https://doi.org/10.17221/231/2018-PSE

Nitrate addition inhibited methanogenesis in paddy soils under long-term managements

Jun WANG[1,3], Tingting XU[1], Lichu YIN[2], Cheng HAN[1,4], Huan DENG[1,4], Yunbin JIANG[1,*], Wenhui ZHONG[1,4]

[1]*Jiangsu Provincial Key Laboratory of Materials Cycling and Pollution Control, Nanjing Normal University, Nanjing, P.R. China*
[2]*College of Resources and Environment, Hunan Agricultural University, Changsha, P.R. China*
[3]*Shandong Provincial Key Laboratory of Water and Soil Conservation and Environmental Protection, Linyi, P.R. China*
[4]*Jiangsu Center for Collaborative Innovation in Geographical Information Resource Development and Application, Nanjing, P.R. China*
**Corresponding author: jiangyunbin89@163.com*

ABSTRACT

Wang J., Xu T.T., Yin L.C., Han C., Deng H., Jiang Y.B., Zhong W.H. (2018): Nitrate addition inhibited methanogenesis in paddy soils under long-term managements. Plant Soil Environ., 64: 393–399.

Rice fields are a major source of atmospheric methane (CH_4). Nitrate has been approved to inhibit CH_4 production from paddy soils, while fertilization as well as water management can also affect the methanogenesis. It is unknown whether nitrate addition might result in shifts in the methanogenesis and methanogens in paddy soils influenced by different practices. Six paddy soils of different fertilizer types and groundwater tables were collected from a long-term experiment site. CH_4 production rate and methanogenic archaeal abundance were determined with and without nitrate addition in the microcosm incubation. The structure of methanogenic archaeal community was analysed using the PCR-DGGE (polymerase chain reaction denaturing gradient gel electrophoresis) and pyrosequencing. The results showed that nitrate addition significantly decreased the CH_4 production rate and methanogenic archaeal abundance in all six paddy soils by 70–100% and 54–88%, respectively. The quantity, position and relative intensity of DGGE bands exhibited differences when nitrate was added. Nitrate suppressed the growth of methanogenic archaeal species affiliated to Methanosaetaceae, unidentified Euryarchaeota, Thaumarchaeota and Methanosarinaceae. The universal inhibition of nitrate addition on the methanogenesis and methanogens can be adopted as a practice of mitigating CH_4 emission in paddy soils under different fertilization and water managements.

Keywords: *Oryza sativa* L.; mineral fertilizer; organic manure; biomethanation; archaeal 16S rRNA gene

Rice fields have been recognized as a major source of atmospheric methane (CH_4), with up to 15~20% of the total global CH_4 emissions to the atmosphere (Conrad 2002, Scheer et al. 2008). A positive balance between methanogenesis and methanotrophy leads to the CH_4 emission from paddy soils. Methanogens, belonging to the domain Archaea, have a limited trophic spectrum comprised of simple substrates, including H_2 + CO_2 and acetate (Boone et al. 1993). Nitrate can act as alternative hydrogen sink and inhibits CH_4 production from anoxic soil, which has been widely observed in paddy soils (Lu et al. 2000, Banger et al. 2012). However, paddy fields of the rice harvest area are under various management practices, which also affects methanogenesis through their effects on methanogens. The inhibitive efficiency of

Supported by the National Key Research and Development Plan of China, Project No. 2016YFD0200302.

https://doi.org/10.17221/231/2018-PSE

nitrate on CH_4 production is worth investigations in paddy soils under complex conditions.

Fertilization and water managements are the main cultural practices that affect the methanogenesis. *In situ* studies have shown that the type of fertilizers differs in CH_4 emission (Snyder et al. 2009, Linquist et al. 2012). Organic matter incorporation markedly increased CH_4 emission, while the effects of mineral fertilizers on CH_4 emission are complex and sometimes contradictory (Cai et al. 1997, Linquist et al. 2012). An increase of CH_4 emission was observed with urea applied in continuously flooded rice fields (Lindau and Bollich 1993). In China, mid-season aeration is one basic practice for raising rice yields and is widely adopted in rice cultivation (Cai et al. 1997). *In situ* studies report a significant decrease of CH_4 emission by rice fields that are drained one or several times during the crop cycle (Yagi et al. 1996, Hadi et al. 2010). In addition, the groundwater table controlling the soil O_2 concentration and redox potential is another non-negligible practice, which affects the methanogenesis and methanogens in paddy fields. Submerged soils maintained at different Eh values differed in methanogenesis (Kludze and DeLaune 1995). Combined with the application of organic fertilizer, an appropriate groundwater table could provide the optimum conditions for methanogenesis. As a competitor for H_2, the inhibition of nitrate on the methanogenesis and methanogens in paddy soils influenced by different practices has not been thoroughly interpreted.

Red soils are widely distributed throughout the tropical and subtropical areas of South China and account for 6.5% of the total arable land. In this context, a total of six red paddy soils under different fertilization and groundwater tables were collected. Nitrate was added in the subsamples of the soils followed by microcosm incubation. The rate of CH_4 production was measured and the abundance and community structure of methanogenic archaea were analysed. The aims of this study were to determine (1) the inhibitive efficiency of nitrate on the methanogenesis and (2) the shifts of nitrate in the methanogens developed under different long-term fertilization and water managements.

MATERIAL AND METHODS

Site description and soil sampling. Surface soil samples from six treatments were collected from a long-term fertilization station: (i) high-water-level (groundwater table –20 cm) mineral fertilizer (A); (ii) low-water-level (groundwater table –80 cm) mineral fertilizer (B); (iii) high-water-level normal amount of organic manure (C); (iv) low-water-level normal amount of organic manure (D); (v) high-water-level high amount of organic manure (E), and (vi) low-water-level high amount of organic manure (F). The fertilization station was established on a Ferralic Cambisol soil derived from the Quaternary red clay in 1982 on the campus of the Hunan Agricultural University, Hunan province, China. The site has a subtropical monsoon climate and a crop succession of early rice, late rice and winter fallow. The nitrogen (N) fertilizer was provided as urea at 150 kg/ha, P as single superphosphate at 33 kg/ha for early rice or 13 kg/ha for late rice, and K as KCl at 125 kg/ha. The seasonal applications of high amount of organic manure were provided as fresh *Lolium perenne* L. at 45 000 kg/ha for early rice and 25 000 kg/ha for late rice. Those of normal amount were half of the high amount, respectively.

There were six replicates for each treatment in the long-term experiment. Three cores from each replicates were randomly sampled to a 0–20 cm depth. Each sample was a composite of eighteen random cores collected from a single treatment and three soil samples were collected for each treatment. The fresh soil was mixed thoroughly, and sieved through 2 mm screens for incubation and further analysis. Basic physicochemical properties of the soil samples were listed in Table 1.

Nitrate addition and incubation. For each treatment, sieved fresh soil (equivalent to 10.0 g DW (dry weight)) was added in a 120 mL serum bottles. Distilled water was added to keep the soil/water ratio at 1:1. Nitrate (NO_3^--N 50 mg/kg in KNO_3 solution) was added into half of the bottles. Then the serum bottles were capped with butyl stoppers and aluminum caps, flushed with N_2 for 6 min, and incubated without shaking at 30°C in the dark. On each of the five different sampling dates, 36 bottles (6 treatments × 2 nitrate incubations × 3 replicates) were sacrificed. The bottles after incubating for 1 h were sampled as day 0.

Chemical measurements. Gas samples (20 mL) were collected from the headspace of the serum bottles using a pressure-lock syringe on the sampling dates. The concentrations of CH_4 was analysed using a gas chromatograph (GC, 7890A, Agilent

https://doi.org/10.17221/231/2018-PSE

Table 1. Basic physicochemical properties of paddy soils under different fertilization and water managements

	pH	Organic C	Total N	Available N†	NH_4^+-N	NO_3^--N
		(g/kg)			(mg/kg)	
A	5.51 ± 0.04^a	14.9 ± 1.27^{ab}	1.41 ± 0.20^c	93.5 ± 6.36^a	8.23 ± 0.80^a	1.20 ± 0.44^b
B	5.50 ± 0.09^a	14.1 ± 1.16^b	1.26 ± 0.19^d	99.6 ± 11.8^a	10.1 ± 3.14^a	1.98 ± 0.12^{ab}
C	5.51 ± 0.02^a	19.4 ± 2.41^a	1.58 ± 0.23^b	107 ± 7.80^a	11.2 ± 1.26^a	1.30 ± 0.35^b
D	5.54 ± 0.03^a	18.0 ± 1.53^{ab}	1.60 ± 0.16^b	116 ± 17.5^a	10.1 ± 0.44^a	4.18 ± 1.43^a
E	5.48 ± 0.10^a	19.7 ± 1.45^a	1.64 ± 0.16^b	115 ± 23.6^a	10.4 ± 0.76^a	1.77 ± 0.49^{ab}
F	5.46 ± 0.12^a	19.5 ± 2.09^a	1.77 ± 0.24^a	104 ± 17.7^a	10.7 ± 0.28^a	3.80 ± 1.85^{ab}

†Available nitrogen refers to the nitrogen easily absorbed and utilized by crops in soil, including ammonium, nitrate, amino, amide and some simple peptides and protein compounds. Mean ± standard error. Different letters within each column indicate significant differences in soils ($P < 0.05$, Tukey's test)

Technologies, Palo Alto, USA) equipped with a flame ionization detector (FID). The concentrations of N was extracted with 2.0 mol/L KCl and determined by a segmented flow analyser (Skalar SAN Plus, Skalar Inc., Breda, The Netherlands).

Soil DNA extraction. After incubation for 7 days, triplicate bottles from each treatment were destructively sampled and the fresh soil was stored at –80°C within 15 min. Total soil DNA was extracted from 0.5 g soil using a bead beating as described in the manufacturer's instructions (MP Biomedicals, Santa Ana, USA). Purity and quantity of DNA were determined using a Nanodrop ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, USA). DNA extracts were stored at –20°C until use.

Quantitative PCR. The abundance of methanogenic archaeal 16S rRNA gene was quantified by qPCR on a CFX96 Optical Real-Time Detection System (Bio-Rad, USA) in three biological replicates and each with three technical replicates using primer pairs 1106F/1378R (Feng et al. 2012). The reaction was performed in a 20 µL mixture containing 10 µL SYBR Premix Ex Taq (Takara, Shanghai, China), 0.5 µmol of each primer, and 1 µL of DNA template. The reaction conditions were as follows: 95°C for 3 min, 40 cycles of 95°C for 30 s, 57°C for 20 s and 72°C for 30 s and followed by plate reads at 83°C. Amplification efficiencies of 96.5–103.0% were obtained with R^2 values of 0.993–0.997.

PCR-DGGE. PCR products used for DGGE were amplified by primer pairs 1106F-GC/1378R (Watanabe et al. 2004), targeting methanogenic archaeal 16S rRNA gene. The reaction was performed in a 50 µL mixture containing 6 µL of 10 × HotStar-Taq buffer, 0.5 µL of each 20 µmol/L primer, 2.5 U HotStar-Taq polymerase (TaKaRa) and 1 µL DNA template. Reaction conditions were as follows: initial denaturation at 94°C for 3 min; 35 cycles consisting of 94°C for 45 s, 55°C for 30 s, and 72°C for 30 s and followed by a final extension at 72°C for 6 min. DGGE was performed using a Dcode Universal Mutation Detection System (Bio-Rad). PCR products (about 200 ng) were loaded onto an 8% polyacrylamide (acrylamide: bisacrylamide = 37.5:1) gel with a linear gradient of 30–70% (100% denaturant contains 7 mol/L urea and 40% formamide) denaturant for methanogenic archaeal 16S rRNA gene. The gel was electrophoresed at 100 V for 14 h with a constant temperature of 60°C.

Cloning, sequencing, and phylogenetic analysis. The dominant bands in the DGGE gel were excised and DNA was eluted by incubating each band in 40 µL of sterilized distilled water over night at 4°C. The re-amplified PCR products using primer pairs 1106F/1378R of the eluted DNA solutions were cloned using pEASY-T1 vector (TransGen Biotech, Beijing, China) according to the instructions of the manufacturer. Three clones containing the correct gene insert fragments from each DGGE band were sequenced (Genscript, Nanjing, China). Phylogenetic analysis was performed with MEGA 4.0 according to the protocol described previously (Tamura et al. 2007).

Statistical analyses. Analysis of variance (ANOVA) followed by the Tukey's post hoc test were performed to assess the differences within the data sets. All data were analysed using a statistical package, SPSS 18.0 and $P < 0.05$ was considered to be statistically significant.

https://doi.org/10.17221/231/2018-PSE

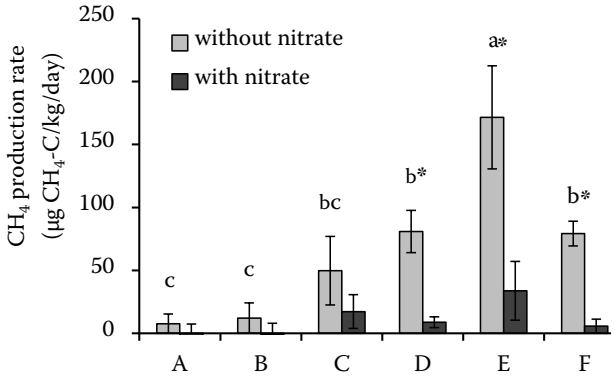

Figure 1. CH_4 production rates in different fertilization and water management of paddy soils with or without nitrate addition. Vertical bars denote a standard deviation of means (n = 3). Different letters above vertical bars indicate a significant difference among different soils without nitrate addition (P < 0.05, Tukey's test). A significant difference between treatment with and without nitrate addition is shown with asterisk

RESULTS

Methanogenesis in paddy soils under long-term fertilization and water managements. The contents of organic C and N in collected paddy soils were shown in Table 1. The nitrate reduction rates of the paddy soils were from 11.91 ± 0.20 mg/kg/day to 14.33 ± 0.28 mg/kg/day. The NO_3^--N contents of the soils all decreased to around 1 mg/kg within 3 days since nitrate added (data not shown).

Without nitrate addition, the CH_4 production rates of organic manure treatments (C, D, E and F) were significantly higher than the mineral fertilizer treatments (A and B) between fertilizer types (Figure 1). Between groundwater tables, only the difference in high amount of organic manure treatments (E and F) reached a significant level. The results of two-way ANOVA concluded that fertilization had significant (P < 0.001) while groundwater table had no significant (P = 0.09) impact on the CH_4 production rate of paddy soils. The results of Pearson's correlation analysis showed that the CH_4 production rate had no significant correlation with soil pH, organic carbon, nitrogen contents or nitrate reduction rate (data not shown).

With nitrate addition, the CH_4 production rate all decreased compared with those without nitrate addition. For the treatments (D, E and F) possessing relatively fast CH_4 production rate, nitrate addition significantly inhibited the methanogenesis. In A and B, approximately no CH_4 was produced after nitrate addition during the incubation. Nitrate addition decreased the CH_4 production rate by 69.6–100%.

Methanogens in paddy soils under long-term fertilization and water managements. Without nitrate addition, the methanogenic archaeal 16S rRNA gene copies of soil C were significantly higher than the other treatments (Figure 2). The results of two-way ANOVA concluded that fertilization (P < 0.001) as well as groundwater table (P < 0.05) both had significant impacts on the methanogenic archaeal abundances. The results of Pearson's correlation analysis showed that the methanogenic archaeal abundance had no significant correlation with soil basic physiochemical properties and nitrate reduction rate (data not shown). With nitrate addition, the methanogenic archaeal 16S rRNA gene copies of all the treatments were significantly lower than those without addition. Nitrate addition decreased the methanogenic archaeal abundance by 53.5–87.9%.

DGGE profile based on the methanogenic archaeal 16S rRNA gene was shown in Figure 3. The

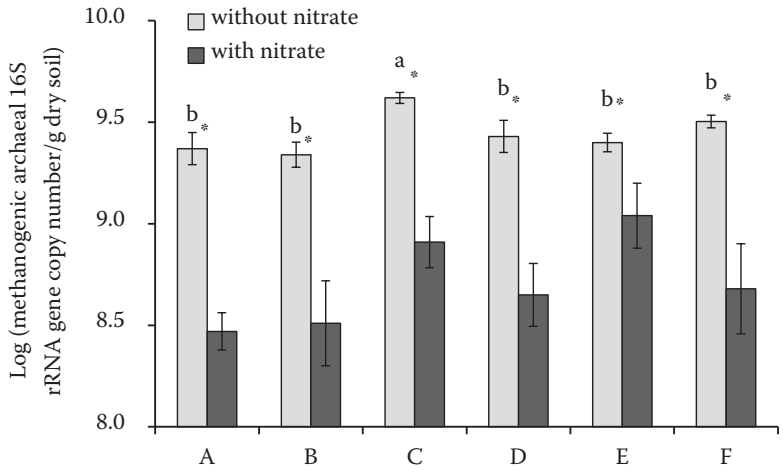

Figure 2. Log numbers of methanogenic archaeal 16S rRNA gene copies in different fertilization and water management of paddy soils with or without nitrate addition. Vertical bars denote a standard deviation of means (n = 3). Different letters above vertical bars indicate a significant difference among different soils without nitrate addition (P < 0.05, Tukey's test). A significant difference between treatments with and without nitrate addition is shown with asterisk

https://doi.org/10.17221/231/2018-PSE

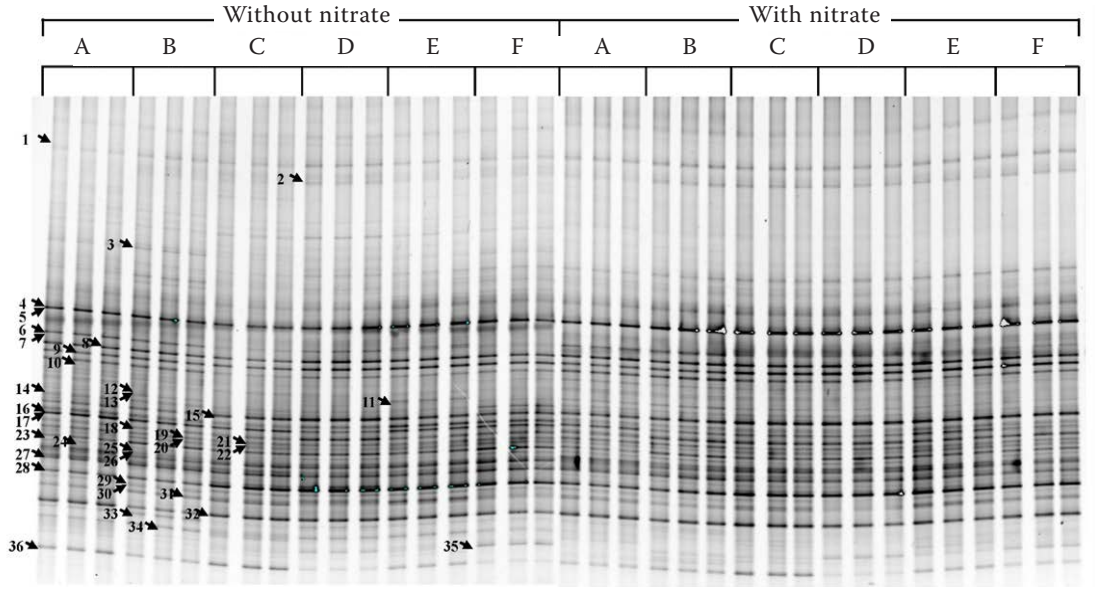

Figure 3. Denaturing gradient gel electrophoresis (DGGE) profiles of amplified methanogenic archaeal 16S rRNA gene obtained from different fertilization and water management of paddy soils with or without nitrate addition

quantity, position and relative intensity of bands had differences among the treatments. The band quantities of mineral fertilizer treatments were higher than those of organic manure treatments. For all treatments, nitrate addition decreased the quantities of DGGE bands.

A total of 36 DGGE bands were recovered and the contained methanogenic archaeal 16S rRNA genes were sequenced (Figure 4). Bands 3 and 11 were detected only in the treatments without nitrate addition, and affiliated to Methanosaetaceae and unidentified Euryarchaeota, respectively. The intensities of bands 33 and 34 were dramatically lower in the treatments with nitrate added than those without nitrate added. They belonged to Thaumarchaeota and Methanosarinaceae, respectively. The intensity of band 10 belonging to Rice Cluster I was higher with nitrate addition.

DISCUSSION

With nitrate addition, the CH_4 production rate was significantly inhibited in all six paddy soils. For mineral fertilizer treatments, CH_4 production rate was completely suppressed. For organic fertilizer treatments, the inhibitive efficiency of nitrate ranged from 70–93%. The inhibition can be efficient in soils with a wide range of methanogenesis rate. For high groundwater table, the CH_4 production rate was decreased on average about 83%, while the inhibitive efficiency was on average about 93% for low groundwater table. Nitrate would be a universal inhibitor for CH_4 production in paddy soils under different groundwater conditions. The application of nitrate might be adopted to mitigate the CH_4 emission in rice fields. Furthermore, nitrate would also increase the crop yields as an N fertilizer for the nitrate-preferred plants (Fageria and Baligar 2005).

The inhibitory effect of nitrate on methanogens could be explained by two mechanisms. On one hand, there would be a lack in H_2 for methanogenesis during the phase of nitrate reduction due to the substrate competition by the nitrate reducers (Roy and Conrad 1999). This effect can also be observed when closed-circuit MFCs were operated in paddy soils, causing the substrate competition with methanogens by the exoelectrogenic bacteria (Zhong et al. 2017). On the other hand, the denitrification intermediates (nitrite, NO and N_2O) could bring toxicity to the methanogens and hence depress their growth and activity (Zumft 1993, Choi et al. 2006).

Based on the PCR-DGGE analysis, nitrate addition decreased the abundance of methanogens affiliated to Methanosaetaceae, unidentified Euryarchaeota, Thaumarchaeota and Methanosarinaceae. Scheid et al. (2003) reported that the methanogenic archaeal community was altered by nitrated addition,

https://doi.org/10.17221/231/2018-PSE

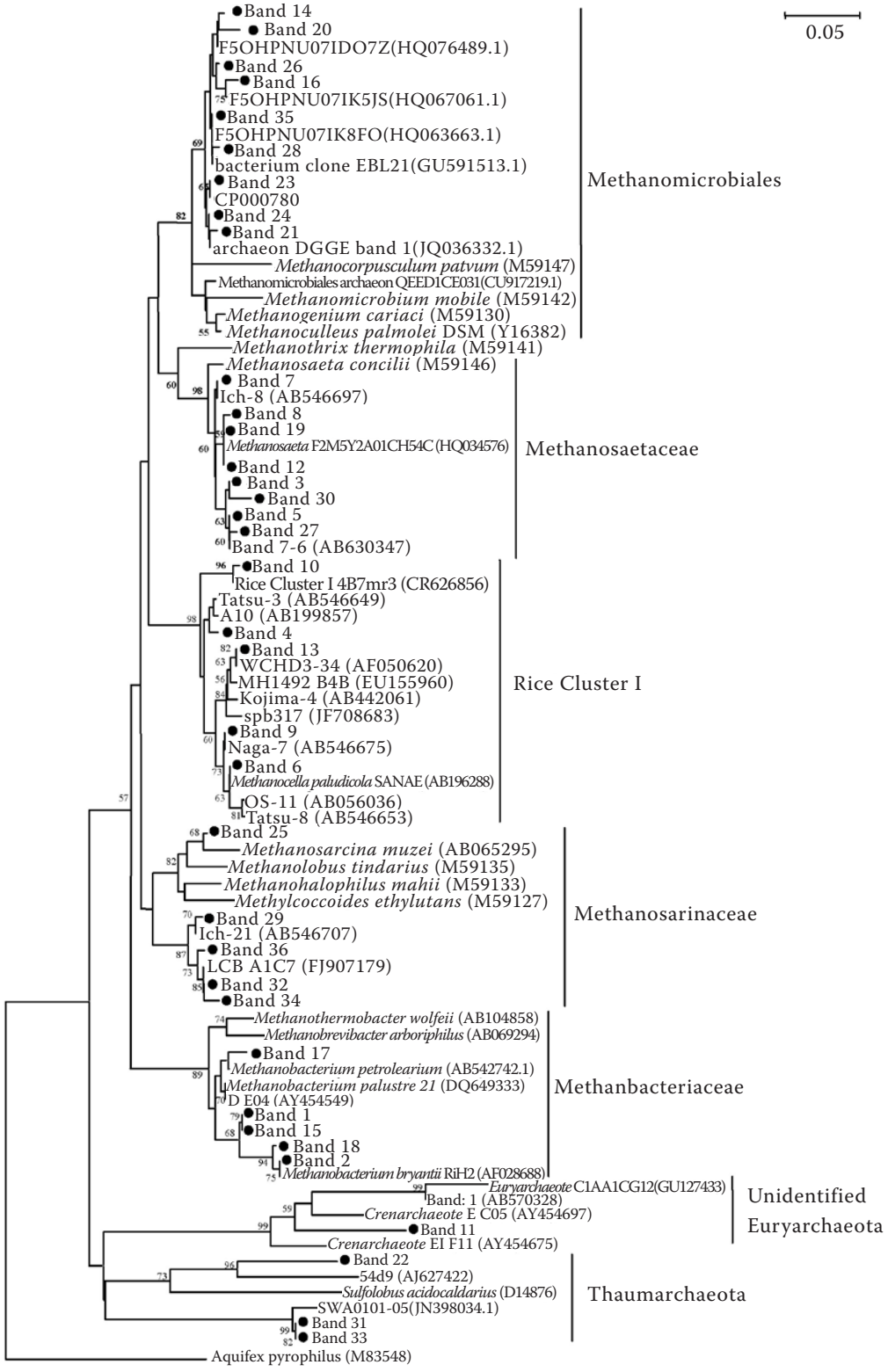

Figure 4. Phylogenetic relationships of methanogenic archaeal 16S rRNA gene sequences derived from different fertilization and water management of paddy soils with or without nitrate addition. The phylogenetic trees were constructed by 1000-fold bootstrap analysis using the neighbour-joining method and maximum composite likelihood model. Bootstrap values (%) are shown at branch points (more than 50%). Aquifex pyrophilus (M83548) was used as the out-group. The scale bars represent 0.05 substitutions per nucleotide

https://doi.org/10.17221/231/2018-PSE

significant for those utilizing H_2. The archaeal groups suppressed would be the major contributors to the CH_4 production in the paddy soil samples collected (Yuan and Lu 2009, Dubey et al. 2014, Hernández et al. 2017). A decrease in their abundance resulted in the falling of CH_4 production rate. Besides, archaeal groups affiliated to Methanomicrobiales and Methanobacteriaceae were not significantly affected by the nitrate addition. Methanogens would be dormant or starved but can still survive in unfavourable conditions (Watanabe et al. 2007). Their activities of CH_4 production may be suppressed by competition or toxicity and the DNA of inactive cells can also be detected. Nevertheless, the growth of one species belonging to Rice Cluster I was slightly stimulated by nitrate addition. Certain methanogenic archaea were resistant to environmental stress and toxicity (Yuan et al. 2009). It might be more favourable for their metabolism when other methanogenic groups are suppressed to be inactive.

REFERENCES

Banger K., Tian H.Q., Lu C.Q. (2012): Do nitrogen fertilizers stimulate or inhibit methane emissions from rice fields? Global Change Biology, 18: 3259–3267.

Boone D.R., Whitman W.B., Rouvière P. (1993): Diversity and taxonomy of methanogens. In: Ferry J.G. (ed.): Methanogenesis. Boston, Springer, 35–80.

Cai Z.C., Xing G.X., Yan X.Y., Xu H., Tsuruta H.R., Yagi K., Minami K. (1997): Methane and nitrous oxide emissions from rice paddy fields as affected by nitrogen fertilisers and water management. Plant and Soil, 196: 7–14.

Choi P.S., Naal Z., Moore C., Casado-Rivera E., Abruña H.D., Helmann J.D., Shapleigh J.P. (2006): Assessing the impact of denitrifier-produced nitric oxide on other bacteria. Applied and Environmental Microbiology, 72: 2200–2205.

Conrad R. (2002): Control of microbial methane production in wetland rice fields. Nutrient Cycling in Agroecosystems, 64: 59–69.

Dubey S.K., Singh A., Watanabe T., Asakawa S., Singla A., Arai H., Inubushi K. (2014): Methane production potential and methanogenic archaeal community structure in tropical irrigated Indian paddy soils. Biology and Fertility of Soils, 50: 369–379.

Fageria N.K., Baligar V.C. (2005): Enhancing nitrogen use efficiency in crop plants. Advances in Agronomy, 88: 97–185.

Feng Y.Z., Xu Y.P., Yu Y.C., Xie Z.B., Lin X.G. (2012): Mechanisms of biochar decreasing methane emission from Chinese paddy soils. Soil Biology and Biochemistry, 46: 80–88.

Hadi A., Inubushi K., Yagi K. (2010): Effect of water management on greenhouse gas emissions and microbial properties of paddy soils in Japan and Indonesia. Paddy and Water Environment, 8: 319–324.

Hernández M., Conrad R., Klose M., Ma K., Lu Y.H. (2017): Structure and function of methanogenic microbial communities in soils from flooded rice and upland soybean fields from Sanjiang plain, NE China. Soil Biology and Biochemistry, 105: 81–91.

Kludze H.K., DeLaune R.D. (1995): Gaseous exchange and wetland plant response to soil redox intensity and capacity. Soil Science Society of America Journal, 59: 939–945.

Lindau C.W., Bollich P.K. (1993): Methane emissions from Louisiana first and Ratoon crop rice. Soil Science, 156: 42–48.

Linquist B.A., Adviento-Borbe M.A., Pittelkow C.M., van Kessel C., van Groenigen K.J. (2012): Fertilizer management practices and greenhouse gas emissions from rice systems: A quantitative review and analysis. Field Crops Research, 135: 10–21.

Lu Y.H., Wassmann R., Neue H.-U., Huang C.Y. (2000): Dissolved organic carbon and methane emissions from a rice paddy fertilized with ammonium and nitrate. Journal of Environmental Quality, 29: 1733–1740.

Roy R., Conrad R. (1999): Effect of methanogenic precursors (acetate, hydrogen, propionate) on the suppression of methane production by nitrate in anoxic rice field soil. FEMS Microbiology Ecology, 28: 49–61.

Scheer C., Wassmann R., Kienzler K., Ibragimov N., Lamers J.P.A., Martius C. (2008): Methane and nitrous oxide fluxes in annual and perennial land-use systems of the irrigated areas in the Aral Sea Basin. Global Change Biology, 14: 2454–2468.

Scheid D., Stubner S., Conrad R. (2003): Effects of nitrate- and sulfate-amendment on the methanogenic populations in rice root incubations. FEMS Microbiology Ecology, 43: 309–315.

Snyder C.S., Bruulsema T.W., Jensen T.L., Fixen P.E. (2009): Review of greenhouse gas emissions from crop production systems and fertilizer management effects. Agriculture, Ecosystems and Environment, 133: 247–266.

Tamura K., Dudley J., Nei M., Kumar S. (2007): MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 24: 1596–1599.

Watanabe T., Asakawa S., Nakamura A., Nagaoka K., Kimura M. (2004): DGGE method for analyzing 16S rDNA of methanogenic archaeal community in paddy field soil. FEMS Microbiology Letter, 232: 153–163.

Watanabe T., Kimura M., Asakawa S. (2007): Dynamics of methanogenic archaeal communities based on rRNA analysis and their relation to methanogenic activity in Japanese paddy field soils. Soil Biology and Biochemistry, 39: 2877–2887.

Yagi K., Tsuruta H., Kanda K.I., Minami K. (1996): Effect of water management on methane emission from a Japanese rice paddy field: Automated methane monitoring. Global Biogeochemical Cycles, 10: 255–267.

Yuan Y., Conrad R., Lu Y. (2009): Responses of methanogenic archaeal community to oxygen exposure in rice field soil. Environmental Microbiology Reports, 1: 347–354.

Yuan Q., Lu Y. (2009): Response of methanogenic archaeal community to nitrate addition in rice field soil. Environmental Microbiology Reports, 1: 362–369.

Zhong W.H., Cai L.C., Wei Z.G., Xue H.J., Han C., Deng H. (2017): The effects of closed circuit microbial fuel cells on methane emissions from paddy soil vary with straw amount. Catena, 154: 33–39.

Zumft W.G. (1993): The biological role of nitric oxide in bacteria. Archives of Microbiology, 160: 253–264.

Received on April 7, 2018
Accepted on June 20, 2018
Published online on June 29, 2018