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ノート

Enhanced Methanogenesis in the Vicinity of Rice Straw Residues in Surface Layers of a Paddy Soil

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Methanogenesis in irrigated rice fields is carried out by methanogenic archaea under strictly anaerobic conditions. In the present study, the effect of rice straw on methanogenesis in surface layers of paddy soil was investigated in a pot experiment with surface application of rice straw. Rice straw application led to a significant development of reduced conditions in the surface soil, and markedly increased methane（CH_4）emissions. Potential methanogenic activity in the straw residues and the soil layer immediately under the residues（0～1 cm depth）was considerably promoted, while that in the deeper soil layers（4～6 cm depth）was not affected by the residues of rice straw. These results indicate that the increased CH_4 emission in the treatment with surface application of rice straw was due to the activation of methanogenesis in the vicinity of the residues. Consequently, in the present study, it was demonstrated that rice straw application enhanced methanogenesis even in the surface layers of paddy soil, which are generally considered to be oxic layers.

Key words：methane emission, methanogenic activity, paddy soil, rice straw application, surface layers

Irrigated rice fields are one of the major anthropogenic sources of methane（CH_4）emission, which is the most significant greenhouse gas following carbon dioxide（Mosier, 1998）. The emission rates of CH_4 from irrigated rice fields are estimated to amount to 60～150 Tg year^{-1}, corresponding to 15～20% of the total annual anthropogenic CH_4 emission on the earth（Aulakh *et al.*, 2001）. The increasing rate is reported to be approximately 1% year^{-1} (Bouwman, 1990).

Most of the atmospheric CH_4 is biologically produced by methanogenic archaea（methanogens）in anaerobic environments. After rice fields are flooded, anaerobic conditions develop associated with the decomposition of organic matter in soil（Takai and Kamura, 1966; Ponnamperuma, 1972）. Thereafter, CH_4 produced by methanogens is released mainly through rice plants（Inubushi *et al.*, 1989; Nouchi *et al.*, 1990; Dannenberg and Conrad, 1999）. In general, since the surface of irrigated rice fields had been considered to consist of oxic layers, methanogenesis in the surface layers had hardly been noticed（Cheng *et al.*, 2000）. However, in recent studies on the vertical profile of the methanogenic population and CH_4-producing activity, it was reported that methanogens are constantly distributed throughout the plough layer of paddy soils, and that CH_4 production may occasionally occur even in the surface layers（Rothfuss and Conrad, 1993; Asakawa and Hayano, 1995）.

Ueki *et al.*（1999）, who measured the potential methanogenic activity in an irrigated rice field where rice straw had been incorporated, reported that the potential methanogenic activity in the topmost layer（0～1 cm depth）was higher than that in the other layers during the continuous flooding period before midseason drainage. This phenomenon suggests that methanogens living in the surface layers may play a significant role in the total CH_4 emission from irrigated rice fields. Considering that cultivation of rice in conservation tillage systems with straw mulching has been recognized as a labor-saving and low-cost agricultural technique over the last decades, greater attention should be focused on methanogenesis in the surface layers.

Our previous laboratory experiments demonstrated that methanogenesis could be initiated in and around rice straw applied onto flooded soil（Harada *et al.* 2005）. These

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preliminary results strongly suggested that rice straw residues may provide methanogens with a niche anoxic enough to support methanogenesis even in the floodwater - soil interface. In the present study, we conducted a pot experiment with and without surface application of rice straw, and compared the changes in the Eh values, CH_4 emission rates and potential methanogenic activity, with a view to determining how rice straw influenced methanogenesis occurring in the surface layers of a paddy soil.

Materials and Methods

Pot preparation

The characteristics of the gray lowland soil and rice straw used in the present study were described previously (Harada *et al.,* 2005). The moist soil corresponding to 3 kg dry weight (dw) was passed through a 4.76 - mm mesh stainless steel sieve, and was put into a 0.02 - m^2 plastic Wagner pot (inside diameter: 159 mm, height: 250 mm) on June 12, 2000. The soil block was 20 cm thick. Mixed with basal fertilizers consisting of $Na_2HPO_4 \cdot 12H_2O$ and KCl at the rates of 0.1 g P_2O_5 and 0.1 g K_2O kg^{-1} dw soil, respectively, the soil in the pots was immediately submerged. On the next day, 10 g of rice straw chopped into 30 ~ 50 mm segments was applied onto the soil surface, and two 24 - day - old seedlings of Japonica type rice (*Oryza sativa* L. cultivar Nihonbare) were transplanted (RS). Control pots without rice straw application were also prepared (CT). Eight pots were prepared for each treatment. These pots were placed in a greenhouse located at The University of Tokyo (Tokyo, Japan), and were maintained under continuous flooding conditions. Heading of rice occurred on August 23 (70 days after transplanting [DAT]), and harvest was performed on October 20 (129 DAT).

Periodic analyses

Using two previously selected pots for each treatment, the soil redox potential (Eh) and CH_4 emission rates were determined at intervals of seven days during the cultivation. Soil Eh was measured at 0.5 and 5 cm depths using platinum electrodes fixed along the internal wall of each pot and a Toa Electronics HM - 20 pH meter (Tokyo, Japan). CH_4 emission rates were measured by the closed chamber method (Harada *et al.,* 2005).

Potential methanogenic activity

Topmost soil layer (0 ~ 1 cm depth), deeper soil layers (4 ~ 6 cm depth) and rice straw residues were destructively sampled from two pots for each treatment at 27, 55, 83 and 129 DAT. Potential methanogenic activity was measured according to the method proposed by Kaku *et al.* (1999) with some modifications. Rice straw residues were chopped into small fragments, after being washed with a 0.5 g l^{-1} L - cysteine·HCl solution as a sterile reducing agent. Five grams of the fragments were put into a 26 - ml serum bottle, and 5 ml of the sterile reducing agent was then added. The soil samples were mixed thoroughly, and aliquots (5 g each) were put into 26 - ml serum bottles with 5 ml of the sterile reducing agent. Each bottle was tightly closed with a butyl rubber septum and an aluminum cap, and the headspace gas was replaced three times with O_2 - free N_2 gas using a Sanshin IP - 8 gas exchanger (Yokohama, Japan). After 4 - h incubation at 30 ℃ in darkness, the bottles were shaken for 1 min vigorously. The amount of CH_4 emitted in the headspace was then measured by gas chromatography (Harada et al. 2001). The dry weight of the rice straw or soil samples was determined for calculation.

Statistical analysis

The difference in the estimated CH_4 emissions between the treatments was analyzed by Student's t - test. To determine the effect of surface application of rice straw on the potential methanogenic activity, two - way ANOVA with treatment as a factor and time as a block was used.

Results and Discussion

Surface application of rice straw in the RS pots changed the redox potential in the topmost layer of soil (Fig. 1). Eh levels measured at a depth of 0.5 cm decreased soon after rice transplantation, and reached a value of - 0.2 V within 28 days, because rice straw was immediately decomposed by the soil bacteria with the consumption of oxygen (Takai and Kamura, 1966; Ponnamperuma, 1972). Such a low Eh level was maintained afterwards. Without rice straw application (CT), high Eh values (>0.2 V) were recorded until 63 DAT, followed by a rapid decrease to - 0.2 V. The reduction of the redox potential in this treatment may have been induced by the degradation of organic matter including dead algae observed in the floodwater. The difference in the Eh values between the treatments was no longer observed after 70 DAT. Eh values measured at a depth of 5 cm changed similarly, regardless of rice straw application. They gradually decreased to - 0.2 V within 49 DAT, and showed negligible changes thereafter. These results indicate that surface application of rice straw accelerated the development of reduced conditions in the topmost layer of soil during the early vegetative stage of rice plants.

Seasonal changes in the CH_4 emission rates and temperature in the greenhouse are presented in Fig. 2. CH_4 emission in CT, which was negligible until 42 DAT, gradually increased thereafter, associated with the decrease

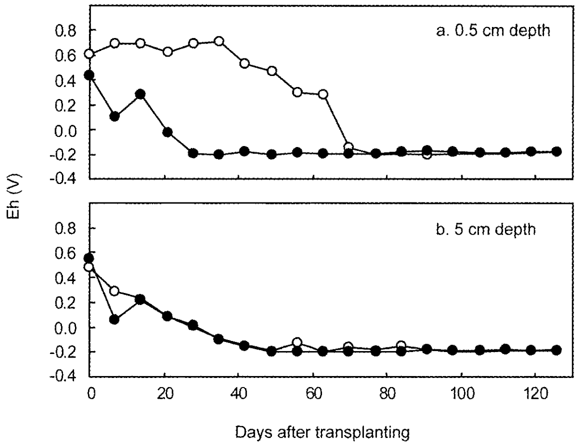

Fig. 1 Changes in the soil Eh values measured at (a) 0.5 and (b) 5 cm depths during the cultivation with and without surface application of rice straw (RS [●] and CT [○], respectively) . Each data point represents the mean of duplicated determinations.

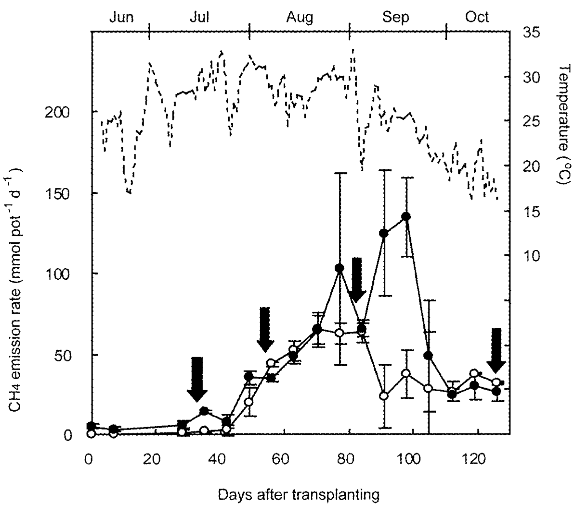

Fig. 2 Seasonal changes in CH_4 emission rates with and without the surface application of rice straw (RS [●] and CT [○], respectively : mean ± deviation, n=2), and average temperatures in the greenhouse (broken line). *Arrows* denote the sampling points for the measurements of the potential methanogenic activity.

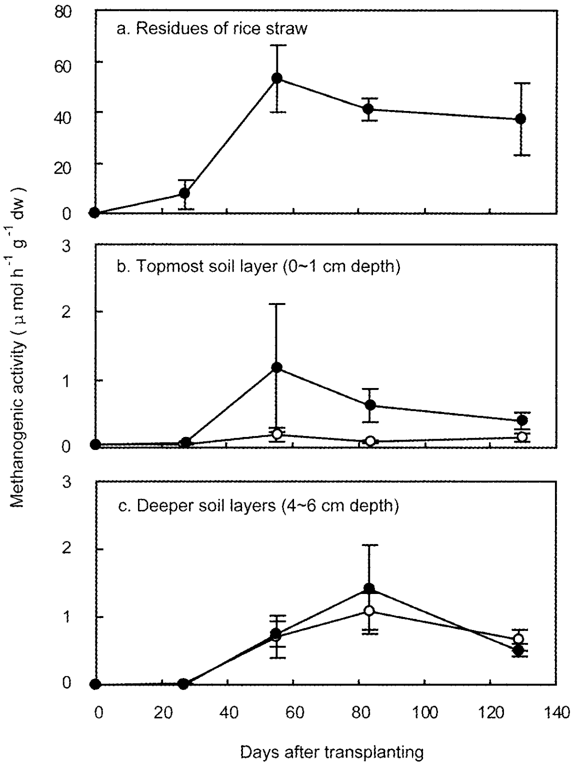

Fig. 3 Changes in the potential methanogenic activity in samples of (a) rice straw residues, (b) topmost soil layer and (c) deeper soil layers collected from the pots (RS [●] and CT [○], respectively). The potential methanogenic activity was defined as the hourly CH_4 emission from the samples determined after 4 - h incubation at 30 ℃ under the dark conditions (mean ± deviation, n=2).

of the Eh values to - 0.2 V recorded at a depth of 5 cm (Fig. 1). The maximum emission rate appeared at 70 DAT, and the CH_4 emission rates then decreased by half. As described in many reports (Yagi and Minami, 1990; Sass *et al.,* 1991; Wassmann *et al.,* 1996; Watanabe and Kimura, 1998; Bossio *et al.,* 1999; Wang *et al.,* 1999), rice straw application in the RS pots markedly enhanced CH_4 emission rates during the early vegetative stage (0 ~ 49 DAT) and, in particular, during the ripening stage of rice plants (77 ~ 105 DAT) (Fig. 2). Assuming that each value represented the average emission rates in a week, cumulative CH_4 emissions during the cultivation were estimated to amount to 5.3 and 3.3 mol pot^{-1} from the RS and CT pots, respectively. The difference in the estimated CH_4 emissions between the treatments was statistically significant (Student's t - test, $P<0.05$).

Figure 3 depicts the changes in the potential methanogenic activity associated with rice straw residues, the topmost soil layer, and deeper soil layers collected from the pots. The rice straw residues displayed a remarkable methanogenic activity, although they were collected from the soil surface (Fig. 3a) . In particular, the potential methanogenic activity on a dry weight basis of the rice straw residues was 500 times higher than that of the deeper soil layers at 27 DAT. The contribution rate of the rice straw residues to the whole methanogenic activity was estimated to exceed 70%, assuming that deeper soil can be representative of bulk soil. This indicates that rice straw applied onto the soil surface immediately provided methanogens with conditions sufficiently reduced to enable them to initiate methanogenesis in the vicinity of the residues, as demonstrated in our previous experiments (Harada *et al.,* 2005) . Under such reduced conditions, methanogens produce CH_4 mainly due to the fermentation of low molecular organic acids formed by the decomposition of rice straw (Gotoh and Onikura, 1971; Krylova *et al.,* 1997; Glissmann and Conrad, 2000) .

Kaku *et al.* (2000), who measured the potential methanogenic activity in samples of soil, living rice roots and plant residues (mainly rice straw plowed into soil) in a wetland rice field during the rice cropping season by a method similar to that used in the present study, demonstrated that the methanogenic activity in the plant residues increased immediately after flooding, and that the activity on a dry weight basis was much higher than that in the soil fraction in the early cropping season. The present results were in agreement with the description of Kaku *et al.* (2000), although the method of rice straw application differed. Methanogenesis occurred even in the oxic topmost layer in rice fields, when hot spots of fresh organic materials were available (Wachinger *et al.*, 2000).

Potential methanogenic activity of the topmost soil layer was markedly promoted in the RS pots (Fig. 3b). Two-way ANOVA indicated that the difference between the treatments was statistically significant ($P<0.05$), although the respective data deviated. Changes in the methanogenic activity in the deeper soil layers were almost the same, regardless of rice straw application (Fig. 3c) (two-way ANOVA, $P>0.05$), indicating that the effect of rice straw application was minimal in the layers at a depth of 4 ~ 6 cm. In both treatments, the potential methanogenic activity of the deeper soil layers was negligible at 27 DAT, presumably because microbial sequential reduction prior to methanogenesis was not completed yet, as shown in Fig. 1. Thereafter, the methanogenic activity increased linearly until 83 DAT, but decreased to approximately half of the maximum value at harvest.

These results indicate that the difference in the CH_4 emission rates between the treatments may be attributed to the activation of methanogenesis associated with the rice straw residues and the soil layer immediately under the residues. However, the increase in the potential methanogenic activity by rice straw application (Fig. 3) was not necessarily concomitant with that of CH_4 emission (Fig. 2). This may be due to the fact that CH_4 emission is likely to be influenced by a number of factors such as methane oxidation, temperature and plant physiology (Sass and Fisher, 1997).

Consequently, in the present study, it was demonstrated that, when rice straw was applied onto the surface of a paddy soil, reduced conditions developed in the vicinity of the residues. This change in the redox potential supported the initiation of methanogenesis in the residues and in the soil layer immediately under the residues. In field studies, Ishibashi *et al.* (2001a) showed that the continuation of no-tilled direct seeding cultivation with surface application of rice straw led to the accumulation of organic matter and immediate decrease of the soil Eh value in the topmost layer of the paddy fields. Such changes increased CH_4 emission from the fields gradually (Ishibashi *et al.* 2001b). Although the method of rice cultivation in the present study differed from that reported by Ishibashi *et al.* (2001a, b), the finding that surface application of rice straw enhanced methanogenesis in the surface layers of a paddy soil is quite common.

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Reference

1) Asakawa S and Hayano K (1995) Populations of methanogenic bacteria in paddy field soil under double cropping conditions (rice-wheat). *Biol. Fertil. Soils,* **20**, 113-117

2) Aulakh MS, Wassmann R and Rennenberg H (2001) Methane emissions from rice fields - Quantification, mechanisms, role of management, and mitigation options. *Adv. Agron.,* **70**, 193-260

3) Bossio DA, Horwath WR, Mutters RG and van Kessel C (1999) Methane pool and flux dynamics in a rice field following straw incorporation. *Soil Biol. Biochem.,* **31**, 1313-1322

4) Bouwman AF (1990) Exchange of greenhouse gases between terrestrial ecosystems and the atmosphere. *In* Soils and the Greenhouse Effect, p.61-127, John Wiley & Sons, New York

5) Cheng W, Chander K and Inubushi K (2000) Effects of elevated CO_2 and temperature on methane production and emission from submerged soil microcosm. *Nutr. Cycl. Agroecosys.,* **58**, 339-347

6) Dannenberg S and Conrad R (1999) Effect of rice plants on methane production and rhizospheric metabolism in paddy soil. *Biogeochemistry,* **45**, 53-71

7) Glissmann K and Conrad R (2000) Fermentation pattern of methanogenic degradation of rice straw in anoxic paddy soil. *FEMS Microbiol. Ecol.,* **31**, 117-126

8) Gotoh S and Onikura Y (1971) Organic acids in a flooded soil receiving added rice straw and their effect on the growth of rice. *Soil Sci. Plant Nutr.,* **17**, 1-8

9) Harada N, Nishiyama M and Matsumoto S (2001) Inhibition of methanogens increases photo-dependent nitrogenase activities in anoxic paddy soil amended with rice straw. *FEMS Microbiol. Ecol.,* **35**, 231-238

10) Harada N, Otsuka S, Nishiyama M and Matsumoto S (2005) Influences of indigenous phototrophs on methane emissions

from a straw-amended paddy soil. *Biol. Fertil. Soils,* **41**, 46-51

11) Inubushi K, Hori K, Matsumoto S, Umebayashi M and Wada H (1989) Methane emission from the flooded paddy soil to the atmosphere through rice plant. *Jpn. J. Soil Sci. Plant Nutr.,* **60**, 318-324 (in Japanese)

12) Ishibashi E, Akai N, Ohya M and Oki K (2001a) The influence of no-tilled direct seeding cultivation on methane emission from three rice paddy fields in Okayama, western Japan. 1. The relationship between the continuation of no-tilled direct seeding cultivation and soil reduction potential. *Jpn. J. Soil Sci. Plant Nutr.,* **72**, 535-541 (in Japanese with English summary)

13) Ishibashi E, Akai N, Ohya M, Ishii T and Tsuruta H (2001b) The influence of no-tilled direct seeding cultivation on methane emission from three rice paddy fields in Okayama, western Japan. 2. The relationship between the continuation of no-tilled cultivation and methane emission. *Jpn. J. Soil Sci. Plant Nutr.,* **72**, 542-549 (in Japanese with English summary)

14) Kaku N, Ueki A, Fujii H and Ueki K (2000) Methanogenic activities on rice roots and plant residue and their contributions to methanogenesis in wetland rice field soil. *Soil Biol. Biochem.,* **32**, 2001-2010

15) Kaku N, Ueki A and Ueki K (1999) Sample preparation for methanogenic activities of heterogeneous flooded rice soils. *Microbes Environ.,* **14**, 151-156

16) Krylova NI, Janssen PH and Conrad R (1997) Turnover of propionate in methanogenic paddy soil. *FEMS Microbiol. Ecol.,* **23**, 107-117

17) Mosier AR (1998) Soil processes and global change. *Biol. Fertil. Soils,* **27**, 221-229

18) Nouchi I, Mariko S and Aoki K (1990) Mechanism of methane transport from the rhizosphere to the atmosphere through rice plants. *Plant Physiol.,* **94**, 59-66

19) Ponnamperuma FN (1972) The chemistry of submerged soils. *Adv. Agron.,* **24**, 29-96

20) Rothfuss F and Conrad R (1993) Vertical profiles of CH_4 concentrations, dissolved substrates and processes involved in CH_4 production in a flooded Italian rice field. *Biogeochemistry,* **18**, 137-152

21) Sass RL and Fisher FM (1997) Methane emissions from rice paddies : a process study summary. *Nutr. Cycl. Agroecosys.,* **49**, 119-127

22) Sass RL, Fisher FM, Harcombe PA and Turner FT (1991) Mitigation of methane emissions from rice fields : Possible adverse effects of incorporated rice straw. *Global Biogeochem. Cy.,* **5**, 275-287

23) Takai Y and Kamura T (1966) The mechanism of reduction in waterlogged paddy soil. *Folia Microbiol.,* **11**, 304-313

24) Ueki A, Nishida S, Kumakura M, Kaku N, Kainuma Y, Hattori C, Fujii H and Ueki K (1999) Effects of organic matter application, temperature, and sunshine duration on seasonal and annual variations of methanogenic activity in wetland rice field soil. *Soil Sci. Plant Nutr.,* **45**, 811-823

25) Wachinger G, Fiedler S, Zepp K, Gattinger A, Sommer M and Roth K (2000) Variability of soil methane production on the micro-scale : spatial association with hot spots of organic material and Archaeal populations. *Soil Biol. Biochem.,* **32**, 1121-1130

26) Wang B, Xu Y, Wang Z, Li Z, Ding Y and Guo Y. (1999) Methane production potentials of twenty-eight rice soils in China. *Biol. Fertil. Soils,* **29**, 74-80

27) Wassmann R, Neue HU, Alberto MCR, Lantin RS, Bueno C, Llenaresas D, Arah JRM, Papen H, Seiler W and Rennenberg H (1996) Fluxes and pools of methane in wetland rice soils with varying organic inputs. *Environ. Monit. Assess.,* **42**, 163-173

28) Watanabe A and Kimura M (1998) Effect of rice straw application on CH4 emission from paddy fields. IV. Influence of rice straw incorporated during the previous cropping period. *Soil Sci. Plant Nutr.,* **44**, 507-512

29) Yagi K and Minami K (1990) Effect of organic matter application on methane emission from some Japanese paddy fields. *Soil Sci. Plant Nutr.,* **36**, 599-610