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

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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 × 62 × 112 cm³ and soil alone chambers 20 × 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 poten-
tials (GWP) of 25, which is greater than CO₂ [1]. Particularly, CH₄ is a major issue in flooded rice culture accounting for 10% - 40% of the global CH₄ 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 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, CH₄ 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 CH₄ 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 ± 3.9 g·kg⁻¹; soil pH (1:5 with H₂O), 6.2 ± 0.32; available P₂O₅, 78.7 ± 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 × 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 methanotrophic 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 μl [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).
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.
3.3. Net CH$_4$ 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 3. mcrA gene as influenced by different cover crop biomass incorporation on 30th and 70th day after rice transplanting.

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

CH₄ emission takes place (Table 1(a)). At later growth stages, easily available carbon sources reduced [26] and O₂ supply increases in the rhizosphere [27] and methanotrophs uses CH₄ as terminal electron acceptor for their energy source [28] [29] [30] [31] resulting in emission of CH₄ low (Table 1(b)) and more CO₂ from paddy field [32]. The abundance of methanotrophs and methanogens (Figure 3, Figure 4) justifies our statement.
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.

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<th>Parameters</th>
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<th>Soil plus plant</th>
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<td>0   3   6   12</td>
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<td>CH₄ emission rate (g∙m⁻²)</td>
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<td>DOC (mg∙kg⁻¹)</td>
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<tr>
<td>HWOC (mg∙kg⁻¹)</td>
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</table>

(b)

<table>
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<th>Parameters</th>
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<th>Soil plus plant</th>
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<td>Biomass application level (Mg∙ha⁻¹)</td>
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<tr>
<td>HWOC (mg∙kg⁻¹)</td>
<td>600d 778c 803b 994a 917d 1022c 1132b 1182a</td>
<td></td>
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</tbody>
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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% CH₄ emission was mediated by rice plants and about 90% - 91% by soil alone when 0 and 3 Mg∙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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