Production Potential and Emission of Methane in Flooded Rice Soil Microcosms after Continuous Application of Straws

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Experiments were conducted to examine the effect of continuous application of different kinds of straw on methane emission and production in flooded rice soil microcosms. Consecutive cultivation for four years revealed that there was no significant difference in the amounts of methane emitted by plots applied with rice straw and those applied with wheat straw, though emissions varied remarkably from year to year. Methane emission from plots with an application of 6 t ha⁻¹ rice straw or wheat straw increased 3.5 times or 3.4 times compared to that from plots without straw, respectively. Continuous application of straw altered the methanogenic characteristics by increasing the response to glucose added to the soil. The production potential of methane and carbon dioxide was measured by laboratory incubation, and was significantly increased in samples taken from plots receiving straw applications when glucose was added, while there was no response to glucose addition in soil from plots without straw application. In contrast, dissolved carbon including organic acids were accumulated in the control plot. These results indicated that the methane production potential in response to glucose was maintained by continuous application of straws.

Key words: Flooded rice soil, Rice straw, Wheat straw, Potential production of methane, Methane emission

Introduction

Application of organic materials to flooded rice soil has been found to enhance methane emission. For rice straw, emissions increased with the quantities and mode of application^{3,8)}, from about 0.5 times the unfertilized plots with an application of 3 t ha⁻¹, to about 2 times the unfertilized plots with an application rate of 5 t ha⁻¹, and to a maximum of about 2.4 times the unfertilized plots⁷⁾ with an ap-

plication of 12 t ha⁻¹. Yagi and Minami⁹⁾ found that addition of rice straw at a rate of 6 t ha⁻¹ increased the emission of methane by a factor of 1.8 to 3.3 compared to those without straw application.

Most reports have focused on the quantity of methane emissions based on straw incorporation. However, the mode of rice straw incorporation, either continued annually or applied for the first time, is also supposed to affect methane production. Organic residues resulting from the decomposition of straws may serve as substrates for methanogenic bacteria or related microorganisms. Nevertheless, little is known about the effect of the

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annual application of straw on the potential production of methane. Furthermore, effects of different kinds of straw applied on the potential production of methane and carbon dioxide are also poorly understood. Thus, in this report we investigated the influences of continuous application of different kinds of straws on the production potential and emission of methane and carbon dioxide.

Materials and Methods

The experiment was conducted in rice field-simulating vinyl containers ($64 \times 44 \times 38$ cm) with 0.07 m^3 soil. The containers were placed outside to allow rice plants to grow under natural conditions. Ayama Gley soil (Typic hydraquent), with 19.7 g kg^{-1} of total carbon and 0.92 g kg^{-1} of total nitrogen, was used. In the control plot, rice was cultivated for four consecutive years (1989–1992) without incorporating any organic material. In the rice straw plot (RS plot) and wheat straw plot (WS plot), rice plants were cultivated every year with rice or wheat straw applied at a rate of 6 t ha^{-1} . At the beginning of each planting season, chopped straw (2–3 cm in length) was incorporated at once, then the plot was flooded and rice plants were transplanted on the following day. Twenty-day old rice seedlings (*Oryza sativa* cv. Yamahikari) were transplanted with 4 seedlings per hill and 20×20 cm spacing. The water level was kept at 5 cm throughout the growing season. Chemical fertilizer was applied every year to all plots at a rate of 40 kg each of N, P_2O_5 and K_2O ha^{-1} . All plots were topdressed with ammonium sulfate (30 kg N ha^{-1}) twice during the cultivation period. To measure the amount of methane emission, a subplot surrounding one hill containing rice plants was made by inserting a plastic pipe into the soil layer of each container. Methane flux during the cropping season was measured weekly using the closed-chamber technique and the concentration increase for 30 min. was determined by a gas chromatograph (Shimadzu GC-8A) using these subplots²⁾.

To examine the effects of continuous incorpora-

tion of straw on the production potential of methane and carbon dioxide, soil samples were taken from each plot after the harvest on the 4th year for laboratory incubation. (We use the phrase “production potential” to refer to the production of methane under “*in vitro*” conditions). Soils were air-dried and screened through a 2 mm sieve, a 5 g sample was placed into a 30 mL-Erlenmeyer flask, then sufficient amounts of deionized water (5 mL) were added to simulate flooded conditions. Soil from each treatment was divided into 4 subtreatments according to amendments; glucose solution, rice straw powder (RS powder), wheat straw powder (WS powder) and no amendment. In all cases, the amount of amendments was equivalent to 11.04 gC kg^{-1} . Each subtreatment was prepared in duplicates. Flasks were then sealed with a double butyl stopper, evacuated 3 times and refilled with pure nitrogen gas in the head space. The samples were incubated at 30°C for 4 weeks with weekly determination of the head space gas concentration²⁾ (CH_4 and CO_2). At the end of incubation, dissolved organic carbon and organic acids were determined using a total organic carbon analyzer (Shimadzu TOC 5000) as described by Yoshikawa and Inubushi¹⁰⁾ and HPLC (Shimadzu LC-6A) as described by Lawongsa *et al.*⁵⁾, respectively.

Results and Discussion

Methane emission during four consecutive years

Total amounts of methane emission during 4 years of cultivation are summarized in Table 1. Despite equal amounts and types of straw application, the same cultivation practices and the same rice variety being planted, the amount of methane emission varied considerably from year to year. Tyler *et al.*⁶⁾ suggested that while paddy fields share many traits, their individual contributions to methane emission can differ markedly depending on local characteristics such as temperatures, nutrients, water management, and soil factors. In our case, rice straw incorporation increased the amount of methane emission 2.6 to 7.7 times, while wheat straw

increased its emission 2.1 to 10.1 times the control plot. On average during 4 years, the amount of methane emission was 3.5 times the control plot for rice straw and 3.4 times the control plot for wheat straw. There was no significant difference in the average amount of methane emission between the plots with rice straw and wheat straw.

Effects of continuous incorporation of straw on methane production potential and organic C

The effects of continuous incorporation of straw

on methane production potential was studied by laboratory incubation of the soil samples. Although the production potential of methane and carbon dioxide did not significantly differ among plots when no amendment was added, glucose, rice straw and wheat straw powder stimulated methane production potential in all plots, except in the control plot where glucose did not stimulate the methane production potential (Figs. 1 and 2). All amendments also enhanced the production potential of carbon dioxide in all plots, although to a lower extent in the control

Table 1. The amounts of methane emission for 4 contiguous years

Treatments	Amount of emitted CH ₄ (g C m ⁻²)				Average*	Index
	1st year	2nd year	3rd year	4th year		
Control plot	5.4	23.7	6.4	29.1	16.2 ^a	100.0
Rice straw plot	41.5	61.4	44.0	80.7	56.9 ^b	351.2
Wheat straw plot	36.6	58.3	64.8	60.7	55.1 ^b	340.1

(*significant difference at $p \leq 0.01$, $n=4$ by F-test)

Fig. 1. Production potential of methane in different plots. Error bars indicate the standard deviation of two replications. The letters a, b and c refer to significant differences at $p \leq 0.01$ within the plot, while different numbers of asterisks (*, **, ***) indicate significant differences at $p \leq 0.01$ between plots.

Fig. 2. Production potential of carbon dioxide in different plots. Definitions of error bars, the letters a, b and c, and asterisks are the same as those described in Fig. 1.

plot when compared to that in the others. These results revealed the different response of methanogenic bacteria or related microorganisms to glucose, in different plots. Thus, it can be said that the characteristics of methanogenic conditions were altered by the continuous application of straws.

The methane production potential of soil was further investigated with the measurement of dissolved C and organic acids in the soil solution. When amended with glucose and incubated for 4 weeks, large amounts of dissolved C and organic acids were detected in the soil solution from the control plot (Table 2). Large accumulations of dissolved C and organic acids were accompanied by low methane production potential as shown in Fig. 1. The main organic acids detected were acetic, propionic, n-butyric and hydroxy benzoic acid. Smaller quantities of dissolved C and/or organic acids were accumulated in RS and WS plots when rice or wheat straw was added. This seems to be due to the transformation of these substrates to methane and carbon dioxide. It is noteworthy that when soil taken from the RS plot was amended with WS

powder, no significant change in potential production was found. The results are also similar for soil taken from the WS plot and *vice versa*.

Contribution of amended C to the production of CH₄, CO₂ and organic C

The percentage of C in the amendments (input) which was converted to C in methane, carbon dioxide, dissolved C and organic acids after 4 weeks of anaerobic incubation was calculated (Table 3). The calculation was made by excluding the amount of C in methane, carbon dioxide and dissolved C in plots without any organic amendments. We assumed that excess C in plots with organic amendments were derived from the amended C. Only 0.84% of glucose C was converted to C of methane for soil from the control plot. A high portion of glucose added to this soil was accumulated as organic acids (21.65%). A greater portion of glucose C was converted to methane and carbon dioxide in soil from continuous straw incorporation plots than in soil from control plot. Organic acids accounted for 31.80% and 62.20% of dissolved C in glucose

Table 2. Dissolved C and organic acids contents in soil samples after amended with various organic materials and incubated at 30°C for 4 weeks

Treatments Amendments	Dissolved C (g C kg ⁻¹)	Organic acid (g C kg ⁻¹)					
		*Mal	Lac	Ace	Pro	n-bu	hydrox
Control plot							
no amendment	0.24	0.05	0.02		0.07		0.004
glucose	3.29			0.23	0.10	0.17	1.89
rice straw powder	0.22						
wheat straw powder	0.17	0.05					
Rice straw plot							
no amendment	0.19						
glucose	0.22	0.05	0.02				
rice straw powder	0.18						
wheat straw powder	0.18						
Wheat straw plot							
no amendment	0.20	0.07	0.05	0.08			
glucose	0.45	0.07	0.02	0.02	0.17		
rice straw powder	0.19						
wheat straw powder	0.19	0.06	0.02				

*Mal; Malic acid, Lac; Lactic acid, Ace; Acetic acid, Pro; Propionic acid, n-bu; n-butyric acid, hydrox; hydroxy benzoic acid

Table 3. Contribution of amended organic material to the production of methane, carbon dioxide and organic acids

Treatments Amendments	Output C/Input C in CH ₄ (%)	Output C/Input C in CO ₂ (%)	Dissolved C Input C (%)	Total org. acids-C# Input C (%)	Org. acids-C Dissolved C (%)
Control plot					
glucose	0.84	6.07	29.80	21.65	72.64
RS powder	4.10	4.89	1.99	*ND	*ND
WS powder	4.66	5.62	1.54	0.45	29.40
Rice straw plot					
glucose	14.51	12.95	1.99	0.63	31.80
RS powder	4.55	6.16	1.63	*ND	*ND
WS powder	4.51	6.61	1.63	*ND	*ND
Wheat straw plot					
glucose	7.74	9.15	4.08	2.54	62.20
RS powder	5.43	5.99	1.72	*ND	*ND
WS powder	6.18	8.79	1.72	0.72	42.10

(ND*=not detectable, #total organic acids=Summation of detected organic acids shown in Table 2 in each amendment)

amended soil from RS and WS plots, respectively. Regardless of plots, C in rice straw powder was converted to C in methane about 4–5% during the incubation time of 4 weeks. About 5–6% of C in wheat straw powder was transformed to methane. In addition, the percentage of C from amendments converted to carbon dioxide was generally higher than that converted to methane, except for glucose-amended soil from the RS plot.

Possible mechanisms of the effect of continuous straw application on methane production

To produce methane, methanogens need partner microorganisms that can provide methanogenic substrates⁴⁾. The results in this study suggested that glucose was metabolized to other forms of dissolved C, mainly organic acids, and accumulated in these forms in the soil from the control plot. Methane and carbon dioxide formation from glucose was greater in the RS and WS plots than in the control plot. Thus, it is reasonable to assume that methanogenic bacteria and related microorganisms in the control plot were less adapted to high amounts of glucose amendment than those in plots receiving continuous applications of straw.

The great response of methane production poten-

tial to glucose addition in RS and WS plots suggested that methanogenic bacteria and related microorganisms were readily responsive to exogenous substrates after continuous applications of straw. Thus, the availability of and affinity to substrates are likely the critical limiting factors in the production potential of methane in this case. From these results, we conclude that continuous annual supply of organic material maintains the ability of soil microorganisms to readily utilize easily decomposable organic material added to paddy soil. In addition, it is possible that products of decomposed straw as organic residue may provide micro-habitats that protect methane-producing bacteria or involved microorganisms from unfavorable conditions, for example, when paddy soils are exposed to draught and oxic conditions after the cropping season. Furthermore, a supply of organic materials may stimulate the activity of nonmethanogenic bacteria which establish suitable redox conditions and supply precursors for methanogenesis as suggested by Conrad¹⁾, when paddy soil is subjected to flooding.

In conclusion, this study revealed that; 1) continuous incorporation of straw enhanced the production potential of methane and carbon dioxide from glucose added to the soil. It seems that annual ap-

plication of straw maintains the ability of soil microorganisms, including methane-producing bacteria, to respond to easily decomposable organic materials added to the soil, 2) no significant difference in methane production potential and emission was noted between rice and wheat straw applications.

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