GLOBAL CHANGE BIOLOGY
BIOENERGY

GCB Bioenergy (2015) **7**, 244–252, doi: 10.1111/gcbb.12117

Electrical energy production from forest detritus in a forested wetland using microbial fuel cells

JIANING DAI, JUN-JIAN WANG, ALEX T. CHOW and WILLIAM H. CONNER

School of Agriculture, Forest, and Environmental Sciences, The Belle W. Baruch Institute of Coastal Ecology & Forest Science, Clemson University, PO Box 596, Georgetown, SC 29442, South Carolina

Abstract

Microbial fuel cell (MFC) technology has shown great potential for harvesting energy from waste organic materials. Here, we explored the potential of MFC-based electricity generation from forest detritus, a large untapped biomass pool. Electricity generation from *in situ* MFCs and relevant environmental parameters (i.e., carbon sources and concentrations, temperature, water depth) in a seasonally flooded freshwater cypress-tupelo wetland were monitored intensively for two flooding periods. Current outputs ranged from 0 to 1.27 mA (mean of 0.40 mA for flooding period) and were highly sensitive to environmental changes, showing seasonal and diel dependences. Excluding the influence of heavy storms, drought, or wetland icing, current output was highly temperature-dependent dielly. Seasonally, current output gradually increased in the first 3–4 months (limited by temperature) and decreased slightly during the last 1–2 months (probably limited by carbon and nutrients) of both flooding periods. Litter extract of baldcypress (*Taxodium distictum*) with lower C/N ratio and aromatic content showed greater stable current outputs (0.57 mA) based on 50 mg l^{-1} biological oxygen demand compared to extracts of water tupelo (*Nyssa aquatica*) and longleaf pine (*Pinus palustris*), suggesting that the current output of *in situ* MFCs could depend on the vegetation within a wetland. Our study highlights the potential application of MFC in generating green and sustainable electricity from forest biomass for powering remote sensors in wetland ecosystems.

Keywords: dissolved organic carbon, humus layer, plant litterfall, power production, swamp, temporal variation

Received 14 May 2013 and accepted 9 July 2013

Introduction

Microbial fuel cell (MFC) technology allows the harvesting of electrical energy via microbial utilization of biodegradable organic substrates like wastewater (He *et al.*, 2005; Rabaey & Verstraete, 2005; Logan *et al.*, 2006; McCarty *et al.*, 2011; Logan & Rabaey, 2012; Sevda *et al.*, 2013). A variety of bio-molecules (e.g., protein and carbohydrates) and complex organic matrices (e.g., sludge from wastewater treatment tank, corn stover biomass, paddy soils, and seafloor sediments) have been proven to be capable of generating electricity via MFCs (Rabaey *et al.*, 2005; Heilmann & Logan, 2006; Xu *et al.*, 2008; Thygesen *et al.*, 2009; Pant *et al.*, 2010). Forest litter represents a sustainable biomass and bioenergy pool, but has not been considered a source to generate electricity directly through MFC technology. In particular, forested wetlands commonly have a thick layer of humus under the water along with well-aerated surface waters (Chow *et al.*, 2013), which can serve as the anaerobic and aerobic compartments of *in situ* MFCs, respectively, thus making this application feasible.

In situ MFCs have been tested to generate electricity in several different natural environments (Donovan *et al.*, 2008; Pant *et al.*, 2010). For example, a benthic MFC installed on the sea floor powered temperature and oxygen sensors with a power density of 44 mW m^{-2} (Gong *et al.*, 2011). An *in situ* MFC installed in a rice paddy field generated 6 mW m^{-2} (Kaku *et al.*, 2008). Notably, the performance of *in situ* MFC was influenced by many environmental factors, such as temperature, nutrient dynamics, and other physical and biogeochemical characteristics of the sites (Liu *et al.*, 2005; Hong *et al.*, 2009; Dunaj *et al.*, 2012). Therefore, performance of *in situ* MFCs in different ecosystems could be site specific, and knowledge gained from a specific ecosystem may not be directly applied to other environments. Detailed studies on the association of electricity generation patterns and environmental conditions are desirable before any full-scale *in situ* MFC application.

Seasonally flooded cypress-tupelo wetlands, dominated by baldcypress (*Taxodium distictum*), water tupelo (*Nyssa aquatica*), and swamp tupelo (*Nyssa sylvatica* var. *biflora*), are widespread in coastal regions of the

Correspondence: Jun-Jian Wang, tel. +843 546 1013, fax +843 546 6296, e-mail: junjiaw@clemson.edu

southeastern United States (Conner *et al*., 2007). Most of the tree species found in wetlands are deciduous species with high inputs of fresh litter between October and December (Busbee *et al*., 2003; Chow *et al*., 2013). Depending on the tree density and composition of a wetland, monthly leaf litterfall can range from 371 to 582 g m^{-2} in the fall (Watt & Golladay, 1999; Busbee *et al*., 2003). Approximately, half of the annual leaf litter is degraded within a year (Battle & Golladay, 2007), suggesting a high proportion of biodegradable carbon from the foliar litter. Indeed, high levels of dissolved organic carbon (DOC) and nutrients like nitrogen and phosphorus have been found in surface and soil waters within cypress-tupelo wetlands (Anderson & Lockaby, 2007; Chow *et al*., 2013), which could provide abundant nutrients for microbial reactivity. Importantly, these foliar litters are replenished naturally every year, suggesting potential and sustainable substrates for *in situ* MFCs.

Here, we hypothesize that the forest detritus in a forested wetland could be used to generate substantial electrical energy directly via MFCs. We installed two *in situ* MFCs in a typical cypress-tupelo wetland, and analyzed the diel and seasonal dynamics of electricity production and associated environmental factors, including temperature, water level, and nutrient concentrations. Also, we developed two types of controlled laboratory MFCs (dual-chamber reactors and column reactors) using litter extract and humus material as substrates to examine the applicability of MFCs in forested wetlands and the individual effects of environmental factors on electricity production.

Materials and methods

Site description and in situ MFC configuration

Two *in situ* MFCs were installed and operated from November 2010 to May 2012 in a freshwater cypress-tupelo forested wetland on Hobcaw Barony near Georgetown, South Carolina. The site, identified as 'Crabhaul' from here on, was chosen because it has been well studied in terms of vegetation types and density, productivity, litterfall, nutrient dynamics, and hydrology (Busbee *et al*., 2003; Chow *et al*., 2013). Water level had been continuously monitored since 2003. Temperature and precipitation had been continuously monitored since November 2010. Grab samples of surface and subsurface (0.5 m below ground) waters were collected and analyzed for inorganic nitrogen, total phosphate, and DOC and dissolved total nitrogen (DTN) weekly in the first flooding period and monthly in the second flooding period. Detailed analytical techniques and nutrient dynamics of the study period in the Crabhaul wetland can be found in Chow *et al.* (2013).

Each *in situ* MFC was composed of an array of graphite rods with a diameter of 0.6 and 30 cm long (Poco EDM; Saturn Industries, Hudson, NY, USA) with a surface area (SA) of 246 cm^2 as the anodes, an array of graphite fiber brushes (Mills-Rose Co., Mentor, OH, USA) as cathodes (SA = 0.66 m^2), and a 100 ohm resistor (Fig. S1, Table S1). The anodes were buried about 5 cm below the soil–water interface, whereas the cathodes floated freely on the water surface.

Column MFC reactors

Two-phase (water and sediment) column reactors mimicking the wetland environment were used to study effects of operating electrode distance (reflecting the water level in field) and temperature on MFC performance. Transparent plexiglass columns (10 cm inside diameter with 30 cm height) were all filled with a 5 cm sand layer at the bottom and a 15 cm soil humus layer on top. The surface humus (0–15 cm) was collected from the studied forested wetland and placed directly into the MFC column reactors without any treatment. To examine the effect of electrode distance, MFC column reactors with different distances from soil–water interface to water surface (H: 5, 10, 35 cm) or anode depth (D: 1, 5, 15 cm; from center of anode to the soil–water interface) were operated under temperature-controlled laboratory conditions (25 ± 2 °C) for 10 days. In these MFC reactors, the anode was a graphite fiber brush (0.88 m^2) buried below the water–soil interface. The cathode was a 10 cm^2 carbon cloth coated with a Pt-catalyst (0.5 mg cm^{-2}, 10% Pt, Fuel Cell Earth Co., Stoneham, MA, USA) floating on the water surface (Table S1). For all the column reactors, internal resistances were measured by polarization curves after 2 days when the voltage became stable. The series of loads used in internal resistance measurement were 100 000, 30 000, 3000, 600, 100, 20, 4, and 0.8 ohm.

To examine the effect of temperature, column MFC reactors with 2 replicates were placed in both an indoor temperature-controlled laboratory (28 ± 2 °C) and in a shaded outdoor environment for 10 days. A 0.5 cm thick piece of graphite felt (Weaverind, Denver, PA, USA) with a surface area of 32 cm^2 buried 3 cm below the water–soil interface were used as anodes instead of graphite fiber brush for this test (Fig. S2). The water level was set at 10 cm above the soil–water interface. Temperature loggers were buried close to the anodes and they recorded data every 30 min. A 10 000 ohm resistor was used to complete the circuit.

Dual-chamber MFC reactors

Dual-chamber reactors with only an aqueous phase as substrate were used to evaluate the electricity production from leaf litter extracts of three plant species in multi-level concentrations. Each of the custom-made plexiglass reactors was composed of an equal volume (0.2 l, holding 0.18 l solution) of anode and cathode chambers (Fig. S3) and a rinsed 16 cm^2 anion exchange membrane (International Membrane Co., Ringwood, NJ, USA) (Kim *et al*., 2007). The MFC circuit contained a rinsed graphite fiber brush (Mills-Rose Co.) with SA of 0.22 m^2 as the anode, a 10 cm^2 10%-Pt/carbon cloth as the cathode, and a 10 000 ohm resistor (Table S1). An inoculum of 0.18 l containing 20% anaerobic digester sludge (by volume)

from the Georgetown South Carolina Wastewater Treatment Plant and nutrient medium (0.5 g l^{-1} sodium acetate + 100 mM phosphorous buffer solution) was fed into the anode compartment. After inoculation and biofilm formation, the nutrient medium was replaced with litter extract. During operation, air was continuously pumped into the cathode chamber, and in each replenishment process, litter extract was purged with N_2 for 15 min to deplete the O_2 in the anode chamber. To evaluate the sustainability of the reactors, litter extract was replenished in the anode chambers for at least 3 cycles.

Water extracts of leaf litter from three common tree species found in coastal South Carolina forested wetlands, including baldcypress, water tupelo, and longleaf pine, were used as organic substrates. Fresh litterfall was collected using litterfall traps in Fall 2010. The leaf litter was separated by species, oven-dried (70 °C), ground, and sieved (1 mm pore size). Then, 12 g of litter was soaked in 0.4 l deionized water at room temperature (18–22 °C) for 4 h, and all extracts were filtered through a 0.45 μm membrane filter (Supor-450 Pall Corporation, Ann Arbor, MI, USA). Original and diluted extracts (diluted by 10, 50, 100, and 125 times with deionized water) were fed into MFCs individually to evaluate concentration effects on the performance of MFCs. In addition, to compare extracts of different plant species, all three extracts were diluted to 50 mg l^{-1} of biological oxygen demand for 5 days (BOD_5) and fed into the dual-chamber reactors. All extracts were used within 48 h after extraction. Small amounts of Na_2HPO_4 and NaH_2PO_4 were added to the diluted samples to bring the phosphorous buffer solution concentration to 100 mM.

The BOD_5 of litter extracts before and after MFC treatment were measured according to standard method #5210 (American Public Health Association, 1992). Dissolved organic carbon and DTN of litter extracts were analyzed from filtered extracts using a TOC-5000 TOC/TN Analyzer (Shimadzu Corporation). Ultraviolet absorbance from 200 to 700 nm was measured using a quartz cell with a 1 cm path length in an UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) and the water extractable organic carbon (WEOC) and water extractable total nitrogen (WETN) in mg g^{-1} were calculated based on the litter weight. Specific ultraviolet absorbance at 254 nm (SUVA) was calculated as UV_{254}/DOC (in l mg-C^{-1} m^{-1}). The spectral slope ratio (S_R) was calculated as the ratio of two spectral slopes between 275–295 nm and 350–400 nm. The E2/E3 ratio was calculated as absorbance at 254 nm divided by absorbance at 365 nm. SUVA, S_R, and E2/E3 ratios are common optical indices representing aromaticity, molecular weight, and reactivity of dissolved organic matter (DOM), respectively (Helms *et al.*, 2008; Dalrymple *et al.*, 2010). The fluorescence excitation-emission matrix (EEM) was measured in a 1 cm quartz cuvette with a spectrofluorometer RF5301 (Shimazu Corporation) for each water sample after its $UV_{254}$was diluted between 0.2 and 0.3 cm^{-1} (Miller *et al.*, 2010; Zhou *et al.*, 2013). The slit width of both excitation and emission was set at 5 nm. Each sample was scanned within the excitation wavelength from 240 to 450 nm with a 5-nm interval and the emission wavelength from 350 to 550 nm with a 1-nm interval.

Voltage recording and current calculation

The voltage output of *in situ* MFCs was recorded every 30 min by voltage loggers VR-71 (T & D Corporation, Nagano, Japan), whereas the dual-chamber and column MFC reactors were recorded every 15 and 10 min, respectively, by voltage loggers Keithley 2700 (Keithley Instruments Inc., Cleveland, OH, USA). The current output was calculated as the voltage output divided by external resistance.

Calculation for BOD utilization

With the differences in BOD before and after MFC treatments, Eqn 1 was proposed to elucidate the utilization of BOD (in mg l^{-1}) during the electricity generation process:

$$BOD_{initial} = BOD_{residue} + BOD_{electricity} + BOD_{growth}, \quad (1)$$

where $BOD_{initial}$ and $BOD_{residue}$ are the BODs of the litter extract before and after feeding into MFC, BOD_{growth} is the BOD for microbial growth and metabolism, and $BOD_{electricity}$ is the BOD for electricity generation calculated by the following:

$$BOD_{electricity} = \frac{MW_{O2}\int_0^t \frac{U}{R}dt}{FN_{ez}V}, \quad (2)$$

where U is the voltage output over the resistor (V), R is resistance (Ω), MW_{O2} is the molecular weight of oxygen gas (g mol^{-1}), F is Faraday's constant (96485 s A mol^{-1}), N_{es} is the number of electrons exchanged per mole of oxygen ($O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$), Δt is the reaction time (s), and V is the volume of litter extract fed into the MFC reactors (m^3).

Total electrical energy generation in dual-chamber MFC reactors (E) was calculated as (Cheng *et al.*, 2006):

$$E = \int_0^n Pdt \approx \sum_{i=1}^{n} P_i\Delta t = \sum_{i=1}^{n}\left(\frac{U_i^2}{R}\right)\Delta t \quad (3)$$

Statistical analyses

The student's t-test was adopted for comparing the difference of electricity output of *in situ* MFC between two flooding periods, and the ANOVA followed by post hoc Tukey's test was used for comparison the water quality of litter extracts. The Pearson's correlation were performed between electricity output and environment factors. All statistical analyses were implemented using SPSS v15.0.

Results

Performance of in situ MFCs in a forested wetland

Current output of the *in situ* MFC, temperature, water level, and water quality of the studied wetland were monitored between November 2010 and May 2012 (Fig. 1). The *in situ* MFCs were able to generate continuous electricity during flooding periods but only pulses

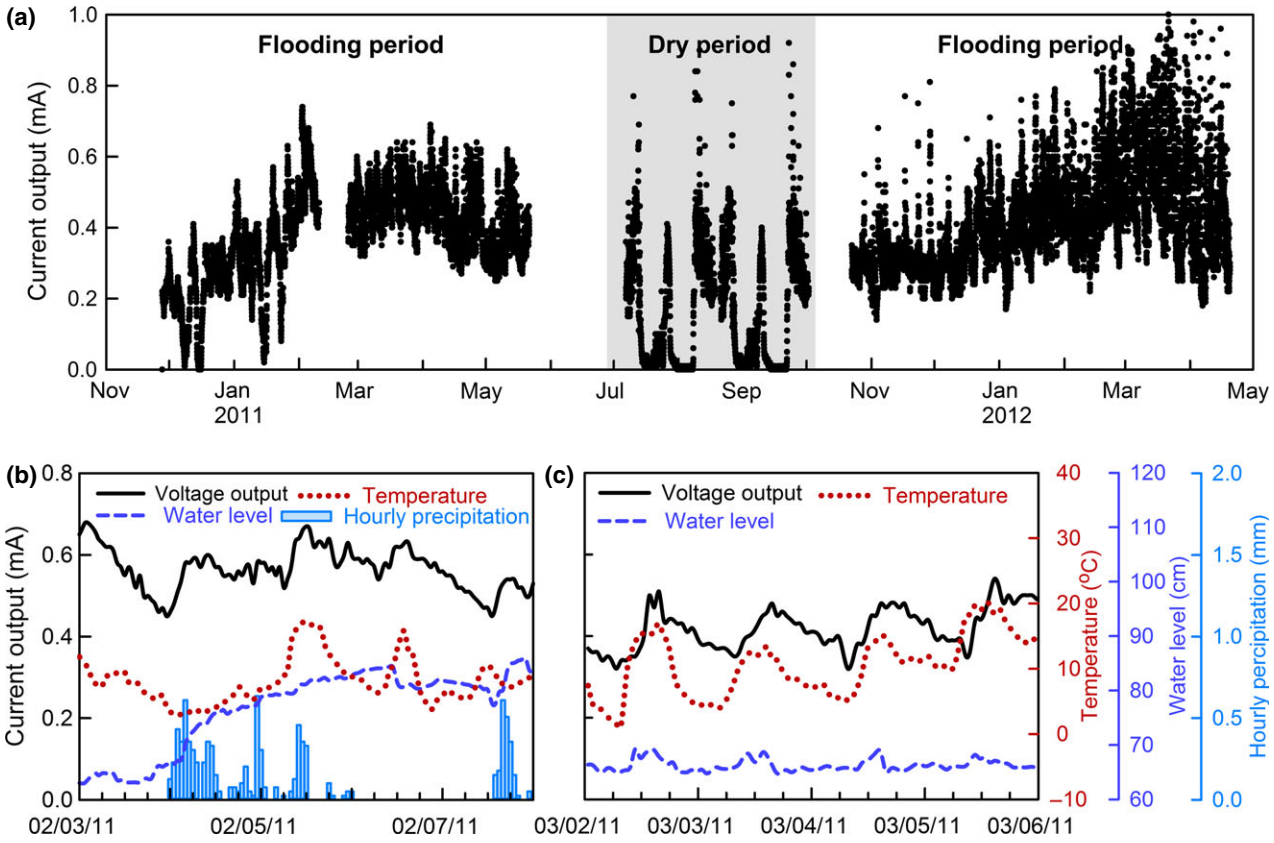

Fig. 1 Current output from *in situ* microbial fuel cells at seasonal scale (a), and its variation compared to those of temperature, water level, and hourly precipitation during raining (b) and ordinary days (c) at diel scale.

of electricity in storm events during the dry season (July to September).

In both flooding periods from November to May, the daily mean current output showed similar trends, that is, roughly increased and doubled in the first 3–4 months, followed afterward by a 1- to 2-month decrease. No significant difference was observed between the two periods ($P > 0.1$), and the average current output of the two flooding periods in was 0.40 mA. There were some zero volt points during the wintertime in the first period but not the second flooding period. The absence of current outputs occurred when the temperature dropped below 0 °C and a thin layer of ice was formed. During long flooding periods, the mean current output, either of 1 day, 3 days or 1 week, did not show any significant correlation with DOC or other nutrient concentrations ($P > 0.1$). During the dry period, electricity generation stopped when there was no surface water to maintain MFC circuit connections. There were several narrow and high current peaks showing the pulses of electricity generation during short periods of standing waters following heavy rainstorms (Fig. 1a).

During short periods of flooding (i.e., within days), current output oscillations significantly depended on temperature and water level. There was commonly a 2–6 h lag response of current output with a temperature change (i.e., temperature peak vs. current peak) (Fig. 1c). During some heavy rain days, the dependence of current on temperature was relatively weakened compared to ordinary days (e.g., the storm event on 4 February 2011, as shown in Fig. 1b). In addition, at the beginning of each storm event, current output increased rapidly for the first several hours.

Response of column MFCs to temperature and electrode distance

In outdoor MFC reactors, current output closely followed diel temperature oscillation but with 1–2 h lag time, even with a narrow temperature fluctuation (25.9–29.9 °C) (Fig. 2). Indoor column MFCs fluctuated between 0.052 and 0.056 mA with an average of 0.054 mA in a temperature range of 4 °C. Outdoor MFCs that experienced a wider temperature fluctuation (20.0–29.0 °C) had a much wider current output range (0.038–0.050 mA) with an average of 0.046 mA.

The electrode distance experiment using MFC column reactors showed that the internal resistance increased

with electrode distance between the cathode and anode (Table 1). As internal resistance increased, current output decreased. Taking the 3k-ohm resistor as an example (Table 1), current outputs (*I*) were 70.8, 67.7, and 27.3 μA, when cathode heights (*H*) were 5, 10, and 35 cm, respectively. The current outputs (*I*) were 70.8, 70.0, and 67.7 μA, when anode depths (*D*) were 1, 5, and 15 cm, respectively. Apparently, water level also played an important role on the performance of MFC.

Response of MFC to different litter substrates

Baldcypress leaf litter showed unique chemical characteristics compared to those of water tupelo and longleaf pine (Table S2). Among the three species, it had the lowest WEOC/WETN ratio (23.45 mol mol^{-1}) and lightest extract color (Fig. S4) with a SUVA of 0.79 l mg-C^{-1} m^{-1} (Table 2). Water tupelo and longleaf pine had similar WEOC/WETN ratios, but water tupelo's extract had the most intensive color (Fig. S4) and the highest SUVA (3.52 l mg-C^{-1} m^{-1}).

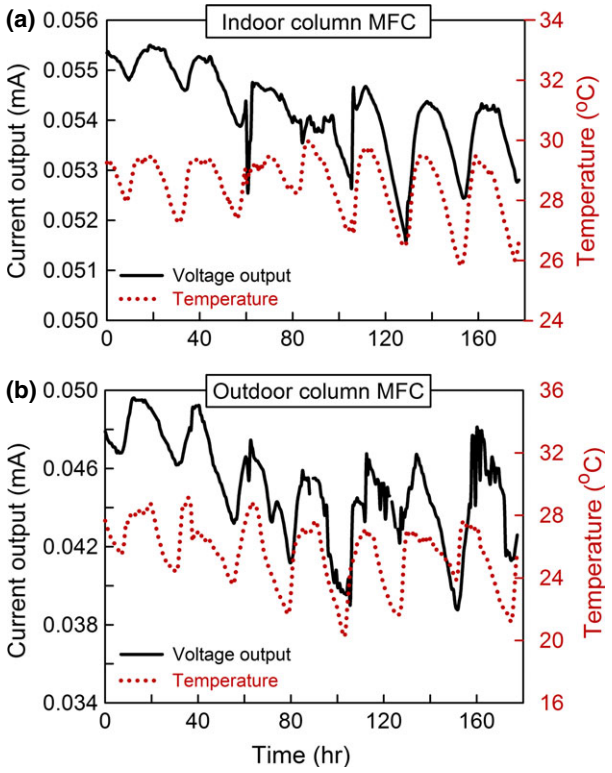

Fig. 2 Current output of indoor (a) and outdoor (b) column microbial fuel cells varied with fluctuating temperature (8 cycles).

As shown in Fig. 3, baldcypress extract generated the highest electrical signal at a stable current level of 0.57 mA, compared to extracts of water tupelo (0.50 mA) and longleaf pine (0.46 mA), or the 2 g l^{-1} sodium acetate (0.56 mA) (Fig. S6). All three extracts were utilized rapidly in the MFCs, and maximum currents appeared within 3–5 h reaction time. Baldcypress extract had the longest stable power output (>30 h) and the shortest 'shut-off' time (from maximum to 0.050 mA) (<5 h). After generating electricity, BOD_5 concentration in baldcypress, water tupelo, and longleaf pine extracts decreased 60%, 46%, and 44%, and DOC concentration decreased 52%, 17%, and 15%, respectively. Greater decreases in BOD_5 compared to DOC showed the consumption of readily bio-degradable DOC in MFC reactions. Also, significant changes in optical properties including SUVA, S_R, E2/E3 ratio and fluorescence EEM spectra were observed (Table 2).

Taking baldcypress extracts as an example, the effect of substrate concentration on electricity generation in MFC was showed in Fig. 4a. With a 20 h reaction time, the original and the 10-times diluted baldcypress extract generated similar current outputs and had the highest stable current levels among other diluted extracts, suggesting the dual-chamber reactor reached its upper limit in electricity generation at about 50 mg l^{-1} BOD_5. With lower concentrations of litter extract, the maximum current outputs of MFCs decreased and no stable current output was able to be achieved. With a dilution factor of 50 (BOD_5 of ca. 10 mg l^{-1}), the maximum current was 0.48 mA, and it dropped rapidly after the peak. Similarly, the maximum currents at dilution factors of 100 (BOD_5 of ca. 5 mg l^{-1}) and 125 (BOD_5 of ca. 4 mg l^{-1}) were only about 0.22 and 0.18 mA, respectively.

Table 1 Effects of electrode distance and matrix on internal resistance of the laboratory column microbial fuel cells (MFCs)

Cathode (cm)	Anode (cm)	Electrode distance (cm)	Internal resistance (kΩ)	Current at 3 kΩ Load (μA)
Fixed anode at 1 cm below soil surface but varied cathode distance in water column (H)				
5	−1	6	7.5	70.8
10	−1	11	8.7	67.7
35	−1	36	29.7	27.3
Fixed cathode at 5 cm above soil surface but varied anode distance in soil matrix (D)				
5	−1	6	7.5	70.8
5	−5	10	8	70.0
5	−15	20	10.9	67.7

Table 2 Characterization of litter extracts before and after electricity generation in a dual-chamber microbial fuel cell reactor (Mean ± SD; n = 4). Means with superscript 'a' indicate no significant difference ($P > 0.05$)

	Baldcypress		Water tupelo		Longleaf pine	
	Before	After	Before	After	Before	After
DOC (mg l^{-1})	51.06 ± 2.36	24.56 ± 0.76	57.40 ± 0.84	47.54 ± 1.31	30.12 ± 0.02	25.46 ± 0.70
DOC/DTN (mol mol^{-1})	23.4 ± 0.3	–	120.7 ± 6.7[a]	–	125.3 ± 3.7[a]	–
BOD_5 (mg l^{-1})	45.77 ± 0.60	18.18 ± 1.59	48.33 ± 0.72	26.04 ± 0.84	43.39 ± 0.97	24.28 ± 1.87
SUVA (l mg^{-1} m^{-1})	0.79 ± 0.01	1.77 ± 0.03	3.52 ± 0.12[a]	3.41 ± 0.07[a]	1.19 ± 0.02	1.81 ± 0.06
S_R	0.67 ± 0.02	0.94 ± 0.03	0.62 ± 0.01	0.85 ± 0.02	1.63 ± 0.03[a]	1.73 ± 0.06[a]
E2/E3	7.81 ± 0.14	6.19 ± 0.22	5.08 ± 0.05	3.60 ± 0.01	13.87 ± 0.32	7.90 ± 0.14

BOD, biological oxygen demand; DTN, dissolved total nitrogen; SUVA, specific ultraviolet absorbance; DOC, dissolved organic carbon.

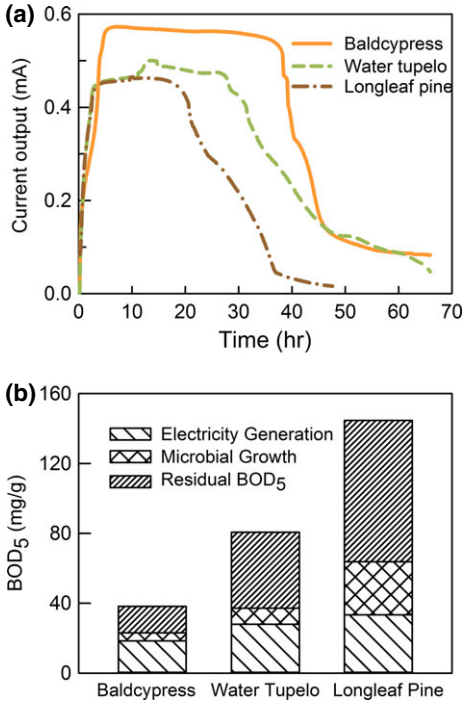

Fig. 3 (a) Current output of dual-chamber Microbial fuel cells (MFCs) (resistor: 1000 ohm) using baldcypress, water tupelo, and longleaf pine extracts as substrates biological oxygen demand ($BOD_5 \approx 50$ mg l^{-1}), and (b) the fractional energy distribution of three types of leaf litter in terms of BOD_5 during MFC treatment (on dry weight basis).

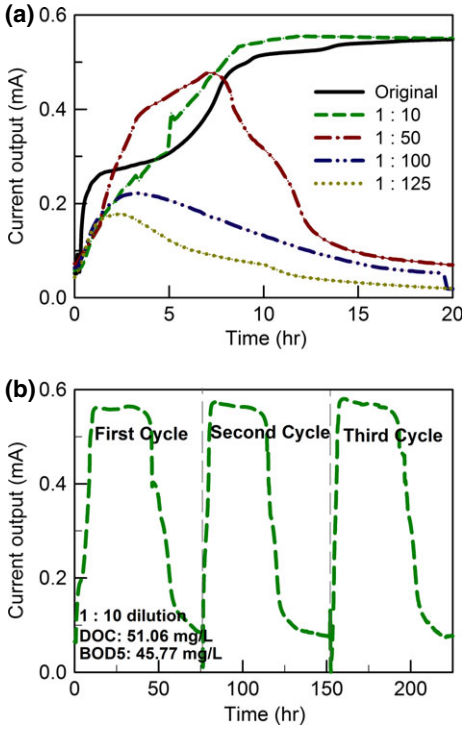

Fig. 4 Current output of dual-chamber Microbial fuel cells (resistor: 1000 ohm) with different substrate's concentrations (a) and in different operation cycles (litter extract of 45.77 mg l^{-1} BOD_5 biological oxygen demand was refilled after each cycle) (b). Taking cypress extracts with original concentration of 457.7 mg l^{-1} BOD_5 (510.6 mg l^{-1} DOC) as example.

Discussion

Electricity generation of in situ MFCs at diel scale

Generally, electricity generation in the field fluctuated dielly throughout the whole flooding duration of the study. Within 1 day, soil and water temperatures in forested wetland of coastal South Carolina could fluctuate over 10 °C (Chow *et al.*, 2013), whereas the variation of water level is relatively small (commonly several cm without precipitation supply). The current output of controlled laboratory MFCs was highly sensitive to temperature variation, which could be attributed to the effects of temperature fluctuation on the enzymatic activity of the bio-component of MFC. Similarly, the temperature was found to be critical to determine the diel variation of current output of *in situ* MFCs (Fig. 1c). This result is also consistent with previous

findings that temperature was a dominant driving factor on MFC performance (Hong *et al.*, 2009). As in the laboratory column MFCs, an increase of water level (i.e., electrode distance) increased the internal resistance of MFCs and decreased the current output. By implication, the water level in the field is likely to slightly counteract the temperature effect, resulting in a larger lag effect of current output after temperature oscillation in the field (2–6 h; Fig. 1c) compared to the laboratory (1–2 h; Fig. 2). In some heavy storm events, the diel variation of current output could be interrupted. The variation of temperature and a rapid increase in water level could not explain the fluctuation of current output during these periods. The inputs of DOM and nutrients from the surrounding watershed (Chow *et al.*, 2013), as well as an increase in turbidity, could be the causes for the increase of current output during the rainstorm.

Electricity generation of in situ MFCs at seasonal scale

Electricity generation behaved differently during flooding and dry periods, highlighting that standing water is an important 'circuit switch'. In the dry period from July to September, the floating electrode laid on the dried soil surface, resulting in the disconnection of the MFC circuit and no electricity generation. When temperature dropped below the freezing point, ice formation also blocked the charge transfer by freezing the cathode at the water surface and discontinued the electrical circuit, causing the pulses of electricity generation of *in situ* MFCs in the flooding wintertime (e.g., December 2010 and February 2011 in Fig. 1a).

Besides these extreme weather conditions, electricity generally increased from November to April but decreased in May. During winter flooding, massive litterfall was available and the DOC amounted to >50 mg l^{-1}, which was close to the ideal DOC concentration in the laboratory dual-chamber MFCs (Fig. 4). However, temperature was a limiting factor (commonly < 10 °C) to microbial reactivity, resulting in a relative low current output (Fig. 1a). As temperature gradually increased in the spring, the MFCs started to utilize the organic matter much faster, thereby enhancing the electricity generation. During a late flooding period in May, however, the low DOC level (15–30 mg l^{-1}) and its less biodegradability [increased DOC/DON (dissolved organic nitrogen) ratio and SUVA after humification (Wang *et al.*, 2012; Chow *et al.*, 2013)] seemed to limit the current output from MFCs, even though the temperature continued to increase. Besides poor nutrient conditions, biofilm fouling may also contribute to decreased current output (Zhang *et al.*, 2011b; Sevda *et al.*, 2013).

During the dry summer, electricity generation from MFCs intermittently recovered after heavy rainstorms. Sharp current signals at these times could be even higher than those in flooding periods. This phenomenon may be caused by the following: 1. Rainwater dissolved and flushed a large amount of DOM and nutrients from soils of the surrounding area and ended up with a high substrate concentration (Chow, 2006; Chow *et al.*, 2013); 2. High temperature in the summer ensures the high activity of the microbes; and 3. There is a shorter electrode distance with relative shallow water level compared to the flooding period.

Foliar litter as substrates in MFC

As our results from dual-chamber MFCs showed, forest litter extracts could be utilized as MFC substrates to generate electricity. After MFC reaction, the baldcypress extract had the largest decrease in BOD_5 and DOC, but the greatest increase in SUVA and fluorescence peaks of fulvic acid-like and humic acid-like regions in EEM spectra (defined in Chen *et al.*, 2003; Zhou *et al.*, 2013), showing largest potential (similar BOD_5 based) being utilized by MFC microorganism.

Compared to water tupelo or longleaf pine, baldcypress litter extract had more protein-like organic matter rather than humic-like organic matter (Fig. S5), lower DOC/DTN ratio (Table 2), and was much less aromatic (indicated by SUVA in Table 2). Humic acid-like substances proved to be more advantageous for electricity generation (Thygesen *et al.*, 2009), but is not as good a fuel source because of its low nitrogen content and being less biodegradable (Qualls, 2004). As the ideal molar C/N ratio for growing bacteria is 100 : 5 (Jana *et al.*, 2001), the low C/N ratio of baldcypress extract was close to this value and suggested an abundance of available nutrients for microbial activity. All these properties were favorable for microbial activity and thus enabled the DOM in original baldcypress extract to generate the highest power. Accordingly, for electricity generation from MFCs, litter materials containing high biodegradable matter and nitrogen content are likely to be ideal substrates.

To evaluate the energy potential of litter materials, another important parameter to be considered is the leachability of DOM from those materials. Under similar BOD_5 concentrations, baldcypress litter extract was the most efficient source for electricity generation, although baldcypress litter was the least extractable and had the lowest BOD_5 of litter examined (Table S2; Fig. 3b). Calculations based on a dry weight basis show that pine litter reserved higher energy (128.15 J g^{-1}) in the form of DOM than tupelo (101.44 J g^{-1}) and baldcypress (79.15 J g^{-1}). This order is totally opposite from

that of the energy potential of DOM per carbon basis. Therefore, when analyzing the relative contribution of plant species on the DOM concentration in wetland, the availability of DOM in litter materials should also be considered.

Energy budget and prospective application

Considering the flooding season in 2010–2011 as example (December 2010 and May 2011), each *in situ* MFC produced 2893 J m^{-2} of energy in an area of 0.075 m^2. As the total area of the studied wetland is 2.4 ha, about 0.69 GJ of electrical energy could be potentially produced annually. If one extrapolated it to the approximately 200 000 ha of tidal freshwater wetlands along the coast of the southeastern United States (Field *et al*., 1991), there would be approximately 58 000 GJ yr^{-1} of energy available. Optimal energy production from litter extracts in this study was 79.15 J g^{-1} of dry baldcypress litter mass and 101.44 J g^{-1} of water tupelo litter. With a make-up of 34% baldcypress and 56% water tupelo, the 833 g m^{-2} of litter fall produced by trees in the study area (Busbee *et al*., 2003) has a potential to produce 16.74 GJ of electrical energy, which means the current *in situ* MFC only harvests 4.12% of the energy available from the foliar litter. Previous studies demonstrated that the configuration of specific MFC reactors could affect the substrate utilization for electricity generation (Li *et al*., 2010), but as the first study of MFC development in a forest wetland, we did not optimize the MFC configuration but used economically available materials to build low-cost MFCs. Higher energy production to utilization ratios of forest detritus could be expected if more effective MFC configurations are applied (like catalytic cathodes and graphite felt anodes).

Although forest biomass has been considered an important energy source as well as a source of biofuel (Parikka, 2004), the majority of this material is decomposed naturally in the environment without energy collection. Based on the results of three types of MFCs, the idea of using forest detritus to directly generate electricity is feasible via low-cost MFC technique, but the main concern is that their current and power output are too low for practical application. For now, one promising application for these *in situ* MFCs is serving as a charging source of field devices [e.g., biosensor and data loggers (Zhang *et al*., 2011a)] in forested wetlands, similar to the proposed application in deep sea areas (Reimers *et al*., 2001; Gong *et al*., 2011). This application would be helpful to support the on-line forest wetland monitoring and intensive data collection, as wetlands were important ecosystems but rather dynamic regarding hydrology and the resulting physiochemical environments (Conner *et al*., 2007; Reddy & DeLaune, 2008). Notably, as revealed in this study, environmental factors including temperature, water level, and water quality should be taken into consideration of *in situ* MFC design and power management systems in specific forested wetlands.

Acknowledgement

Portions of this study were supported by the US Geological Survey Climate and Land Use Change Research and Development Program, the USEPA under grant number SU834703, by NIFA/USDA under project numbers SC1700409 and SC1700424, and by the NSFC-GD Joint Foundation of the Key Projects of China (no. U1201234). Jun-Jian Wang thanks for the financial support from China Scholarship Council (CSC[2011] 3010). Technical contribution number 6155 of the Clemson University Experiment Station.

References

American Public Health Association (1992) *Standard Methods for the Examination of Water and Wastewater*. APHA Publication Office, Washington, D.C.

Anderson C, Lockaby BG (2007) Soils and biogeochemistry of tidal freshwater forested wetlands. In: *Ecology of Tidal Freshwater Forested Wetlands of the Southeastern United States* (eds Conner WH, Doyle TW, Krauss KW), pp. 65–88. Springer, The Netherlands.

Battle JM, Golladay SW (2007) How hydrology, habitat type, and litter quality affect leaf breakdown in wetlands on the gulf coastal plain of Georgia. *Wetlands*, **27**, 251–260.

Busbee WS, Conner WH, Allen DM, Lanham JD (2003) Composition and aboveground productivity of three seasonally flooded depressional forested wetlands in coastal South Carolina. *Southeastern Naturalist*, **2**, 335–346.

Chen W, Westerhoff P, Leenheer JA, Booksh K (2003) Fluorescence excitation - Emission matrix regional integration to quantify spectra for dissolved organic matter. *Environmental Science & Technology*, **37**, 5701–5710.

Cheng S, Liu H, Logan BE (2006) Increased power generation in a continuous flow MFC with advective flow through the porous anode and reduced electrode spacing. *Environmental Science & Technology*, **40**, 2426–2432.

Chow AT (2006) Disinfection byproduct reactivity of aquatic humic substances derived from soils. *Water Research*, **40**, 1426–1430.

Chow AT, Dai J, Conner WH, Hitchcock DR, Wang J-J (2013) Dissolved organic matter and nutrient dynamics of a coastal freshwater forested wetland in Winyah Bay, South Carolina. *Biogeochemistry*, **112**, 571–587.

Conner WH, Doyle TW, Krauss KW (2007) *Ecology of Tidal Freshwater Forested Wetlands of the Southeastern United States*, Springer, New York.

Dalrymple RM, Carfagno AK, Sharpless CM (2010) Correlations between dissolved organic matter optical properties and quantum yields of singlet oxygen and hydrogen peroxide. *Environmental Science & Technology*, **44**, 5824–5829.

Donovan C, Dewan A, Heo D, Beyenal H (2008) Batteryless, wireless sensor powered by a sediment microbial fuel cell. *Environmental Science & Technology*, **42**, 8591–8596.

Dunaj SJ, Vallino JJ, Hines ME, Gay M, Kobyljanec C, Rooney-Varga JN (2012) Relationships between soil organic matter, nutrients, bacterial community structure, and the performance of microbial fuel cells. *Environmental Science & Technology*, **46**, 1914–1922.

Field DW, Reyer AJ, Genovese PV, Shearer BD (1991) *Coastal Wetlands of the United States: An Accounting of a Valuable National Resource*. Strategic Assessment Branch, Ocean Assessments Division, Office of Oceanography and Marine Assessment, National Ocean Service, National Oceanic and Atmospheric Administration, Rockville, MD.

Gong YM, Radachowsky SE, Wolf M, Nielsen ME, Girguis PR, Reimers CE (2011) Benthic microbial fuel cell as direct power source for an acoustic modem and seawater oxygen/temperature sensor system. *Environmental Science & Technology*, **45**, 5047–5053.

He Z, Minteer SD, Angenent LT (2005) Electricity generation from artificial wastewater using an upflow microbial fuel cell. *Environmental Science & Technology*, **39**, 5262–5267.

Heilmann J, Logan BE (2006) Production of electricity from proteins using a microbial fuel cell. *Water Environment Research*, **78**, 531–537.

Helms JR, Stubbins A, Ritchie JD, Minor EC, Kieber DJ, Mopper K (2008) Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnology and Oceanography*, **53**, 955–969.

Hong SW, Chang IS, Choi YS, Chung TH (2009) Experimental evaluation of influential factors for electricity harvesting from sediment using microbial fuel cell. *Bioresource Technology*, **100**, 3029–3035.

Jana BB, Chakraborty P, Biswas JK, Ganguly S (2001) Biogeochemical cycling bacteria as indices of pond fertilization: importance of CNP ratios of input fertilizers. *Journal of Applied Microbiology*, **90**, 733–740.

Kaku N, Yonezawa N, Kodama Y, Watanabe K (2008) Plant/microbe cooperation for electricity generation in a rice paddy field. *Applied Microbiology and Biotechnology*, **79**, 43–49.

Kim JR, Cheng S, Oh SE, Logan BE (2007) Power generation using different cation, anion, and ultrafiltration membranes in microbial fuel cells. *Environmental Science & Technology*, **41**, 1004–1009.

Li FX, Sharma Y, Lei Y, Li BK, Zhou QX (2010) Microbial fuel cells: the effects of configurations, electrolyte solutions, and electrode materials on power generation. *Applied Biochemistry and Biotechnology*, **160**, 168–181.

Liu H, Cheng SA, Logan BE (2005) Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration. *Environmental Science & Technology*, **39**, 5488–5493.

Logan BE, Rabaey K (2012) Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies. *Science*, **337**, 686–690.

Logan BE, Hamelers B, Rozendal RA *et al.* (2006) Microbial fuel cells: methodology and technology. *Environmental Science & Technology*, **40**, 5181–5192.

McCarty PL, Bae J, Kim J (2011) Domestic wastewater treatment as a net energy producer-Can this be achieved? *Environmental Science & Technology*, **45**, 7100–7106.

Miller MP, Simone BE, McKnight DM, Cory RM, Williams MW, Boyer EW (2010) New light on a dark subject: comment. *Aquatic Sciences*, **72**, 269–275.

Pant D, Van Bogaert G, Diels L, Vanbroekhoven K (2010) A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. *Bioresource Technology*, **101**, 1533–1543.

Parikka M (2004) Global biomass fuel resources. *Biomass and Bioenergy*, **27**, 613–620.

Qualls RG (2004) Biodegradability of humic substances and other fractions of decomposing leaf litter. *Soil Science Society of America Journal*, **68**, 1705–1712.

Rabaey K, Verstraete W (2005) Microbial fuel cells: novel biotechnology for energy generation. *Trends in Biotechnology*, **23**, 291–298.

Rabaey K, Ossieur W, Verhaege M, Verstraete W (2005) Continuous microbial fuel cells convert carbohydrates to electricity. *Water Science and Technology*, **52**, 515–523.

Reddy KR, DeLaune RD (2008) *Biogeochemistry of Wetlands: Science and Applications*. CRC Press, New York.

Reimers CE, Tender LM, Fertig S, Wang W (2001) Harvesting energy from the marine sediment-water interface. *Environmental Science & Technology*, **35**, 192–195.

Sevda S, Dominguez-Benetton X, Vanbroekhoven K, De Wever H, Sreekrishnan TR, Pant D (2013) High strength wastewater treatment accompanied by power generation using air cathode microbial fuel cell. *Applied Energy*, **105**, 194–206.

Thygesen A, Poulsen FW, Min B, Angelidaki I, Thomsen AB (2009) The effect of different substrates and humic acid on power generation in microbial fuel cell operation. *Bioresource Technology*, **100**, 1186–1191.

Wang JJ, Ng TW, Zhang Q, Yang XB, Dahlgren RA, Chow AT, Wong PK (2012) Technical note: reactivity of C1 and C2 organohalogens formation – from plant litter to bacteria. *Biogeosciences*, **9**, 3721–3727.

Watt KM, Golladay SW (1999) Organic matter dynamics in seasonally inundated, forested wetlands of the Gulf Coastal Plain. *Wetlands*, **19**, 139–148.

Xu C, Franks AE, Nevin KP, Russell TP, Lovely DR (2008) Structured anode materials to optimize power production of microbial fuel cells. *Abstracts of the General Meeting of the American Society for Microbiology*, **108**, 597.

Zhang F, Tian L, He Z (2011a) Powering a wireless temperature sensor using sediment microbial fuel cells with vertical arrangement of electrodes. *Journal of Power Sources*, **196**, 9568–9573.

Zhang L, Zhu X, Li J, Liao Q, Ye DD (2011b) Biofilm formation and electricity generation of a microbial fuel cell started up under different external resistances. *Journal of Power Sources*, **196**, 6029–6035.

Zhou J, Wang JJ, Baudon A, Chow AT (2013) Improved fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. *Journal of Environmental Quality*, **42**, 925–930.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 *In situ* MFC was installed in a freshwater wetland at Hobcaw Barony, Georgetown, SC.Graphite rods (anode) were buried 5 cm below soil surface and graphite brushes (cathode) floated freely on water surface.
Fig. S2 Configuration of column reactor for measurement of temperature and electrode distance effects.
Fig. S3 Configuration of the dual-chamber microbial fuel cell reactor for measurement of substrates' effect and change.
Fig. S4 The litter materials used for feeding the dual-chamber reactors.
Fig. S5 Fractional fluorescence excitation-emission matrix of the feeding extracts organic matter before and after the reaction in dual-chamber microbial fuel cell reactors fed with 50 mg l^{-1} biological oxygen demand BOD_5 diluted litter extracts.
Fig. S6 Two gram per liter sodium acetate was used as the substrate in controlled dual-chamber reactors.
Table S1 Configuration of microbial fuel cells.
Table S2 Chemical properties of three types of leaf litters on dry weight basis (mean ± SD; $n = 4$).