



Methane production from rice straw carbon in five different methanogenic rice soils: rates, quantities and microbial communities

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Abstract The input of organic substances (e.g., rice straw) in rice field soils usually stimulates the production and emission of the greenhouse gas methane (CH₄). However, the amount of CH₄ derived from the applied rice straw, as well as the response of bacterial and archaeal communities during the methanogenic phase, are poorly understood for different rice field soils. In this study, samples of five different rice soils were amended with ¹³C-labeled rice straw (RS) under methanogenic conditions. Immediately after RS addition, the RS-derived CH₄ production rates were higher in soils (Uruguay, Fuyang) that possessed a stronger inherent CH₄ production potential compared with other soils with lower inherent potentials (Changsha, the Philippines, Vercelli). However, soils with higher inherent potential did not necessarily produce higher amounts of CH₄ from the RS applied, or vice versa. Quantitative PCR

showed copy numbers of both bacteria and methanogens increased in straw-amended soils. High-throughput sequencing of 16S rRNA genes showed distinct bacterial communities among the unamended soil samples, which also changed differently in response to RS addition. Nevertheless, RS addition generally resulted in all the rice field soils in a relative increase of primary fermenters belonging to *Anaerolineaceae* and *Ruminococcaceae*. Meanwhile, RS addition also generally resulted in a relative increase of *Methanosarcinaceae* and/or *Methanocellaceae*. Our results suggest that after RS addition the total amounts of RS-derived CH₄ are distinct in different rice field soils under methanogenic conditions. Meanwhile, there are potential core bacterial populations that are often involved in primary fermentation of RS under methanogenic conditions.

Keywords ¹³C-labeled rice straw · Methane production · Rice field soil · Microbial community

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1 Introduction

The plant organic substances, such as rice straw, are often applied in large amounts (up to 12 Mg/ha annually) to rice field soil so as to maintain soil fertility (Liu et al. 2014; Sass et al. 1991; Schütz et al. 1989; Yagi and Minami 1990). Rice straw is typically added at the beginning of the season before rice planting, but other types of plant organic matter are continuously added during the rice-growing season since organic substances such as root exudates, sloughed-off leaves, and roots are released into soils during the life of rice plants (Dannenbergh and Conrad 1999; Lu et al. 2002). Such organic matter input eventually results in a steady increase of soil organic matter in rice field soils (Kalbitz et al. 2013; Liu et al. 2006, 2014; Mitra et al.

2005). However, incorporation of plant organic matter also increases the production and emission of CH₄, a potent greenhouse gas (Denier van der Gon and Neue 1995; Gao et al. 2016; Haque et al. 2013; Kim et al. 2012, 2015; Kimura et al. 2004; Schütz et al. 1989; Yagi and Minami 1990). The contribution of rice fields to atmospheric CH₄ is about 10% (Conrad 2009). Methane is produced during the anaerobic decomposition of organic substances by bacterial populations involved in hydrolysis, fermentation, syntrophy and homoacetogenesis together with methanogenic archaea (Conrad 1999; Schink and Stams 2013). The methane production capacity of the rice field soil without organic amendment was considered as its inherent methane production potential (Lu et al. 2000), which was mainly controlled by the availability of degradable organic substrates in soil (Yao and Conrad 1999; Yao et al. 1999).

The more rice straw is added to rice field soil, the higher is the production of CH₄ (Bao et al. 2014; Denier van der Gon and Neue 1995; Naser et al. 2007). Meanwhile, the addition of organic substances, including root exudates and rice straw seems to trigger a faster and higher response of CH₄ production rates when the soils possess a high inherent CH₄ production potential compared to those with low production potential (Lu et al. 2000). This is consistent with the observation that identical amounts of straw incorporation can cause different timing and different shapes of emission peaks of CH₄ in different rice field soils (Yagi and Minami 1990). There are numerous studies of the influence of RS addition on the methanogenic archaeal communities, concluding that members of these communities respond differently to the type of organic residues and incubation temperature and duration (Conrad and Klose 2006; Conrad et al. 2012; Lu et al. 2015; Peng et al. 2008). For example, the incorporation of rice straw selectively enhanced the growth of *Methanosarcinaceae* and suppressed rice cluster I (*Methanocellaceae*) methanogens (Conrad and Klose 2006). However, the response of the bacterial community to rice straw (RS) addition has only rarely been investigated (Ji et al. 2018; Rui et al. 2009; Wegner and Liesack 2016).

Little attention has been paid to the total amount of CH₄ that is derived from the plant organic substances during the methanogenic phase in different rice soils, although it is very important for the evaluation of the greenhouse gas emissions by adding organic matter in rice field soils. Rice straw, which mainly consists of polysaccharides plus some lignin, is relatively easily degradable, so that after one season typically 80–90% of the added rice straw has disappeared (Neue and Scharpenseel 1987). Therefore, a significant percentage of rice straw should be converted to CH₄ and CO₂ during several weeks of incubation. However, CH₄ is produced not only from the degradation of rice straw but also from soil organic matter and root exudates

(Kimura et al. 2004). For quantification of the RS-derived CH₄, it is, therefore, better to measure the CH₄ produced from isotopically labeled RS (Yuan et al. 2012a). In addition, at the beginning of flooding (fresh, non-methanogenic soils) there will be a succession of bacterial and archaeal communities along with the sequential reduction of inorganic electron acceptors (including SO₄²⁻ and Fe(III)) (Lueders and Friedrich 2000; Yao et al. 1999; Yuan et al. 2012a; Yuan and Lu 2009). Later on, when methanogenic conditions are established the microbial community in rice field soil becomes relatively stable (Yuan et al. 2012a; Yuan and Lu 2009). In order to account for the methanogenic conversion of RS, straw should preferentially be added when the methanogenic community has already established. The responses of the microbial communities to RS addition might then be different from that at the beginning of flooding. In-depth knowledge of the amount of plant organic substances-derived CH₄ production and the response of the microbial community to plant organic substances is of particular importance for the development of options to mitigate CH₄ emissions.

In the present study, five flooded rice soil samples were amended with ¹³C-labelled rice straw after preincubation for 40 days under anaerobic conditions, so as to (1) determine the amount of RS-derived CH₄ and CO₂ production, and (2) identify the response of bacterial and archaeal community in different soils after RS amendment under methanogenic conditions. We hypothesized that in different rice field soils, the total amounts of RS-derived CH₄ are different and there are core microbial populations involved in the decomposition of RS under methanogenic conditions.

2 Materials and methods

2.1 Soil samples

Five different paddy soils were collected from China (Fuyang and Changsha), the Philippines, Italy (Vercelli) and Uruguay. The Fuyang soil, a clay loam, was collected from a rice field at the China National Rice Research Institute in Hangzhou (Rui et al. 2009). The Changsha soil, a sandy clay loam, was collected in a typical double rice cropping field of Changsha County in Hunan Province, China. Uruguay soil, a silt clay soil, was sampled from a field 70 km from the Instituto Nacional de Investigación Agropecuaria (INIA) at the city Treinta-y-Tres, Uruguay (Fernandez Scavino et al. 2013). The Vercelli soil is a silt loam that originated from a rice field at the Italian Rice Research Institute in Vercelli, Italy (Yuan et al. 2012b). The Philippine's rice field soil is a silt loam collected at the

International Rice Research Institute in Los Banos, the Philippines (Breidenbach and Conrad 2015). The rice soil samples were air-dried, sieved (< 0.5 mm) and stored at room temperature (Ma et al. 2010). The main characteristics are given in Table 1. The storage of dry soil samples at room temperature does not significantly influence its CH₄ production capacity (Mayer and Conrad 1990).

2.2 Soil incubation

For the soil incubation, 4 g dry weight soil was mixed with 6 ml anoxic water in each 26-ml pressure tube as described before (Yuan et al. 2014). Each tube was sealed with a butyl rubber stopper together with aluminum crimp, then flushed with pure N₂ for 3 min and incubated statically at 25 °C under dark condition. The production of CH₄, CO₂ and Fe(II) were measured during the incubation (Yao et al. 1999). After 40 days of preincubation, during which any available ferric iron or sulfate was reduced, each tube was amended with 8 mg (0.2%) of RSI or RSII powder so as to prepare RS treatments I and II. An unamended control was run in parallel. The amount of 8 mg RS is equivalent to 267 μmol C. The application of 0.2% RS equals field conditions when the rice straw is applied at roughly 3.6 Mg/ha (considering soil bulk density is around 1.2 g/cm³). The δ¹³C values of RSI (596.1‰) and RSII (885.0‰) were achieved by mixing the desired amount of ¹³C-labeled (δ¹³C = 1859.9‰) and unlabeled (δ¹³C = -27.6‰) RS. All the RS originated from rice plants cultivated in the greenhouse, ¹³C-labeled RS was obtained by cultivating the rice plants in the chamber amended with ¹³CO₂ (Yuan et al. 2012b). The rice plants were harvested at the late vegetative stage, then dried at 60 °C and ground to powder. The tubes were sealed again and flushed with N₂ immediately after amended with RS, then shook and re-flushed with N₂ so as to remove the residual CH₄ and CO₂. At last, the tubes were incubated under the conditions as described above for 22 days. Triplicates were prepared for all the treatments.

2.3 CH₄ and CO₂ analyses

The analyses of CH₄ and CO₂ were the same as described before (Yuan et al. 2018). In brief, every 2 to 5 days, gas samples taken with gas-tight syringe from the headspace of the tubes were injected into a gas chromatograph (GC) equipped with flame ionization detector (FID), so as to analyze the concentrations of CH₄ and CO₂ (Bodelier et al. 2000). The CO₂ was measured after conversion to CH₄ with a methanizer (nickel catalyst at 350 °C) (Penning and Conrad 2007). At the end of incubation, the liquid was analyzed for pH after the tubes were opened. Total inorganic carbon (TIC) was defined as the sum of gaseous, dissolved and bicarbonate CO₂ (Stumm and Morgan 1981) and determined at the end of incubation.

The δ¹³C values of CH₄ and CO₂ were determined using gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) (Finnigan, Bremen, Germany), as described in an earlier study (Penning and Conrad 2007). The δ¹³C values of gaseous and bicarbonate CO₂ were calculated from the δ¹³C of gaseous CO₂ based on the corresponding fractionation factors (Stumm and Morgan 1981), all these δ¹³C values were used to calculate δ¹³C_{TIC} according to the mole fractions of each CO₂ species (Penning and Conrad 2006).

2.4 Calculation of CH₄ and CO₂ derived from the RS applied

The calculations of CH₄ and CO₂ derived from the RS applied were the same as described earlier (Yuan et al. 2014; 2018). In brief, since the treatments RSI and RSII are only different in the δ¹³C of the RS, the fraction of CH₄ derived from RS (*f*_{RS}) could be calculated by:

$$f_{RS} = (\delta^{13}C_{CH_4-I} - \delta^{13}C_{CH_4-II}) / (\delta^{13}C_{RS-I} - \delta^{13}C_{RS-II}) \quad (1)$$

of which the δ¹³C values were measured during the incubation experiment. The δ¹³C_{CH₄-I} and δ¹³C_{CH₄-II} were the δ¹³C values of the CH₄ produced in the tubes of RS treatment I and II, respectively; the δ¹³C_{RS-I} (596.1‰) and

Table 1 Characteristics of the soil samples

Soil	pH	OC (%)	TN (%)	Fe(II) (μmol/g d.w.)	Soil texture	Soil type
Vercelli	7.01	1.39	0.11	88	Silt loam	Fluvisol
Fuyang	7.14	2.38	0.23	123	Clay loam	Hydrargic anthrosol
Changsha	6.92	1.54	0.16	86	Sandy clay loam	Ultisol
Philippines	7.19	1.97	0.15	116	Silt loam	Mollisol
Uruguay	6.64	1.57	0.14	32	Silt clay	Planosol

OC organic carbon, TN total nitrogen

$\delta^{13}\text{C}_{\text{RS-II}}$ (885.0‰) are $\delta^{13}\text{C}$ values of the RS carbon in RS treatment I and II, respectively.

Next, the amount of CH_4 derived from RS ($p_{\text{RS,CH}_4}$) was calculated from the f_{RS} and the total amount of CH_4 produced (p_{CH_4}) in the tube:

$$p_{\text{RS,CH}_4} = f_{\text{RS}} p_{\text{CH}_4} \quad (2)$$

Analogous equations are valid for calculation of the fractions and amounts of CO_2 derived from RS.

2.5 DNA extraction of soil samples and quantification of microbial abundance

The soil samples collected after the incubation were used for DNA extraction with the FastDNA® Spin kit for soil (Qbiogene, Germany). For the quantifications of copy numbers of bacterial 16S rRNA, the primer pair (519f and 907r) and parameters were applied according to the protocol described before (Stubner 2002); for *mcrA* gene, which is marker gene for methanogenic archaea, the primer pair (mlas-mod and mcrA-rev) and parameters were performed according to the protocol described in (Angel et al. 2011). The quantitative PCR was done on iCycler thermocycler (Bio-Rad, Germany).

2.6 Pyrosequencing of the bacterial and archaeal communities

Tagged pyrosequencing of the bacterial and archaeal 16S rRNA genes was performed using primer pairs F515/R806 (Bates et al. 2011) and Arch344F/Arch915, respectively (Casamayor et al. 2002; Yu et al. 2008). The forward primer of each pair contained a unique 6-bp barcode (Hernandez et al. 2015). Sequencing of the pooled PCR products was carried out at the Max Planck Genome Centre in Cologne with a Roche 454 Genome Sequencer GS FLX+.

The raw sequence data were analyzed with Mothur (v. 1.27) software package (<http://www.mothur.org/>) (Schloss et al. 2009). Sequences were removed from further analysis in case they were less than 200 bp, harbored ambiguous bases or homopolymers longer than 6 bp. Chimeras were removed with the UCHIME (Edgar et al. 2011). 97% sequence similarity was used to define the operational taxonomic unit (OTU) from the good sequences. OTU table was created using the UPARSE pipeline (Edgar 2013). Rarefaction, Shannon index, and Chao1 estimators of OTUs were calculated in RDP (<http://pyro.cme.msu.edu/>). The phylogenetic affiliation of each bacterial and archaeal 16S rRNA gene sequence was analyzed with the RDP Classifier at a confidence level of 80%. Resampling to the same sequence depth was conducted before the downstream analysis. The original pyrosequencing data

were deposited in the NCBI Sequence Read Archive (SRA) under the study number SRP058834.

2.7 Statistical analysis

The redundancy analysis (RDA) was performed to measure the influence of environmental variables on bacterial and archaeal communities using the R package Vegan (<http://cran.r-project.org/web/packages/vegan/index.html>). Linear discriminant analysis (LDA) effect size (LEfSe) was used to detect bacterial taxa differentiating the different rice soil samples (Segata et al. 2011). Two-tailed independent *t* test and one-way analysis of variance (ANOVA) were used to test the significance of the differences between and among the treatments on various variables, respectively.

3 Results

3.1 CH_4 production rates in control and RS-amended rice field soils

Anoxic incubation of soils with RS and without (control) resulted in linear accumulation of CH_4 with time. Averaged CH_4 production rates were calculated from the total amount of CH_4 accumulated during the whole incubation period (22 days) in control and RS treatment. The results of the control soils showed that Uruguay soil had the highest inherent potential for CH_4 production, and Fuyang soil also had apparently higher CH_4 production potential compared with other soils (Fig. 1a). The RS addition substantially stimulated the CH_4 production rates in all soil samples. After RS addition, the CH_4 production rates in these soil samples were 2 to 6 times of those in the control.

3.2 RS-derived CH_4 and CO_2 production in different rice field soils

The accumulated total amounts of CH_4 derived from the added RS were calculated with Eq. 2. On day 5 after RS addition, the amounts of RS-derived CH_4 in Uruguay and Fuyang soil were about 3 and 2 times, respectively, larger than those in the other three soils (Fig. 1b). In most soils, the accumulated amounts of RS-derived CH_4 reached the maximum at day 15. In Vercelli soil, however, the amount of RS-derived CH_4 still increased steadily afterward. The total amounts of RS-derived CH_4 were in a range of 35–55 μmol (Fig. 1b). Eventually, the amounts of RS-derived CH_4 in Vercelli and Uruguay soils were by 21 to 46% higher ($P < 0.01$) than those in the other soils. The total amounts of RS-derived CO_2 (shown as TIC) were around 80 μmol at the end of incubation (Fig. 1c). They were relatively lower in Changsha soil than in the other

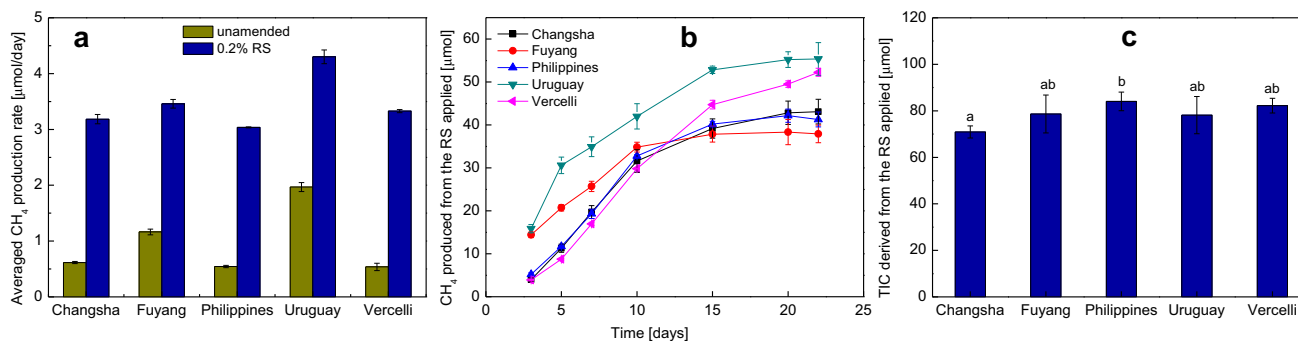


Fig. 1 Averaged CH₄ production rate in control and rice straw (RS) treatment of each soil sample (a). Accumulation of RS-derived CH₄ in each soil sample amended with RS (b). Amounts of RS-derived total inorganic carbon (TIC) in RS amended soil samples at the end of incubation (day 22) (c). The data shown are from treatment RSI since they were almost identical to the results from treatment RSII. Data are mean \pm SD (n = 3). The differences between different soil samples were tested by one-way analysis of variance (ANOVA), different letter on the top of the bar between the column indicated a significant difference ($P < 0.05$) between the soils

soils. The total amounts of RS carbon mineralized to CH₄ + CO₂ in soil from Changsha, Fuyang, the Philippines, Uruguay and Vercelli were 115.7 ± 2.6 , 116.5 ± 10.2 , 125.3 ± 5.7 , 133.5 ± 11.8 , and 134.5 ± 3.1 μmol, respectively. These amounts are equivalent to 42–54% of the added RS carbon.

3.3 Abundance and composition of bacterial and archaeal communities in control and RS-amended rice field soils

The results of quantitative PCR showed that in unamended control soils, the copy numbers of bacteria and methanogenic archaea ranged from 4.4×10^9 to 9.0×10^9 and from 2.5×10^7 to 1.3×10^8 , respectively. RS addition led to a significant increase in the abundance of bacteria only in Fuyang and Philippines soils, while the abundance of methanogenic archaea significantly increased in almost all soils after the RS amendment (Table 2). The final ratio between the *mcrA* gene and bacterial 16S rRNA gene copy

numbers (Fig. 2) was lower in most of the control soils (0.005–0.01) than in Fuyang soil (0.023). After RS addition, however, this ratio decreased in Fuyang soil while it substantially increased in the other soils.

Pyrosequencing analysis of bacterial 16S rRNA genes indicated that *Acidobacteria*, *Actinobacteria*, *Clostridia*, *Chloroflexi*, and *Deltaproteobacteria* were the dominant bacterial groups in each soil sample (Fig. 3a). Among them, the relative abundance of *Clostridia* was obviously higher in Fuyang soil than in the other soil samples. After RS addition, the relative abundance of *Clostridia* apparently increased in the soils from Uruguay, the Philippines and Changsha. The relative abundance of *Chloroflexi* was elevated in Changsha, Fuyang and Vercelli soils after RS addition.

Sequencing of archaeal 16S rRNA genes indicated that the *Methanosarcinaceae* and *Methanocellaceae* were the

Table 2 Increase of copy numbers of bacterial 16S rRNA gene and *mcrA* gene in each soil given by the difference before and after RS addition; mean \pm SD (n = 3)

	Bacterial 16S rRNA genes	<i>mcrA</i> genes
Changsha	$0.3 \times 10^9 \pm 0.8 \times 10^9$	$3.5 \times 10^7 \pm 0.4 \times 10^7$ *
Fuyang	$3.7 \times 10^9 \pm 0.6 \times 10^9$ *	$5.3 \times 10^7 \pm 5.0 \times 10^7$ *
Philippines	$0.8 \times 10^9 \pm 0.4 \times 10^9$ *	$5.1 \times 10^7 \pm 0.6 \times 10^7$ *
Uruguay	$-3.0 \times 10^9 \pm 3.4 \times 10^9$	$1.1 \times 10^7 \pm 0.7 \times 10^7$ *
Vercelli	$-0.9 \times 10^9 \pm 2.6 \times 10^9$	$2.5 \times 10^7 \pm 2.2 \times 10^7$

Two-tailed independent t-test was applied to examine the increase in copy number of each gene after RS addition in each soil sample, indicated by * when $P < 0.05$. Data of Fuyang and Uruguay soil samples were calculated from the bacterial 16S rRNA gene and *mcrA* gene copy numbers presented in Yuan et al. (2018)

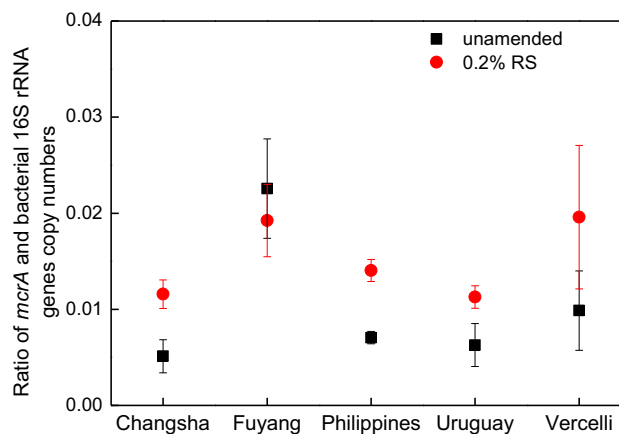


Fig. 2 Ratio between *mcrA* gene and bacterial 16S rRNA gene copy numbers in soils amended and unamended with RS at the end of incubation (day 22); mean \pm SD (n = 3). Results of Fuyang and Uruguay soils were calculated from data presented in Yuan et al. 2018

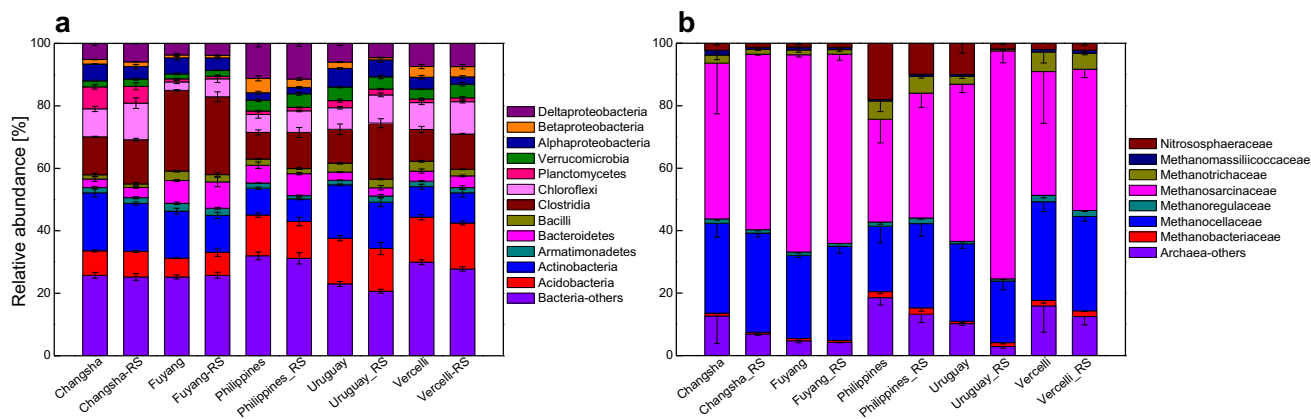


Fig. 3 Relative abundances of bacterial phyla or classes (a), and archaeal families (b) in control and RS treatment of each soil sample. Major taxa with average relative sequence abundances higher than 1% are displayed; mean \pm SD ($n = 3$). Relative abundances of all the rare phyla or families ($< 1\%$ in all samples), candidate divisions and unclassified sequences were combined and indicated with the column “Bacteria-others” or “Archaea-others”

dominant archaeal families in each soil sample (Fig. 3b). The relative abundance of *Methanocellaceae* was slightly elevated in Fuyang, Changsha and the Philippines soil samples, while *Methanosarcinaceae* were substantially higher (by 22.6%) in the Uruguay soil sample, and also slightly higher in the other soils compared to the Fuyang soil. Besides, the relative abundances of *Nitrososphaeraceae* were higher in the control soils from the Philippines and Uruguay compared with the other soils but apparently decreased in both soils after RS addition.

Redundancy analysis (RDA) was conducted to analyze the relationship between environmental variables and microbial communities. The RDA ordination showed that specific environmental conditions shaped the variations in bacterial and archaeal community compositions (Fig. 4). The bacterial community of Fuyang soil was strongly affected by both soil organic carbon (SOC) and total nitrogen (TN) contents (Fig. 4a). As for the archaeal community, the relatively large vector size indicated that TN was more strongly correlated with the archaeal community composition than with the other tested factors (Fig. 4b).

To detect the bacterial groups that most likely explain the differences among the different soil samples, we used the linear discriminant analysis effect size (LEfSe), a tool for biomarker discovery. LEfSe detected many bacterial groups with statistically significant differences ($P < 0.001$) among the soil samples (Fig. 5). For example, four bacterial families belonging to the class *Clostridia* were over-represented in Fuyang soil, while the order *Bacillales* and the families *Beijerinckiaceae* and *Bradyrhizobiaceae* were significantly more abundant only in Uruguay soil. The family *Chitinophagaceae* and several families affiliated with *Deltaproteobacteria*, including *Geobacteraceae* and *Desulfobacteraceae* were more abundant in the Philippines

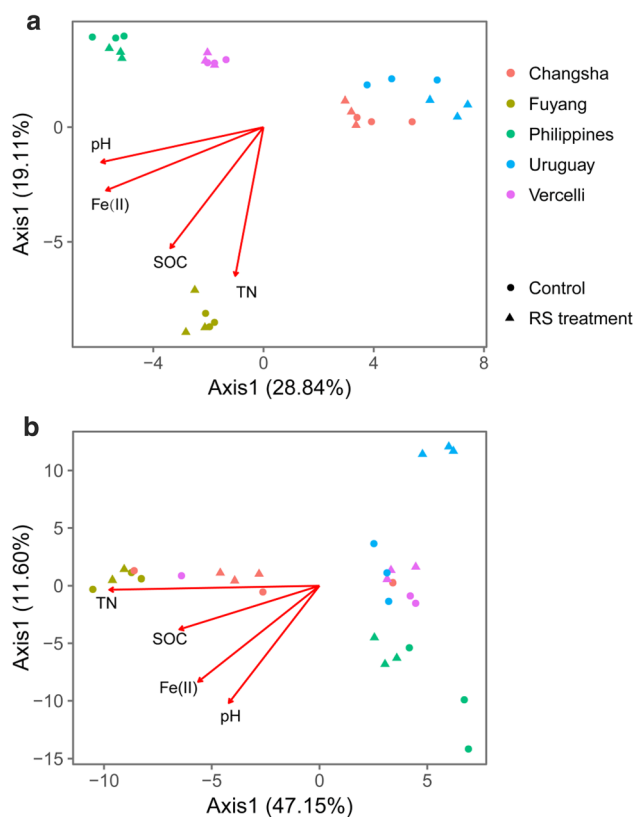


Fig. 4 Ordination diagrams from redundancy analysis (RDA) of bacterial (a) and archaeal (b) communities and environmental parameters. Arrows indicate the direction and magnitude of environmental parameters associated with bacterial and archaeal community structures. Each sample is represented by a colored circle (control) and triangle (RS treatment). *TN* total nitrogen, *SOC* soil organic carbon

soil, but the family *Planctomycetaceae* was relatively higher in Changsha soil.

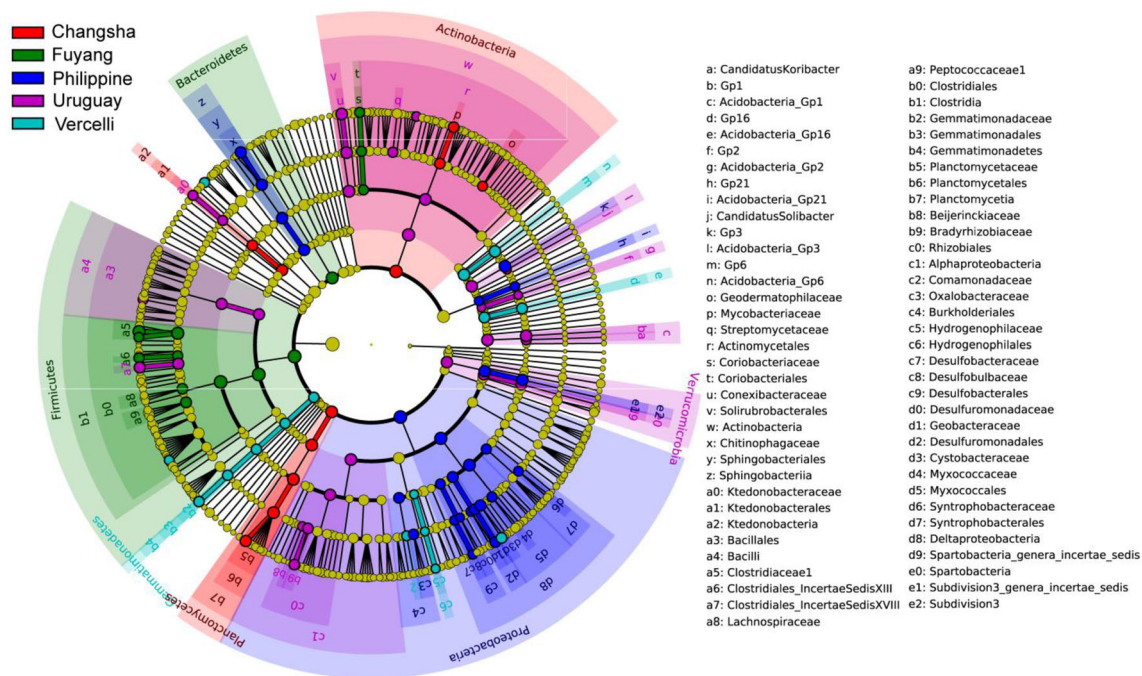


Fig. 5 Taxonomic cladogram obtained from LEfSe analysis of bacterial taxa (relative abundance > 0.5% in at least one sample). The LEfSe analysis identified the most differentially ($P < 0.001$) abundant taxa among the soil samples (highlighted by small circles and by shading); color denotes the different soils and that the colored bacterial taxon was significantly overrepresented in the respective soil. The diameter of each dot is proportional to the relative abundance of the taxon. Yellow circles represent non-significant differences in abundance among the soil samples for that particular taxonomic group

3.4 Key bacterial groups responsive to RS addition in rice field soils

In order to identify the key bacterial groups responsive to the addition of RS, we summarized the significant increase of members of the bacterial community at a lower taxonomic rank (Table 3). There were about 17 bacterial families that significantly increased (albeit to different extents) after RS addition, most of them being affiliated with *Firmicutes*, *Acidobacteria*, *Bacteroidetes*, *Chloroflexi* and *Verrucomicrobia*. The Uruguay soil exhibited a higher increase (10.8%) in the relative abundance of bacterial families after RS addition than the other soils. This observation was consistent with the increase of several *Firmicutes* only in Uruguay soil after RS treatment, such as the Clostridiaceae 1 and Clostridiales_Incertae Sedis XVIII. Upon RS addition, there was a general increase in the relative abundances of both *Ruminococcaceae* and *Anaerolineaceae* in all soil samples, while *Opiutaceae* increased to a less extent in most of the soil samples.

4 Discussion

4.1 Methanogenic decomposition of RS in rice field soils with different inherent CH_4 production potential

Rice straw typically contains > 85% easily degradable organic matter (i.e., soluble material, polysaccharides, lipids) and only low amounts of recalcitrant lignin (about 5–15%) (Watanabe et al. 1993). The decomposition of RS started immediately after RS addition. The slow-down of CH_4 accumulation after day 15 indicated that the fast phase of RS decomposition was finished probably because the easily degradable organic carbon in RS was exhausted (Lu et al. 2003). A previous study also showed that the decomposition rate of RS was very high in the early stage after RS addition, while the decomposition rate strongly decreased after 22 days (Watanabe et al. 1998). These observations are consistent with the finding that turnover times of cellulose degradation are in a range of 7–14 days in fresh and methanogenic soil (Chidthaisong and Conrad 2000). Since the RS applied was ground to powder for accelerating the decomposition rate, the easily degradable organic carbon of the RS applied was probably almost completely decomposed at the end of the incubation. In Uruguay and Fuyang soil, the apparently higher RS-

Table 3 Increase in relative abundances of bacterial families and unclassified bacterial groups in each soil after RS application

Taxa	Fuyang (%)	Uruguay (%)	Vercelli (%)	Changsha (%)	Philippines (%)
<i>Clostridia Clostridiales Ruminococcaceae</i>	1.519	1.42	0.90	2.35	1.92
<i>Clostridia Clostridiales Lachnospiraceae</i>	0.56				0.19
<i>Clostridia Clostridiales Clostridiaceae</i> 1		2.09			
<i>Clostridia Clostridiales Clostridiales_Incertae Sedis XVIII</i>		1.63			
<i>Bacilli Bacillales Bacillaceae</i> 1		0.30			
Unclassified <i>Firmicutes</i>		0.67			
<i>Chloroflexi Anaerolineae Anaerolineales Anaerolineaceae</i>	2.75	2.00	1.55	3.05	1.74
<i>Verrucomicrobia Opitutae Opitutales Opitutaceae</i>	0.29		0.48	0.27	0.53
<i>Verrucomicrobia</i> unclassified Subdivision3			0.91	0.35	
<i>Verrucomicrobia</i> unclassified <i>Spartobacteria</i>		0.93			
<i>Actinobacteria Acidimicrobiales Acidimicrobinae_incertae_sedis</i>			0.10		1.12
<i>Actinobacteria Actinomycetales Cellulomonadaceae</i>			0.54	0.08	
<i>Bacteroidetes Bacteroidia Bacteroidales Prolixibacteraceae</i>			0.46		
Unclassified <i>Bacteroidetes</i>	1.50				
Unclassified <i>Acidobacteria_Gp3</i>	1.12				
Unclassified <i>Acidobacteria_Gp1</i>		0.82			
Unclassified BRC1		0.98			
Total	7.74	10.84	4.94	6.10	5.50

The bacterial group was shown when its relative abundance was significantly higher in RS treatment compared with that in control ($P < 0.05$)

derived CH₄ production rates at the beginning of RS decomposition could be attributed to the higher inherent potential for CH₄ production in both soils compared with other soil samples. Previous studies have reported higher production rates of RS-derived CH₄ in soils of stronger inherent CH₄ production potential (Lu et al. 2000).

However, our results indicated that soils with higher inherent CH₄ production rates did not necessarily produce higher total amounts of RS-derived CH₄ or vice versa. The higher total amounts of RS-derived CH₄ in Uruguay and Vercelli soil samples were consistent with their relative higher total amounts of RS-derived CO₂ compared with other soil samples (Fig. 1b, c). Thus, methanogenic decomposition of the same amount of RS resulted in more end products (CH₄ and CO₂) in Uruguay and Vercelli soils compared to the other soils. Still, the RS-derived CH₄ and CO₂ represented at most 54% of the applied RS carbon. Hence, a large part of the non-recalcitrant RS carbon must have become converted to organic matter that was no longer easily available to methanogenic degradation, perhaps microbial biomass or new soil organic carbon, especially in Fuyang, Changsha and the Philippines soils. This conclusion is consistent with the increase in abundance of bacterial 16S rRNA genes and/or methanogenic *mcrA* genes that was higher in Fuyang, Changsha and the Philippines soils than in the other soil samples (Table 2). Furthermore, the results suggest that in these three paddy soils straw application may result in a relatively higher

elevation of SOC content than in the other soils. This increase possibly also causes higher soil fertility, since increasing SOC content has been found to exhibit a positive correlation with the responses of soil macro-aggregates and crop yield to straw return (Liu et al. 2014).

Meanwhile, the ratios of RS-derived CO₂ to CH₄ were between 1.4 and 2.1 in these soil samples, although complete mineralization of polysaccharides (e.g. rice straw) under methanogenic conditions should give equal amounts of CO₂ and CH₄ (Conrad et al. 2010; Zinder 1993). This was partly because hydrogenotrophic methanogenesis from SOC-derived CO₂ was enhanced by H₂ released during RS decomposition (Yuan et al. 2014) so that the RS-derived CH₄ production was reduced accordingly. In addition, although acetate carbon was predominantly consumed by acetoclastic methanogens, part of it could also have been assimilated as a C source by a small and heterogeneous community of bacteria, thus resulting in a decrease of CH₄ production (Hori et al. 2007; Schwarz et al. 2007).

4.2 Responses of bacterial and archaeal communities to RS addition in methanogenic rice field soils

The bacterial communities were apparently different on the phylum level among the different rice field soils (Fig. 3a), being consistent with LEfSE analysis of the different soils (Fig. 5). The differences in bacterial communities were

well explained by distinct controlling factors in the different soils, including pH and the contents of iron, soil organic carbon and total nitrogen (Fig. 4). For example, the higher SOC content in Fuyang soil probably explained its obviously higher abundances of bacterial families affiliated with *Clostridia*, which are usually involved in hydrolysis and fermentation of organic matter (Kim and Liesack 2015; Rui et al. 2015). In addition, the responses of the bacterial communities to RS addition were also largely different among the different soils.

First, there were differential increases of bacterial families affiliated with *Acidobacteria*, *Bacteroidetes*, and *Verrucomicrobia* among the different soils, although these different taxonomic bacterial groups were probably all involved in hemicellulose breakdown after RS addition (Wegner and Liesack 2016). Moreover, several families affiliated with *Clostridia* displayed a strong increase after RS addition (Table 3). Among them, *Clostridiaceae*, *Lachnospiraceae* etc. were found to predominantly express glycosyl hydrolase transcripts that are involved in cellulose and chitin breakdown after RS addition to Vercelli soil (Wegner and Liesack 2016). However, the relative abundance of *Clostridiaceae* increased only in Uruguay soil (Table 3), while it decreased in the other soils including Fuyang soil (data are not shown), in which *Clostridiaceae* had the highest relative abundance (Fig. 5). In contrast, a previous report indicated that RS addition in Fuyang soil at the beginning of flooding largely stimulated the abundance of species affiliated with *Clostridiaceae* (Rui et al. 2009). These results suggested that the role of *Clostridiaceae* may change in different soils and under different soil physico-chemical conditions.

Common to all the different soils was the observation that *Ruminococcaceae* and *Anaerolineaceae* substantially increased. The *Ruminococcaceae* are known for plant polymer breakdown in gut environments (Jalanka-Tuovinen et al. 2011; Turnbaugh et al. 2009) and rice field soil (Wegner and Liesack 2016). Similarly, most species of the *Anaerolineaceae* are capable of fermentative metabolism, they could utilize carbohydrates and proteinaceous carbon substrates under anaerobic conditions (Sekiguchi et al. 2003; Sun et al. 2016; Yamada et al. 2006). They are likely important primary fermenters in anaerobic environments, such as anaerobic digester (McIlroy et al. 2017; Meng et al. 2017), but their role in the paddy soils was hardly reported previously. Besides, *Opitutaceae* increased to a less extent in most of the soil samples. The genomic analysis of *Opitutaceae* bacterium strain isolated from wood-feeding termites showed that it harbors genes coding for lignocellulosic degradation (Isanapong et al. 2012). And OTUs belonging to *Opitutaceae* were identified consumers of plant-derived carbon in the rice rhizosphere (Breidenbach et al. 2016; Hernandez et al. 2015). On the other hand, RS

addition just resulted in a relative increase of *Methanosarcinaceae* and/or *Methanocellaceae* (Fig. 3b), probably because both families were the only dominant methanogenic archaeal groups in each soil samples.

5 Conclusion

Our results suggest that after RS addition the rates of CH₄ production and the total amounts of RS-derived CH₄ are distinct in different rice field soils under methanogenic conditions. Two potential core populations consisting of *Ruminococcaceae* and *Anaerolineaceae* were identified, suggesting that they may be indispensable during the primary fermentation of RS under methanogenic conditions. These results are probably important for evaluating the greenhouse effect of rice residues, which will help to improve process-based modeling of CH₄ emission from flooded rice fields. Meanwhile, these results suggest that the amount of straw return should be reduced in the rice field soils because of its adverse effects on CH₄ production.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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