

*Full Length Research Paper*

# Effects of different fertilizers on methane emission from paddy field of Zhejiang, China

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**Methane (CH<sub>4</sub>) emissions from Chinese paddy soil (Zhejiang province) were measured over the rice growing seasons. Different fertilizers (organic and chemical) were applied, emissions of methane were high during two periods (05 days after peak tillering and 07 days after heading flowering stage) and significant effects of fertilizers were observed. Methanogenic activities in soils treated with organic manure were obviously higher than those with chemical fertilizers. Among the organic manure fields the maximum methane emission from green manure, biogas residue and beef manure treatment were 52, 20 and 19 times, respectively of that given by control, and among chemical fertilizers it was NH<sub>4</sub>HCO<sub>3</sub> > CO(NH<sub>2</sub>)<sub>2</sub> > (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> > NH<sub>4</sub>Cl > NaNO<sub>3</sub> with 2.4, 2, 1.5, 1.3, and 0.2 times, respectively of that from control.**

**Key words:** Methane emission, field, methanogenic flora flooded rice, organic and chemical fertilizers.

## INTRODUCTION

The rapid increase of the world population over the past 50 to 100 years has led to increased atmospheric concentrations of carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O). These gases along with additional trace gas species (green house gases) are causing an increase in global temperature. CH<sub>4</sub>, following CO<sub>2</sub>, is the second important gas contributing to the radiative forcing of the atmosphere (Peter, 2012). In terms of the potential of increasing temperature, CH<sub>4</sub> contributes 15% to the greenhouse effect and atmospheric methane increases at a rate of 1% every year (Huang et al., 2014).

Agricultural soils are a primary source of anthropogenic

trace gas emissions. Rice paddies have been identified as a major source of atmospheric CH<sub>4</sub> and N<sub>2</sub>O. The CH<sub>4</sub> emission from global rice fields was estimated to be 25.6 Tg year<sup>-1</sup> (Yan et al., 2009). In flood-irrigated rice fields, anaerobic soil conditions lead to CH<sub>4</sub> generation as the final product of organic compost decomposition by methanogenic bacteria. Soils can also act as a significant sink for CH<sub>4</sub>, via oxidation by methanotrophic bacteria, and the net efflux is the balance between production and oxidation (Dalal et al., 2008). Soil C: N ratio is an important parameter affecting CH<sub>4</sub> production in flooded rice soil and methane production and emission decreased when the C-content and the C:N ratio of the

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incorporated material decreased (Das and Adhya, 2014). A high C: N ratio usually corresponds to an organic material rich in labile C and thus easily usable by the methanogenic microbes and methane production (Le Mer and Roger, 2001). Different manure + urea-N application significantly increased methane emission (Das et al., 2014). Report on the application of rice straw in paddy soil proved that decomposing rice straw is not only a substrate of methane production, but in addition stimulates methane production from soil organic and root organic carbon (Yuan et al., 2014).

Report on effect of organic matter level on methane emission in acid sulphate soil from Indonesia proved that the intensity of reduction processes in submerged soils depended on the content and the nature of organic matter (Wahida et al., 2014). Lindau et al. (1990) measured CH<sub>4</sub> fluxes from rice fields in Louisiana, USA and proved that CH<sub>4</sub> emission from Louisiana rice fields increased as the application rate of urea-N was increased. They also demonstrated that methane emission increased as soil redox potential decreased.

FAO estimated that rice production must increase by 40% the end of 2030 (FAO, 2009). This significant increase in rice may require a higher application of fertilizers to paddy fields, which can lead to increased methane emission to the atmosphere (Kim et al., 2014; Roy et al., 2014; Haque et al., 2015). CH<sub>4</sub> emission from every rice growing country needs to be measured and assessed from different conditions. In this study, we aimed to determine methane emission rates during rice growing season in the Zhejiang paddy field, China and the effects different fertilizers produce.

## MATERIALS AND METHODS

### General condition for the field test

The nine treatments were set up as follows: the first five treatments contained the same quantity of nitrogen from chemical fertilizers NH<sub>4</sub>HCO<sub>3</sub>, CO(NH<sub>2</sub>)<sub>2</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, NaNO<sub>3</sub> and NH<sub>4</sub>Cl. For the next three treatments, 130 g of green manure, animal manure and biogas residues were added respectively. For the last treatment, no fertilizer was added for the control (CK). The tests were carried out in pots 30 cm in diameter and 45 cm in height, the soil in the pots was submerged with tap water. Before transplanting the rice seedlings, 1 g of urea was applied as basal fertilizers and 1 g of urea as dressing fertilizer 15 days after transplanting in pot. Three pots for each treatment were planted with rice seedling named Zhe 852. Three hills of rice seedling (6 rice seedling for each hill) were planted in each pot. CK was also planted with rice.

### Methane determination

#### Collection of methane gas in situ

Glass cylinders with a capacity of 2600 ml were used to cover various representative rice hills for 24 h. Gas samples were taken by syringe from a late pipe fixed on the glass cylinders. First, the air

inside the glass cylinders was mixed well by using the syringe, then the sample gas was withdrawn and the needle of the syringe was sealed by a rubber cap to prevent the escape of the sample gas from the syringe.

### Determination of methane and hydrogen

Methane was measured by 102 GC type of gas chromatograph with a hydrogen flame ionization detector. Under the following conditions: carrier GD-X-102, air speed 700 ml/min, H<sub>2</sub> 40 ml/min, N<sub>2</sub> 25 ml/min, chromatographic room temperature 40°C, standard time for appearance of methane peaked at 17 s.

Hydrogen was measured by 102 GC type of gas chromatograph with thermal conductivity detector under the following condition: carrier (h) × 104 N<sub>2</sub> 40 ml/min, chromatographic room temperature 40°C, standard time for appearance of hydrogen peaked at 15 s. All the data in this paper are average values of triplicates

### Composition and preparation of media for determination and isolation of methanogenic flora

Medium composition for determination of hydrolytic fermentative bacteria is as follows (g l<sup>-1</sup>): glucose, 10; beef extract, 3; peptone, 5; NaCl, 3; cystein, 0.5; resazurin, 0.002; pH 7.2 to 7.4, distilled water.

Medium composition for determination of hydrogen-producing acetogenic bacteria is as follows (g l<sup>-1</sup>): CH<sub>3</sub>CH<sub>2</sub>COONa, 30 mmoles; CH<sub>3</sub>(CH<sub>2</sub>)<sub>2</sub>COONa, 30 mmoles; sodium lactate, 30 mmoles; sodium succinate, 30 mmoles; CH<sub>3</sub>CH<sub>2</sub>OH, 30 mmoles; yeast extract, 2; MgCl<sub>2</sub>, 0.1; NH<sub>4</sub>Cl, 1; K<sub>2</sub>HPO<sub>4</sub>, 0.4; KH<sub>2</sub>PO<sub>4</sub>, 0.4; cystein, 0.5; resazurin, 0.002; trace element solution, 10 ml; soil extract solution, 300 ml; pH, 7.0 to 7.3. The composition and preparation of trace element solution and soil extract solution are same as that used in medium for determination of methanogens.

Medium composition for determination of methanogens is as follows (g l<sup>-1</sup>): NH<sub>4</sub>Cl, 1; MgCl<sub>2</sub>, 0.1; K<sub>2</sub>HPO<sub>4</sub>, 0.4; KH<sub>2</sub>PO<sub>4</sub>, 0.4; KH<sub>2</sub>PO<sub>4</sub>, 0.4; yeast extract, 1; cystein, 0.5; HCOONa, 5; CH<sub>3</sub>COONa, 5; CH<sub>3</sub>OH, 5 ml; H<sub>2</sub>/CO<sub>2</sub>(80/20,v/v); soil extract, 300 ml trace element solution 10 ml.

Preparation of soil extract is as follows: take several kg paddy soil and add tap water (soil water about 1:1.5), stir it and then let it stand still for 24 h, filter the supernatant with filter paper, sterilise the filtrate and store it in a refrigerator.

Composition of trace element solution is as follows (g l<sup>-1</sup>) ; N(CH<sub>2</sub>COOH)<sub>3</sub>, 4.5; FeCl<sub>2</sub>·4H<sub>2</sub>O, 0.4; MnCl<sub>2</sub>·H<sub>2</sub>O, 0.1; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.12; ZnCl<sub>2</sub>, 0.1; AlK(SO<sub>4</sub>)<sub>2</sub>, 0.01; NaCl, 1; CaCl<sub>2</sub>, 0.02; Na<sub>2</sub>MoO<sub>4</sub>, 0.01; H<sub>2</sub>BO<sub>3</sub>, 0.01; and distilled water 11. Store it in a refrigerator.

The media was prepared according to Hungate's anaerobic technique. Before the medium for methanogens was used, 0.1 ml anaerobic sterilised Na<sub>2</sub>S (10 g kg<sup>-1</sup>)/NaHCO<sub>3</sub>(50 g kg<sup>-1</sup>) mixed solution was added into each tube with 4.5 ml medium to decrease further redox of the medium and then 0.1 ml of 160000 units ml<sup>-1</sup> of penicillin to inhibit eubacteria.

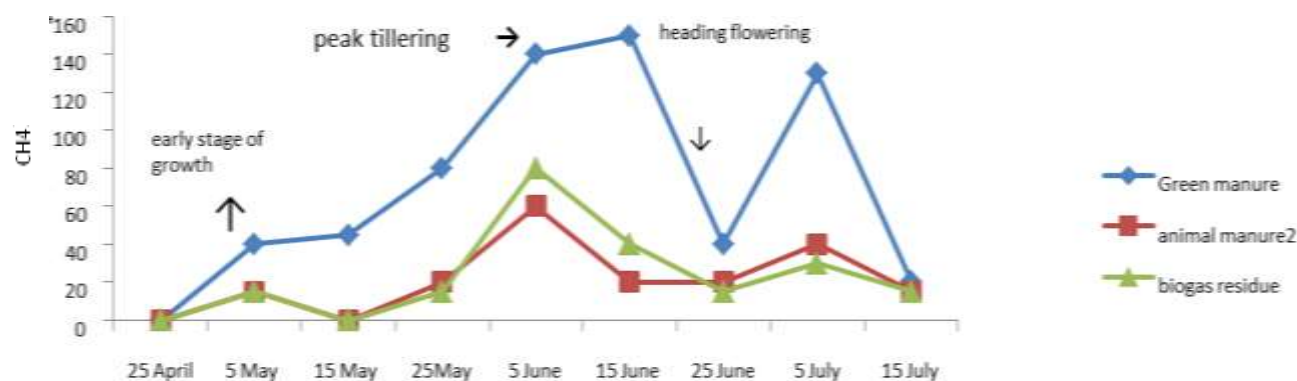
The population of each group of methanogenic flora was measured by MPN method with triplicate. The formation of H<sub>2</sub> and CH<sub>4</sub> were used as the indexes of amounts for hydrogen-producing acetogenic and methanogenic bacteria, respectively.

## RESULTS AND DISCUSSION

### Methane emission

#### General trend of methane emission

The emission of methane by rice plants at different



**Figure 1.** Effect of different organic fertilizers application on methane emission during growth stage of rice plan. (Methane emitted,  $10^{-5}$  mol/(pot /day)).

**Table 1.** The differences of methane fluxes emitted from rice soil with different fertilizers.

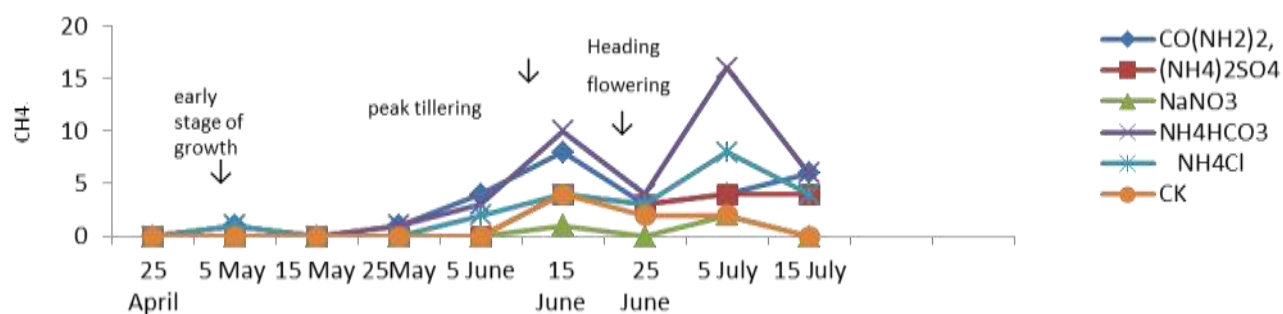
Treatment	Total flux of CH <sub>4</sub> emission $10^{-5}$ mol/(Pot/Day)	Differences (fold)
CO(NH <sub>2</sub> ) <sub>2</sub>	20.8	201.2
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	15.9	153.7
NaNO <sub>3</sub>	2.7	26.2
NH <sub>4</sub> HCO <sub>3</sub>	25.7	248.4
NH <sub>4</sub> Cl	13.7	132.2
Green manure	537.8	5191.1
Animal manure	195.0	1881.9
Biogas residue	206.6	1993.9
Control (CK)	10.4	100.0

stages of growth was affected by different fertilizers treatment. The effect of application of fertilizers is shown in Figure 1. There was a common trend that emission of methane was lower during the early growth stages. It then increased gradually and peaked 5 days after the peak tillering stage and decreased during the end tillering period in all eight treatments, it increased again 1 week after the heading flowering stage as shown in Figure 2.

#### Effects of different fertilizers on methane emission

There were pronounced differences among the fluxes of different fertilizers as shown in Table 1. The amounts of methane emission were significantly higher in pots with organic manures than those treated with chemical fertilizers. The result confirmed again previous observations that when organic amendments were added to flooded soils, methane production and emission were increased by lowering the *Eh* and providing more carbon sources. This agrees with many studies (Neue et al., 1996; Wahida et al., 2014). The order of the amount of

methane emitted from organic fertilizers was green manure, biogas residue and animal (cow) manure, with 52, 20, and 19 times to the CK, respectively. The emission order of the chemical fertilizers treated pots was NH<sub>4</sub>HCO<sub>3</sub>, CO(NH<sub>2</sub>)<sub>2</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, NH<sub>4</sub>Cl and NaNO<sub>3</sub> with 2.4, 2.0, 1.5, 1.3, and 0.2 times, respectively (There is no large difference in fluxes between the addition of NH<sub>4</sub>HCO<sub>3</sub> and CO (NH<sub>2</sub>)<sub>2</sub>). The amounts of methane emission were higher in rice pots with NH<sub>4</sub>HCO<sub>3</sub>, CO (NH<sub>2</sub>)<sub>2</sub> fertilizers than that from rice pots with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, this might be due to the competition for substrate between sulfate reducers and methanogens. Sulfate reducers (in presence of SO<sub>4</sub><sup>2-</sup>) can out-compete methanogens for substrates due their high affinity for acetate and hydrogen (Min, 1993) and due the increase of soil redox potential (Wahida et al., 2014); also, the decomposition of NH<sub>4</sub>HCO<sub>3</sub> and CO(NH<sub>2</sub>)<sub>2</sub>, in flooded conditions can liberate carbon dioxide and methanogens can easily produce methane from carbon dioxide reduction. This reason, along with the quick decomposition of NH<sub>4</sub>Cl in flooded conditions during a long rice growing period, can also be explained the lower



**Figure 2.** Effect of different chemical fertilizers application on methane emission during growth stage of rice plan (Methane emitted,  $10^{-5}$  mol/(pot.day)).

**Table 2.** The amount of methanogens in paddy rice soil with application of different fertilizers during growth stages of rice ( $10^4$  cells/g dry soil).

Microbial group	Growth stage of rice	Difference treatments								
		1	2	3	4	5	6	7	8	9
HFB	Early stage of growth	159	804	160	126	152	184	344	170	761
	Peak tillering stage	119	31	69	21	4920	473	415	491	163
	Heading lowering stage	20	39	766	200	70	1620	1650	707	67
HPAB	Early stage of growth	335	715	756	128	21	4310	3350	268	43
	Peak tillering stage	151	311	69	30	295	14	518	268	27
	Heading lowering stage	2.7	14.7	7.2	0.7	0.7	6.6	2.3	1.2	2.2
MB	Early stage of growth	7.5	1.7	4.2	76.7	32.0	32.0	775.0	17.0	1.6
	Peak tillering stage	1.2	0.7	0.4	0.1	0.1	0.05	90	4.9	0.4
	Heading lowering stage	0.9	1.9	0.2	1.9	0.9	1.9	4.0	0.9	0.2

HFB: Hydrolytic fermentative bacteria; HPAB: Hydrogen-producing acetogenic bacteria; MB: Methanogenic bacteria; 1: CO(NH<sub>2</sub>)<sub>2</sub>; 2: (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; 3: NaNO<sub>3</sub>; 4: NH<sub>4</sub>HCO<sub>3</sub>; 5: NH<sub>4</sub>Cl; 6: Green manure; 7: Animal manure; 8: Biogas residue; 9: CK. The populations obtained by MPN method.

by methane emission from rice pots with NH<sub>4</sub>Cl fertilizer as compared with that from rice pots with NH<sub>4</sub>HCO<sub>3</sub> and CO(NH<sub>2</sub>)<sub>2</sub> fertilizers. CH<sub>4</sub> emission was very low in the control treatment likely from inhibition of microbes due to lack of nutrients and suitable reducing substrates. However, the amount of methane emission was significantly lower in rice pots with NaNO<sub>3</sub> fertilizer (lower than that from control). This is because when NaNO<sub>3</sub> fertilizer was applied in flooded soils, there was an increase in soil Eh and methanogens were very sensitive to high Eh. Nitrate reducers out compete methanogens for soils electrons (NO<sub>3</sub> reduction). Methane is exclusively produced by methanogenic bacteria that can metabolize only in the strict absence of free oxygen and at redox potentials of less than -150 mV (Huang et al., 2014). However, the second peak of the methane emission from those pots can be explained by the fact that initially high

NO<sub>3</sub> concentration decreased with time.

### Methanogenic microflora

The amounts of methanogenic microbes, including hydrolytic fermentative, hydrogen producing acetogenic and methanogenic bacteria, were measured during the different growth stages of rice, as presented in Table 2. The results showed that the order of amount of hydrolytic fermentative bacteria was at  $10^5$  to  $10^8$  g dry soil, about  $10^2$  times more than the amounts of hydrogen-producing acetogenic bacteria ( $10^3$  to  $10^6$  g<sup>-1</sup> dry soil), and about  $10^3$  times more than the amounts of methanogenic bacteria ( $10^2$  to  $10^3$  g<sup>-1</sup> dry soils) treated by different fertilizers. The amounts of hydrolytic fermentative bacteria increased or decreased with the growth of rice in different treated

soils, but that of hydrogen-producing acetogenic bacteria and methanogenic decreased with the growth of rice.

## Conclusion

The emission of methane by rice plants at different stages of growth was affected by different fertilizers treatment. The amounts of methane emission were significantly higher in pots with organic manures than those treated with chemical fertilizers. The amount of methanogenic bacteria was smaller in soils with chemical fertilizers than that in soils with organic fertilizers. To conclude, it is important to show that the application of organic fertilizers created a suitable condition for reproduction and methane formation of methanogenic bacteria.

## Conflict of interests

The author has not declared any conflict of interests.

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