

Contribution of Rice Plants and Cover Crop Biomass Amended Soil on Methane Emission

Md Mozammel Haque^{1,2*}, Jatish Chandra Biswas², Muhammad Ashraful Alam¹, Pil Joo Kim^{1,3*}

¹Division of Applied Life Science, Gyeongsang National University, Jinju, South Korea ²Soil Science Division, Bangladesh Rice Research Institute, Gazipur, Bangladesh ³Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, South Korea Email: *mhaquesoil@yahoo.com

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Abstract

Rice plant and soil are playing vital role for produce of methane (CH₄) emission from flooded rice soil. Contribution of rice plants and cover crop biomass amended soil on methane emission has not been yet studied under different cover crop biomass incorporated in paddy fields. Closed-chamber method was used to estimate CH₄ emission rates during rice cultivation under soil plus rice plants and soil alone condition. Soil plus rice plants chambers 62 \times 62 \times 112 cm³ and soil alone chambers 20 \times 20 cm² were placed at the same time during rice cultivation (0 days after rice transplanting). Therefore, to evaluate the contribution of soil plus rice plants and soil alone on methane (CH₄) emission under different rates of cover crop biomass incorporated soil during rice cultivation. Methane emission from soil plus rice plants increased up to 53 days after transplanting (DAT) and then it's decreased and continued till harvesting. It was found that ca. 47% - 52% CH₄ was mediated by rice plants and ca. 48% - 53% through rice soil alone under 12 Mg·ha⁻¹ cover crop biomass incorporated treated plots. Whereas, only ca. 9% - 10% CH4 emission was mediated by rice plants and ca. 90% - 91% by rice soil alone when 0 and 3 Mg·ha⁻¹ cover crop biomass was incorporated. Therefore, it could be concluded that rice soil alone was more influenced for CH₄ emission than rice plants in paddy fields.

Keywords

Rice Plant, Rice Soil, Methane Emission, Green Manure

1. Introduction

Methane, a major component of natural gas is the second most important greenhouse gas (GHG) and the concentration of atmospheric CH₄ was 700 -1774 ppb in 2005 [1]. It is the most potent GHG gas with global warming potentials (GWP) of 25, which is greater than CO_2 [1]. Particularly, CH_4 is a major issue in flooded rice culture accounting for 10% - 40% of the global CH_4 emissions [2] [3] [4] and will continue to be a major source as global rice production needs to be increased to feed an ever increasing population, especially in Asian countries [5]. To meet up future demand, annual rice production must to be increased from 520 million tons to at least 880 million tons by 2025 [6].

Methane produced in rice fields by methanogenic bacteria is thought to be released into the atmosphere by different pathways: molecular diffusion at water-air interfaces, ebullition of gas bubbles and plant mediated transport [7]. However, CH_4 emission contribution from rice fields have not been yet studied under different cover crop biomass incorporated field conditions. Therefore, the objective of this study was to find out the contribution of rice plants and soil amended with cover crop biomass incorporation rates on CH_4 emission in mono rice culture.

2. Methods

2.1. Experimental Field Preparation and Rice Cultivation

In Korean paddy soil, 140 and 90 kg·ha⁻¹ of barley and hairy vetch seeds are recommended as a winter cover crop, respectively [8]; but a mixture of 75% barley and 25% vetch seeds were sown after rice harvest in 2010 and 2011 at the experimental farm of Gyeongsang National University (36°50'N and 128°26'E), Jinju, South Korea. The selected soil was silt loam in texture and classified as typic Haplaquents with somewhat impeded drainage and organic matter content of 20.4 \pm 3.9 g·kg⁻¹; soil pH (1:5 with H₂O), 6.2 \pm 0.32; available P₂O₅, 78.7 \pm 3.1 mg·kg⁻¹.

In early June of 2011 and 2012, the above-ground biomass of cover crop was harvested manually and yield properties were recorded. Cover crop biomass productivity was 12 Mg·ha⁻¹, which was composed of 3 and 9 Mg·ha⁻¹ barley and hairy vetch in both the years, respectively. The cover crop mixture contained 42.20% (wt·wt⁻¹ on dry weight base) total organic C, 2.42% total N, 17.44 C/N ratio, cellulose 29.08%, lignin 18.43%, protein 17.06% and ash 8.4%. Cover crop was chopped into 5 - 10 cm size manually and applied at 0, 3, 6, and 12 Mg·ha⁻¹ as treatments followed by mechanical mixing with surface soil. Randomized complete block design was utilized and treatments were repeated thrice.

Twenty one days old rice (Japonica type) seedlings were transplanted at 15 cm \times 30 cm spacing on 11th and 8th June of 2011 and 2012. The recommended dose of chemical fertilizers (N – P – K = 90 – 20 – 48 kg·ha⁻¹) were applied one day before rice transplanting [9]. Soil was flooded right after biomass incorporation at 5 - 7 cm depth, and then this level was maintained during rice cultivation. Water was drained at 21 days before rice harvesting on 21 and 18 October, 2011 and 2012, respectively.

2.2. CH₄ Gas Sampling and Analysis

A closed-chamber method [10] [11] was used to estimate CH₄ emission rates

during rice cultivation. Closed acrylic column chambers with 20 cm diameter and 20 cm height were placed inner soil surface by 20 cm between rice hills for estimating CH_4 emission rates from soil during rice cultivation [12] [13] [14].

2.3. Soil Sampling and Analysis

2.3.1. Estimation of Dissolve Carbons

Dissolved organic carbon from fresh soil was determined using hot water as described by Ghani *et al.* [15].

2.3.2. mcrA and pmoA gene Copy Numbers

Fresh soil samples were collected at 30 and 70 days after transplanting (DAT) during rice cultivation to compare methanogenic and metanotrophic activities. Soil samples were lyophilized by a Pilot Lyophilizer (PVTFD50A, Ilsin, Korea) and stored at -70°C for analysis. DNA was extracted from the lyophilized soils by a Fast DNA SPIN Kit (MP Biomedical, Santa Ana, CA, USA) following the manufacturer's instruction and was used as a template for quantitative analysis. The real-time quantitative PCR (qPCR) was performed in a BioRad CFX96 real-time thermo-cycler (BioRad Laboratories, Hercules, CA, USA). Reaction mixtures contained 5 µl of qPCR ROX & Go Green (qBiogene, Illkirch, France), 1.5 µg bovine serum albumin (Sigma-Aldrich, Germany), 5 pmol of each primer [16], 5% dimethyl sulfoxide (Sigma-Aldrich, Steinheim, Germany), and 0.5 µl DNA template and water was added to make the final volume up to $25 \mu [17]$. The amplification was carried out as follows: initial denaturation at 95°C for 10 min and 40 cycles at 94°C for one min, 52°C for one min and 72°C for one min. Standard curves were constructed using 10-fold serial dilutions of plasmids containing a partial sequence of Methanosarcina mazei mcrA gene and Methylocystis sp. SD5 pmoA gene. Amplification efficiencies of the PCRs were calculated using data from the standard curves with the formula: efficiency [10 (-1/slope)]- 1. To minimize the inhibitory effects of co-extracted substances with DNA, amplifications of serial diluted standards were performed for samples of each plot. Four independent assays were run for each sample. The quality of the amplification was evaluated by the generation of melting curves of the PCR products.

2.4. Statistical Analysis

Statistical analyses were conducted using SAS software [18]. A one-way ANOVA was carried out to compare the means of different treatments. Fisher's protected least significant difference (LSD) was calculated at 0.05 probability level for making treatment mean comparisons.

3. Results

3.1. Methane Emission through Rice Plants and Soil

Methane flux was low with 0 and 3 Mg·ha⁻¹ biomass incorporated plots, which was comparable to typical CH_4 emission pattern of a general paddy soil (Figure 1).



Figure 1. Changes of CH_4 emission rates from single soils, and rice planted soils under different cover crop biomass applied condition during rice cultivation.

Methane emission rate was comparatively lower at initial rice growing stage and then increased significantly with the development of soil reductive conditions and plant growth. Higher CH_4 emission rates were observed from paddy field due to incorporation of 6 Mg·ha⁻¹ or more cover crop biomass (**Figure 1**). However, the highest peak of CH_4 emission was observed at 30 DAT with organic amended soils. For example, more than 77% of total CH_4 was emitted within 50 DAT when 12 Mg·ha⁻¹ biomass was incorporated.

3.2. Methane Emission through Rice Soil Alone

In rice soil, CH_4 emission rates were lower up to 53 DAT and then gradually increased CH_4 in all treatments. Among the treatments, low CH_4 emissions were observed in 0 and 3 Mg·ha⁻¹ from rice soil and pattern was not comparable to typical CH_4 emission trend of a general paddy field (**Figure 1**). However, application of higher amount of biomass was responsible for increased CH_4 emission. Most CH_4 was emitted from rice soil after 53 DAT and the rate sharply increased due to biomass application levels. For example, about 62% of the total CH_4 was emitted under 12 Mg·ha⁻¹ biomass applications after 53 DAT.



Figure 2. Comparison of total CH₄ fluxes during rice cultivation under different cover crop biomass applied condition.

3.3. Net CH₄ Emission

The contribution of rice plants plus soil on total CH_4 flux was 181 - 186 and 354 - 367 kg·ha⁻¹ and the contribution of rice soil alone was 165 - 171 and 324 - 334 kg·ha⁻¹ with 0 and 3 Mg·ha⁻¹ biomass incorporation in 2011 and 2012, respectively (**Figure 2**). About 9% - 10% CH_4 emission was mediated by rice plants and about 90% - 91% from rice soil alone in 0 and 3 Mg·ha⁻¹ treated plots. However, emission rates were 47% - 52% through rice plants and 48% - 53% from soil alone because higher rate of biomass incorporation in both the years.

4. Discussion

At initial rice growth stages, most CH_4 emission took place from soil plus rice plants but after 53 DAT its emission was larger from rice soil alone. However, many authors claim that majority of CH_4 gas produced in the rice field is emitted through aerenchyma channels and only a little portion is diffused through the soil-water inter-phase of flooded soils [19] [20]. Our results showed that rice plants plus soil emitted CH_4 until 53 DAT and then its decreased and continued up to harvesting (**Figure 1**). At early growth stages, rice roots released more carbon substrates for methanogen activity [21] [22] [23] [24] [25] and thus more







Figure 4. pmoA gene as influenced by different cover crop biomass incorporation on 30th and 70th day after rice transplanting.

 CH_4 emission takes place (**Table 1(a)**). At later growth stages, easily available carbon sources reduced [26] and O_2 supply increases in the rhizosphere [27] and methanotrophs uses CH_4 as terminal electron acceptor for their energy source [28] [29] [30] [31] resulting in emission of CH_4 low (**Table 1(b)**) andmore CO_2 from paddy field [32]. The abundance of methanotrophs and methanogens (**Figure 3**, **Figure 4**) justifies our statement.

		(a)						
Parameters	Soil alone				Soil plus plant			
Biomass application level (Mg·ha ⁻¹)	0	3	6	12	0	3	6	12
CH_4 emission rate (g·m ⁻²)	0.32d	0.76c	1.50b	4.12a	1.05d	3.61c	20.19b	28.78a
DOC (mg·kg ⁻¹)	217c	242b	242b	252a	244d	251c	271b	274a
HWOC (mg·kg ⁻¹)	595d	602c	742b	888a	914d	994c	1057b	1152a
		(b)						
Parameters	Soil alone				Soil plus plant			
Biomass application level (Mg·ha ⁻¹)	0	3	6	12	0	3	6	12
CH_4 emission rate (g·m ⁻²)	3.54d	3.84c	13.77b	15.74a	1.16d	1.38c	2.41b	3.78a
DOC (mg·kg ⁻¹)	262d	298c	338b	420a	320d	352c	380b	484a
HWOC (mg·kg ⁻¹)	600d	778c	803b	994a	917d	1022c	1132b	1182a

Table 1. (a) Soil characteristics as influenced by different cover crop biomass incorporation on 30th day after rice transplanting; (b) Soil characteristics as influenced by different cover crop biomass incorporation on 70th day after rice transplanting.

Note: DOC and HWOC mean, dissolved, and hot water extractable organic carbon, respectively.

5. Conclusion

Methane emission increased up to 53 DAT from soil plus rice plants and then it decreased and continued up to rice harvesting stage. Our results predicted that about 9% - 10% CH_4 emission was mediated by rice plants and about 90% - 91% by soil alone when 0 and 3 $Mg \cdot ha^{-1}$ of cover crop biomass was incorporated.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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