Methane Production Potential of Soil Profile in Organic Paddy Field

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Abstract


The use of organic fertilizers in the organic paddy/rice field can increase methane (CH\textsubscript{4}) production, which leads to environmental problems. In this study, we aimed to determine the CH\textsubscript{4} production potential (CH\textsubscript{4}-PP) by a soil profile from samples using flood incubation. Soil properties (chemical, physical, and biological) were analyzed from soil samples of three different paddy farming systems (organic, semi-organic, and conventional), whilst soil from teak forest was used as the control. A significant relationship was determined between soil properties and CH\textsubscript{4}-PP. The average amount of CH\textsubscript{4}-PP in the organic rice field profile was the highest among all the samples (1.36 µg CH\textsubscript{4}/kg soil/day). However, the CH\textsubscript{4} oxidation potential (CH\textsubscript{4}-OP) is high as well, as this was a chance of mitigation options should focus on increasing the methanotrophic activity which might reduce CH\textsubscript{4} emissions to the atmosphere. The factor most influencing CH\textsubscript{4}-PP is soil C-organic (C\textsubscript{org}). C\textsubscript{org} and CH\textsubscript{4}-PP of the top soil of organic rice fields were 2.09% and 1.81 µg CH\textsubscript{4}/kg soil/day, respectively. As a consequence, here the mitigation options require more efforts than in the other farming systems. Soil with various amounts of C\textsubscript{org} reached a maximum point of CH\textsubscript{4}-PP at various time after incubation (20, 15, and 10 days for the highest, medium, and the lowest amounts of C\textsubscript{org}, respectively). A high amount of C\textsubscript{org} provided enough C substrate for producing a higher amount of CH\textsubscript{4} and reaching its longer peak production than the low amount of C\textsubscript{org}. These findings also provide guidance that mitigation option reduces CH\textsubscript{4} emissions from organic rice fields and leads to drainage every 10–20 days before reaching the maximum CH\textsubscript{4}-PP.

Keywords: emission; horizon; methane; mitigation; soil
rice-consuming countries. Ca. 78% of methane emissions produced in Indonesia, China, India, Thailand, Vietnam, and Myanmar stem from rice cultivation. Therefore, there is a need to do an assessment on GHG emissions from agricultural land with a high potential of methane emissions. The present study is a potential investigation, especially to determine the most appropriate mitigation options based on key factors.

Methane emissions are the result of CH$_4$ cumulation from bottom-to-top horizon with the soil profile. It can reach the surface and eventually be emitted to the atmosphere. This study aims to determine the potential of each soil horizon to produce and oxidize CH$_4$. The CH$_4$ production potential (CH$_4$-PP) and CH$_4$ oxidation potential (CH$_4$-OP) were determined by measuring the amount of CH$_4$ production from soil samples with a flood incubation treatment in the laboratory. The results will be useful in order to find mitigation options for CH$_4$ emissions, especially in organic paddy fields.

**MATERIAL AND METHODS**

**Soil samples.** The horizon was determined based on the Soil Survey Staff (2014). The soil was taken from the horizon in the soil profile of organic (P1), semi-organic (P2), conventional (P3) paddy field, and the teak forest land-use (P4) as a control. Soil samples were air-dried and sieved for diameter 0.5 mm and 2 mm. All soil samples were collected from soil profiles of organic paddy fields in Sukorejo, Sambirejo District, Sragen Regency, in Central Java. The sample organic rice fields have been certified organic by INOFICE (2008) as a producer of organic food (organic rice). The criterion for organic rice field selection is the use of organic fertilizers, for semi-organic the use of chemical and organic fertilizers, for conventional the use of most types of fertilizers (including both chemical and only occasionally or periodically), and for the teak forest as a control it is the land-use type of teak forest.

**Soil properties.** The soil properties were analyzed by texture (pipette method), H$_2$O pH (soil:water = 1:2.5; pH meter), C-organic (1 N K$_2$Cr$_2$O$_7$ oxidation), total of N (concentrated H$_2$SO$_4$ destruction), C/N ratio, available P (0.5 M NaHCO$_3$ extraction), total of P (HCl 25% extraction), available K (1 M NH$_4$OAc percolation), total of K (HCl 25% extraction), cation exchange capacity (percolation 1 M NH$_4$OAc and NaCl 10%), and base saturation (1 M NH$_4$OAc percolation) (EVIATI & SULAEUMAN 2009). Microbial biomass C was observed using the CHCl$_3$ fumigation method, with 0.5 M K$_2$SO$_4$ extraction after 0.05 M K$_2$SO$_4$ pre-extraction (MUELLER et al. 1992). The difference between fumigated and non-fumigated C was the number of microbial biomass C.

**CH$_4$-PP and CH$_4$-OP.** CH$_4$-PP was measured by incubating soil samples in the laboratory, then the gas produced was analyzed by a gas chromatograph (Shimadzu GC-14A; Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector (FID). Twenty grams of 0.5 mm diameter soil samples were air-dried, then put into a glass incubator, and added with 40 ml of distilled water, mixed gently, and then put into the incubator at 30°C. The gas production protocol was adopted from SUSILOWATI (2007) with the modification of adding acetylene as an inhibitor of methane oxidation (WATANABE et al. 1995, 1997; CHAN & PARKIN 2000). Fifty ml of acetylene was added into each 1000 ml of air in the headspace room of the glass incubator (WATANABE et al. 1995). Gas samples were measured at hour 0 (C$_0$) and hour 24 (C$_{24}$). The measurements were taken eight times, at five-day intervals.

After getting the concentration at times C$_0$ and C$_{24}$, the gas production (in µg CH$_4$/kg soil/day) was calculated using the following formula:

$$\text{CH}_4\text{ production} = (C_{24} - C_0) \times \frac{\text{Vhs}}{\text{WS}} \times \frac{\text{MW}}{\text{Vm}} \times \frac{\text{Tst}}{(\text{Tst} + \text{T})}$$

where:

- $C_0$ – CH$_4$ concentration at hour 0 (ppm)
- $C_{24}$ – CH$_4$ concentration at hour 24 (ppm)
- Vhs – headspace volume (ml)
- WS – weight of soil sample (g)
- MW – molecular weight of CH$_4$ (16.123 g)
- Vm – CH$_4$ volume at standard conditions (273.2 K) = 22.41 l
- Tst – temperature standard conditions (273.2 K)
- T – air temperature of incubation (°C)

The measurement of CH$_4$-OP used the same procedure as the measurement of CH$_4$-PP above, excluding the addition of acetylene into the samples (WATANABE et al. 1995, 1997; CHAN & PARKIN 2000). The difference between CH$_4$ production with and without the inhibitor is the amount of CH$_4$ oxidized (CH$_4$-OP).

**Statistical analysis.** The correlations between soil properties, CH$_4$-PP, and CH$_4$-OP were determined using the correlation analysis (STEELE & TORIE 1980), the Pearson’s correlation coefficient was calculated as well. The differences between soil properties,
CH$_4$-PP, and CH$_4$-OP between all horizon depths were determined using one-way analysis of variance (ANOVA) with the Duncan's Multiple Range test. All statistical analyses were performed with SPSS Statistics 17.0 software.

RESULTS AND DISCUSSION

CH$_4$-PP and CH$_4$-OP. The description of the soil profile shows that P1 consists of four horizons, P2 consists of four horizons, P3 consists of five horizons, and P4 consists of six horizons. Table 1 shows the horizons profile, selected soil properties, CH$_4$-PP, and CH$_4$-OP. The horizons of P1 profile (organic) have the highest average CH$_4$-PP and CH$_4$-OP followed by P2 (semi-organic), P4 (teak forest), and P3 (conventional).

From the result, CH$_4$-PP and CH$_4$-OP have a significant correlation ($r = 0.92$, $P < 0.001$, $n = 19$). It means that the soil has a high activity of methanogens (CH$_4$ production) and methanotrophs (CH$_4$ oxidation). This correlation is quite obvious as the first group (methanogens) produces a substrate (CH$_4$), which is used by the second group (methanotrophs) (Joulian et al. 1997). Brzezińska et al. (2012) also found that if the soil has a high level of CH$_4$ production, it also has a high level of CH$_4$ consumption (oxidation). Soil that has a high potential of CH$_4$ production will not necessarily release high emissions of CH$_4$. Zhu et al. (2012) stated that around 50% of the methane produced in wetlands is consumed before it reaches the atmosphere. In the laboratory condition, less than a half of the CH$_4$ was oxidized in the soil, while almost all of it was oxidized around a root area (Zang et al. 2013). By employing the methanothroph bacteria through some oxidation mechanisms, CH$_4$ could be converted into CO$_2$ (Nieder & Benbi 2008; Thaurer et al. 2008). CH$_4$ is oxidized by O$_2$ into 

### Table 1. Horizons P1–P4, selected soil properties, methane (CH$_4$) production potential (CH$_4$-PP), and oxidation potential (CH$_4$-OP)

<table>
<thead>
<tr>
<th>Profile</th>
<th>Horizon (notation)</th>
<th>Soil depth (cm)</th>
<th>Epipedon Endopedon soil classification*</th>
<th>Organic C Mean</th>
<th>Microbial biomass C Mean</th>
<th>CH$_4$-PP Mean</th>
<th>CH$_4$-OP Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>I (Apg) 0–18/22</td>
<td>Umbric Argillic/Sombric Umbric Epiaqualf</td>
<td>2.09</td>
<td>498.39</td>
<td>1.67</td>
<td>277.91</td>
<td>1.81</td>
</tr>
<tr>
<td></td>
<td>II (Bt) 18/22–52/58</td>
<td>1.31</td>
<td>84.98</td>
<td>1.05</td>
<td>0.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III (Bw1) 52/58–78/100</td>
<td>1.30</td>
<td>110.24</td>
<td>0.92</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV (Bw2) &gt; 150</td>
<td>1.30</td>
<td>110.24</td>
<td>0.92</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>I (Apg) 0–19/22</td>
<td>Umbric Cambic Epiaquept</td>
<td>1.78</td>
<td>475.42</td>
<td>1.62</td>
<td>292.26</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>II (Bw1) 19/22–46/62</td>
<td>1.56</td>
<td>172.26</td>
<td>0.82</td>
<td>0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III (Bw2) 46/62–91/108</td>
<td>1.48</td>
<td>321.54</td>
<td>0.66</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV (Bw3) &gt; 150</td>
<td>0.78</td>
<td>266.42</td>
<td>0.53</td>
<td>0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>I (Apg) 0–27/32</td>
<td>Umbric Cambic Epiaquept</td>
<td>1.83</td>
<td>468.53</td>
<td>1.58</td>
<td>324.99</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>II (Bw1) 27/32–56/75</td>
<td>1.88</td>
<td>443.27</td>
<td>1.12</td>
<td>0.47</td>
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<td></td>
</tr>
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<td></td>
<td>III (Bw2) 56/75–75/96</td>
<td>1.63</td>
<td>271.02</td>
<td>0.80</td>
<td>0.36</td>
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<tr>
<td></td>
<td>IV (Bw3) 75/96–102/108</td>
<td>1.52</td>
<td>282.50</td>
<td>0.67</td>
<td>0.22</td>
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<td></td>
<td>V (Bw4) &gt; 150</td>
<td>1.35</td>
<td>197.52</td>
<td>0.73</td>
<td>0.46</td>
<td></td>
<td></td>
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<tr>
<td>P4</td>
<td>I (Ap) 0–23/33</td>
<td>Umbric Cambic Epiaquept</td>
<td>1.83</td>
<td>468.53</td>
<td>1.58</td>
<td>324.99</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>II (Bw1) 23/33–50/70</td>
<td>1.63</td>
<td>271.02</td>
<td>0.80</td>
<td>0.36</td>
<td></td>
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<tr>
<td></td>
<td>III (Bw2) 50/70–80/95</td>
<td>1.52</td>
<td>282.50</td>
<td>0.67</td>
<td>0.22</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>IV (Bw3) 80/95–100/123</td>
<td>1.35</td>
<td>197.52</td>
<td>0.73</td>
<td>0.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>V (Bw4) 100/123–143/168</td>
<td>1.24</td>
<td>287.09</td>
<td>0.61</td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VI (Bw5) 143/168–180</td>
<td>1.24</td>
<td>287.09</td>
<td>0.61</td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P1 – organic; P2 – semi-organic; P3 – conventional; P4 – teak forest; *soil classification according to Soil Survey Staff (2014); Sub group, data not shown.
CO₂ + H₂O (Nieder & Benbi 2008), by NO₃⁻ into N₂ + CO₂, and by SO₄²⁻ into CO₂ + H₂S (Thauer et al. 2008).

**CH₄-PP and soil C₇org**. The soil property which has the closest relationship with CH₄-PP is C-organic (C₇org) (r = 0.80, P < 0.001, n = 19) (see Figure 1, left). When the C₇org in soil increases, the potential CH₄ production goes up. These results are in line with those obtained by Joulian et al. (1996), Oelbermann and Schiff (2008), and Liu et al. (2011) that CH₄-PP is strongly influenced by C₇org. Higher C₇org leads to the higher amount of CH₄-PP.

The decomposition of organic matter through the oxidation-reduction process is terminated by the formation of CO₂ and CH₄: C₆H₁₂O₆ → 3 CO₂ + 3 CH₄ (Le Mer & Roger 2001; Nieder & Benbi 2008). Sanchez (1976) stated that the decompositions of organic matter in the flooded and unflooded soil are the same until the stage of the pyruvic acid formation. The pyruvic acid and the other intermediate products formed in unflooded soil further oxidize to the final product of CO₂, NO₂⁻, SO₄²⁻ and resistant humic material, and the pyruvic acid formed in flooded soil with anaerobic conditions is further reduced to alcohol, organic acids, etc., and finally to the final product of CO₂ and CH₄.

The group of methanogens in the soil can produce CH₄ from either the reduction of CO₂ and H₂ into CH₄, the fermentation of CH₃COOH (acetic acid) into CH₄ and CO₂ (Nieder & Benbi 2008; Thauer et al. 2008), or from the reduction of CH₃OH (methanol) into CH₄ (Reddy & DeLaune 2008). Figure 1 (left) shows that the CH₄-PP has a quadratic function with C₇org. When C₇org increases, the CH₄-PP significantly rises, which is defined by the equation $Y$ function (CH₄-PP) = 0.91$x^2$ − 1.82$x$ + 1.53 ($R^2 = 0.81$). Brzeziniska et al. (2012) proved that there is a significant correlation between C₇org and CH₄ production through the equation model $Y$ = 0.127$x^2$ + 0.07 and $R^2 = 0.90$.

P1 has the highest average of C₇org content (1.67%) and CH₄-PP (1.36 µg CH₄/kg soil/day) if compared to other soil profiles (see Table 1). C₇org content in the soil profiles of organic fields, especially in horizons I and II (up to depths of ca. 50 cm), is assessed into moderate category (2–3%) and is significantly higher than in other soil profiles of the same horizon that are categorized as “low” and “very low” (< 2%). The organic rice system practices since 2001, with the average use of 6 t of manure during each planting season, are strongly suspected as the primary factor causing higher amounts of C₇org in organic fields if compared to semi-organic, conventional, and teak forest areas.

**CH₄-PP and microbial biomass C**. The populations of methanogens and methanotrophs were observed by analyzing the microbial biomass C to describe the microbial population in the soil. The correlation between microbial biomass C and C₇org is significant (r = 0.52, P < 0.05, n = 19). Figure 1 (right) shows the quadratic regression of the equation ($R^2 = 0.53$). The higher the C₇org content in the soil, the higher the microbial population observed. This result is supported by a previous research by Dalai et al. (2011).

Microbial biomass C correlates significantly with CH₄-PP (r = 0.56, P < 0.05, n = 19), but not with CH₄-OP (r = 0.35, P > 0.05, n = 19). Even though the correlation between microbial biomass C and CH₄-OP is not significant, it has a tendency of increasing in the amount of microbial biomass C that is also followed by rising amounts of CH₄-OP. The soil profile with a high population of microbial biomass C has
high CH$_4$-PP and CH$_4$-OP as well. These correlations are presented in quadratic regressions with the value of $R^2 = 0.74$ and 0.67 respectively (see Figure 2). Five soil horizons of P3 contain a low amount of C$_{org}$ and a lower amount of microbial biomass C (Figure 1, right). This condition caused the amount of CH$_4$-PP to decrease (Figure 2). However, it can be assumed that the increasing amount of microbial biomass C is followed by the number of microbes that produce and oxidize CH$_4$. Dar et al. (2008) argue that methanogens have the range of 10% of the total microbes in the laboratory scale bioreactor experiments.

**CH$_4$-PP and soil depth.** There was no significant difference between CH$_4$-PP and C$_{org}$ between all horizon depths observed (one-way ANOVA $F = 2.225$, $P > 0.05$; one-way ANOVA $F = 1.159$, $P > 0.05$, respectively). However, there was a significant variation in microbial biomass C (one-way ANOVA $F = 3.587$, $P < 0.05$). Microbial biomass C in horizon I was the highest among all the other samples. Although there was no significant difference in CH$_4$-PP and C$_{org}$ between all horizon depths analyzed, microbial biomass C correlates significantly with C$_{org}$ ($r = 0.52$, $P < 0.05$, $n = 19$) and CH$_4$-PP ($r = 0.56$, $P < 0.05$, $n = 19$), as C$_{org}$ correlates significantly with CH$_4$-PP ($r = 0.80$, $P < 0.001$, $n = 19$). The significant variation in microbial biomass C between the horizon depths and the three correlations shows that there is a strong presumption significantly affecting the depth of horizon for CH$_4$-PP through its influence on the C$_{org}$ and microbial biomass C. In the upper horizon, C$_{org}$ and microbial biomass C were high as it would increase the CH$_4$-PP.

Figure 2. Regression lines of microbial biomass C with CH$_4$ production potential (CH$_4$-PP) and CH$_4$ oxidation potential (CH$_4$-OP)

Figure 3. CH$_4$ production potential (CH$_4$-PP) patterns of soil with high C$_{org}$
Whalen and Reeburg (2000) and Brzezińska et al. (2012) investigated the correlation between soil depth, C$_{org}$ and CH$_4$-PP. C$_{org}$ content in the upper horizon was higher than in the lower horizon, so as the CH$_4$-PP. Freitag et al. (2010) concluded that the depth of the soil is one of the factors that control the amount of CH$_4$ emissions.

**CH$_4$-PP and incubation time**. The level of C$_{org}$ determines both CH$_4$-PP and CH$_4$ production patterns based on the time of flood incubation. The soil with high C$_{org}$ (P1 horizon I and II, P2 horizon I, and P4 horizon I and II), moderate C$_{org}$ (P1 horizon IV, P2 horizon II, III, and IV, and P4 horizon IV and V), and low C$_{org}$ (P3 horizon II, III, IV, and V, and P4 horizon VI) reached the maximum average of CH$_4$ production at various times after incubation, i.e. on day 20 (Figure 3), day 15 (Figure 4), and day 10 (Figure 5), respectively.

Soil with high C$_{org}$ has enough C substrates to produce high amounts of CH$_4$ and it will reach a longer peak production period. Meanwhile, soil with low C$_{org}$ has less C substrate, produces lower amount of CH$_4$, and shows faster peak production periods than the others. These results were strengthened by previous findings by Brzezińska et al. (2012) and Yuan et al. (2014), which measured CH$_4$ production after flooding the soil samples. Soil with higher C$_{org}$ experienced a longer peak time of production than soil with lower C$_{org}$.

**CONCLUSION**

The CH$_4$ production in soil is reflected in its chemical, physical, and biological properties. This study showed that the CH$_4$ production potential in organic rice fields is high because of the presence of high amounts of C$_{org}$. In consequence, the mitigation options need more efforts than required in other cultivation (farming) systems. The CH$_4$ oxidation
potential in organic rice fields is high as well, as the mitigation options should focus on increasing the methanotrophic activity which might reduce CH$_4$ emissions to the atmosphere. Because C$_{\text{org}}$ has an important role in the production of CH$_4$, it determines the dynamics of CH$_4$-PP and CH$_4$-OP. So using particular kinds of organic matter as C$_{\text{org}}$ sources would manage the composition of methanogens and methanotrophs so as to reduce CH$_4$-PP and to increase CH$_4$-OP.

C$_{\text{org}}$ also affected the pattern of the CH$_4$ production potential by the time of flood incubation. High amounts of C$_{\text{org}}$ reached a maximum CH$_4$-PP on day 20 after incubation, while moderate C$_{\text{org}}$ on day 15, and low C$_{\text{org}}$ on day 10. Higher amounts of C$_{\text{org}}$ provide enough C substrates to produce higher amounts of CH$_4$ and reach longer peak times of production than lower C$_{\text{org}}$. This finding provides guidance that mitigation option reduces CH$_4$ emissions from organic rice fields and experiences drainage every 10–20 days before reaching the maximum CH$_4$-PP.

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