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Effect of Paddy-Upland Rotation on Methanogenic Archaeal Community Structure in Paddy Field Soil

Dongyan Liu · Hiroki Ishikawa · Mizuhiko Nishida · Kazunari Tsuchiya · Tomoki Takahashi · Makoto Kimura · Susumu Asakawa

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Abstract Methanogenic archaea are strict anaerobes and demand highly reduced conditions to produce methane in paddy field soil. However, methanogenic archaea survive well under upland and aerated conditions in paddy fields and exhibit stable community. In the present study, methanogenic archaeal community was investigated in fields where paddy rice (*Oryza sativa* L.) under flooded conditions was rotated with soybean (*Glycine max* [L.] Merr.) under upland conditions at different rotation histories, by polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) and real-time quantitative PCR methods targeting 16S rRNA and *mcrA* genes, respectively. Soil samples collected from the fields before flooding or seeding, during crop cultivation and after harvest of crops were analyzed. The abundance of the methanogenic archaeal populations decreased to about one-tenth in the rotational plots than in the consecutive paddy (control) plots. The composition of the methanogenic archaeal community also changed. Most members of the methanogenic archaea consisting of the orders *Methanosarcinales*, *Methanocellales*, *Methanomicrobiales*, and *Methanobacteriales* existed autochthonously in both the control and rotational plots, while some were strongly affected in the rotational plots, with fatal effect to some members belonging to the *Methanosarcinales*. This study revealed that the upland conversion for one or longer than 1 year in the rotational system affected the methanogenic archaeal community structure and was fatal to some members of methanogenic archaea in paddy field soil.

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D. Liu (✉) · H. Ishikawa · M. Kimura · S. Asakawa
Soil Biology and Chemistry, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya, Aichi 464-8601, Japan
e-mail: liu.dongyan@f.mbox.nagoya-u.ac.jp

M. Nishida · K. Tsuchiya · T. Takahashi
NARO Tohoku Agricultural Research Center, Daisen, Akita 014-0120, Japan

Introduction

Methane is an important greenhouse gas and paddy rice fields are one the of major emission sources of methane [17]. Methane is produced by methanogenic archaea under anoxic conditions in paddy field soil. Methanogenic archaea are strict anaerobes and demand highly reduced conditions for methanogenesis. However, methanogenic archaea survive well under aerated conditions in paddy fields and exhibit stable community. Population of methanogenic archaea did not change conspicuously throughout 2 years including flooded and drained periods in paddy field soils, which were determined by the MPN method [4, 5] and archaeal ether lipid analysis [5]. Methanogenic archaea showed remarkably stable communities irrespective of flooded and drained conditions in a year in paddy field soil by the polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA [30, 31] and *mcrA* [32] genes.

Rice feeds about a half of the world population and especially is the most important crop in Asian countries. In Asian countries, rotational cultivation of paddy rice (*Oryza sativa* L.) and upland crops has been recommended for many reasons such as the multiple use of land, improvement of nutrient imbalance in the plant and soil, increase of crop production and mitigation of the methane emission. Various upland crops, e.g. soybean (*Glycine max* [L.] Merr.), maize (*Zea mays* L.), pasture and vegetables, are cultivated in drained paddy fields

[19, 20, 24]. In Japan, as rice production has exceeded domestic demand, the government has implemented the rotation policy about 40 years ago and nowadays paddy rice-upland crop rotation systems have been used as a major land management to reduce rice production and improve rates of self-sufficiency of the upland crops [28].

In the paddy-upland rotational fields, upland crops such as soybean are cultivated under drained conditions in summer for one to several years, followed by paddy rice cropping for one to several years. The soil conditions are kept oxic during the upland periods in these fields. In the previous studies mentioned above [4, 5, 30–32], methanogenic archaeal community in paddy fields with double cropping of rice and wheat were investigated. The double cropping is distinct from paddy-upland rotation. Paddy rice is always cropped in summer and an upland crop such as wheat (*Triticum aestivum* L.) or barley (*Hordeum vulgare* L.) is cultivated in winter in the double-cropping system. Methanogenic archaea survived well without any conspicuous changes in the population and community composition even in the upland periods under wheat cultivation for 7 months in a year. The question is whether the upland periods longer than 1 year affect methanogenic archaeal community in the rotational paddy field. Methanogenic archaea sustain the stable community structure as shown in the pasture-based field with irrigated rice cultivation by Scavino et al. [24]. In addition, methanogenic archaea were detected in upland and various aerated soils even in desert soil [1, 2, 11, 24]. On the other hand, the community structure may be changed because drainage practices influenced methanogenic archaeal communities in paddy field soil [16, 33].

Thus, the objective of the present study was to clarify the effect of paddy-upland rotational system, i.e., upland periods longer than 1 year, on methanogenic archaeal community structure. We used experimental paddy fields under paddy-upland rotation at different intervals cropped with rice or soybean for more than 20 years. Soil samples were collected from the fields at three time points, i.e., before flooding or seeding of soybean, during crop cultivations and after harvest of crops, and structure of methanogenic archaeal community was analyzed by PCR-DGGE and real-time quantitative PCR methods.

Materials and Methods

Sampling Fields and Soil Samples

Soil samples were collected from experimental paddy fields located in NARO Tohoku Agriculture Research Center, Daisen (Omagari), Akita, Japan (39° 29′ N, 140° 29′ E) in 2008, 2009 and 2011. There were two paddy fields, E and 114. The soil type in these paddy fields is classified as Gray Lowland soil (Typic Fluvaquents) [26]. The paddy field E consisted of rotational plots and consecutive paddy rice plots (as a control) with two replicates and the 114 field consisted of consecutive paddy rice plots (as a control) and rotational plots under two kinds of rotational intervals, short- and long-term rotational plots, with two replicates. The rotational history of paddy rice and soybean in the E and 114 fields is detailed in Tables 1 and 2. Paddy fields were puddled in early May and rice plants were transplanted in mid May after flooding and harvested in mid September after drainage in early September. Soybean seeds were sown in early June just after plowing and harvested in early to mid October. Soil samples were taken from the plow layer at the depth of 5–10 cm at 4 points in each plot on April 30/June 4 (before flooding [rice] or seeding [soybean]), July 9 (during soybean or rice cultivation) and November 20 (after rice or soybean harvest) in 2008, April 28/June 3, July 14 and October 19 in 2009 and also August 2 in 2011, and mixed well to make a composite sample for each replicate plot. The information of the fields has already been reported by Shirato et al. [26] and the soil physical and chemical properties are summarized in Table 2. Soil moisture content was determined by drying the soil sample at 105 °C for 16 h and is shown in Supplemental Table 1. The soil samples were brought to the laboratory in polyethylene bags, stored at 4 °C, then passed through a 2-mm mesh sieve and stored at −20 °C until use.

DNA Extraction and PCR Amplification

DNA for PCR-DGGE analysis and real-time quantitative PCR was extracted from 0.5 g soil samples in duplicate for each sample using the ISOIL for Beads Beating Kit for soil (NIPPON GENE, Tokyo, Japan) by the bead-beating method based on the manufacturer's instruction (4,600 rpm for 30 s). Extracted DNA was diluted 40-fold with TE (10 mM Tris, 1 mM EDTA, pH 8.0) buffer and used for PCR-DGGE and real-time quantitative PCR. PCR amplification was carried out using the primer set 1106F-GC/1378R [30], which targeted methanogenic archaeal 16S rRNA gene. The PCR program had an initial denaturation at 95 °C for 90 s, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 90 s, and a final extension at 72 °C for 6 min.

DGGE, Cluster and Sequencing Analysis

Approximately 150–200 ng PCR products were used for DGGE analysis. DGGE was performed with a Dcode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA) and the denaturant gradient of the gel ranged from 32 to 62 %, in which 100 % denaturant contained 7 M urea and 40 % formamide. Electrophoresis was run for 14 h at 60 °C and 100 V. The gels were stained for 20 min with SYBR Green I nucleic acid gel stain (Lonza, Rockland, ME,

Table 1 Rotational history of E and 114 fields

Field	Management	1981	1982–89	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	*2008*	*2009*	2010	*2011*	2012	2013
E	Control	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	*R*	*R*	R	*R*	R	R
	Rotation	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	R	R	R	*S*	*S*	S	*R*	R	S
114	Control	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	*R*	*R*	R	*R*	R	R
	ST-rotation	R	R	S	R	R	R	S	R	S	R	R	S	R	S	S	R	R	S	R	R	*S*	*R*	R	*R*	S	R
	LT-rotation	R	R	S	S	S	R	S	S	S	R	R	S	S	S	S	R	S	S	S	R	*S*	*S*	S	*R*	S	S

ST short term; *LT* long term; *R* paddy rice; *S* soybean

Sampling year and site are presented in italics

USA). The stained gel was immediately scanned with an image analyzer (Typhoon 9400, Amersham Bioscience/GE Healthcare UK, Buckinghamshire, England). The cluster analysis by the Ward method was performed for the data obtained from the DGGE band patterns based on the intensity of DGGE bands (0, no band; 1, weak; 2, moderate; 3, strong) by visual inspection using the Black Box Program [3]. Representative DGGE bands were sequenced as detailed by Liu et al. [14] with an ABI 3130 automated sequencer (Applied Biosystems, Foster City, CA, USA). Close relatives and phylogenetic affiliation of the obtained sequences were determined by using the BLAST search program at the DNA Data Bank of Japan (DDBJ) web site. Phylogenetic trees of the sequences of methanogenic archaea in these two fields were constructed by the neighbor-joining method with the software of MEGA 5.1 [29].

Real-Time Quantitative Polymerase Chain Reaction

Abundance of methanogenic archaeal population was estimated in the soil samples by real-time quantitative PCR targeting *mcrA* gene which encodes a key enzyme for methanogensis (methyl-coenzyme M reductase α subunit). The real-time quantitative PCR was performed by using a Thermal Cycler Dice Real Time System (TaKaRa, Otsu, Japan) and the primers (mcrA-f: GGT GGT GTM GGA TTC ACA CAR TAY GCW ACA GC and mcrA-r: TTC ATT GCR TAG TTW GGR TAG TT) [15]. The real-time quantitative PCR reaction mixture in a total volume of 25 μL contained 12.5 μL of SYBR Premix ExTaq, 0.1 μL each of primer set mcrA-f/mcrA-r (50 mM), 1 μL template DNA, and 11.3 μL sterilized ultrapure water. The PCR program was as follows: initial denaturation at 95 °C for 30 s, followed by 45 cycles of amplification reactions of denaturation at 95 °C for 40 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min. The mixture of known numbers (10^1 to 10^7 copies) of *mcrA* gene fragments from three methanogenic archaeal strains were used as the standard references according to Watanabe et al. [34]. The measurement was duplicated for each DNA extract and blanks were always run with water as the template instead of soil DNA extracts. Standard curve was obtained with a slope of −3.481, efficiency of 95.3 %, average y intercept of 38.67, and R^2 of 0.997. Amplification of non-specific DNA fragments was not detected by a dissociation curve analysis.

Statistical Analysis

The copy number of *mcrA* gene was logarithmically transformed and subjected to two-way ANOVA and Tukey's test to determine a significant difference for each effect with Microsoft Excel 2013 for Windows (Microsoft Corp., Tokyo,

Table 2 Summary of managements, soil sampling, and soil properties in E and 114 fields

Field	Management	Sampling year			pH	Total-C	Total-N
		2008 (BF/BS, CG, AH)	2009 (BF/BS, CG, AH)	2011 (CG)	(H_2O)	(g kg^{-1})	(g kg^{-1})
E	Control (paddy)	Rice	Rice	Rice	5.2	22.5	1.8
	Rotation	Soybean	Soybean	Rice	5.6	17.3	1.4
114	Control (paddy)	Rice	Rice	Rice	5.3	39.2	2.7
	Short-term rotation	Soybean	Rice	Rice	5.5	35.2	2.5
	Long-term rotation	Soybean	Soybean	Rice	5.8	35.5	2.5

ST short term; *LT* long term. Sampling seasons are shown in the parentheses: *BF/BS* before flooding of paddy rice or before seeding of soybean; *CG* crop (rice/soybean) growing period; *AH* after crop (rice/soybean) harvest

Japan) and Ekuseru-Toukei 2012 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results

DGGE Analysis of Methanogenic Archaeal Community in Paddy-Upland Rotational Field

The composition of methanogenic archaeal community was determined by DGGE analysis targeting 16S rRNA genes in the E and 114 field soils. Identical DGGE band patterns were obtained in the duplicates of DNA extraction and also between the two replicate plots in both the E and 114 fields (Supplemental Fig. 1). Thus, one of the patterns in the replicates was used as the representative sample, and all the samples at seven time sampling points in 2008, 2009, and 2011 were subjected to the DGGE analysis in the E and 114 fields (Fig. 1). In the E field, the DGGE band patterns were different between the control and rotational plots and similar among seven time sampling points in each plot. In total, 31 and 26 bands were observed in the control and rotational plots, respectively, and 23 bands were commonly present in both plots (Table 3). Bands E-5, 9, and 19 were present solely in the rotational plot, while bands E-1, 2, 12, 18, 20, 25, 29, and 31 were found exclusively in the control plot (Fig. 1a). In the 114 field, the DGGE band patterns were also different between the control and rotational plots (short- and long-term rotational plots) and did not show any conspicuous difference among seven time sampling points in both control and rotational plots. There were 20 common bands in all the plots, and 26 and 23 bands in total were present in the control and rotational (short- and long-term rotational plots) plots, respectively (Table 3). Bands 114-10, 11, and 21 were observed only in both the short- and long-term rotational plots, while bands 114-1, 2, 16, and 26 were present solely in the control plots (Fig. 1b).

Cluster analysis of the DGGE band patterns further confirmed that methanogenic archaeal communities in the control

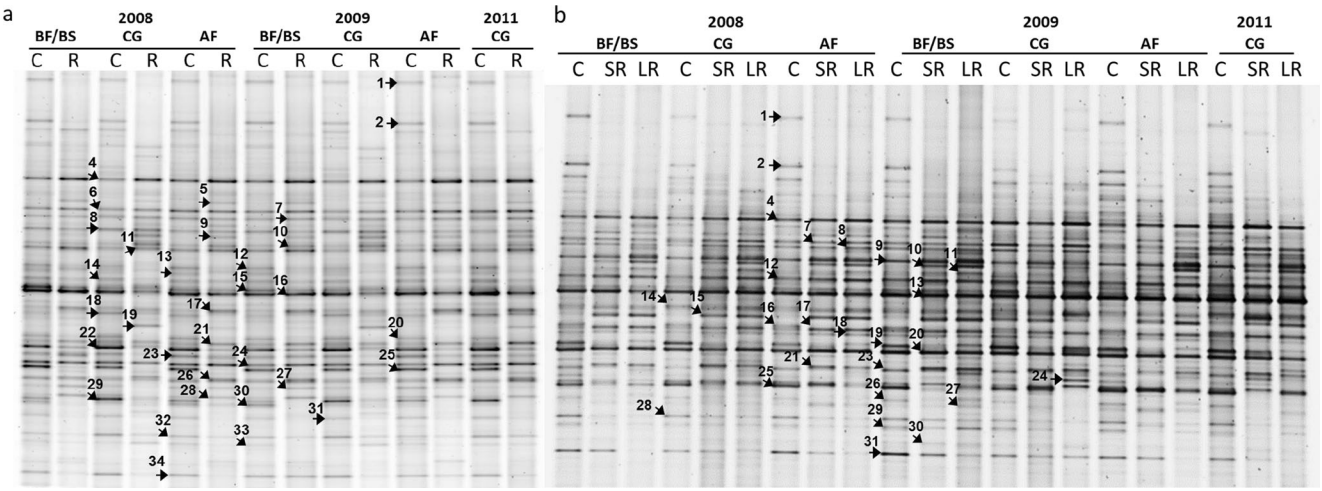

Fig. 1 DGGE band patterns of methanogenic archaeal 16S rRNA genes derived from the E (**a**) and 114 (**b**) fields at seven time sampling points in 2008, 2009, and 2011. *BF/BS* before flooding of paddy rice or before seeding of soybean; *CG* crop (rice/soybean) growing period; *AF* after crop (rice/soybean) harvest, *C* control plot; *R* rotational plot; *SR* short-term rotational plot; *LR* long-term rotational plot

Table 3 Taxonomic affiliation of DGGE bands in the E and 114 fields

Field	Close relative	Common bands	Specific bands	
E			Control	Rotation
	Methanosarcinales			
	Methanosaetaceae	Bands 13, 16, 17, 24	Bands 1, 2, 12, 20, 25	
	Methanosarcinaceae	Bands 23, 32, 33		
	ZC-I	Band 28	Bands 29, 31	
	Methanocellales	Bands 4, 6, 7, 8, 11		Band 5
	Methanomicrobiales	Bands 21, 22, 26, 27		
	Methanobacteriales	Band 14		Band 19
	Thaumarchaeota	Bands 10, 15, 34	Band 18	Band 9
	Crenarchaeota	Band 30		
114			Control	Rotation
	Methanosarcinales			
	Methanosaetaceae	Bands 13, 20	Bands 1, 2, 16	Band 21
	Methanosarcinaceae	Bands 14, 19, 29, 30		
	ZC-I	Bands 25, 28	Band 26	
	Methanocellales	Bands 4, 7, 8, 9		Bands 10, 11
	Methanomicrobiales	Bands 17, 18, 23, 24		
	Thaumarchaeota	Bands 12, 15, 31		
	Crenarchaeota	Band 27		

Band numbers are same as those in Fig. 1. Band 3 common for both the control and rotational plots in E field and bands 3 and 22 specific for the control plots in 114 field were not sequenced

plots were different from the community in the rotational plots in the both fields even in 2011, when all the plots were flooded under paddy rice cultivation (Fig. 2). Only small differences were found among the patterns at seven time sampling points in each plot.

Sequencing Analysis of Methanogenic Archaeal DGGE Bands

Phylogenic affiliation of methanogenic members was determined by sequencing the DGGE bands excised from the gels

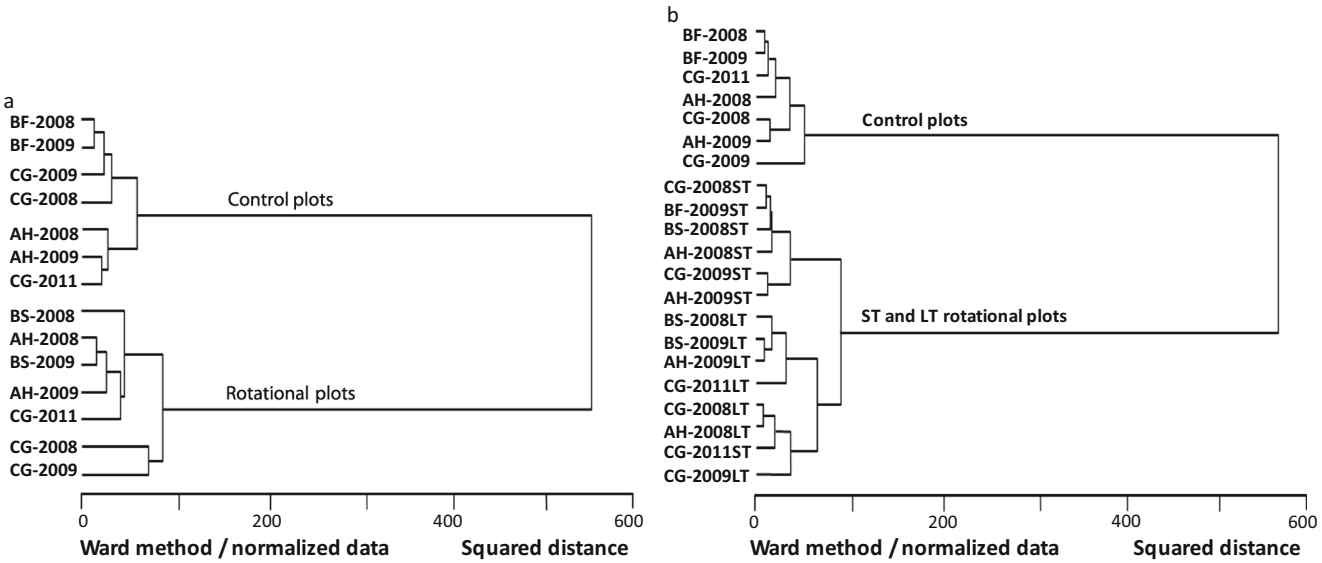

Fig. 2 Cluster analysis of DGGE band patterns of methanogenic archaeal 16S rRNA genes in the E (**a**) and 114 (**b**) fields in 2008, 2009, and 2011. ST and LT mean short- and long-term, respectively. *BF/BS* before flooding of paddy rice or before seeding of soybean; *CG* crop (rice/soybean) growing period; *AF* after crop (rice/soybean) harvest, *C* control plot; *R* rotational plot; *SR* short-term rotational plot; *LR* long-term rotational plot

in the E and 114 fields (Fig. 3). In the E field, 15, 5, 4, 1, 4, and 1 sequences in the control plot and 8, 6, 4, 2, 4, and 1 sequences in the rotational plot were closely related to *Methanosarcinales*, *Methanocellales*, *Methanomicrobiales*, *Methanobacteriales*, *Thaumarchaeota*, and *Crenarchaeota*, respectively (Fig. 3a). In the 114 field, 12, 4, 4, 3, and 1 sequences in the control plot and 9, 6, 4, 3, and 1 sequences in the rotational plots were affiliated with *Methanosarcinales*, *Methanocellales*, *Methanomicrobiales*, *Thaumarchaeota*, and *Crenarchaeota*, respectively (Fig. 3b). The DGGE bands of methanogenic archaea specific to the control plots belonged to *Methanosarcinales*. The bands of methanogenic archaea found exclusively in the rotation plots were affiliated with *Methanocellales* and *Methanobacteriales* for the E field and with *Methanosarcinales* and *Methanocellales* for the 114 field (Table 3).

Quantification of Methanogenic Archaeal *mcrA* Gene

The numbers of methanogenic archaeal *mcrA* genes in the E field ranged from 9.1×10^6 to 4.0×10^7 and 7.6×10^7 to 1.1×10^8 copies g^{-1} dry soil in the rotational and control plots, respectively (Fig. 4a). In the 114 field, the number of *mcrA* ranged from 1.3×10^7 to 3.5×10^7, 5.2×10^6 to 1.4×10^7, and 9.1×10^7 to 1.2×10^8 copies g^{-1} dry soil in the short-term, long-term rotational and control plots, respectively (Fig. 4b). The two-way ANOVA test (Table 4) showed that the gene copy numbers were significantly lower, less than one-tenth in some cases, in the rotational plots than that in the control plots in both the fields ($P<0.01$). All the data were compared among the groups each for the E or 114 field by the Tukey test because the interaction between the factors was significant (Table 4). Some significant differences were also found between short- and long-term rotational plots and among the sampling points (Fig. 4).

Discussion

In this study, the abundance of methanogenic archaeal population was significantly lower in the rotational plots than that

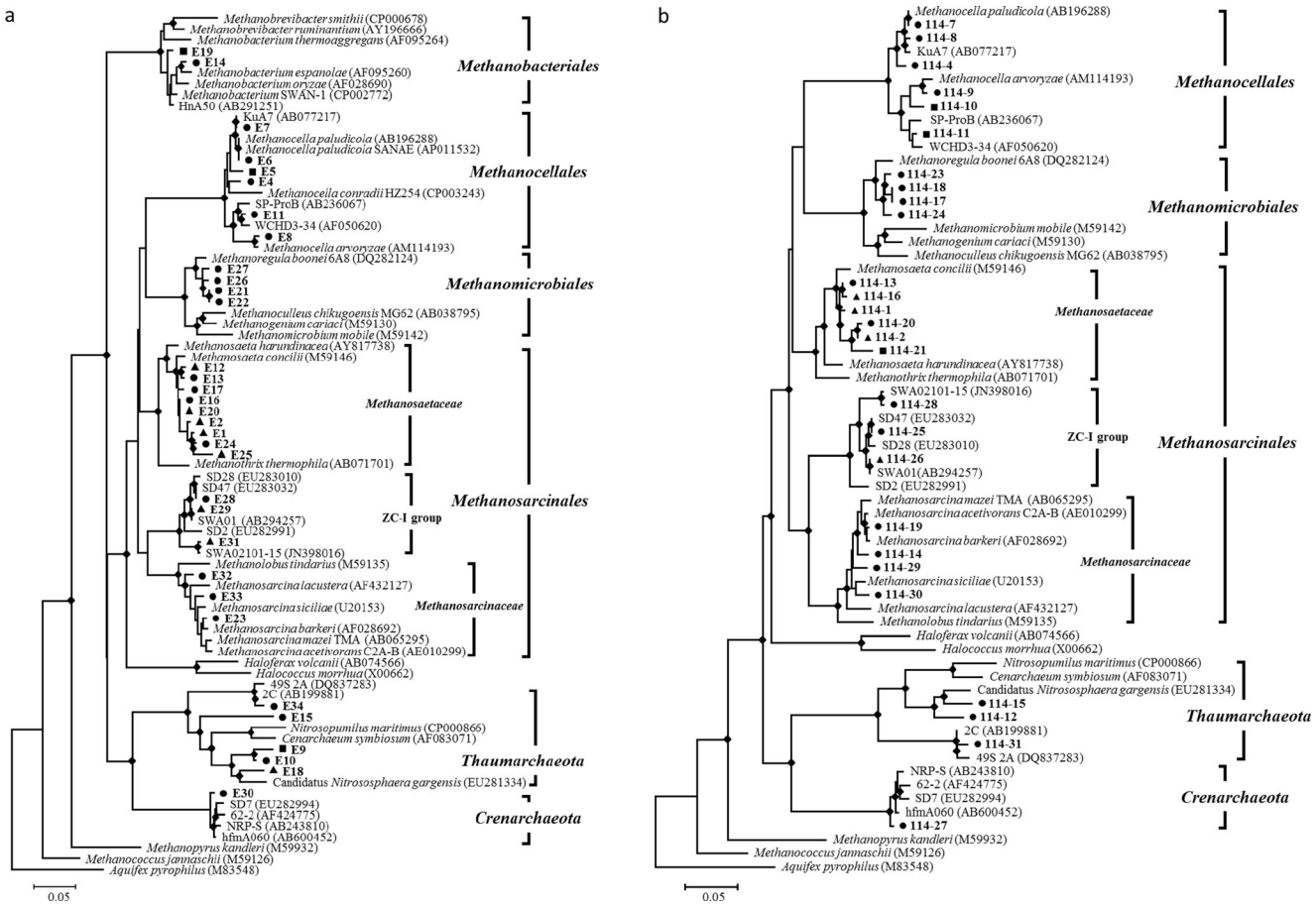

Fig. 3 Phylogenetic relationships of methanogenic archaeal 16S rRNA gene sequences retrieved from DGGE gels in the E (**a**) and 114 (**b**) fields. The sequences obtained in the present study are shown with *symbols plus numbers*. *Closed circles* indicate common bands in both control and rotational plots; *triangles and squares* indicate specific bands in the control and rotational plots, respectively. *Diamonds at branch points* show bootstrap values greater than 50 %. *Aquifex pyrophilus* was used as an outgroup

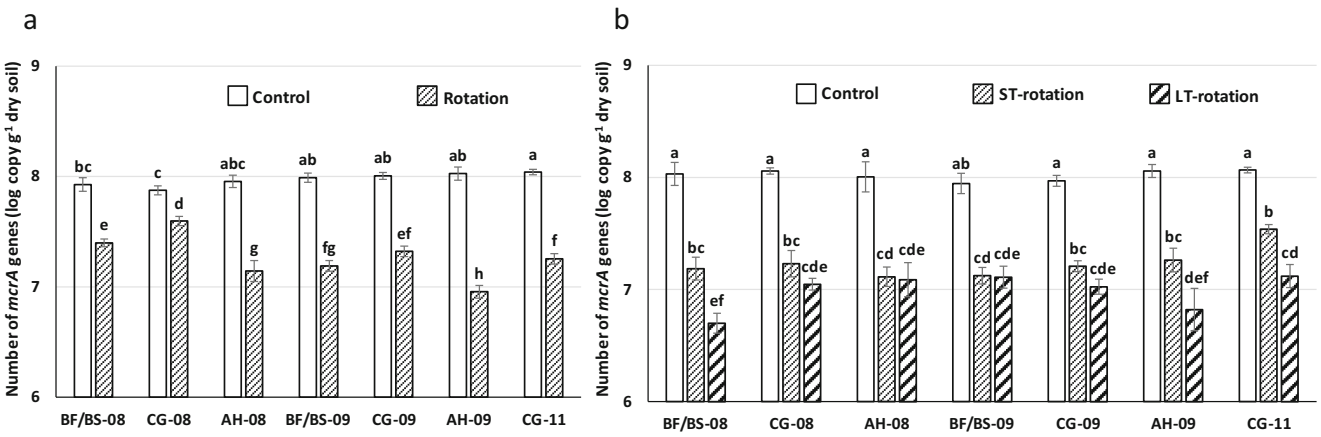

Fig. 4 Copy number of *mcrA* genes of methanogenic archaea in the E (**a**) and 114 (**b**) fields at seven times sampling points in 2008, 2009, and 2011. The number stands for log10 value. ST and LT mean short- and long-term, respectively. *BF/BS* before flooding of paddy rice or before seeding of soybean; *CG* crop (rice/soybean) growing period; *AF* after crop (rice/soybean) harvest. *Bars* represent ranges of mean values of duplicate measurements from two replicate plots. *Different letters* indicate statistically significant differences ($P<0.05$). Standard curve was obtained with a slope of −3.481, efficiency of 95.3 %, average *y* intercept of 38.67, and R^2 of 0.997. Amplification of nonspecific DNA fragments was not detected by a dissociation curve analysis

in the control plots (Fig. 4). In the E field, the abundance in the rotational plot was slightly lower in 2009 and 2011 than that in 2008, just after paddy rice cultivation (Fig. 4a). This means that methanogenic archaea decreased the population influenced by a relatively long duration of upland conditions. In the 114 field, the abundance was significantly lower ($P<0.05$) in the long-term rotational plots than that in the short-term rotational plots at three sampling points (Fig. 4b). Longer duration of upland conditions for 16 years in the long-term rotational plot had greater influences on the methanogenic archaea than the upland periods for 8 years in the short-term rotational plot (Table 1). On the other hand, recovery of the methanogenic archaeal population by paddy rice cultivation after upland cropping for years was not clearly demonstrated. Eusufzai et al. [9] reported that the population of methanogenic archaea was higher in the field after 19 years of conversion from soybean to paddy rice than in the fields after 1 and 2 years of the conversion. In addition, some studies showed that methanogenic archaea existed in upland or even in the desert soils increased the population by incubating the soils under flooded conditions [1, 22, 24]. Further monitoring of the dynamics of methanogenic archaea as for recovery of the abundance by 2 to 3 years of paddy rice cultivation after soybean cultivation will be needed in the rotational plots. These information may help to understand effects of paddy-upland rotation on methane production because Nishimura et al. [21] reported that methane emission was very low 1 year after conversion from upland crop to paddy rice, but increased conspicuously 2 years after the conversion.

Abundance of the methanogenic archaeal population decreased, but still stayed relatively large (10^6 to 10^7 copies of *mcrA* gene g^{-1} dry soil) in the rotational plots. There must be some mechanisms that these methanogenic archaea were adapted to dry-oxic conditions in the soil. It was reported that methanogenic archaea could survive well under the presence of oxygen and desiccation stress for several hours up to days in the pure culture and soil [10, 13, 16]. In addition, some members of methanogenic archaea possess detoxification enzymes such as superoxide dismutase and catalase to protect them from poisonous products derived from oxygen [6, 7, 25]. Besides the tolerance and detoxification ability of methanogenic archaea, anoxic microsites may have concealed methanogenic archaea and contributed the survivability. Methanogenic archaea might have obtained substrates for their survival from some facultative anaerobes which could degrade monosaccharides to acetate, CO_2, H_2, and formate in the anoxic microzones inside the aerated soils [8]. Further investigations are needed to elucidate the detailed mechanisms for the

Table 4 Two-way analysis of variance of copy number of *mcrA* genes in the E and 114 fields

Field	Factor	Degrees of freedom	*F*	*P*
E	Season	6	40.0	0.000**
	Rotation	1	4.98×10^3	0.000**
	Interaction	6	88.3	0.000**
	Error	14		
	Total	27		
114	Season	6	3.93	0.009**
	Rotation (ST and LT)	2	3.93×10^2	0.000**
	Interaction	12	2.80	0.019*
	Error	21		
	Total	41		

ST short term; *LT* long term

**$P<0.01$; *$P<0.05$

methanogenic archaea to survive with relatively high population in the soil under dry-oxic conditions.

Methanogenic archaeal community structure in the soil was affected by summer soybean cultivation under upland conditions for one or longer than 1 year in both the E and 114 fields (Figs. 1 and 2). These results did not correspond with the previous findings in the paddy fields under double cropping of summer paddy rice and winter wheat [4, 5, 30–32]. Methanogenic archaeal community and population were stable in the double-cropping fields throughout the year irrespective of flooded or drained conditions under winter wheat cultivation. Relatively shorter period of drainage for 7 months in the double-cropping fields than that in the rotational fields may have been less harmful to methanogenic archaea. One possible reason is that oxygen fully diffused into soil by plowing soil several times under upland conditions during the soybean cultivation. This may have been fatal to some of methanogenic archaea and they failed to survive. Another possible factor is low water content in the soils. For instance, methanogenic archaeal diversity was very low in soil with extremely low water content and only two main groups of methanogenic archaea, *Methanosarcinales* and *Methanocellales*, were detected [1, 2]. In the present study, the soil conditions presumably with low water content and increased oxygen availability for longer period may have changed the community structure of methanogenic archaea in the rotational plots. Watanabe et al. [33] showed that methanogenic archaeal community was affected by an alternated wetting and drying water-saving management in paddy field soil. Ma and Lu [16] reported that multiple drainages decreased methanogenic archaeal population in soil in a microcosm experiment.

Dominant groups of methanogenic archaea in the E and 114 fields (Fig. 3) corresponded with the previous findings in paddy field soils from other regions in Japan [14, 30] and also in Korea recently reported by pyrosequencing analysis [12]. Those members of methanogenic archaea existed in both the control and rotational plots (Table 3), indicating that they could be prevalent in the soils and well adapted to the upland conditions for years. In addition, some members of methanogenic archaea belonging to *Methanocellales* and *Methanobacteriales* in the E field and belonging to *Methanosarcinales* and *Methanocellales* in the 114 field were observed only in the rotational plots (Table 3). These members may have had tenacious resistance to dry-oxic soil conditions and survived very well for a long time. Thus, those common members and some specific members in the rotational plots might be inherent and had adaptation to dry-oxic conditions in the soils. Some studies also pointed out that some methanogenic archaea could survive in upland soils; e.g., *Methanosarcinales* and *Methanocellales* were detected in upland pasture and barley soils [18, 23]. Angel et al. [2] reported that groups of *Methanosarcinales*, *Methanocellales*, and also less dominantly *Methanobacteriales* were detected in various aerated soils and even in the desert soils collected globally.

On the other hand, some members of methanogenic archaea belonging to *Methanosarcinales* were exclusively present in the control plots and not found in the rotational plots in the E and 114 fields. It is interesting that these members of methanogenic archaea survived during drained periods such as before flooding and after harvest of rice in the control plots, but their DGGE bands disappeared in the rotational plots. Most of those damaged members of methanogenic archaea in the rotational plots belonged to *Methanosarcinales*, especially *Methanosaetaceae*, although some of these members were phylogenetically close with the members only observed in the rotational plots (Table 3 and Fig. 3). Simankova et al. [27] also reported that phylogenetically close relationships of methanogenic archaeal isolates from cold terrestrial habitats with mesophilic strains did not correlate with their ecophysiological traits in the case of psychrotolerance. In addition to effects of dry-oxic conditions of the soils mentioned above, a shortage of substrates supply might have affected survivability of these *Methanosaetaceae* members who use acetate as the sole substrate for growth because acetate is produced in the anaerobic decomposition of organic matter.

In conclusion, the composition of methanogenic archaeal community was changed and the population decreased about one-tenth in the paddy-upland rotational field. This study revealed that methanogenic archaeal community structure in paddy field soil was affected by the paddy-upland rotation unlike in the field under double cropping. Some methanogenic archaea still survived in the rotational field. The next question is which member that remained in the methanogenic archaeal community sustains activity and what degree is the activity. Further investigation on the methanogenic archaeal community based on the RNA analysis will be necessary in the future.

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