Journal of Applied and Natural Science
Special Issue: 13 (SI), 26 - 34 (July, 2021)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

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

S. K. Rajkishore*
Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore - 641003 (Tamil Nadu), India
M. Maheswari
Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore - 641003 (Tamil Nadu), India
K. S. Subramanian
Director of Research, Tamil Nadu Agricultural University, Coimbatore - 641003 (Tamil Nadu), India
R. Prabhu
School of Post Graduate Studies, Tamil Nadu Agricultural University, Coimbatore - 641003 (Tamil Nadu), India
G. Vanitha
School of Post Graduate Studies, Tamil Nadu Agricultural University, Coimbatore - 641003 (Tamil Nadu), India

*Corresponding author. Email: rajkishoresk@gmail.com

Article Info
https://doi.org/10.31018/jans.v13iSI.2773
Received: March 22, 2021
Revised: April 17, 2021
Accepted: May 8, 2021

How to Cite
Rajkishore, S. K. *et al.* (2021). Influence of elevated carbon dioxide concentrations on methane emission and its associated soil microflora in rice ecosystem. *Journal of Applied and Natural Science*, 13 (SI), 26 - 34. https://doi.org/10.31018/jans.v13iSI.2773

Abstract
The dynamics of methane emission and its associated soil microflora in rice ecosystem as a response to elevated CO_2 concentrations were studied in open top chamber (OTC) conditions. The treatments consisted of three levels of CO_2 (396, 550 and 750 µmol mol^{-1}) and three levels of nitrogen (0, 150 and 200 kg ha^{-1}) and replicated five times in a completely randomized design. The data showed that elevated [CO_2] significantly ($P \leq 0.01$) increased the DOC throughout the cropping period with the values ranging from 533 to 722 mg L^{-1} and 368 to 501 mg L^{-1} in C_{750} and C_{amb}, respectively. Methane emission rates were monitored regularly during the experiment period and it was revealed that elevated [CO_2] had increased the methane emissions regardless of stages of crop growth. It was observed 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}. Consistent with the observed increases in methane flux, the enumeration of methanogens showed a significant ($P \leq 0.01$) increase under elevated [CO_2] with the population ranging from 5.7 to 20.1 x 10^4 CFU g^{-1} of dry soil and 5.1 to 16.9 x 10^4 CFU g^{-1} of dry soil under C_{750} and C_{amb} concentrations, respectively. Interestingly, even though higher methanotrophs population was recorded under elevated [CO_2], it could not circumvent the methane emission. Overall, the results of OTC studies suggest that methane mitigation strategies need to be explored for the future high CO_2 environments.

Keywords: Elevated CO_2, Methane, Methanogens, Methanotrophs, Nitrogen

INTRODUCTION

Rice fields are considered important sources of atmospheric methane (CH_4), contributing about 5-19 per cent of total global CH_4 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 CO_2 on a 100-year horizon (Intergovernmental Panel on Climate Change , 2013). Projections to the end of this century suggest that atmospheric [CO_2] 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 [CO_2] 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_4 emitted from rice fields to the atmosphere is the balance of two opposite processes, i.e., CH_4 production and oxidation in the soil. In the global CH_4 cycle, a substantial amount of CH_4 is consumed by biological processes. The only known biological sink for atmospheric CH_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_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_2 *viz.,* 550 µ mol mol $^{-1}$ CO_2 and 750 µ mol mol^{-1} CO_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^{-1}), low in available nitrogen (110.3 mg kg^{-1}), medium in available phosphorus and potassium (6.8 mg kg^{-1}and 118.0 mg kg^{-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^{-1} (41.6 g pot^{-1}) and NPK at 150:50:60 kg ha^{-1} (500, 166.6, 200 mg NPK pot^{-1}) were applied in the form of urea, single super phosphate and muriate of potash, respectively. Zinc sulphate at rate of 25 kg ha^{-1} (83.3 mg pot^{-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^{-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_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_{amb} - Ambient CO_2 concentration (396 µ mol mol^{-1} CO_2)

C_{550} - 550 µ mol mol^{-1} CO_2

C_{750} - 750 µ mol mol^{-1} CO_2

Factor 2:

N_0 - 0 kg N ha^{-1}

N_{100} - 150 kg N ha^{-1}

N_{200} - 200 kg N ha^{-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_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 $K_2Cr_2O_7$, 10.0 mL of 98% H_2SO_4, and 5.0 mL of 88% H_3PO_4 in a tube and digested for 30 min at 150^{0}C. Upon cooling, the solutions were transferred to 150 mL Erlenmeyer flasks and titrated with 0.005 M Fe $(NH_4)_2(SO_4)_2$ .6 H_2O in 0.4 M H_2SO_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^{-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 incubate 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, napthol 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, panicle 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_3 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 CO_2 on soil microbial population is linked to the nutrient status of the soil. Under nitrogen limited conditions, effects of elevated CO_2 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 CO_2 and nitrogen levels on dissolved organic carbon (DOC).

Treatments		DOC (mg L^{-1})					
		Tillering		Flowering		Harvest	
C_{amb}	N_0	368		427		350	
	N_{150}	418		485		397	
	N_{200}	430		501		409	
C_{550}	N_0	464		538		440	
	N_{150}	529		614		508	
	N_{200}	548		639		523	
C_{750}	N_0	533		618		517	
	N_{150}	607		703		573	
	N_{200}	624		722		590	
Mean	C_{amb}	405		471		385	
	C_{550}	514		597		490	
	C_{750}	588		681		560	
	N_0	455		528		436	
	N_{150}	518		601		493	
	N_{200}	534		621		507	
		SEd	CD	SEd	CD	SEd	CD
C		4.1	8.4**	4.8	9.8	3.9	8.0**
N		4.1	8.4**	4.8	9.8	3.9	8.0**
C x N		7.2	NS	8.3	NS	6.8	NS

CO_2 levels : C_{amb} - 396 µ mol mol^{-1} CO_2; C_{550} - 550 µ mol mol^{-1} CO_2; C_2 - 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

Table 2. Effect of elevated CO_2 and nitrogen levels on soil methanogens and methanotrophs population.

Treatments		Methanogens (× 10^4 CFU g^{-1} of dry soil)						Methanotrophs (× 10^3 CFU g^{-1} of dry soil)					
		Tillering		Flowering		Harvest		Tillering		Flowering		Harvest	
C_{amb}	N_0	7.60		10.1		5.1		11.4		11.9		12.5	
	N_{150}	11.80		15.1		8.2		7.3		7.7		7.9	
	N_{200}	13.10		16.9		9.1		6.3		6.5		6.9	
C_{550}	N_0	8.10		11.3		5.3		12.4		12.8		13.1	
	N_{150}	13.30		17.6		8.6		8.6		9.0		9.5	
	N_{200}	14.60		19.5		9.5		6.7		7.2		7.6	
C_{750}	N_0	8.90		12.4		5.7		12.9		13.1		13.6	
	N_{150}	14.20		18.9		9.0		10.2		10.7		11.0	
	N_{200}	14.90		20.1		9.7		7.1		7.9		8.3	
Mean	C_{amb}	10.8		14.0		7.5		8.4		8.7		9.1	
	C_{550}	12.0		16.1		7.8		9.2		9.7		10.1	
	C_{750}	12.7		17.1		8.1		10.1		10.5		11.0	
	N_0	8.2		11.3		5.4		12.2		12.6		13.1	
	N_{150}	13.1		17.2		8.6		8.7		9.1		9.5	
	N_{200}	14.2		18.8		9.4		6.7		7.2		7.6	
		SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD
C		0.09	0.20**	0.13	0.27**	0.06	0.13**	0.08	0.16**	0.08	0.16**	0.06	0.12**
N		0.09	0.20**	0.13	0.27**	0.06	0.13**	0.08	0.16**	0.08	0.16**	0.06	0.12**
C x N		0.17	0.35*	0.23	0.46*	0.11	NS	0.13	0.27**	0.14	0.28**	0.10	0.21**

CO_2 levels : C_{amb} - 396 µ mol mol^{-1} CO_2; C_{550} - 550 µ mol mol^{-1} CO_2; C_2 - 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

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 10^4 CFU g^{-1} of dry soil, while the lowest population ranged from 5.1 to 16.9 x 10^4 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 10^4 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 methanogen load (9.1 to 20.1 x 10^4 CFU g^{-1} of dry soil) was observed in $N_{200,}$ while the lowest population (5.1 to 12.4 x 10^4 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 tillering 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.

Treatments		Methane emission (mg pot^{-1} d^{-1})						Average methane emission (mg pot^{-1} d^{-1})	Total methane emission (g pot^{-1})
		Tillering		Flowering		Harvest			
	N_0	1.43		4.13		0.50		2.02	0.22
C_{amb}	N_{150}	2.00		5.27		0.66		2.64	0.29
	N_{200}	2.19		5.54		0.83		2.85	0.31
	N_0	1.79		5.41		0.61		2.60	0.29
C_{550}	N_{150}	2.54		6.96		0.80		3.43	0.38
	N_{200}	2.83		7.38		1.00		3.74	0.41
	N_0	2.04		6.35		0.67		3.02	0.33
C_{750}	N_{150}	2.88		8.15		0.87		3.97	0.44
	N_{200}	3.19		8.52		1.09		4.27	0.47
	C_{amb}	1.87		4.98		0.66		2.51	0.28
	C_{550}	2.39		6.58		0.80		3.26	0.36
Mean	C_{750}	2.70		7.67		0.88		3.75	0.41
	N_0	1.75		5.30		0.59		2.55	0.28
	N_{150}	2.47		6.79		0.78		3.35	0.37
	N_{200}	2.74		7.15		0.98		3.62	0.40
		SEd	CD	SEd	CD	SEd	CD		
C		0.019	0.039**	0.053	0.108**	0.007	0.014**		
N		0.019	0.039**	0.053	0.108**	0.007	0.014**		
C x N		0.034	0.069**	0.093	0.118*	0.012	0.024*		

CO_2 levels : C_{amb} - 396 µ mol mol^{-1} CO_2; C_{550} - 550 µ mol mol^{-1} CO_2; C_2 - 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 [CO_2]. 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 [CO_2]. This suggests that the availability of O_2 and the concentration of CH_4 jointly determine the methanotrophic activity (van Bodegom *et al.,* 2001).

Effect of elevated CO_2 on methane emissions

Methane emission rate

Elevated CO_2 levels significantly increased the methane emission rate irrespective of stages of observation (Table 3). CO_2 concentration @ 750 µ mol mol^{-1} CO_2 recorded the highest methane emission rate (0.67 to 8.52 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 C_{750} and the lowest values (1.87, 4.98 and 0.66 mg $pot^{-1}d^{-1}$) in C_{amb} were recorded at tillering, flowering and harvest stages, respectively. C_{750} increased the methane emission rate by 44.4, 54.0 and 33.3 per cent over C_{amb} at tillering, flowering and harvest stages, respectively. Under C_{550} levels, the methane emission rate increased by 27.8, 32.1 and 21.2 per cent over C_{amb} at tillering, flowering and harvest stages, respectively. The methane emission rate increased under C_{750} to the tune of 13.0, 16.6 and 10per cent over C_{550} at tillering, flowering and harvest stages, respectively.

Addition of nitrogen significantly increased the methane emission rate regardless of CO_2 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 N_{200} and N_0 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} CO_2, concentrations, respectively (Table 3). The mean values are 2.51, 3.26 and 7.75 mg $pot^{-1}d^{-1}$ under C_{amb}, C_{550} and C_{750} levels, respectively. With respect to nitrogen levels, the mean values are 2.55, 3.35 and 3.62 mg $pot^{-1}d^{-1}$ in N_0, N_{150} and N_{200}, respectively.

Total methane emission

The total methane emission was highest under 750 µ mol mol^{-1} CO_2 followed by 550 µ mol mol^{-1} CO_2 and the lowest under 396 µ mol mol^{-1} CO_2 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 C_{amb}, C_{550} and C_{750} levels, respectively. The highest mean value (0.41 g pot^{-1}) was observed in $C_{750,}$ while the lowest value (0.28 g pot^{-1}) in C_{amb}. C_{750} increased the total methane emission by 46.4 per cent and 13.9 per cent over the C_{amb} and C_{550} levels, respectively. The total methane emission increase was to the tune of 28.6 per cent in C_{550} level over the ambient CO_2 concentration.

Total methane emission increased with increasing doses of nitrogen fertilizers. N_{200} increased the total methane emission by 42.9 per cent and 8.1 per cent over the N_0 and N_{150} levels, respectively. The total methane emission increase was to the tune of 32.1 per cent in N_{150} over the control (N_0).

The data clearly indicated that methane emissions

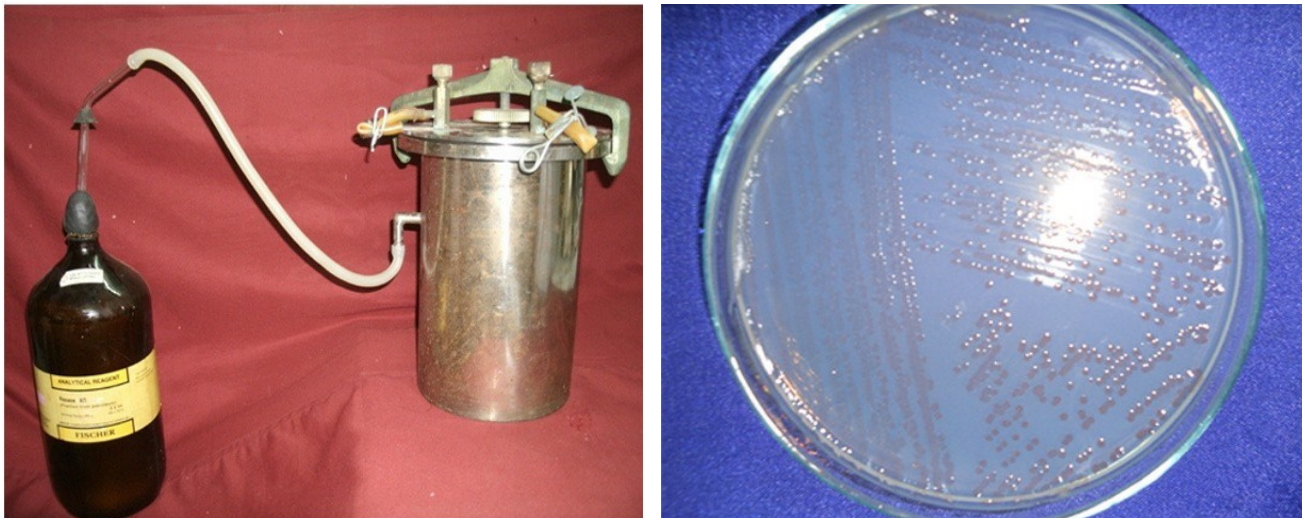

Plate 1. *Mcintosh jar assembly and colonies of methanotrophs.*

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 CH4 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

1. Allen, L.H., Stephan, L., Albrecht, W., Colo´n-Guasp, S.A., Covell, J.T., Baker, D., Pan & Boote, K.J. (2003). Methane emissions of rice increased by elevated carbon dioxide and temperature. *J. Environ. Qual.*, 32, 1978-1991.
2. Anderson, T.H. & Domsch, K.H. (1986). Carbon link between microbial biomass and soil organic matter. In: *Proceedings of the Fourth International Symposium on Microbial Ecology*. Eds. F Megusar and M Gantar. Ljubljana, Yugoslavia. pp. 471-476.
3. Cai, C., Yin, X., He, S., Jiang, W., Si, C., Struik, P.C., Luo, W., Li, G., Xie, Y., Xiong, Y. & Pan, G. (2016). Responses of wheat and rice to factorial combinations of ambient and elevated CO_2 and temperature in FACE experiments. *Global Change Biol.*, 22, 856-874.
4. Cardon, Z.G., Hungate, B.A., Cambardella, C.A., Chapin, F.S., Field, C.B., Holland, E.A., & Mooney, H.A. (2001). Contrasting effects of elevated CO_2 on old and new soil carbon pools. *Soil Biol. Biochem.*, 33, 365-373.
5. Cheng, W. & Johnson, D.W. (1998). Elevated CO_2, rhizosphere processes and soil organic matter decomposition. *Plant Soil*, 202, 167-174.
6. Cheng, W., Yagi, K., Sakai, H. & Kobayashi, K. (2006). Effects of elevated atmospheric CO_2 concentrations on CH_4 and N_2O emission from rice soil: an experiment in controlled-environment chambers. *Biogeochemistry*, 77, 351-373.
7. Farrar, J., Hawes, M., Jones, D. & Lindow, S. (2003). How roots control the flux of carbon to the rhizosphere. *Ecol.*, 84, 827-837.
8. Farrar, J.F. & Jones, D.L. (2003). The control of carbon acquisition by and growth of roots. In: Kroon *H de, Visser EJW. Ed: Root Ecology*. Springer, Berlin Heidelberg, New York. pp. 90-124.
9. Gomez, K.A. & Gomez, A.A. (1984). *Statistical Procedures for Agricultural Research.* John Wiley and Sons, New Delhi, p.680.
10. Hou, A.X., Chen, G.X., Wang, Z.P., Van Cleemput, O. & Patrick, W.H. (2000). Methane and nitrous oxide emissions from a rice field in relation to soil redox and microbiological processes. *Soil Sci. Soc. Am. J.*, 64, 2180-2186.

11. Hungate, R.S. (1957). Microorganisms in the rumen of cattle fed at a constant ration. *Can. J. Microbiol.*, 3, 289-311.
12. Ineson, P., Cotrufo, M.F., Bol, R., Harkness, D.D. & Blum, H. (1996). Quantification of soil carbon inputs under elevated CO2: C_3 plants in a C_4 soil. *Plant Soil.*, 187, 345-350.
13. Inubushi, K., Cheng, W. & Chander, K. (1999). Carbon dynamics in submerged soil microcosms as influenced by elevated CO_2 and temperature. *Soil Sci. Plant Nutr.*, 45, 863-872.
14. Inubushi, K., Cheng, W., Aonuma, S., Hoque, M.M., Kobayashi, K. & Miura, S. (2003). Effects of free-air CO_2 enrichment (FACE) on CH_4 emission from a rice paddy field. *Global Change Biol.*, 9, 1458-1464.
15. Inubushi, K., Cheng, W., Mizuno, T., Lou, Y., Hasegawa, T., Sakai, H. & Kobayashi, K. (2011). Microbial biomass carbon and methane oxidation influenced by rice cultivars and elevated CO_2 in a Japanese paddy soil. *E. J. Soil Sci.*, 62, 69-73.
16. Intergovernmental Panel on Climate Change (2013). Climate Change. The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (Eds. T.F. Stocker, D. Qin, G.K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley), Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, p. 1535.
17. Intergovernmental Panel on Climate Change (2018). Summary for Policymakers. In: Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty (Eds. V. Masson-Delmotte, P. Zhai, H.O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J.B.R. Matthews, Y. Chen, X. Zhou, M.I. Gomis, E. Lonnoy, T. Maycock, M. Tignor and T. Waterfield), In Press.
18. Kimball, B.A., Mauney, J.R., Nakayama, F.S. &Idso, S.B. (1993). Effects of increasing CO_2 on vegetation. *Vegetation*, 104/105, 65-75.
19. Korner, C., Diemer, M., Schappi, B., Niklaus, P.A. & Arnone, J.A. (1997). The responses of alpine grassland to four seasons of CO_2 enrichment: a synthesis. *Acta Oecol.*, 18, 165-176.
20. Kruger, M., Frenzel, P. & Conrad, R. (2001). Microbial processes influencing methane emission from rice fields. *Global Change Biol.*, 7, 49-63.
21. Lamborg, M.R. (1983). Microbial effects. In: Lemon, E.R. (Ed.), The Response of Plants to Rising Levels of Atmospheric Carbon Dioxide. Westview Press, Boulder, pp. 131-176.
22. Li, Z., Yagi, K., Sakai, H. & Kobayashi, K. (2004). Influence of elevated CO_2 and nitrogen nutrition on rice plant growth, soil microbial biomass, dissolved organic carbon and dissolved CH_4. *Plant Soil*, 258, 81-90.
23. Li, S., Song, L., Gao, X., Jin, Y., Liu, S., Shen, Q. & Zou, J. (2017). Microbial abundances predict methane and nitrous oxide fluxes from a windrow composting system. *Front. Microbiol.,* 8, 409.
24. Liu, Y., Liu, X., Cheng, K., Li, L., Zhang, X., Zheng, J., Zheng, J. & Pan, G. (2016). Responses of methanogenic and methanotrophic communities to elevated atmospheric CO_2 and temperature in a paddy field. *Front. Microbiol.,* 7, 1895.
25. Lu, Y., Wassmann, R.H., Neue, U. & Huang, C. (2000). Dynamics of dissolved organic carbon and methane emissions in a flooded rice soil. *Soil Sci. Soc. Am. J.*, 64, 2011-2017.
26. Mah, R.S. (1980). Isolation and characterization of Methanococcus mazei. *Curr. Microbiol.*, 3, 321-326.
27. Montealegre, C.M., Van Kessel, C., Russelle, M.P. & Sadowsky, M.J. (2002). Changes in microbial activity and composition in a pasture ecosystem exposed to elevated atmospheric carbon dioxide. *Plant Soil*, 243, 197-207.
28. Nelson, D.W. & Sommers, L.E. (1996). Total carbon, organic carbon and organic matter. In: D.L. Sparks (ed.) Methods of soil analysis. Part 3. SSSA Book Ser. 5. SSSA, Madison, WI. pp. 961-1010.
29. Rajkishore, S.K., Doraisamy, P., Subramanian, K.S. & Maheswari, M. (2013). Methane emission patterns and their associated soil microflora with SRI and conventional systems of rice cultivation in Tamil Nadu, India. *Taiwan Water Conservancy*, 61(4), 126-134.
30. Ramasamy, K., Kalaichelvan, G. & Nagamani, B. (1992). Working with anaerobes: methanogens. A Laboratory manual. pp. 94.
31. Rogers, H.H., Runion, G.B. & Krupa, S.V. (1994). Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environ. Pollut.*, 83, 155-189.
32. Satpathy, S.N. (1997). Factors affecting methane emission in tropical rice soil. Ph.D. Thesis, Utkal University, Bhubaneswar.
33. Schimel, J. (2000). Global change: Rice, microbes and methane. *Nature*, 403, 375-377.
34. Schortemeyer, M., Hartwig, U.A., Hendrey, G.R. & Sadowsky, M.J. (1996). Microbial community changes in the rhizospheres of white clover and perennial ryegrass exposed to free air carbon dioxide enrichment (FACE). *Soil Biol. Biochem.*, 28, 1717-1724.
35. Seiler, W., Holzapfel-Pschorn, A., Conrad, R. & Scharffe, D. (1984). Methane emission from rice paddies. *J. Atmos. Chem.*, 1, 241-268.
36. Singh, J.S. (2011). Methanotrophs: the potential biological sink to mitigate the global methane load. *Curr. Sci.*, 100 (1), 29-30.
37. Smith, J.L. & Paul, E.A. (1990). The significance of soil microbial biomass estimates. In Soil Biochemistry. Eds. J Bollag and G Stotsky. Mercel Dekker, New York. pp. 357-393.
38. Tiensing, T., Preston, S., Strachan, N. & Paton, G.I. (2001). Soil solution extraction techniques for microbial toxicity testing: A comparative evaluation. *J. Environ. Monit.*, 3(1), 91-96.
39. Tokida, T., Fumoto, T., Cheng, W., Matsunami, T., Adachi, M., Katayanagi, N., Matsushima, M., Okawara, Y., Nakamura, H., Okada, M., Sameshima, R. & Hasegawa, T. (2010). Effects of free-air CO_2 enrichment (FACE) and soil warming on CH_4 emission from a rice paddy field: impact assessment and stoichiometric evaluation. *Biogeosci.*, 7, 2639-2653.
40. USDA classification (1999). Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil

Surveys. Eds. Soil Survey Staff. United States Department of Agriculture, Agriculture Handbook 436. PP. 1-886.
41. van Bodegom, P., Stams, F., Mollema, L., Boeke, S. & Leffelaar, P. (2001). Methane Oxidation and the Competition for Oxygen in the Rice Rhizosphere. *App. Environ. Microbiol.*, 67(8), 3586-3597.
42. van Groenigen, K., Craig, V., Osenberg, W. & Hungate, B.A. (2011). Increased soil emissions of potent greenhouse gases under increased atmospheric CO_2. *Nature*, 475, 214-216.
43. van Veen, J.A., Liljeroth, E., Lekkerkerk, L.J.A. & van de Geijn, S.C. (1991). Carbon fluxes in plant soil systems at elevated atmospheric CO_2 levels. *Ecol. Appl.*, 1, 175-181.
44. Wang, C., Jin, Y., Ji, C., Zhang, N., Song, M., Kong, D., Liu, S., Zhang, X., Liu, X., Zou, J., Li, S. & Pan G. (2018). An additive effect of elevated atmospheric CO_2 and rising temperature on methane emissions related to methanogenic community in rice paddies. *Agriculture, Ecosystems and Environment*, 257, 165-174.
45. Xu, Z., Zheng, X., Wang, Y., Han, S., Huang, Y., Zhu, J. & Butterbach-Bahl, K. (2004). Effects of elevated CO_2 and N fertilization on CH4 emission from paddy rice fields. *Glob. Change Biol.*, 18, 3009.
46. Yue, J., Liang, W., Wu, J., Shi, Y. & Huang, G. (2003). CH_4 and N_2O emissions from phaeozem rice field and their mitigative measures. *Chin. J. Appl. Ecol.*, 14, 2015-2018.
47. Yue, J., Shi, Y., Zheng, X., Huang, G. & Zhu, J. (2007). The influence of free-air CO_2 enrichment on microorganisms of a paddy soil in the rice-growing season. *Applied Soil Ecol.*, 35, 154-162.
48. Ziska, L.H., Moya, T .B., Wassmann, R., Namuco, O. S., Lantin, R.S., Aduna, J.B., Bao Jr. E.A., Bronson, K.F., Neue, H.U. & Olszyk, D. (1998). Long-term growth at elevated carbon dioxide stimulates methane emission in tropical paddy rice. *Global Change Biol.*, 4, 657-665.