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Yield-scaled N_2O and CH_4 emissions as affected by combined application of stabilized nitrogen fertilizer and pig manure in rice fields

Kaikuo Wu[1,2], *Ping Gong*[1,3], *Lili Zhang*[1,3]*, *Zhijie Wu*[1,3], *Xueshi Xie*[4], *Hengzhe Yang*[4], *Wentao Li*[1,2], *Yuchao Song*[1,3], *Dongpo Li*[1,3]

[1]*Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, P.R. China*
[2]*University of Chinese Academy of Sciences, Beijing, P.R. China*
[3]*National Engineering Laboratory for Soil Nutrient Management, Shenyang, P.R. China*
[4]*Stanley Agriculture Group Co., Ltd., Shandong, P.R. China*
**Corresponding author: llzhang@iae.ac.cn*

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Abstract: A field experiment was conducted to study the effects of stabilized nitrogen fertilizer combined with pig manure on rice yield and nitrous oxide (N_2O) and methane (CH_4) emissions. Four treatments were established: urea (U); pig manure (PM); PM and urea (PM + U); PM and stabilized nitrogen fertilizer (urea plus 1% NBPT (N-(n-butyl) thiophosphoric triamide), 1% PPD (phenylphosphorodiamidate) and 2% DMPP (3,4-dimethylpyrazole phosphate)) (PM + U + I). In this study, compared with PM, PM + U significantly increased cumulative N_2O emission, but PM + U + I showed no significant difference from PM on N_2O cumulative emission, indicating that stabilized nitrogen fertilizer combined with PM is effective at reducing N_2O emissions. The cumulative emission of CH_4 from PM + U + I treatment was significantly lower than that from PM and PM + U, indicating that stabilized nitrogen fertilizer combined with PM can effectively reduce CH_4 emissions as well. The yields of PM + U and PM + U + I were not significantly different from those of U and PM, indicating that local conventional nitrogen application and returns of PM can provide sufficient nitrogen for rice growth. For yield-scaled emissions (YSE), PM was the highest, while PM + U + I significantly decreased YSE. Concomitant application of stabilized nitrogen fertilizer can achieve the goal of reducing YSE when PM is returned to the field.

Keywords: global warming potential; static chamber method; *Oryza sativa* L.; nitrification

Rice production plays a pivotal role in ensuring China's food security. In China, over 60% of its 1.4 billion people consume rice daily. Rice yield is about 10 000 kg/ha with a conventional nitrogen fertilizer at a typical application level, while the highest yields of rice can reach 15 000 kg/ha (Tang and Cheng 2018), so there is still great potential for rice yield to increase. Annual global CH_4 emissions from rice fields are about 5–19% of global CH_4 emissions and agricultural N_2O emissions account for 60% of global anthropogenic N_2O emissions. Besides, the two gases are reactive chemicals, i.e., CH_4 affects the chemistry and oxidation capacity of the atmosphere, and elevated atmospheric N_2O is involved in stratospheric ozone depletion (Li et al. 2011). Therefore, it is necessary to have an in-depth understanding of N_2O and CH_4 emission patterns in rice fields in China and to adopt corresponding measures to increase production and reduce emissions.

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In China, the total amount of pig manure produced per year is 208 million tons, and pig manure can be a pollutant as well as a source of nutrients (Li et al. 2015). Application of pig manure can increase soil organic carbon storage (Zhang et al. 2009), and increase rice yield (Maris et al. 2016), but at the same time promote N_2O and CH_4 emissions because soil N_2O and CH_4 emissions are moderated by multiple factors such as soil inorganic nitrogen (N) and organic carbon (C) availability (Zhou et al. 2017). Maris et al. (2016) assert that the use of large amounts of pig manure can actually reduce yield-scaled emissions (YSE), and this was attributed to the formation of phytotoxic substances in the soil at high organic C contents. The term 'stabilized fertilizers' refers to fertilizers with urease inhibitors and/or nitrification inhibitors added in the production process. The application of stabilized fertilizers is known to increase rice yield (Yin et al. 2017) and reduce N_2O and CH_4 emissions by inhibiting nitrification and denitrification (Zhang et al. 2010, Vitale et al. 2018). Therefore, this experiment aimed to explore whether the application of stabilized fertilizer with pig manure can promote the increase of rice yield and reduce N_2O and CH_4 emissions.

MATERIAL AND METHODS

Field site. The field experiment was set up at the Shenyang Experimental Station of the Institute of Applied Ecology, Liaoning province, China (43°32'N, 123°23'E). The mean annual air temperature is 7–8°C; the mean annual precipitation is about 700 mm and the frost-free period is 147–164 days. The test soil was an Alfisol with an organic C content of 13.18 g/kg and a total N of 1.24 g/kg. Before this experiment, paddy fields had been planted for 27 years. The field was set up in spring 2017, and the experiment was launched in spring 2018. The farming system is continuous rice with one season per year.

Test design. Four treatments were established: urea (U) (180 kg N/ha, 53 kg P/ha, 124 kg K/ha); pig manure (PM) (266 kg N/ha, 81 kg P/ha, 194 kg K/ha); pig manure and urea (PM + U) (266 + 180 kg N/ha, 81 + 53 kg P/ha, 194 + 124 kg K/ha); pig manure and stabilized nitrogen fertilizer (urea with 1% NBPT (N-(n-butyl) thiophosphoric triamide), 1% PPD (phenylphosphorodiamidate) and 2% DMPP (3,4-dimethylpyrazole phosphate)) (PM + U + I) (266 + 180 kg N/ha, 81 + 53 kg P/ha, 194 + 124 kg K/ha). The local PM application rate was 12 600 kg/ha, containing 21.11 g N/kg, 6.40 g P/kg, and 15.40 g K/kg. Fertilizers were urea (46% N), triple superphosphate (19% P), and KCl (50% K), respectively. The inhibitors NBPT, PPD and DMPP, were applied at a rate of 1, 1 and 2% of urea nitrogen, respectively. A randomized block design with three replicates was arranged. The area of each plot was 30 m^2 (5 m × 6 m). Fertilization and irrigation were carried out on May 25. On May 29, rice seedlings (cv. Meifeng 9) were transplanted at a density of 15 cm × 30 cm and 3–5 rice seedlings per hole. Field management was in line with local traditions. Soil properties before fertilization in 2018 are shown in Table 1. Harvest took place on October 16th.

Sample collection and analysis. Soil pH was measured in a 1:2.5 soil/water suspension with a combination electrode. Available N was extracted using 2 mol/L KCl solution, available P was extracted using 0.5 mol/L $NaHCO_3$, and available K was extracted using neutral 1 mol/L NH_4OAc (Zhao et al. 2004). *In-situ* N_2O and CH_4 emissions were measured in the rice field in May 2018 by the static chamber method (Li et al. 2018). Static boxes made of transparent plexiglass with sealed tops contained two parts, namely the base and body. The base was 30 cm in diameter, with a groove in the middle and a height of 10 cm. The body was 30 cm in diameter and 50 cm in height. Small fans were installed inside the static boxes. Gas samples were collected every 2 days in

Table 1. Soil properties (0–20 cm soil layer) before fertilization in 2018

Treatment	Organic C	Total N	Ammonium N	Nitrate N	Available P	Available K	pH
	(g/kg)		(mg/kg)				
U	13.23 ± 0.48	1.30 ± 0.04	6.24 ± 0.13	14.16 ± 2.08	10.14 ± 1.07	78.95 ± 2.42	6.95 ± 0.07
PM	15.20 ± 0.97	1.47 ± 0.12	8.38 ± 0.29	28.38 ± 6.09	21.31 ± 2.60	62.56 ± 4.50	7.08 ± 0.14
PM + U	15.07 ± 0.50	1.49 ± 0.05	8.71 ± 0.76	33.95 ± 0.81	26.47 ± 4.00	66.83 ± 4.63	7.22 ± 0.17
PM + U + I	13.70 ± 0.68	1.32 ± 0.07	9.89 ± 0.66	35.03 ± 5.59	22.80 ± 0.91	67.58 ± 2.88	7.17 ± 0.04

U – urea; PM – pig manure; U + I – stabilized nitrogen fertilizer the first week after fertilization, and then every 7 days in the first month. In the case of rain, the sampling time was adjusted appropriately, and gas was collected every 2 weeks in other months. N_2O was collected a total of 7 times during 31 days, and CH_4 was collected a total of 12 times in 109 days. Gas samples were collected with a 50-mL syringe and immediately transferred into 200-mL gasbags at 0, 15, 30 and 45 min after the cap was placed on the chamber. Sampling time and temperature in the chamber were recorded. The temperature inside the chamber was measured by a thermocouple 20 cm from the top of the body. Gas samples were analyzed by gas chromatograph (Agilent 7890B, Gas Chromatograph, Delaware, USA).

Calculation and statistical analysis. The calculation of N_2O and CH_4 emission fluxes were as follows (Li et al. 2018):

$$F = \rho \times h \times dc/dt \times 273/(273 + T)$$

Where: F – N_2O flux (μg/m^2/h) or CH_4 flux (mg/m^2/h); ρ – their standard-state density (N_2O 1.964 kg/m^3 and CH_4 0.714 kg/m^3); h – chamber height above soil (m); c – N_2O/CH_4 concentration; dc/dt – slope of the gas concentration curve, estimated using a linear regression model (Vitale et al. 2017); 273 – gas constant; T – average air temperature inside the chamber during gas collection (°C).

The cumulative emissions (CE) of N_2O (kg N_2O/ha) and CH_4 (kg CH_4/ha) were calculated by summing the products of the average of two neighboring measurement fluxes by their interval time (Vitale et al. 2018).

N_2O and CH_4 emissions were used to calculate their combined emission as CO_2 equivalents (NCE kg CO_2 eq/ha). The following equation was used:

$$NCE = GWP\ (CH_4) \times CE\ (CH_4) + $$
$$+ GWP\ (N_2O) \times CE\ (N_2O)$$

Where: GWP (CH_4) and GWP (N_2O) – global warming potential (GWP) of CH_4 and N_2O relative to CO_2, which are 25- and 298-fold greater than that of CO_2 on a 100-year horizon, respectively.

Yield-scaled emissions (YSE) were calculated using the following equation:

$$YSE = NCE/yield$$

Where: YSE – combined emission of CH_4 and N_2O per unit of rice yield (kg CO_2 eq/kg grain yield).

Statistical evaluation of data was performed by SPSS Statistics 16.0 (SPSS Inc., Chicago, USA). The data were checked by one-way ANOVA, followed by Duncan's test. Correlation analysis was used to analyze the relationship between NH_4^+-N/NO_3^--N in the soil and water layers and N_2O fluxes. Data are shown as mean ± standard error.

RESULTS AND DISCUSSION

N_2O flux. N_2O flux peaked on the first day after fertilization, then decreased gradually (Figure 1a). Most of the emissions occurred in the first week after fertilization with relatively low emissions later in the season, likely due to stable moisture and temperature conditions (Ruser and Schulz 2015). The cumulative N_2O emission followed the order of PM + U (0.144 kg N_2O/ha) > PM + U + I (0.072 kg N_2O/ha) > U (0.069 kg N_2O/ha) > PM (0.051 kg N_2O/ha) (Figure 2a), reflecting the difference in total fertilizer N inputs. It has been suggested that N fertilizer posed the greatest impacts when applied at rates higher than crop demands (Pittelkow et al. 2014). Vitale et al. (2017) reported lower N_2O fluxes in soil treated with organo-mineral amendments

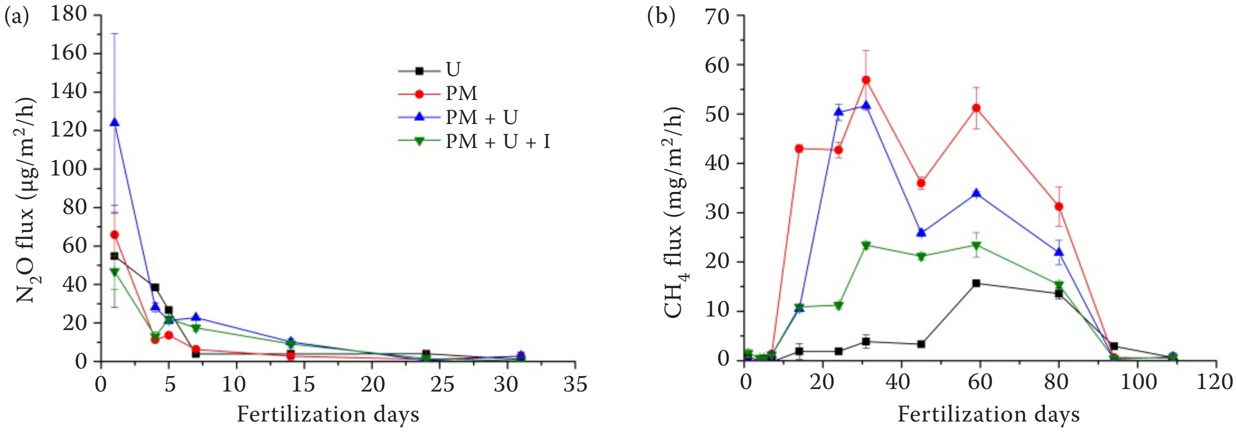

Figure 1. Effects of different treatments on N_2O and CH_4 fluxes in paddy fields. U – urea; PM – pig manure; U + I – stabilized nitrogen fertilizer

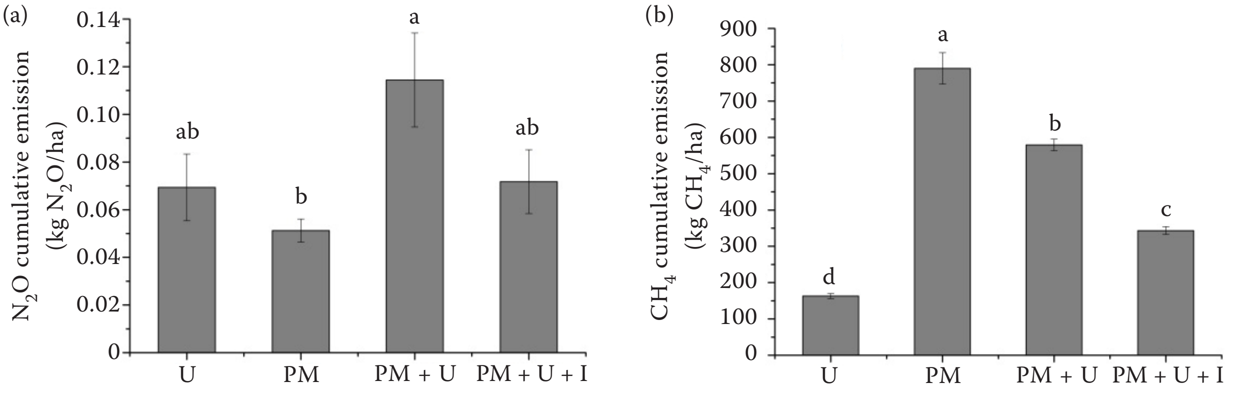

Figure 2. Effects of different treatments on cumulative emissions of N_2O and CH_4 in paddy fields. Different letters within treatments indicate significant differences ($P < 0.05$). U – urea; PM – pig manure; U + I – stabilized nitrogen fertilizer

compared to soils treated with mineral fertilizers. Conversely, our results show that the application of PM + U promotes N_2O emission compared with U, this may be due to the high content of easily decomposable N and C in the manure (Sosulski et al. 2017). Compared with PM, PM + U had significantly higher cumulative N_2O emissions ($P < 0.05$), possibly due to more nitrogen provided by PM + U, but the cumulative emission difference between PM + U + I and PM was not significant, indicating that the addition of inhibitors effectively suppressed N_2O emissions (Ruser and Schulz 2015, Yin et al. 2017). The reduction in cumulative N_2O emissions maybe because nitrification and denitrification are important biochemical processes that produce N_2O (Ruser and Schulz 2015). This may be due to the delay in urea hydrolysis caused by urease inhibitors (Yin et al. 2017), making more NH_4^+-N available in synchrony with plant demand (Zhang et al. 2010) and thus reducing nitrification substrates. The nitrification inhibitor further inhibited nitrification, directly reducing the formation of NO_3^--N, the substrate for denitrification (Yin et al. 2017). Meanwhile, NO_3^--N may increase with time to reduce the ratio of DOC/NO_3^--N and, therefore, reduce denitrification, which may also be an important reason for our observations (Sosulski et al. 2016). We also found that N_2O flux was significantly correlated with NH_4^+-N and NO_3^--N in the paddy water layer (Table 2). This was in line with the fact that NH_4^+-N and NO_3^--N are important substrates for nitrification and denitrification (Gregorutti and Caviglia 2017).

CH_4 flux. The CH_4 emission differed from N_2O (Figure 1b), with a strong increase 10 days after irrigation. This may be a result of decreased CH_4 oxidation after an irrigation (Banger et al. 2012). The low CH_4 production in the first 10 days may be due to the relatively high oxygen content in the water, i.e., high CH_4 oxidation, as discussed by Hao et al. (2019). Large amounts of CH_4 are typically produced during the rice-growing season (Shang et al. 2011). As seen in Figure 2b, the cumulative emission in the PM treatment was larger than in the U treatment ($P < 0.05$), indicating that the CH_4 emission potential of pig manure is greater than that of urea. This is likely because pig manure is a rich source of carbon and nitrogen, providing more substrates for CH_4 production (Linquist et al. 2012). Compared with U, PM + U increased the cumulative emissions of CH_4 ($P < 0.05$), which is consistent with other work (Linquist et al. 2012). However, cumulative emissions

Table 2. Correlation analysis of N_2O flux and NH_4^+-N/NO_3^--N in soil/water layer

Variable		U		PM		PM + U		PM + U + I	
		R^2	P	R^2	P	R^2	P	R^2	P
Soil	NH_4^+-N	0.144	0.758	–0.719	0.069	0.506	0.247	–0.015	0.975
	NO_3^--N	–0.305	0.506	–0.019	0.968	0.077	0.870	0.054	0.908
Water	NH_4^+-N	0.924	0.003**	0.612	0.144	0.828	0.021*	0.817	0.025*
	NO_3^--N	0.996	0.000**	0.865	0.012*	0.844	0.017*	0.706	0.076

*$P < 0.05$; **$P < 0.01$; U – urea; PM – pig manure; U + I – stabilized nitrogen fertilizer

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Table 3. Global warming potential, rice yield and yield-scaled gas emissions from each treatment

Treatment	N_2O cumulative emission (kg N_2O/ha)	CH_4 cumulative emission (kg CH_4/ha)	NCE (kg CO_2 eq/ha)	Yield (kg/ha)	YSE (kg CO_2 eq/kg)
U	0.069 ± 0.014^{ab}	163 ± 7^{d}	4100 ± 173^{d}	10 160 ± 493^{ab}	0.40 ± 0.02^{d}
PM	0.051 ± 0.005^{b}	790 ± 43^{a}	19 762 ± 1079^{a}	10 383 ± 237^{ab}	1.90 ± 0.09^{a}
PM + U	0.114 ± 0.020^{a}	579 ± 16^{b}	14 514 ± 400^{b}	10 840 ± 405^{a}	1.34 ± 0.08^{b}
PM + U + I	0.072 ± 0.013^{ab}	343 ± 11^{c}	8601 ± 279^{c}	9330 ± 367^{b}	0.93 ± 0.06^{c}

Date are expressed as mean ± standard error, *n* = 3; Different letters within treatments indicate significant differences ($P < 0.05$). U – urea; PM – pig manure; U + I – stabilized nitrogen fertilizer; NCE – N_2O and CH_4 emissions; YSE – yield-scaled emissions

of CH_4 in the PM + U were lower than those in the PM treatment ($P < 0.05$). Compared with PM + U, PM + U + I significantly reduced cumulative CH_4 emission ($P < 0.05$), indicating that urea addition compromised CH_4 emissions in manure, and the inclusion of inhibitor further reduced CH_4 emissions (Ruser and Schulz 2015). The application of urea increased available nitrogen, and the addition of inhibitors further influenced the existing forms of nitrogen; this is beneficial to the growth of methanotrophic organisms and results in increased CH_4 oxidation (Maris et al. 2016).

Rice yield and YSE. Table 3 shows that the yields of PM + U and PM + U + I were not significantly different from those of U and PM, indicating that the conventional urea application and local practice of returning pig manure to the field can meet the nitrogen demand of rice; higher inputs of nitrogen would not increase rice yield. YSE in the PM treatment was significantly greater than in the U treatment ($P < 0.05$), which is consistent with the research of Zhou et al. (2017). This phenomenon can be attributed to the fact that CH_4 emission is the main contributor to NCE in paddy fields (Shang et al. 2011), accounting for more than 99% of NCE in all treatments (Table 2). PM application may provide more nitrogen, promote the abundance of methanogens and provide more DOC directly or indirectly, which is an important determinant of CH_4 production (Zhang et al. 2018), thus leading to the increase of YSE. When we compared the YSE of the PM + U and PM treatments, we found the PM + U treatment to be significantly lower than the PM treatment ($P < 0.05$). In the PM + U treatment, application of extra inorganic nitrogen afforded nutrition for soil microorganisms, but made carbon a limiting factor for microbial reproduction and activity (Chen et al. 2014). This increased microbial demand for carbon likely led to the lower YSE. Between the PM + U + I and PM + U treatments, YSE was significantly less in the PM + U + I treatment ($P < 0.05$). Inhibitors delay urea hydrolysis and nitrification (Yin et al. 2017), leaving more NH_4^+-N in the paddy fields, which can result in the stimulation of methanotrophic activity and CH_4 oxidation, leading to the observed results (Maris et al. 2016).

It can be concluded that under the condition of PM returns to the field, concomitant application of stabilized nitrogen fertilizer (PM + U + I) can not only guarantee rice yield but also reduce N_2O and CH_4 emissions, which is an effective way to develop environmentally-friendly management and maximize the benefit of pig manure in paddy fields.

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