Dynamics of sulfate reduction and sulfate-reducing prokaryotes in anaerobic paddy soil amended with rice straw

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Abstract Incorporation of rice straw to soil is a common agricultural practice in rice cultivation. In anaerobic paddy soil, the complete mineralization of organic matter to CH₄ and CO₂ is accomplished by the sequential reduction of nitrate, ferric iron, sulfate, and methanogenesis. In order to estimate the temporal changes of sulfate-reducing prokaryotes (SRP) as decomposers of organic matters, the effects of rice straw amendment on the dynamics of sulfate reduction and SRP were investigated by combining the monitoring of CH₄, sulfate, and organic acids with molecular tools such as soil DNA extraction, real-time PCR, cloning, sequencing, and phylogenetic analysis. The incorporation of rice straw into paddy soil significantly increased concentrations of sulfate, formate, acetate, propionate, and lactate and CH₄ production. The rate of sulfate reduction in the straw-amended slurries was significantly higher than that in the unamended slurries. The *dsrAB* gene copy numbers of SRP in the straw-amended soil slurries ranged from 4.26×10^6 to 1.96×10^8 per gram of dry soil, which were significantly higher than those in the unamended control ranging from 1.99×10^6 to 7.90×10^7 per gram of dry soil. Significant correlations were observed between SRP *dsrAB* gene copy numbers and the concentrations of sulfate and acetate. Cloning and sequencing analyses showed a clear shift of

SRP community structure between treatments and time. In the straw-amended slurries, Clostridia-like SRP significantly increased, while Deltaproteobacteria-like SRP (*Syntrophobacter*, *Desulfobacterium*, *Desulfovibrio*, and *Desulfomonile*) decreased during the incubation period. Novel uncultured SRP were abundant in the straw-amended slurries and changed during the incubation period.

Keywords Sulfate-reducing prokaryotes · Paddy soil · Acetate · Quantification · Real-time PCR · Community structure · Clone libraries

Introduction

In paddy soil, methanogenesis is not the exclusive final step in anaerobic organic matter mineralization. Reductions of nitrate, ferric iron, and sulfate play important roles in the mineralization process, especially during the early phase of flooding of the rice fields when nitrate, ferric iron, and sulfate have not been completely depleted (Yao et al. 1999). Rice straw is one of the main carbon sources and commonly incorporated into the paddy soil to enhance soil fertility and crop productivity (Ponnumperuma 1984). After addition of rice straw into the paddy soil, the C/N ratio changed from 18:1 to 24:1, indicating a relative large increase of soil carbon content and C/N ratio (Conrad and Klose 2006). Rice straw increased concentration of available nutrients and microbial biomass (Shibahara and Inubushi 1997). It also increased copies of bacterial and archaeal 16S rRNA in anaerobic slurries of Italian paddy soil (Weber et al. 2001; Conrad and Klose 2006), suggesting microorganisms might have been actively involved in the mineralization of rice straw.

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The incorporation of rice straw into paddy soil has been a prevalent agronomic practice in southeast China (Shen et al. 2007). Rice straw consists of different biopolymers, including hemicellulose (26% to 35%), cellulose (38% to 41%), lignin (15%), and water-soluble polysaccharides (8%) (Grant 1998). There is a complex community with microbial populations interacting with each other for degrading these biopolymers in paddy soil. These microbial groups include hydrolytic, cellulolytic, fermenting, homoacetogenic, and syntrophic bacteria and acetate- and H_2 -utilizing sulfate-reducing prokaryotes (SRP) and methanogenic archaea (Stams 1994; Chidthaisong and Conrad 2000a). Hydrolytic microorganisms convert the macropolymers to monomers and oligomers such as cellobiose and glucose firstly. These hydrolysates are then fermented by cellulolytic and associated saccharolytic organisms, yielding various organic acids, alcohols, H_2 , and CO_2 . The next step involves the conversion of organic acids to H_2 , CO_2 , and acetate. The final step in organic carbon mineralization involves the conversion of acetate, H_2 , and CO_2 either into CH_4 in methanogenic environments or to CO_2 in the environments in which electron acceptors such as sulfate or ferric iron and the bacterial populations of sulfate reducers and iron reducers are present. Quantitatively, acetate is the main substrate for methanogenesis in freshwater sediments and for sulfate reduction in marine sediments (Ward and Winfrey 1985). Sulfate reducers (i.e., sulfate-reducing prokaryotes) can potentially compete with methanogens for acetate in freshwater sediments and have been shown to oxidize a part of the available acetate even at very low sulfate concentration (Smith and Klug 1981). There was also an association of methane oxidation with sulfate reduction under anaerobic conditions whereas it has not been documented in paddy fields (Barnes and Goldberg 1976; Panganiban et al. 1979).

However, so far, all information about the microbial community involved in this anaerobic degradation processes was obtained either by the investigation of bacterial community or by the characterization of methanogen community (Weber et al. 2001; Conrad and Klose 2006). Detailed information on the response of sulfate reduction and sulfate-reducing prokaryotes in rice straw-amended conditions is still lacking. In this study, we hypothesized that: (1) the application of rice straw could result in the temporal change of sulfate reduction in paddy soil slurries and (2) the application of rice straw might cause dynamic shifts in SRP abundance and community structure during the incubation period. Therefore, we tested this hypothesis by anaerobic incubation of paddy soil amended with rice straw and determination of the dynamics of sulfate reduction, formate, acetate, propionate, lactate, and CH_4 production. The changes of SRP abundance and community structure during the degradation of rice straw in paddy soil slurries should be

determined using combined real-time PCR, clone library, and sequencing analyses.

Materials and methods

Soil and straw samples

Soil samples were collected from the Taoyuan long-term Paddy Soil Experimental Station of Chinese Academy of Sciences (28°55'49.8"N, 111°26'25.7"E), Hunan Province, China. It is a reddish clayey paddy soil, and the main characteristics have been described previously (Zheng et al. 2008; Liu et al. 2009). The soil samples were air-dried and sieved at <2 mm. Air-dried straw, originated from rice plants named *Oryza sativa* L cv. Jiahua-1 (Chen et al. 2008), was cut into pieces of 2 cm in length.

Preparation and incubation of slurries

The experimental setup was that described by Weber et al. (2001) and reported in Table 1: glass bottles (150-ml) were filled with 40 g of dry soil, 0.5 g of rice straw, and 40 ml of deionized water. The bottles were gassed with N_2 for 2 min and closed with butyl rubber stoppers. Then, the bottles were vigorously shaken by hand to homogenize the soil straw mixture and incubated for 4 weeks at 25°C in the dark. In control, rice straw was incubated as the above procedure. Gas and soil pore water samples from triplicate tubes of each treatment were collected at days 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28 for chemical analysis. Triplicate soil samples were collected at day 1 and day 28 to extract DNA for molecular microbial analysis.

Analytical techniques

At the sampling days, gas samples (0.5 ml) were taken from the headspace with a pressure-lock syringe after vigorously shaking the bottles by hands. The concentrations of CH_4 were measured by a gas chromatograph equipped with a flame ionization detector and a molecular sieve 5 Å column (Dionex, USA). Pore water samples (3 ml) were taken with sterile syringe equipped with long needles. The pore waters were transferred to 5-ml tubes and centrifuged for 15 min at $16,000\times g$ at 4°C. The supernatants were collected and stored at -20°C until further analysis. Prior to analysis, the samples were filtered through a 0.2- μm membrane filter. Sulfate concentration was measured by ion chromatography equipped with an AS14 column and an ECD50 conductivity detector (Dionex, USA). Organic acids such as formate, acetate, propionate, and lactate were analyzed by high-performance liquid chromatography (Dionex, USA).

Table 1 The setup and sampling method of the paddy soil slurry experiment

Treatment	Sampling		Nomination	Content		ddH ₂ O (40ml)
	Type	Time		Dry soil (40g)	Rice straw (0.5g)	
Control	Gas and soil pore water	Days 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28	/	–	+	+
Unamended paddy soil slurry	Soil	Day 1	SUS1	+	–	+
		Day 28	SUS28			
Straw-amended paddy soil slurry	Gas and soil pore water	Days 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28	/			
	Soil	Day 1	SAS1	+	+	+
		Day 28	SAS28			
	Gas and soil pore water	Days 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28	/			

Soil DNA extraction

Soil DNA was extracted from 0.5 g of fresh paddy soil slurry with the FastDNA[®] SPIN Kit for Soil (MP Biomedicals, USA) as recommended by the manufacturer. The extracted DNA was evaluated on a 1% agarose gel, and the concentration and purity of the extracts were estimated by spectrophotometry (NanoDrop, USA).

Quantification analysis of *dsrAB* genes by real-time PCR

Abundances of SRP were determined by quantitative real-time PCR analysis based on dissimilatory (bi)sulfite reductase genes (*dsrAB*) α -subunit on an iCycler iQ5 thermocycler (Bio-Rad, USA) according to the protocols described previously (Liu et al. 2009). In short, quantitative PCR was carried out with the DSRp2060F and DSR4R primers as follows: for initial denaturation, 2 min at 95°C; 40 cycles of 10 s at 95°C, 30 s at 50°C, and 30 s at 72°C. The DNA concentration was 5 to 10 ng for each reaction. Triplicates for standard and unknown DNA samples were performed on a single plate. The results were analyzed using the iQTM5 software (Bio-Rad, USA). Copy numbers were log-transformed to normalize the values prior to statistical analysis.

Construction of the *dsrAB* gene clone library and RFLP analysis

Amplifications of the *dsrAB* gene were processed with the primer set DSRAB_F (5'-ACSCACTGGAAGCACG GYGG-3') and DSRAB_R (5'-GTAGCAGTTWCCGCAG WACAT-3') as described previously, which produced a 1.9-kb fragment (Schmalenberger et al. 2007). The 50- μ l reaction mixture consisted of 5 μ l 10 $\times$ Ex Taq buffer, 4 μ l dNTPs (2.5 mM), 0.3 μ l Ex Taq HS (5U μ l⁻¹; Takara, Japan), 0.5 μ l bovine serum albumin (25 mM), 0.5 μ l of

each primer (10 μ M), 3 μ l of template DNA (5–10 ng), and 36.2 μ l distilled H₂O. PCR amplification was carried out with the following conditions: three minutes at 94°C; and 35 cycles of 1 min at 94°C, 1 min at 60°C, and 3 min at 72°C; and a final extension at 72°C for 10 min. The PCR products were subjected to electrophoresis on a 1% agarose gel, and the expected fragment was gel-purified by the Wizard[®] SV Gel and PCR Clean-Up System (Promega, USA). Four clone libraries of *dsrAB* gene were constructed from the DNA extractions at days 1 and 28 from the straw-amended and unamended paddy soil slurries. The purified PCR products were ligated into the pGEM-T Easy Vector (Promega, USA) and transformed into the *Escherichia coli* JM109 competent cells (Takara, Japan) according to the manufacturer's instructions. The expected PCR products were screened by the vector-specific primer set SP6 and T7. Approximate 100 positive colonies were randomly selected from each library for restriction fragment length polymorphism (RFLP) analyses using the restriction enzyme *Mbo* I. The digested products were analyzed by 2.5% agarose gel electrophoresis at 37°C for 2 h. The RFLP patterns were analyzed by the software Quantity One (Bio-Rad, USA) and grouped according to the different band patterns.

Sequencing and phylogenetic analysis

At least one clone representative of each RFLP pattern was selected. The part encoding the α -subunit (800 bp) of the dissimilatory sulfate reductase was sequenced on the ABI3700 sequencer (Applied Biosystems, USA). The homology analysis of deduced amino acid sequences of dissimilatory sulfate-reductase α -subunit (DsrA) was performed with the software DNAMAN version 6.0. (Lynnon Biosoft, USA). The sequences showing more than 80% amino acid identity were grouped into the same operational taxonomy unit (OTU). The representative

sequences of each OTU were aligned and analyzed using Clustal X 1.81 (Thompson et al. 1997). Phylogenetic tree was constructed with MEGA version 4.0 using neighbor-joining method with bootstrap value of 1,000 (Tamura et al. 2007). The sequences in different OTUs were deposited in the GenBank nucleotide sequence database under accession numbers from FJ472864 to FJ472887.

Statistical analysis

The differences in concentrations of SO_4^{2-} , organic acids, and CH_4 between treatments were statistically evaluated using one-way analysis of variance under significant differences $P < 0.05$ using SPSS 13.0 statistical package. Bivariate correlation analyses were carried out to link SRP

dsrAB gene copy numbers and concentrations of sulfate, organic acids, and CH_4 .

Results

Reduction of sulfate

The sulfate concentration in the straw-amended slurries was significantly higher ($P < 0.05$) than that in the unamended slurries from 1 to 14 days (Fig. 1a). The rates of sulfate reduction calculated during the active period of sulfate utilization were 0.12 mM d^{-1} and 0.10 mM d^{-1} in the straw-amended and unamended slurries, respectively. Sulfate of the unamended paddy soil slurries was completely

Fig. 1 Temporal changes of sulfate (a), formate (b), acetate (c), propionate (d), lactate (e), and CH_4 (f) in rice straw-amended (filled circle) and unamended (open circle) paddy soil slurries (bars=SD, $n=3$)

reduced at about day 7. Application of rice straw delayed the completion of sulfate reduction until day 14. The sulfate concentration was approximately 1 mM after 1 day of incubation in the straw-amended slurries, while initial sulfate concentration in the unamended slurries changed from 0.2 to 0.4 mM in the first 3 days. There was a 6-day lag before sulfate started decreasing in the amended slurries compared with a 3-day lag in unamended slurries.

Accumulation of organic acids and CH₄ production

Acetate was found in high concentrations compared with the concentrations of other organic acids in all samples (Fig. 1c). Acetate concentration in the straw-amended slurries was significantly higher ($P<0.05$) than that in the unamended slurries from day 1 to day 7. In the unamended slurries, the acetate concentration decreased from 1.5 mM at day 1 to approximately 0 at day 14. In the straw-amended slurries, up to 10.3 mM acetate transiently accumulated with a maximum at day 6, but thereafter, acetate turnover progressed more rapidly than in the unamended slurries. Thus, acetate depletion was also complete at day 14. Formate, propionate, and lactate concentrations in the straw-amended slurries were significantly higher ($P<0.05$) than those in the unamended control from day 1 to day 28 (Fig. 1b, d, and e). Application of rice straw stimulated significant increment ($P<0.05$) of CH₄ production compared with the unamended slurries after day 7 (Fig. 1f). The rate of CH₄ production in straw-amended slurries was 0.96 mmol l⁻¹ d⁻¹, which was six times higher than in the unamended slurries with 0.16 mmol l⁻¹ d⁻¹.

Abundance of *dsrAB* gene

The *dsrAB* gene copy numbers of SRP in the straw-amended paddy soil slurries, ranging from 4.26×10^6 to 1.96×10^8 per gram of dry soil, was significantly higher ($P<0.05$) than those in the unamended slurries ranging from 1.99×10^6 to 7.90×10^7 per gram of dry soil at day 1, 14, 21, and 28 (Table 2). After 1 day incubation, the *dsrAB* gene copy numbers of SRP in the straw-amended slurries were greater than that in the unamended control. The *dsrAB* gene copy numbers of SRP in both straw-amended and unamended slurries decreased significantly ($P<0.05$) from day 1 to day 14, while increased significantly ($P<0.05$) from day 14 to day 28. Bivariate correlation analyses demonstrated that the SRP *dsrAB* gene copy numbers had significant correlations with concentrations of sulfate and acetate (Table 3).

Community structure of SRP

Four clone libraries were constructed from the SAS1, SAS28, SUS1, and SUS28. Phylogenetic trees of SRP

Table 2 Temporal changes of sulfate-reducing prokaryotes *dsrAB* gene copy numbers in anaerobic Chinese paddy soil slurries amended and unamended with rice straw

	<i>dsrAB</i> gene copy number (copies g ⁻¹ dry soil)		Contrast (SAS vs SUS)
	Straw-amended slurry (SAS)	Unamended slurry (SUS)	
Day 1	$1.96 \times 10^8 \pm 6.26 \times 10^7$ a ^a	$7.90 \times 10^7 \pm 4.76 \times 10^7$ a	**
Day 14	$4.26 \times 10^6 \pm 1.81 \times 10^6$ b	$1.99 \times 10^6 \pm 9.69 \times 10^5$ b	**
Day 21	$6.55 \times 10^6 \pm 1.30 \times 10^6$ bc	$1.72 \times 10^6 \pm 2.82 \times 10^5$ b	**
Day 28	$1.85 \times 10^7 \pm 5.50 \times 10^6$ c	$8.27 \times 10^6 \pm 1.77 \times 10^6$ c	**

^a Mean $\pm$ SD, $n=3$. Values within the same column followed by the same letter do not differ at $P<0.05$

**Values differ significantly at $P<0.05$ between straw-amended slurry and unamended slurry

dsrAB gene sequences and related sequences are shown in Fig. 2. All SRP *dsrAB* gene sequences fell within six clusters, affiliated to Deltaproteobacteria, Clostridia/*Archaeoglobus*, and uncultured SRP. More than half of the *dsrAB* gene sequences (51.5% of the total clone numbers, Table 4) were closely related to uncultured SRP. Cluster 2 and cluster 3 (34.9% of the total numbers, Table 4) belonged to Gram-positive SRP of Clostridia or *Archaeoglobus*. Cluster 1 (13.6% of the total numbers, Table 4) was affiliated to Deltaproteobacteria, composed of the families Syntrophobacteraceae, Syntrophaceae, Desulfobacteraceae, and Desulfovibrionaceae. Phylogenetic analysis of *dsrAB* gene sequences revealed pronounced changes between treatments and time in the relative abundance of different SRP clusters (Table 4). In the straw-amended slurries, uncultured SRP of cluster 4 were the dominant SRP groups. In the unamended slurries, however, members of uncultured SRP of cluster 5 and cluster 2 of Clostridia became abundant. The proportions of uncultured SRP of clusters 5 and 6 increased during the incubation period in both straw-amended and unamended slurries, while the abundance of clusters 1, 2, and 4 decreased during the incubation period.

Table 3 Correlation analyses between the SRP *dsrAB* gene copy numbers and concentrations of sulfate, organic acids, and CH₄ in paddy soil

	<i>P</i> value	Pearson correlation coefficient
SO ₄ ²⁻	0.029	0.759
Formate	NS	-0.177
Acetate	0.009	0.839
Propionate	NS	-0.172
Lactate	NS	0.033
CH ₄	NS	-0.698

NS not significant $P>0.05$

Fig. 2 Phylogenetic trees based on the protein sequences of the dissimilatory (bi)sulfite reductase α -subunit (DsrA) retrieved from the straw-amended and unamended paddy soil slurries. Bootstrap values of >50% based on 1,000 replicates are indicated by the numbers at the nodes

Discussion

Temporal change of sulfate

In our experiment, the productions of sulfate, formate, acetate, propionate, lactate, and CH_4 were significantly higher in the straw-amended slurries than in the unamended control, which was in accordance with previous observation

that application of organic carbon into freshwater wetlands could stimulate sulfate reduction, organic acids, and CH_4 emission (Feng and Hsieh 1998; Glissmann and Conrad 2000). The sulfate concentration in the straw-amended slurries after 1 d incubation was approximately 0.9 mM, which was much higher than that of 0.3 mM in the unamended slurries. It was due to the high sulfate concentration of 0.5 mM measured in the control treatment

Table 4 Relative abundance (%) of major phylogenetic SRP clusters in *dsrAB* gene clone libraries in both straw-amended and unamended paddy soil slurries

Phylogenetic group		OTU	Clone library (%) ^a				Total
			SAS1	SAS28	SUS1	SUS28	
Cluster 1	Deltaproteobacteria	OTU-9, 21–24	20.9	13.3	16.6	4.0	13.6
Cluster 2	Clostridia/ <i>Archaeoglobus</i>	OTU-13, 14, 15, 16	25.0	23.3	33.3	20.0	25.2
Cluster 3	Clostridia/ <i>Archaeoglobus</i>	OTU-17, 18, 19, 20	8.3	13.3	4.2	12.0	9.7
Cluster 4	Uncultured SRP	OTU-1–8,12	33.3	26.7	16.7	12.0	22.4
Cluster 5	Uncultured SRP	OTU-11	8.3	16.7	29.2	48.0	25.2
Cluster 6	Uncultured SRP	OTU-10	4.2	6.7	0.0	4.0	3.9

^a Clone library: SAS1 straw-amended paddy soil slurry at day 1, SAS28 straw-amended paddy soil slurry at day 28, SUS1 straw-unamended paddy soil slurry at day 1, SUS28 straw-unamended paddy soil slurry at day 28

only with rice straw in our experiment. Because of the significantly higher sulfate concentration from straw amendment, soil redox potential (Eh) might become elevated. This made sulfate reduction thermodynamically endergonic. Hence, it is reasonable that sulfate started decreasing after a 6-day lag in the straw-amended slurries compared with a 3-day lag in the unamended slurries.

Temporal change of organic acids

In paddy soil, decomposition of rice straw served as an important source of organic matter for SRP growth in the anaerobic process (Chidthaisong and Conrad 2000b). Application of rice straw stimulated the immediate accumulation of fermentation products during the incubation period. In the detected organic acids, acetate was in most abundance during the decomposition of rice straw, whereas propionate showed the lowest concentration. This result was different from a previous study that found propionate was also an important intermediate following acetate (Glissmann and Conrad 2000). The unamended slurries also exhibited a lower amount of organic acids than the amended slurries, suggesting that, even without straw, the organic matter of paddy soil can be degraded with production of organic acids during anaerobic incubation.

Abundance of SRP in straw-amended paddy soil slurries

Addition of rice straw also resulted in a significantly higher SRP *dsrAB* gene copy numbers in the straw-amended paddy soil slurries compared with the unamended slurries, indicating the important roles of carbon substrate to stimulate the growth of *dsrAB*-containing SRP. The SRP *dsrAB* gene copy numbers had significant correlations with acetate concentration and sulfate concentration, respectively. This suggested that acetate and sulfate concentrations were important factors in controlling SRP abundance in the paddy soil slurries, and acetate was the most active intermediate

organic acid in terms of its concentration. The higher copy numbers of *dsrAB* gene in the straw-amended slurries than that in the unamended slurries may be due to the large SRP *dsrAB* gene copy numbers in the straw itself. The decrease of the *dsrAB* gene copy numbers from day 1 to day 14 was paralleled by the depletion of sulfate. Then, the *dsrAB* gene copy numbers increased from day 14 when the paddy soil slurries were in methanogenic steady state. Possible explanations for these results include: (1) sulfate was not the limiting factor to SRP growth; (2) SRP can survive by fermentation in syntrophism with methanogens under low-sulfate condition (Oude-Elferink et al. 1994; Hadas and Pinkas 1995). The *dsrAB* gene copy numbers at day 28 both in the straw-amended and unamended paddy soil slurries were one order of magnitude less than those at day 1. This trend was similar to the shift of the bacterial 16S rRNA gene copies both in the straw-amended and unamended slurries detected by using FISH probe Univ1392 targeting all organisms (Weber et al. 2001). It might imply that SRP grow syntrophically with many other eubacteria as well as archaea.

Dynamics of SRP community structure in straw-amended soil slurries

In addition to significantly decreasing in SRP abundance from day 1 to day 28, a corresponding shift was observed in SRP community composition between day 1 and day 28 in straw-amended paddy soil slurries. Most pronounced was a temporal increase of Clostridia in cluster 3 (8.3% to 13.3%) and uncultured SRP in clusters 5 and 6 (12.5% to 23.4%). Gram-positive sulfate reducers, Clostridia, have been mostly isolated from aquifers, rice root, and freshwater environments and grew well in low sulfate concentration (Ikegaga et al. 2003; Leloup et al. 2006). It was observed that Gram-positive SRP could grow well in the low-sulfate condition by fermentation together with methanogens (Sass et al. 1997). In our study, the sulfate concentration

decreased and CH₄ production enhanced during the incubation period. It was therefore reasonable that more abundant Gram-positive SRP were present. Cluster 1 was similar to *Syrophobacter*, *Desulfobacterium*, *Desulfovibrio*, and *Desulfomonile* in Deltaproteobacteria. *Syrophobacter*-like and *Desulfobacteraceae*-like SRP were acetoclastic, which lived by acetotrophic syntrophism with methanogens by the degradation of various aromatic hydrocarbons and other organic substrates (Boone and Bryant 1980; Schnell et al. 1989; Kniemeyer et al. 2003). These acetate-utilizing sulfate reducers could not outcompete with acetoclastic methanogens at high concentration of acetate (Achtnich et al. 1995). Considering the rapid decrease in acetate concentration and the stimulation in CH₄ production after day 7, these acetate-utilizing sulfate reducers could not outcompete with methanogens, and the relative abundance of cluster 1 decreased during the incubation period. Novel uncultured SRP with high relative abundance in clone libraries affiliated with those retrieved from the freshwater environment such as acidic fen (Schmalenberger et al. 2007), estuary mudflats (Leloup et al. 2006), low-sulfate hot spring microbial mat (Dillon et al. 2007), and soil (Hansel et al. 2008). The reason of increasing relative abundances of clusters 5 and 6 and decreasing relative abundance of cluster 4 during the incubation period is not clear. Considering the significantly higher concentration of lactate in straw-amended slurries in this study, the lactate-utilizing SRP may play a role in the later incubation period (Qatibi et al. 1990). In a word, it deserves further studies to elucidate if these novel uncultured SRP have the similar lifestyle to Gram-positive sulfate reducers Clostridia and acetate-utilizing sulfate reducers, or they possessed different growth mechanisms (Liu et al. 2009).

Conclusions

In conclusion, our results have demonstrated the potential effect of rice straw on the dynamics of sulfate reduction and sulfate-reducing prokaryotes in the paddy soil slurries. The application of rice straw significantly enhanced concentrations of sulfate, formate, acetate, propionate, and lactates and stimulated CH₄ production. The *dsrAB* gene copy numbers in the straw-amended paddy soil slurries were significantly higher than that in the unamended slurries. The *dsrAB* gene copy numbers had significant correlation with concentrations of sulfate and acetate. Sulfate and acetate concentrations were the important factors controlling SRP population size, but sulfate was not the limiting factor for SRP growth. Significant differences in SRP community structure between treatments and time were detected. Novel uncultured SRP were abundant in the straw-amended paddy soil slurries and

shifted remarkably during the incubation period. Their growth mechanisms deserve further studies to better understand their functions in the degradation of organic matters in the paddy soil slurries.

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