Methanogenic responses to exogenous substrates in anaerobic rice soils

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Abstract

Soils collected from rice fields in the Philippines differed in their inherent potential for methane in vitro production and were tested for their response to organic amendments. Two soils were amended with either acetate or glucose (experiment I), root exudates (experiment II), and three soils were amended with rice straw (experiment III). Addition of acetate, glucose, or root exudates stimulated CH\textsubscript{4} production in soil with high production capacity (Pangil) (16.0 \textmu mol CH\textsubscript{4} g\textsuperscript{−1}) as well as low production capacity (Maahas) (0.171 \textmu mol CH\textsubscript{4} g\textsuperscript{−1}). However, the response triggered by a given amendment was more pronounced in Pangil soil than in Maahas soil. Similarly, application of rice straw triggered the fastest response in the soil with high inherent production potential (Pila) (peaking at 2 weeks after incubation at 25°C) as compared to the soil with moderate (Luisiana) (peaking at 3 weeks) and low production potential (Maahas) (peaking at 4 weeks). In all experiments, soils with an inherently high production (Pangil, Pila) showed a faster and higher response than those with suppressed production (Luisiana, Maahas). The net increments of production rates were used to calculate the transformation efficiencies, i.e. the stochiometric rate of CH\textsubscript{4} produced from a given substrate amendment. The transformation efficiencies of added substrates decreased in the order of glucose > acetate > root exudates > straw. High transformation efficiencies of acetate, glucose and root exudates indicated a priming effect, i.e. enhanced decomposition of soil organic matter through added substrate. This priming effect due to the reactivation of fermentative microflora by adding substrate C may also increase the effects of root exudates under field conditions. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Methanogenesis; Exogenous substrate; Anaerobic incubation; Flooded soil; Priming effects

1. Introduction

The emission of methane from irrigated rice fields is the result of complex interactions between rice plants and the microbial community of the soil (Neue and Sass, 1994; Conrad, 1996). The flooding of a rice field initiates a series of events resulting in CH\textsubscript{4} production as the final step (Ponnamperuma, 1972). Soil organic matter, applied organic materials, and root-derived carbon provide carbon sources for CH\textsubscript{4} production and emission.

The CH\textsubscript{4} production from irrigated rice can be divided into two phases, based on the nature of the methanogenic substrate. In the initial phase, CH\textsubscript{4} production results from mineralisation of inherent soil organic matter and previously incorporated organic materials such as plant residues and manure (Yagi and Minami, 1990; Sass et al., 1991; Cicerone et al., 1992;
In the second phase, CH$_4$ production results from newly-generated plant material, in particular, decaying roots and root exudates (Holzapfel-Pschorn et al., 1986; Lindau et al., 1991; Kimura, 1997). The soil type and the properties of the organic substrates influence CH$_4$ production and emission in both phases. Identical amounts of straw incorporation may cause different timing and shapes of emission peaks in the initial phase (Yagi and Minami, 1990), while the quantity of root-derived materials alone cannot explain pronounced differences in CH$_4$ production among different cultivars (Neue et al., 1997). Our objectives were to determine the methanogenic responses of anaerobic soils to exogenous substrates, including acetate, glucose, root exudates and rice straw, and to evaluate effects of soil and substrate types on CH$_4$ production in flooded soils.

2. Materials and methods

2.1. Soil samples

The four soils were sampled in rice fields of Laguna Province (Philippines) within a radius of approximately 15 km. Soil samples were taken from the plow layer (0–20 cm) during the fallow period and were air-dried, ground, and sieved (1 mm). Details of the soil properties are given in Table 1. The soils represented a broad range regarding pH (4.0–7.8) and organic matter content (1.57–3.76%).

2.2. Organic amendments

The solutions of glucose and sodium acetate used in experiment I had concentrations of 3.47 and 10.4 mM, respectively. These substrate solutions were mixed with 25 mM KH$_2$PO$_4$–Na$_2$HPO$_4$ buffer solution to obtain an initial pH of 6.8 in all samples. Root exudates used in experiment II were collected from a separate experiment that provided three different types of exudates of the cultivar IR72 grown under different amounts of P-nutrition (Lu et al., 1999). In brief, rice plants were grown in nutrient culture with high P (10 µg ml$^{-1}$) and low P (0.5 µg ml$^{-1}$) supplies. Root exudate was collected at the tillering and flowering stages in 500 ml sterilized deionized water. Collected solutions were filtered in the laboratory and subsequently concentrated 20-fold through freeze-drying in a lyophilizer. The concentrated exudates were mixed with 25 mM KH$_2$PO$_4$–Na$_2$HPO$_4$ buffer solution and adjusted to pH 6.8. The final solutions had total carbon concentrations of 1.05, 1.49 and 2.49 mmol C ml$^{-1}$, respectively, for the A, B and C samples of exudates. All samples were kept frozen until the experiment on CH$_4$ production rates started.

For experiment III, fresh rice straw was washed in water, dried at 60°C in oven, ground to 0.2–0.5 mm, and stored until use. The dried straw had a carbon content of 40%.

2.3. Response to glucose and acetate (experiment I)

The experimental procedure consisted of the following steps:

1. Mixing and starting. Anaerobic incubation was preceded by mixing 10 g of air-dried soil with 16 ml deionized water in incubation vessels. Each incubation vessel consisted of a 100 ml spoutless beaker with a magnetic bar at the bottom and a rubber stopper seal equipped with gas outlet and inlet. The soil suspensions were purged with N$_2$ for 3 min to remove O$_2$ from the liquid and gas phases in the beaker.

2. Conditioning incubation. The soil suspensions without amendments were incubated at 30°C for 14 days to ensure the development of anaerobic conditions for methanogenesis. The incubation scheme shown in Fig. 1 encompassed recording phases of 12 h and connecting phases of different duration. Recording

<table>
<thead>
<tr>
<th>Soil</th>
<th>Maahas</th>
<th>Pangil</th>
<th>Luisiana</th>
<th>Pila</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:1 water)</td>
<td>6.40</td>
<td>4.40</td>
<td>4.50</td>
<td>7.4</td>
</tr>
<tr>
<td>Org. C (%)</td>
<td>1.57</td>
<td>3.96</td>
<td>1.84</td>
<td>2.08</td>
</tr>
<tr>
<td>Tot. N (%)</td>
<td>0.174</td>
<td>0.283</td>
<td>0.180</td>
<td>0.182</td>
</tr>
<tr>
<td>Avail P (Olsen) (mg kg$^{-1}$)</td>
<td>10.0</td>
<td>2.20</td>
<td>5.9</td>
<td>24</td>
</tr>
<tr>
<td>CEC (meg 100 g$^{-1}$)</td>
<td>37.3</td>
<td>49.9</td>
<td>24.9</td>
<td>27.2</td>
</tr>
<tr>
<td>Active Mn (%)</td>
<td>0.119</td>
<td>0.0340</td>
<td>0.109</td>
<td>0.058</td>
</tr>
<tr>
<td>Active Fe (%)</td>
<td>2.27</td>
<td>5.91</td>
<td>4.63</td>
<td>0.800</td>
</tr>
<tr>
<td>Inherent capacity (µmol g$^{-1}$ soil)$^a$</td>
<td>0.171</td>
<td>16.0</td>
<td>3.44</td>
<td>30.5</td>
</tr>
<tr>
<td>Texture</td>
<td>Clay</td>
<td>Clay</td>
<td>Clay</td>
<td>Silt</td>
</tr>
<tr>
<td>Soil order</td>
<td>Mollisol</td>
<td>Inceptisol</td>
<td>Entisol</td>
<td>Alfisol</td>
</tr>
</tbody>
</table>

$^a$ Inherent capacity is the cumulative methane production during 28 days incubation without organic amendment.
phases started with the flushing of N$_2$ for 3 min as described earlier. Gas samples were collected at the end of each recording phase whereas connecting phases were not used for sampling.

3. Addition and ensuing incubation. Addition solutions were purged with N$_2$ for 1 h before addition to remove O$_2$ from the solutions. An aliquot of 4 ml addition solution of glucose or acetate was added into four replicate samples for each soil. Four control samples per soil were treated with 4 ml of 25 mM KH$_2$PO$_4$–Na$_2$HPO$_4$ buffer solution. The incubation was continued for another 14 days while CH$_4$ production rates were determined eight times (Fig. 1).

4. Sampling and analysis. 1 ml gas samples were withdrawn through a septum in the headspace for analysis. The CH$_4$ concentration was determined in a gas chromatograph equipped with a flame ionization detector using Porapak N column (100/200 mesh, 2 m length $\times$ 0.2 cm i.d.). N$_2$ was used as carrier gas. The column and detector temperatures were 60 and 150°C, respectively.

2.4. Response to root exudates (experiment II)

The anaerobic incubation with root exudates corresponded to the procedure described for experiment I. 4 ml of three types of concentrated exudate solutions were added to the anaerobic soil suspensions which had been conditioned for 14 days before addition. After addition the incubation was continued for another 14 days for sampling.

2.5. Response to straw amendment (experiment III)

Dried straw (0.2 g) was added to 20 g air-dried soil at the start of the experiment (without prior incubation). Three different soils (Maahas, Luisiana and Pila) were incubated at 25, 30 and 35°C over 56 days (Wassmann et al., 1998). Production rates were determined at weekly intervals with a procedure identical to that described earlier; the incubation interval of one recording phase was set to 24 h. As in experiment I and II, the experiment encompassed four replications per amendment and soil as well as four controls per soil.

2.6. Calculation of CH$_4$ production rate

Methane production rates were computed using the following equation

$$P = \frac{dc}{dt} \times V_{hs} \times MV \times W_s$$

where,

$$P = \text{methane production rate (} \mu\text{mol CH}_4 \text{ g}^{-1} \text{d.w. soil day}^{-1})$$,

$$dc = \text{increment in methane concentration in the headspace (} \mu\text{l} \text{l}^{-1})$$,

$$dt = \text{incubation time (=0.5 day)}$$,

$$V_{hs} = \text{volume of headspace (l)}$$,

$$MV = \text{molar volume of methane at 30°C (=40.2 mmol l}^{-1})$$,

$$W_s = \text{dry weight of soil (g)}$$.

3. Results

3.1. Inherent methane production

The four soils tested encompassed a wide variation in soil physical–chemical properties (Table 1). Pangil had high contents of organic C and total N, high CEC and low pH. Pila showed silt structure and a relatively high pH. Maahas and Luisiana were low in organic C and total N contents.

The inherent CH$_4$ production differed significantly among these soils (Table 1). Pila and Pangil showed a substantially higher CH$_4$ production potential than Luisiana and Maahas. The cumulative CH$_4$ production within 28-day incubation was 9 and 178 times higher for Pila and 5 and 94 times higher for Pangil than Luisiana and Maahas, respectively. Temporal patterns of CH$_4$ production showed immediate development for Pila and Pangil, delayed development for Luisiana and suppressed development for Maahas (Wassmann et al., 1998). However, there was no simple correlation between CH$_4$ production potentials and any of the soil properties listed.

3.2. Response to acetate and glucose

Addition of acetate or glucose (at identical rates of 8.33 $\mu$mol substrate-C g$^{-1}$ soil) stimulated CH$_4$ pro-
duction in Pangil and Maahas, but response patterns of these soils were different. In Pangil, the addition of these substrates triggered an immediate increase in CH$_4$ production, with maximum values of 3.1 and 5.7 μmol CH$_4$ g$^{-1}$ dry weight soil day$^{-1}$ recorded at 2 and 4 days, respectively, for acetate and glucose (Fig. 2(a)). In Maahas, CH$_4$ production gradually increased until maximum of 1.2 μmol CH$_4$ g$^{-1}$ dry weight soil day$^{-1}$ (acetate) and 3.4 μmol CH$_4$ g$^{-1}$ dry weight soil day$^{-1}$ (glucose) at 11 day (Fig. 2(b)). Pangil responded faster and stronger to glucose amendment than Maahas.

The transformation efficiency ($= \mu$mol of CH$_4$–C produced $\mu$mol$^{-1}$ of substrate–C) was derived from the carbon content in the net CH$_4$ production, i.e. CH$_4$–C production with amendment deducted by CH$_4$–C production in control, and the carbon content of the amended substrate. Transformation efficiencies were 1.88 and 1.54 μmol CH$_4$–C $\mu$mol$^{-1}$ substrate–C for glucose in Pangil and Maahas, respectively, and the corresponding values were 0.94 for acetate in both soils. Derived from the stochiometry, 1 μmol of substrate–C (acetate or glucose) can generate a maximum of 0.5 μmol CH$_4$ (plus 0.5 μmol CO$_2$). However, the measured transformation efficiency largely exceeded the theoretical maxima. Furthermore, glucose yielded higher transformation efficiencies than acetate. Although the total added carbon for acetate and glucose was identical (8.33 μmol substrate–C g$^{-1}$ soil), the accumulated CH$_4$ production over the 15-day incubation was higher for glucose than for acetate (Table 2).

3.3. Response to root exudates

The effects of root exudates on methanogenesis are shown in Fig. 3. In Pangil, CH$_4$ production rates sharply increased and reached maximum of 1.3–1.7 μmol CH$_4$ g$^{-1}$ dry weight soil day$^{-1}$ at 2 day after addition of root exudates (Fig. 3(a)). Then, CH$_4$ production decreased steadily in all trials to uniform rates in samples with or without amendments. In Maahas, stimulation of methanogenesis was less pronounced; only exudate type C resulted in a distinct peak after 6 days, which was 0.1 μmol CH$_4$ g$^{-1}$ dry weight soil day$^{-1}$ (Fig. 3(b)). The absolute values of the stimulated methanogenesis by root exudates in Maahas were much lower than in Pangil.

Root exudates with high concentrations of organic carbon (type C) yielded high production rates while low concentrations resulted in less production (types A and B) (Table 2). The small difference in carbon content of types A and B was only detectable against a low rate of inherent production (Maahas) while the differences were negligible in Pangil, i.e. the soil with a high inherent production (Table 2).

Transformation efficiencies of root exudates were 1.94, 1.28 and 1.22 μmol CH$_4$–C $\mu$mol$^{-1}$ root exudate–C.

Fig. 2. Methane production rates of (a) Pangil and (b) Maahas soils after amendment of acetate or glucose. Control left without amendment. Bars represent standard deviation. Arrows indicate time of acetate or glucose addition.

Fig. 3. Methane production rates of (a) Pangil and (b) Maahas soils after amendment with root exudates. Control left without amendment. Three types of root exudates differed in carbon contents (A < B < C). Bars represent standard deviation. Arrows indicate time of root exudate addition.
for types A, B and C, respectively, in Pangil, and 0.50, 0.48 and 0.44, respectively, in Maahas. The measured transformation efficiencies in Pangil were much higher than the theoretical maxima, while those in Maahas were approximately equal to the theoretical maxima.

3.4. Response to rice straw

Incorporation of rice straw enhanced CH$_4$ production in all soils (Fig. 4), but the response patterns of soils to straw amendments also differed significantly.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Total added C ($\mu$mol C g$^{-1}$ soil)</th>
<th>Pangil soil</th>
<th>Maahas soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH$_4$ production ($\mu$mol CH$_4$–C g$^{-1}$ soil)</td>
<td>Net production ($\mu$mol CH$_4$–C g$^{-1}$ soil)</td>
<td>CH$_4$ production ($\mu$mol CH$_4$–C g$^{-1}$ soil)</td>
</tr>
<tr>
<td>Experiment I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.75</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>8.33</td>
<td>16.58</td>
<td>7.83</td>
</tr>
<tr>
<td>Glucose</td>
<td>8.33</td>
<td>24.42</td>
<td>15.67</td>
</tr>
<tr>
<td>Experiment II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.39</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Exudate A</td>
<td>0.56</td>
<td>5.47</td>
<td>1.08</td>
</tr>
<tr>
<td>Exudate B</td>
<td>0.80</td>
<td>5.41</td>
<td>1.02</td>
</tr>
<tr>
<td>Exudate C</td>
<td>1.33</td>
<td>6.02</td>
<td>1.63</td>
</tr>
</tbody>
</table>

* Accumulated CH$_4$ production were calculated from interpolating and averaging their rates after 15-day incubation after substrate addition in experiment I and 7-day incubation in experiment II.
* Control left without amendment.
* Net CH$_4$ production is the amended rate minus the control rate.

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Fig. 4. Effects of temperature on methane production rates of three soils with (+RS) or without (–RS) rice straw amendment. Straw was added at day 0. Bars represent standard deviation.
among soils. In correspondence with the inherent potential (Fig. 4(d)–(f)), Pila showed the fastest response to amendments amongst three soils (Fig. 4(a)–(c)). For Pila, the production peaks were recorded after 1–2 weeks whereas Maahas and Luisiana developed these values after 2–4 weeks.

An increase in temperature enhanced CH$_4$ production both with or without rice straw amendments (Fig. 4). At higher temperature, maximum CH$_4$ production appeared earlier and reached higher values. The total CH$_4$ production over 56 days of incubation at 35°C was 36, 60 and 66% higher than at 25°C, respectively, for Pila, Luisiana and Maahas soils (Table 3). The transformation efficiencies of rice straw were 0.2–0.3 in these soils, i.e. straw amendment did not trigger a priming effect in rice soils.

4. Discussion

4.1. Differences among soils

The responses of CH$_4$ production in anoxic soils to exogenous substrates can be grouped into two patterns: (1) with a rapid development of CH$_4$ production rates as observed in Pangil and Pila and (2) with a lag in development as observed in Maahas and Luisiana. This grouping was observed irrespective of the substrate type, i.e. acetate and glucose (experiment I), exudates (experiment II) and rice straw (experiment III). Apparently, response patterns were related to inherent production potential; soils with a high potential react rapidly while soils with a lower potential show a delay in the development of CH$_4$ production.

However, the reasons for the correlation between response pattern and inherent production potential are not clear. For experiments I and II, a possible explanation for this finding can be seen in the different types of development of methanogenic activity during the initial 14 days of incubation. At the time when the additional substrate was supplied, CH$_4$ production rates were already high derived from the inherent substrate of Pangil, while production was low in Maahas. However, rice straw was amended at the start of the incubation in experiment III, and again, the soil with high inherent production showed fastest development.

4.2. Differences among substrates

In Pangil and Maahas, the addition of acetate and glucose yielded much higher transformation efficiencies than the theoretical maxima. Root exudates also showed high transformation efficiency in a soil with high CH$_4$ production potential (Pangil). Glucose stimulated higher CH$_4$ production than acetate. A possible explanation for the high transformation efficiencies is the priming effect of added substrates on CH$_4$ production. This priming could be caused by an enhanced decomposition of native soil organic matter facilitated by reactivation of fermentative microflora (Chahal and Wagner, 1965; Behera and Wagner, 1974; Mary et al., 1993), or an enhanced turnover of microflora (Chahal and Wagner, 1965; Dalenberg and Jager, 1989). Wu et al. (1993) reported that a marked priming effect existed with large addition of glucose (5 mg C g$^{-1}$ soil) but not with low addition (0.5 mg C g$^{-1}$ soil). Shen and Bartha (1996, 1997) observed that the priming effect of glucose could reach 100% of utilized substrate-C, while its structural analogs, fructose and inositol, failed to elicit any priming. The priming effect of glucose in this experiment appeared extremely high and the reason for this is not known.

Root-derived materials contributed to CH$_4$ production during the latter growth periods (Lindau et al., 1991; Minoda and Kimura, 1994; Neue and Sass, 1994; Chidthaisong and Watanabe, 1997). Our study demonstrated that root exudates were rapidly converted to CH$_4$ (Fig. 3). Transformation efficiency of root exudate was also high, especially in the soil with high inherent capacity. Thus, root exudates seemed not only to provide a carbon source for CH$_4$ formation but also promoted decomposition of soil endogenous organic matter.

A cross-comparison among all substrates can be accomplished by citing the experiments with Maahas at 30°C in experiments I, II, and III. The transformation efficiencies of added substrates decreased in the order of glucose > acetate > exudates > straw. This observation indicates that the quality of substrate affects CH$_4$ generation in rice soil. Maximum production rates occurred after 4–7 days for root exudates and 21–28 days for rice straw. The low transformation efficiency and the delay in the CH$_4$ developed from rice straw can be explained by the relatively slow break-down of rather inert constituents of plant tissue. This time span of 21–28 days until maximum CH$_4$
production corresponds to the temporal pattern of CH$_4$ emission in the field after straw application (Wassmann et al., 1993a,b, 1996).

Increasing temperature from 25°C to 35°C stimulated faster and higher CH$_4$ production (Fig. 3). Higher temperatures could enhance soil microbial activity, which then cause faster CH$_4$ production from amended as well as non-amended soils (Sexstone and Mains, 1990; Dunfield et al., 1993; Kimura et al., 1993). The dependence of methanogenesis to temperature may largely regulate seasonal fluctuation of CH$_4$ production in the fields.

5. Conclusions

In our experiments the different types of substrate amendments corresponded to those made under field conditions. Rice straw is generally incorporated before field preparation and is present at the start of the flooding period. The results of experiment III indicate an effect of the soil type on the pattern of CH$_4$ production rates in the early stage of the growing season. Soils with a low inherent production potential cause a delayed response to straw amendment. Thus, the development of methanogenesis in these soils will be substantially retarded as compared to soils with a fast response and high inherent production.

Root exudation is generally low during the younger stages of rice growth and reaches maximum during the flowering stage. Although wetland rice fields may undergo temporary drainage periods, it seems likely to assume that the bulk of root exudates are released when the soil is anaerobic. The results of experiment II showed that the root exudates are readily available for methanogenesis and the transformation efficiency is high. The effects of root exudates on CH$_4$ emissions might be magnified by a priming of soil organic matter. This priming effect depends on the composition of root exudates; glucose is a very effective priming agent. Thus, quantity and quality of root-derived materials would largely control CH$_4$ production and emission during the latter stage of the growing season.

References


