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Methane Production/Oxidation Potential and Methanogenic Archaeal Diversity in Two Paddy Soils of Japan

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ABSTRACT

Soil characteristics regulate various belowground microbial processes and consequently affect the structure and function of microbial communities due to change in soil type which in turn influences the CH_4 production/oxidation potential of soils. Thus, two different soil types (Andosol and Regosol) were studied to assess their CH_4 production/oxidation potential and also the methanogenic archaeal diversity in Japanese paddy soils. Andosol produced significantly higher concentration of CH_4 and CO_2 under waterlogged incubation. It also consumed CH_4 quicker than Regosol soil and also produced significantly higher concentration of CO_2 under aerobic incubation. The cumulative CH_4 production was found to be significantly correlated with soil's physico-chemical properties. Denaturing Gradient Gel Electrophoresis showed differences in methanogenic archaeal banding pattern in both soils. The present study suggested that CH_4 production/oxidation potential of different soils depends on physico-chemical properties and microbial communities.

Key Words: Methane Oxidation; Carbon Dioxide Production; Soil Characteristics; DGGE· Methanogens; Andosol; Regosol

INTRODUCTION

Among various sources, rice cultivation is considered as one of the most important anthropogenic sources that accounts for 10-15% of the global methane (CH_4) emission to the atmosphere (Cheng et al. 2008). The global average CH_4 emission from rice cultivation is approximately 60 Tg CH_4 yr^{-1} which may increase further due to the expansion of rice cultivation for the rising world population (Cheng et al. 2008). After carbon dioxide (CO_2), methane (CH_4) is a major greenhouse gas (GHG) with almost 25-folds higher global warming potential than CO_2 (IPCC 2007). The concentration of CH_4 in the atmosphere has increased from the pre-industrial level of 715 ppb to about 1,774 ppb in 2005 (IPCC 2007). Therefore, characterizing rice ecosystems in relation to the CH_4 production potential of soils has attracted scientific investigators to address the issue and suggest possible mitigation strategies. Rice productivity and C storage in paddy soils are major areas of concern which mainly depend on the field management practices such as tillage operations, crop residue management, soil amendments with desirable nutrients, fertilizer applications and irrigation water, which ultimately affect CH_4 emission (Singla and Inubushi 2014 a, b).

Spatio-temporal variability of CH_4 production has also been observed in different studies (Wachinger et al. 2000; Mitra et al. 2002). A considerable diversity of methanogenic archaeal community has been observed in different soil types of Japan, China, Italy and Thailand using "culture-independent" techniques such as T-RFLP, DNA-SIP and DGGE (Watanabe et al. 2009; Wang et al. 2010; Conrad et al. 2012) and different reasons are responsible for diversity in methanogenic archaeal community structure in different soil types. Our previous studies showed that different kinds of soil amendments in one soil type can influence GHG in different manner (Singla and Inubushi 2013, 2014 a, c, Singla et al. 2013).

Our another study with Indian Paddy soils showed that CH_4 production potential of soils could be different depending on the soil type, soil chemical properties as well as changes in methanogenic archaeal diversity (Dubey et al. 2014). However, this study didn't provide any evidence that how CH_4 oxidation potential could be different with variations in soil types.

Based on previous reports, it was hypothesized that CH_4 production as well as oxidation potential should be different with variations in soil type. A laboratory incubation experiment with two different paddy soils of Japan was conducted to assess the CH_4 production/ oxidation potential and associated methanogenic archaeal diversity in both soils.

MATERIALS AND METHODS

Experimental Site and Soil Analyses

Soil samples (non-rhizosphere) were collected from rice fields located at Chiba (Regosol) and Nagano (Andosol), Japan. Samples were taken from flooded fields. At each sampling site, five soil samples were collected randomly from different locations and pooled together to make a composite sample to represent the overall characteristics of the site studied (Vishwakarma et al. 2010). Composite samples were further divided into two sub-samples, one of the fresh samples was stored at 4°C for molecular biological analyses within a week, and the other was air dried for physico-chemical characteristics of soils. All analyses were done in triplicate. All the soil samples were collected (0-10 cm depth) using a 5 cm diameter soil corer within a week, sieved through 2 mm mesh.

The texture of collected soils was measured using Hydrometer method (Day 1965). Air-dried soil (10 g) was mixed with 25 mL distilled water to measure soil pH (Singla and Inubushi 2014b). Total C (TC) and total N (TN) were estimated after combustion using a CN analyzer (MT-700, Yanaco Analytical Instruments Co., Kyoto, Japan). Soil samples were analysed for NH_4^+-N contents after fresh soil extraction with 1M KCl solution (1:5; soil: solution) by using the nitroprusside method (Anderson and Ingram 1989). Permanganate oxidizable carbon (POXC) was measured as per the procedure described by Weil et al. (2003).

CH_4 Production Potential

The CH_4 production potential was determined as described by Singla and Inubushi (2013). Twenty five grams of the soil was taken in 100 mL glass bottle. All the bottles were kept under waterlogged conditions. Initially, the air of head space of each bottle was replaced by N_2 gas and sealed with rubber stoppers. All the bottles were incubated at 30 °C in three replicates.

CH_4 Oxidation Potential

Twenty five grams of each soil was taken in 100 mL glass bottle and incubated under aerobic conditions. The water holding capacity of the soils was adjusted at 60 % prior to the incubation and sealed with rubber stoppers. A known amount of CH_4 (1150 ppm) was also injected at day 0 of incubation (DOI) in the incubated bottles. All the bottles were incubated at 25 °C in three replicates.

Gas Sampling and Measurement

Head space gases were taken directly from sealed bottles at three days intervals, and measured for CO_2 and CH_4 using gas chromatographs (GC) (Shimadzu GC 14B, Kyoto, Japan) equipped with thermal conductivity detector (TCD) and flame ionization detector, respectively (Singla and Inubushi 2013). For TCD, column temperature was kept at 40 °C and the injector and detector at 50 °C . Helium was provided as carrier gas. For FID, all the three temperatures were followed as 60, 100 and 100 °C. Nitrogen was used as carrier gas and hydrogen was used as flame gas for FID. Porapak Q column (80-100 mesh) was used in TCD; while it was Porapak R (80-100 mesh) in FID.

DNA Extraction from Soils and PCR Amplification

Total genomic DNA was extracted from soils (0.5 g) as per the protocol given in Fast DNA® Spin Soil Kit (M P Biomedicals, Solon, Ohio, USA) using bead-beating method (Dubey et al. 2014). The DNA concentration and purity were evaluated spectrophotometrically. DNA samples were amplified for 16S rRNA gene of methanogenic archaea using specific primer pairs 1106 F-GC (5'-TTW AGT CAG GCA ACG AGC -3') and 1378 R (5'-TGT GCA AGG AGC AGG GAC-3') (Watanabe et al. 2006). The PCR reaction mixture (50 µl) contained 5 µL 10X reaction buffer , 5 µL dNTPs (each 2.5 mM), 0.5 µL 50 µM of each primer, 0.25 µL (5 U μL^{-1}) Ex *Taq* polymerase (TaKaRa, Otsu, Japan) and 1.0 µL of 20-fold diluted DNA template.

The PCR was performed using a 96-well-Thermal Cycler (PCR Thermal Cycler Dice, TaKaRa, Otsu, Japan), under the following conditions: an initial denaturation time of 90 s at 95 °C followed by 35 cycles of denaturation at 95 °C (30 s), annealing at 55 °C (30 s), and elongation at 72 °C (90 s). The last cycle was followed by extension at 72 °C (6 min). At completion, the PCR products were resolved by electrophoresis in 2.0 % (w/v) agarose (Funakoshi, Tokyo, Japan) in 1X TAE buffer stained with ethidium bromide (0.5 μg mL^{-1}). The images were digitized with FAS-III (Toyobo, Osaka, Japan) and the DNA fragment lengths identified using 100 bp DNA ladder (New England BioLabs, Ipswich, UK) as the molecular weight standard. Amplified PCR products were purified with NucleoSpin®Gel and PCR clean-up Kit (MN GmbH, Duren, Germany) and quantified using UV-2450 (Shimadzu, Kyoto, Japan) and stored at -20 °C for further analysis.

DGGE Analysis

DGGE was performed with the DCode™ Universal Mutation Detection System (Bio-Rad laboratories, Hercules, CA, USA) as described by Muyzer et al. (1993). PCR products (approx. 200 ng) were loaded on to 8 % (w/v) polyacrylamide gel immersed in 1X TAE buffer, and electrophoresed for 14 h at 60 °C under a constant voltage (100 V). Polyacrylamide gel was prepared with denaturing gradients in the range of 32 to 62 % (100 % denaturant was achieved by using 7 mol L^{-1} urea and 40 % formamide). After electrophoresis, the gel was stained with the SYBR Green I nucleic acid gel stain (1:10000 dilution) (Lonza, Molecular Application, Rochland ME, USA), rinsed using distilled water, and photographed on an UV-trans-illuminator (Printgraph, ATTO Corporation, Type-GX 430251, Tokyo, Japan) at 312 nm with the SYBR Green gel stain photographic filter (Lonza).

Statistical Analyses

Data were subjected to analysis of variance (ANOVA) in order to determine the effect of sampling site. Tukey's HSD test (at $P < 0.05$) was applied for the differences in mean values. Correlation analysis was used to determine the relationship between CH_4 production potential of Regosol and Andosol soils and other variables. All such analyses were done using SPSS Statistics 20 (IBM, New York, USA).

RESULTS AND DISCUSSION

The physico-chemical properties of each soil are mentioned in Table 1.

Table 1. The physico-chemical properties of Regosol and Andosol soils used in the study

Parameters	Regosol	Andosol
Location	Chiba, Japan	Nagano, Japan
Soil texture	Sandy sand 97.3%; silt 2.7%, clay <0.01%	Sandy loam sand 56.3%; silt 7.3%, clay 36.4%
pH (H_2O)	5.6	6.4
Total C (%)	0.85 ± 0.07	2.36 ± 0.36
Total N (%)	0.12 ± 0.01	0.29 ± 0.05
C/N ratio	7.1	8.1
NH_4-N (μg g^{-1} ds)	6.57 ± 0.50	9.76 ± 1.06
POXC (μg g^{-1} ds)	508.3 ± 8.9	859.9 ± 4.4

CH_4 and CO_2 Production Potential

The production of CH_4 increased with incubation time in both soils (Figure 1a). It attained almost stationary phase 18 DOI in Regosol; while it reached in stationary phase 21 DOI in Andosol. The cumulative CH_4 production over 30 DOI was significantly higher in Andosol (3.44 ± 0.10 mg kg^{-1} ds) compared to Regosol (1.40 ± 0.08 mg kg^{-1} ds). The production of CO_2 also increased with the incubation time and reached almost stationary phase 24 DOI in both soils (Figure 1b). The cumulative CO_2 production over 30 DOI was also significantly higher in Andosol (1.88 ± 0.05 g kg^{-1} ds) compared to Regosol soil (1.07 ± 0.13 g kg^{-1} ds). The cumulative production of both gases showed that CO_2 production was higher than CH_4 production in both soils. The cumulative CH_4 production was significantly correlated with cumulative CO_2 production and the soil's chemical properties (Table 2). POXC showed the strongest correlation; while NH_4^+-N was found to be the weakest among the parameters.

Liu et al. (2011) reported that the soil organic C significantly increased CH_4 production potential in wetland soils. Glatzel et al. (2004) demonstrated a significant positive correlation between CH_4 and CO_2 production in peat soils, whereas a positive correlation

Table 2 Statistical correlation and level of significance of various soil parameters for CH_4 production in Regosol and Andosol soils under waterlogged incubation

Parameters	R^2	P
CO_2	0.947	0.001
Total C	0.936	0.002
Total N	0.926	0.002
POXC	0.997	0.000
NH_4^+-N	0.841	0.01

between cumulative CH_4 and CO_2 production has also been demonstrated in 16 rice paddy soils from 3 countries following anaerobic incubations (Yao et al. 1999). It could be due to dominating acetoclastic pathways for CH_4 production which is favored for CO_2 production along with CH_4 production (Figure 1a, b) rather than hydrogenotrophic pathway under water-logged incubation (Conrad and Klose 1999). POXC indicates the availability of active C in the soil, and is therefore, considered as the early indicator of C change in soils (Culman et al. 2012). The availability of labile C in the soil can influence methanogenesis directly due to being the sole C source for methanogens. Bhattacharyya et al. (2012) also reported high CH_4 and CO_2 emission in presence of high POXC in Indian paddy fields. The presence of NH_4^+-N in the soil is one of the important factors controlling the CH_4 production under water-logged conditions. It has been demonstrated that the existence of NH_4^+ can stimulate CH_4 emission from rice

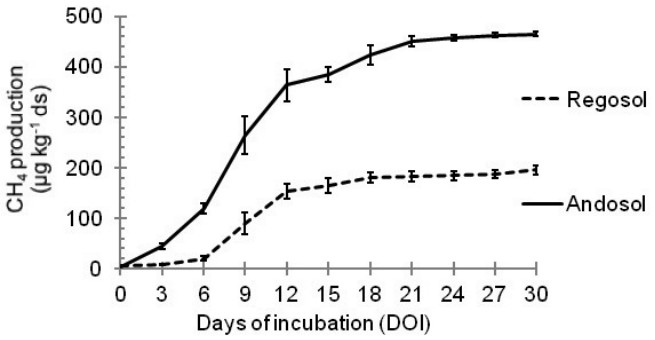

Figure 1a. CH_4 production pattern in Regosol and Andosol soil under 30 days of waterlogged incubation

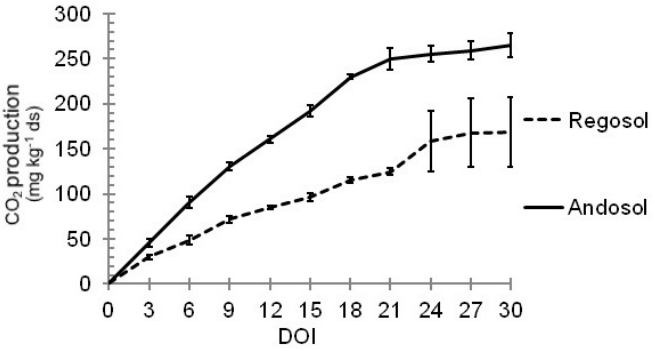

Figure 1b. CO_2 production pattern in Regosol and Andosol soil under 30 days of waterlogged incubation

paddy fields due to the competition of NH_4^+ for the oxidation with CH_4 by methanotrophs (Mosier et al. 1991). Generally, CH_4 emission should increase with the increase in NH_4^+-N content of the soil as also indicated in the present study (Figure 1a, Table 1).

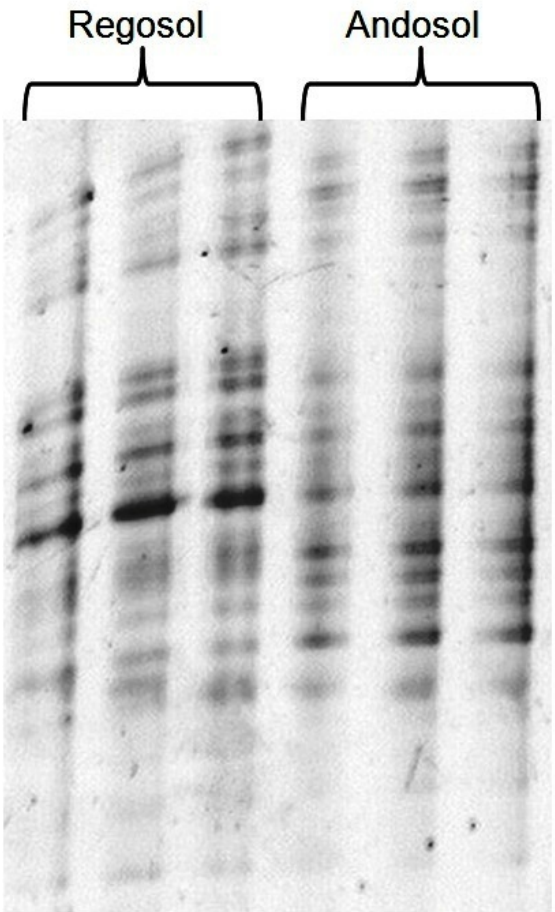

Figure 2. DGGE fingerprinting profiles of methanogenic archaeal communities belonging to two paddy soils of Japan

Another reason for differences in CH_4 production potential of Andosol and Regosol could be the variations among methanogenic archaeal diversity in both soils as indicated by DGGE banding pattern (Figure 2). Such community level variations are possibly due to variations in the physico-chemical properties of soils. Hoshino et al. (2011) suggested that the soil properties affect the archaeal communities in agricultural soils. The effect of soil properties like pH on the soil archaeal community at various soil profiles is also determined by Cao et al. (2012). Watanabe et al. (2009) also reported a distinctive community of methanogenic archaea in the four reclaimed paddy fields of Japan. According to Hoshino et al. (2011), soil chemical properties and soil type mostly overlapped in their effects on the archaeal community. Our study also confirms interactions between soil physico-chemical properties and methanogenic community inhabiting the experimental fields. However, methanogenic archaeal community structure analysis was not done in the present study but our previous study showed that changes in DGGE banding pattern could also give an indication for the changes in methanogenic archaeal community in different soils (Dubey et al. 2014).

CH_4 Oxidation and CO_2 Production Potential

The consumption of CH_4 occurred in both soils with the incubation time. Rapid consumption occurred during first 3 DOI in both soils (Figure 3a). A significant difference between both soils was observed for CH_4 oxidation at each measurement. As similar with CH_4 production (Figure 1a), Andosol soil consumed CH_4 more rapidly than Regosol soil. The cumulative CH_4 consumption over 24 DOI was significantly higher in Andosol (1044 $\pm$ 4 mg kg^{-1}) than Regosol soil (904 $\pm$ 9 mg kg^{-1}). On the other hand, CO_2 production in both soils increased with incubation time (Figure 3b). It was also significantly higher in Andosol compared to Regosol soil at each measurement. As similar with waterlogged incubation (Figure 1b), the cumulative CO_2 production over 24 DOI was higher in Andosol (1.34 $\pm$ 0.07 g kg^{-1} ds) compared to Regosol soil (0.73 $\pm$ 0.05 g kg^{-1} ds).

CH_4 oxidizing bacteria, also known as methanotrophs, are the only known biological sink of CH_4. They utilize CH_4 as a sole source of C and energy. Methanotrophs are categorized into two broad groups based on their affinity for CH_4. The first group, known to conduct "high affinity oxidation", occurs at CH_4 concentrations close to that of the atmosphere (<2 ppm). It is estimated that high affinity oxidation contributes 10 % of total CH_4 consumption. The second group, capable of "low affinity oxidation" occurs at CH_4 concentrations higher than 40 ppm. CH_4 oxidation in high methanogenic environments (rice fields, peat soils, landfills, etc.) has low-affinity activity (Chowdhury and Dick 2013). These low-affinity methanotrophs once getting the favourable conditions for CH_4 oxidation can oxidize the much higher amount of CH_4 within the short time as similar with the present study (Figure 3a).

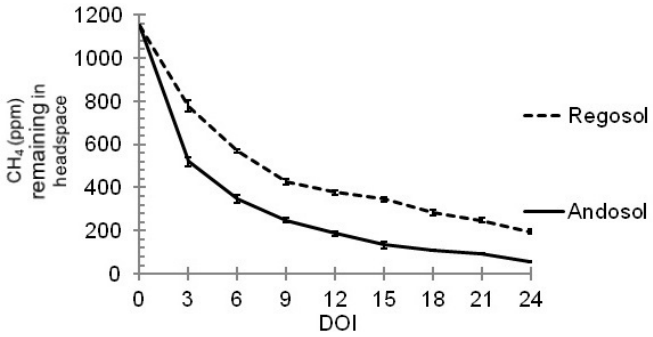

Figure 3a. CH_4 oxidation pattern in Regosol and Andosol soil under 24 days of aerobic incubation

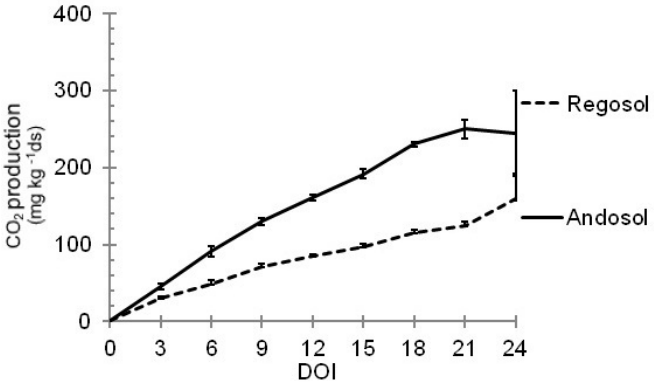

Figure 3b. CO_2 production pattern in Regosol and Andosol soil under 24 days of aerobic incubation

Muramatsu and Inubushi (2009) have already demonstrated that in rice paddy soils, CH_4 production and oxidation is always in a balanced stage. So, the soils having high CH_4 production potential are also expected to have high CH_4 oxidation potential. This could be the reason that Andosol had high CH_4 production potential as well as high CH_4 oxidation potential than Regosol soil

(Figure 1a, 3a). Oxidation of CH_4 is accompanied with CO_2 production. So, high CH_4 oxidation could be the reason for high CO_2 production in Andosol under aerobic incubation (Figure 3a, b).

CONCLUSIONS

Our study showed that methane production potential of paddy soils could vary due to different soil types. It will also depend on the physico-chemical properties as well as on the microbiological circumstances of soils. The soils having the higher methane production potential will also be expected to have the higher oxidation potential for methane once getting the favorable conditions for methane oxidation.

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