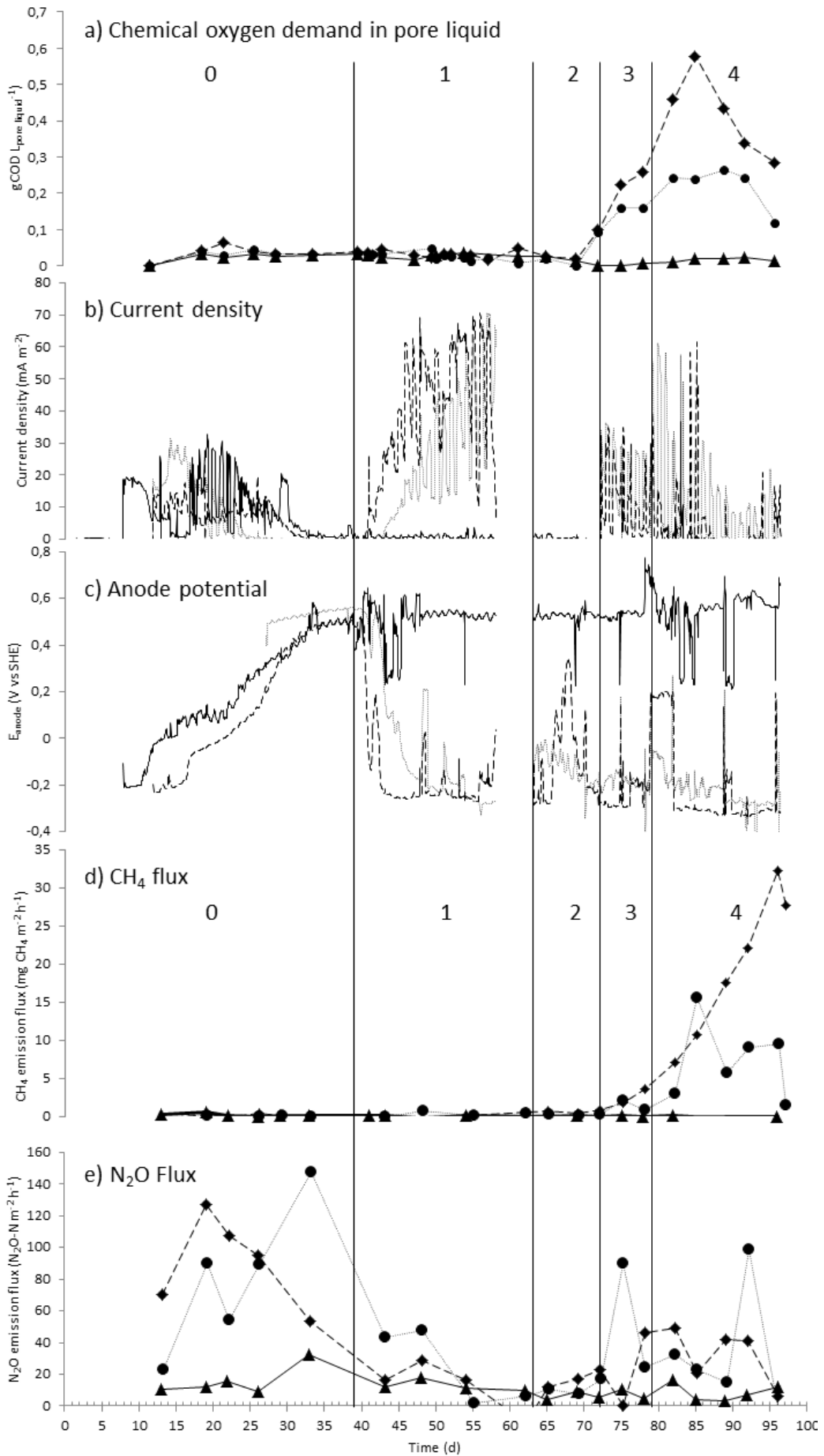
c) Anode potential

d) CH₄ flux

e) N₂O Flux

Figure 2: overview of a) chemical oxygen demand, b) current density, c) anode potentials, d) methane flux, multiply value by 25 to arrive at CO_2 equivalents and e)nitrous oxide flux, multiply value by 298 to arrive at CO_2 equivalents during the rice growth period. M1: ▲ *M3:* ◆ *M4:* ●*. M2 is not plotted as data are similar to M1. For continuous measurements, b) and c), symbols are omitted. Experimental stages: 0: initial plant growth and MFC start-up 1: addition of organic carbon 2: open circuit period 3: closed circuit period, 500 Ω 4: external resistance lowered from 500 Ω to 100 Ω for M3 and M4. No electrochemical data available for day 58-63.*
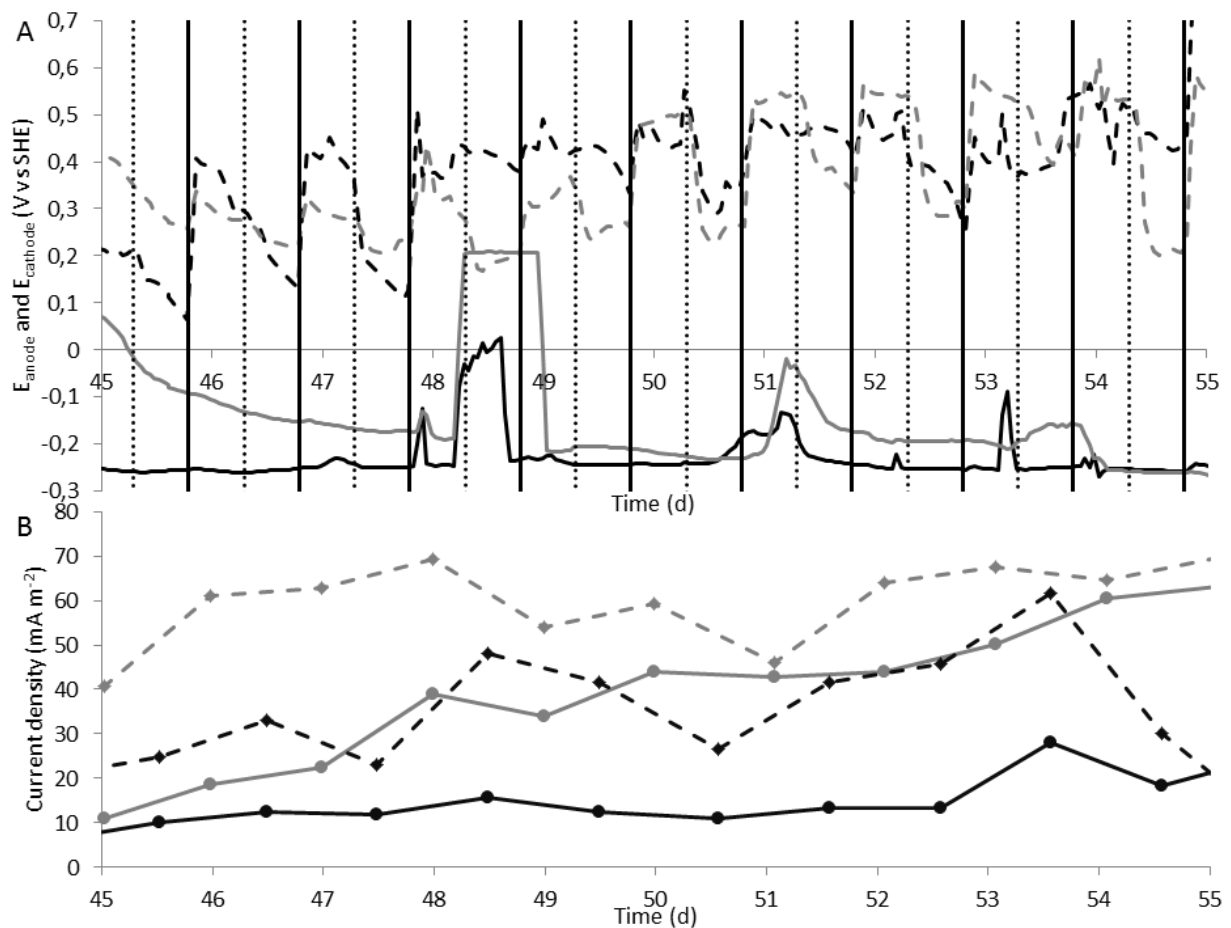

Figure 3: Close up of data between day 45-55 showing the diurnal variation. a) anode and cathode potentials during day 45-55. Day 48: anode reference electrodes disconnected. Vertical solid lines; 7 am, lights on. Vertical dotted lines; 7 pm, lights off. M3 anode: ——— *M3 cathode:* — — *M4 anode:* ——— *M4 cathode:* — — *b) Current density resulting from the varying electrode potentials day 45-55. M3 daily max.:* ◇ *M3 Nightly min. :* ◆ *M4 daily max.:* ○ *M4 Nightly min. :* ●

3.4 Current was generated after addition of organic carbon.

The overall availability of organic carbon in the pore liquid was low during the first part of the experiment (35.3 ± 10.5 mgCOD L^{-1} for M3 and 31.8 ± 9.3 mgCOD L^{-1} for M4 till day 55; Figure 1a). To

compensate for the low reducing power provided by the plants, soluble organic carbon was added to mimic the rhizodeposition process. The added COD was immediately used by the electroactive microbial community present on the anode, as can be seen by the almost instantaneous increase in current (Figure 2b). The organic carbon was efficiently oxidized as only on day 70 it started to appear in the pore liquid of the bulk (Figure 2a). No short chain fatty acids were detected in the bulk liquid of the microcosms (data not shown). The efficient use of exogenous organic matter was not only for current generation, as current generation only accounted for maximum 15% of the organic carbon ([~60 mA m^{-2}] / [401 mA m^{-2}] *100%). The organic carbon that was not used for current generation accumulated in the system or was metabolised using alternative electron acceptors such as nitrate and sulphate or O_2 due to radial oxygen loss from the rice roots. For M3 and M4 a decrease of 69 % and 75 % COD equivalents was noticed for nitrite, nitrate and sulphate combined after day 39. The substrate limited situation of all microcosms could also be derived from the fact that M2 received the same nutrient solution as M3 & M4 but without organic carbon. The addition of only Hoagland solution did not result in an increase of current, nor CH_4 emissions nor organic carbon concentration in the pore liquid. M1 did not receive any extra organic carbon or liquid, which also did not result in an increase of available organic carbon in the pore liquid.

Due to current generating metabolism, a drop in pH could be expected but this was not detected in the bulk pore liquid. The pH of all pore liquid samples was 7.79 ± 0.44 over the whole experimental period.

3.5 Anode biofilm redox potentials in relation to greenhouse gas emissions

Methane was produced when favourable redox conditions existed for the producing microbial community (i.e. < -150 mV vs. SHE; Hou et al., 2000; Yu and Patrick, 2003; Johnson-Beebout et al., 2009). The potentials measured in the reference electrode/ bioanode combination could be related with bulk gas emissions for CH_4 (Figure 4a). N_2O emissions showed a less clear relationship with the redox potentials as measured with the reference electrode/ bioanode combination (figure 4b).
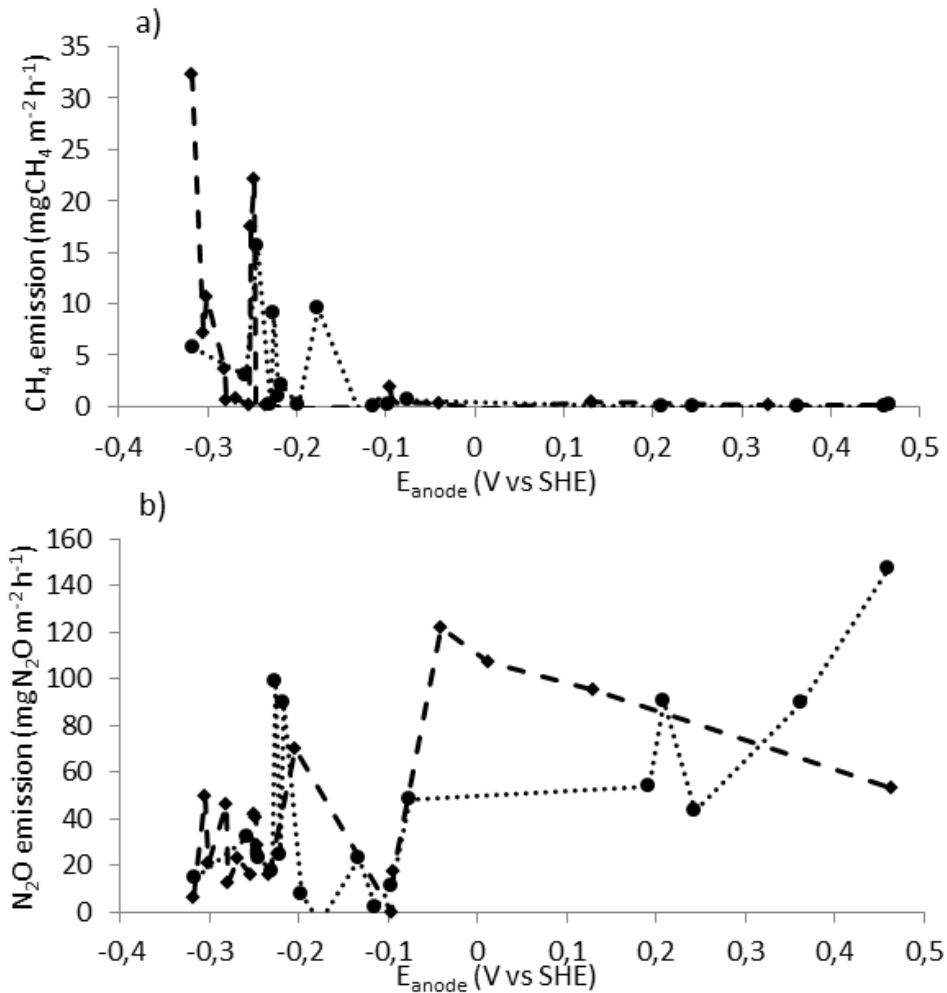
Figure 4: Greenhouse gas emissions in relation to measured anode potential. a) CH_4 multiply value by 25 to arrive at CO_2 equivalents b) nitrous oxide flux, multiply value by 298 to arrive at CO_2 equivalents. M3: ◆ − *M4:* ••●•••.

3.6 Methane emissions lagged behind current generation.

Organic carbon was efficiently metabolised to current, to other sinks or accumulated, but was not metabolised to methane. Methane only appeared in the headspace measurements of M3 & M4 after the current flow was interrupted by removing the external resistor from the electrical circuit for 9 days (day 63-72). Towards the end of this period (day 69; Figure 2a), organic carbon concentrations started to increase in the pore liquid (up to 577 mgCOD L^{-1} for M3, day 85 and 265 mgCOD L^{-1} for M4, day 89). This increase was followed by an increase in CH_4 emissions (starting on day 75; Figure 2d). Closing the electrical circuit with a 500 Ω resistor on day 72 did not result in a decrease in CH_4 emissions. Conversely, CH_4 emissions increased even further. Methane release from the microcosms did not show any relation with either closing the electrical circuit or lowering the external resistance to 100Ω (day 79; Figure 2b and 2d). The effect of the electrical current on the CH_4 emissions after re-closing the external circuit remains to be determined as the current was markedly lower compared

to the period before the electrical circuit was opened. This was the result of the low activity of the (bio)cathode resulting in an overall low current density (Figure 2b and § 3.3). Methane emissions in these microcosms were indeed facilitated by the aerenchyma of the rice plants. This was established by harvesting the above-ground biomass at the end of the study period and subsequently determining the CH_4 flux. Cutting the biomass in M3 and exposing the aerenchyma to the air resulted in a 14% lower emission the next day (32.3 to 27.8 mgCH$_4$ m^{-2} h^{-1}). Cutting the biomass in M4 and raising the water level to above the aerenchyma resulted in a 85% decrease of emissions the next day (9.5 to 1.4 mgCH$_4$ m^{-2} h^{-1}).

3.7 Bacterial community structure is driven by available organic carbon

Based on the DGGE-profile of the 16S rRNA gene of the bacterial community, the main parameter affecting the enrichment of the various microbial communities was the amount of available electron donor (organic carbon) present at the various locations. This was confirmed by two methods of cluster analysis, Jaccard band based clustering (Figure 5) and via a Pearson correlation clustering (not shown). M3 and M4 were able to support a bacterial community with a higher community organisation (Co) and a higher richness (R) compared to M1 and M2. The higher richness indicates a more developed microbial community. It can be reasoned that this is related to a metabolic network where fermentation of substrates to H_2 plays a key role however this could not be validated with the used technique.

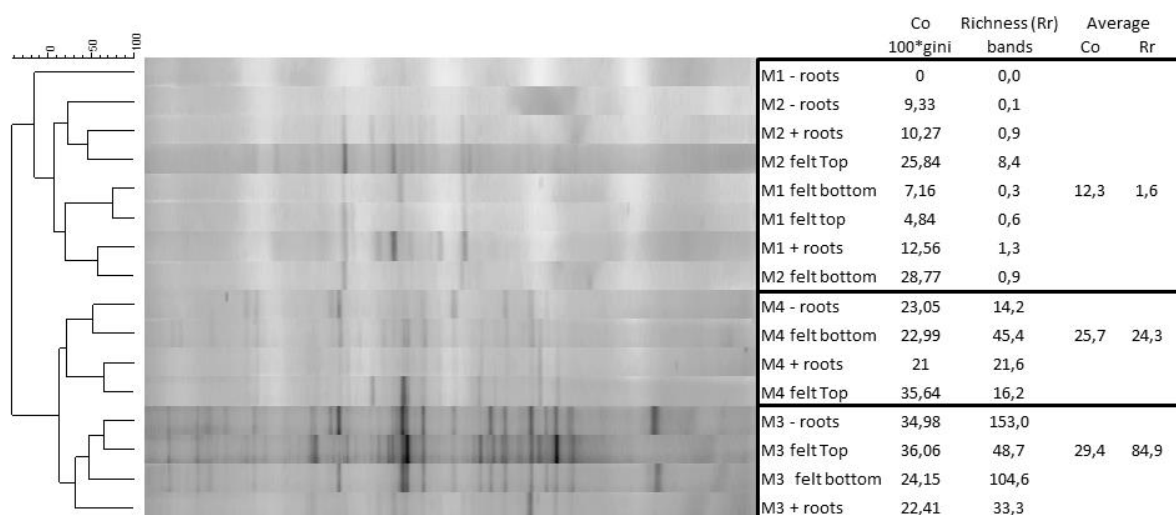|	Co 100*gini	Richness (Rr) bands	Average Co	Average Rr
M1 - roots	0	0,0		
M2 - roots	9,33	0,1		
M2 + roots	10,27	0,9		
M2 felt Top	25,84	8,4		
M1 felt bottom	7,16	0,3	12,3	1,6
M1 felt top	4,84	0,6		
M1 + roots	12,56	1,3		
M2 felt bottom	28,77	0,9		
M4 - roots	23,05	14,2		
M4 felt bottom	22,99	45,4	25,7	24,3
M4 + roots	21	21,6		
M4 felt Top	35,64	16,2		
M3 - roots	34,98	153,0		
M3 felt Top	36,06	48,7	29,4	84,9
M3 felt bottom	24,15	104,6		
M3 + roots	22,41	33,3		

Figure 5: Jaccard bandbased clustering of 16s rRNA-gene based microbial community. Microbial Resource Management (MRM) parameters community organization (Co) and range weighted richness (Rr) are indicated (Read et al. 2011).

3.8 Archaeal community structure

The results of the qPCR analysis of the archaeal microbiome at the end of the experiment indicates that H_2 based methanogenesis (orders *Methanobacteriales* & *Methanomicrobiales*) was likely the dominant process compared to acetate based methanogenesis (Family *Methanosaetaceae*). M1 (low COD concentration in the pore liquid, and resulting low CH_4 emissions) showed a slight enrichment in H_2 consuming *Methanomicrobiales* compared to acetate consuming methanogens (3.9 vs. 3.8 log copies g $^{-1}$, other groups were below detection) at the roots of the rice plants. The microcosms with a higher COD-load (M3 & M4) showed at all sample locations (with and without roots, top and bottom felt) a higher abundance of H_2-consuming methanogens. *Methanosaetaceae* (acetate dependent) were present in a lower abundance compared to *Methanosarcinaceae* (mixotrophic) at the bottom anode (5.2 vs. 6.0 log copies g $^{-1}$ for M3 & 4.7 vs. 5.1 log copies g $^{-1}$ M4) whereas they were more abundantly present at the top anode (5.0 vs. 4.7 log copies g $^{-1}$ for M3 & 4.7 log copies g $^{-1}$ vs. < 3.7 log copies g $^{-1}$ for M4). All archaeal groups were below detection limit at all sample locations for M2, correlating with the low COD concentrations in the rhizosphere. The same was valid for M1 where archaea were only detected in samples with roots. The copy numbers of all four groups of methanogens added up to the copy numbers obtained by means of the total archaea qPCR for all microcosms. This indicates that no other groups were involved in methanogenesis in these microcosms.

4. Discussion

4.1 Anode/rhizosphere organic carbon and redox potential

The low current density recorded for M1 and M2 over the whole experimental period and for M3 and M4 for the first 40 days indicate that exudation of low molecular weight organic acids or sugars were not directly responsible for current generation at the anode. Moreover, no short chain fatty acids were detected in the bulk liquid, indicating that other organic components were the major components of the organic material present. These results are in line with previous research where long start-up times for Plant-MFCs were recorded with pristine anodes (De Schamphelaire et al. 2008; Helder et al. 2010; Helder et al. 2012). The long start-up times indeed suggest that components derived from rhizodeposition other than small molecules released via direct exudation are more important in current generation by plant-sediment MFCs. The model put forward by Timmers et al. (2011) showed that a low amount of reducing equivalents available for current generation was corroborated by the presence of alternative electron acceptors and could possibly be related to radial oxygen loss into the rhizosphere (Liesack et al. 2000). In this work, redox potentials as measured at the anode electrode of M3 and M4 decreased over time, with subsequent increase in bulk gas emissions of CH_4, with increased current generation and a decrease in alternative electron

acceptor concentrations. For both CH_4 and N_2O, favourable redox potentials are needed, i.e. for N_2O E_h >180 mV vs. SHE via denitrification and for CH_4 E_h < -150 mV vs. SHE (Hou et al. 2000; Johnson-Beebout et al. 2009; Yu and Patrick 2003). Bulk methane emissions can be related to redox potentials as determined with the bioanodes. A clear relation was not observed for N_2O emission and bulk anode potentials (Figure 4). This can be attributed to the position of the reference electrode in relation to the location of microbial activity. The top of the microcosm which was exposed to ambient air, while the bulk microcosm was waterlogged.

4.2 Current producing metabolism precedes methanogenic metabolism.

As stated before, organic carbon was instantaneously metabolised to current and not to CH_4. Methane emissions became apparent after interrupting current flow by removing the external load. During this period, organic carbon became available in the pore liquid and CH_4 emissions started to increase. An exploratory study already revealed that at higher organic carbon loads, no difference in methane emissions could be observed between the open and closed circuit conditions. In accordance with the findings in this work, Freguia et al. (2008) have also shown that methanogenesis is a robust process largely operating independent of electrode based processes. This was established in a reactor type anode with a packed bed of similar granular electrode material. Kaku et al. (2008) were also not able to detect decreased CH_4 emissions from Plant-MFCs in closed circuit in a rice field. On the other hand, it was shown that a closed circuit MFC was able to release 10 times less methane compared to an open circuit MFC using a reactor type setup and rice paddy soil as anode inoculum (Ishii et al. 2008). Cabezas de Rosa (2010) showed that it was possible to reduce methane emissions with electrodes in sediment systems by 47%. These seemingly contradicting observations indicate that the electrical circuit and the current generating microorganisms were only able to outcompete methanogenic microorganisms at low organic carbon concentrations (electron-donor) and high electron acceptor concentrations (anode in closed circuit). This concept is supported by the fact that the affinity (Ks) of *Geobacter sulfurreducens* (a known current generating microorganism) for acetate can be as low as 10 µM whereas the lowest affinity for acetate reported for *Methanosaetaceae* is 160 µM (Esteve-Núñez et al. 2005; Qu et al. 2009). For *Methanosarcinaceae* this value is even higher, 3 mM (Qu et al. 2009).

Competition for substrate seemed to be most important as acidification of the bulk pore liquid was not detected. With an average current density of 35 mA m^{-2} (day 45-55 Figure 2b), it can be calculated that about 0.5 mmol of protons are produced per day. This amount of protons produced locally at the anode can indeed cause local acidification. The results indicate however that local buffer capacity, in companion with proton diffusion to the cathode, were able to prevent bulk acidification in the studied microcosms. Lowering the resistance in the external circuit from 500 Ω to

100 Ω did not result in a higher current. This is in conjunction with the observations of Helder et al. on an a rooftop MFC (Helder et al. 2013). Although they were not able to indicate a cause for this phenomena, from this work it could be observed that it was mainly due to a deterioration of cathode performance. The low cathode potentials were most likely caused by an accumulation of organic detritus (e.g. degraded leaves, algae, roots) on the cathode electrode, leading to a low O_2 availability for electrochemical reduction.

Overall, a sequence of steps can be distinguished; initially, during early waterlogging conditions and plant development, a high redox potential due to the presence of O_2 and other soluble electron acceptors is noticed. Gradually organic carbon becomes available in the rhizosphere, electron acceptors are being depleted except for the electrode and redox potential decreases leading to current generation. Over time, more organic carbon becomes available than the current generating bacteria can metabolise and CH_4 production starts and cannot be stopped anymore.

4.3 Archaeal communities in the rhizosphere of plant-MFCs

Few microbial communities of anodes of Plant-MFCs have been elucidated (Cabezas da Rosa 2010; De Schamphelaire et al. 2010; Kaku et al. 2008; Timmers et al. 2012). Of the microbial communities studied, focus has been placed on the anode bacterial community and not on the archaeal microbial community. In the current study methanogenic community slightly dominated by H_2-dependent methanogens was detected. This was also the case in several other studies and sometimes even more pronounced in closed circuit compared to open circuit conditions. De Schamphelaire et al. (2010) have seen a relative enrichment of H_2 utilizing methanogens in Plant-MFCs consisting of a soil or vermiculite rhizosphere with graphite felt electrodes, which only covered a small part of the rhizosphere. Timmers et al., (2012) characterized the microbial populations in the anode of a high current (167 ± 65 mA m^{-2}) vs. a low current (67 ± 58 mA m^{-2}) producing Plant-MFC (*Glyceria maxima*) with the complete anode compartment filled with electrode material. The relatively high current indicated that in that case sufficient organic substrate was present in the anode under reducing conditions. In their study, a selection towards a H_2-based metabolism was detected after 225 days of operation where 95% of the archaea could be related to the genus *Methanobacterium* in the high current producing Plant-MFC vs. a more diverse archaeal community in the low current MFC. The bacterial community supported these results as H_2 producing clostridia were abundantly found in the high current producing systems (Timmers et al. 2012).

Using a sediment MFC, Cabezas da Rosa (2010) found that H_2-based methanogenesis was more important compared to acetate based methanogenesis at the archaeal community level, i.e. *Methanosaetaceae* were less abundant compared to *Methanosarcinaceae* and *Methanomicrobiaceae* in the current generating systems. This result was corroborated by the stable

isotope ratios of CH_4 indicating lower acetoclastic CH_4 production in closed circuit systems (Cabezas da Rosa 2010).

In the microcosms studied in this work, an alternative electron acceptor was introduced in the rhizosphere aimed at lowering the activity of the methanogenic microbial community. As seen in other (plant) MFC based anodes, also here hydrogenotrophic methanogens were most abundantly detected. This indicates that to effectively lower methane emissions by introducing an anode, the focus should be on stimulating hydrogen to current conversions.

4.4 Outlook & challenges

From this longitudinal study several challenges for the control of methane emissions from rice paddies or other wetlands on a larger than lab-scale by means of a bioelectrochemical system can be envisioned. Condensing the limited information that is known on (competitive) inhibition of CH_4 formation in anodes of (sediment) bioelectrochemical systems, it shows that contradicting data are present in the existing literature, because of 1) different inocula, 2) a varying electrode configuration, 3) a varying organic loading rate and 4) a different electrochemical control (Cabezas da Rosa 2010; Freguia et al. 2008; Ishii et al. 2008; Kaku et al. 2008).

A first challenge is the effective range of the anode and the technical application of the bioelectrochemical system. In order to achieve an effect, carbon granules were applied in a 2/3 ratio vs. the amount of vermiculite matrix. Combining these dispersed granules with a suitable current collector and a reference system poses a great challenge. On top of that, agricultural practices(e.g. plowing) can interfere with the bioelectrochemical system. A solution to this challenge might be the use of electron shuttle molecules (phenazines, humic substances etc). However the effectiveness of these molecules in electron transfer processes in soils and sediments needs to be established. Moreover, these molecules can also interfere with other processes than electron transfer.

The second challenge is the question of electrochemical control. In this work, electrochemical control was applied by using an external resistor coupled to an O_2-reducing (bio)cathode. Visual inspection, polarization curves and electrochemical monitoring of the cathode potential indicated deterioration of cathode performance over time. A probable cause for this deterioration is the amount of organic carbon that accumulated on the cathode electrode over time due to growth of algae and dead (plant)biomass. Another option for electrochemical control, is the polarization of the anode electrode at a certain potential (e.g. 0 vs. SHE). This might however lead to large energy investments due to the internal resistance of a sediment-BES configuration (see Supplementary information S2, Figure S1).

A third challenge is the durability of CH_4 emissions mitigation over a long period of time. In this work, it was established that CH_4 emissions could be postponed by introducing a bioanode. However, the

effectiveness on a time scale longer than one growing season needs to be determined. A longer effective working period can possibly be obtained in combination with an anode of conductive biochar in the rhizosphere. As Feng et al. (2012) has shown, biochar is able to increase the abundance of methanotropic bacteria. During an anaerobic period, a conductive biochar can act as an anode, while during an aerobic period it can act as a methanotrophic stimulant. The application of agricultural practices such as mid-season drainage (Xu et al. 2007) to provide a competitive advantage for the current generating microbial community (that is able to withstand O_2 (Freguia et al. 2008; Nevin et al. 2011)) in conjunction with methanotropic stimulation is another option.

In conclusion, It was shown that placing an anode of a bioelectrochemical system in a waterlogged rhizosphere led to current generation before methane emissions started, possible leading to postponement of methane emissions from these anoxic systems in the short term. However, in the long-term (i.e. period of complete rice cropping season), low methane emissions could not be maintained due to an excess of organic matter in the rhizosphere. Based on qPCR analysis of the archaeal community in the rhizosphere/anode plane, H_2 was indicated as the most important precursor for methane production. This shows that there was an effective competition on the level of acetoclastic methanogenesis, but that the competition for H_2 needs to be enhanced to achieve a more effective and sustainable mitigation of methane emissions by means of a bioelectrochemical system. The effect of local acidification due to anodic metabolism on methanogenic metabolism could not be established in this work. To achieve a practical, large scale application of a bioelectrochemical system to mitigate methane emissions, a combined strategy with other methane mitigation approaches is envisioned.

References

Appels L, Baeyens J, Degrève J, Dewil R (2008) Principles and potential of the anaerobic digestion of waste-activated sludge. Prog Energ Combust 34(6):755-781

Arends JBA, Blondeel E, Tennison S, Boon N, Verstraete W (2012) Suitability of granular carbon as an anode material for sediment microbial fuel cells. J Soils Sediments 12(7):1197-1206

Arends JBA, Verstraete W (2012) 100 years of microbial electricity production: three concepts for the future. Microbial Biotechnology 5(3):333-346

Aulakh MS, Wassmann R, Bueno C, Kreuzwieser J, Rennenberg H (2001) Characterization of Root Exudates at Different Growth Stages of Ten Rice (Oryza sativa L.) Cultivars. Plant Biology 3(2):139-148

Aulakh MS, Wassmann R, Rennenberg H, Fink S (2000) Pattern and Amount of Aerenchyma Relate to Variable Methane Transport Capacity of Different Rice Cultivars. Plant Biology 2(2):182-194

Bazhin N (2010) Theory of methane emission from wetlands. Energ Environ Sci 3(8):1057-1072

Boon N, Goris J, De Vos P, Verstraete W, Top EM (2000) Bioaugmentation of Activated Sludge by an Indigenous 3-Chloroaniline-Degrading Comamonas testosteroni Strain, I2gfp. Appl Environ Microbiol 66(7):2906-2913

Cabezas da Rosa A (2010) Diversity and function of the microbial community on anodes of sediment microbial fuel cells fueled by root exudates. Dissertation. Philipps-Universität Marburg

Conrad R (2002) Control of microbial methane production in wetland rice fields. Nutrient Cycling in Agroecosystems 64(1):59-69

Counce PA, Keisling TC, Mitchell AJ (2000) A Uniform, Objective, and Adaptive System for Expressing Rice Development. Crop Sci 40(2):436-443

De Schamphelaire L, Cabezas A, Marzorati M, Friedrich MW, Boon N, Verstraete W (2010) Microbial Community Analysis of Anodes from Sediment Microbial Fuel Cells Powered by Rhizodeposits of Living Rice Plants. Appl Environ Microbiol 76(6):2002-2008

De Schamphelaire L, van den Bossche L, Dang HS, Hofte M, Boon N, Rabaey K, Verstraete W (2008) Microbial fuel cells generating electricity from rhizodeposits of rice plants. Environ Sci Technol 42(8):3053-3058

Denman KL, G. Brasseur, A. Chidthaisong, P. Ciais, P.M. Cox, R.E. Dickinson, D. Hauglustaine, C. Heinze, E. Holland, D. Jacob, U. Lohmann, S Ramachandran, P.L. da Silva Dias, Wofsy SC, Zhang X (2007) Couplings Between Changes in the Climate System and Biogeochemistry. In: Solomon S, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor, Miller HL (eds) Climate Change 2007: The Physical Science Basis Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge United Kingdom and New York, NY, USA

Donovan C, Dewan A, Peng H, Heo D, Beyenal H (2011) Power management system for a 2.5 W remote sensor powered by a sediment microbial fuel cell. J Power Sources 196(3):1171-1177

Esteve-Núñez A, Rothermich M, Sharma M, Lovley D (2005) Growth of Geobacter sulfurreducens under nutrient-limiting conditions in continuous culture. Environ Microbiol 7(5):641-648

Feng Y, Xu Y, Yu Y, Xie Z, Lin X (2012) Mechanisms of biochar decreasing methane emission from Chinese paddy soils. Soil Biology & Biochemistry 46:80-88

Forster P, V. Ramaswamy, P. Artaxo, T. Berntsen, R. Betts, D.W. Fahey, J. Haywood, J. Lean, D.C. Lowe, G. Myhre, J. Nganga, R. Prinn, G. Raga, Schulz M, Dorland RV (2007) Changes in Atmospheric Constituents and in Radiative Forcing. In: Solomon S, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor, Miller HL (eds) Climate Change 2007: The Physical Science Basis Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge United Kingdom and New York, NY, USA

Freguia S, Rabaey K, Yuan ZG, Keller J (2008) Syntrophic processes drive the conversion of glucose in microbial fuel cell anodes. Environ Sci Technol 42(21):7937-7943

Gogoi N, Baruah KK, Gogoi B, Gupta PK (2005) Methane emission characteristics and its relations with plant and soil parameters under irrigated rice ecosystem of northeast India. Chemosphere 59(11):1677-1684

Helder M, Strik DPBTB, Hamelers HVM, Kuhn AJ, Blok C, Buisman CJN (2010) Concurrent bio-electricity and biomass production in three Plant-Microbial Fuel Cells using *Spartina anglica*, *Arundinella anomala* and *Arundo donax*. Bioresour Technol 101(10):3541-3547

Helder M, Strik DPBTB, Hamelers HVM, Kuijken RCP, Buisman CJN (2012) New plant-growth medium for increased power output of the Plant-Microbial Fuel Cell. Bioresour Technol 104:417-423

Helder M, Strik DPBTB, Timmers RA, Raes SMT, Hamelers HVM, Buisman CJN (2013) Resilience of roof-top Plant-Microbial Fuel Cells during Dutch winter. Biomass and Bioenergy 51(0):1-7

Hong S, Chang I, Choi Y, Kim B, Chung T (2009) Responses from freshwater sediment during electricity generation using microbial fuel cells. Bioprocess and Biosystems Engineering 32(3):389-395

Hou AX, Chen GX, Wang ZP, Van Cleemput O, Patrick WH (2000) Methane and nitrous oxide emissions from a rice field in relation to soil redox and microbiological processes. Soil Science Society of America Journal 64(6):2180-2186

Ishii Si, Hotta Y, Watanabe K (2008) Methanogenesis versus electrogenesis: Morphological and phylogenetic comparisons of microbial communities. Biosci Biotechnol Biochem 72(2):286-294

Johnson-Beebout SE, Angeles OR, Alberto MCR, Buresh RJ (2009) Simultaneous minimization of nitrous oxide and methane emission from rice paddy soils is improbable due to redox potential changes with depth in a greenhouse experiment without plants. Geoderma 149(1–2):45-53

Kaku N, Yonezawa N, Kodama Y, Watanabe K (2008) Plant/microbe cooperation for electricity generation in a rice paddy field. Appl Microbiol Biotechnol 79(1):43-49 doi:10.1007/s00253-008-1410-9

Liesack W, Schnell S, Revsbech NP (2000) Microbiology of flooded rice paddies. Fems Microbiol Rev 24(5):625-645

Logan BE, Hamelers B, Rozendal R, Schröder U, Keller J, Freguia S, Aelterman P, Verstraete W, Rabaey K (2006) Microbial Fuel Cells: Methodology and Technology. Environ Sci Technol 40(17):5181-5192

Majumdar D (2003) Methane and nitrous oxide emission from irrigated rice fields: Proposed mitigation strategies. Current Science 84(10):1317-1326

Nevin KP, Pei Z, Franks AE, Woodard TL, Lovley DR (2011) Anaerobes unleashed: Aerobic fuel cells of Geobacter sulfurreducens. J Power Sources 196(18)

Ovreas L, Forney L, Daae FL, Torsvik V (1997) Distribution of bacterioplankton in meromictic Lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. Appl Environ Microbiol 63(9):3367-3373

Qu X, Vavilin VA, Mazéas L, Lemunier M, Duquennoi C, He PJ, Bouchez T (2009) Anaerobic biodegradation of cellulosic material: Batch experiments and modelling based on isotopic data and focusing on aceticlastic and non-aceticlastic methanogenesis. Waste Management 29(6):1828-1837

Rabaey K, Clauwaert P, Aelterman P, Verstraete W (2005) Tubular microbial fuel cells for efficient electricity generation. Environ Sci Technol 39(20):8077-8082

Rabaey K, Verstraete W (2005) Microbial fuel cells: novel biotechnology for energy generation. Trends Biotechnol 23(6):291-298

Read S, Marzorati M, Guimaraes BCM, Boon N (2011) Microbial Resource Management revisited: successful parameters and new concepts. Appl Microbiol Biotechnol 90(3):861-871

Reimers CE, Tender LM, Fertig S, Wang W (2001) Harvesting energy from the marine sediment-water interface. Environ Sci Technol 35(1):192-195

Rozendal RA, Hamelers HVM, Buisman CJN (2006) Effects of membrane cation transport on pH and microbial fuel cell performance. Environ Sci Technol 40(17):5206-5211

Rozendal RA, Hamelers HVM, Rabaey K, Keller J, Buisman CJN (2008) Towards practical implementation of bioelectrochemical wastewater treatment. Trends Biotechnol 26(8):450-459

Singh S, Singh JS, Kashyap AK (1999) Methane flux from irrigated rice fields in relation to crop growth and N-fertilization. Soil Biology and Biochemistry 31(9):1219-1228

Strik D, Hamelers HVM, Snel JFH, Buisman CJN (2008) Green electricity production with living plants and bacteria in a fuel cell. International Journal of Energy Research 32(9):870-876

Tender LM, Gray SA, Groveman E, Lowy DA, Kauffman P, Melhado J, Tyce RC, Flynn D, Petrecca R, Dobarro J (2008) The first demonstration of a microbial fuel cell as a viable power supply: Powering a meteorological buoy. J Power Sources 179(2):571-575

Timmers RA, Rothballer M, Strik DPBTB, Engel M, Schulz S, Schloter M, Hartmann A, Hamelers B, Buisman C (2012) Microbial community structure elucidates performance of Glyceria maxima plant microbial fuel cell. Appl Microbiol Biotechnol 94(2):537-548

Timmers RA, Strik DPBTB, Arampatzoglou C, Buisman CJN, Hamelers HVM (2011) Rhizosphere anode model explains high oxygen levels during operation of a Glyceria maxima PMFC. Bioresour Technol 108 60-67

Xu SP, Jaffe PR, Mauzerall DL (2007) A process-based model for methane emission from flooded rice paddy systems. Ecol Model 205(3-4):475-491

Yu KW, Patrick WH (2003) Redox range with minimum nitrous oxide and methane production in a rice soil under different pH. Soil Science Society of America Journal 67(6):1952-1958

Yu Y, Lee C, Kim J, Hwang S (2005) Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnology and Bioengineering 89(6):670-679

Zhang A, Bian R, Pan G, Cui L, Hussain Q, Li L, Zheng J, Zheng J, Zhang X, Han X, Yu X (2012) Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: A field study of 2 consecutive rice growing cycles. Field Crops Research 127:153-160

Supplementary information: Greenhouse gas emissions from rice microcosms amended with a plant microbial fuel cell.

Jan B. A. Arends[1], Jonas Speeckaert[1], Evelyne Blondeel[1#], Jo De Vrieze[1], Pascal Boeckx[2], Willy Verstraete[1], Korneel Rabaey[1], Nico Boon[1]*

[1]Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, B-9000 Gent, Belgium.
[2]Isotope Bioscience Laboratory (ISOFYS), Ghent University, Coupure Links 653, B-9000 Gent, Belgium.
Currently at: EnBiChem, HOWEST, Graaf Karel de Goedelaan 5, B-8500 Kortrijk, Belgium

*Corresponding author. Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, B-9000 Ghent, Belgium;
phone: +32 (0)9 264 59 76;
fax: +32 (0)9 264 62 48;
E-mail: Nico.Boon@ugent.be;
Webpage: www.labmet.Ugent.be.

This supplementary information contains:

Supplementary information S1. Example calculation to convert current density, chemical oxygen demand removal rate and current in the same units

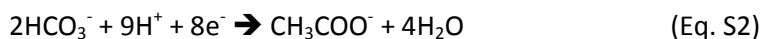Electrical current density (A m^{-2}), chemical oxygen demand removal rate (gCOD d^{-1}) and methane emissions (mgCH$_4$ m^{-2} h^{-1}) can all be recalculated into the same units. All three parameters express, in essence, the movement of electrons. Here some example calculations are presented to clarify these conversions.

Electrical current density J (A m^{-2}), can be expressed as the amount of charge Q, expressed in coulomb (C) per area A (m^2) per time t (s)

$$Q/(t*A) = J \ (A \ m^{-2}) \quad\quad\quad\quad (Eq. \ S1)$$

The amount of charge can be expressed as mole of electrons by conversion with the Faraday constant (96485 C mol (e$^-$)$^{-1}$). Thus electrical current density is a measure of the amount of charge that moves in a given time through a certain area.

Organic matter removal can be expressed as chemical oxygen demand (COD) removal per day. This can also be expressed as the movement of charge in a given time. For example acetate oxidation with O$_2$ can be written according to two half reactions, the first is the oxidation of acetate and the second the reduction of O$_2$ to water.

$$2HCO_3^- + 9H^+ + 8e^- \rightarrow CH_3COO^- + 4H_2O \quad\quad (Eq. \ S2)$$
$$2O_2 + 8e^- + 8H^+ \rightarrow 2H_2O \quad\quad\quad\quad (Eq. \ S3)$$

From these two half reactions it can be established that 1.1 g of O$_2$ (32 g mol^{-1}) is needed for the oxidation of 1 g of acetate (59 g mol^{-1}). Moreover, as can be seen in S2 and S3, electrons are being

displaced. Accordingly, the oxidation of organic matter per area per time can be expressed as a current density. For example oxidizing 1 g organic matter per m^2 per h results in a current density of 3.4 A m^{-2}.

$$1 \text{ (gCOD m}^{-2})/32 \text{ (g mol}^{-1} O_2) * 4 \text{ (mol (e}^-)\text{mol}^{-1} O_2) *1/3600 \text{ (h s}^{-1})*96485 \text{ (C mol (e}^-)^{-1}) = 3.4 \text{ A m}^{-2}$$

Methane removal or production can also be expressed as a current density when applying the same reasoning.

$$1HCO_3^- + 9H^+ + 8e^- \rightarrow 1CH_4 + 3H_2O \qquad \text{(Eq. S4)}$$
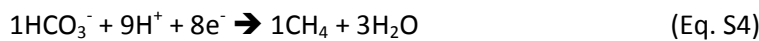
For example producing 10 mgCH$_4$ per m^2 per h results in an equivalent current density of 0.13 A m^{-2}

$$10*10^{-3} \text{ (gCH}_4 \text{ m}^{-2})/16 \text{ (g mol}^{-1} CH_4) * 8 \text{ (mol (e}^-) \text{ mol}^{-1} CH_4) *1/3600 \text{ (h s}^{-1})*96485 \text{ (C mol (e}^-)^{-1}) = 0.13 \text{ A m}^{-2}$$

Conversely, current density can be expressed in equivalent units such as gCOD d^{-1} or mg CH_4 m^{-2} h^{-1} enabling fast comparison of the various metablic rates. See also Arends et al. (2012)

Supplementary information S2. Estimation of electrical operating costs for a poised anode in a wetland

To estimate the amount of electrical power and thus the electrical operating cost per m^2 of plant-MFC in case of anode potentiostatic control, several parameters influence the outcome of this estimation. One important parameter is the distance between the reference electrode and the anode electrode. This causes an ohmic voltage drop that must be compensated for to achieve a good potentiostatic control of the anode. This ohmic voltage drop is dependent on the current that flows through the system (this can be understood as the methane mitigation rate) and the resistivity of the matrix (in this case rice or wetland soil). Here an estimation is made for the following case, a wetland soil with 1 reference electrode, a maximum effective anode-reference distance of 1 m and an energy price of € 0.20 kwh^{-1}. Soil resistivity was varied between 0 and 50 Ω m (Domínguez-Garay et al., 2013) and current density between 10 and 300 mA m^{-2} which is equivalent to 0.13-3.9 mgCH$_4$ m^{-2} h^{-1} (Eq. S4). Within these parameters 55 scenarios were calculated which are depicted in Figure S1. From this estimation it can be seen that when aiming for an appreciable methane mitigation rate, a considerable monthly cost is incurred. Options to reduce this cost are placing more reference electrodes (extra capital cost) or opting for less sophisticated electrochemical control by using an external resistance.
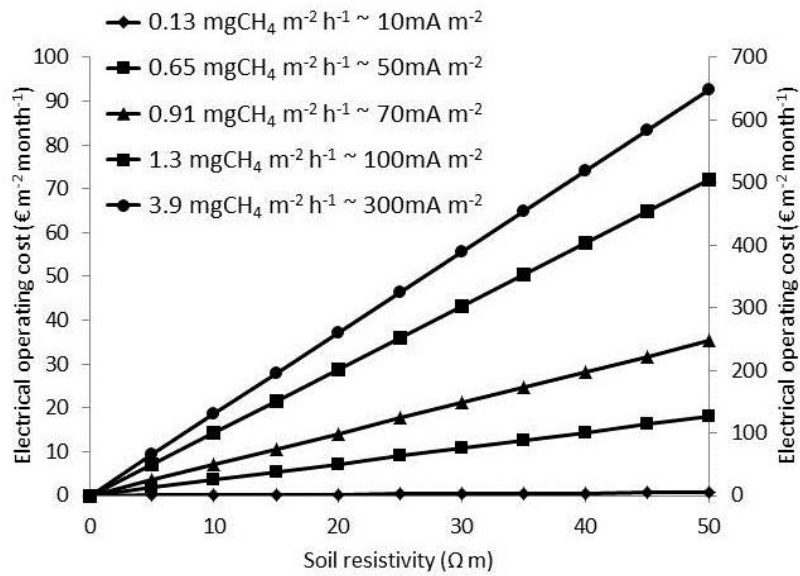
Figure S1: Estimation of electrical operating costs for a poised anode in a wetland based on soil resistivity and current density. Scenario of 3.9 mg CH$_4$ m^{-2} h^{-1} ~ 300 mA m^{-2} is depicted on the right axis.

Other capital costs such as anode and cathode materials, electrical wiring and control periphirals have already been described elsewhere for BESs (Foley et al. 2010; Rozendal et al. 2008). On the profit side a small amount of electrical power can be noted (when using MFC mode) and one can possibly enter in a CO$_2$-certificate trading scheme when emissions are succesfully lowered.
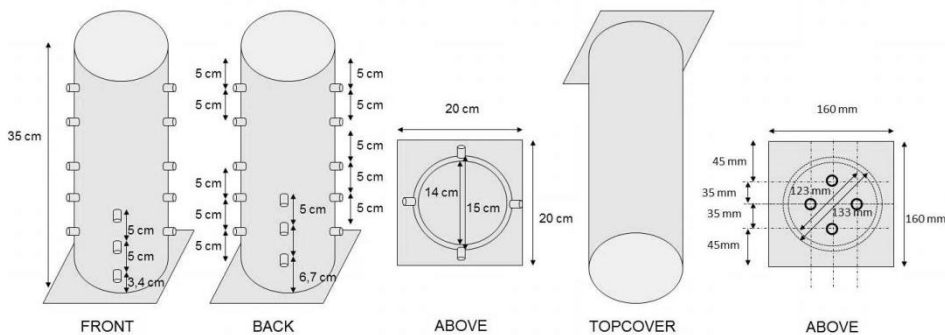

Figure S2: Left 3 schematics: bottom part of the microcosm used for rice growth. The top ledge contained a 2 cm wide rim (not shown) with a 1 cm wide slot to accommodate the closed chamber for headspace gas measurements. Right 2 schematics: Chamber to allow headspace gas measurements on the microcosms.
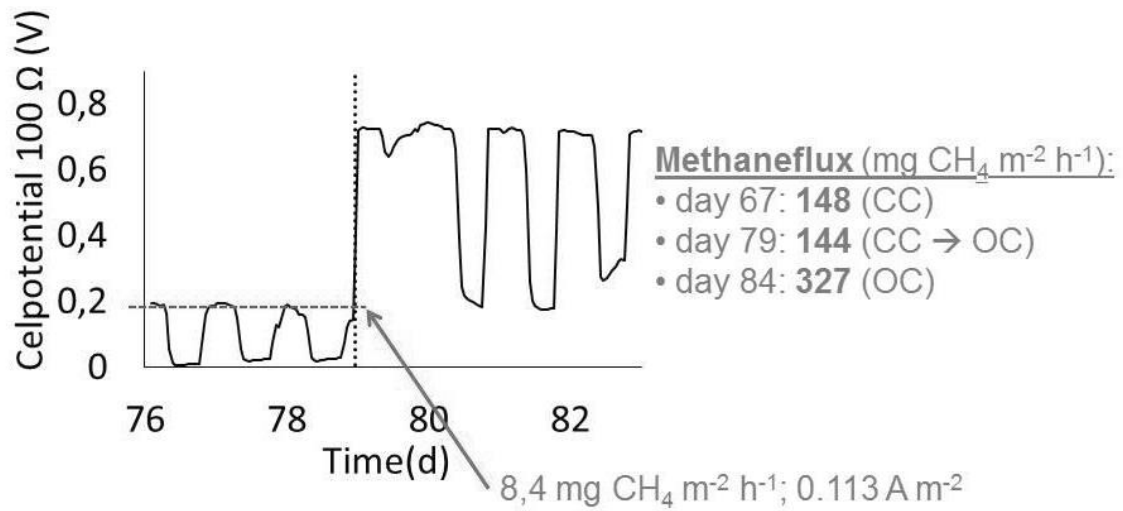
Figure S3: Result from the exploratory study where removing the external resistance resulted in an increased CH₄-flux 22 times higher than can be estimated based on the current in closed circuit. CC: Closed Circuit, OC: Open Circuit.

Table S1: Overview of all experiments. 1) Composition see main text. 2) amount of granules based on previous work (Arends et al., 2012) 3) only current collectors present in the anode compartment. 4) non-planted systems are considered as sediment-MFC 5) liquid was added here without organic carbon. OC: open circuit. CC: closed circuit over 500 Ω external resistance. A: anodic effluent of a well performing MFC in the lab 10 vol-% total anode compartment. M: anaerobic methanogenic sludge from WWTP 'Ossemeersen' Gent, Belgium 1 vol-% of total anode compartment.

#	# of plants	Seedling age at transplanting (week)	Added organic carbon (kg m^{-2})[1]	OC/CC	Inoculum (A and/or M)	Granules in anode [2]	Plant growth area (m^2)
Exploratory study							
E1	2	7	2	OC	A & M	Y	0.015
E2	2	7	2	CC	A & M	Y	0.015
E3	2	7	2	CC	A & M	No [3]	0.015
E4	-[4]	-	2	OC	A & M	Y	0.015
E5	-	-	2	CC	A & M	Y	0.015
E6	2	12	2	OC	A & M	Y	0.032
E7	2	12	2	CC	A & M	Y	0.032
E8	2	12	No	CC	A & M	Y	0.032
E9	-	-	2	CC	A & M	Y	0.032
E10	-	-	2	CC	A	Y	0.032
Detailed study		(kg m^{-2} d^{-1})[1]					
M1	2	3	No	CC	A	Y	0.015
M2	2	3	No[5]	CC	A	Y	0.015
M3	2	3	0.013	CC	A	Y	0.015
M4	2	3	0.013	CC	A	Y	0.015

Table S2: Quality control parameters for qPCR analysis. Parameters as obtained during analysis with StepOnePlus software V2.2.2. Detection limit calculated to copies/gram wet weight of the sample taking dilution and extraction efficiency into account.

	Amplification plot		Standard Curve			
	Threshold (ΔRn)	Baseline	Slope	R^2	Eff (%)	Detection limit (copies g^{-1})
Total Bacteria	3.1	3	-3.3	0.98	100	3.1×10^5
Total Archaea	4.5	3	-3.2	0.97	100	4.4×10^3
Methanobacteriales	2.9	3	-3.6	0.99	88	3.9×10^3
Methanomicrobiales	2.6	3	-4.0	0.99	78	3.6×10^3
Methanosarcinaceae	4.5	3	-3.7	1.00	86	4.8×10^3
Methanosaetaceae	2.6	3	-3.6	0.99	91	5.1×10^3

Supplementary references

Arends, J.B.A., Verstraete, W., 2012. 100 years of microbial electricity production: Three concepts for the future. Microbial Biotechnology 5, 333-346.

Domínguez-Garay, A., Berná, A., Ortiz-Bernad, I., Esteve-Núñez, A., 2013. Silica colloid formation enhances performance of sediment microbial fuel cells in a low conductivity soil. Environmental Science & Technology.

Foley, J.M., Rozendal, R.A., Hertle, C.K., Lant, P.A., Rabaey, K., 2010. Life cycle assessment of high-rate anaerobic treatment, microbial fuel cells, and microbial electrolysis cells. Environmental Science & Technology 44, 3629-3637.

Rozendal, R.A., Hamelers, H.V.M., Rabaey, K., Keller, J., Buisman, C.J.N., 2008. Towards practical implementation of bioelectrochemical wastewater treatment. Trends in Biotechnology 26, 450-459.