Influence of elevated carbon dioxide concentrations on methane emission and its associated soil microflora in rice ecosystem

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INTRODUCTION

Rice fields are considered important sources of atmospheric methane (CH₄), contributing about 5-19 per cent of total global CH₄ emissions to the atmosphere (Intergovernmental Panel on Climate Change, 2018). Methane is the potent greenhouse gas next to carbon dioxide, which is 25 times greater in global warming potential than CO₂ on a 100-year horizon (Intergovernmental Panel on Climate Change, 2013). Projections to the end of this century suggest that atmospheric [CO₂] will top 700 ppm or more (Intergovernmental Panel on Climate Change, 2018). Photosynthesis, a major process of sequestration and turnover of the total carbon on the planet is strongly influenced by the elevated atmospheric carbon dioxide concentrations. Crops sense and respond directly to rising [CO₂] through photosynthesis and stomatal conductance and there is a
need to assess the likely influence of changing atmospheric carbon dioxide concentrations on methane emission and its associated microflora. The amount of CH\textsubscript{4} emitted from rice fields to the atmosphere is the balance of two opposite processes, i.e., CH\textsubscript{4} production and oxidation in the soil. In the global CH\textsubscript{4} cycle, a substantial amount of CH\textsubscript{4} is consumed by biological processes. The only known biological sink for atmospheric CH\textsubscript{4} is its oxidation in aerobic soils by methanotrophs or methane-oxidizing bacteria (MOB), which can contribute up to 15 per cent to the total global CH\textsubscript{4} destruction (Singh, 2011).

With this background, experiments were conducted by employing carbon dioxide enrichment facility to understand the response of elevated carbon dioxide concentrations on methane emission and its associated biological activities, especially methanogens and methanotrophs.

**MATERIALS AND METHODS**

**Open top chambers (OTCs)**

The influence of elevated levels of CO\textsubscript{2} viz., 550 µmol mol\textsuperscript{-1} CO\textsubscript{2} and 750 µmol mol\textsuperscript{-1} CO\textsubscript{2} on rice crops on methane emission were investigated by employing Open Top Chambers with a dimension of 3x3x3 m.

**Pot experiment**

The soil used was sandy clay which belongs to Noyyal series and classified taxonomically as *Typic Ustochrept* according to USDA classification, 1999. The soil was slightly alkaline (pH = 8.21) with low soluble salts (EC = 0.35). The soil was high in organic carbon content (6.78 g kg\textsuperscript{-1}), low in available nitrogen (110.3 mg kg\textsuperscript{-1}), medium in available phosphorus and potassium (6.8 mg kg\textsuperscript{-1} and 118.0 mg kg\textsuperscript{-1}), respectively. Seven kilograms of soil transferred into a syntex pot was used in this study. The FYM at the recommended dose of 12.5 t ha\textsuperscript{-1} (41.6 g pot\textsuperscript{-1}) and NPK at 150:50:60 kg ha\textsuperscript{-1} (500, 166.6, 200 mg NPK pot\textsuperscript{-1}) were applied in the form of urea, single super phosphate and muriate of potash, respectively. Zinc sulphate at rate of 25 kg ha\textsuperscript{-1} (83.3 mg pot\textsuperscript{-1}) was applied and was thoroughly mixed with the soil. The N and K was applied in four splits and P was applied basally before transplanting. Rice crop was treated with three different levels of nitrogen viz., 0, 150, 200 kg N ha\textsuperscript{-1} and the N was applied in four splits on soil weight basis. Nursery was raised in the wetland farm and 14 days old paddy (ADT 45) seedlings were transplanted into the pots. After establishment, two healthy seedlings were allowed to grow in each pot. Twenty five days old rice crop was subjected to different CO\textsubscript{2} atmospheric conditions. The pots were maintained under flooded conditions (cyclic submergence) throughout the crop period.

**Treatments**

| Design: Factorial Competely Randomized Design (FCRD) |
| Replications: Five |

**Factor 1:**

- C\textsubscript{amb} - Ambient CO\textsubscript{2} concentration (396 µmol mol\textsuperscript{-1} CO\textsubscript{2})
- C\textsubscript{550} - 550 µmol mol\textsuperscript{-1} CO\textsubscript{2}
- C\textsubscript{750} - 750 µmol mol\textsuperscript{-1} CO\textsubscript{2}

**Factor 2:**

- N\textsubscript{0} - 0 kg N ha\textsuperscript{-1}
- N\textsubscript{100} - 150 kg N ha\textsuperscript{-1}
- N\textsubscript{200} - 200 kg N ha\textsuperscript{-1}

**Estimation of methane flux**

Gas samples were collected from the pots using static closed chamber technique and the gas chambers (250 mm diameter and 890 mm height) were fabricated in such a way that it fits the pot. The other specifications and components are similar to the chambers used for field measurements. Gas samples were collected at active tillering, flowering and harvest using tedlar bags and the protocol was followed for collection and estimation were carried out as per the standard procedure (Rajkishore et al., 2013).

**Redox potential**

Measurements for redox potential were done with each set of CH\textsubscript{4} flux measurement. The redox potential (Eh) of the field soil was measured by inserting a combined waterproof ORP/ redox meter (Eutech Instruments, USA) to the soil and measuring the potential difference in mV (Satpathy, 1997). The Eh of soil was measured (rhizosphere to bulk soil interface) in the morning and afternoon at different points near the flux measurement setup and averaged for the day.

**Dissolved organic carbon (DOC)**

Equilibrated soil solution samples were collected by zero tension sampling using soil water samplers (Tiensing et al., 2001). The DOC was estimated by adopting the protocol of Nelson and Sommers (1996) with a slight modification as described by Lu et al. (2000). 2 mL of the soil solution was mixed with 3.0 mL of deionized water, 5.0 mL of 0.0175 M K\textsubscript{2}Cr\textsubscript{2}O\textsubscript{7}, 10.0 mL of 98% H\textsubscript{2}SO\textsubscript{4}, and 5.0 mL of 88% H\textsubscript{3}PO\textsubscript{4} in a tube and digested for 30 min at 150°C. Upon cooling, the solutions were transferred to 150 mL Erlenmeyer flasks and titrated with 0.005 M Fe (NH\textsubscript{4})\textsubscript{2}(SO\textsubscript{4})\textsubscript{2}·6 H\textsubscript{2}O in 0.4 M H\textsubscript{2}SO\textsubscript{4} solution and sucrose was used as a standard.

**Methanotrophs**

Nine ml of phosphate buffer solution were taken in test tubes representing up to 10\textsuperscript{6} dilution. Then the tubes
were sterilized in autoclave at 15psi for 20 minutes. One gram of soil sample was taken and serially diluted. Dilutions of $10^3$ were used for plating. Pour plate technique was performed using Noble Agar Medium and the plates were incubated in the Macintosh jar assembly (Plate 1) with provisions for attaching a bladder containing methane. Methane was provided as the carbon source for the growth of methanotrophs in the chamber and the plates were incubated for 7 to 15 days. Methane gas was replenished once in two days through the bladder. After fifteen days of incubation, the plates were removed, and the o-dianisidine dye test was performed to assess the methanotrophs activities in the presence of naphthalene crystals. Few naphthalene crystals were sprinkled on the plate lid and stored inverted for 15 minutes. Then the plates were opened and freshly prepared o-dianisidine (tetrazotized; zinc chloride) was sprayed and incubated for 15 minutes in the presence of the dye. Methanotrophs exhibits methane mono oxygenase (MMO) activity and hence, naphthal was produced and purple red colour colonies were observed. Methanotrophs colonies (Purple red) colonies were counted and expressed as CFU g$^{-1}$ of dry soil.

**Methanogens**

Methanogens were enumerated by adopting the roll tube technique (Hungate, 1957). Soil samples were collected at active tillering, panicule initiation, flowering and harvest stages and enumerated for the population of anaerobic micro-flora. The samples were collected at the lower horizon (10 cm depth) under anaerobic conditions (Ramasamy et al., 1992). The population of methanogens was estimated by using Mah’s medium (Mah, 1980). The colonies were identified by their bluish fluorescence under UV light.

**Statistical analysis**

The data were statistically analyzed, as suggested by Gomez and Gomez (1984). Wherever the treatment differences were found significant, the critical difference (CD) were worked out at the 5 per cent level of significance with mean separation by least significant difference and denoted by the symbol * (** for 1%). Treatment differences that were not significant were denoted as ‘NS’.

**RESULTS AND DISCUSSION**

**Redox potential**

Redox potential remained unaltered throughout the growth phase of rice crop regardless of CO$_2$ concentrations or nitrogen levels. In general, the redox potential ranged between -276 mV and -281 mV and the values were non-significant. The interaction effect was non-significant.

**Dissolved organic carbon (DOC)**

DOC was significantly highest under elevated levels of CO$_2$ (Table 1). The highest DOC was observed under C$_{750}$ levels and the lowest under C$_{amb}$ (ambient) conditions. DOC ranged from 533 to 722 mg L$^{-1}$ and 368 to 501 mg L$^{-1}$ in C$_{750}$ and C$_{amb}$, respectively. Incremental levels of nitrogen addition significantly increased the DOC regardless of the stages of measurements. Highest DOC contents were recorded in N$_{200}$ and the mean values were 534, 621 and 507 mg L$^{-1}$ at tillering, flowering and harvest stages, respectively. Control (N$_0$) registered the lowest DOC contents and the mean values are 455, 528 and 436 mg L$^{-1}$ at tillering, flowering and harvest stages, respectively. Among the stages, flowering recorded the highest DOC contents and lowest at the harvest stage. The interaction effect was non-significant.

Our results revealed that the elevated [CO$_2$] significantly increased the DOC throughout the cropping period. Enhanced photosynthesis and plant growth under elevated [CO$_2$] had led to increased C input to the soil (including cortical cell sloughing, root exudation and mortality) (Ineson et al., 1996; Cheng and Johnson, 1998). As soil microorganisms are often C limited (Anderson and Domsch, 1986; Smith and Paul, 1990), more C input will directly contribute for increased soil microbial biomass and activities. Moreover, in submerged rice soils enhanced algal growth in response to CO$_2$ enrichment was reported to increase microbial biomass of the surface soil (Inubushi et al., 1999; Inubushi et al., 2011).

Increasing atmospheric [CO$_2$] is unlikely to directly influence soil microorganisms because CO$_2$ concentrations in soils are already 10–50 times higher than in the atmosphere (Lamborg, 1983; Schortemeyer et al., 1996), even though it usually stimulates plant (especially C$_4$ plant) productivity due to higher net carbon assimilation (Kimball et al., 1993). Nevertheless, elevated atmospheric [CO$_2$] may indirectly affect soil microbial populations (Montealegre et al., 2002; Wang et al., 2018), since root biomass, total rhizodeposition, and chemical composition of plant tissues and root exudates probably change when atmospheric CO$_2$ is enriched (Rogers et al., 1994; Schortemeyer et al., 1996; Cai et al., 2016). Our results are in conformity with Li et al. (2004) and Wang et al. (2018), who reported that DOC had a positive relationship with elevated [CO$_2$]. Nitrogen fertilization significantly increased DOC irrespective of the [CO$_2$] and this is attributed to the fact that addition of nitrogen favours increased plant biomass and in turn it contributes for more root exudates which serves as a source of energy for soil microbial population. On the other hand, low N supplement limited the enhancement of root growth by elevated [CO$_2$], leading consequently to the diminished response of DOC to CO$_2$ enrichment (Li et al., 2004). According to Cardon et al. (2001), the
influence of elevated CO₂ on soil microbial population is linked to the nutrient status of the soil. Under nitrogen limited conditions, effects of elevated CO₂ on plants were generally found to be much smaller (Korner et al., 1997). Besides, it is also suggested that poor N supply limited the microbial utilization of C compounds (van Veen et al., 1991).

Methanogens

The present study on the mechanisms associated with

Table 1. Effect of elevated CO₂ and nitrogen levels on dissolved organic carbon (DOC).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Tillering</th>
<th>Flowering</th>
<th>Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DOC (mg L⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N₀</td>
<td>N₁₅₀</td>
<td>N₂₀₀</td>
</tr>
<tr>
<td>Camburg</td>
<td>750 µ mol mol⁻¹ CO₂</td>
<td>455</td>
<td>573</td>
</tr>
<tr>
<td>C₅₅₀</td>
<td>529</td>
<td>614</td>
<td>508</td>
</tr>
<tr>
<td>C₇₅₀</td>
<td>607</td>
<td>703</td>
<td>573</td>
</tr>
<tr>
<td>Mean</td>
<td>405</td>
<td>471</td>
<td>385</td>
</tr>
</tbody>
</table>

CO₂ levels: C₃₅₀ - 396 µ mol mol⁻¹ CO₂; C₅₅₀ - 550 µ mol mol⁻¹ CO₂; C₇₅₀ - 750 µ mol mol⁻¹ CO₂

Nitrogen levels: N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹; *P ≤ 0.05; **P ≤ 0.01; NS - Non significant

Table 2. Effect of elevated CO₂ and nitrogen levels on soil methanogens and methanotrophs population.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Methanogens (× 10⁶ CFU g⁻¹ of dry soil)</th>
<th>Methanotrophs (× 10⁵ CFU g⁻¹ of dry soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tillering</td>
<td>Flowering</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N₀</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>N₁₅₀</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>N₂₀₀</td>
<td>10.7</td>
</tr>
<tr>
<td>Mean</td>
<td>10.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

CO₂ levels: C₃₅₀ - 396 µ mol mol⁻¹ CO₂; C₅₅₀ - 550 µ mol mol⁻¹ CO₂; C₇₅₀ - 750 µ mol mol⁻¹ CO₂

Nitrogen levels: N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹; *P ≤ 0.05; **P ≤ 0.01; NS - Non significant
methane flux under elevated [CO$_2$], the population dynamics of methanogens and methanotrophs showed that the Methanogen population was significantly ($P \leq 0.05$) highest under elevated CO$_2$ levels regardless of stages of observation (Table 2). C$_{750}$ had a significantly highest methanogen population, ranging from 5.7 to 20.1 x 10$^3$ CFU g$^{-1}$ of dry soil, while the lowest population ranged from 5.1 to 16.9 x 10$^3$ CFU g$^{-1}$ of dry soil under ambient (C$_{amb}$) concentration. At flowering, the mean values were 14.0, 16.1 and 17.1 x 10$^3$ CFU g$^{-1}$ of dry soil under 396, 550 and 750 µ mol mol$^{-1}$ CO$_2$ concentrations, respectively. Nitrogen significantly increased the methanogen population irrespective of CO$_2$ levels or stages. The highest mean values (12.2, 12.6 and 13.1 x 10$^3$ CFU g$^{-1}$ of dry soil) were observed in N$_{200}$, while the lowest population (5.1 to 12.4 x 10$^3$ CFU g$^{-1}$ of dry soil) was recorded in N$_0$. Among the stages, flowering registered the highest methanogen population and the lowest at the harvest stage irrespective of CO$_2$ levels. The interaction effect was significant only at tillering and flowering stages.

**Methanotrophs**

Elevated CO$_2$ levels significantly increased the soil methanotrophs population irrespective of stages of observation (Table 2). The highest methanotrophs population ranging from 7.1 to 13.6 x 10$^3$ CFU g$^{-1}$ of dry soil was observed in C$_{750}$ while the lowest population ranging from 6.3 to 12.5 x 10$^3$ CFU g$^{-1}$ of dry soil in ambient CO$_2$ concentration. At harvest, the mean values are 9.1, 10.1 and 11.0 x 10$^3$ CFU g$^{-1}$ of dry soil under 396, 550 and 750 µ mol mol$^{-1}$ CO$_2$ concentrations, respectively. Incremental levels of nitrogen addition significantly decreased the methanotrophs population regardless of CO$_2$ concentrations or stages of observation. The highest mean values (12.2, 12.6 and 13.1 x 10$^3$ CFU g$^{-1}$ of dry soil) were observed in N$_0$ while the lowest population (6.7, 7.2 and 7.6 x 10$^3$ CFU g$^{-1}$ of dry soil) was recorded in N$_{200}$ at tillering, flowering and harvest stages, respectively. Among the stages, harvest registered the highest methanotrophs population and the lowest at the harvest stage irrespective of CO$_2$ levels. The interaction effect was significant.

Consistent with the observed increases in methane flux, the enumeration of methanogens showed a significant increase under elevated [CO$_2$]. This positive effect of elevated [CO$_2$] may be attributed to stimulated rice above ground and below ground biomasses (Ziska et al., 1998; Liu et al., 2016) which might have provided more carbon substrates for methanogens (Hou et al., 2000; Inubushi et al., 2003; Yue et al., 2003; Yue et al., 2007; Liu et al., 2016; Li et al., 2017). Root exudation accounts for approximately 0.5–5.0 per cent of net fixed C (Farrar and Jones, 2003) and provides 10 and 50 per cent of the carbon substrate needed for methanogenesis (Seiler et al., 1984). In addition, the cells are sloughed from the cortices of living roots and lysates consisting of polymeric C compounds and enzymes.

**Table 3. Effect of elevated CO$_2$ and nitrogen levels on methane emission.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Methane emission (mg pot$^{-1}$ d$^{-1}$)</th>
<th>Average methane emission (mg pot$^{-1}$ d$^{-1}$)</th>
<th>Total methane emission (g pot$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tillering</td>
<td>Flowering</td>
<td>Harvest</td>
</tr>
<tr>
<td>C$_{amb}$</td>
<td>N$_0$ 1.43</td>
<td>4.13</td>
<td>0.50</td>
</tr>
<tr>
<td>C$_{amb}$</td>
<td>N$_{150}$ 2.00</td>
<td>5.27</td>
<td>0.66</td>
</tr>
<tr>
<td>C$_{amb}$</td>
<td>N$_{200}$ 2.19</td>
<td>5.54</td>
<td>0.83</td>
</tr>
<tr>
<td>C$_{amb}$</td>
<td>N$_0$ 1.79</td>
<td>5.41</td>
<td>0.61</td>
</tr>
<tr>
<td>C$_{750}$</td>
<td>N$_{150}$ 2.54</td>
<td>6.96</td>
<td>0.80</td>
</tr>
<tr>
<td>C$_{750}$</td>
<td>N$_{200}$ 2.83</td>
<td>7.38</td>
<td>1.00</td>
</tr>
<tr>
<td>C$_{750}$</td>
<td>N$_0$ 2.04</td>
<td>6.35</td>
<td>0.67</td>
</tr>
<tr>
<td>C$_{750}$</td>
<td>N$_{200}$ 2.88</td>
<td>8.15</td>
<td>0.87</td>
</tr>
<tr>
<td>C$_{750}$</td>
<td>N$_{200}$ 3.19</td>
<td>8.52</td>
<td>1.09</td>
</tr>
<tr>
<td>Mea</td>
<td>C$_{amb}$ 1.87</td>
<td>4.98</td>
<td>0.66</td>
</tr>
<tr>
<td>Mea</td>
<td>C$_{750}$ 2.39</td>
<td>6.58</td>
<td>0.80</td>
</tr>
<tr>
<td>Mea</td>
<td>C$_{750}$ 2.70</td>
<td>7.67</td>
<td>0.88</td>
</tr>
<tr>
<td>C$_{750}$</td>
<td>N$_0$ 1.75</td>
<td>5.30</td>
<td>0.59</td>
</tr>
<tr>
<td>C$_{750}$</td>
<td>N$_{150}$ 2.47</td>
<td>6.79</td>
<td>0.78</td>
</tr>
<tr>
<td>C$_{750}$</td>
<td>N$_{200}$ 2.74</td>
<td>7.15</td>
<td>0.98</td>
</tr>
<tr>
<td>C x N</td>
<td>0.019</td>
<td>0.039**</td>
<td>0.053</td>
</tr>
<tr>
<td>C x N</td>
<td>0.019</td>
<td>0.039**</td>
<td>0.053</td>
</tr>
<tr>
<td>C x N</td>
<td>0.034</td>
<td>0.069**</td>
<td>0.093</td>
</tr>
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</table>

**CO$_2$ levels :** C$_{amb}$- 396 µ mol mol$^{-1}$ CO$_2$; C$_{150}$ - 550 µ mol mol$^{-1}$ CO$_2$; C$_{750}$ - 750 µ mol mol$^{-1}$ CO$_2$. **Nitrogen levels :** N$_0$ - 0 kg N ha$^{-1}$; N$_{150}$ - 150 kg N ha$^{-1}$; N$_{200}$ - 200 kg N ha$^{-1}$; $^*$P $\leq$ 0.05, $^{**}$P $\leq$ 0.01, NS - Non significant
enter the rhizosphere, providing further substrate for microorganisms (Farrar et al., 2003). This fact also supports our observations on methanotrophs, which was found to be higher under elevated [CO₂]. Interestingly, the data showed that the highest methanotrophic population was found at the harvest stage and these observations are in accordance with (Yue et al., 2007), who reported enhanced populations of methanotrophs at maturing periods under elevated [CO₂]. This suggests that the availability of O₂ and the concentration of CH₄ jointly determine the methanotrophic activity (van Bodegom et al., 2001).

Effect of elevated CO₂ on methane emissions

Methane emission rate
Elevated CO₂ levels significantly increased the methane emission rate irrespective of stages of observation (Table 3). CO₂ concentration @ 750 μ mol mol⁻¹ CO₂ recorded the highest methane emission rate (0.67 mg pot⁻¹ d⁻¹) while the lowest rate (0.50 to 5.54 mg pot⁻¹ d⁻¹) was observed under ambient concentration. The highest mean methane emission values (2.70, 7.67 and 0.88 mg pot⁻¹ d⁻¹) in C₇₅₀ and the lowest values (1.87, 4.98 and 0.66 mg pot⁻¹ d⁻¹) in C₅₅₀ were recorded at tillering, flowering and harvest stages, respectively. C₇₅₀ increased the methane emission rate by 44.4, 54.0 and 33.3 per cent over C₅₅₀ at tillering, flowering and harvest stages, respectively. Under C₅₅₀ levels, the methane emission rate increased by 27.8, 32.1 and 21.2 per cent over C₅₅₀ at tillering, flowering and harvest stages, respectively. The methane emission rate increased under C₇₅₀ to the tune of 13.0, 16.6 and 10 per cent over C₅₅₀ at tillering, flowering and harvest stages, respectively.

Addition of nitrogen significantly increased the methane emission rate regardless of CO₂ levels or stages of observation. The highest mean values (2.74, 7.15 and 0.98 mg pot⁻¹ d⁻¹) and the lowest values (1.75, 5.30 and 0.59 mg pot⁻¹ d⁻¹) were recorded in N₂₀₀₀ and N₀ at tillering, flowering and harvest stages, respectively. At the flowering stage, the highest methane emission rate was recorded while harvest registered the lowest. The interaction effect was significant.

Average methane emission
The average methane emissions ranged from 2.02 to 2.85 mg pot⁻¹ d⁻¹, 2.60 to 3.74 mg pot⁻¹ d⁻¹ and 3.02 to 4.27 mg pot⁻¹ d⁻¹ under 396, 550 and 750 μ mol mol⁻¹ CO₂ concentrations, respectively (Table 3). The mean values are 2.51, 3.26 and 7.75 mg pot⁻¹ d⁻¹ under C₇₅₀, C₅₅₀ and C₇₅₀ levels, respectively. With respect to nitrogen levels, the mean values are 2.55, 3.35 and 3.62 mg pot⁻¹ d⁻¹ in N₀, N₁₅₀ and N₂₀₀₀, respectively.

Total methane emission
The total methane emission was highest under 750 μ mol mol⁻¹ CO₂ followed by 550 μ mol mol⁻¹ CO₂ and the lowest under 396 μ mol mol⁻¹ CO₂ concentrations (ambient) (Table 3). Total methane emission ranged from 0.22 to 0.31 g pot⁻¹, 0.29 to 0.41 g pot⁻¹ and 0.33 to 0.47 g pot⁻¹ under C₇₅₀, C₅₅₀ and C₇₅₀ levels, respectively. The highest mean value (0.41 g pot⁻¹) was observed in C₇₅₀, while the lowest value (0.28 g pot⁻¹) in C₅₅₀ increased the total methane emission by 46.4 per cent and 13.9 per cent over the C₇₅₀ and C₅₅₀ levels, respectively. The total methane emission increase was to the tune of 28.6 per cent in C₅₅₀ level over the ambient CO₂ concentration.

Total methane emission increased with increasing doses of nitrogen fertilizers. N₂₀₀₀ increased the total methane emission by 42.9 per cent and 8.1 per cent over the N₀ and N₁₅₀ levels, respectively. The total methane emission increase was to the tune of 32.1 per cent in N₁₅₀ over the control (N₀).

The data clearly indicated that methane emissions...
were significantly higher under [CO$_2$] of 750 µmol mol$^{-1}$ by 33 to 54 per cent over the ambient [CO$_2$] of 396 µmol mol$^{-1}$]. Our results are in line with ample literature which reported that elevated [CO$_2$] enhanced the methane emissions by 49 to 60 per cent @ 650 µmol mol$^{-1}$ (Ziska et al., 1998), 38 to 51 per cent @ 550 µmol mol$^{-1}$ (Inubushi et al., 2003), 58 per cent @ 700 µmol mol$^{-1}$ (Cheng et al., 2006), 26 per cent @ 580 µmol mol$^{-1}$ (Tokida et al., 2010) and 28-120 per cent @ 500 µmol mol$^{-1}$ (Wang et al., 2018) over the ambient [CO$_2$]. These facts are further supported by a recent meta-analysis report (van Groenigen et al., 2011), indicating that [CO$_2$] between 463 to 780 µmol mol$^{-1}$ stimulated CH$_4$ emissions an average by 43.4 per cent. CH$_4$ is the dominant terminal degradation product of soil organic materials in submerged rice fields (Kruger et al., 2001), therefore, increased C input to the soil in response to elevated [CO$_2$] leads likely to enhanced CH$_4$ production (Ziska et al., 1998; Li et al., 2004; Cai et al., 2016). Moreover, the positive correlations between CH$_4$ emissions and above-ground or root biomass also agree well with the results of previous studies suggesting that greater assimilation of carbon under high [CO$_2$] leads to higher rates of rhizo-deposition (root exudation and autolysis products), which is an important source of substrates for CH$_4$ production (Inubushi et al., 2003; Xu et al., 2004; Tokida et al., 2010; Cai et al., 2016; Wang et al., 2018). Further, the positive correlation of DOC with methane emissions also adds strength to our results. Among the different stages of observation, the flowering stage recorded the highest methane flux irrespective of [CO$_2$]. This period generally corresponds with increased availability of root sloughing or exudates due to peak photosynthetic activity and advanced root senescence and might probably provide more substrate for methanogenesis (Allen et al., 2003; Tokida et al., 2010; Li et al., 2017). The results from the present experiment also demonstrated that nitrogen fertilization increased the methane flux irrespective of [CO$_2$]. Inference from our observations is in line with Schimel (2000), who have reported that the application of N fertilizers enhances rice biomass and contributed for enhanced root exudates that favours methane production.

**Conclusion**

Elevated CO$_2$ levels favoured methane emission as a result of enhanced carbon assimilation and production of energy rich root exudates that stimulated microbial activities in the soil rhizosphere of rice ecosystem. Consistent with the observed increases in methane flux, the enumeration of methanogens showed a significant ($P \leq 0.01$) increase under elevated [CO$_2$]. In addition, the methanotrophs population was also significantly ($P \leq 0.01$) highest under elevated CO$_2$ levels regardless of stages of observation. Incremental levels of nitrogen addition significantly increased the methanogens but decreased the methanotrophs population regardless of CO$_2$ concentrations or stages of observation. This study unequivocally demonstrated that even though higher methanotrophs population was recorded under elevated [CO$_2$], it could not circumvent the methane emission, thereby showcasing the knowledge gap and suggesting that methane mitigation strategies need to be explored for the future high CO$_2$ environments by duly optimizing the dosage of nitrogenous fertilizer.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**REFERENCES**


