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Response of syntrophic bacterial and methanogenic archaeal communities in paddy soil to soil type and phenological period of rice growth

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ABSTRACT

Methanogenesis and syntrophy are the most important microbial metabolic processes for the anaerobic decomposition of organic matters in paddy soils. However, the diversity and abundance of syntrophic communities and their ecological determinants remain largely unexplored. In this study, we aimed to unravel the impacts of soil type and rice phenology on both syntrophic and methanogenic communities. The relative abundances of syntrophic bacteria and methanogenic archaea were significantly affected by soil type and rice growth stages, with 0.39−1.66% and 1.68−3.95% for syntrophic bacteria and 0.49−6.04% and 9.29−13.69% for methanogenic archaea, respectively. Besides, the populations of short-chain fatty acid-degrading syntrophs (SFAS) varied across soil types (range: 8.21×10^6–7.83×10^7 16S rRNA gene copies g^{-1} dry soil) and rice growth stages (range: 2.27×10^7–3.88×10^7 16S rRNA gene copies g^{-1} dry soil). Moreover, the populations of syntrophic propionate-oxidizing bacteria were 1.2–6.2 times higher than those of butyrate-oxidizing bacteria in the 10 paddy soils during the off-rice season, indicating that propionate is the main intermediate product during anaerobic decomposition of organic matter. However, *Syntrophomonas* was the most dominant throughout the rice growing season, implying that syntrophic pathways may vary between off-rice and growing seasons. Interestingly, the populations of SFAS and methanogens showed a significantly positive correlation ($P < 0.05$). This suggests that syntrophic bacteria, in cooperation with methanogenic archaea, can affect methane production in paddy soils. Furthermore, C/N ratio, soil moisture, soil pH, oxalate, citrate, and their interaction accounted for 65.20% of the changes in syntrophic communities. Altogether, our findings indicate that soil type, rice phenological stages and soil properties can shape the distribution of syntrophic communities in rice paddy fields.

1. Introduction

Methane (CH_4) is the second most abundant anthropogenic greenhouse gas in the atmosphere (Alam et al., 2019). Rice paddy fields are the important anthropogenic sources of atmospheric CH_4 (Wang et al., 2019a,b), accounting for approximately 21% of global CH_4 emissions (Conrad, 2007). There are three major sources of organic matter in paddy fields, including rice straw, plant photosynthesis and sloughed-off root cells or decaying roots, which can be eventually converted to CH_4 and thus contributes significantly to CH_4 emissions. About 20% of total CH_4 emissions are derived from the anaerobic degradation of soil organic carbon, followed by straw or produced plant carbon (Conrad, 2007).

Complex organic matters, such as soil organic carbon, can be metabolized to CH_4 and CO_2 through the cooperation of anaerobic microorganisms, including fermentative bacteria, fatty acid-oxidizing bacteria and methanogens (Sieber et al., 2019; Zhang

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et al., 2019). Methanogenic archaea have been viewed to take the main responsibility for CH_4 production in rice fields (Kim et al., 2013). Experimental investigations have been conducted on the role of methanogenic archaea in CH_4 production (Conrad and Klose, 2006), and their microbial abundances in paddy soils (Conrad, 2007; Yuan et al., 2018). Syntrophic metabolism is an important intermediate step for the complete conversion of organic matter to CH_4 (McInerney et al., 2010). In methanogenic environments, syntrophic metabolism contributes to most of the carbon fluxes (Kim et al., 2013; McInerney et al., 2010), and approximately 20–30% of carbon is converted into intermediate products (Mackie and Bryant, 1981). Propionate and butyrate are two crucial intermediary products resulted from the degradation of organic residues in paddy soils (Rui et al., 2009). Propionate accounted for 18.2–27.7% of CH_4 production by syntrophic bacteria in paddy soil incorporated with rice straw (Glissmann and Conrad, 2000). Syntrophic fatty acids may be directly used as substrates for methanogenesis by methanogens during the anaerobic decomposition of soil organic carbon (Zhong et al., 2020; Sieber et al., 2019). Therefore, apart from methanogenic archaea, syntrophic bacteria also play a key role in regulating CH_4 production in paddy soil.

Syntrophic bacteria have become a research hotspot in the field of anaerobic digesters. The majority of studies have focused on their diversity, abundance, substrate degradation pathway, electron transfer mechanisms and so on (Sieber et al., 2012; Rotaru et al., 2012; Kobayashi et al., 2020; Nozhevnikova et al., 2020). Recently, the abundance of syntrophic fatty acid oxidizing bacteria in paddy soil system has gained increasing attention (Huang et al., 2020; Zhuang et al., 2017; Holmes et al., 2017). Soil temperature, nutrient and pH have been demonstrated to affect the diversity and abundance of syntrophs and methanogens (Gan et al., 2012; Chauhan et al., 2004, 2006; Hao et al., 2019). Moreover, soil type has been considered as the main determinant factor influencing the diversity and abundance of soil bacterial community (Girvan et al., 2003). Soil type also plays important roles in regulating CH_4 production and methanogenic communities (Bao et al., 2014). However, little is known on the natural abundance and distribution of syntrophic bacteria in different paddy soils at large spatial scales.

Besides, the source strength of CH_4 in soil system is related to rice variety, phenological stages, growth variability, soil fertilization and nutrient management (Kim et al., 2013; Conrad and Klose, 2006). During the early period of rice planting, the rate of CH_4 production is generally low; with the growth of plants and enhancement of anaerobic conditions, the flux may increase gradually (Ali et al., 2009). Numerous studies have investigated the changes in CH_4 production and methanogenic community during rice growth (Jürgen et al., 2012; Singh and Dubey, 2012), and the trend of CH_4 production was very similar to that of methanogenic population with increasing rice growth. Additionally, the cumulative production of CH_4 was positively correlated with the population size of methanogens (Dubey et al., 2013). In recent years, few researches have paid attention to the shift of syntrophic community structure and abundance in rice plants at different growth stages. However, the relationships between environmental factors (e.g. pH values, ammonia concentration, C/N ratio, etc.) and the abundance of syntrophic bacteria in soil system at different rice phenological stages remain largely unexplored.

Here, we speculate that soil type can determine the changes in syntrophic community during the off-rice season, and rice phenological periods may contribute to the association between syntrophs and methanogens throughout the rice growth season. Thus, the objectives of this study covered two major aspects: (i) to investigate the abundance of syntrophs in different types of rice paddy soils in Southern China, and their distribution at different rice phenological stages; and (ii) to determine the effects of environment variables on syntrophic community structure and abundance. High-throughput sequencing was conducted to evaluate the abundance and diversity of syntrophic and methanogenic communities based on 16S rRNA genes. Quantitative polymerase chain reaction (qPCR) was employed to characterize the populations of specific syntrophic bacteria and methanogenic archaea. Previous studies on the abundance of syntrophs in paddy soils were mainly conducted at a laboratory scale or a selected rice field (Zhong et al., 2020; Kobayashi et al., 2020; Ji et al., 2018a, 2018b). To our knowledge, this is the first study to investigate the distribution of syntrophic bacteria in different paddy soils at large spatial scales. The findings would provide basic fundamental knowledge for the ecological diversity of syntrophic bacteria and methanogenic archaea in paddy soil system.

2. Materials and methods

2.1. Soil sampling and physicochemical properties

It has been reported that the yield-scaled emissions of CH_4 increased significantly from northern to southern regions in China (Guo et al., 2017). During the off-rice season, soil samples were collected from 10 sites in Southern China, covering from inland to southeast coastal areas, at the same month. These soil samples were mainly classified into 6 different types: laterite, red soil, yellow soil, purple soil, eel blood paddy soil and southern paddy soil, according to the soil information service platform (http://www.soilinfo.cn/map/index.aspx; Table 1). A map showing the sampling locations is presented in Fig. 3a. The sampling sites of these different soil types showed large-scale variations in soil parent matter, pH value and soil organic matter. All samples were taken at a depth of 5–20 cm with three replicates (0.5 kg) in July 2013 from the major rice-producing areas in Southern China during the off-rice season. In each sample site, five sub-samples (four from corners and one from a center point of paddy field), were collected and then mixed into a single sample. The mixed samples were placed in sterile plastic bags, and subsequently transferred to the laboratory on ice in a styrofoam box. All replicate samples were divided, and the sub-samples were sifted through a 2.0-mm sieve for further measurement of major physicochemical parameters. The remaining sub-samples were kept in the freezer at −80 °C for subsequent microbiological analysis, as referred to Yang et al. (2015). Particle sizes were determined using a particle analyzer (MS2000; Malvern, United Kingdom), and the 2.0-mm sieve was used for the removal of gravel and rice roots. Electrical conductivity of the soil samples was measured using a conductivity meter (BEC520; Philips, Holland), which serves as an indicator for reflecting the conditions of soil salinity. The measurements of pH values, total carbon (TC), total nitrogen (TN), and total organic carbon (TOC) were performed as described in Yang et al. (2015). The levels of NO_3^--N, NH_4^+-N, SO_4^{2-}, Fe (II), total Fe and organic acids (i.e. lactate, acetate, propionate, formate, succinate, oxalate and citrate) were detected by using an ion chromatography (ICS-3000; Dionex). All experiments were carried out in triplicate.

2.2. Soil characteristics and pot experiment

To determine the changes in syntrophic community at different rice growth stages, a pot experiment was conducted. Compared to other sampling sites in Southern China, Hunan (HN) was located in the middle part, and the relative abundance of syntrophs was maintained at a medium level (described in Section 3.2). Thus, the soil samples for pot experiments were taken from HN (28.91°N, 111.43°E), a southern province in China. Urea-N, calcium superphosphate and KCl were used as basic fertilizers, with the doses of 0.25, 0.06 and 0.1 g kg^{-1}, respectively (Li et al., 2016). These

Table 1
Physical properties and locations of paddy soils.

Soil type	Samples	Province	Latitude	Longitude	Parent material	% of sample with indicated particle size			Soil moisture (% of dry weight)	Electrical conductivity ±SD (μS/cm)
						<2 μm	2 μm–50 μm	>50 μm		
Laterite	GD	Guangdong	21.30°N	110.46°E	river alluvium	3.21	53.28	43.51	64.26 ± 6.32	123.53 ± 4.21
Red soil	JX	Jiangxi	28.25° N	116.99°E	basalt	2.04	29.59	68.37	35.08 ± 1.79	174.83 ± 6.49
Yellow soil	GZ	Guizhou	26.61°N	106.73°E	the Quaternary red clay	8.65	80.94	10.41	47.20 ± 1.23	39.44 ± 5.07
Yellow soil	HN	Hunan	28.91°N	111.43°E	littoral sediment	3.06	61.57	35.37	56.52 ± 4.02	995.50 ± 17.90
Purple soil	SC	Sichuan	31.00°N	105.47°E	the Quaternary red clay	2.62	39.04	58.34	57.58 ± 2.00	127.83 ± 4.16
Eel blood paddy soil	ZJ	Zhejiang	30.85°N	120.73°E	purple sandy shale	0.67	39.89	59.44	79.17 ± 5.90	251.87 ± 3.52
Eel blood paddy soil	JS	Jiangsu	31.68°N	120.82°E	river alluvium	3.68	61.99	34.33	49.78 ± 1.07	239.13 ± 12.44
Southern paddy soil	HB	Hubei	30.01°N	112.65°E	quartz sandstone	25.13	71.89	2.98	53.76 ± 3.85	168.23 ± 7.04
Southern paddy soil	FJ	Fujian	26.29°N	119.08°E	loess-like lacustrine soil	1.55	56.32	42.13	66.96 ± 1.66	167.60 ± 2.54
Southern paddy soil	FST	Guangdong	22.00°N	112.54°E	lacustrine deposits	2.56	55.59	41.85	57.66 ± 3.61	249.90 ± 6.46
Pot experiment										
Yellow soil	HN	Hunan	28.91°N	111.43°E	littoral sediment	3.04	61.51	35.45	46.37 ± 2.12	990.40 ± 20.65

Note: The particle size and soil moisture had been published in Yang et al. (2015).

fertilizers were homogeneously mixed with soils at the beginning of the experiment. The rice plants were grown in a greenhouse for 122 days, involving five phenological stages of seeding (12th day), tillering (37th day), booting (62nd day), flowering (82nd day) and ripening (122nd day), with a temperature difference of 5 °C between day (30 ± 1 °C) and night (25 ± 1 °C). Rhizosphere pore water and rhizosphere soil were collected at each growth stage, by using a soil moisture sampler (Rhizon SMS, Netherlands). The soil samples were divided into two parts: one part was used to extract genomic DNA and measure TN, TC and SOC; while another part was stored for the evaluation of physicochemical properties such as electrical conductivity, pH and NH_4^+. Organic acids were analyzed in pore water as referred to Li et al. (2016).

2.3. DNA extraction and qPCR analysis

Metagenomic DNA was extracted from 0.25 g soil samples using the PowerSoil DNA isolation kit (MoBio, Solana Beach, CA, USA) according to the maximum yield protocol and manufacturer's instructions. Subsequently, the DNA samples were eluted with 100 μL sterile elution butter and stored at −20 °C for further molecular analysis.

qPCR was conducted by using the16S rRNA gene-targeted primer sets. Previous studies have revealed that *Syntrophobacter* spp., *Pelotomaculum* spp., *Smithella* spp. and *Syntrophomonas* spp. are involved in the syntrophic oxidation of propionate and butyrate in rice paddy soils (Lueders et al., 2004; Imachi et al., 2002). Therefore, these bacterial groups were quantified to assess the abundance of short-chain fatty acids-oxidizing syntrophs (SFAS) in paddy soils (Mathai et al., 2016). In addition, the quantification of methanogens was performed with methanogen-specific short primer (Met630F and Met803R). The characteristics of primers and amplification conditions are summarized in Table 2 qPCR was conducted on a real-time PCR detection system (LightCycler 480; Roche Diagnostics GmBH, Germany) as described previously (Yu et al., 2010).

2.4. High-throughput sequencing

The V4 variable region in the 16S rRNA gene was amplified by a 'universal' primer set 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). The barcodes used for multiplexing were tagged on the forward primer (Caporaso et al., 2012). All samples were amplified with PCR, and then purified using the MinElute Gel Extraction Kit (Qiagen, Valencia, CA, USA). The amplicons were mixed at a constant molality, and subjected to sequencing by Shanghai Majorbio (Shanghai, China) using a PE250 platform (Illumina Inc., San Diego, CA, USA). The free online platform of Majorbio I-Sanger Cloud Platform (www.i-sanger.com) was used for data testing and analysis. The raw sequencing data of 10 paddy soil samples and soil samples at different growth stages were deposited in the NCBI Sequence Read Archive database (accession no. PRJNA512120; SRP 095149).

2.5. Statistical analysis

For qPCR and Illumina sequencing results, the means ± standard deviation (SD) of three replicates were calculated. R^2 value of qPCR standard curves was larger than 0.99 and amplification efficiency was higher than 1.8. The correlation analysis between the populations of methanogens and SFAS, as well as statistical significance tests were carried out using SPSS version 18.0. Spearman's rank correlation between syntrophs and environmental factors was conducted using R software (Sunagawa et al., 2015). To assess their relationship with syntrophic community structure, the environmental parameters were transformed by ln (x + 1) and standardized in the Mantel experiment (Yang et al., 2017). Constrained canonical analysis of principal coordinates (CAP) was conducted using R version 3.4.0 with the vegan package. Network analysis was executed on the free online platform of Majorbio I-Sanger Cloud Platform (www.i-sanger.com). OriginPro 8.5 was used to generate other plots.

3. Results and discussion

3.1. Physicochemical properties

Different types of paddy soils collected from the 10 sampling sites in Southern China showed the spatial variability of soil physical and chemical properties related to parent matter, pH value, soil organic matter, soil moisture, electrical conductivity, etc. (Table 1). Three soil samples (JS, FJ and SC) exhibited relatively high soil moisture contents (64.26–79.17%), and SC sample had the highest moisture content due to the perennial wet weather in Sichuan province. As shown in Table 3, six of the soil samples (ZJ, GZ, GD, JS, HB, and SC) were maintained at nearly neutral pH values (6.40–7.36), and the remaining four samples (JX, HN, FJ and FST) displayed significantly lower pH values (5.11–5.96; $P < 0.05$). The concentrations of NO_3^--N (0.0003–0.005 g kg^{-1}) was about 10 times lower than those of NH_4^+-N (0.003–0.015 g kg^{-1}). FST and GZ samples demonstrated an extremely high concentration of SO_4^{2-}. This is mainly due to the fact that FST soil samples are collected from an acid reflux field or better known as coastal acid sulfate soils. Guizhou province is the most serious acid rain affected region

Table 2
Characteristics of the16S rRNA gene-targeted qPCR primer sets and real-time amplification conditions.

Target group	Primer[a]	Sequence[b] (5′-3′)	Amplicon size (bp)	Real-time amplification conditions	Reference
Bacteria	BAC338F BAC805R	ACTCC TACGG GAGGC AG GACTA CCAGG GTATC TAATC C	468	Initial incubation 94 °C,10 min; Denaturation 94 °C, 10 s;	Yu et al. (2010)
Archaea	ARC787F ARC1059R	ATTAG ATACC CSBGT AGTCC GCCAT GCACC WCCTC T	273	Annealing extension 60 °C, 30 s; 45cycels[d]	
Methanogens	Met630F Met803R	GGATTAGATACCCSGGTAGT GTTGARTCCAATTAAACCGCA	174	Initial incubation 95 °C, 5 min; Denaturation 95 °C, 10 s; Annealing extension 60 °C, 30 s; 45cycels[d]	Yu et al. (2010)
Syntrophobacter	SBC695F SBC844R	ATTCGTAGAGATCGGGAGGAATACC TGRKTACCCGCTACACCTAGTGMTC	150	Initial incubation 95 °C, 3 min; Denaturation 95 °C, 10 s;	Mathai et al. (2016)
Smithella	SMI732F SMI831R	GRCTTTCTGGCCCDATACTGAC CACCTAGTGAACATCGTTTACA	100	Annealing extension 60 °C, 30 s; 45cycels[d]	
Pelotomaculum	PEL622F PEL877R	CYSDBRGMSTRCCTBWGAAACYG GGTGCTTATTGYGTTARCTAC	256	[c]Initial incubation 95 °C, 3 min; Denaturation 95 °C, 10 s; Annealing extension 60 °C, 60 s; Extension 72 °C, 60 s; 45cycels[d]	
Syntrophomonas	SMS637F SMS757R	TGAAACTGDDDDTCTTGAGGGCAG CAGCGTCAGGGDCAGTCCAGDMA	121	The same condition to *Syntrophobacter* amplification.	

[a] F, Forward primer; R, Reverse primer.
[b] R = A/G, K = G/T, M = A/C, D = A/G/T, Y=C/T, S = G/C, B=C/G/T, W = A/T.
[c] The amplification condition for *Pelotomaculum* was modified in this study.
[d] The cycle procedures includes denaturation and annealing extension (extension).

Table 3
Chemical properties of paddy soils.

	pH	Amt (μmol g^{-1}, ±SD) of dry paddy soil)			Amt (mg kg^{-1}, ±SD) of dry paddy soil			Amt (g kg^{-1}, ±SD) of dry paddy soil		C/N
		Fe (II)	Total Fe	Ferric iron	NO_3^--N	NH_4^+-N	SO_4^{2-}	SOC	TN	
10 paddy soils										
JX	5.11	41.54 ± 8.62	51.25 ± 5.60	9.70	2.61 ± 0.18	10.95 ± 0.37	32.28 ± 2.57	26.81 ± 1.17	2.28 ± 0.08	11.81
HN	5.44	30.33 ± 1.28	34.32 ± 5.49	3.99	1.45 ± 0.82	7.55 ± 1.03	13.63 ± 3.33	21.45 ± 0.74	2.03 ± 0.01	10.60
FJ	5.85	49.66 ± 7.31	54.91 ± 7.15	5.25	0.87 ± 0.41	14.06 ± 1.08	17.23 ± 1.67	18.41 ± 0.56	1.63 ± 0.01	11.34
FST	5.96	23.52 ± 11.82	45.23 ± 8.81	21.71	2.15 ± 0.48	ND	110.55 ± 15.73	20.12 ± 1.57	1.86 ± 0.00	10.84
ZJ	6.40	46.04 ± 15.71	67.10 ± 2.07	21.06	0.34 ± 0.08	2.88 ± 0.15	31.81 ± 3.03	27.58 ± 0.60	2.37 ± 0.00	11.67
GZ	6.51	22.35 ± 6.96	42.05 ± 12.17	19.71	1.23 ± 0.47	3.67 ± 0.24	182.95 ± 1.07	24.94 ± 0.84	1.96 ± 0.00	12.76
GD	6.75	9.96 ± 2.20	42.65 ± 6.85	32.70	1.13 ± 0.04	3.83 ± 0.67	44.44 ± 4.18	15.51 ± 0.63	1.29 ± 0.04	12.12
JS	6.77	77.84 ± 46.17	88.30 ± 18.12	10.46	0.28 ± 0.01	5.83 ± 2.72	6.76 ± 1.18	35.32 ± 0.98	3.00 ± 0.01	11.79
HB	7.25	68.20 ± 27.38	76.00 ± 8.65	7.81	1.06 ± 0.30	7.11 ± 1.51	46.17 ± 9.28	27.04 ± 2.08	2.31 ± 0.05	14.03
SC	7.36	64.25 ± 15.47	65.04 ± 14.80	0.79	0.32 ± 0.08	5.30 ± 0.10	2.41 ± 0.65	30.43 ± 1.14	2.66 ± 0.01	17.52
Pot experiment										
Seeding	6.45	ND	ND	ND	8.38 ± 0.81	176.44 ± 21.64	ND	21.15 ± 0.18	2.44 ± 0.02	8.66 ± 0.08
Tillering	6.09	ND	ND	ND	48.92 ± 11.88	183.88 ± 11.88	ND	21.46 ± 0.40	2.32 ± 0.12	9.24 ± 0.42
Booting	5.04	ND	ND	ND	22.35 ± 1.73	77.72 ± 35.06	ND	22.75 ± 0.31	2.14 ± 0.06	10.60 ± 0.15
Flowering	5.57	ND	ND	ND	1.59 ± 0.04	49.58 ± 5.42	ND	22.83 ± 0.26	2.08 ± 0.02	10.94 ± 0.04
Ripening	5.19	ND	ND	ND	1.73 ± 0.40	12.75 ± 2.72	ND	23.14 ± 0.26	2.13 ± 0.06	10.82 ± 0.22

Notes: SD, standard deviations; ND, not detected; the pH values, concentrations of NH_4^+-N, NO_3^--N, SOC, TN and C/N in 10 paddy soils had been published in Yang et al. (2015); the pH values, concentrations of NH_4^+-N, NO_3^--N, SOC and C/N with rice growth had been published in Li et al. (2016).

Table 4
Organic acids in rhizosphere at different growth periods of rice plant.

Phenological period	Units: mg L^{-1} for pore water						
	Lactate	Acetate	Propionate	Formate	Succinate	Oxalate	Citrate
Seedling	1.96 ± 0.20	5.76 ± 0.50	6.52 ± 1.00	3.82 ± 1.02	3.12 ± 1.03	12.11 ± 1.53	1.69 ± 0.35
Tillering	4.63 ± 0.50	57.81 ± 14.87	6.65 ± 1.30	3.03 ± 0.80	1.25 ± 0.40	7.70 ± 1.77	0.16 ± 0.24
Booting	3.39 ± 0.46	5.69 ± 0.33	0.01 ± 0.00	2.15 ± 0.62	0.47 ± 0.40	1.36 ± 0.50	0.09 ± 0.00
Flowering	2.14 ± 1.00	3.59 ± 1.20	13.18 ± 1.70	5.99 ± 2.25	6.25 ± 1.76	4.42 ± 1.49	0.47 ± 0.21
Ripening	0.64 ± 0.24	0.34 ± 0.16	0.22 ± 1.41	0.73 ± 0.24	99.47 ± 0.73	0.32 ± 0.18	0.04 ± 0.00

in China, and its specific topographical and meteorological conditions may induce the dispersion and dilution of pollutants (e.g. SO_X and NO_X) in the atmosphere. The concentrations of SOC and TN varied from 15.51 to 35.32 g kg^{-1} and from 1.29 to 3.00 g kg^{-1}, respectively, leading to a range of C/N ratios of 10.60–17.52. Among the 10 samples, JS exhibited the highest SOC and TN levels, while SC displayed the highest C/N ratio.

The pH values in HN were ranged from 5.04 to 6.45 across different rice growth stages and decreased throughout the growth stages, except for the flowering stage. The concentrations of ammonia were ranged from 0.013 to 0.184 g kg^{-1} and exhibited the same trend with pH values. The levels of SOC increased with rice growing time, with the highest value of 23.14 g kg^{-1} achieved at the ripening stage. The ratios of C/N also increased with rice growth

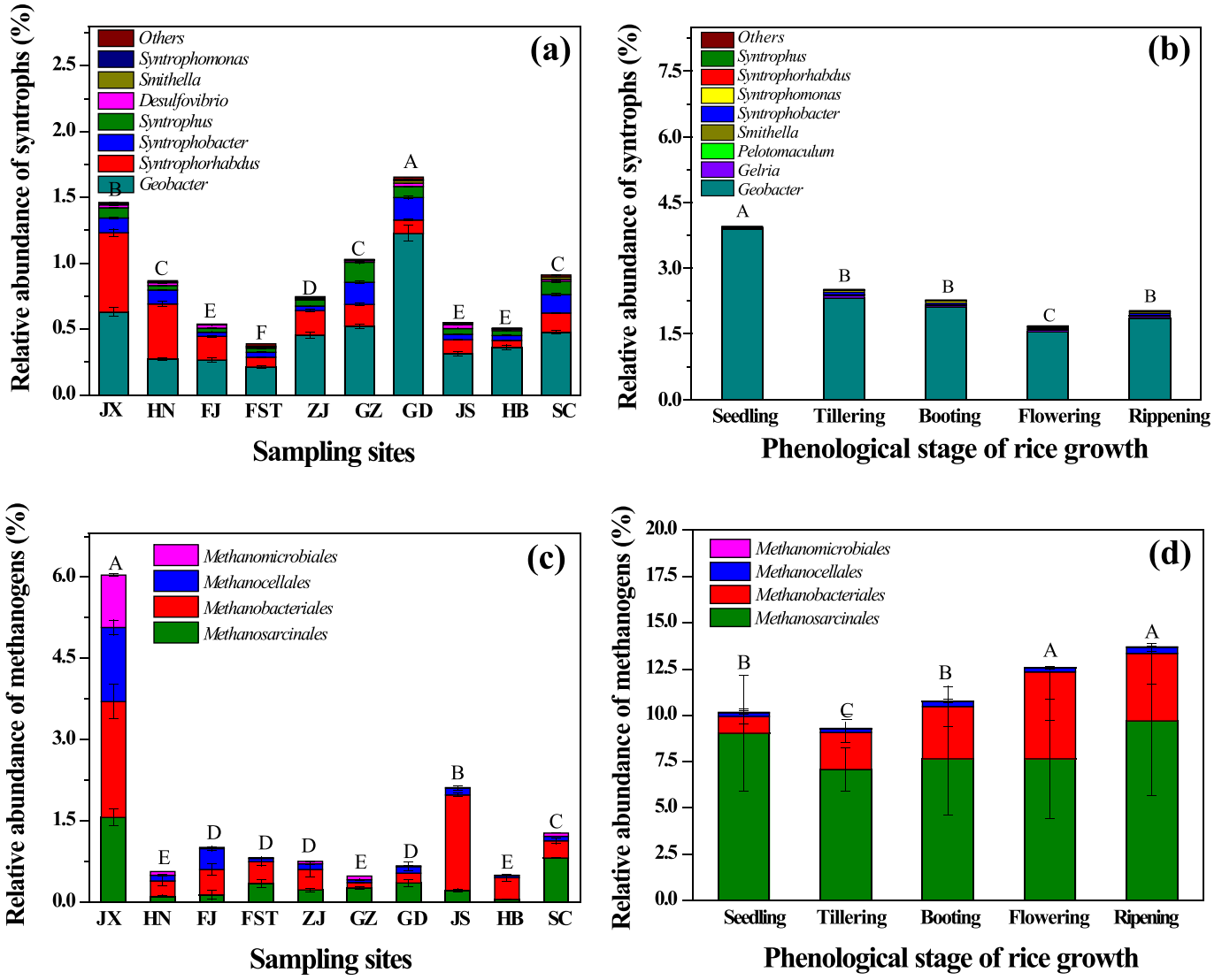

Fig. 1. Relative abundance and composition of (a, b) syntrophs and (c, d) methanogens in the 10 paddy soils (a, c) at different rice growth stages (b, d). Letters in each figure denote the significant differences between sample groups ($P < 0.05$). A, B, C, D, E and F in plot (a) indicate the total relative abundance of syntrophs, while A, B, C, D and E in plot (c) represent the differences between 10 paddy soils. A, B and C in plot (b) indicate the total syntrophs, while A, B and C in plot (d) represent the differences between each phenological stage of rice growth.

and reached the highest ratio of 10.82 at the ripening stage. Similar findings have been reported by Li et al. (2016). The quantities of organic acids excreted by rice roots were detected with several dominant components, including formate, acetate, propionate and succinate (Table 4). Different levels of organic acids were observed at the developmental stage, indicating that their composition and quantity vary with rice growth. The concentrations of propionate and formate were elevated at the flowering stage. Such a changing pattern was consistent with the total numbers of SFAS and hydrogenotrophic methanogens detected at the flowering stage (Section 3.3), suggesting that rice phenology may affect syntrophic-methanogenic associations by altering soil chemical properties.

3.2. Relative abundance and diversity of syntrophs and methanogens

3.2.1. Responses of syntrophic and methanogenic communities to soil type

Syntrophy exists between H_2/formate-producing bacteria and H_2/formate-utilizing archaea throughout the microbial world. Among the putative syntrophs, seven genera of syntrophs were detected in the 10 paddy soils and eight genera during the off-rice season (Fig. 1a and b). The relative abundances of total detected syntrophic bacteria were ranged from 0.39 to 1.66%, with the following order: GD (lateritic soil) > JX (red soil) > GZ, HN (yellow soil) > SC (purple soil) > ZJ (eel blood paddy soil) > JS (eel blood paddy soil), HB, FJ, FST (southern paddy soil) (Fig. 1a, Table A1). The results indicated that soil type significantly affected the abundance of syntrophic bacteria in paddy soils. In addition, these findings are consistent with those of previous studies showing that syntrophic short-acid-oxidizing bacteria constitute a small percentage (<2%) of total microorganisms in the anaerobic digestion system (Sundberg et al., 2013; McMahon et al., 2004). Moreover, the impact of syntrophic fatty acid-oxidizing bacteria on the structure and function of microbial community is far greater than their relative abundance (Power et al., 1996). In this study, *Syntrophobacter* was the most dominant syntrophic propionate-oxidizing bacteria (SPOB), with the relative abundances of 0.05–0.23% in the 10 paddy soils; while *Syntrophomonas*, the most dominant syntrophic butyrate-oxidizing bacteria (SBOB), only accounted for 0.01% or lower in most soil

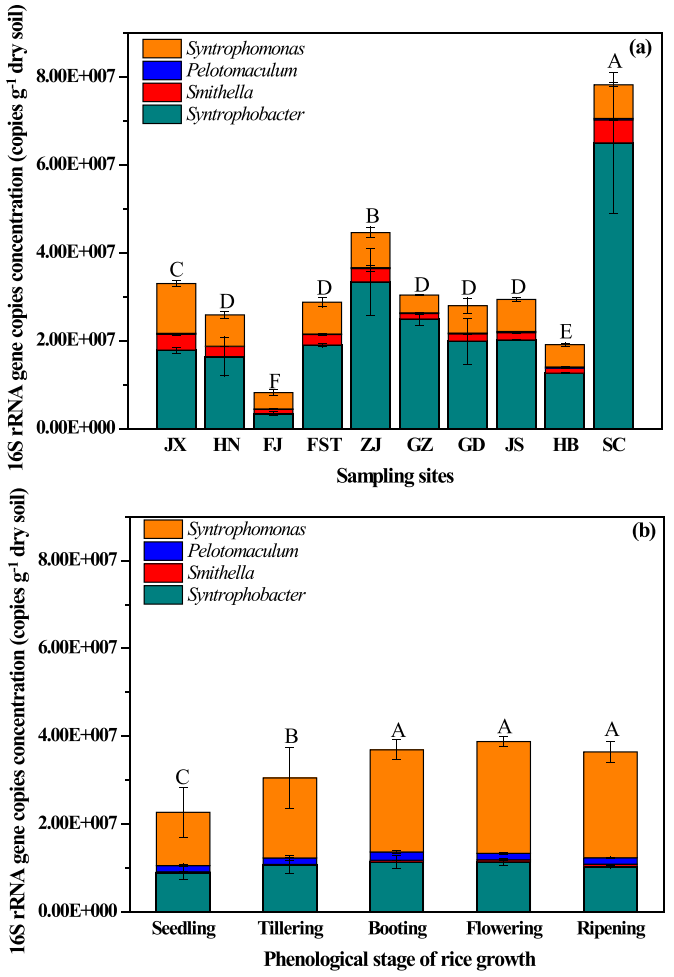

Fig. 2. Total 16S rRNA gene copies of syntrophic fatty acid-oxidizing bacteria in the 10 paddy soils (a) at different rice growth stages (b). Letters in each figure indicate the significant differences between sample groups ($P < 0.05$). A, B, C, D, E and F in plot (a) denote the differences in total quantified syntrophs among the 10 paddy soils, while A, B and C in plot (b) represent the differences in total quantified syntrophs between each phenological stage of rice growth.

samples. This indicates that syntrophic propionate oxidation may play an even more important role than butyrate oxidation in the studied soil systems.

Hydrogenotrophic methanogens (e.g. *Methanobacteriales*, *Methanocellales,* and *Methanomicrobiales*) and acetoclastic methanogens (e.g. *Methanosarcinales*) were the main orders in the 10 soil samples. These four groups of methanogens accounted for 0.49–6.04% of total microbial communities in the 10 paddy soils (Table A2), with the order of JX > JS > SC > FJ, FST, ZJ, GD > HN, GZ, HB. This suggests that the red and purple soils exhibit relatively higher methanogen abundance than yellow and southern paddy soils. In addition, it was found that JS and ZJ showed significantly different relative abundance of methanogens, even though they were both eel blood soil. Therefore, soil type may serve as one of the key factors that alter methanogenic communities. Besides, soil pH is another important factor (Hao et al., 2019). In all acidic soils, hydrogenotrophic *Methanobacteriales* were the most dominant group in most of the samples (JX, HN, FJ, FST, ZJ, JS, and HB). Besides, the acetoclastic methanogen *Methanosarcinales was* dominated in neutral soil samples (SC, GZ and GD). Similarly, *Geobacter* demonstrated a high relative abundance in these three samples. As reported previously (Holmes et al., 2017), *Methanosaeta* spp. could reduce CO_2 via direct interspecies electron transfer from *Geobacter* in paddy soils. Anaerobic fermentation is in favor of acetate accumulation, which might be attributed to the fact that soil acidification is dominated by hydrogenotrophic methanogens (Duddleston et al., 2002).

3.2.2. Responses of syntrophic and methanogenic communities to rice growth

The relative abundances of syntrophs were ranged from 1.68 to 3.95% and decreased with rice growth. This might be related to the changes in soil oxygen conditions during rice growth. Syntrophic bacteria exhibited a higher relative abundance at different rice growth stages compared to the off-rice season, indicating that syntrophs are highly active throughout the growing season and the available organic matters may not be sufficient during the off-rice season. In addition, *Geobacter* could serve as a dominant active syntrophic bacterium in paddy soils. A recent study has reported that the paddy fields and lakeside soils are rich in *Geobacter*, accounting for 0.26–7.70% of the total bacteria (Wang et al., 2019a,b). This might be attributed to the fact that *Geobacter* spp. has undergone alternative degradation pathways and grow with specific substrates or through anaerobic respiration by using electron acceptors, including SO_4^{2-} and Fe(III) (Sieber et al., 2012). *Geobacter* spp. plays an important role during the direct interspecies electron transfer in terrestrial methanogenic environments and is thus considered as the most metabolically active bacteria in methanogenic rice paddy soils (Hori et al., 2010; Kim and Liesack, 2015). Although *Syntrophobacter* could serve as the most dominant SPOB at different rice growth stages, the relative abundance of *Syntrophobacter* was quite low, ranging from 0.003 to 0.04%. Furthermore, *Smithella* and *Pelotomaculum* were found to exist in the studied soil samples.

The relative abundances of methanogens ranged from 9.29 to 13.69% in HN at different rice growth stages (Fig. 1c and d). Contrary to syntrophic bacteria, the abundance of methanogens was increased with rice growth. This may be due to the fact that plant photosynthesis is becoming an increasingly important source for CH_4 production during rice growth, and photosynthetically derived carbon can contribute to more than 60% of total CH_4 emissions (Conrad, 2007). Moreover, CH_4 production is positively correlated with the abundance of methanogens (Dubey et al., 2013). High abundances of methanogens at flowering and ripening stages were significantly observed, except for the above mentioned reasons, it might be partially explained by the elevated levels of SOC (Table 3). The amount of sloughed-off root cells or decaying roots could be increased in this stage, which represent the alternative carbon sources for bacteria and archaea (Conrad, 2007).

In addition, *Methanosarcinales* was the most predominant methanogenic group in HN soil sample at different rice growth stages compared to the off-rice season. It was also observed that the relative abundance of *Methanobacteriales* was significantly increased with rice growth. *Methanocellales*, a mesophilic hydrogenotrophic methanogen isolated from an anaerobic propionate-degrading enrichment culture (Sakai et al., 2008), was also found in all paddy soil samples and exhibited a relatively higher abundance compared to *Methanomicrobiales*. These results indicate that the dominance of methanogen groups may shift according to the cultivation states of paddy soils.

3.3. Abundance and distribution of specific syntrophs and methanogens

3.3.1. Effects of soil type on syntrophic bacterial population

The populations of target syntrophic groups (SPOB: *Syntrophobacter*, *Smithella* and *Pelotomaculum*; SBOB: *Syntrophomona*) varied significantly ($P < 0.05$) in the 10 paddy soils (Fig. 2a). A significant increase in the population of syntrophs was observed during the

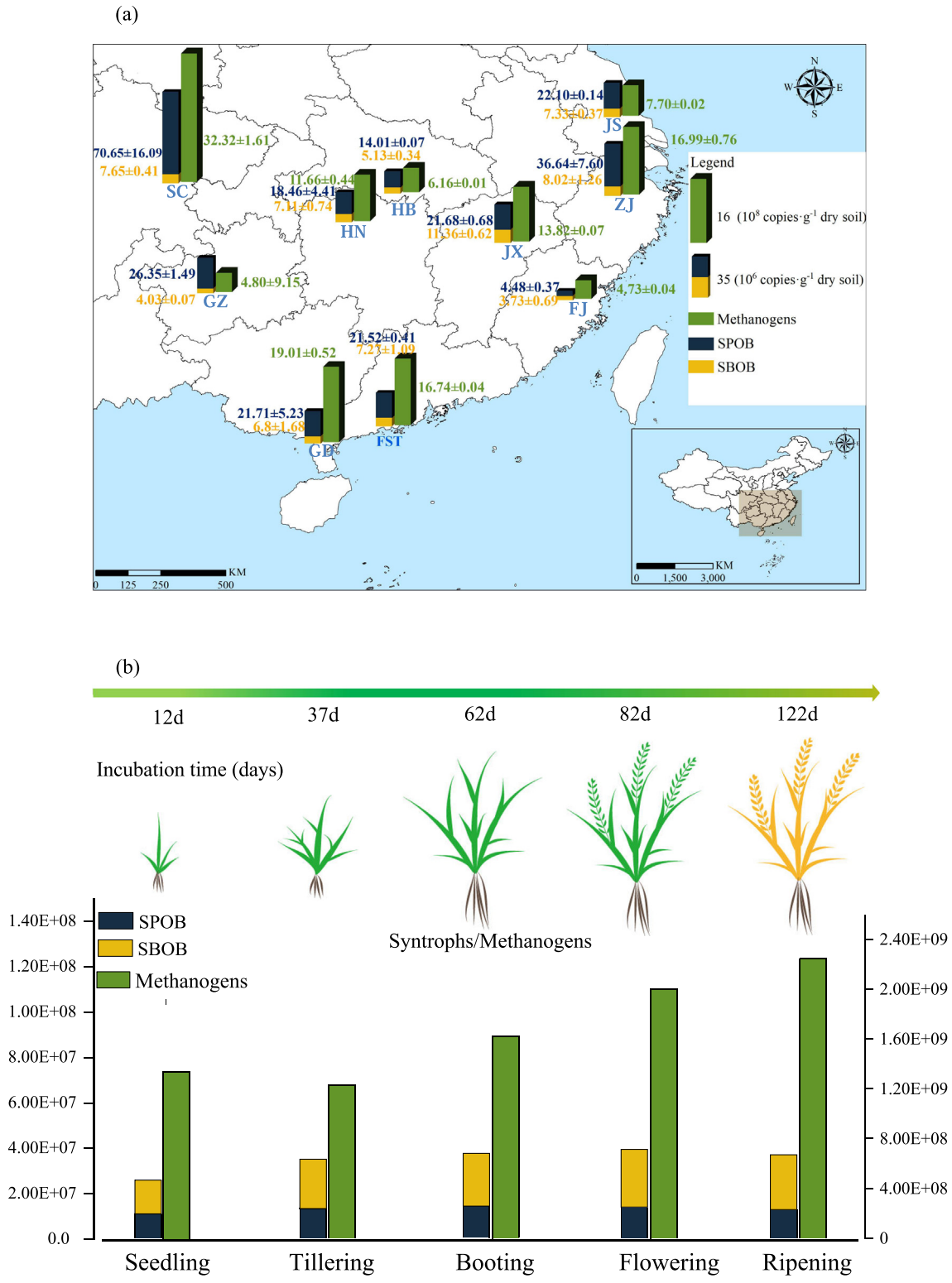

Fig. 3. Distributions of syntrophs (SPOB and SBOB) and methanogens in the 10 paddy soils (a) at different rice growth stages (b).

first three growth stages of the rice plant (Fig. 2b). In overall, the population of SFAS ranged from 8.21×10^6 to 7.83×10^7 copies g^{-1} dry soil in the 10 paddy soils.

The 16S rRNA gene copies of SPOB were varied in the 10 paddy soils, ranging from 1.40×10^7 to 7.06×10^7 copies g^{-1} dry soil. Among them, SC (purple soil), ZJ (eel blood soil), and JX (red soil) exhibited highest abundance of SPOB, while HB and FJ (southern paddy soil) showed the lowest abundance. In contrast, the population of SBOB was one order of magnitude lesser than that of SPOB, ranging from 3.73×10^6 to 7.65×10^6 copies g^{-1} dry soil. These values were approximately 10-fold higher than those of SPOB (4.63×10^5 copies g^{-1} dry soil) and SBOB (4.63×10^5 copies g^{-1} dry

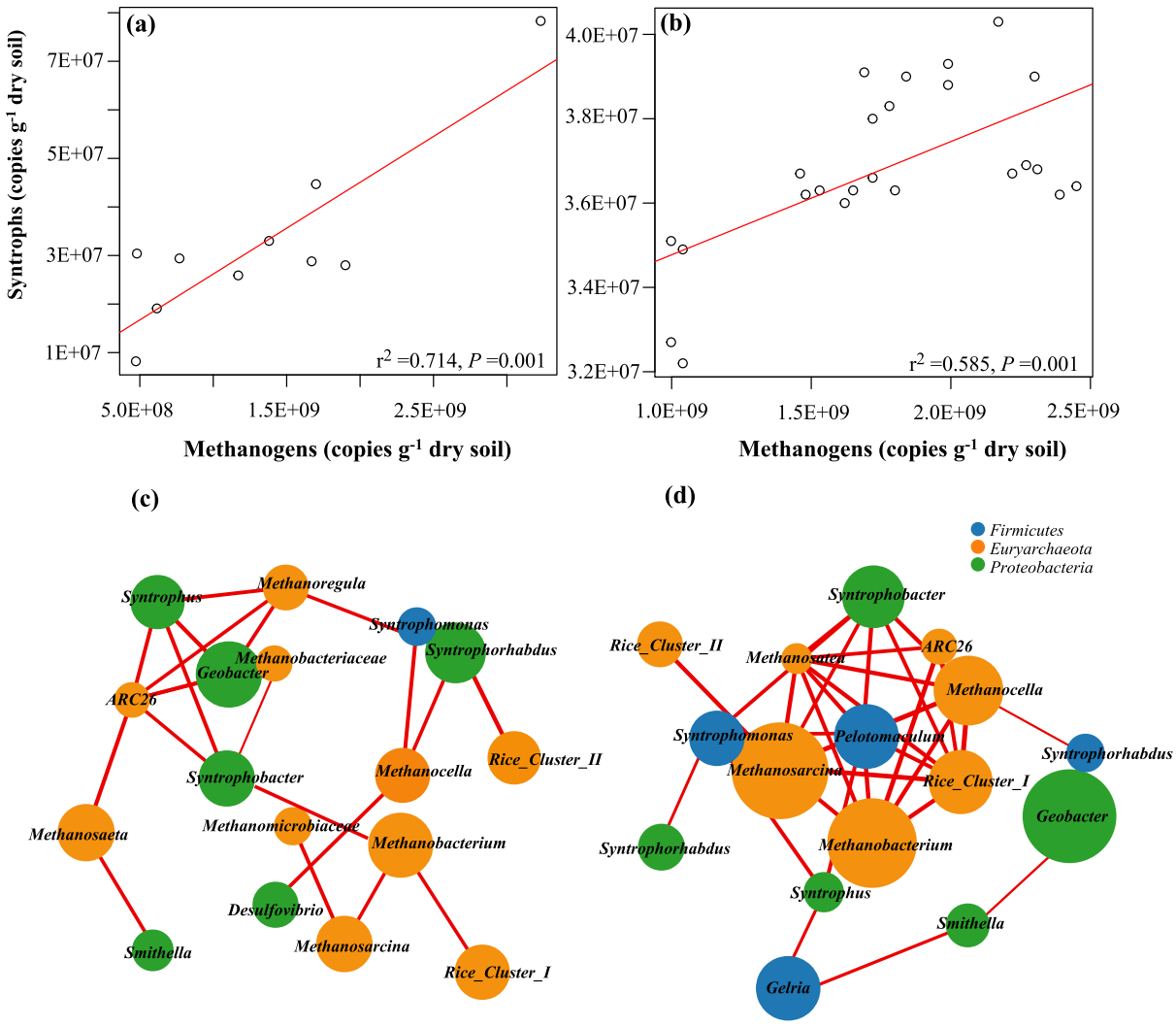

Fig. 4. Relationship between the 16S rRNA gene copy number of methanogens and total quantified syntrophs (both SPOB and SBOB) in the 10 paddy soils (a) at different rice growth stages (b). Network analysis of syntrophs and methanogens in the 10 paddy soils (c) at different rice growth stages (d). The connection reveals a significant correlation ($P < 0.01$). Node size is relative to the abundance of genera. Node color stands for taxonomic classification. Edge color reflects positive (red) correlations, and the thickness of edge indicates the correlation values. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

soil) detected in Everglades marsh suffered from eutrophication (Chauhan et al., 2004). It can be deduced that propionate is the major intermediary product resulted from the anaerobic metabolism of organic matter in the studied paddy fields during the off-season. Moreover, the abundance of SPOB was partially affected by soil type. A high conversion rate of propionate has been reported, which accounts for up to 30% of organic matter transformation in paddy soils (Glissmann and Conrad, 2000). The existence of *Syntrophomonas* in the propionate-enriched culture is most probably due to the presence of *Smithella*. Previous research has shown that *Smithella* spp. activates a non-randomizing pathway, in which propionate is converted to acetate and butyrate at first, and then the butyrate is available for *Syntrophomonas*, and ultimately metabolized to acetate via β-oxidation (de Bok et al., 2001).

3.3.2. Effects of rice phenology on syntrophic bacterial population

The total populations of SFAS varied from 2.27×10^7 to 3.88×10^7 copies g^{-1} dry soil across different rice growth stages. Notably, SBOB was the most dominant SFAS in the 10 paddy soils during the rice-growing season compared to the off-rice season, with a significant increase in abundance ($P < 0.05$) starting from the seedling stage until the booting stage (first 62 days). Concurrently, an increase in the abundance of *Smithella* spp. has been observed at later periods (day 60, 30 °C) of rice growth (Gan et al., 2012), and the degradation rate of soil organic matter in the initial periods (0–60 days) differs greatly from that in the later periods (60–120 days). Available carbon is utilized by microbes at the early periods of decomposition, while the accumulated N is used during the later periods (Esperschutz et al., 2013). These findings reveal that the degradation pathway of syntrophic fatty acids may vary from rice planting until harvesting. However, SPOB exhibited no significant variation in their total population throughout the rice growing season (Fig. 2b). Additionally, *Syntrophobacter* was the most dominant genus of SPOB in all paddy soils, while *Pelotomaculum* accounted for a lower percentage. This may be due to the fact that *Syntrophobacter* spp. is recognized as a highly active syntrophic group in paddy soil, whereas the activities of *Pelotomaculum* spp. are related to soil temperature, especially at 50 °C (Gan et al., 2012; Lin et al., 2016). It should be pointed out that all paddy soil samples were collected in July under mesophilic conditions.

3.3.3. Distributions and relationships of SFAS and methanogens in the 10 paddy soils at different periods of rice growth

The distributions of SFAS and methanogens are mapped in Fig. 3. The 16S rRNA gene copy numbers of methanogens ranged from

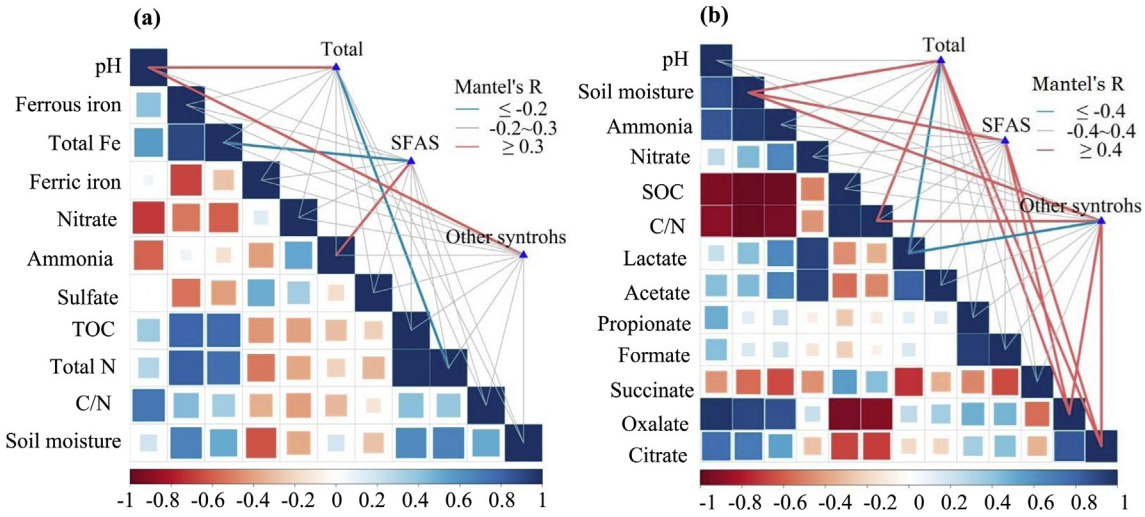

Fig. 5. Environmental drivers of syntrophic bacteria composition in the 10 paddy soils (a) at different rice growth stages (b). Pairwise comparisons of environmental parameters and bacterial communities are demonstrated, and the color gradient indicates Spearman's correlation coefficients. Total syntrophs and SFAS were correlated to each environmental factor, as revealed by the partial Mantel tests. The edge color and width represent the statistical significance and Mantel's R statistic, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

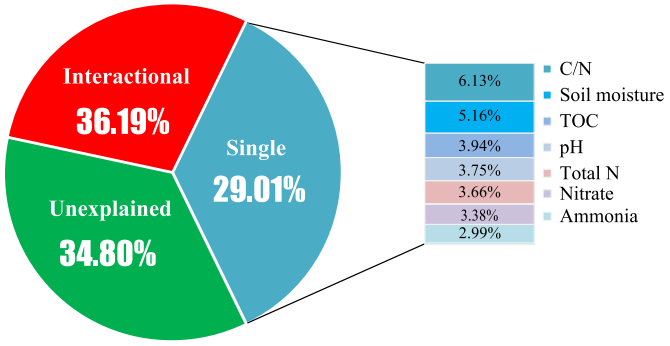

Fig. 6. Contributions of soil properties, soil type and rice phenology to syntrophic community composition calculated by constrained canonical analysis of principal coordinates (CAP). Interactional explanation denotes the co-contribution of soil properties to syntrophic community composition.

4.73×10^8 to 3.23×10^9 and 1.22×10^9 to 2.24×10^9 copies g^{-1} dry soil in the 10 paddy soils during the off-rice season and in HN sample across different rice growth stages, respectively. Among the 10 paddy soils, the highest value was found in SC (purple soil) sample, while the lowest value was in FJ (southern paddy soil) sample (Fig. 3a). Besides, GZ, HB and JS samples demonstrated lower levels of methanogen populations, ranging from 4.80×10^8 to 7.70×10^8 copies g^{-1} dry soil. Such findings can be explained by different environmental conditions, including soil type, soil pH, SOC, etc. (discussed in Section 3.4). Furthermore, a significant increase in the abundance of methanogens ($P < 0.05$) was observed throughout the entire period of rice growth (Fig. 3b). These results were in good agreement with the changes in the relative abundance of methanogens (discussed in Section 3.2.2).

In additions, the populations of syntrophic bacteria demonstrated a similar pattern with those of methanogens. As shown in Fig. 4a and b, the quantities of total syntrophic bacteria (the sum of SPOB and SBOB) exhibited a positive correlation with those of methanogens ($r^2 = 0.714$ vs. $r^2 = 0.585$, $P = 0.001$). These findings indicate that syntrophic bacteria, in cooperation with methanogens, can affect soil methane production in paddy soil system. A similar result was observed in an anaerobic digester, UASB (Upflow Anaerobic Sludge Blanket), by domesticating syntrophs using the mixture of formate, propionate and butyrate as substrates for SFAS (Lv et al., 2020).

The OTUs of 16S rRNA were applied to construct two co-occurrence networks, and the results showed a significant interaction between methanogens and symbiotic bacteria (Fig. 4c and d). In the 10 paddy soils, *Syntrophobacter* was positively correlated with the genera of *Methanobacterium* and *Methanobacteriaceae*, while *Syntrophomonas* showed a positive correlation with *Methanocella*. However, at different rice growth stages, *Syntrophobacter* was closely related to the methanogenic genera of *Methanocella*, *Methanosarcina* and *Methanosatea*, while *Syntrophomonas* had positive association with *Methanobacterium* and *Methanosatea*. These findings revealed that syntrophs and methanogens were associated with each other for the metabolic degradation of volatile fatty acids. *Smithella* demonstrated no significant relationship with hydrogenotrophic methanogens. *Pelotomaculum* was closely related to both syntrophs (e.g. *Syntrophobacter*) and methanogens (e.g. *Methanobacterium*, *Methanocella*, *Methanosatea* and *Methanosarcina*). Lueders and Pommerenke (2004) have found that *Methanosarcinaceae* and *Methanocellales* are the predominant methanogens involved in butyrate degradation, while *Methanobacterium*, *Methanosarcina* spp. and *Methanocellales* are closely associated with syntrophic propionate oxidation. Therefore, there is no specific selectivity in the symbiotic relationship between syntrophs and methanogens, and their association might be dependent on several environmental factors in paddy soils. To the best of our knowledge, our findings provide new and reasonable insights into the association between syntrophic propionate/butyrate oxidation and methanogenesis, which may serve as a significant pathway for the anaerobic degradation of organic matter in paddy soils (McInerney et al., 2010).

3.4. Effects of environmental variables on syntrophic community composition

Synthrophic community structure differs greatly in various particle sizes, with a higher bacterial diversity in small-sized (<200 μm) fractions than in coarse-sized (200-2000 μm) fractions (Ling et al., 2014). However, in this study, the soil particle size was smaller than 200 μm. Therefore, compared to other properties (e.g.

soil pH, C/N, etc.), particle size might not be a determinative factor for bacteria communities and was not being considered. Different syntrophic bacteria showed significantly different responses to environmental factors. Both pH value and total N were significant environmental drivers for shaping the total syntrophic community in the 10 paddy soils. It has been reported that soil pH can affect the diversity and abundance of microbial communities (Fierer and Jackson, 2006). Besides, the concentrations of ammonia and total Fe were closely associated with SFAS, including SPOB and SBOB (Fig. 5a). Moreover, oxalate and citrate significantly correlated with total syntrophs and SFAS. Given that oxaloacetate is an important intermediate product in the process of syntrophic propionate oxidation, it can be directly utilized by SPOB (e.g. *Syntrophobacter* and *Pelotomaculum*) (McInerney et al., 2010). Oxalate can be degraded anaerobically to acetate, formate and CO_2, which are all methanogenic precursors (Daniel et al., 2007). Citrate usually serves as a chelating agent for anaerobic bacteria (Rotaru et al., 2014). Our results indicated that the ecological determinants of syntrophic community might differ between the off-rice season and the rice-growing season. During rice growing period, soil moisture, C/N ratio, oxalate and succinate were the decisive factors for shaping the structure and diversity of syntrophic communities (Fig. 5b).

As discussed before, apart from soil type and rice growth stages, soil properties also affected the diversity and abundance of syntrophic community. Constrained canonical analysis of principal coordinates (CAP) revealed that the compositions of syntrophs were dependent on soil properties (Fig. 6), such as C/N ratio and soil moisture, which explained by 6.13% and 5.16% of the changes in syntrophic community, respectively, followed by TOC (3.94%) and pH (3.75%). The measured soil properties accounted for 65.20% (including 36.19% interaction) of the changes in the active microbial communities between soils (Fig. 6). Therefore, it can be concluded that soil type, rice phenology and soil properties together influence the syntrophic bacteria in the soil system. These results were in agreement with a previous study (Bao et al., 2014) showing that the combination of biological and abiotic factors in paddy soil could affect methanogenic archaeal community and CH_4 production.

4. Conclusions

The distribution of syntrophs and methanogens was investigated in the paddy soils obtained from Southern China. The relative abundances of syntrophs at different rice growth stages (1.68–3.95%) were increased compared to off-rice season (0.39–1.66%) in the 10 paddy soils. Soil type and rice phenology significantly affected the relative abundance of syntrophs. SPOB were the most abundant SFAS in the 10 paddy soils during off-rice season, while syntrophic butyrate oxidation was dominant throughout the rice growing season. The main pathways for the syntrophic degradation of fatty acids might vary depending on rice growth. Different soil parameters, such as pH value, ammonia concentration, soil moisture, oxalate and citrate, could be viewed as the ecological determinants affecting the abundance and diversity of syntrophs. Overall, syntrophic bacteria, in cooperation with methanogens, could regulate CH_4 production in paddy soil system.

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CRediT authorship contribution statement

Xiaofang Pan: Investigation, Validation, Writing - original draft. **Hu Li:** Conceptualization, Resources, Data curation. **Lixin Zhao:** Investigation, Writing - review & editing. **Xiaoru Yang:** Investigation, Methodology. **Jianqiang Su:** Writing - review & editing. **Shaoqing Dai:** Software, Visualization. **Jing Ning:** Writing - review & editing, Visualization. **Chunxing Li:** Writing - review & editing. **Guanjing Cai:** Supervision, Formal analysis. **Gefu Zhu:** Conceptualization, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2020.123418.

References

Alam, M.K., Bell, R.W., Biswas, W.K., 2019. Increases in soil sequestered carbon under conservation agriculture cropping decrease the estimated greenhouse gas emissions of wetland rice using life cycle assessment. J. Clean. Prod. 224, 72–87. https://doi.org/10.1016/j.jclepro.2019.03.215.

Ali, M.A., Lee, C.H., Lee, Y.B., Kim, P.J., 2009. Silicate fertilization in no-tillage rice farming for mitigation of methane emission and increasing rice productivity. Agric. Ecosyst. Environ. 132, 16–22. https://doi.org/10.1016/j.agee.2009.02.014.

Bao, Q., Xiao, K., Chen, Z., Yao, H., Zhu, Y., 2014. Methane production and methanogenic archaeal communities in two types of paddy soil amended with different amounts of rice straw. FEMS Microbiol. Ecol. 88, 372–385. https://doi.org/10.1111/1574-6941.12305.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624. https://doi.org/10.1099/ijs.0.02212-0.

Chauhan, A., Ogram, A., Reddy, K.R., 2004. Syntrophic-methanogenic associations along a nutrient gradient in the Florida Everglades. Appl. Environ. Microbiol. 70, 3475–3484. https://doi.org/10.1128/AEM.70.6.3475-3484.2004.

Chauhan, A., Reddy, K.R., Ogram, A.V., 2006. Syntrophic-archaeal associations in a nutrient-impacted freshwater marsh. J. Appl. Microbiol. 100, 73–84. https://doi.org/10.1111/j.1365-2672.2005.02751.x.

Conrad, R., 2007. Microbial ecology of methanogens and methanotrophs. Adv. Agron. 96, 1–63. https://doi.org/10.1016/S0065-2113(07)96005-8.

Conrad, R., Klose, M., 2006. Dynamics of the methanogenic archaeal community in anoxic rice soil upon addition of straw. Eur. J. Soil Sci. 57, 476–484. https://doi.org/10.1111/j.1365-2389.2006.00791.x.

Daniel, S.L., Pilsl, C., Drake, H.L., 2007. Anaerobic oxalate consumption by microorganisms in forest soils. Res. Microbiol. 158, 300–309. https://doi.org/10.1016/j.resmic.2006.12.010.

de Bok, F.A.M., Stams, A.J.M., Dijkema, C., Boone, D.R., 2001. Pathway of propionate oxidation by a syntrophic culture of *Smithella propionica* and *Methanospirillum hungatei*. Appl. Environ. Microbiol. 67, 1800–1804. https://doi.org/10.1128/AEM.67.4.1800-1804.2001.

Dubey, S.K., Singh, A., Singh, R.S., Upadhyay, S.N., 2013. Changes in methanogenic population size and CH_4 production potential in response to crop phenology in tropical rice field. Soil Biol. Biochem. 57, 972–978. https://doi.org/10.1016/j.soilbio.2012.07.001.

Duddleston, K.N., Kinney, M.A., Kiene, R.P., Hines, M.E., 2002. Anaerobic microbial biogeochemistry in a northern bog: acetate as a dominant metabolic end product. Global Biogeochem. Cycles 16, 1063. https://doi.org/10.1029/2001gb001402.

Esperschutz, J., Zimmermann, C., Dumig, A., Welzl, G., Buegger, F., Elmer, M., 2013. Dynamics of microbial communities during decomposition of litter from

pioneering plants in initial soil ecosystems. Biogeosciences 10, 5115–5124. http://hdl.handle.net/10388/8903.
Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. Unit. States Am. 103, 626–631. https://doi.org/10.1073/pnas.0507535103.
Glissmann, K., Conrad, R., 2000. Fermentation pattern of methanogenic degradation of rice straw in anoxic paddy soil. FEMS Microbiol. Ecol. 31, 117–126. https://doi.org/10.1016/s0168-6496(99)00091-4.
Gan, Y., Qiu, Q., Liu, P., Rui, J., Lu, Y., 2012. Syntrophic oxidation of propionate in rice field soil at 15 and 30°C under methanogenic conditions. Appl. Environ. Microbiol. 78, 4923–4932. https://doi.org/10.1128/AEM.00688-12.
Girvan, M.S., Bullimore, J., Pretty, J., Osborn, A.M., Ball, A.S., 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl. Environ. Microbiol. 69, 1800–1809.
Guo, J., Song, Z., Zhu, Y., Wei, W., Li, S., Yu, Y., 2017. The characteristics of yield-scaled methane emission from paddy field in recent 35-year in China: a meta-analysis. J. Clean. Prod. 161, 1044–1050. https://doi.org/10.1016/j.jclepro.2017.06.073.
Hao, X., Jiao, S., Lu, Y., 2019. Geographical pattern of methanogenesis in paddy and wetland soils across eastern China. Sci. Total Environ. 651, 281–290. https://doi.org/10.1016/j.scitotenv.2018.09.167.
Holmes, D.E., Shrestha, P.M., Walker, D.J.F., Dang, Y., Nevin, K.P., Woodard, T.L., 2017. Metatranscriptomic evidence for direct interspecies electron transfer between Geobacter and Methanothrix species in methanogenic rice paddy soils. Appl. Environ. Microbiol. 83 https://doi.org/10.1128/AEM.00223-17 e00223-17.
Hori, T., Muller, A., Igarashi, Y., Conrad, R., Friedrich, M.W., 2010. Identification of iron-reducing microorganisms in anoxic rice paddy soil by ^{13}C-acetate probing. ISME J. 4, 267–278. https://doi.org/10.1038/ismej.2009.100.
Huang, J., Ma, K., Xia, X., Gao, K., Lu, Y., 2020. Biochar and magnetite promote methanogenesis during anaerobic decomposition of rice straw. Soil Biol. Biochem. 143, 107740. https://doi.org/10.1016/j.soilbio.2020.107740.
Imachi, H., Sekiguchi, Y., Kamagata, Y., Hanada, S., Ohashi, A., Harada, H., 2002. *Pelotomaculum thermopropionicum gen. nov. sp. nov.* an anaerobic, thermophilic, syntrophic propionate-oxidizing bacterium. Int. J. Syst. Evol. Microbiol. 52, 1729–1735. https://doi.org/10.1099/ijs.0.02212-0.
Ji, Y., Liu, P., Conrad, R., 2018a. Change of the pathway of methane production with progressing anoxic incubation of paddy soil. Soil Biol. Biochem. 121, 177–184. https://doi.org/10.1016/j.soilbio.2018.03.014.
Ji, Y., Liu, P., Conrad, R., 2018b. Response of fermenting bacterial and methanogenic archaeal communities in paddy soil to progressing rice straw degradation. Soil Biol. Biochem. 124, 70–80. https://doi.org/10.1016/j.soilbio.2018.05.029.
Jürgen, K., Hans, J.H., Gömmel, M., Ammon, C., Berg, W., 2012. Effects of climatic factors and soil management on the methane flux in soils from annual and perennial energy crops. Biol. Fertil. Soils 48, 1–8. https://doi.org/10.1007/s00374-011-0603-z.
Kim, S.Y., Pramanik, P., Kim, P.J., 2013. Impact of methanogens originated from cattle manure on increasing CH_4 emission in paddy soil during rice cultivation. In: Xu, J., Wu, J., He, Y. (Eds.), Functions of Natural Organic Matter in Changing Environment. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-5634-2-160.
Kim, Y., Liesack, W., 2015. Differential assemblage of functional units in paddy soil microbiomes. PLoS One 10, e0122221. https://doi.org/10.1371/journal.pone.0122221.
Kobayashi, T., Kuramochi, H., Xu, K., Maeda, K., 2020. Simple solvatochromic spectroscopic quantification of long-chain fatty acids for biological toxicity assay in biogas plants. Environ. Sci. Pollut. Res. 27, 17596–17606. https://doi.org/10.1007/s11356-019-06532-w.
Li, H., Yang, X., Weng, B., Su, J., Nie, S., Gilbert, J.A., Zhu, Y., 2016. The phenological period of rice growth determines anaerobic ammonium oxidation activity in the rhizosphere soil. Soil Biol. Biochem. 100, 59–65. https://doi.org/10.1016/j.soilbio.2016.05.015.
Lin, Q., He, G., Rui, J., Fang, X., Tao, Y., Li, J., 2016. Microorganism-regulated mechanisms of temperature effects on the performance of anaerobic digestion. Microb. Cell Factories 15, 96–113. https://doi.org/10.1186/s12934-016-0491-x.
Ling, N., Sun, Y., Ma, J., Guo, J., Zhu, P., Peng, C., Yu, G., Ran, W., Guo, S., Shen, Q., 2014. Response of the bacterial diversity and soil enzyme activity in particle-size fractions of Mollisol after different fertilization in a long-term experiment. Biol. Fertil. Soils 50, 901–911. https://doi.org/10.1007/s00374-014-0911-1.
Lueders, T., Pommerenke, B., 2004. Friedrich, M.W. Stable-isotope probing of microorganisms thriving at thermodynamic limits: syntrophic propionate oxidation in flooded soil. Appl. Environ. Microbiol. 70, 5778–5786. https://doi.org/10.1128/AEM.70.10.5778-5786.2004.
Lv, N., Zhao, L., Wang, R., Ning, J., Pan, X., Li, C., Cai, G., Zhu, G., 2020. Novel strategy for relieving acid accumulation by enriching syntrophic associations of syntrophic fatty acid-oxidation bacteria and H_2/formate-scavenging methanogens in anaerobic digestion, Biores. Technol. 313, 123702. https://doi.org/10.1016/j.biortech.2020.123702.
Mackie, R.I., Bryant, M.P., 1981. Metabolic activity of fatty acid-oxidizing bacteria and the contribution of acetate, propionate, butyrate, and CO_2 to methanogenesis in cattle waste at 40 and 60 degrees C. Appl. Environ. Microbiol. 41, 1363–1373 https://doi.org/doi:, https://doi.org/.
Mathai, P.P., Zitomer, D.H., Maki, J.S., 2016. Quantitative detection of syntrophic fatty acid degrading bacterial communities in methanogenic environments. Microbiology 161, 1189. https://doi.org/10.1099/mic.0.000085.
McMahon, K.D., Zheng, D., Stams, A.J., Mackie, R.I., Raskin, L., 2004. Microbial population dynamics during start-up and overload conditions of anaerobic digesters treating municipal solid waste and sewage sludge. Biotechnol. Bioeng. 87, 823–834. https://doi.org/10.1002/bit.20192.
McInerney, M.J., Struchtemeyer, C.G., Sieber, J., Mouttaki, H., Stams, A.J., Schink, B., 2010. Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism. Ann. NY. Acad. Sci. 1125, 58–72. https://doi.org/10.1196/annals.1419.005.
Nozhevnikova, A.N., Russkova, Y.I., Litti, Y.V., Parshina, S.N., Nikitina, A.A., 2020. Syntrophy and interspecies electron transfer in methanogenic microbial communities. Microbiology 89, 129–147. https://doi.org/10.1134/S0026261720020101.
Power, M.E., Tilman, D., Estes, J.A., Menge, B.A., Bond, W.J., Mills, L.S., 1996. Challenges in the quest for keystones. Bioscience 46, 609–620. https://doi.org/10.2307/1312990.
Rotaru, A.E., Shrestha, P.M., Liu, F., Shrestha, M., Shrestha, D., Embree, M., Zengler, K., Wardman, C., Nevin, K.P., Lovley, D.R., 2014. A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to *Methanosaeta* for the reduction of carbon dioxide to methane. Energy Environ. Sci. 7, 408–415. https://doi.org/10.1039/C3EE42189A.
Rotaru, A.E., Shrestha, P.M., Liu, F., Ueki, T., Nevin, K., Summers, Z.M., 2012. Interspecies electron transfer via hydrogen and formate rather than direct electrical connections in cocultures of *Pelobacter carbinolicus* and *Geobacter sulfurreducens*. Appl. Environ. Microbiol. 78, 7645–7651. https://doi.org/10.1128/AEM.01946-12.
Rui, J., Peng, J., Lu, Y., 2009. Succession of bacterial populations during plant residue decomposition in rice field soil. Appl. Environ. Microbiol. 75, 4879–4886. https://doi.org/10.1128/AEM.00702-09.
Sakai, S., Imachi, H., Hanada, S., Ohashi, A., Harada, H., Kamagata, Y., 2008. *Methanocella paludicola gen. nov. sp. nov.* a methane-producing archaeon, the first isolate of the lineage 'rice cluster i', and proposal of the new archaeal order *Methanocellales ord. nov.* Int. J. Syst. Evol. Microbiol. 58, 929–936. https://doi.org/10.1099/ijs.0.65571-0.
Sieber, J.R., Mcinerney, M.J., Gunsalus, R.P., 2012. Genomic insights into syntrophy: the paradigm for anaerobic metabolic cooperation. Annu. Rev. Microbiol. 66, 429–452. https://doi.org/10.1146/annurev-micro-090110-102844.
Sieber, J.R., Michael, J.M., Nicolai, M., Bernhard Schink, R.P.G., Caroline, M.P., 2019. Methanogens : syntrophic metabolism. In: Stams, A.J.M., Diana, S. (Eds.), Biogenesis of Hydrocarbons. Springer, Cham, pp. 1–31. https://doi.org/10.1007/978-3-319-53114-4_2-1, 978-3-319-53114-4. Available under.
Singh, A., Dubey, S.K., 2012. Temporal variations in methanogenic community structure and methane production potential of tropical rice ecosystems. Soil Biol. Biochem. 48, 162–166. https://doi.org/10.1016/j.soilbio.2012.01.022.
Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K., Salazar, G., 2015. Structure and function of the global ocean microbiome. Science 348, 1261359. https://doi.org/10.1126/science.1261359.
Sundberg, C., Al-Soud, W.A., Larsson, M., Alm, E., Yekta, S.S., Svensson, B.H., 2013. 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. FEMS Microbiol. Ecol. 85, 612–626. https://doi.org/10.1111/1574-6941.12148.
Wang, J., Deng, H., Wu, S.S., Deng, Y.C., Liu, L., Han, C., Jiang, Y.B., Zhong, W.H., 2019a. Assessment of abundance and diversity of exoelectrogenic bacteria in soil under different land use types. Catena 172, 572–580. https://doi.org/10.1016/j.catena.2018.09.028.
Wang, T., Li, Z., Chen, X., Long, X.E., 2019b. Effects of nickel and cobalt on methane production and methanogen abundance and diversity in paddy soil. Microbiology 7, e6274. https://doi.org/10.7717/peerj.6274.
Yang, J., Zhang, X., Xie, Y., Song, C., Sun, J., Zhang, Y., 2017. Ecogenomics of zooplankton community reveals ecological threshold of ammonia nitrogen. Environ. Sci. Technol. 51, 3057–3064. https://doi.org/10.1021/acs.est.6b05606.
Yang, X.R., Li, H., Nie, S.A., Su, J.Q., Weng, B.S., Zhu, G.B., 2015. Potential contribution of anammox to nitrogen loss from paddy soils in southern China. Appl. Environ. Microbiol. 81, 938–947. https://doi.org/10.1128/AEM.02664-14.
Yu, Y., Lee, C., Kim, J., Hwang, S., 2010. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol. Bioeng. 89, 670–679. https://doi.org/10.1002/bit.20347.
Yuan, J., Yuan, Y., Zhu, Y., Cao, L., 2018. Effects of different fertilizers on methane emissions and methanogenic community structures in paddy rhizosphere soil. Sci. Total Environ. 627, 770–781. https://doi.org/10.1016/j.scitotenv.2018.01.233.
Zhang, L., Guo, B., Zhang, Q., Florentino, A., Liu, Y., 2019. Co-digestion of blackwater with kitchen organic waste: effects of mixing ratios and insights into microbial community. J. Clean. Prod. 236, 117703. https://doi.org/10.1016/j.jclepro.2019.117703.
Zhong, D., Li, J., Ma, W., Qian, F., 2020. Clarifying the synergetic effect of magnetite nanoparticles in the methane production process. Environ. Sci. Pollut. Res. 27, 17054–17062. https://doi.org/10.1007/s11356-020-07828-y.
Zhuang, L., Ma, J., Tang, J., Tang, Z., Zhou, S., 2017. Cysteine-accelerated methanogenic propionate degradation in paddy soil enrichment. Microb. Ecol. 73, 916–924. https://doi.org/10.1007/s00248-016-0882-x.