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Research article

Winter drainage and film mulching cultivation mitigated CH_4 emission by regulating the function and structure of methanogenic archaeal and fermenting bacterial communities in paddy soil

Yang Ji [a], Yongji Xu [a], Mengying Zhao [a], Guangbin Zhang [b,*], Ralf Conrad [c], Pengfei Liu [d], Zhaozhong Feng [a], Jing Ma [b], Hua Xu [b]

[a] *College of Applied Meteorology, Nanjing University of Information Science & Technology, Nanjing 210044, China*
[b] *State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Sciences, Chinese Academy of Sciences, East Beijing Road 71, Nanjing, 210008, China*
[c] *Max-Planck-Institute for Terrestrial Microbiology, Marburg, 35043, Germany*
[d] *Center for the Pan-Third Pole Environment, Lanzhou University, Lanzhou, 730000, China*

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Abstract: Winter flooding of harvested rice fields is a typical cropping system in mountainous areas, which emits considerable amounts of CH_4. Plastic film mulching cultivation is recognized as an important rice cultivation practice in paddy field for water-saving irrigation. However, the effects of these managements on CH_4 emissions in paddy soil and the underlying microbial mechanism are unclear. A field experiment was carried out with the application of winter drainage followed by traditional rice cultivation (WD), winter drainage followed by plastic film mulching cultivation (MC), as well as winter flooding followed by traditional rice cultivation (WF) as control in hilly paddy fields. We investigated the CH_4 emissions, functional (CH_4 production rate, ^{13}C isotope) and structural (abundance, structure) responses of soil methanogenic archaeal and fermenting bacterial communities during rice season. Shifting the fields from WF into WD and MC substantially mitigated CH_4 emissions by 62.3% and 59.2%, respectively, paralleled with the enhancement of soil Eh and the reductions of soil DOC content. Compared with WF, WD and MC both significantly decreased CH_4 production rates and the copy numbers of *mcrA* gene. Moreover, an increasing contribution of hydrogenotrophic methanogenesis (from 30.7% to 50.0%) to total CH_4 production was observed during the conversion from WF to MC under an anaerobic incubation, paralleled with the decreased acetate content and increased $\delta^{13}C$ values of acetate-methyl and total acetate. The communities of methanogenic archaea and fermenting bacteria strongly responded to the shift from WF to WD, while MC only showed significant effects on the methanogenic archaeal communities. Compared with WF, WD and MC significantly increased the relative abundance of *Methanothrix*, *Methanosarcina* and *Methanocella*, while those of *Methanoregula, Massilia* and *Geobacter* were decreased. The co-occurrence networks showed that WD and MC induced the loss of mixed methanogenic fermentation modules, indicating the decrease in functional biodiversity and redundancy of fermenting bacterial and methanogenic archaeal communities.The findings suggest that WD and MC approach mitigate CH_4 emission by regulating the function and structure of methanogenic archaeal and fermenting bacterial communities in paddy soil, which represent the effective management strategies considering the water availability and CH_4 mitigation in paddy-field agriculture.

1. Introduction

Rice fields are one of the major sources in the global methane (CH_4) budget and contribute in the range of 25–300 Tg per year (Bridgham et al., 2013; Tariq et al., 2018). Methane and CO_2 are the end products of the degradation of organic matter under anaerobic conditions and are produced by a complex microbial community consisting of hydrolytic and fermentative bacteria and methanogenic archaea (Conrad et al., 2012). It is usually produced by acetoclastic and hydrogenotrophic methanogenic archaea with acetate or H_2/CO_2 as the two major products of organic matter fermentation (Conrad et al., 2009). Although CH_4 production generally requires wet and anoxic conditions, potential activity can well survive drainage, desiccation and aeration of the soil (Conrad, 2020; Deng et al., 2020). Thus, CH_4 production can be

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* Corresponding author.
E-mail address: gbzhang@issas.ac.cn (G. Zhang).

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activated in drained soils as well, although the structures (abundance and composition) of the methanogenic microbial communities are different (Angel et al., 2012; Tariq et al., 2018).

Winter flooding (WF) of harvested rice fields is an environmentally friendly agricultural practice in mountainous areas without irrigation throughout East Asia (Negri et al., 2020). In the WF system, the soil is flooded during winter fallow periods after rice harvest to conserve water for following season, which can promote soil anoxic conditions and production of methanogenic substrates resulting in substantial CH_4 production (Xu et al., 2020; Zhang et al., 2018). Proper water management is considered to be one of the most important options for regulating CH_4 emission from paddy fields (Oliveira et al., 2020; Wu et al., 2019). Drainage, relative to flooding, in the winter fallow season not only prevents CH_4 emission from the fields directly in the current season, but also sharply reduces CH_4 emission indirectly during the following rice-growing season (Tariq et al., 2018; Zhang et al., 2018). However, its effect on the processes of CH_4 production, especially the methanogenic microbial community, remains poorly documented.

The application of plastic film mulching cultivation (MC) is recognized as a useful method of improving crop production by increasing water and nitrogen use efficiencies in agriculture in East Asian countries (Kim et al., 2017; Qin et al., 2015; Zhang et al., 2018). Approximately 20 million hectares of farmland are covered with plastic film on the global scale (Yao et al., 2017). Previous studies showed that the MC cultivation in agriculture strongly affected the decomposition of soil organic carbon by influencing soil biogeochemical processes (Yu et al., 2021; Zhang et al., 2018) and reduced the release of CH_4 by maintaining unsaturated conditions in paddy soil (Kreye et al., 2007; Yao et al., 2017). However, how the CH_4-producing archaea and fermenting bacteria adapt to such conditions is poorly known. Therefore, We hypothesized that activity and structure of the methanogenic archaeal and fermenting bacterial communities involved in CH_4 production would respond differently to the winter water management and rice cultivation practice.

Accordingly, a field experiment was carried out with the application of winter drainage followed by traditional rice cultivation (WD), and winter drainage followed by film mulching rice cultivation (MC), as well as winter flooding followed by traditional rice cultivation (WF) as control to investigate the CH_4 emissions in paddy fields of Central Sichuan hilly areas. Furthermore, we investigated the functional (CH_4 production rate, ^{13}C isotope) and structural (abundance, structure) responses of soil methanogenic archaeal and fermenting bacterial communities during rice season. We determined methanogenic function by rates in the presence and absence of methyl fluoride (CH_3F), an inhibitor of aceticlastic methanogenesis (Janssen and Frenzel, 1997), and characterized the methanogenic archaeal and fermenting bacterial community structures by the abundance and taxonomic composition of the *mcrA* gene and the bacterial 16s rRNA gene, respectively. Ultimately, the goal of this research is to better understand the impact of WD and MC approach on the function and structure of CH_4-cycling microbial communities thereby affecting CH_4 emisssions in paddy field.

2. Material and methods

2.1. Field experimental design

Soil samples were taken from the experimental field station located at Ziyang City, Sichuan Province, China (30° 05'N, 104° 34'E) in 2018. The soil properties are as follows: total carbon (C) of 3.4%, total N of 0.17%, initial pH of 8.2, $\delta^{13}C$ of soil organic carbon (SOM) of −27‰, and the soil parent material is purple shale. Three treatments (WF, WD, MC) were established during the rice season since the year 2008 (Zhang et al., 2018), i.e. winter fallow under continuous flooding followed by traditional rice cultivation (WF), winter fallow with drainage management followed by traditional rice cultivation (WD), and winter fallow with drainage management followed by plastic film mulching rice cultivation (MC). MC plots were designed with 4 raised ridges (5 m × 1.45 m) and mulched by utilizing transparent plastic films (0.004 mm), which were water-saving irrigated throughout the rice season, only with irrigation in the ditches but no standing water on the ridge surface. Detailed descriptions of field experimental design can be found in Table S1.

2.2. CH_4 measurements and soil sampling

CH_4 fluxes were monitored by the static chamber technique during rice season, which was calculated by the slopes of linearly increasing of CH_4 concentration at each sampled time (0, 15, 30 and 45 min with chamber closure). Redox potential (Eh) at 10 cm depth was simultaneously measured.

Fresh soil samples of different treatments were collected on the same day at each typical rice-growing stage from the upper 10-cm layer in each plot with three replicate locations. This was done by taking six cores from each of the three replicate locations. Soil water content was determined gravimetrically after drying at 105 °C for 8 h. The soil DOC was extracted by 0.5 M K_2SO_4, followed the procedure of Jones and Willett (2006). Fresh soil collected at the rice vegetative stage from the upper 10-cm layer was kept on ice and later stored at −20 °C for the following lab incubation and amplicon sequencing analysis.

2.3. Incubation experiments and isotopic analysis

Fresh soil samples collected at the rice vegetative stage were used for the anaerobic incubation experiments to determine the functional responses of methanogenic microbial communities to different managements. Soil slurries were prepared in 26-ml pressure tubes using 5 g dry soil and 5 ml anoxic sterile water incubated at 25 °C. The tubes were anaerobically incubated until CH_4 production was constant. Inhibition of aceticlastic methanogenesis was achieved by the addition of 3% CH_3F to the gas phase (Janssen and Frenzel, 1997). CH_4 was analyzed from the headspace at regular time intervals. The liquid was examined for the acetate concentration, $\delta^{13}C$ of acetate-methyl and total acetate at the end of all incubations. CH_4, acetate, and $\delta^{13}C$ isotope were analyzed through GC, HPLC and HPLC-C-IRMS, respectively. The off-line pyrolysis was used to determine the $\delta^{13}C$ values of the acetate-methyl group. Values of $\delta^{13}C$ are presented in permil relative to the standard of Vienna Pee Dee Belemnite (Ji et al., 2018).

2.4. Amplicon sequencing and processing

Genomic DNA extraction was carried out from 0.5 g fresh soil collected at the rice vegetative stage by implementing NucleoSpin™ Soil Kit (Macherey-Nagel, Germany). The extracted DNA was used for qPCR and ILLUMINA sequencing. The numbers of archaeal and bacterial 16S rRNA gene copies, methanogenic *mcrA* gene copies were ascertained by quantitative PCR by utilizing two technical replicate reactions (Angel et al., 2012).

ILLUMINA Miseq sequencing was executed for the methanogenic *mcrA* gene and bacterial 16S rRNA gene. Sequence datasets are available with the number of accession SRP103721 within the NCBI Sequence Read Archive. A two-step PCR approach was used for the barcoded PCR amplicons, and two independent libraries were created through pooling equal quantities of individual barcoded amplicons of *mcrA* and 16S rRNA genes, accordingly (Herbold et al., 2015). The sequencing of the library of *mcrA* gene was done by utilizing a 2 × 300 cycle combination mode *via* Microsynth on an ILLUMINA MISEQ system (Balgach, Switzerland); the library of bacterial 16S rRNA gene was sequenced using a 2 × 250 cycle combination mode on a system of ILLUMINA HISEQ 2000 through Max Planck-Genome-Centre (Cologne, Germany). The procedure of high-throughput sequencing analysis was described in Ji et al. (2018).

2.5. Statistical analyses and data exploration

The significance of the factors (winter water management and rice cultivation management) was examined by using a two-way analysis of variance (ANOVA) in SPSS for Windows (version 19.0, SPSS Inc., USA). Tukey HSD was employed to evaluate the significant differences between treatments with agricolae package (version 1.2–1; Mendiburu, 2015) in R (version 4.0.5; R Development Core Team).

An illustration of a heatmap for the relative abundance of OTUs between different samples was created by employing ggplot version 2.2.1.0 in R version 4.0.5 (Wickham, 2009). PCA was constructed applying prcomp for the OTUs selection to explain most of the differences between samples. A Hellinger transformation of the OTU counts was conducted applying the vegan package version 2.2–1 (Oksanen et al., 2015). A total of 37 unique OTUs were obtained for the heatmap construction. The abundances of OTU were converted to reads percentage from all considered samples, and the distances of Manhattan were measured. PerMANOVA was performed by exerting package vegan version 2.2–1 'adonis' function by bray-curtis dissimilarities (Oksanen et al., 2015).

Based on the abundance of 16S rRNA and *mcrA* genes OTUs, two co-occurrence networks were constructed according to the pipelines explored developed by Williams et al. (2014). In general, OTU table without singletons was initially rarefied to the least specimen depth (herein 4317 and 10,322 reads for *mcrA* gene and 16S rRNA gene, respectively). The correlation coefficient of Pairs with Spearman $0.70 \leq \rho \leq 0.9$ and FDR corrected p-value ≤ 0.01 were implemented for the construction of co-occurrence networks. Modules within the networks were calculated by employing the multilevel modularity algorithm optimization (Blondel et al., 2008). Detailed descriptions of OTUs filtering and network construction could be found in Ji et al. (2018).

3. Results

3.1. Soil Eh, DOC and CH_4 emissions

WD and MC managements both induced substantially lower soil water content (Fig. 1A) and higher Eh values (Fig. 1B) during rice growth season compared with that of WF management, with an average Eh value of −148, −132 and −189 mV, respectively. A similar seasonal pattern was observed in soil DOC content (Fig. 1C), showing decreasing values by 25.7% and 32.6% in WD and MC treatments, respectively. The CH_4 fluxes during rice season increased gradually to the peaks, ranging from 18.8 mg m^{-2} h^{-1} for WD and 39.1 mg m^{-2} h^{-1} for WF treatment, and then decreased sharply (Fig. 1D). The seasonal variations of CH_4 flux were negatively correlated with Eh value ($r = -0.391$, $p < 0.01$) and positively with soil DOC content ($r = 0.584$, $p < 0.05$) across the three treatments. Compared with WF treatment, winter drainage and plastic film mulching managements sharply decreased the total CH_4 emissions during rice season by 62.3% and 59.2%, respectively (Table 1).

3.2. Methanogenesis rates and isotopic signatures

Production of gaseous CH_4 was measured during the incubation course both in the absence and the presence of CH_3F, an inhibitor of acetoclastic methanogenesis. Typical time courses for each soil are shown in Fig. S1. CH_4 accumulation showed the lowest value in MC treatment compared to the other two treatments. A similar pattern was observed for the CH_4 production rates (Table 1). Compared with WF treatment, WD and MC significantly decreased CH_4 production rates during rice season ($p < 0.05$), similarly as our observation of CH_4 emission under field conditions (Fig. 1D, Table 1). Amendment of CH_3F led to strong inhibition of CH_4 production due to the specific inhibition of acetoclastic methanogenesis (Table 1). Hence, the residual activity is due to hydrogenotrophic methanogenesis, which accounted for 30.7% of total CH_4 production in WF and WD treatments, but for 50.0% in MC treatment (Table 1). The CH_4 production rates were significantly correlated with the copy numbers of archaeal 16s rRNA genes (Fig. 2, $p < 0.05$) and the total CH_4 emissions under field conditions (Table 1, $p < 0.05$).

Furthermore, at the end of the incubation, CH_3F addition also considerably raised the acetate concentration (Table 1). In the inhibited samples, $\delta^{13}C$ values could be determined in which accumulated acetate had high concentrations, but not in the uninhibited treatments with much lower concentrations. Theoretically, the $\delta^{13}C$ values of the residual acetate in the absence of CH_3F are much higher than the $\delta^{13}C$ of acetate in the presence of CH_3F. $\delta^{13}C$ values of the acetate-methyl part ranged from −40‰ to −60‰ which were about 2–7‰ lower than those of total acetate. Generally, the concentration of acetate was considerably lower in MC than that of WF and WD treatments, while the $\delta^{13}C$ values of acetate and the methyl part were significantly higher in WD and MC than that of WF treatments.

3.3. Abundance of microorganisms

The copy numbers of bacterial 16S rRNA gene were generally higher by one order of magnitude in comparison to the *mcrA* and archaeal 16S rRNA genes (Fig. 2). Statistical comparison of the three genes showed a similar pattern among different treatments. Compared with WF treatment, winter drainage and plastic film mulching managements decreased the copy numbers of the three genes during the rice growth season by 1.1–22.3% and 25.1–43.3%, respectively, while significant differences were only found for the archaeal and methanogen numbers ($p < 0.05$).

3.4. Diversity and composition of methanogenic archaeal communities

The samples from WF, WD and MC treatments were sequenced for the rice vegetative growth stage. A total of 73,365 reads targeting the methanogenic *mcrA* gene were obtained from 9 soil samples, resulting in 4317–9507 high-quality sequences for each sample after quality trimming, frameshift correction and chimera removal, equivalent to about 86–102 *mcrA* OTUs. Seven taxa of the methanogenic communities were found assigned to the genera *Methanothrix, Methanosarcina*, *Methanocella*, *Methanomassiliicoccus, Methanoregula, Methanobacterium,* the order *Methanomicrobiales*, and to *mrtA* gene (Fig. 3A). *Methanothrix, Methanosarcina, Methanoregula and Methanocella* were the dominant groups, accounting for 88.2–93.5% in all the different treatments.

Six α-diversity indices were calculated based on the obtained *mcrA* OTUs, including OTU richness (Observed OTUs), estimated richness (Chao1), ACE, Shannon, Simpson and Fisher (Table 2). Statistical analyses showed that winter drainage and plastic film mulching managements significantly increased the values of Shannon and Simpson indices in comparison with those of WF treatment ($p < 0.05$).

Compared with WF, winter drainage and plastic film mulching managements significantly increased the relative abundance of acetoclastic *Methanothrix* and *Methanosarcina*, hydrogenotrophic *Methanobacterium* and *Methanocella*, while that of hydrogenotrophic *Methanoregula* was decreased (Fig. 3A). The analysis of Principal coordinates (Pcoa) demonstrated that the compositions of archaeal *mcrA* gene constructed distinct clusters for different managements (Fig. S2A). Assessments through perMANOVA illustrated that the clustering was substantially influenced by winter water management ($p < 0.05$) and rice cultivation practice (Table S2, $p < 0.05$).

3.5. Diversity and composition of bacterial communities

ILLUMINA sequencing of the bacterial 16S rRNA genes resulted in 10,322–18,043 high-quality sequences, equal to around 2020–2457 bacterial OTUs. α-diversity indices based on the obtained bacterial OTUs were compared among different treatments (Table 2). Statistical analyses showed that WD treatment significantly increased the values of

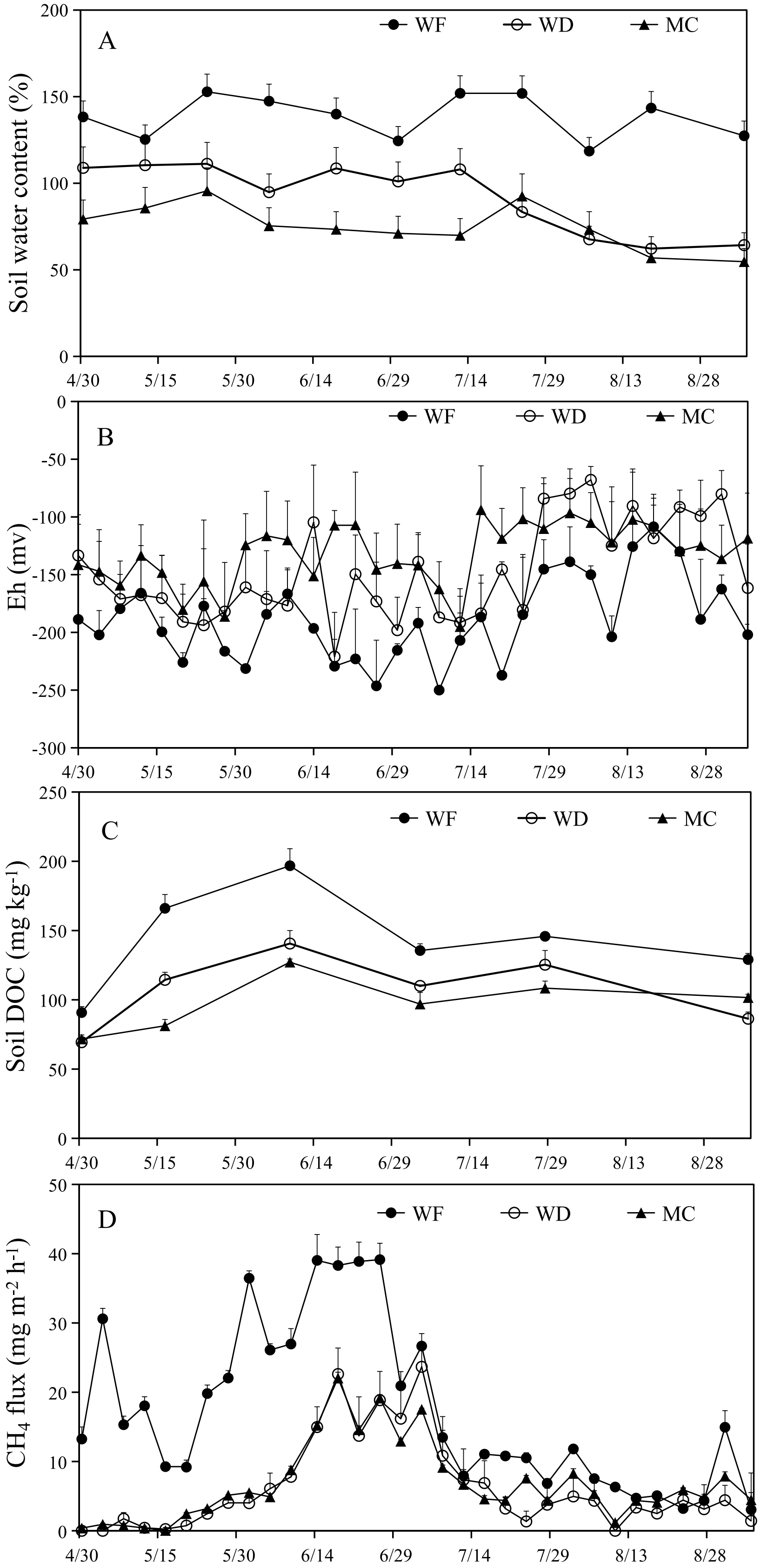


Fig. 1. Temporal variation in the (A) soil water content, (B) soil Eh, (C) soil DOC and (D) CH_4 flux during rice season from winter flooding management (WF), winter drainage management (WD) and plastic film mulching rice cultivation (MC).

Table 1
Total CH_4 emission during rice growth, CH_4 production rates in the presence/absence of methylfluoride (CH_3F, inhibiting aceticlastic methanogenesis), concentrations of acetate, $\delta^{13}C$ values of acetate-methyl and total acetate at the end of incubation with CH_3F, and contribution of hydrogenotrophic methanogenesis from the soils during rice season in winter flooding management (WF), winter drainage management (WD) and plastic film mulching rice cultivation (MC) treatments.

	CH_4 emission (kg CH_4 ha^{-1} d^{-1})	CH_4 production rate (nmol h^{-1} gdw^{-1})	Inhibited CH_4 production rate (nmol h^{-1} gdw^{-1})	Hydrogenotrophic methanogenesis (%)	Acetate (mM)	$\delta^{13}C$ of total acetate (‰)	$\delta^{13}C$ of acetate-methyl (‰)
WF	4.04 ± 0.32a	6.20 ± 0.61a	2.09 ± 0.23a	33.71 ± 5.12b	2.90 ± 0.32 ab	−53.50 ± 0.92c	−60.10 ± 2.52c
WD	1.52 ± 0.53b	4.99 ± 0.36b	1.53 ± 0.50a	30.66 ± 10.05b	3.32 ± 0.08a	−42.01 ± 2.19 ab	−44.93 ± 1.77 ab
MC	1.65 ± 0.08b	2.48 ± 0.77c	1.24 ± 0.12a	50.00 ± 16.31a	2.18 ± 0.17b	−38.28 ± 0.81a	−39.65 ± 1.20a

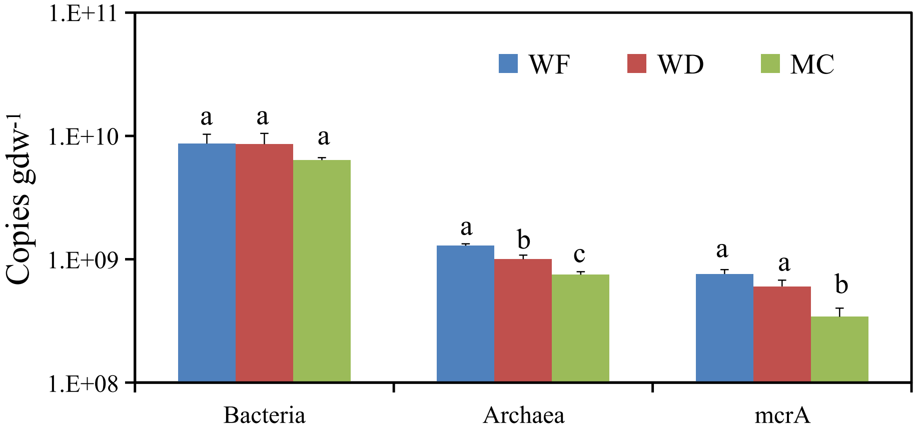


Fig. 2. Copy numbers of archaeal and bacterial 16S rRNA genes and *mcrA* gene in the soils during rice season from winter flooding management (WF), winter drainage management (WD) and plastic film mulching rice cultivation (MC).

Observed OTUs, chao1, ACE, Fisher indices in comparison with those of WF treatment ($p < 0.05$), while a less effect was found in MC cultivation, indicating that winter water management had a more significant effect on α-diversity of bacterial communities than did rice cultivation practices.

The dominate bacterial phyla include *Proteobacteria* (29–49%), *Firmicutes* (13–22%), *Chloroflexi* (9–17%), *Bacteroidetes* (5–10%), *Acidobacteria* (4–6%), *Actinobacteria* (2–4%), and *Cyanobacteria* (1–10%), which were observed in all the treatments (Fig. 3B). The relative abundance of phyla *Gemmatimonadedetes* and *Chloroflexi* of WD and MC treatments were significantly higher than that from WF treatment, while that of phylum *Proteobacteria* showed an opposite pattern. Principal coordinates (Pcoa) analysis showed that the 16S rRNA gene composition formed separate clusters for each treatment (Fig. S2B). The perMANOVA analysis showed that winter water management (WD), rather than rice cultivation practice (MC), significantly affected the compostion of bacterial communities ($p < 0.05$, Table S2).

A heatmap was constructed based on 37 selected OTUs to display the differences in the relative abundance of bacterial OTUs (Fig. 4). The heatmap showed that WF and the other two treatments were clearly separated, while WD treatment clustered with the OTUs from MC treatment. ANOVA identified 9 rare OTUs with low (<10%) abundance that were substantially distinct in WF and the other two managements. Seven of them were affiliated to *Betaproteobacteria* (OTU5, 29), three to *Gammaproteobacteria* (OTU41, 78, 56), two to *Sphingobacteria* (OTU61, 143), one to *Deltaproteobacteria* (OTU13), and the other one to *Cyanobacteria* (OTU12). OTU5, affiliated to the genus *Massilia*, was the most abundant OTU, accounting for 1.43–10.41% of all bacterial groups among all samples (Fig. S3). The abundance decreased from 10.4% in WF treatment to 1.43–1.76% in WD and MC treatments. A similar pattern was detected in the OTUs associated with the genera *Geobacter*, *Pseudomonas, Thiobacillus*, WCHB1-69, *Arenimonas* and *Chitinophagaceae* unclassified.

3.6. Co-occurrence network analysis of methanogenic archaeal and fermenting bacterial communities

Two correlation-based co-occurrence networks were constructed based on the OTUs of *mcrA* and bacterial 16S rRNA genes for the treatments with traditional rice cultivation practice (WF and WD, Fig. 5A) and those with winter drainage and MC cultivation (WD and MC, Fig. 5B), respectively, to reveal the interactions between different methanogenic microbial groups. Generally, the two networks included 146 nodes and 166 nodes, respectively. Five major methanogenic fermentation modules (co-occurrence of CH_4-producing archaea with putatively fermenting bacteria) were found in the network of the samples with traditional rice cultivation practice (WF and WD, Fig. 5A), named TM1 to TM5. TM1 and TM2 were the hydrogenotrophic methanogenesis fermentation modules, in which the genus *Methanoregula* was the key methanogenic genus, mainly co-occurred with OTUs affiliated to the genus *Clostridium*, Bacteroidetes vadinHA17 group and the orders *Burkholderiales* and *Anaerolineales*. In contrast, TM3, TM4 and TM5 were all acetoclastic plus hydrogenotrophic fermentation methanogenesis modules, with *Methanosarcina*, *Methanothrix*, *Methanocella, Methanobacterium* as the key methanogenic OTUs, mainly co-occurred with the taxa *Clostridium*, *Gaiella,* Acidobacterial subgroup 6, and *Anaerolineaceae*.

In contrast to the network TM, the network for the samples with winter drainage and MC cultivation (WD and MC, Fig. 5B) was mainly composed of primary fermentation functional modules, including abundant *Bacteroidetes*, *Clostridia* and *Burkholderiales*. Only three methanogenic fermentation modules (DM1 to DM3) were identified, which were all situated at the periphery of the network. The three DM methanogenic modules were either hydrogenotrophic or acetoclastic methanogenic modules. For example, DM1 was an acetoclastic methanogenic module, with the genus *Methanothrix* as the key methanogenic OTU, mainly co-occurred with the family *Nitrospiraceae, Coriobacteriaceae* and

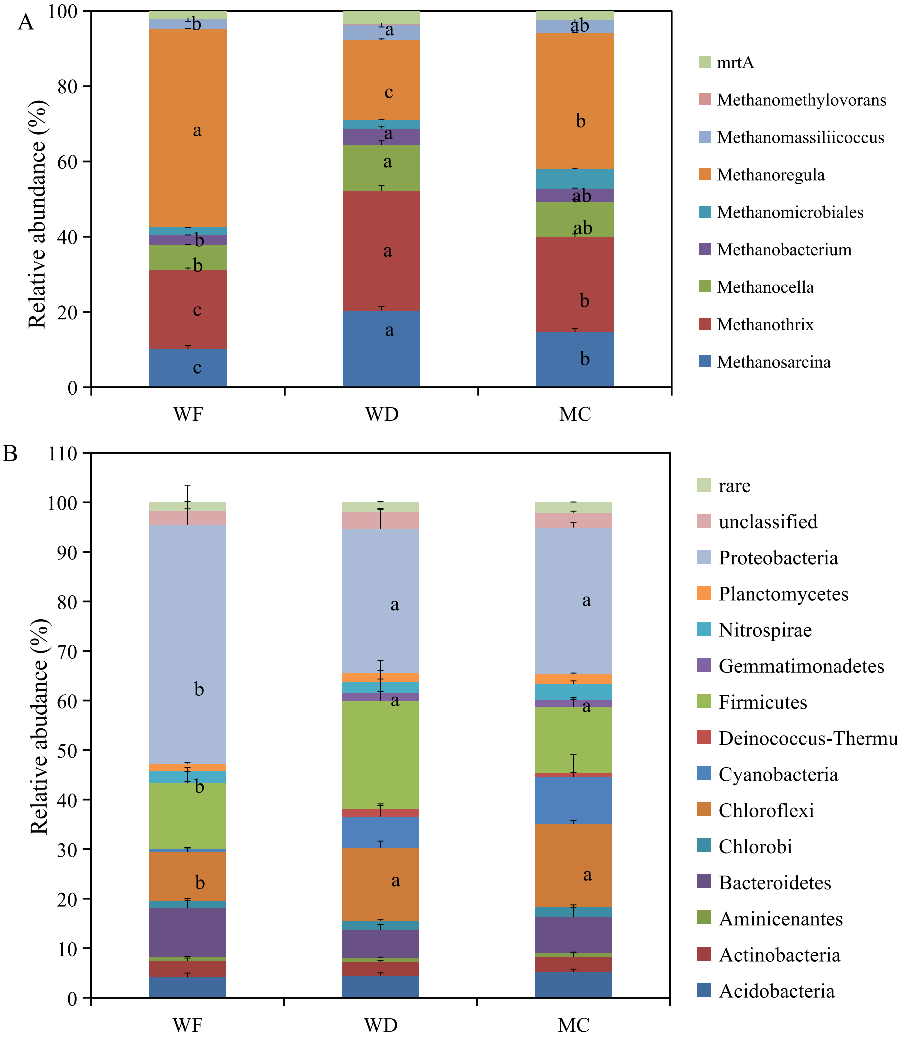


Fig. 3. Relative contribution of (A) the taxonomic groups of *mcrA* gene and (B) the bacterial phyla (16S rRNA gene) in the soils during rice season from winter flooding management (WF), winter drainage management (WD) and plastic film mulching rice cultivation (MC).

Table 2
The α-diversity indices of methanogenic and bacterial communities from the soils during rice season in winter flooding management (WF), winter drainage management (WD) and plastic film mulching rice cultivation (MC) treatments.

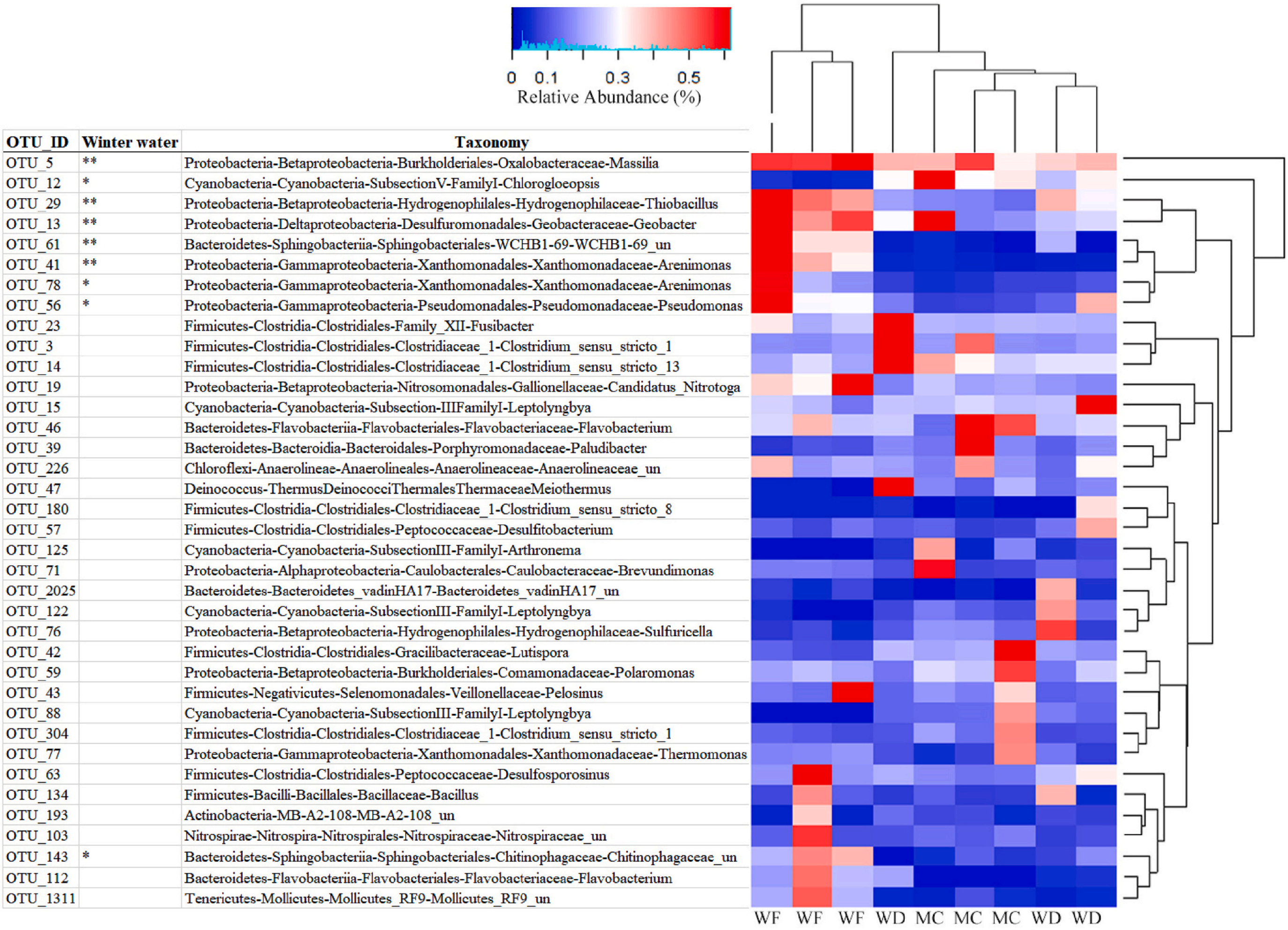
Treatment		Observed OTU	Chao1	ACE	Shannon	Simpson	Fisher
Methanogen	WF	92.33 ± 2.07a	111.11 ± 8.73a	108.60 ± 5.75a	2.37 ± 0.04c	0.73 ± 0.01c	14.58 ± 0.68a
	WD	96.00 ± 5.13a	110.97 ± 8.40a	111.42 ± 9.15a	3.04 ± 0.02a	0.91 ± 0.00a	15.60 ± 0.24a
	MC	95.67 ± 1.80a	109.85 ± 1.95a	110.99 ± 2.41a	2.80 ± 0.01b	0.86 ± 0.00b	15.02 ± 0.31a
Bacteria	WF	2020 ± 25b	4004 ± 27c	4130 ± 55b	5.72 ± 0.30a	0.98 ± 0.01a	662 ± 28c
	WD	2457 ± 182a	4628 ± 246 ab	4723 ± 273a	6.14 ± 0.38a	0.98 ± 0.01a	836 ± 49a
	MC	2374 ± 117 ab	4652 ± 115a	4671 ± 85 ab	6.15 ± 0.16a	0.99 ± 0.01a	800 ± 39 ab

the order *Myxococcales*. DM2 and DM3 were both hydrogenotrophic methanogenic modules. In the case of DM2, the key methanogenic genera was *Methanoregula*, which co-occurred with *Nitrospiraceae* and *Syntrophomonas*. *Methancella* was the key methanogenic genus in the case of DM3, mainly co-occurred with the genus *Desulfitobacterium* and unclassified group of *Burkholderiales*.

4. Discussion

4.1. Effect of winter drainage and film mulching cultivation on CH_4 emission and production in paddy soil

In this field experiment, winter drainage and plastic film mulching, relative to WF, remarkably depressed CH_4 emission and production rates in the following rice growing season. The application of WD and MC decreased the water content, leading to much higher soil Eh values during the following rice season, and a negative correlation was found between CH_4 emission and soil Eh. Soil DOC is an important carbon source for methanogenesis, which favors CH_4 production and emission



OTU_ID	Winter water	Taxonomy
OTU_5	**	Proteobacteria-Betaproteobacteria-Burkholderiales-Oxalobacteraceae-Massilia
OTU_12	*	Cyanobacteria-Cyanobacteria-SubsectionV-FamilyI-Chlorogloeopsis
OTU_29	**	Proteobacteria-Betaproteobacteria-Hydrogenophilales-Hydrogenophilaceae-Thiobacillus
OTU_13	**	Proteobacteria-Deltaproteobacteria-Desulfuromonadales-Geobacteraceae-Geobacter
OTU_61	**	Bacteroidetes-Sphingobacteriia-Sphingobacteriales-WCHB1-69-WCHB1-69_un
OTU_41	**	Proteobacteria-Gammaproteobacteria-Xanthomonadales-Xanthomonadaceae-Arenimonas
OTU_78	*	Proteobacteria-Gammaproteobacteria-Xanthomonadales-Xanthomonadaceae-Arenimonas
OTU_56	*	Proteobacteria-Gammaproteobacteria-Pseudomonadales-Pseudomonadaceae-Pseudomonas
OTU_23		Firmicutes-Clostridia-Clostridiales-Family_XII-Fusibacter
OTU_3		Firmicutes-Clostridia-Clostridiales-Clostridiaceae_1-Clostridium_sensu_stricto_1
OTU_14		Firmicutes-Clostridia-Clostridiales-Clostridiaceae_1-Clostridium_sensu_stricto_13
OTU_19		Proteobacteria-Betaproteobacteria-Nitrosomonadales-Gallionellaceae-Candidatus_Nitrotoga
OTU_15		Cyanobacteria-Cyanobacteria-Subsection-IIIFamilyI-Leptolyngbya
OTU_46		Bacteroidetes-Flavobacteriia-Flavobacteriales-Flavobacteriaceae-Flavobacterium
OTU_39		Bacteroidetes-Bacteroidia-Bacteroidales-Porphyromonadaceae-Paludibacter
OTU_226		Chloroflexi-Anaerolineae-Anaerolineales-Anaerolineaceae-Anaerolineaceae_un
OTU_47		Deinococcus-ThermusDeinococciThermalesThermaceaeMeiothermus
OTU_180		Firmicutes-Clostridia-Clostridiales-Clostridiaceae_1-Clostridium_sensu_stricto_8
OTU_57		Firmicutes-Clostridia-Clostridiales-Peptococcaceae-Desulfitobacterium
OTU_125		Cyanobacteria-Cyanobacteria-SubsectionIII-FamilyI-Arthronema
OTU_71		Proteobacteria-Alphaproteobacteria-Caulobacterales-Caulobacteraceae-Brevundimonas
OTU_2025		Bacteroidetes-Bacteroidetes_vadinHA17-Bacteroidetes_vadinHA17_un
OTU_122		Cyanobacteria-Cyanobacteria-SubsectionIII-FamilyI-Leptolyngbya
OTU_76		Proteobacteria-Betaproteobacteria-Hydrogenophilales-Hydrogenophilaceae-Sulfuricella
OTU_42		Firmicutes-Clostridia-Clostridiales-Gracilibacteraceae-Lutispora
OTU_59		Proteobacteria-Betaproteobacteria-Burkholderiales-Comamonadaceae-Polaromonas
OTU_43		Firmicutes-Negativicutes-Selenomonadales-Veillonellaceae-Pelosinus
OTU_88		Cyanobacteria-Cyanobacteria-SubsectionIII-FamilyI-Leptolyngbya
OTU_304		Firmicutes-Clostridia-Clostridiales-Clostridiaceae_1-Clostridium_sensu_stricto_1
OTU_77		Proteobacteria-Gammaproteobacteria-Xanthomonadales-Xanthomonadaceae-Thermomonas
OTU_63		Firmicutes-Clostridia-Clostridiales-Peptococcaceae-Desulfosporosinus
OTU_134		Firmicutes-Bacilli-Bacillales-Bacillaceae-Bacillus
OTU_193		Actinobacteria-MB-A2-108-MB-A2-108_un
OTU_103		Nitrospirae-Nitrospira-Nitrospirales-Nitrospiraceae-Nitrospiraceae_un
OTU_143	*	Bacteroidetes-Sphingobacteriia-Sphingobacteriales-Chitinophagaceae-Chitinophagaceae_un
OTU_112		Bacteroidetes-Flavobacteriia-Flavobacteriales-Flavobacteriaceae-Flavobacterium
OTU_1311		Tenericutes-Mollicutes-Mollicutes_RF9-Mollicutes_RF9_un

Fig. 4. Heatmap illuminating the relative abundance of the selected 37 bacterial OTUs in the soils during rice season from winter flooding management (WF), winter drainage management (WD) and plastic film mulching rice cultivation (MC); p-values of the ANOVA: $^*p < 0.05$, $^{**}p < 0.01$.

(Said-Pullicino et al., 2016; Xu et al., 2020).

In the present study, two aspects simultaneously contributed to the lower CH_4 emission in WD and MC plots during rice season. One direct reason was probably that small amounts of substrates due to enhanced aerobic decomposition during the precedent winter drainage, confirmed by the results that the acetate concentration was considerably lower in MC than that of WF treatments in the present study (Table 1). Soil moisture in winter significantly affects the decomposition rate of labile organic materials which are returned or left in soils, and the content of labile organic materials before rice transplanting decreased with decreasing soil moisture from flooded condition to 25% WHC during winter season (Kudo et al., 2017; Xu et al., 2003). Indeed, significant correlation were found between CH_4 emission and soil DOC content in this study, indicating that the application of WD and MC mitigated CH_4 emission and production by soil Eh enhancement and soil DOC decrease under the field condition. Another reason may be the higher Eh during rice season which is not suitable for CH_4 production. When a permanently flooded rice field is drained in the fallow season, its soil is exposed directly to air, leading to an increase in soil Eh, which in turn affects the activity of methanogens and inhibits CH_4 production (Zhang et al., 2020). Moreover, drainage improves soil aeration and increased the production of oxidants, such as Fe^{3+} or sulphate, along with the increase in soil Eh (Kruger et al., 2001). As a result, the growth and activity of methanogens was probably out-competed by iron- or sulphate-reducing bacteria. In the present study, the relative abundance of iron-reducing bacteria *Geobacter* (OTU13) and *Pseudomonas* (OTU56) in WD and MC treatments significantly differed from that of WF treament (Fig. 4), probably indicating the higher production of oxidants in WD treatment due to the lower water content during winter fallow season. Therefore, drainage in the winter fallow season significantly increased soil Eh and obviously decreased methanogenesis in paddy soil compared to flooding, and hence depressed the CH_4 emission and production during rice season.

However, WD did not affect the relative contribution of methanogenic pathways (hydrogenotrophic/acetoclastic methanogenesis) to total CH_4 production in the following rice season based on the lab incubation. This is concluded from the relative inhibition of acetoclastic methanogenesis by CH_3F. Hydrogenotrophic methanogenesis contributed about 30% to the total CH_4 production, which is theoretically expected when polysaccharides serve as the main substrate (Conrad, 2020). By contrast, MC cultivation increased the relative contribution of hydrogenotrophic methanogenesis to about 50% of the total production. Since total CH_4 production was lowest in MC, we assume that the availability of organic matter was the rate-limiting step. Under these conditions, the contribution of hydrogenotrophic methanogenesis increases in relative to that of acetoclastic methanogenesis (Ji et al., 2018). Also, acetate accumulated to lower concentrations in MC than in WD when acetoclastic methanogenesis was inhibited, indicating a decreased contribution of this methanogenic pathway.

WD and MC also affected the isotopic composition of the produced acetate, with the $\delta^{13}C$ of acetate becoming increasingly larger compared to WF, indicating a decreased contribution of chemolithotrophic

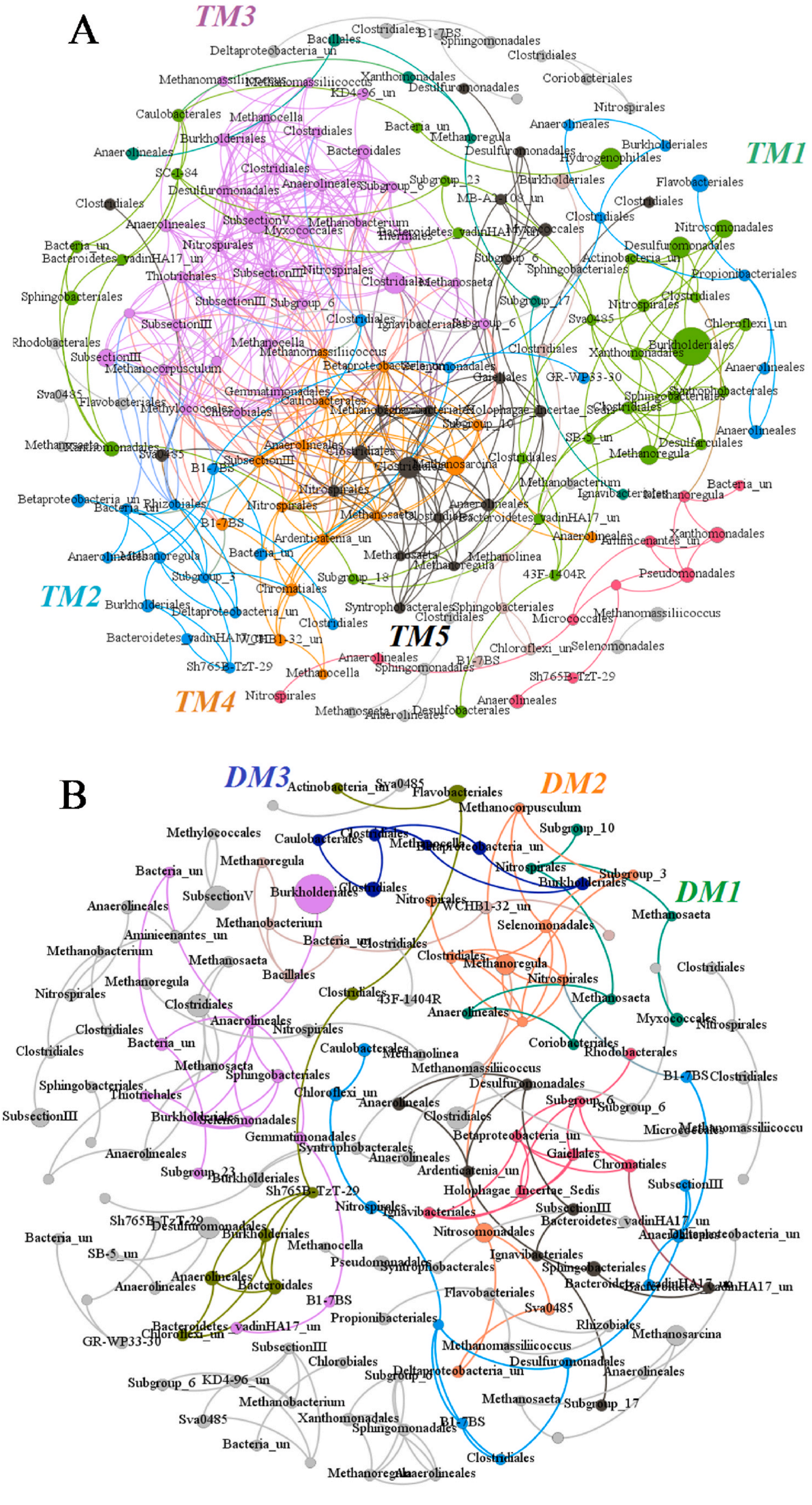


Fig. 5. Correlation networks based on the OTUs of *mcrA* and 16S rRNA genes of the samples with traditional rice cultivation practice (WF and WD, A) and those with winter drainage and MC cultivation (WD and MC, B). A node indicates one OTU. A connection represents a significant (Spearman's $0.70 \leq \rho \leq 0.90$, FDR corrected p-value$\leq$0.01) correlation. The size of all nodes is proportionate to the connections number. Nodes were specified through different modules. The phylogenetic affiliation of the nodes which down to the genus level is demonstrated by the label. Abbreviations: un_ for the unclassified. Names of the methanogenesis fermentation modules are illustrated.

acetogenesis to total acetate production (Fu et al., 2018). The acetate in the addition of CH_3F was possibly because of the formation of microbial acetate by fermentation or chemolithotrophic acetogenesis, while the remaining acetate following the consumption of microbial acetate was shown in the absence of CH_3F, probably by acetoclastic methanogens. In the addition of CH_3F, the $\delta^{13}C$ of acetate should only be affected by the production pathway. Since acetate produced from H_2/CO_2 (chemolithotrophic acetogenesis) is strongly depleted in ^{13}C (Fu et al., 2018), while that produced from organic carbon (fermentative acetate production) is only little depleted (Conrad, 2020), WD and MC treatments may have enhanced the relative contribution of fermentative acetate production.

4.2. Effect of winter drainage and film mulching cultivation on methanogenic archaeal community in paddy soil

In paddy soils, the methanogenic microbial communities have been found to change only moderately upon seasonal drainage but more substantially after long-term upland conditions (Breidenbach et al., 2016; Tariq et al., 2018). In this study, drainage, in comparison to flooding, in the winter fallow season, decreased the number of methanogenic archaea. Therefore, the suppressed CH_4 emissions were probably attributed to the decreasing numbers and activities of methanogens (Oliveira et al., 2020; Sacco et al., 2012).

Moreover, WD influenced the composition of methanogenic microbial communities, which significantly increased the abundance of acetoclastic *Methanothrix* and *Methanosarcina*, hydrogenotrophic *Methanobacterium* and *Methanocella*, while that of hydrogenotrophic *Methanomicrobiales* was decreased. We summarized the relative abundance of putative hydrogenotrophic and acetoclastic methanogens according to Fig. 3, showing that WD significantly affected the relative abundance of the above two methanogens. In the controlled treatment, the dominant groups were putative hydrogenotrophic methanogens rather than putative acetoclastic methanogens, while WD increased the relative abundance of putative acetoclastic methanogens, resulting in the balance between the two groups (Table S2). WD significantly increased the relative abundance of hydrogenotrophic *Methanocella* and acetoclastic *Methanosarcina*, confirming that methanogens contain diverse genes encoding for enzymes with detoxification oxygen application, such as *Methanocellaceae* and *Methanosarcinaceae* (Angel et al., 2012) permitting them to retrieve the oxygen following the drainage.

Similar patterns were also found in MC treatment. Previous studies have found that CH_4 production potential in wetlands is mainly affected by the quality and quantity of methanogenic substrates, which, in turn, would affect the methanogenic archaeal structure and the methanogensis pathway (Rooney-Varga et al., 2007; Zhang et al., 2018). In this study, a lower seasonal pattern of soil DOC content was observed in MC treatments than that of WF treatment under field condition (Fig. 1C), indicating that MC induced the functional shift in methanogenic pathways (decreasing contribution of acetoclastic methangenesis and increasing contribution of hydrogentrophic methanogenesis) by changing the DOC quantity, finally resulting in the lower CH_4 production and emission in paddy soil. In contrast, Malyan et al. (2016) and Yao et al. (2017) found that film mulching facilitated rice growth and increased the amount of roots and rhizodeposition available as a source of organic matter for methanogenesis and enhanced CH_4 production. However, the film mulching greatly increased the activity of CH_4-oxidizing bacteria by maintaining aerobic conditions under non-flooded water conditions, which greatly exceeded the increase in CH_4 production (Zhang et al., 2018).

4.3. Effect of winter drainage and film mulching cultivation on bacterial community in paddy soil

The bacterial community is important for anaerobic degradation of organic matter to yield the main methanogenic precursors H_2, CO_2 and acetate (Conrad, 2020). In this study, the community of bacteria exhibited a stronger response to winter water management rather than rice cultivation practice. Similar as the archaeal community structure, the phylum-level composition of the bacterial community also revealed a dynamic pattern affected by winter drainage, increasing the relative abundance of phyla *Gemmatimonadedetes* and *Chloroflexi*, while decreasing that of phylum *Proteobacteria*.

A few OTUs on lower taxonomic levels were also observed to change with water management in winter. Thus, OTU5 (affiliated to the genus *Massilia*), the most abundant OTU, significantly decreased after drainage in winter. *Massilia* had been detected in the rhizosphere and roots of many plant species in recent years (Ofek et al., 2012). Li et al. (2014) reported that high proliferation rates might be attributed to the dominance of *Massilia* when adequate energy and carbon sources are available. Therefore, the loss of dominance of *Massilia* in this study may be ascribed to the decrease in quality and quantity of organic carbon in soils during the conversion of flooding to drainage in the winter fallow season.

Another example was the higher relative abundance of OTU13 (affiliated to the genera *Geobacter*). *Geobacter* was found to be the agent for coupling the oxidation of organic matter to the reduction of insoluble Mn(IV) and Fe(III) oxides in various sediments and soils (Lovley et al., 2011). Kusel et al. (2008) reported that temporal soil drying in methanogenic peatlands could divert the flow of reductants from CH_4 formation to other electron-accepting process by alternating the electron acceptor, espcecially changing the Fe (III)-reducing microbial activities, which was consistent with the results in this study.

4.4. Networks of fermenting bacterial and CH_4-producing archaeal communities

Two correlation-based co-occurrence networks were constructed based on the OTUs of bacterial 16S rRNA and archaeal *mcrA* genes to reveal the interactions between the fermenting bacterial and methanogenic archaeal communities in the present study. Both of the two networks were well organized by functional modules, including hydrogenotrophic methanogenic modules, acetoclastic methanogenic modules and hydrogenotrophic plus acetoclastic methanogenic modules, indicating strong interactions between the fermenting bacterial and methanogenic archaeal communities. Methanogenesis is the last metabolic procedure in CH_4 production. Acetoclastic *Methanothrix* and hydrogenotrophic *Metharegula, Methanocella* were the most frequently dominated methanogens in the functional modules, and tended to co-occur with fermenting bacterial order *Clostridiales*, *Nitrospirales* as well as *Anaerolineales*, which probably participate in fermentation and hydrolysis of SOM and finally produce the methanogenic substrates (formate, H_2/CO_2, and acetate).

Nitrospira, which contains some nitrite oxidizers, is capable to utilize pyruvate, hydrogen, and lactate as electron donors (Cheng et al., 2013). In the present study, *Nitrospira*-related OTUs frequently co-occurred with hydrogenotrophic methanogens, which is consistent with previous studies showing that some species of *Nitrospira* could grow in co-culture with hydrogenotrophic methanogens as syntrophic partners (Roest et al., 2005).

High functional diversity and redundancy are critical for sustaining ecosystem function, and analyses of community functional diversity changes in response to environmental change have the potential to connect species loss to shifts in ecosystem function (Fetzer et al., 2015). Notably, we found that the mixed methanogenic fermentation modules were all unique for the network from the samples with traditional rice cultivation practice (WF and WD), characterized by *Methanothrix*, *Metharegula* and *Methanocella* as the key acetoclastic methanogens, while the methanogenic fermentation modules in the network from those with winter drainage and MC technology were either acetoclastic or hydrogenotrophic modules. The loss of the mixed methanogenic fermentation modules indicated that the functional biodiversity and redundancy apparently decreased after winter drainage and MC technology during rice growth. These results suggest that the changes in species composition resulting from winter drainage and MC technology can have significant impacts on the loss of functional diversity and redundancy, and thus on ecosystem function of CH_4 production (Teixidó et al., 2018).

5. Conclusion

Consistent with our hypothesis, our field observation shows that shifting the fields from flooding during winter fallow and rice seasons into winter drainage and MC cultivation substantially mitigated CH_4 emissions in paddy soils of Central Sichuan hilly areas, paralleled with the reductions of soil DOC content and enhancement of soil Eh values. The anaerobic incubation in the absence and the presence of CH_3F showed that, winter drainage significant affects the functions and structures of fermenting bacterial and methanogenic archaeal communities in the following rice season, while plastic film mulching only showed an additional effect on those of methanogenic archaeal communities to a less content. Plastic film mulching increased the relative contribution of hydrogenotrophic methanogenesis to total CH_4 production, perhaps since organic matter decomposition became the rate-limiting step. Network analysis based on *mcrA* and 16S rRNA genes showed that winter drainage and plastic film mulching induced the loss of mixed methanogenic fermentation modules, indicating the decrease in functional biodiversity and redundancy of fermenting bacterial and methanogenic archaeal communities. Considering that water stress limits crop production and food security, our findings suggest that winter drainage and plastic film mulching represent the effective management strategies to mitigate CH_4 emission by regulating the function and structure of methanogenic archaeal and fermenting bacterial communities in paddy field.

Credit author statement

Yang Ji: Conceptualization; Resources; Investigation; Visualization; Methodology; Funding acquisition; Writing – original draft and Editing. Yongji Xu: Investigation; Visualization; Methodology; Writing-Reviewing and Editing. Mengying Zhao: Investigation; Visualization; Methodology; Writing-Reviewing and Editing. Guangbin Zhang: Conceptualization; Resources; Writing-Reviewing and Editing; Supervision. Ralf Conrad: Conceptualization; Resources; Writing-Reviewing and Editing. Pengfei Liu: Investigation; Methodology; Writing-Reviewing and Editing. Zhaozhong Feng: Writing-Reviewing and Editing. Jing Ma: Conceptualization; Writing-Reviewing and Editing; Hua Xu: Conceptualization; Writing-Reviewing and 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.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

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References

Angel, R., Claus, P., Conrad, R., 2012. Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. ISME J. 6, 847–862.

Blondel, V.D., Guillaume, J.L., Lambiotte, R., Lefebvre, E., 2008. Fast unfolding of communities in large networks. J. Statistical Mechanics P10008.

Breidenbach, B., Blaser, M.B., Klose, M., Conrad, R., 2016. Crop rotation of flooded rice with upland maize impacts the resident and active methanogenic microbial community. Environ. Microbiol. 18, 2868–2885.

Bridgham, S.D., Cadillo-Quiroz, H., Keller, J.K., Zhuang, Q., 2013. Methane emissions from wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales. Global Change Biol. 19, 1325–1346.

Cheng, L., He, Q., Ding, C., Dai, L.R., Li, Q., Zhang, H., 2013. Novel bacterial groups dominate in a thermophilic methanogenic hexadecane-degrading consortium. FEMS Microbiol. Ecol. 85, 568–577.

Conrad, R., 2020. Methane production in soil environments-anaerobic biogeochemistry and microbial life between flooding and desiccation. Microorganisms 8 (6), 881.

Conrad, R., Klose, M., Lu, Y., Chidthaisong, A., 2012. Methanogenic pathway and archaeal communities in three different anoxic soils amended with rice straw and maize straw. Front. Microbiol. 3, 4.

Conrad, R., Klose, M., Noll, M., 2009. Functional and structural response of the methanogenic microbial community in rice field soil to temperature change. Environ. Microbiol. 11, 1844–1853.

Deng, O.P., Li, X., Deng, L.J., Zhang, S.R., Gao, X.S., Lan, T., Zhou, W., Tian, D., Xiao, Y. L., Yang, J., Ou, D.H., Luo, L., 2020. Emission of CO_2 and CH_4 from a multi-ditches system in rice cultivation region: flux, temporal-spatial variation and effect factors. J. Environ. Manag. 270, 110918.

Fetzer, I., Johst, K., Schawe, R., Banitz, T., Chatzinotas, A., 2015. The extent of functional redundancy changes as species' roles shift in different environments. Proc. Natl. Acad. Sci. USA 112, 14888–14893.

Fu, B., Conrad, R., Blaser, M., 2018. Potential contribution of acetogenesis to anaerobic degradation in methanogenic rice field soils. Soil Biol. Biochem. 119, 1–10.

Herbold, C.W., Pelikan, C., Kuzyk, O., Hausmann, B., Angel, R., Berry, D., Loy, A., 2015. A flexible and economical barcoding approach for highly multiplexed amplicon sequencing of diverse target genes. Front. Microbiol. 6, 731.

Janssen, P.H., Frenzel, P., 1997. Inhibition of methanogenesis by methyl fluoride- studies of pure and defined mixed cultures of anaerobic bacteria and archaea. Appl. Environ. Microbiol. 63, 4552–4557.

Ji, Y., Liu, P.F., Conrad, R., 2018. Response of fermenting bacterial and methanogenic archaeal communities in paddy soil to progressing rice straw degradation. Soil Biol. Biochem. 124, 70–80.

Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved, organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem. 38, 991–999.

Kim, G.W., Das, S., Hwang, H.Y., Kim, P.J., 2017. Nitrous oxide emissions from soilsn amended by cover-crops and under plastic film mulching: fluxes, emission factors and yield-scaled emissions. Atmos. Environ. 152, 377–388.

Kreye, C., Dittert, K., Zheng, X.H., Zhang, X., Lin, S., Tao, H.B., Sattelmacher, B., 2007. Fluxes of methane and nitrous oxide in water-saving rice production in North China. Nutrient Cycl. Agroecosyst. 77, 293–304.

Kruger, M., Frenzel, P., Conrad, R., 2001. Microbial processes influencing methane emission from rice fields. Global Change Biol. 7, 49–63.

Kudo, Y., Noborio, K., Shimoozono, N., Kurihara, R., Minami, H., 2017. Greenhouse gases emission from paddy soil during the fallow season with and without winter flooding in central Japan. Paddy Water Environ. 15, 217–220.

Kusel, K., Blothe, M., Schulz, D., Reiche, M., Drake, H.L., 2008. Microbial reduction of iron and porewater biogeochemistry in acidic peatlands. Biogeosciences 5, 1537–1549.

Li, X.Z., Rui, J.P., Mao, Y.J., Yannarell, A., Mackie, R., 2014. Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. Soil Biol. Biochem. 68 (1), 392–401.

Lovley, D.R., Ueki, T., Zhang, T., Malvankar, N.S., Shrestha, P.M., Flanagan, K.A., Aklujkar, M., Butler, J.E., Giloteaux, L., Rotaru, A., Holmes, D.E., Franks, A.E., Orellana, R., Risso, C., Nevin, K.P., 2011. Geobacter: the microbe electric's physiology, ecology, and practical applications. Adv. Microb. Physiol. 59, 1–100.

Mendiburu, F., 2015. Agricolae: Statistical Procedures for Agricultural Research ver.1.2-1. http://cran.fyxm.net/web/packages/agricolae/.

Malyan, S.K., Bhatia, A., Kumar, A., Gupta, D.K., Singh, R., Kumar, S.S., Tomer, R., Kumar, O., Jain, N., 2016. Methane production, oxidation and mitigation: a mechanistic understanding and comprehensive evaluation of influencing factors. Sci. Total Environ. 572, 874–896.

Negri, C., Chiaradia, E., Rienzner, M., Mayer, A., Facchi, A., 2020. On the effects of winter flooding on the hydrological balance of rice areas in Northern Italy. J. Hydrol. 590, 125401.

Ofek, M., Hadar, Y., Minz, D., 2012. Ecology of root colonizing *Massilia* (oxalobacteraceae). PLoS One 7, e40117.

Oksanen, J., Blanchet, G.F., Kindt, R., Legendre, R., Minchin, P.R., ÓHara, R.B., 2015. vegan: community ecology package ver.2.3-0. http://cran.r-project.org/web/packages/vegan/index.html.

Oliveira, B.G., Mendes, L.W., Smyth, E.M., Tsai, S.M., Feigl, B.J., Mackie, R.I., 2020. Assessment of microbial diversity associated with CH_4 emission from sugarcane vinasse storage and transportation systems. J. Environ. Manag. 269, 110748.

Qin, W., Hu, C., Oenema, O., 2015. Soil mulching significantly enhances yields and water and nitrogen use efficiencies of maize and wheat: a meta-analysis. Sci. Rep. 5, 16210.

Rooney-Varga, J.N., Giewat, M.W., Duddleston, K.N., Chanton, J.P., Hines, M.E., 2007. Links between archaeal community structure, vegetation type and methanogenic pathway in Alaskan peatlands. FEMS Microbiol. Ecol. 60, 240–251.

Roest, K., Altinbas, M., Paulo, P., Heilig, H., Akkermans, A., Smidt, H., de Vos, W., Stams, A., 2005. Enrichment and detection of microorganisms involved in direct and indirect methanogenesis from methanol in an anaerobic thermophilic bioreactor. Microb. Ecol. 50, 440–446.

Sacco, D., Cremon, C., Zavattaro, L., Grignani, C., 2012. Seasonal variation of soil physical properties under different water managements in irrigated rice. Soil Tillage Res. 118, 22–31.

Said-Pullicino, D., Miniotti, E.F., Sodano, M., Bertora, C., Lerda, C., Chiaradia, E.A., Romani, M., Maria, S.C.D., Sacco, D., Celi, L., 2016. Linking dissolved organic carbon cycling to organic carbon fluxes in rice paddies under different water management practices. Plant Soil 401, 273–290.

Tariq, A., Jensen, L.S., Sander, B.O., de Tourdonnet, S., Ambus, P.L., Thanh, P.H., Trinh, M.V., de Neergaard, A., 2018. Paddy soil drainage influences residue carbon contribution to methane emissions. J. Environ. Manag. 225, 168–176.

Teixidó, N., Gambi, M.C., Parravacini, V., Kroeker, K., Micheli, F., Villéger, S., Ballesteros, E., 2018. Functional biodiversity loss along natural CO_2 gradients. Nat. Commun. 9, 5149.

Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis. Springer, Dordrecht, The Netherlands.

Williams, R.J., Howe, A., Hofmockel, K.S., 2014. Demonstrating microbial co-occurrence pattern analyses within and between ecosystems. Front. Microbiol. 5, 358.

Wu, X.H., Wang, W., Xie, K.J., Yin, C.M., Hou, H.J., Xie, X.L., 2019. Combined effects of straw and water management on CH_4 emissions from rice fields. J. Environ. Manag. 231, 1257–1262.

Xu, H., Cai, Z.C., Tsuruta, H., 2003. Soil moisture between rice-growing seasons affects methane emission, production, and oxidation. Soil Sci. Soc. Am. J. 67 (4), 1147–1157.

Xu, X.Y., Zhang, M.M., Xiong, Y.S., Yuan, J.F., Shaaban, M., Zhou, W., Hu, R.G., 2020. The influence of soil temperature, methanogens and methanotrophs on methane emissions from cold waterlogged paddy fields. J. Environ. Manag. 264, 110421.

Yao, Z., Zheng, X., Liu, C., Lin, S., Zuo, Q., Butterbach-Bahl, K., 2017. Improving rice production sustainability by reducing water demand and greenhouse gas emissions with biodegradable films. Sci. Rep. 7, 39855.

Yu, Y.X., Zhang, Y.X., Xiao, M., Zhao, C.Y., Yao, H.Y., 2021. A meta-analysis of film mulching cultivation effects on soil organic carbon and soil greenhouse gas fluxes. Catena 206, 105483.

Zhang, G.B., Ma, J., Yang, Y.T., Yu, H.Y., Song, K.F., Dong, Y.J., Lv, S.H., Xu, H., 2018. Achieving low methane and nitrous oxide emissions with high economic incomes in a rice-based cropping system. Agric. For. Meteorol. 259, 95–106.

Zhang, G.B., Yang, Y.T., Huang, Q., Ma, J., Yu, H.Y., Song, K.F., Dong, Y.J., Lv, S.H., Xu, H., 2020. Reducing yield-scaled global warming potential and water use by rice plastic film mulching in a winter flooded paddy field. Eur. J. Agron. 114, 126007.