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ORIGINAL PAPER

Responses of archaeal, bacterial, and functional microbial communities to growth season and nitrogen fertilization in rice fields

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Abstract

Fertilization provides excess N to soil microorganisms, thus possibly affecting soil microbial abundance, activity, and community composition during rice cultivation. The abundance and diversity of archaeal, bacterial, and functional microbial communities in rice soils upon different N fertilization regimes (no fertilizer, urea, and controlled-release fertilizer) were investigated by sampling four seasonal growth stages (seedling, tillering, vegetative, maturing) under field conditions. The abundance of bacteria was significantly affected by fertilization and seasonal time, while that of the archaea was not significantly affected. Analysis of terminal restriction fragment polymorphism (T-RFLP) of 16S rRNA genes showed no effect of N fertilization on the archaeal and bacterial community composition, but changes with plant growth time. This result was confirmed by the patterns of pyrosequencing of bacterial 16S rRNA genes. The function of the methanogenic microbial community was assayed at maturing plant growth stage by determining CH_4 production rates and stable isotope fractionation in the absence and presence of methyl fluoride, an inhibitor of acetoclastic methanogenesis. N fertilization had a pronounced effect on the CH_4 production rate but not on the pathway of CH_4 formation. Additionally, the abundance of functional microbial communities related to CH_4 and N_2O emissions was measured by qPCR of functional genes. Similarly to the taxonomic composition, rice growth season showed a significant effect on the abundance of the functional microbial communities represented by the *mcrA*, *pmoA*, *nirK*, *nirS*, and *nosZ* genes, while N addition had usually no significant effect. A similar result was also obtained by correlation analysis between CH_4 and N_2O emission rates and abundances of the functional microbial gene copies. In summary, rice growth time had pronounced effects on abundance, composition, and function of microbial communities in the rice soil, while the effect of N fertilization was negligible on the level of both specific functional genes and taxonomic 16S rRNA genes.

Keywords Archaea · Bacteria · Functional gene · N fertilization · Growth season · Methanogenesis · Nitrous oxide

Introduction

Irrigated rice fields represent an important anthropogenic biological source of atmospheric CH_4, accounting for ca. 5% of the global CH_4 emission (IPCC 2014). Of the global anthropogenic N_2O emission, approximately 65% is attributed to agricultural soils (Beauchamp 1997; Mosier and Kroese 2000). Irrigated rice fields have the potential to emit both CH_4 and N_2O, but the magnitude of these emissions depends on agricultural management (Cai et al. 1997). Linquist et al. (2012) estimated that the combined emission of CH_4 and N_2O in rice production systems is approximately four times higher than that of either upland wheat or maize systems.

Application of N fertilizers in rice cultivation has been commonly adopted to improve N availability and achieve

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high grain yields (Cassman et al. 1998; Dong et al. 2011). It provides excess N to soil microorganisms, thus affecting soil microbial abundance, activity, and diversity (Chen et al. 2012; Ge et al. 2017; Islam et al. 2011; Marschner et al. 2003; Nemergut et al. 2008; Tao et al. 2018; Yin et al. 2015). Production of CH_4 and N_2O is biological processes, which are affected directly or indirectly by N in the soil (Schimel 2000). Many studies have been conducted to understand the effects of N fertilizer applications on the soil bacterial communities in rice soils (Lüdemann et al. 2000; Lu et al. 2006; Kikuchi et al. 2007; Watanabe et al. 2010; Wu et al. 2011, 2018). Although these studies have provided useful information about the overall changes caused by N fertilization, the techniques used for bacterial community analyses (16S rRNA gene clone library analysis and fingerprinting methods) are thought to be insufficient for assessing the full extent of soil bacterial diversity (Bartram et al. 2011; Roesch et al. 2007) and for providing detailed information about individual bacterial groups. Concerning archaeal communities in rice soil, studies mostly focused on specific functional groups such as methanogens (Conrad et al. 2009; Lueders and Friedrich 2000; Wang et al. 2010) or nitrifiers (Wu et al. 2009).

Recently, we reported that CH_4 and N_2O emissions from rice fields were influenced by different N fertilization (Ji et al. 2013, 2014). Therefore, we hypothesized that soil bacterial and archaeal communities in rice soil will also be influenced by different N fertilization. Since a comprehensive seasonal record of microorganisms was lacking, we investigated the soil archaeal and bacterial communities under field conditions by sampling four distinct seasonal growth stages (seedling, tillering, vegetative, maturing). In addition, we also investigated an upland wheat field at maturing stage. The microbial composition and abundance were assessed by fingerprinting with terminal-restriction fragment length polymorphism (T-RFLP) and quantitative PCR (qPCR) targeting the archaeal and bacterial 16S rRNA genes. In order to identify changes in the lower taxonomic groups, the bacterial 16S rRNA genes were deeply sequenced by 454 pyrosequencing. Finally, specific functional groups of microorganisms were analyzed, which are involved in CH_4 and N_2O emission. Function of methanogens was analyzed by carbon isotope fractionation during CH_4 production and by qPCR of the *mcrA* gene coding for the methyl CoM reductase. Methanotrophs were analyzed by qPCR of the *pmoA* gene coding for the particulate methane monooxygenease; bacterial (AOB) and archaeal (AOA) nitrifiers by qPCR of the bacterial and archaeal *amoA* genes coding for the ammonia monooxygenease; and denitrifiers by qPCR of the *nirS*, *nirK*, and *nosZ* genes coding for the two types of nitrite reductases and the N_2O reductase, respectively.

Material and methods

Experimental design and field sampling

Soil samples were taken from the experimental field station located at Baitu Town, Jurong City, Jiangsu Province, China (31°58′N, 119°18′E). The soil of the experimental paddy field is classified as Typic Haplaquepts (Soil Survey Staff 1975). Detailed site description can be found in Ji et al. (2013, 2014). The experiment followed a cropping rotation system of one single-cropping rice in summer and one upland crop (usually wheat) in winter.

Detailed description of rice cultivation can be found in Ji et al. (2013, 2014). Intermittent irrigation was adopted in the experiment as the water management practice. The paddy field in this study was continuously flooded for about 1 month after rice transplanting (3 Jul~28 Jul), exposed to aeration for 10~14 days (29 Jul~13 Aug, midseason aeration, MSA), reflooded after MSA (14 Aug~25 Aug), subjected to intermittent irrigation before the crop was mature (26 Aug~7 Oct), and then kept dry for 1 month before rice harvesting (7 Oct~4 Nov).

Three treatments were adopted in this experiment, i.e., treatment CK (no N fertilizer), treatment U (conventional N fertilizer-urea), and treatment CRF (controlled-release fertilizer, CRF). Treatments were laid out in a split-plot, randomized complete block design with triplicates. The plot area was 180 m2 (15 m by 12 m). For treatment U, urea was applied at 240 kg N ha^{-1} for three times, 50% as basal fertilizer, 25% as tilling fertilizer, and 25% as panicle initiating fertilizer, and for treatment CRF, CRF was applied at 240 kg N ha^{-1} only once as basal. The CRF used in this experiment is thermoplastic resin-coated urea, a product of the Shandong Kingenta Ecological Engineering CO., LTD, China, containing 4% coating material, 42% N. P-fertilizer [$Ca(H_2PO_4)_2$] and K-fertilizer [KCl] were both applied as basal together with N fertilizer at the rate of 450 kg ha^{-1} and 225 kg ha^{-1}, respectively.

Briefly, soil samples were taken at the seedling (23 Jul), tillering (3 Aug), vegetative (11 Aug), and maturing (4 Nov) growth stage of the rice plants (variety: Zhendao 624) from the three different treatments (CK, U, CRF) described above. Additionally, soil samples were also taken from an upland wheat field at maturing stage before the beginning of the rice growing season. The wheat field had been flooded and cultivated with rice in the previous season. About 100 g soil was taken from the upper 10-cm soil layer. This was done by taking six cores from each of the three replicate locations. Soil samples were kept on ice and later stored at −20 °C for the following further analysis.

Molecular analyses

DNA was extracted from 0.5 g of soil using the NucleoSpin® Soil Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions (Vestergaard et al. 2017). The extracted DNA was used for qPCR, T-RFLP, and pyrosequencing. The abundance of bacterial and archaeal 16S rRNA gene copies was determined by qPCR done using an iCycler instrument (CFX ConnectTM, Bio-Rad) and the primers listed in Table 1. The reaction solution in a total volume of 25 μl contained 12.5 μl of SybrGreen Jumpstart TaqReadyMix (Sigma), 0.25 μM concentrations of the primers, 0.5 μl of bovine serum albumin (Sigma), and 5 μl of 50-fold or 1000-fold diluted DNA for gene copy quantification. Clone libraries were constructed and DNA standards were prepared as described earlier (Ma et al. 2012).

The analysis of T-RFLP of 16S rRNA genes was done as described using the primer combinations 109f/915r for Archaea (Grosskopf et al. 1998) with the reverse primer labeled with FAM (6-carboxyfluorescein) and 27f/907r for Bacteria (Lane 1991; Weisburg et al. 1991) with the forward primer labeled with FAM. The 16S rRNA gene amplicons were digested with *Taq*I and *Msp*I for Archaea and Bacteria, respectively, and the products were size-separated in an ABI 3130 DNA sequencer (Applied Biosystems, Darmstadt, Germany). Normalization and standardization of the T-RFLP profiles were done according to the method from Dunbar et al. (2001). The relative abundance was calculated from the ratio between the height of the fluorescence signal and the total height of all signals in one sample.

For tagged pyrosequencing of bacterial 16S rRNA gene fragments, we used the primers F515 and R806 described by Bates et al. (2011). Each primer contained a unique 8-pb barcode. The PCR products from replicates of each soil were pooled and sequenced at the Max Planck Genome Centre in Cologne using a Roche 454 Genome Sequencer GS FLX+ (Scholer et al. 2017). All analyses described in the current study were performed within version 1.22 of the mothur software package (http://www.mothur.org/) (Schloss et al. 2009). The pyrosequencing-based analysis resulted in recovery of about 10,000 high-quality sequences with a minimum read length of 200 bp across all samples. The raw sequences were sorted by primer sequence using appropriate commands. Those that did not match the primer sequences, were less than 200 bp long, or contained any ambiguities were excluded from further analysis. For phylotype analyses, the remaining sequences were denoised (Schloss et al. 2011) and aligned against the SILVA bacterial 16S rRNA gene database (Release 115) (Pruesse et al. 2007) in mothur. A distance matrix was constructed and processed in mothur to define bacterial operational taxonomic units (OTU) with 3% dissimilarity employing the SOP pyrosequencing pipeline (Schloss et al. 2011). Bacterial OTUs which were in total less than 10 reads across all samples or were detected in < 20% of the samples were filtered out (Berry and Widder 2014). Sequence data were deposited under the study accession number SRP1589784 for bacterial sequences in the NCBI Sequence Read Archive (SRA).

To evaluate the effects of N fertilization and season on the functional microbial communities in rice soil, we also analyzed the abundance of specific functional groups of microorganisms involved in CH_4 and N_2O emission via qPCR, including methanogenic functional gene *mcrA*, methanotrophic functional gene *pmoA*, nitrifier functional gene *amoA*, denitrifier functional genes *nirS*, *nirK*, and *nosZ*. The qPCR conditions and the primers are described in the references given in Table 1. The concentrations of the DNA standards ranged from 1.0×10^2 to 1.0×10^7 copies μl^{-1}. Each measurement was performed in duplicate.

Incubation experiments

Soil samples taken from the maturing phase before rice harvesting (4 Nov) were used for the incubation experiments to determine the functional responses of methanogenic microbial communities to N fertilizer application (CH_4 production pathways and rate) via stable isotopic analysis. The incubation procedure was the following. 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 closed with black

Table 1 Primers and references used in qPCR

Targeted gene	Forward primer	Reverse primer	Reference
Archaeal 16S rRNA	364f	934b	Burggraf et al. (1997), Grosskopf et al. (1998)
Bacterial 16S rRNA	519f	907r	Lane (1991)
mcrA	mlas-mod	mcrA-rev	Angel et al. (2011), Angel et al. (2012)
pmoA	A189F	mb661R	Costello and Lidstrom (1999)
amoA	amoA-1F	amoA-2R	Jia and Conrad (2009)
nirs	qCd3af	qR3cd	Kandeler et al. (2006)
nirk	qnirK876	qnirK1040	Henry et al. (2004)
nosZ	nosZ2F	nosZ2R	Henry et al. (2006)

rubber stoppers, flushed with N2, pressurized to 0.5 bar overpressure, and incubated until CH_4 production was constant. Inhibition of acetoclastic methanogenesis was achieved by the addition of 3% methyl fluoride (Janssen and Frenzel 1997). At regular time intervals, the headspace was analyzed for CH_4, CO_2, H_2 and for the $\delta^{13}C$ of CH_4 and CO_2 during the course of incubation. At the end of all incubations, the tubes were opened, and the liquid was analyzed for pH, acetate, and $\delta^{13}C$ of acetate and the methyl group.

The chemical analysis of gas and liquid samples was done as described before (Conrad 2009; Conrad et al. 2014). Briefly, CH_4 and CO_2 were analyzed by gas chromatography (GC), acetate by high-pressure liquid chromatography (HPLC), and the $\delta^{13}C$ by either GC combustion isotope ratio mass spectrometry (GC-C-IRMS) or HPLC-C-IRMS. The $\delta^{13}C$ of the methyl group of acetate was determined after off-line pyrolysis. The $\delta^{13}C$ of organic matter was analyzed by the Centre for Stable Isotope Research and Analysis (KOSI) at the University of Göttingen using an elemental analyzer coupled to an IRMS. Values of $\delta^{13}C$ are given in permil relative to Vienna Pee Dee Belemnite standard. The fraction (f_{H2}) of CH_4 production by hydrogenotrophic methanogenesis was calculated by mass balance as described before (Conrad et al. 2010) using

$$f_{H2} = (\delta^{13}C_{CH4} - \delta^{13}C_{CH4}\text{–ma}) / (\delta^{13}C_{CH4}\text{–mc} - \delta^{13}C_{CH4}\text{–ma}) \quad (1)$$

with $\delta^{13}C_{CH4} = \delta^{13}C$ of total CH_4 produced, $\delta^{13}C_{CH4}$-mc = $\delta^{13}C$ of CH_4 produced from hydrogenotrophic methanogenesis, which is equivalent to the CH_4 produced in the presence of CH_3F, and $\delta^{13}C_{CH4}$-ma = $\delta^{13}C$ of CH_4 produced from acetotrophic methanogenesis. The $\delta^{13}C_{CH4}$-ma was assumed to be equal to $\delta^{13}C_{ac\text{-}methyl}$ if there is no fractionation during the reduction of acetate-methyl to CH_4. The $\delta^{13}C$ of total acetate was measured at the end of the incubation in the presence and absence of CH3F, that of acetate-methyl only in the presence of CH_3F.

Statistical analyses

For qPCR data, gene copy numbers were log-transformed to satisfy the normality assumptions, and the analysis of variance was performed to test significant differences between treatments using the Tukey's honestly significant difference post hoc test (Tukey HSD) within package agricolae version 1.2-1 (Mendiburu 2015) in R version 3.2.1 (R Development Core Team 2011). A two-way ANOVA was used to test the effect of growth time, of fertilization, and of fertilization*time on the gene copy numbers. A Pearson correlation analysis was done between CH_4 and N_2O emission rates and the copy numbers of 16S rRNA genes and functional genes along the rice growth using SPSS (version 18.0) for Windows (SPSS Inc., USA). Permutational multivariate analysis of variance (perMANOVA) was performed based on Bray-Curtis dissimilarities using package vegan version 2.2-1 'adonis' function (Oksanen et al. 2015) to determine which variables were important for cluster formation of archaeal and bacterial T-RFs.

A heatmap representation of the relative abundance of OTUs between samples was constructed using R. A Hellinger transformation of the OTU counts was performed using the decostand function in the vegan package version 2.2-1 (Oksanen et al. 2015). Principal component analysis (PCA) was performed using prcomp to select the OTUs explaining most of the differences between samples. A total of 60 unique OTUs were obtained for the heatmap construction. The OTU abundances were converted to percentage of reads from each sample and Manhattan distances were calculated and the heatmap constructed using ggplot version 2.2.1.0 (Wickham 2009). The taxonomy of the selected OTUs was added separately.

Results

Bacterial and archaeal 16S rRNA gene copy numbers

The copy numbers of Bacteria (Fig. 1a) were generally one order of magnitude higher than those of Archaea (Fig. 1b). The bacterial numbers were higher in the flooded rice fields than in the upland wheat field (Fig. 1a), but the archaeal numbers were not significantly different (Fig. 1b). A two-way ANOVA showed that the copy number of bacterial 16S rRNA gene was significantly affected by fertilization ($p < 0.05$), growth time ($p < 0.01$), and the interactions of the two factors ($p < 0.01$), while no significant effect was found for the archaeal 16S rRNA gene (Table S4).

Composition of the archaeal communities

The archaeal community composition was determined by analysis of terminal restriction fragment length polymorphism (T-RFLP) targeting archaeal 16S rRNA genes. The archaeal communities in the wheat and rice cultivated soils were dominated by T-RFs of 184 bp (23~55% relative abundance), 391 bp (11~26%), and 737 bp (6~21%) (Fig. 2). During the rice growth season, the relative abundance of T-RF of 184 bp significantly decreased from 55% at the seedling stage to 23% at the maturing stage, while that of 737 bp significantly increased from 9 to 21%. A similar trend was detected for the relative abundance of T-RF of 391 bp increasing from 11% at the seedling stage to 26% at the maturing stage, but the change was statistically not significant. Although the relative abundance of T-RF of 71 bp was relatively small, it significantly increased from 1 to 9% during the rice growth season.

Non-metric multidimensional scaling (NMDS) analysis of T-RFLP showed that the archaeal 16S rRNA gene

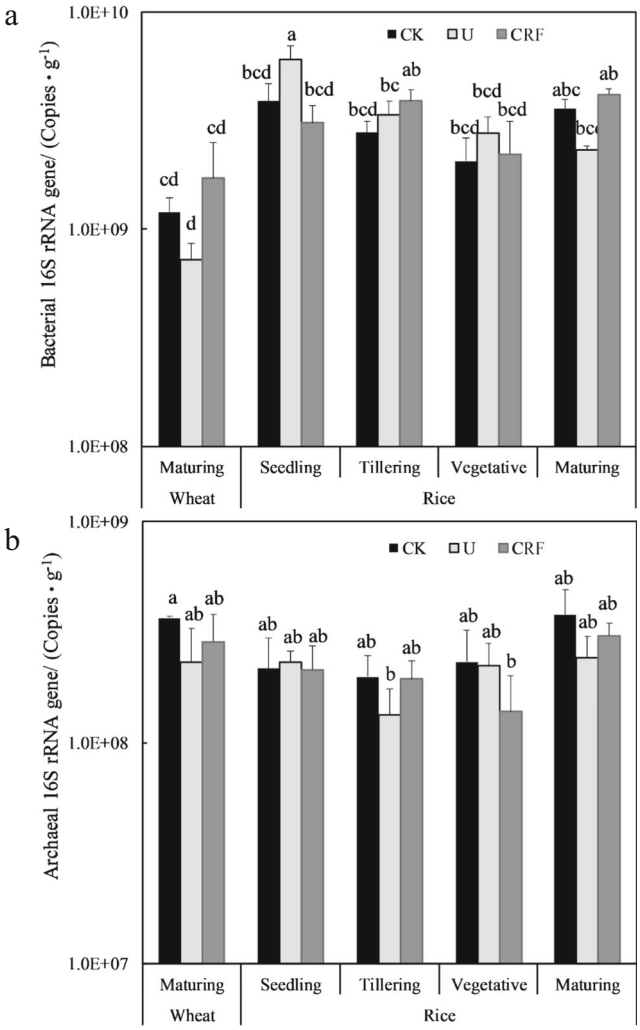


Fig. 1 Copies of bacterial and archaeal 16S rRNA genes per gram dry soil from unamended soil samples (CK), urea fertilizer amended samples (U), and controlled-release fertilizer-amended samples (CRF) during rice growth; mean ± SE ($n = 3$). Different letters indicate significant difference ($p < 0.05$) between copy numbers of the same gene type in different treatments and growth season

composition formed separate clusters for different samples (Fig. S1), and analysis by perMANOVA showed that the clustering of the archaeal gene was significantly affected by time ($p < 0.001$, Table S1), while N fertilizer types (CK, U, CRF) had no significant effect ($p > 0.05$, Table S1), neither alone nor in interaction with time.

Composition of the bacterial communities

The bacterial community composition was also determined by analysis of terminal restriction fragment length polymorphism (T-RFLP) targeting bacterial 16S rRNA genes. Similarly to the results of archaeal gene, NMDS analysis that the bacterial16S rRNA gene composition formed separate clusters for different samples (Fig. S2), and analysis by perMANOVA showed that the clustering of the bacterial genes was also significantly affected by time ($p < 0.001$), while N fertilizer types (CK, U, CRF) had no significant effect ($p > 0.05$, Table S1), neither to the interaction effect between time and fertilization.

Pyrosequencing targeting the bacterial 16S rRNA was conducted to characterize the main bacterial phylotypes in the soil samples. The samples from CK, U, and CRF treatments were sequenced for the wheat field and the rice fields at tillering and maturing growth stages, resulting in 1103 to 2515 high-quality sequences for each sample, equivalent to about 629 to 718 bacterial OTUs. The sequences were assigned to different bacterial phyla. Those with the highest relative abundance were *Proteobacteria* (25–33%), *Actinobacteria* (11~16%), *Acidobacteria* (9~16%), *Chloroflexi* (6~10%), *Firmicutes* (6~9%), and *Planctomycetales* (3~6%), which were found in all the different samples (Fig. 3). The bacterial community composition on the phylum level did not change dramatically with seasonal time.

A heatmap was constructed to depict the relative abundance of bacterial OTUs that best represented the dissimilarity between the bacterial communities by selecting 60 with the largest loadings of the PCA. Among these 60 OTUs, 42 could be classified to family level or lower and are depicted in a heatmap (Fig. 4). Comparison of the dominant OTUs retrieved from different N fertilization treatments showed a rather uniform distribution, while the soil samples from different seasonal time points clustered separately. Comparison of the soils from the tillering and maturing growth stages showed differences among several bacterial OTUs. For example, OTU 39 (affiliated to *Actinomycetales*), OTU 109 (affiliated to *Sulfuricella*), OTU 173 (affiliated to *Oxalobacteraceae*), OTU 71 (affiliated to Acidobacteria_Gp16), and OTU 186 (affiliated to *Clostridiales*) were more abundant at the tillering than maturing growth stages, while OTU 283 (affiliated to Acidobacteria_Gp18), OTU 69 (affiliated to *Chthonomonadaceae*), OTU 79 (affiliated to Acidobacteria_Gp1), OTU 287 (affiliated to *Betaproteobacteria*), OTU 15 (affiliated to *Anaerolineaceae*), and OTU 45 (affiliated to *Lysinibacillus*) were relatively more abundant at the maturing growth stages.

Rates, isotopic analysis, and pathway of methanogenesis

The functional responses of the methanogenic microbial communities to N fertilization were determined in incubation experiments using soil samples from the rice maturing growth stage. The maturing stage was chosen to allow sufficient time in the field for the different treatments to affect the methanogenic communities. The main chemical characteristics of these soils are shown in Table S2. Compared with the control

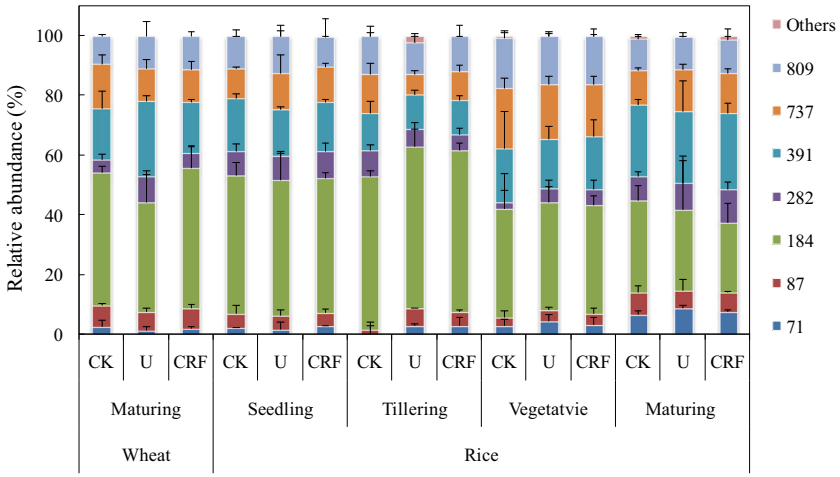


Fig. 2 T-RFLP patterns of archaeal 16S rRNA genes from unamended soil samples (CK), urea fertilizer-amended samples (U), and controlled-release fertilizer-amended samples (CRF) during plant growth; mean ± SE ($n = 3$)

(CK), the concentrations of chloride, nitrate, and sulfate were higher in the N-addition treatments, but these differences were only statistically significant for nitrate. The concentration of nitrate was significantly higher from CRF than that from U treatment. The total C and N contents were similar between U and CRF treatments. The three treatments had a similar $\delta^{13}C$ values of organic matter.

Methane production in the soils started after a lag phase of 22 days in CK and CRF treatments and 30 days in U treatment. The lag was probably due to inhibition of the methanogenic microbial community by the sulfate/nitrate-reducing microbial communities competing for the same substrates (Conrad et al. 2009). Accumulation of CH_4 was similar in control and CRF treatments, but was lower in U treatment (Fig. S3). A similar pattern was observed for CH_4 production rates (Table 2). Hence, N fertilization affected rates of CH_4 production, which was inconsistent with the result that CH_4 emission under field conditions was hardly affected by N addition (Ji et al. 2013, 2014) (the rates of CH_4 and N_2O emission from these publications are compiled in Table S3 for comparison). Moreover, we found that the CH_4 production rates were significantly correlated with the copy numbers of archaeal 16S rRNA genes during the maturing stage ($p < 0.05$) and were significantly affected by N fertilization.

Addition of CH_3F strongly inhibited of CH_4 production due to the inhibition of acetoclastic methanogenesis. The $\delta^{13}C$ of CH_4 in the presence of CH_3F is characteristic for hydrogenotrophically produced CH_4. The $\delta^{13}C$ values of CO_2 were unaffected, but those of CH_4 were more negative in the inhibited than the uninhibited samples (Fig. S4). The averages of the $\delta^{13}C$ values of CH_4 and CO_2 were similar among the three fertilizer treatments (Table 2).

Acetate concentrations in the uninhibited samples were too low to be detected. However, addition of CH_3F strongly increased the concentrations of acetate at the end of the incubation (Table 2). The acetate accumulated in the presence of CH_3F was most probably due to microbial formation, while in the absence of CH_3F, it was most likely consumed by acetoclastic methanogens. The $\delta^{13}C$ values of acetate-methyl ranged between − 40 and − 43‰ and were generally by about 7–9‰ lower than those of total acetate. In general, the concentration of acetate was significantly higher in CK and CRF

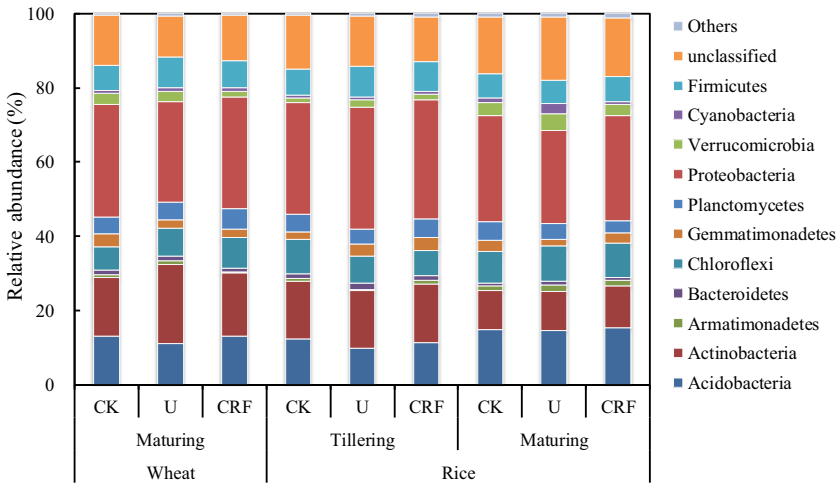
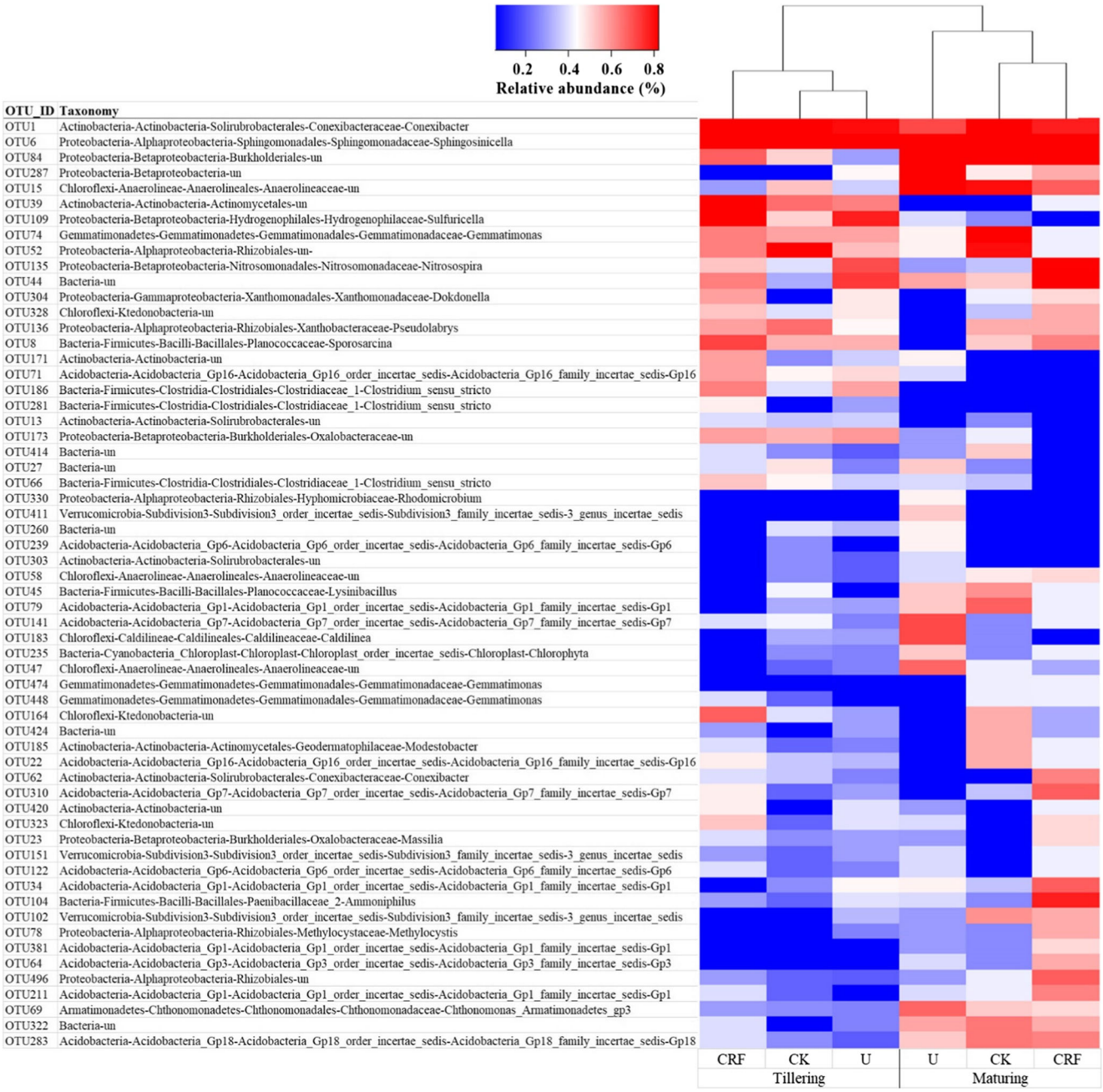


Fig. 3 Pyrosequencing-based relative abundance of bacterial phyla (16S rRNA gene) from unamended soil samples (CK), urea fertilizer-amended samples (U), and controlled-release fertilizer-amended samples (CRF) during plant growth



OTU_ID	Taxonomy
OTU1	Actinobacteria-Actinobacteria-Solirubrobacterales-Conexibacteraceae-Conexibacter
OTU6	Proteobacteria-Alphaproteobacteria-Sphingomonadales-Sphingomonadaceae-Sphingosinicella
OTU84	Proteobacteria-Betaproteobacteria-Burkholderiales-un
OTU287	Proteobacteria-Betaproteobacteria-un
OTU15	Chloroflexi-Anaerolineae-Anaerolineales-Anaerolineaceae-un
OTU39	Actinobacteria-Actinobacteria-Actinomycetales-un
OTU109	Proteobacteria-Betaproteobacteria-Hydrogenophilales-Hydrogenophilaceae-Sulfuricella
OTU74	Gemmatimonadetes-Gemmatimonadetes-Gemmatimonadales-Gemmatimonadaceae-Gemmatimonas
OTU52	Proteobacteria-Alphaproteobacteria-Rhizobiales-un-
OTU135	Proteobacteria-Betaproteobacteria-Nitrosomonadales-Nitrosomonadaceae-Nitrosospira
OTU44	Bacteria-un
OTU304	Proteobacteria-Gammaproteobacteria-Xanthomonadales-Xanthomonadaceae-Dokdonella
OTU328	Chloroflexi-Ktedonobacteria-un
OTU136	Proteobacteria-Alphaproteobacteria-Rhizobiales-Xanthobacteraceae-Pseudolabrys
OTU8	Bacteria-Firmicutes-Bacilli-Bacillales-Planococcaceae-Sporosarcina
OTU171	Actinobacteria-Actinobacteria-un
OTU71	Acidobacteria-Acidobacteria_Gp16-Acidobacteria_Gp16_order_incertae_sedis-Acidobacteria_Gp16_family_incertae_sedis-Gp16
OTU186	Bacteria-Firmicutes-Clostridia-Clostridiales-Clostridiaceae_1-Clostridium_sensu_stricto
OTU281	Bacteria-Firmicutes-Clostridia-Clostridiales-Clostridiaceae_1-Clostridium_sensu_stricto
OTU13	Actinobacteria-Actinobacteria-Solirubrobacterales-un
OTU173	Proteobacteria-Betaproteobacteria-Burkholderiales-Oxalobacteraceae-un
OTU414	Bacteria-un
OTU27	Bacteria-un
OTU66	Bacteria-Firmicutes-Clostridia-Clostridiales-Clostridiaceae_1-Clostridium_sensu_stricto
OTU330	Proteobacteria-Alphaproteobacteria-Rhizobiales-Hyphomicrobiaceae-Rhodomicrobium
OTU411	Verrucomicrobia-Subdivision3-Subdivision3_order_incertae_sedis-Subdivision3_family_incertae_sedis-3_genus_incertae_sedis
OTU260	Bacteria-un
OTU239	Acidobacteria-Acidobacteria_Gp6-Acidobacteria_Gp6_order_incertae_sedis-Acidobacteria_Gp6_family_incertae_sedis-Gp6
OTU303	Actinobacteria-Actinobacteria-Solirubrobacterales-un
OTU58	Chloroflexi-Anaerolineae-Anaerolineales-Anaerolineaceae-un
OTU45	Bacteria-Firmicutes-Bacilli-Bacillales-Planococcaceae-Lysinibacillus
OTU79	Acidobacteria-Acidobacteria_Gp1-Acidobacteria_Gp1_order_incertae_sedis-Acidobacteria_Gp1_family_incertae_sedis-Gp1
OTU141	Acidobacteria-Acidobacteria_Gp7-Acidobacteria_Gp7_order_incertae_sedis-Acidobacteria_Gp7_family_incertae_sedis-Gp7
OTU183	Chloroflexi-Caldilineae-Caldilineales-Caldilineaceae-Caldilinea
OTU235	Bacteria-Cyanobacteria_Chloroplast-Chloroplast-Chloroplast_order_incertae_sedis-Chloroplast-Chlorophyta
OTU47	Chloroflexi-Anaerolineae-Anaerolineales-Anaerolineaceae-un
OTU474	Gemmatimonadetes-Gemmatimonadetes-Gemmatimonadales-Gemmatimonadaceae-Gemmatimonas
OTU448	Gemmatimonadetes-Gemmatimonadetes-Gemmatimonadales-Gemmatimonadaceae-Gemmatimonas
OTU164	Chloroflexi-Ktedonobacteria-un
OTU424	Bacteria-un
OTU185	Actinobacteria-Actinobacteria-Actinomycetales-Geodermatophilaceae-Modestobacter
OTU22	Acidobacteria-Acidobacteria_Gp16-Acidobacteria_Gp16_order_incertae_sedis-Acidobacteria_Gp16_family_incertae_sedis-Gp16
OTU62	Actinobacteria-Actinobacteria-Solirubrobacterales-Conexibacteraceae-Conexibacter
OTU310	Acidobacteria-Acidobacteria_Gp7-Acidobacteria_Gp7_order_incertae_sedis-Acidobacteria_Gp7_family_incertae_sedis-Gp7
OTU420	Actinobacteria-Actinobacteria-un
OTU323	Chloroflexi-Ktedonobacteria-un
OTU23	Proteobacteria-Betaproteobacteria-Burkholderiales-Oxalobacteraceae-Massilia
OTU151	Verrucomicrobia-Subdivision3-Subdivision3_order_incertae_sedis-Subdivision3_family_incertae_sedis-3_genus_incertae_sedis
OTU122	Acidobacteria-Acidobacteria_Gp6-Acidobacteria_Gp6_order_incertae_sedis-Acidobacteria_Gp6_family_incertae_sedis-Gp6
OTU34	Acidobacteria-Acidobacteria_Gp1-Acidobacteria_Gp1_order_incertae_sedis-Acidobacteria_Gp1_family_incertae_sedis-Gp1
OTU104	Bacteria-Firmicutes-Bacilli-Bacillales-Paenibacillaceae_2-Ammoniphilus
OTU102	Verrucomicrobia-Subdivision3-Subdivision3_order_incertae_sedis-Subdivision3_family_incertae_sedis-3_genus_incertae_sedis
OTU78	Proteobacteria-Alphaproteobacteria-Rhizobiales-Methylocystaceae-Methylocystis
OTU381	Acidobacteria-Acidobacteria_Gp1-Acidobacteria_Gp1_order_incertae_sedis-Acidobacteria_Gp1_family_incertae_sedis-Gp1
OTU64	Acidobacteria-Acidobacteria_Gp3-Acidobacteria_Gp3_order_incertae_sedis-Acidobacteria_Gp3_family_incertae_sedis-Gp3
OTU496	Proteobacteria-Alphaproteobacteria-Rhizobiales-un
OTU211	Acidobacteria-Acidobacteria_Gp1-Acidobacteria_Gp1_order_incertae_sedis-Acidobacteria_Gp1_family_incertae_sedis-Gp1
OTU69	Armatimonadetes-Chthonomonadetes-Chthonomonadales-Chthonomonadaceae-Chthonomonas_Armatimonadetes_gp3
OTU322	Bacteria-un
OTU283	Acidobacteria-Acidobacteria_Gp18-Acidobacteria_Gp18_order_incertae_sedis-Acidobacteria_Gp18_family_incertae_sedis-Gp18

Fig. 4 Heatmap showing the relative abundance of selected bacterial OTUs from unamended soil samples (CK), urea fertilizer-amended samples (U), and controlled-release fertilizer-amended samples (CRF) during rice growth

treatments than U treatment, while no significant difference was found in the $\delta^{13}C$ values of total acetate and acetate-methyl among the three treatments.

These above data sets allowed the calculation of the percentage (f_{H2}) of CH_4 produced by hydrogenotrophic methanogenesis (the remainder being due to acetoclastic methanogenesis). The value was initially ca. 46–55% and then decreased with incubation time until a constant value was reached (Fig. S5; Table 2). The decrease in f_{H2} was probably caused by the relatively large contribution from acetoclastic methanogenesis during the phase when the transiently accumulated acetate was again consumed. At the end of incubation, all treatments reached a similar value of 35–37% (Table 2), indicating that N addition (U/CRF) had no effect on the CH_4 formation pathway of methanogenic microbial communities.

Quantification of *mcrA*, *pmoA*, *nirK*, *nirS*, and *nosZ* genes

The wheat fields contained similar numbers of methanogenic *mcrA* and methanotrophic *pmoA* copies compared with the

Table 2 Average rates, $\delta^{13}C$ values of potential CH_4 production in soils in the absence and presence of CH_3F, concentrations of acetate and $\delta^{13}C$ values of acetate and acetate-methyl accumulated until the end of incubation, and the percentage (f_{H2}) of CH_4 produced by hydrogenotrophic methanogenesis from unamended soil samples (CK), urea fertilizer-amended samples (U), and controlled-release fertilizer-amended samples (CRF) during rice growth; mean ± SE ($n = 3$)

	CH_4 production rates (nmol h^{-1} gdw^{-1})	$\delta^{13}C_{CH_4}$ (permil)	$\delta^{13}C_{CO_2}$ (permil)	Acetate (mmol)	δ^{13}Cacetate (permil)	δ^{13}Cmethyl-acetate (permil)	f_{H2} (%)
CK	4.10 ± 1.40a	− 64.0 ± 2.7a	− 23.2 ± 3.3a	–	–	–	36.2 ± 0.3a
U	2.47 ± 0.39b	− 68.8 ± 2.5a	− 25.1 ± 0.6a	–	–	–	36.1 ± 1.8a
CRF	3.92 ± 0.50a	− 64.8 ± 6.1a	− 23.9 ± 2.2a	–	–	–	35.2 ± 0.9a
CK-CH_3F	0.95 ± 0.26 cd	− 101.8 ± 0.4b	− 25.3 ± 1.9a	5.57 ± 2.09a	32.4 ± 3.0a	− 40.3 ± 0.7a	–
U-CH_3F	0.43 ± 0.07d	− 105.9 ± 1.3b	− 27.3 ± 0.4ab	3.34 ± 0.88b	33.1 ± 0.2a	− 42.7 ± 1.7a	–
CRF-CH_3F	1.08 ± 0.14bc	− 104.7 ± 3.1b	− 26.5 ± 1.9ab	6.72 ± 0.2a	31.0 ± 3.6a	− 40.6 ± 1.8a	–

"–" not detected; values within a row followed by the same letter are not significantly different at $p < 0.05$ using the LSD test

fields cultivated with rice (Fig. 5a, b). The response of both *mcrA* and *pmoA* copy numbers in rice soil to seasonal time and N addition was complex (Fig. 5). A two-way ANOVA showed that both of the copy numbers of *mcrA* and *pmoA* genes were significantly affected by time ($p < 0.05$), while fertilization showed no significant affect (Table S4).

The nitrifier *amoA* copy numbers derived from Bacteria (AOB) (Fig. 6a) were generally higher than those from Archaea (AOA) (Fig. 6b). The abundance of AOA was similar between the different cultivated soils and was not affected by fertilization or season (Fig. 6b). The abundance of AOB subjected to different N fertilization regimes and over the seasonal time was more complex. A two-way ANOVA showed that growth time significantly affected the copy numbers of AOB-*amoA* ($p < 0.01$) and AOA-*amoA* genes ($p < 0.05$), while fertilization significantly affected only those of AOB-*amoA* gene ($p < 0.05$) (Table S4).

The copy numbers of denitrifer genes (*nirS*, *nirK*, *nosZ*) were generally one order of magnitude lower than those of AOA or AOB (Fig. 6c–e). Compared with the fields cultivated with wheat, the values of *nirK* and *nosZ* genes were higher in the fields cultivated with rice (Fig. 6d, e). All of the three denitrifier genes were detected with lowest copies during the time of the vegetative stage of rice. A two-way ANOVA showed growth time significantly affected copy numbers of *nosZ* ($p < 0.01$) and *nirK* genes ($p < 0.05$), while fertilization showed no significant effect on the three denitrifier genes (Table S4).

Finally, a Pearson correlation analysis was done between CH_4 and N_2O emission rates (Table S3) and the copy numbers of 16S rRNA genes and functional genes along the seasonal time (Table 3). There was no significant correlation in the control fields. However, the N-fertilized (U, CRF) fields showed significant positive correlations between CH_4 emission rates and number of archaeal 16S-rRNA, *mcrA* and *pmoA* gene copies. The combined (control, U, CRF) rates of CH_4 and N_2O emission were positively correlated ($p < 0.05$) with the numbers of *mcrA*, *pmoA*, and *nosZ* genes.

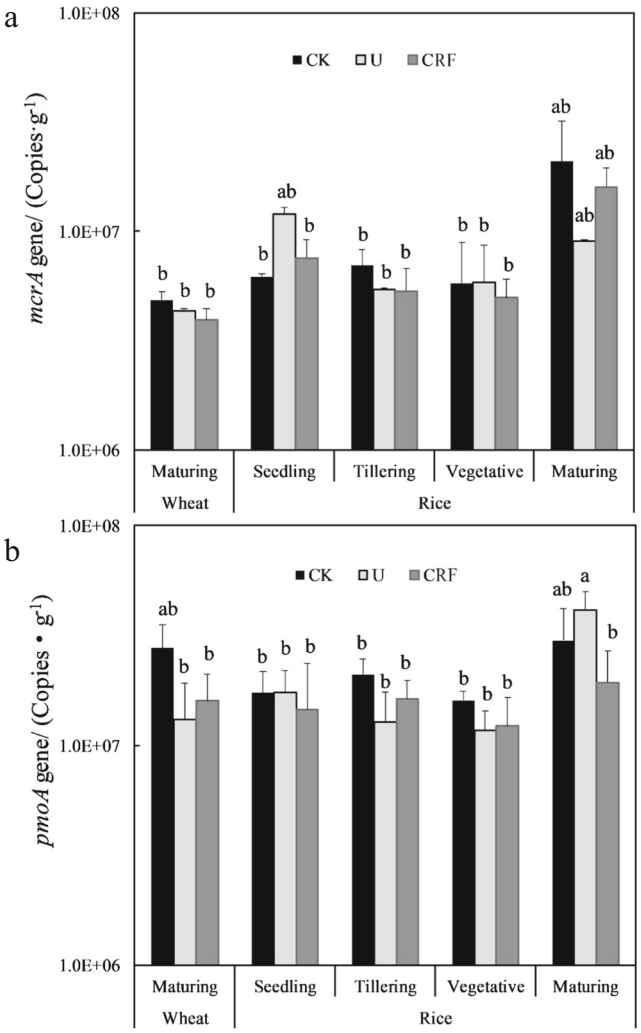


Fig. 5 Copies of *mcrA* and *pmoA* genes per gram dry soil from unamended soil samples (CK), urea fertilizer-amended samples (U), and controlled-release fertilizer-amended samples (CRF) during rice growth; mean ± SE ($n = 3$). Different letters indicate significant difference ($p < 0.05$) between copy numbers of the same gene type in different treatments and growth season

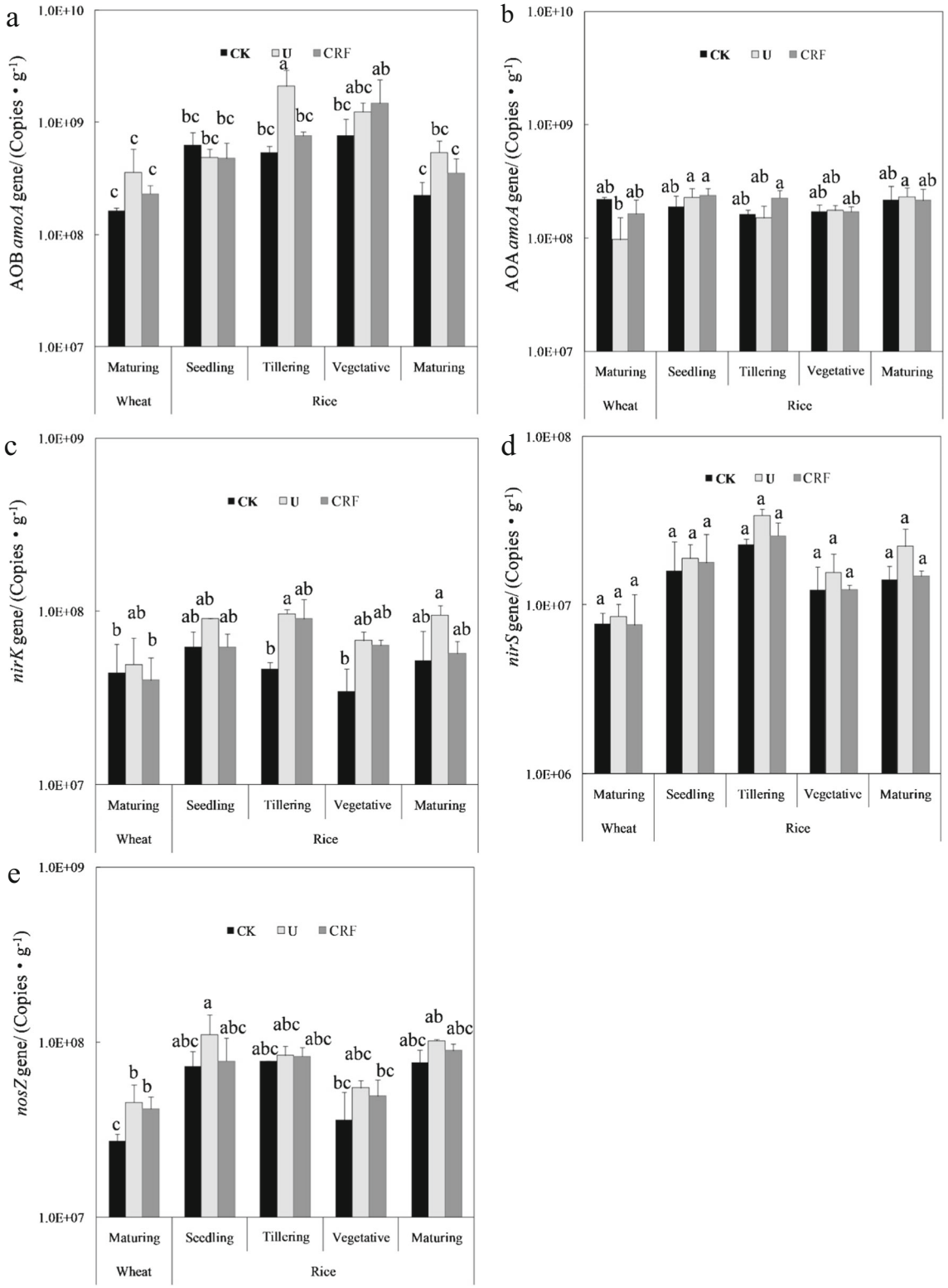


Fig. 6 Copies of bacterial and archaeal *amoA* and dentrifier *nirK*, *nirS*, and *nosZ* genes per gram dry soil from unamended soil samples (CK), urea fertilizer-amended samples (U), and controlled-release fertilizer-amended samples (CRF) during rice growth; mean ± SE ($n = 3$). Different letters indicate significant difference ($p < 0.05$) between copy numbers of the same gene type in different treatments and growth season

Table 3 Correlation between CH_4 and N_2O emission and the abundance of soil microbial communities (Pearson two-tailed test) from unamended soil samples (CK), urea fertilizer-amended samples (U), and controlled-release fertilizer-amended samples (CRF) during rice growth

Pearson correlation		Bacteria	Archaea	*mcrA*	*pmoA*	*AOB*	*AOA*	*nirS*	*nirK*	*nosZ*
Total	CH_4	−0.040	0.073	0.436*	0.485*	0.036	0.194	0.088	−0.198	0.139
$n = 36$	N_2O	0.252	−0.120	0.259	0.167	−0.019	0.147	0.169	0.158	0.759**
CK	CH_4	0.329	−0.116	0.507	0.360	−0.051	0.537	−0.146	−0.510	−0.214
$n = 12$	N_2O	−0.191	−0.303	−0.084	−0.243	0.080	−0.246	−0.381	−0.148	0.126
U	CH_4	−0.475	0.200	0.087	0.698*	0.148	0.070	0.573	0.263	0.087
$n = 12$	N_2O	−0.011	−0.330	0.327	0.299	0.067	0.195	0.254	0.068	0.315
CRF	CH_4	0.444	0.682*	0.723**	0.473	−0.332	0.067	−0.100	−0.160	0.406
$n = 12$	N_2O	0.759*	0.354	0.586*	0.265	−0.460	0.227	−0.029	0.170	0.265

*Correlation is significant at the 0.05 level (two-tailed)

**Correlation is significant at the 0.01 level (two-tailed)

Discussion

Microbial abundance and diversity over the rice-growing season

Seasonal changes of 16S rRNA gene copies were only observed for bacteria but hardly for archaea. Many other rice fields also showed stable archaeal but unstable bacterial 16S rRNA copies throughout the rice growth season (Breidenbach and Conrad 2015; Hussain et al. 2011; Wang et al. 2016). In contrast, many functional genes associated with CH_4 and N_2O emissions were significantly affected by time in this case. These functional gene copies included the methanogenic *mcrA* gene, the methanotrophic *pmoA* gene, the nitrifier *amoA* gene from bacteria (AOB) and archaea (AOA), and the denitrifier *nirK* and *nosZ* genes. Growth time also significantly affected the emission rates of both CH_4 and N_2O.

The microbial communities at the different rice growth time were probably influenced by the plant-driven root exudation (Aulakh et al. 2001; Lu et al. 2006; Pump and Conrad 2014; Watanabe et al. 2006), but also by the water management in our field experiment. As a typical water management in China, rice paddy fields are continuously flooded for about 1 month after rice transplanting, exposed to aeration/drainage for 10–15 days (named MSA), instead of continuous flooding, to inhibit ineffective tillers, remove toxic products of anaerobic metabolism, and improve root activity, and then subjected to intermittent irrigation before the crop is ready for harvest (Li et al. 2011; Yan et al. 2005). The redox potential oscillation caused by periodic flooding and drainage has a significant influence on the soil microbial community, their metabolism, and thus short-term biogeochemical processes (Atere et al. 2017; Bossio and Scow 1995; Kögel-Knabner et al. 2010; Noll et al. 2005). In this field experiment, the soil was continuously flooded during the seedling growth stage, resulting in anaerobic condition. The soil redox potential ranged from −100 to −270 mV (Ji et al. 2014), which is beneficial for the activity of obligate/facultative anaerobic microorganisms such as methanogens and denitrifiers. During the tillering stage, the flooding water was drained to inhibit ineffective tillers, and the rice soil was exposed to air parallel with increasing redox potentials. Therefore, the activity of anaerobes was suppressed, while the aerobes (e.g., AOB) were stimulated. After the MSA period, during the vegetative stage, the soil was reflooded and then subjected to intermittent irrigation, resulting in increasing abundance of obligate and faculatative anaerobes (*mcrA*, *nirK*, *nirS*, and *nosZ*) and decreasing abundance of aerobes (*amoA*). However, the abundance of aerobic methanotroph (*pmoA*) already decreased during the tillering stage after drainage, probably because most of the ammonia was oxidized to nitrate/nitrite during the MSA period, and the relative high concentration of nitrate/nitrite inhibited the methanotrophs (Boer et al. 1988).

The taxonomic composition of the archaeal and bacterial communities detected by T-RFLP analysis were also significantly affected by time. Our archaeal T-RFLP data showed a significant decrease in relative abundance of the dominating 184-bp TRF (probably acetoclastic *Methanosarcinaceae*) along with rice growth, while an increase in relative abundance of the 391-bp TRF (probably hydrogenotrophic *Methanocellales*). Similar results had been obtained in a Korean rice field (Ahn et al. 2012). Three other T-RFs (71 bp, 184 bp, and 737 bp) also showed statistically significant responses to time. The bacterial community consisted of seven major phyla, *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Firmicutes*, and *Planctomycetales*, which have consistently been detected in rice soils (Breidenbach and Conrad 2015; Ji et al. 2015). NMDS analysis based on T-RFLP data showed that the bacterial 16S rRNA gene composition was significantly affected by time. In order to identify changes in the lower taxonomic groups, the bacterial 16S rRNA genes were deeply sequenced by pyrosequencing. The pyrosequencing in the different soils was not replicated, since it was not the intention to obtain a

statistically valid comparison, which was basically achieved by the T-RFLP analysis. On lower taxonomic level, a few OTUs were found to change along time. Thus, OTU173 (affiliated to *Burkholderiales*) was significantly more abundant at the tillering than the maturing stage. Soils from the tillering stage were sampled following water management, which possibly stimulated nitrification and denitrification. Another example was the relatively higher abundance of OTU186, affiliated to *Clostridiales*, at the tillering stage than the maturing stage. The *Clostridiales* are endospore-forming bacteria and seem to play a central role in the resuscitation cascade of dry soil (Angel and Conrad 2013) and dried lake sediments (Conrad et al. 2014) upon reflooding. Finally, OTU71 was more abundant in the tillering than the maturing stages, while the trend of OTU283 was vice versa. These two OTUs belong to the most abundant subgroups of *Acidobacteria*, groups Gp16 and Gp18, respectively (Janssen 2006). We suggest that the decrease of subgroup 16 and increase of subgroup 18 along time are probably caused by the seasonal changes of soil properties, e.g., carbon and water content.

The wheat field showed a similar composition of the archaeal and bacterial communities compared with the rice field at maturing stage. Although the number of total bacteria was significantly lower in the wheat field than that in the rice field, that of total archaea and of the *mcrA* gene was similar (except at maturing stage). This observation is consistent with previous observations showing that microbial communities are relatively stable once established and are resistant to environmental perturbation such as drainage or crop rotation (Breidenbach et al. 2016; Fernandez Scavino et al. 2013; Krüger et al. 2005; Watanabe et al. 2006). They are also consistent with the fact that methanogens such as *Methanosarcinaceae* and *Methanocellaceae* possess various genes encoding for oxygen-detoxifying enzymes (Angel et al. 2011; Erkel et al. 2006) allowing them to survive exposure to oxygen after drainage.

Microbial community composition affected by N fertilization

Fertilizer applications provide excess N and organic matter to soil microorganisms, thus possibly affecting soil microbial abundance, activity, and community composition (Ahamadou et al. 2009; Chen et al. 2012; Marschner et al. 2003; Nemergut et al. 2008). Indeed, the abundances of total bacteria at the seedling stage were significantly higher with the addition of urea than with CRF or in the unfertilized control (CK), probably because urea improved N nutrient supply for the microbial communities and the plant at the early stage of plant growth, while CRF released N slowly (Ji et al. 2014; Sun et al. 2016). Similar observations have been made by Enwall et al. (2005) which reported that N fertilization induced a significantly higher bacterial abundance than the controlled treatment.

However, N addition (U, CRF) had no significant effect on the taxonomic composition of archaeal communities (i.e., 16S rRNA genes) compared to the control treatment, although an influence on the methanogenic *mcrA* gene copies was detected. Similar results were also documented by previous studies showing that microbial communities are relatively stable once established and are resistant to environmental perturbation in rice field (Nicol et al. 2004; Wu et al. 2011). Addition of N also did not significantly affect the rates of CH_4 emission or the pathway of CH_4 production, as all samples showed about 2/3 acetoclastic and 1/3 hydrogenotrophic methanogenesis, which is in accordance with the theoretical degradation of polysaccharides to CH_4 and CO_2 (Conrad 1999). However, a pronounced effect on the rates of CH_4 production was found, showing a lower value in the CRF treatments than U or controlled treatments. A similar pattern was found on the acetate concentration in the CH3F-added samples, indicating that the quantity of substrates plays a more important role in the CH_4 production rather than CH_4 emission. The influence of N fertilizer on the bacterial community composition was also not detectable. While our study did not find an N fertilizer effect during the period of the rice growing season, changes in the bacterial communities by long-term fertilization, possibly caused by pH, are possible (Ahn et al. 2012; Lauber et al. 2008; Rousk et al. 2010).

Similarly to the taxonomic compositions (archaea and bacteria), ANOVA analysis showed that most of the copy numbers of the functional genes were generally hardly affected by different N fertilizers throughout the rice growth, except those of the bacterial *amoA* gene, while the interaction of time and N fertilization showed significant effects on the copy numbers of *pmoA*, AOB, and *nirK* genes. Strong responses of N fertilization were only detected at particular growth stages. For example, CRF application decreased the *pmoA* copies at the maturing stage compared with urea application. Methanotrophs are known to respond differently to the concentrations of ammonia and nitrate/nitrite (Oneill and Wilkinson 1977). In rice soil, ammonium (or urea) can either stimulate (Bodelier et al. 2000), inhibit (Hutsch et al. 1994), or have no effect (Shrestha et al. 2010) on methanotrophs. Similarly to methanogenic and methanotrophic functional genes, there was also an occasional response of ammonia-oxidizing and denitrifying functional genes to N fertilization, but these responses were seasonally restricted and not uniform with respect to being positive or negative. Such contrasting responses have also been documented in the literature for soil ammonia oxidation (Chen et al. 2011; Jia and Conrad 2009; Shen et al. 2012; Leininger et al. 2006; Wessén et al. 2010) and denitrification (Cui et al. 2016; Harter et al. 2013; Palmer et al. 2012; Wolsing and Priemé 2004). In general, however, copy numbers of functional genes did not significantly respond to different N fertilizers, but rather to seasonal changes, similarly as found for the response of the taxonomic community composition.

Correlation between CH_4 and N_2O emissions and abundance of soil microbial communities

Methane is produced by methanogens during organic matter decomposition and oxized by methanotrophs once exposed to aerobic conditions, and N_2O is produced microbiologically in soils mainly through nitrification and denitrification (Braker and Conrad 2011; Mosier et al. 1998). Numerous studies have been done to detect the relationship between the emission rates of CH_4 and N_2O and soil microbial communities in paddy fields (Conrad 2007; Enwall et al. 2005; Maeda et al. 2010; Miller et al. 2008; Qin et al. 2018; Wang et al. 2017). In this study, CH_4 and N_2O emission rates were significantly ($p < 0.001$) affected by time, while N fertilization only showed significant effects on the emission rates of N_2O and the production rates of CH_4 (Table S5; Ji et al. 2013, 2014). Pearson correlation analysis showed no significant correlation either between CH_4 and N_2O emissions and the abundance of related functional genes in the control fields or between N_2O emissions and the abundance of related functional genes in U treatment. Similar results were also found by Fan et al. (2016), showing that the correlation between CH_4 and N_2O emissions and the abundance of related functional genes was not significant, while environmental factors, such as soil redox potential, were more related to CH_4 and N_2O emissions.

Conclusion

The taxonomic archaeal and bacterial community compositions did not change upon different N fertilization regimes, although these treatments strongly affected production rates of CH_4 and emission rates of N_2O. By contrast, a comparatively strong change in the taxonomic community composition was observed over seasonal time. Besides the influence of rice plants and their root exudation, it is probably the changes in the water management (intermitted drainage) during rice growth that caused the changes in the community composition of bacteria and archaea. Similarly to the taxonomic communities, seasonal time showed a significant effect on the abundance of functional microbial populations related to CH_4 and N_2O emissions. However, N addition showed no significant effect over the entire rice growth season. The above results were consistent with our previous studies showing that CH_4 and N_2O emissions dramatically changed with time, while N addition only affected N_2O emission (Ji et al. 2013, 2014). We conclude that rice growth time and water management have pronounced effects on abundance, composition, and function of the soil microbial communities, while N fertilizer effect is negligible on the level of either specific functional genes or taxonomic 16S rRNA genes.

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