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Original Article

# Effects of Sediment Microbial Fuel Cells on $CH_4$ and $CO_2$ Emissions from Straw Amended Paddy Soil

**Adhena Tesfau Bekele [a], Morihiro Maeda [a], Satoshi Akao [b], Hiroaki Somura [a], Chiyu Nakano [c], Yuta Nishina [d]**

[a] *Graduate School of Environmental and Life Science, Okayama University, Okayama, Japan*
[b] *Faculty of Science and Engineering, Doshisha University, Kyoto, Japan*
[c] *Organization for Research Strategy and Development, Okayama University, Okayama, Japan*
[d] *Research Institute for Interdisciplinary Science, Okayama University, Okayama, Japan*

## ABSTRACT

Straw returning into paddy soil enhances soil organic matter which usually promotes the emission of greenhouse gases to the atmosphere. The application of sediment microbial fuel cells (SMFCs) to paddy soil activates power-generating microorganisms and enhances organic matter biodegradation. In the present study, rice straw addition in SMFCs was examined to determine its effect on $CH_4$ and $CO_2$ emissions. Columns (height, 25 cm; inner diameter, 9 cm) with four treatments: soil without and with rice straw under SMFC and without SMFC conditions were incubated at 25°C for 70 days. Anodic potential values at 7 cm depth sediment were kept higher by SMFCs than those without SMFCs. Cumulative $CH_4$ emission was significantly reduced by SMFC with straw amendment ($p < 0.05$) with no significant effect on $CO_2$ emission. 16S rRNA gene analysis results showed that Firmicutes at the phylum, Closteridiales and Acidobacteriales at order level were dominant on the anode of straw-added SMFC, whereas Methanomicrobiales were in the treatment without SMFC, indicating that a certain group of methanogens were suppressed by SMFC. Our results suggest that the anodic redox environment together with the enrichment of straw-degrading bacteria contributed to a competitive advantage of electrogenesis over methanogenesis in straw-added SMFC system.

**Keywords:** straw, methane mitigation, SMFC, microorganisms, current generation

## INTRODUCTION

Rice straw left in paddy fields is a significant substrate for greenhouse gases (GHGs) production [1,2]. Microbial activity in the anaerobic soil and oxygen-rich water layers plays a significant role in determining the net emission of GHGs [3]. Methane gas is naturally produced through anaerobic decomposition of organic matter in soil under flooded conditions. Anaerobic oxidation of $CH_4$ takes place in the sediment [4] and aerobic oxidation in the aerated water layer by methanotrophs, emitting $CO_2$ into the atmosphere [5]. The anaerobic condition in the soil is favorable for methanogenic microorganisms to undertake organic matter degradation. This leads to significant $CH_4$ emissions to the atmosphere. Thus, the magnitude of the emission is determined by several factors such as plants and abiotic factors including soil structure, moisture, temperature, pH, nutrients and organic carbon contents, and other electron acceptors in soil [6–8].

Many studies have focused on the mitigation of GHG emissions, especially $CH_4$ from paddy soil using methods such as biochar application [9–11], and water management [12,13]. Biochar reduces $CH_4$ emission from paddy soil by improving soil aeration and facilitating $CH_4$ oxidation [14], altering the original microbial community and structure [15], and limiting carbon availability for microorganisms by enhancing carbon sequestration [16] while water management through intermittent flooding. However, inconsistent and variable effects have been reported from these methods due

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Corresponding Author: Morihiro Maeda, E-mail: mun@cc.okayama-u.ac.jp
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to biochar characteristics, application rate, soil conditions, and the side effects on other GHG emissions. Moreover, the application of organic matter such as rice straw could further complicate the soil conditions. So, the application of sediment microbial fuel cells (SMFCs) to paddy soil can offer a more consistent and efficient alternative by removing organic matter from the sediment. SMFC has emerged as one of the recent and promising microbial technologies to harvest electricity from soil organic matter [17]. During the operation of SMFCs, bacteria undertake anaerobic decomposition of organic matter to gain their energy and produce $CO_2$, electrons, and protons [18]. The operation allows the diversion of electrons naturally used for methanogenesis in the sediment [19]. Consequently, competition among microbial groups takes place due to electrochemical changes in the soil [20]. It has previously been observed that SMFC reduced $CH_4$ emission by outcompeting methanogenic archaea by electrogenic ones for organic matter in soil [21] and by changing the electrochemical conditions of the sediment for the activity of methanogens by promoting organic matter oxidation [20]. Moreover, bacterial growth and development in the sediment is determinant for efficient SMFC operation and suppression of methanogenesis [22], however little has been researched about the application of rice straw and its effect on SMFC. Recent studies have reported the possible coexistence of electrogens and methanogens under organic matter-rich sediments [23] and therefore this study aims to determine the effect of rice straw application on microbial composition and efficiency of SMFCs to reduce $CH_4$ emission. Moreover, research emphasizes the effect of SMFC application on $CH_4$ emission and there are no clear studies regarding the fate of $CO_2$ emission during SMFC operation in straw-applied paddy soil.

The objectives of this study were, therefore, a) to investigate the effect of SMFCs on $CH_4$ and $CO_2$ emissions from paddy soil treated with rice straw; b) to evaluate electricity production performance in different SMFC treatments; and c) to analyze the relative abundance and diversity of bacterial and archaeal community structure. The use of rice straw in SMFC is expected to undergo an alternative degradation process that leads to the production of electricity rather than $CH_4$. This diversion of organic material towards electricity generation could either reduce the availability of substrates for methanogens or change the soil condition for the methanogenesis activity, thereby decreasing $CH_4$ emission. Besides, less $CH_4$ oxidation due to cathodic oxygen competition could balance the effect of $CO_2$ emission from organic matter oxidation in SMFCs.

## MATERIALS AND METHOD

### Soil and straw preparation

Topsoil layer (0–10 cm) was collected from a paddy field in Aioi city, Hyogo, Japan, then air dried at room temperature and sieved through 2 mm mesh before use. Rice straw was used as a soil amendment. The straw was cut into pieces (~2 cm in length), oven-dried at 70°C for 24 h, and ground into powder. The properties of soil and rice straw used for the experiment are listed in **Table 1**.

### Construction and operation of SMFCs

Lab-scale microcosms were set up using acrylic cylindrical columns (height, 25 cm; inner diameter, 9 cm). The experiment consisted of four SMFC treatments: paddy soil without and with rice straw addition (1.5%), with SMFC (+SMFC), and without SMFC (−SMFC) conditions in triplicate. Rectangular graphite felt electrode (7 cm × 3 cm) and carbon rod (length, 12.5 cm; diameter, 5 mm) were used as anode and cathode materials, respectively. The column was filled with 650 g soil and 1.2 L distilled water (**Fig. 1**). The anode was set 4 cm below the soil-water interface whereas the cathode was positioned 1 cm below the surface of the water column. The anode and cathode were connected with a copper wire having a 100 Ω resistor for treatments with SMFC operation whereas, those were not in treatments without SMFC operation. Gas and water samples were collected at 7-day intervals. Water withdrawn from the systems due to sampling and evaporation was compensated with deionized water. The operation was run for 70 days at 25°C.

### Electrochemical measurements and calculations

Dissolved oxygen (DO) at 2 cm surface water and anodic

**Table 1** Initial soil and rice straw properties.

| | EC ($\mu$S cm$^{-1}$) (1:10) a) | pH (1:10) | Moisture content (%) | C (g kg$^{-1}$) | N (g kg$^{-1}$) | C/N |
|---|---|---|---|---|---|---|
| Soil | 198.2 | 7.1 | 4.02 | 19.2 | 1.8 | 11 |
| Rice straw | - | - | - | 384.3 | 4.8 | 80 |

a) Soil EC and pH were determined in the solution at a dry soil/water ratio of 1:10.

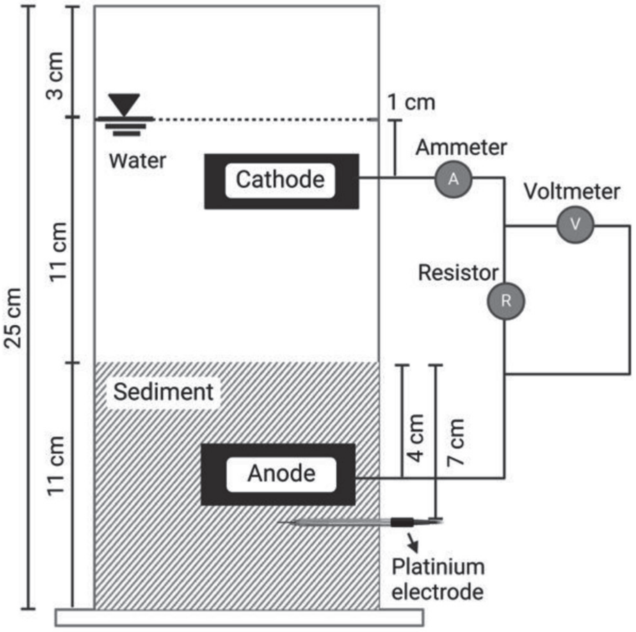

**Fig. 1** Single chamber SMFC setup.

potential (Eh) at 7 cm deep sediment were measured using a digital DO (DO-31P, DKK-TOA, Tokyo, Japan) and Eh meter (PRN-41, DKK-TOA), respectively. The value of pH and EC in the top 2 cm of overlying water were measured using a digital pH meter (F-23, Horiba, Kyoto, Japan) and a digital EC meter (DS-14, Horiba), respectively. Water samples were collected from the overlying water, filtered (0.45 µm), and measured for total organic carbon (TOC) using a total organic carbon analyzer (TOC-L, Shimadzu, Kyoto, Japan). TOC removal rate by SMFC treatments was calculated based on equation (1).

$$\text{TOC removal rate}(\%) = \frac{\text{TOC}_{\text{initial}} - \text{TOC}_{\text{final}}}{\text{TOC}_{\text{initial}}} \times 100\% \tag{1}$$

Where $\text{TOC}_{\text{initial}}$ and $\text{TOC}_{\text{final}}$ represent the concentration of TOC (mg $L^{-1}$) at the beginning and end of the experiment, respectively.

Voltage and current were also recorded at 10 min intervals with a voltmeter (LR8431, Memory Hilogger, Hioki, Nagano, Japan) and an ammeter (PC720M, Digital Multimeter, Sanwa, Tokyo, Japan), respectively. Electrons recovered (ER) by SMFC treatments were calculated based on equation (2).

$$\text{ER} = \frac{\sum(It)}{F} \tag{2}$$

$I$: current (A)
$F$: the Faraday constant (96,485 C $\text{mol}^{-1}$)
$t$: time interval (s)

Carbon degradation by SMFCs was calculated from the recovered electrons assuming that 4 mol of electrons were produced from 1 mol of carbon in the anodic organic matter reaction as follows.

$$CH_2O + H_2O \rightarrow CO_2 + 4H^+ + 4e^- \tag{3}$$

Coulombic efficiency (CE) was calculated according to equation (4), and it showed the ratio of the total ER for electricity generation to the theoretical electrons generated from the consumed organic matter. Theoretical electrons were calculated based on the TOC change in the overlying water over time.

$$\text{CE} = \frac{\sum(It)}{nF\mathit{\Delta}\text{TOC}} \tag{4}$$

$n$: number of electrons transferred per mole of carbon
$\mathit{\Delta}$TOC: TOC removed (mol)

## Gas measurements and flux calculations

The chamber method was employed to collect gas samples from the experimental SMFCs [21]. Gas samples were collected using a closed chamber (height, 5 cm; diameter, 10 cm) weekly. A sample of 30 mL was collected from the headspace of each treatment and transferred to vacuumed 20 mL glass vials. The concentrations of $CH_4$ and $CO_2$ were measured by gas chromatography (GC-2014 and GC-8A, Shimadzu, respectively), equipped with a flame ionization detector (for $CH_4$) (AGE-1000, Shimadzu) and thermal conductivity detector (for $CO_2$). Equation (5) was used to calculate $CH_4$ and $CO_2$ emission fluxes (g C $m^{-2}$ $h^{-1}$) from the SMFC treatments [5,21,24]

$$E = H\left(\frac{M_W}{M_V}\right)\left(\frac{273}{273+T}\right)\left(\frac{\mathit{\Delta}c}{\mathit{\Delta}t}\right) \tag{5}$$

$E$: $CH_4$ or $CO_2$ emission flux (g C $m^{-2}$ $h^{-1}$)
$H$: height of the chamber (m)
$M_W$: molar mass of C (12.01 g $\text{mol}^{-1}$)
$M_V$: molar volume of $CH_4$ or $CO_2$ at standard temperature and pressure ($22.4 \times 10^{-3}$ $m^3$ $\text{mol}^{-1}$)
$T$: in determination temperature (25°C)
$\mathit{\Delta}c/\mathit{\Delta}t$: change in concentration of $CH_4$ or $CO_2$ (ppm × $10^{-6}$) per unit of time (h).

## DNA extraction and 16S rRNA gene analysis

### *Sample collection and preparation*

At the end of the experiment (day 70), the anode electrodes were carefully withdrawn from the sediment. A sterile scalpel was used to scrape the sediment attached to the anode. The scraped anodic sediment was transferred to plastic bags and then stored at −21°C for DNA extraction.

### *DNA extraction, purification, and 16S rRNA gene analysis*

Sediment samples were extracted and purified following the protocols of Fast DNA SPIN Kit for Soil (MP Biomedicals, Irvine, USA) and DNA Clean & Concentrator-25 kit (Zymo Research, Irvine, USA), respectively. The extracted DNA samples were kept at −20°C before further analyses. The V3-V4 region of 16S rRNA gene was amplified using the primer set of 341f (5′-CCTACGGGNGGCWGCAG-3′) and 805r (5′-GACTACHVGGGTATCTAATCC-3′) followed by an amplicon sequencing by an external analytical laboratory (Bioengineering Lab., Sagamihara, Japan). The Illumina MiSeq platform (2 × 300 bp) was undertaken for high-throughput sequencing of multiple amplicons. The FASTX-toolkit (ver. 0.0.14) and FLASH (ver. 1.2.11) [25] were used to process the raw sequencing reads and representative sequences and then amplicon sequences variant tables (ASV) were prepared after removing chimeric and noisy sequences with the dada2 plugin [26] in Qiime2 (ver. 2023.7) [27]. Taxonomy assignment of the ASVs was performed using the q2-feature-classifier-plugin against the EzbioCloud 16S databases [28,29].

## Statistical analysis

A two-way analysis of variance was carried out to make comparisons on the effects of straw and SMFC application on $CH_4$ and $CO_2$ emissions and other parameters. Multiple comparisons of means (Tukey's HSD post hoc) were performed to test significance differences among treatments. A paired *t*-test was conducted to detect differences in parameters at the beginning and end of the experimental period. The data were normally distributed, as assessed by Shapiro-Wilk's test. There were no extreme outliers in the data, as assessed by the boxplot method. Statistical significance was considered at $p < 0.05$.

# RESULTS

## Electrochemical properties

Sedimentary Eh values in SMFCs were slightly higher than in treatments without SMFCs but none of these differences were statistically significant (**Fig. 2**, $p > 0.05$). The value of Eh in the sediment was decreased in all treatments for the first 20–30 incubation days, then slightly increased and stabilized in SMFC treatments. The straw application did not show any differences in sedimentary Eh. On the other hand, SMFCs with straw addition reduced DO concentration in the overlying water (**Fig. 2d**). While DO levels in no-straw treatments remained above 3 mg $L^{-1}$, fluctuations were observed in straw-added treatments with DO concentrations lower than 1.5 mg $L^{-1}$ during the 1st 7 days, then increased up to 3 mg $L^{-1}$.

The variations in pH, EC, and TOC among treatments are presented in **Table 2**. Results showed that pH did not differ with and without SMFCs but significantly decreased with straw application ($p < 0.05$). On day 70, pH values in SMFCs were slightly lower (7.95 and 7.69, without and with straw addition, respectively) than those without SMFCs. Besides, EC values in SMFC treatments also showed a significant increase from the beginning (day 0) to the end (day 70). The value of EC in straw-added SMFCs was significantly higher (577 µS $cm^{-1}$) than SMFCs without straw (309 µS $cm^{-1}$). Similarly, TOC concentration in the overlying water was highest in straw-amended treatment and decreased over time

**Table 2** Chemical properties of the overlying water at the beginning (day 0) and the end (day 70) of the experiment (mean ± standard deviation, $n = 3$).

| Treatment | Condition *i)* | pH *ii)* | | EC (µS $cm^{-1}$) *ii)* | | TOC (mg $L^{-1}$) *ii)* | |
|---|---|---|---|---|---|---|---|
| | | Day 0 | Day 70 | Day 0 | Day 70 | Day 0 | Day 70 |
| No straw | +SMFC | $6.9 \pm 0.1^{bA}$ | $7.9 \pm 0.02^{aA}$ | $204 \pm 8.6^{bC}$ | $309 \pm 12^{aB}$ | $10.9 \pm 0.8^{aB}$ | $3.8 \pm 2.2^{aC}$ |
| | −SMFC | $7 \pm 0.1^{bA}$ | $8.0 \pm 0.07^{aA}$ | $181 \pm 10^{aC}$ | $205 \pm 13.5^{aC}$ | $10.6 \pm 0.3^{aB}$ | $7.9 \pm 0.8^{aBC}$ |
| With straw | +SMFC | $6.3 \pm 0.1^{bB}$ | $7.6 \pm 0.06^{aB}$ | $558 \pm 13^{aA}$ | $577 \pm 4.7^{aA}$ | $172 \pm 18.7^{aA}$ | $14.4 \pm 6.3^{bAB}$ |
| | −SMFC | $6.4 \pm 0.01^{bB}$ | $7.7 \pm 0.05b^{aB}$ | $524 \pm 6.2^{aB}$ | $541 \pm 20.1^{aA}$ | $177.1 \pm 16.9^{aA}$ | $24.3 \pm 3.8^{bA}$ |

*i)* +SMFC and −SMFC represent treatments with and without SMFC application, respectively. *ii)* Different small letters indicate significant differences between the beginning (day 0) and the end (day 70) by the *t*-test, and different capital letters indicate significant differences among treatments and SMFC conditions by Tukey's HSD test.

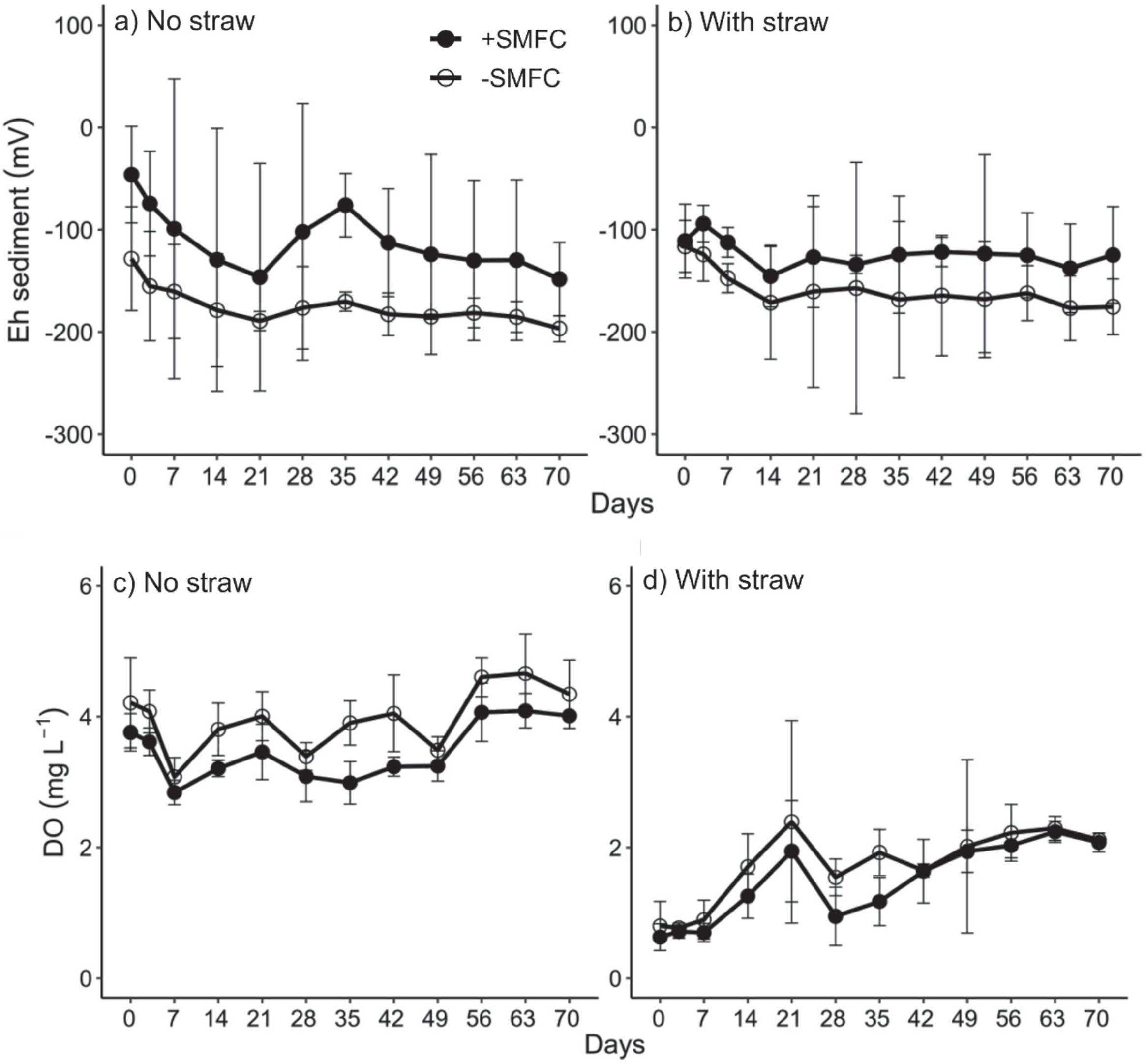

**Fig. 2** The Sedimentary Eh (a and b), and DO (c and d) in overlying water of SMFCs. Error bars represent standard deviation ($n$ = 3): +SMFC and −SMFC represent treatments with and without SMFC application, respectively.

in all treatments. A higher TOC removal rate was observed in the overlying water of no-straw and straw-added SMFC (65.1% and 91.6%, respectively) treatments than without SMFC (25.4% and 86.2%, respectively). This showed only a slight improvement in organic matter degradation performance following the operation of SMFC.

## $CH_4$ and $CO_2$ emission and performance of SMFCs

Lower $CH_4$ emission flux was observed consistently throughout the operational period in straw-added SMFC treatment than without SMFC, in which a significant variation was observed on days 42 and 49 combined (**Fig. 3b**). Straw addition significantly increased $CH_4$ emission flux in both treatments ($p < 0.05$). The highest average $CH_4$ emission flux (180 mg m$^{-2}$ h$^{-1}$) was observed in straw added without SMFC treatment, while the lowest average emission (0.04 mg m$^{-2}$ h$^{-1}$) was observed in SMFC treatment without straw addition. Cumulative $CH_4$ emission was significantly lower by 26.2% in straw-added SMFC treatment than without SMFC (**Table 3**, $p < 0.05$). Like $CH_4$ emission, cumulative $CO_2$ emission also significantly increased with straw application; however, no significant difference was observed among SMFCs and without SMFC treatments (**Table 3**, $p > 0.05$).

The performance of SMFCs in electricity generation showed variations through time course by different treat-

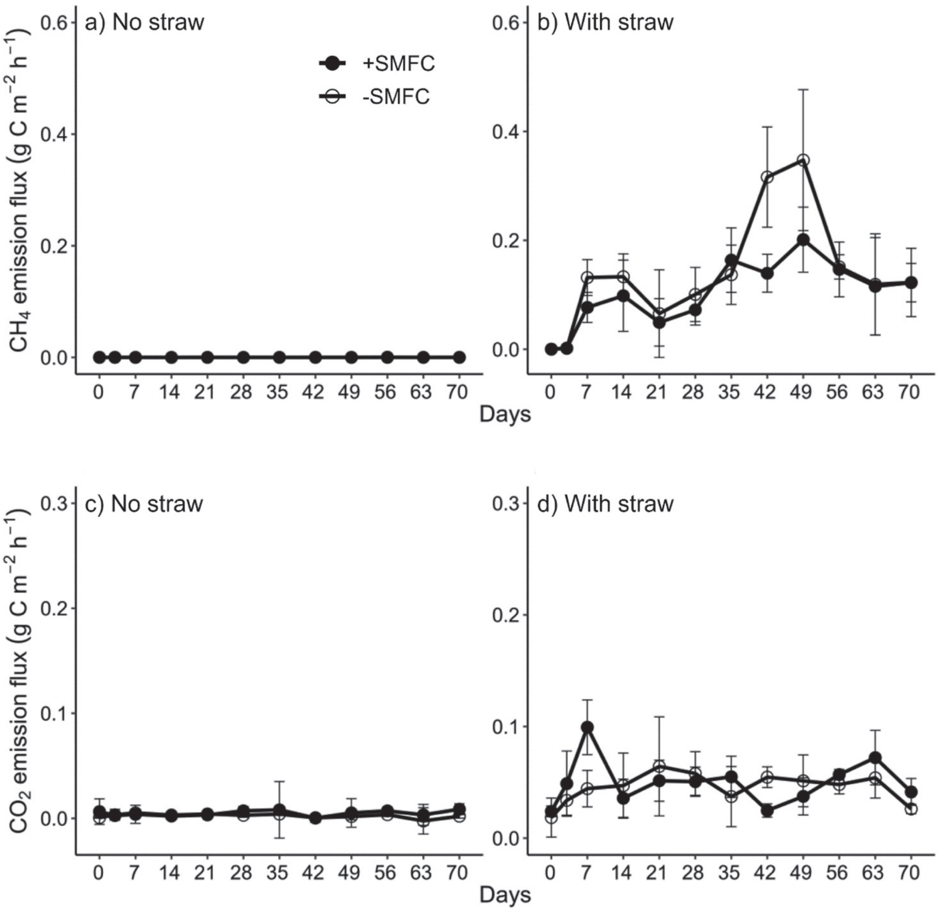

**Fig. 3** $CH_4$ (a and b) and $CO_2$ (c and d) emission flux. Error bars represent standard deviation ($n = 3$): +SMFC and −SMFC represent treatments with and without SMFC application, respectively.

**Table 3** Cumulative $CH_4$ and $CO_2$ emissions (70 days) of different treatments (mean ± standard deviation, $n = 3$).

| Treatment | Condition [i] | Cumulative $CH_4$ emission (g C $m^{-2}$) [ii] | Cumulative $CO_2$ emission (g C $m^{-2}$) [ii] |
|---|---|---|---|
| No straw | +SMFC | $0.05 \pm 0.004^c$ | $7.5 \pm 2.6^b$ |
| | −SMFC | $0.08 \pm 0.02^c$ | $4.1 \pm 0.7^b$ |
| With straw | +SMFC | $194.7 \pm 11.8^b$ | $80.2 \pm 2.7^a$ |
| | −SMFC | $263.9 \pm 27.2^a$ | $73.6 \pm 12.6^a$ |

*i*) +SMFC and −SMFC represent treatments with and without SMFC application, respectively. *ii*) Different small letters indicate significant differences among treatments and SMFC conditions by Tukey's HSD test.

ments. Until day 30, the average current in the straw-added SMFC treatment showed less performance (**Fig. 4a**). Averaged currents in no-straw and straw-added SMFC treatments until day 30 were significantly different, *i.e.*, 124 and 85 µA, respectively. Then, currents became similar between no-straw and straw-added SMFC treatments.

Voltage ranged from 0.28–1.04 V in treatments without SMFCs (**Fig. 4b**). Significantly lower voltage was recorded in straw-amended treatments during the 1st two weeks, but it progressively increased and stabilized. Current production took place and voltage was abruptly lowered to < 0.1 V in SMFC treatments. As a result, during the 1st three weeks, current production was lower in straw-added SMFC treatment, then increased and stabilized following the resistor substitution from 1,000 to 100 Ω on day 30th (**Fig. 4a**). This led to a further reduction of voltage but brought stability and a slight increase in current output of SMFC treatments.

The CE of SMFCs in no-straw and with straw addition is 256.9 and 10.8%, respectively. As presented in **Table 4**, the mole of ER by no-straw SMFC treatment was around 5% higher than the straw-added one. The carbon consumed for the generation of electricity by SMFCs was 15 × $10^{-4}$ and 14 × $10^{-4}$ mol in no-straw and straw-added treatments, respectively. However, the TOC removal rate in SMFC with no-straw (65%) is lower than with straw (91%). Whereas the actual $CH_4$ reduction and $CO_2$ increase by SMFC was higher with straw addition.

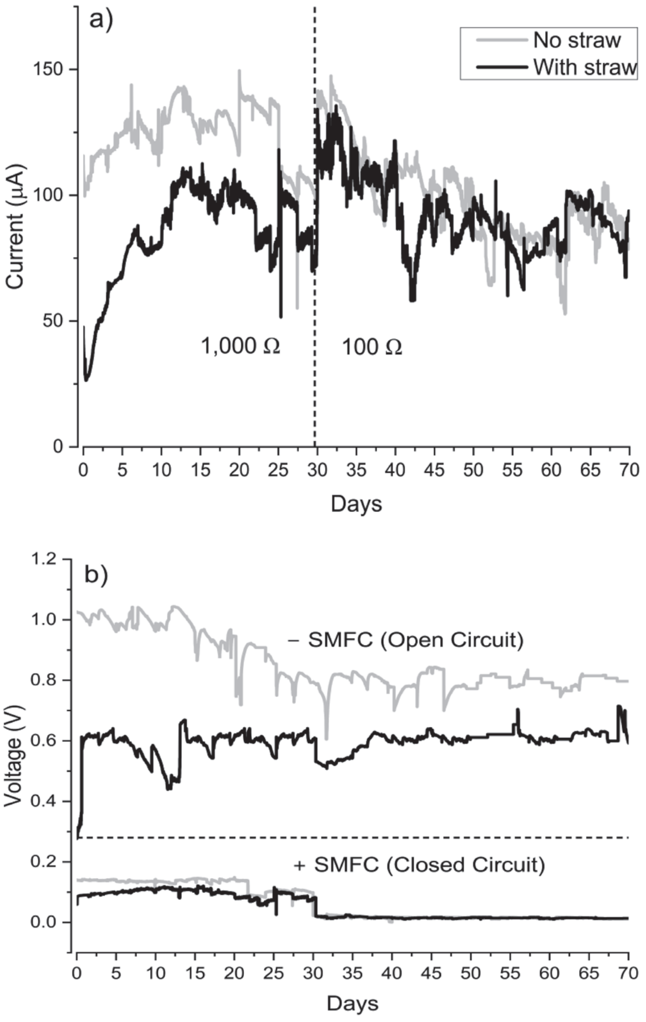

**Fig. 4** Electricity generation; time course of current changes (a) and time course of voltage trend (b): +SMFC and −SMFC represent treatments with and without SMFC application, respectively.

## Microbial diversity and structure

According to 16S rRNA gene analysis results, the dominant phyla identified on the anode in the SMFC treatments (without straw; with straw) belonged to Proteobacteria (24.6%; 19.6%), Actinobacteria (23.4%; 19.2%), Firmicutes (7.02%; 23.01%), Acidobacteria (14.2%; 8.9%), Chloroflex (14.1%; 10.7%), and Bacteroidetes (2.1%; 5.9%) (**Fig 5a**). Notably, Firmicutes and Bacterioidetes were enriched in straw-added SMFC, whereas Proteobacteria, Acidobacteria, and Chloroflex were found to be lesser.

At the Order level, Clostridales and Rhizobiales were the most enriched taxa in straw-added SMFC, accounting for 18.4% and 8.4% of the total relative abundance of bacterial sequences, respectively (**Fig. 5**). They showed around 1% increase in SMFC compared to those without SMFC treatment. At the genus level, *Clostridium* (11.1%) was found in the straw-added SMFC treatment, and *Bacillus* (2.2%) in the no-straw SMFC treatments were the abundant genera under phylum Firmicutes. Although most Bacterioidetes were not identified at the genus level, *Flavisolibacter* and *Lentimicrobium* were identified in low abundance in both no-straw and straw-added SMFC treatments. Some electrogenic bacteria have a slightly higher relative abundance in SMFC with straw application than without SMFC (**Fig. 5 and 6**). Among the identified Euryarcheota, Methanosarcinales were the most predominant order of methanogens, which accounted for 28–45% of the total archaea in sediment both with and without SMFCs. Methanocarcinales in treatment without straw and Methanomicrobials in the straw-added treatment were relatively less abundant in SMFC treatments than without SMFC. According to graphics of linear discriminant analysis (LDA) test (**Fig. 6**), bacterial groups Acidobacteriales, Blastocatellales, Physcispherales, and an unidentified group of Actinobacteria were significantly abundant in

**Table 4** Mass balance in SMFC treatments during the entire experimental period.

| Treatments | ER by SMFC (mol) | C oxidation by SMFC (mol) | Actual $CH_4$ reduction by SMFC (mol) | Actual $CO_2$ emission increase by SMFC (mol) | TOC decrease by SMFC (mol) |
|---|---|---|---|---|---|
| No Straw | 60 × $10^{-4}$ | 15 × $10^{-4}$ | 0.07 × $10^{-4}$ | 7.1 × $10^{-4}$ | 3.3 × $10^{-4}$ |
| With Straw | 57 × $10^{-4}$ | 14 × $10^{-4}$ | 240 × $10^{-4}$ | 23 × $10^{-4}$ | 8.2 × $10^{-4}$ |

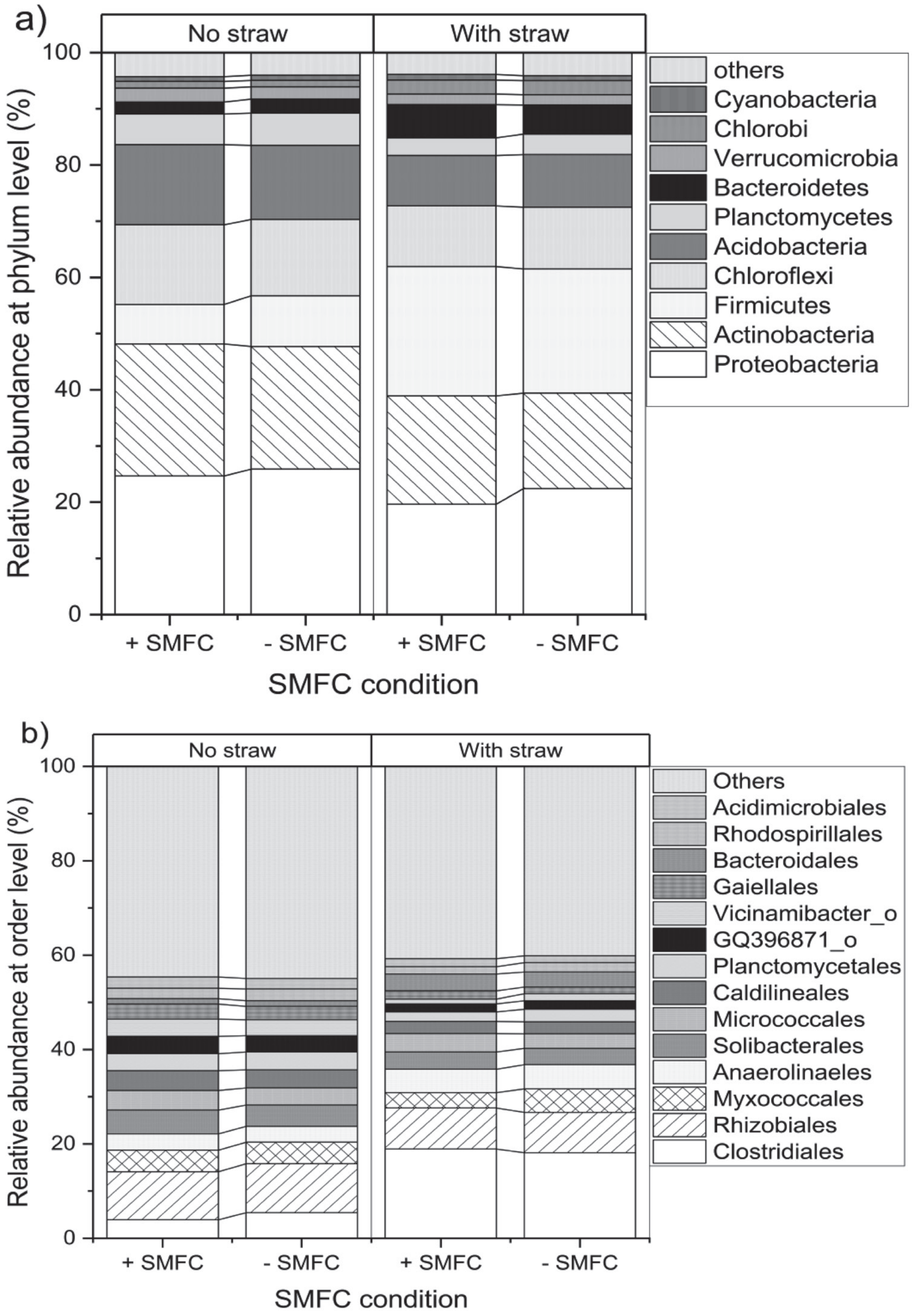

**Fig. 5** Relative abundance of bacterial taxonomic levels of different treatments at the phylum level (a) and at the order level (b): +SMFC and −SMFC represent treatments with and without SMFC application, respectively.

straw-added SMFC treatment whereas Methanomicrobiales, a methanogenic archaeon, was dominant in treatments without SMFC. Whereas, without straw addition, neither of the above bacteria and archaea was significantly abundant in SMFC, rather some sulfate-reducing bacteria such as Desulfovibronales and Myxococcales were significantly enriched.

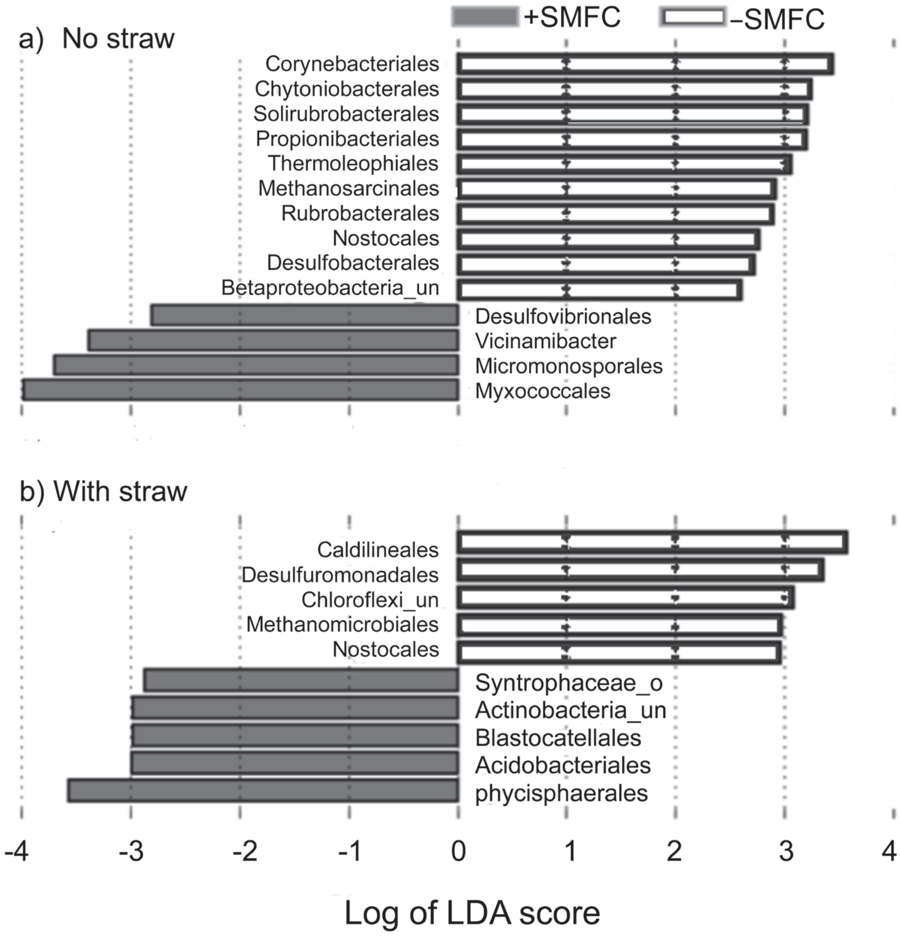

**Fig. 6** Graphics of LDA effect size performed on the order level microbial community relative abundance data. The taxon of bacteria with statistically significant change ($p < 0.05$) in the relative abundance is written alongside the horizontal lines: +SMFC and −SMFC represent treatments with and without SMFC application, respectively.

## DISCUSSION

### Effects of SMFC application on $CH_4$ and $CO_2$ emissions

Previous studies evaluating the effect of SMFC observed inconsistent results on whether SMFC can reduce $CH_4$ emission using different organic amendments. The current investigation found that cumulative emission of $CH_4$ in paddy soil with rice straw addition was significantly reduced by SMFC (**Table 3**, $p < 0.05$). A possible explanation for the effectiveness of SMFC in reducing $CH_4$ emission in this treatment might be due to the establishment of some exoelectrogenic bacteria and the decrease of some methanogenic archaea following the Eh change in the anodic sediment [30,31]. The results of this study show that electrochemically active and straw-degrading bacterial populations in the anode, mainly Firmicutes and Bacteroidetes, were relatively abundant in the straw-added SMFC treatment, likely due to the availability of straw (carbon source) that can be converted into easily accessible molecules by microorganisms. In our study, Firmicutes and Bacteroidetes have significantly increased from 7% and 2.1% in treatments with no straw to 23% and 5.9% in straw-added treatments, respectively. *Bacillus* and *Clostridium* were the most abundant genera in phylum Firmicutes, contributing to methanogenesis suppression by playing roles in electron transfer processes essential for current generation. In this study, specific electrogenic genera from the Bacteroidetes phylum were not detected, the phylum itself was reported to increase in proportion in the anode of SMFCs engaging in the degradation of complex components of rice straw [20,32]. These fermentative and electron-transferring bacteria have competed and succeeded in sediment organic matter under SMFC, which consequently suppressed methanogenesis [33]. Similarly, this led to the under-competition of methanogens for electrons, which resulted in the reduction of $CH_4$ emission from SMFC amended with straw in this study.

It was seen that some archaeal methanogens also showed different responses in treatments without and with straw-added SMFCs (**Fig. 6**). With the application of straw, Methanomicrobiales (hydrogenotophic archaea) were found to be significantly abundant in the treatment without SMFC, showing that these groups of archaea were suppressed by SMFC. However, there was no difference in the abundance of most methanogens among SMFC and without SMFC treatments which may corroborate the concept that SMFC mainly suppresses the activity of methanogens (methanogenesis), not all methanogens [23]. This is due to the redox condition created in the anodic sediment also varying among treatments. In treatment with rice straw addition, Eh in the deep sediment increased up to −100 mV in SMFC (**Fig. 2b**). This has perhaps influenced the growth and activity of some methanogens. Methanogenesis becomes more active with lower sediment Eh which then results in the highest $CH_4$ emission [19]. A recent study also reported the suppression of methanogenesis in sedimentary Eh between +200 and −200 mV [34]. Redox conditions created in the sediment are key to the effectiveness of SMFC in restricting the growth of methane-producing bacteria thereby suppressing methanogenesis [35]. Hence, it is understood that the relationship between electrogens (electrochemically active bacteria) and methanogens in SMFCs can be further influenced by factors such as the availability of organic matter, and the specific microbial community in the system which in turn determines the magnitude of $CH_4$ production and release from the SMFCs systems to the atmosphere.

In straw-added SMFC, the actual $CH_4$ emission reduction by SMFC was higher than the calculated carbon used for electricity generation (**Table 4**). This also showed that although the microbial community in the anode was efficient at converting organic matter, electrons were perhaps indirectly consumed for the reduction of other alternative electron acceptors and fermentation purposes rather than direct current production due to weak cathode performance. This condition might have enhanced anaerobic $CH_4$ oxidation by extracellular electron transfer mechanisms, thereby reducing $CH_4$ emission.

Another finding of this study is that SMFC application did not exert a significant effect on $CO_2$ emission in all treatments in this study ($p > 0.05$). The literature revealed that $CO_2$ is produced during the anaerobic degradation process of organic matter in SMFC [36]. A study by Zhu *et al.* [33] also showed the increase of $CO_2$ emission fluxes over time in constructed wetlands after the integration of microbial fuel cells. Despite that, the production of $CO_2$ in the sediment might be balanced due to the inhibition of $CH_4$ oxidation in the overlying water in SMFCs due to cathodic oxygen consumption [5]. Moreover, hydrogenotrophic methanogens were also reported to use hydrogen and $CO_2$ as substrates [37]. In this study, hydrogenotrophic archaeal groups Methanobacterium and Methanomicrobium were identified in all treatments showing that they were using $CO_2$ as a main substrate for cellular respiration. This perhaps also played a role in reducing the emission of $CO_2$ due to bacterial consumption in SMFC systems. The application of SMFC in this study did not affect pH values in the overlying water (7.6–8.0), which is favorable for microbial activities, resulting in no significant impact on $CO_2$ emission compared to without SMFC treatment.

## Effect of straw addition in SMFCs on current generation

Differences in current output were observed over a time course in SMFCs with and without straw addition (**Fig. 4**). In SMFC treatment with no-straw addition, slightly higher moles of electrons were recovered (**Table 4**), showing the contribution of carbon calculated from TOC removal was only approximately 14%. This is because TOC in the overlying water is just part of the total carbon in the sediment responsible for current generation by SMFC.

Contrary to expectations, this study did not find a significant difference in the current generation between straw-added and without-straw SMFC treatments, rather lesser output was recorded in the former. Although the effectiveness of an SMFC is mainly determined by the development of microbial community structure and composition [38], some other factors could explain the observation. Firstly, in this study, once the SMFC setups had a stable voltage of around 500 mV (after 72 hours of pre-incubation) and the electrodes were connected the voltage dropped automatically resulting in low current output at the beginning of the operation in straw-added SMFC treatment. This probably happened because of weak electron transfer due to high internal resistance [36] as well as the delay in the establishment of electroactive microorganisms in the systems following straw addition, which leads to an initial dominance of fermentative and hydrolytic microbes [35]. Suspended straw particles in the cathode of straw-added SMFC would have reduced DO in the overlying water (**Fig. 2d**) and perhaps increased cathodic resistance, thereby producing low electricity. The current was kept low for up to two weeks when the average DO concentration was <1.5 mg $L^{-1}$, further proving that fewer electrons were consumed by the terminal electron acceptor, limiting the rate of oxygen reduction reaction in the cathode. This has slowed down the overall electron flow from the anode to the cathode, thereby restricting the power generation efficiency of the SMFC system. Other studies have also shown that increasing oxygen at the cathode enhanced power output over less supply of cathodic oxygen [39,40]. Delayed enrichment of electrogenic bacteria and less availability of reduced sugars in the anode could be also other reasons for the less power generation in straw-amended treatments. However, current output in all treatments started to increase and become stabilized following the substitution of a resistor from 1,000 to 100 Ω on the 30th day. This has facilitated the transfer of electrons from the anode through an external circuit to the cathode, resulting in the enhancement of current output in both with and without straw-added SMFC treatments.

In previous studies, most organic amendments were taken as a measure to enhance electricity production in SMFC [5,20,36]. The complexity in the degradation of rice straw due to the high content of lignocellulose was also reported as a limitation in SMFC application [41–43]. This could be another possible reason why the straw application did not exhaustively trigger the current output in the current study. The CE of straw-added SMFC was lower than the no-straw one. This mainly shows the mass loss of electrons from the degradation of readily available organic matter due to weak cathodic performance. Besides, the lignocellulosic nature of rice straw is also less biodegradable by electrogenic microorganisms, thereby hardly contributing to electricity generation. On the other hand, the over-estimated CE in no-straw SMFC treatment showed that TOC change in the water was small since it only accounts for the dissolved organic carbon and does not include the organic matter present in the sediment, which is mainly responsible for the current generation in SMFC. A study by Hassan *et al.* [41] also reported a decrease in the efficiency of microbial fuel cells from 54.3% to 45.1% when the rice straw rate was increased from 0.5 g $L^{-1}$ to 1 g $L^{-1}$. Song *et al.* [35] also corroborated that the slower degradation rate of lignocelluloses in straw-fed SMFC systems and lower current production are expected in the early operational stages. Since our study was run for 70 days, the establishment of microorganisms that can degrade the complex structure of rice straw perhaps delayed resulting in less availability of reduced sugars in the sediment, then lower current output. This was further explained by Hassan *et al.* [41] for two major reasons. Primarily, power generation from rice straw is delayed due to the different composition of lignocellulose. Secondly, more application of rice straw and large anode surface area and anodic chamber volume ratio could reduce CE due to mass loss of electrons in which similar situations might have led to less power generation in the current study, too.

The results are further supported by the abundance of bacteria in the SMFC systems. Proteobacteria, which have been reported to occupy anodic sediment in SMFC systems and have a role in transferring electrons to an external electrode [44], were comparatively less dominant in straw-added SMFCs resulting in lesser current output than in SMFC without straw. Dos Passos *et al.* [45] also reported the less dominancy of Proteobacteria in anodic biofilm fed with acetate compared to native sediment.

## Effects of straw addition and SMFCs on bacterial and archaeal community

In this study differences in the relative abundance of some bacteria and archaea were found following rice straw addition and SMFC application (**Fig. 6**) which may indicate that both straw incorporation and SMFC application have influenced the soil microbial community and their activities by altering the soil environment. An example of this is that the addition of straw has significantly lowered the pH value in SMFCs (**Table 2**, $p < 0.05$), which might have inhibited the growth of some bacteria. On the other side, the slight change in bacterial and archaeal relative abundances among SMFC and without SMFC treatments may be due to the conditions within the SMFC and the competition for resources [46,47]. The distinguished respiration pathway in SMFC favors selective enrichment of distinct microbial communities in the anode [48], but no significant differences were observed in the relative abundances of most bacteria and archaea among SMFC and without SMFC treatments, that can also show that the possible suppression of methanogenesis while most methanogens are coexisting with electrogens.

At the order level, Closteridales were the most enriched group following the application of straw with slightly higher abundance in SMFC than without SMFC (**Fig. 5b**). This was also supported by previous observations which reported these bacteria as a dominating group in the easily degradable component of rice straw [19,49]. Minor groups of Bactriodiales and micrococcales also showed a slight increase in straw-added SMFC compared to those without SMFC. Among the methanogenic archaea, Methanosarcinales were relatively enriched in straw-added treatments and are said to be responsible for $CH_4$ production in organic matter-rich flooded paddy soil [24,50]. In this study, no methanogen was significantly abundant in straw-added SMFC treatment whereas Methanomicrobiales were significantly abundant in treatment without SMFC (**Fig. 6**), showing that a specific group of methanogens was eliminated following the application of SMFC and perhaps this has reduced $CH_4$ emission. Another finding is that Acidobacteriales, a straw-degrading bacteria, were significantly abundant in straw-added SMFC treatment (**Fig. 6**), showing that straw-degrading bacteria were also enriched under SMFC that breakdown the complex components of the straw. This perhaps brought a commensal relationship by providing a source of organic substrates that can then facilitate the growth and activity of electrogenic bacteria in the anode.

## CONCLUSION

The present study was designed to determine the effect of SMFCs on $CH_4$ and $CO_2$ emissions from straw-added paddy soil. The main finding to emerge from this study is that $CH_4$ emissions in paddy soil with straw addition were reduced significantly by the application of SMFC with no significant effect on $CO_2$ emission. In addition, in this study, the over-suspension of particles on the cathode showed an inhibitory effect on the performance of current generation in straw-added SMFC treatment. The abundance of some straw-degrading bacteria was higher in SMFC treatments with straw addition, showing that the combined role of fermentative and electrogenic bacteria in the anode favored electrogenesis over methanogenesis. Consequently, some methanogen groups were found to be significantly less abundant (suppressed) in straw-added SMFC treatment, resulting in lesser $CH_4$ emission. In general, $CH_4$ emissions in SMFCs with straw amendments may involve complex interactions between microbial processes, electron transfer, and sediment electrochemical conditions, and further research is needed to fully elucidate these relationships.

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