RESEARCH ARTICLE

Methane and nitrous oxide emissions from rice paddy soil as influenced by timing of application of hydroquinone and dicyandiamide

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Abstract A pot trial and a field experiment were conducted to study the effect of timing of application of nitrification inhibitor dicyandiamide (DCD) on N_2O and CH_4 emissions from rice paddy soil. Four treatments including Treatment CK_1, DCD-1 (application of DCD with basal fertilizer), DCD-2 (DCD with tillering fertilizer) and DCD-3 (DCD with panicle initiation fertilizer), were designed and implemented in pot experiment. Total N_2O and CH_4 emissions from DCD-treated soils were decreased profoundly when compared with that from urea alone ($P < 0.05$). Application of DCD together with basal fertilizer, tillering fertilizer and panicle initiation fertilizer reduced N_2O emission by 8, 30 and 2%, respectively, while those for CH_4 were 21, 8 and 1%. The field experiment with four treatments was carried out subsequently, and a kind of urease inhibitor hydroquinone (HQ) was also incorporated with DCD simultaneously. The combined use of HQ and DCD with basal fertilizer, tillering fertilizer and panicle initiation fertilizer decreased N_2O emissions by 24, 56 and 17%, respectively, while those for CH_4 were 35, 19 and 12%. N_2O emission was effectively reduced by the inhibitor(s) applied with tillering fertilizer before midseason aeration, while CH_4 emission was effectively decreased by the combined use of inhibitor(s) with basal fertilizer before rice transplanting. Furthermore, an increase in rice yield and a reduction of total global warming potential (GWP) of CH_4 and N_2O could be achieved by using inhibitor(s) in rice paddy field.

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Introduction

Rice cultivation is the leading anthropogenic methane (CH_4) emission source since it provides the suitable conditions for methanogenesis and a huge land area is given to meet the increasing food demand (Khalil and Rasmussen 1983; Wang 1999; Jacobson 2005). As a greenhouse gas, CH_4 is second only to carbon dioxide (CO_2) in volume, but 25 times greater than CO_2 on a mass basis in global warming potential (IPCC 2007a). CH_4 concentration in the atmosphere has more than doubled over the last two centuries

(Towprayoon et al. 2005). Annual global CH$_4$ emissions from rice fields are reported to be 31–112 Tg, about 5–19% of the global CH$_4$ emissions (IPCC 2007b).

Nitrous oxide (N$_2$O), another important greenhouse gas associated with rice farming, is produced naturally in soil via denitrification and/or nitrification processes after chemical or organic fertilization (Tsuruta 2002). N$_2$O is 298 times more potent as a greenhouse gas than CO$_2$ and the atmospheric concentration of N$_2$O continues to rise at a rate of approximate 0.26% year^{-1} and has reached a concentration of 319 ppb (10^{-9} mol mol^{-1}) until 2005 (IPCC 2007a). Globally, agricultural N$_2$O emissions have increased by nearly 17% from 1990 to 2005, and they account for about 60% of global anthropogenic N$_2$O emissions (IPCC 2007c).

Urease inhibitor and nitrification inhibitor, which slows down urea hydrolysis to NH$_4^+$–N and retards nitrification process of NH$_4^+$–N, respectively, jointly inhibit the subsequent formation of N$_2$O, directly by reducing nitrification, and indirectly by reducing the availability of NO$_3^-$–N for denitrification. Conventionally, urease and nitrification inhibitors are usually applied into soil together with basal fertilizer before rice transplanting or seeding. Urease inhibitor hydroquinone and nitrification inhibitor dicyandiamide are a pair that has currently been attracting more and more attention from researchers in this field. It indicated that DCD alone or in combination with HQ, could substantially reduce CH$_4$ and N$_2$O emissions during rice growth season (Xu et al. 2000a, 2002) and effectively regulate the behavior of applied urea–N in a soil-plant system (Xu et al. 2000b, 2001). The combined use of DCD and urea was useful as it simultaneously mitigated N$_2$O emission by 53% and CH$_4$ emission by 22% and improved crop quality when compared with the use of urea alone (Ghosh et al. 2003). Zu et al. (2002) observed that lowering emissions of N$_2$O and CH$_4$ occurred during rice growth season using a combination of DCD and HQ. Boeckx et al. (2005) found that application of U (urea) with HQ, U with DCD, U with HQ plus DCD decreased N$_2$O emissions by 11, 47 and 62%, respectively, and CH$_4$ emission by 30, 53 and 58%. However, controversial report was available on their effects on CH$_4$ production and emission, stating that CH$_4$ emission from rice fields in China was promoted in the presence of

nitrification inhibitor thiourea (Cai et al. 1994). Malla et al. (2005) also indicated that application of DCD reduced CH$_4$ emission by 12%, but application of HQ recorded 12% higher CH$_4$ emission compared to urea alone. Effect of application of HQ and DCD on CH$_4$ and N$_2$O emissions from rice paddy field needed to be further studied.

The conventional way of rice cultivation in China has rice paddy fields flooded continuously for about 1 month after basal fertilizer incorporation, then exposed to aeration for 7–10 days (called midseason aeration, MSA) which is followed by intermittent irrigations. Nitrification inhibitor may not be highly efficient in continuous flooding period, as standing water over the field makes the soil system anaerobic, thus suppressing the nitrification process (Majumdar et al. 2000, 2002). Rapid drainage and drying–wetting alternation may keep the upper layers of the soil aerobic in rice paddy field. Consequently, a considerable amount of N$_2$O is produced via nitrification as well as denitrification and emitted from soil into the atmosphere. Tillering and panicle initiation fertilizers were always applied into soil before drainage of standing water during rice growth season. Nitrification inhibitor may play a great role in controlling N$_2$O emission from rice paddy fields if applied into soil with tillering fertilizer before midseason aeration or with panicle initiation fertilizer before wetting–drying cycles. Although the combined use of HQ and DCD has been considered to be a potential management strategy to reduce N$_2$O and CH$_4$ emissions from rice paddy fields, little information is available on the effect of timing of HQ and DCD application on N$_2$O and CH$_4$ emissions. The objectives of the study were to (1) measure N$_2$O and CH$_4$ emissions from rice paddy soil as affected by application of HQ and DCD, (2) study the effect of timing of application of HQ and DCD on N$_2$O and CH$_4$ emissions, and (3) evaluate mitigation efficiency of HQ and DCD in terms of GWP.

Materials and methods

Two experiments were undertaken to study the influence of timing of application of HQ and DCD on N$_2$O and CH$_4$ emissions from rice paddy soil during rice growth season in pot experiment and field experiment.

Experimental site and soil characteristics

The pot experiment of was carried out in the greenhouse of Jiangsu Polytechnic College of Agriculture and Forestry, Jurong city, Jiangsu province, China (31°37′N, 119°82′E). The soil (0–20 cm) was classified as a Typic Epiaquepts (USDA Taxonomy 1975) with a loamy texture (24.5% clay, 42.9% silt and 32.6% sand) and its properties were: initial pH (H$_2$O) 6.23, total N 0.13%, total C content 1.26%. Air-dried soil sample that passed through a 2 mm sieve was used in pot experiment. The field experiment was conducted in a rice paddy field located at Baitu Town, Jurong City, Jiangsu Province, China (31°99′N, 119°32′E). The soil of the paddy field was classified as Typic Haplaquepts (USDA Taxonomy 1975) with a loamy texture (clay 14.2%, silt 68.7% and sand 17.1%) and its properties were: initial pH 6.91, total N 0.15%, total C 1.19%.

Treatments

Four treatments in triplicates were carried out in pot trial and field experiment to study the effect of timing of application of inhibitor(s) on N$_2$O and CH$_4$ emissions from rice paddy soil. In pot experiment of 2005, four treatments including Treatment CK$_1$ (urea-fertilized, without DCD but the same fertilization rate as in other treatments), DCD-1 (early application of DCD together with basal fertilizer), DCD-2 (postponed application of DCD together with tillering fertilizer before midseason aeration), and DCD-3 (late application of DCD together with panicle initiation fertilizer before intermittent irrigation), were implemented. Six kilograms of soil was thoroughly mixed with basal fertilizer (1.87 g urea, 0.84 g K$_2$HPO$_4$ and 0.24 g KCl) and placed into each pot on June 10. Urea of 0.935 g was applied as tillering fertilizer and panicle initiation fertilizer on June 27 and August 4, respectively. Application rate of DCD was 4% of the applied urea (w/w). In field experiment of 2007, four treatments including Treatment CK$_2$, HQ/DCD-1 (HQ and DCD with basal fertilizer), HQ/DCD-2 (HQ and DCD with the tillering fertilizer), and HQ/DCD-3 (HQ and DCD with panicle initiation fertilizer), were carried out to study the effect of HQ and DCD on N$_2$O and CH$_4$ emissions. Basal application of Ca(H$_2$PO$_4$)$_2$ at 450 kg ha^{-1}, KCl at 225 kg ha^{-1} and urea at 150 kg N ha^{-1} were applied to all the treatments on June 18, fully as a basal dose. Application rate of urea as tillering fertilizer applied on July 11 and panicle initiation

fertilizer applied on August 12 was 75 kg N ha^{-1}. HQ and DCD were thoroughly mixed with urea, and their application rates were 0.3 and 5% (w/w) of the applied urea after physical mixing with urea. All treatments were arranged in a completely randomized block design (RBD). Rice (variety huajing 3) was transplanted during monsoon season (June–October, during which rice is cultivated widely for local farmers due to high precipitation and high temperature required by rice). Thirty-day old seedlings were transplanted on 11 June 2005 in pot experiment and 19 June 2007 in field experiment. Midseason aerations were carried out on 12 July 2005 and 16 July 2007, respectively, during rice growth season. The soils were kept dry for 1 month before rice harvesting in field experiment and the rice was harvested at maturity on 139 DAT (days after transplanting) in pot trail and 123 DAT in field experiment.

Sampling and analysis

The closed-chamber method was used for collecting gas samples. In pot experiment, the cross-sectional area and the height of the chamber were 0.09 m^2 (0.3 × 0.3 m) and 1 m, respectively. The plexiglass chamber was placed on channels jointed to the pot. To make the arrangement airtight, water was poured into the channels. Four gas samples were collected from a chamber using 18-ml vacuumed vials at an interval 2–3 days during the first 10 days after fertilizer application, at an interval of 5 days or so in the other period except the last 2 months of rice growth when an interval of 7 days was chosen. During midseason aeration and the followed re-flooded periods N$_2$O and CH$_4$ were observed daily. Collection of gas samples in field experiment was also carried out by the closed-chamber technique and the removable boardwalks (about 2 m long) were installed early before gas sampling to avoid disturbances of the soil system. The chamber (0.5 × 0.5 × 1 m) could be adjusted to the increasing plant height. The sampling frequency of the field experiment was similar to the pot experiment. All chambers were equipped with a fan inside to ensure complete gas mixing. The gas concentrations were analyzed by gas chromatograph (Shimadzu GC-12A) equipped with flame ionization detector for CH$_4$ analyses and by gas chromatograph (Shimadzu GC-14B) with electron capture detector for N$_2$O analyses.

When CH_4 and N_2O fluxes were monitored, the redox potentials (Eh) of soils were simultaneously measured by using Pt-tipped electrodes (Hirose Rika Co. Ltd. Japan) and an oxidation-reduction potential meter (Toa PRN-41). For the measurements of soil Eh, the electrodes were inserted into the soil at a depth of 10 cm and maintained there throughout rice growth season. All soil Eh measurements of each plot were made in triplicates. The data of soil Eh from 16 DAT to 28 DAT were lost due to malfunction of the apparatus in pot experiment. Water layer depth was measured manually with a ruler, and soil temperatures of 0, 5, 10, 15 cm depth were measured with a hand-carried digital thermometer while gas sampling. In pot experiment, soil solution was collected to a vacuum plastic bottle by a polyvinyl chloride (PVC) pipe from the bottom of the pot when sampling and stored at 4°C freezer for later measurements of NH_4^+–N and $(NO_3^- + NO_2^-)$–N. In field experiment, fresh soil samples were collected from the 0 to 15 cm soil layer at three locations of each plot using a core sampler. Fresh soil samples (30 g, each) were extracted with 2.0 mol l^{-1} KCl (100 ml) in 250 ml Erlenmeyer flasks under shaking on a rotary shaker for 1 h, then the extracts were filtered, and the filtrates were kept in a freezer (4°C) until analysis. A segment flow analyzer of Skalar (SAN++, the Netherlands) was employed to determine the concentrations of NH_4^+–N and $(NO_3^- + NO_2^-)$–N.

Calculation and statistical analysis

N_2O fluxes were calculated according to the following equation:

$$F = \rho \times \frac{V}{A} \times \frac{\Delta c}{\Delta t} \times \frac{273}{273 + T}$$

where F stands for N_2O flux in µg N_2O–N m^{-2} h^{-1} or CH_4 flux in mg CH_4 m^{-2} h^{-1}, ρ for density of N_2O–N or CH_4 under the standard state, V for effective volume of chamber (m^3), A for area from which N_2O or CH_4 was emitted into the chamber (m^2), $\frac{\Delta c}{\Delta t}$ for rate of accumulation in ppbv N_2O–N h^{-1} or ppmv CH_4 h^{-1}, and T for chamber temperature in celsius. Mean N_2O or CH_4 fluxes during the rice-growing period were the average of triplicate fluxes weighted by an interval of two measurements. Significant differences between the means were analyzed using SPSS (version 12.0), with a confidence interval of 95%.

GWP of CH_4 and N_2O emitted in different treatments were calculated using the following equation:

$$GWP = F_{CH_4} \times 25 + \frac{F_{N_2O-N}}{28} \times 44 \times 298$$

where GWP stands for global warming potential of CH_4 and N_2O in mg CO_2-eq m^{-2} h^{-1}, F_{CH_4} for CH_4 flux in mg CH_4 m^{-2} h^{-1}, F_{N_2O-N} for N_2O–N flux in mg N_2O–N m^{-2} h^{-1}.

Results

Soil Eh

Soil Eh during experimental period showed a similar pattern and ranged from −264 to 800 mV in pot experiment and from −142 to 753 mV in field experiment (Fig. 1). Water levels were highly variable during rice growth season due to midseason aeration and re-flooding, and the alternation of wetting and drying during the late stage of rice growth. In general, soil Eh values decreased from positive values under the
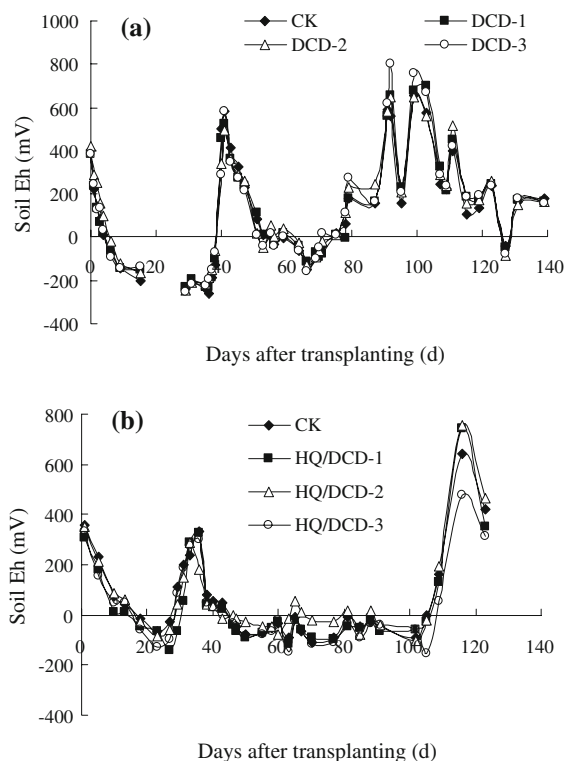

Fig. 1 Seasonal variations of soil Eh during rice growth season in 2005 (a) and 2007 (b)

initial flooding soil conditions to near negative and high negative values under submergence. Soil Eh almost remained negative during the period from re-flooding after midseason aeration to water drainage before rice harvesting in field experiment, but fluctuated at positive values on the days during drying-wetting cycles in pot experiment.

Temperature

Figure 2 indicated seasonal variations of mean soil temperature of different depths and air temperature on sampling days. Mean soil temperature ranged from 12.3 to 32.6°C in pot experiment and from 14.9 to 30.9°C in field experiment. Variation patterns of soil temperature were similar to that of air temperature and temporal variation of mean soil temperature in pot experiment was higher than that of field experiment. In addition, seasonal air temperature tended to be higher in pot experiment than in field experiment due to the extra warming effect of the greenhouse.
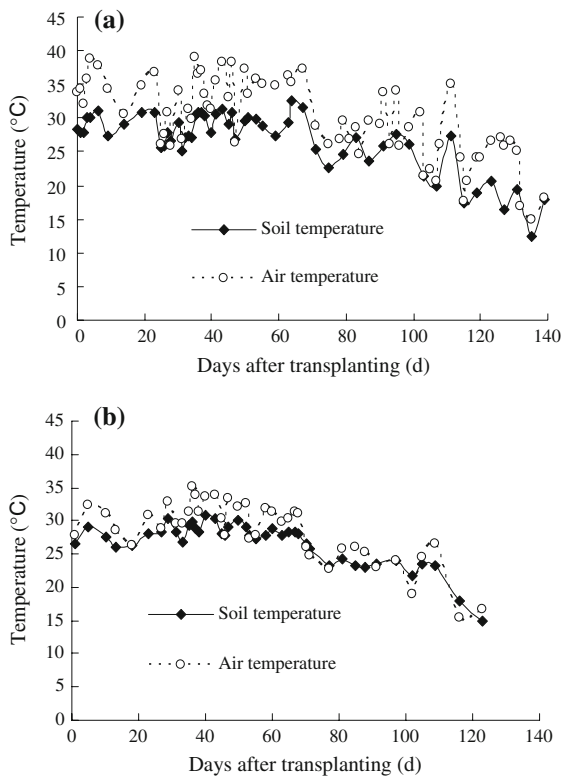
N$_2$O emission

N$_2$O fluxes from treatments with inhibitor(s) had almost the same patterns as from CK treatments in pot experiment and field experiment (Fig. 3). N$_2$O fluxes were low during submergence and increased substantially during drainage of standing water. Application of urea as panicle initiation fertilizer resulted in appearance of small peaks of N$_2$O in all the treatments. N$_2$O fluxes were hardly detectable in any of the treatments after 70 days of rice transplanting.

Two seasonal peaks of N$_2$O flux occurred during midseason aeration and re-flooding periods in pot experiment. Seasonal peaks of N$_2$O in Treatment DCD-2 (2.10 and 1.59 mg N$_2$O–N m^{-2} h^{-1}) were much lower than the peaks in Treatment CK$_1$ (2.31 and 2.29 mg N$_2$O m^{-2} h^{-1}). Soil temperature at different depths was the main factor affecting N$_2$O emissions in pot experiment ($r = 0.40$–0.47, $P < 0.01$). In filed experiment, seasonal peaks of N$_2$O flux were 1.66, 1.31, 0.73 and 1.53 mg N$_2$O–N m^{-2} h^{-1} from Treatment CK$_2$, HQ/DCD-1, HQ/DCD-2 and HQ/DCD-3, respectively (Fig. 3). Apparently, the highest N$_2$O flux

Fig. 2 Seasonal variations of *soil temperature* and *air temperature* during rice growth season in 2005 (**a**) and 2007 (**b**)
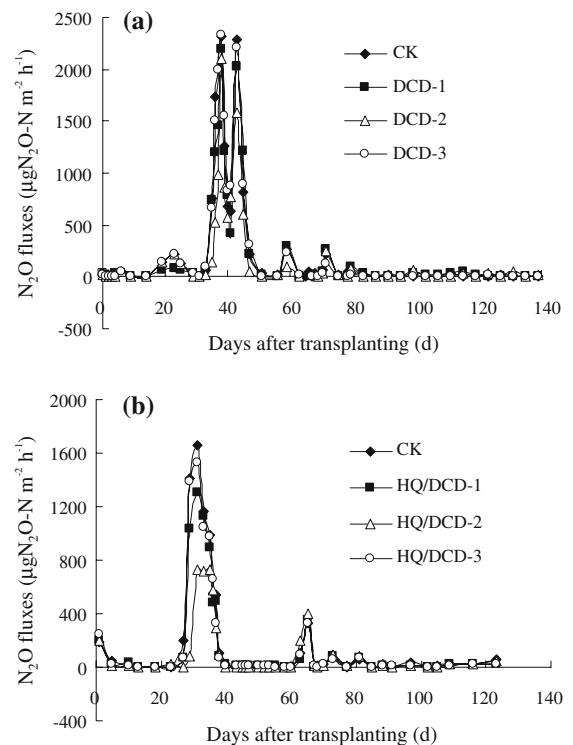

Fig. 3 Seasonal variations of N$_2$O fluxes during rice growth season in 2005 (**a**) and 2007 (**b**)

was observed in Treatment CK_2 which was at par with Treatment HQ/DCD-3, and the lowest N_2O flux in Treatment HQ/DCD-2 where HQ and DCD applied in combination with tillering fertilizer before drainage. Furthermore, Treatment HQ/DCD-1 was less effective than Treatment HQ/DCD-2 in inhibiting N_2O flux, indicating that inhibitors with tillering fertilizer before MSA could sharply decreased N_2O emission. Significant negative correlation between N_2O fluxes and water layer depths was observed the in field experiment ($r = -0.69$ to -0.75, $P < 0.01$) (data not shown).

Mean N_2O fluxes of pot experiment were 156, 143, 109 and 153 µg N_2O–N m^{-2} h^{-1} from Treatment CK_1, DCD-1, DCD-2 and DCD-3, respectively, while those for field experiment were 132, 101, 58.8 and 110 µg N_2O–N m^{-2} h^{-1} from Treatment CK_2, HQ/DCD-1, HQ/DCD-2 and HQ/DCD-3 (Table 1). We observed that reductions of total N_2O emission in pot experiment by application of DCD together with basal fertilizer, tillering fertilizer and panicle initiation fertilizer were 8, 30 and 2%, respectively, while those for field experiment were 24, 56 and 17% from HQ/DCD with basal fertilizer, tillering fertilizer and panicle initiation fertilize, respectively. In general, N_2O emissions were always lower in the presence than in the absence of inhibitor(s) and the combined use of urea and inhibitor(s) with tillering fertilizer before midseason aeration decreased N_2O emission profoundly ($P < 0.05$).

CH$_4$ emission

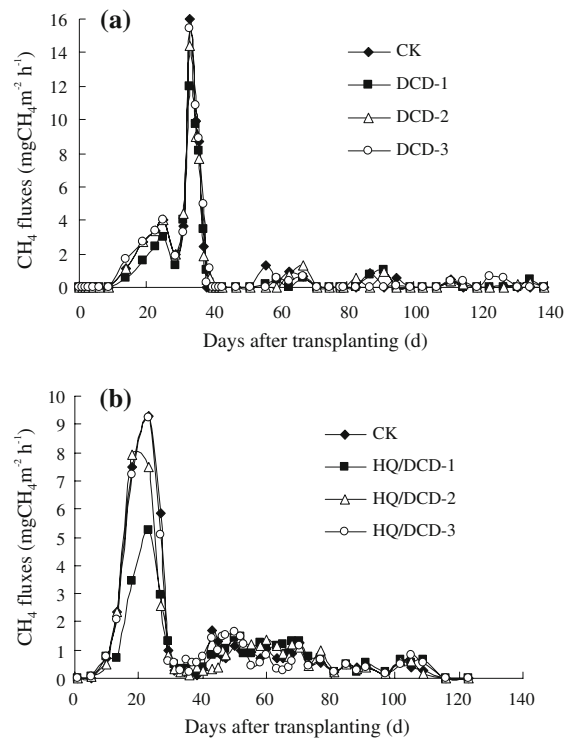Patterns of CH_4 fluxes of pot experiment were much more irregular than that of field experiment (Fig. 4).

Fig. 4 Seasonal variations of CH_4 fluxes during rice growth season in 2005 (**a**) and 2007 (**b**)

CH_4 fluxes increased gradually and reached the seasonal peaks during continuous flooding period and decreased markedly at the beginning of natural drainage of flooding water in field experiment. But in pot experiment, CH_4 fluxes increased gradually during continuously flooded period after rice transplanting, reached the seasonal peaks after drainage of standing water and decreased sharply to near nil during midseason aeration. Significant negative

Table 1 Mean fluxes (±SD) of N_2O and CH_4 and total emissions (±SD) of N_2O and CH_4 during rice growth season in 2005 and in 2007

Years	Treatments	N_2O fluxes (µg N_2O–N m^{-2} h^{-1})	N_2O emissions (kg N_2O–N ha^{-1})	CH_4 fluxes (mg CH_4 m^{-2} h^{-1})	CH_4 emissions (kg CH_4 ha^{-1})
2005	CK_1	156 ± 8.01a	5.19 ± 0.35a	0.95 ± 0.14a	31.69 ± 4.09a
	DCD-1	143 ± 6.24b	4.78 ± 0.52b	0.75 ± 0.03c	25.02 ± 2.10c
	DCD-2	109 ± 7.52c	3.62 ± 0.17c	0.87 ± 0.06b	29.02 ± 5.55b
	DCD-3	153 ± 9.21a	5.11 ± 0.20a	0.94 ± 0.17a	31.39 ± 3.12a
2007	CK_2	132 ± 14.1a	3.90 ± 0.18a	1.49 ± 0.21a	43.39 ± 3.89a
	HQ/DCD-1	101 ± 7.21b	2.98 ± 0.22b	0.95 ± 0.16c	28.04 ± 1.57b
	HQ/DCD-2	58.8 ± 5.75c	1.73 ± 0.15c	1.19 ± 0.25b	35.13 ± 5.20ab
	HQ/DCD-3	110 ± 8.38b	3.23 ± 0.27ab	1.29 ± 0.32b	38.08 ± 1.19a

Different letters indicate significantly different means between treatments at $P < 0.05$

correlations between CH_4 emission and soil Eh were discovered during experimental period in 2005 and 2007 ($r = -0.36$ to -0.51, $P < 0.01$) and the fact that soil Eh was the main factor affected CH_4 emission was found. Application of inhibitor(s) with basal fertilizer decreased the seasonal peak of CH_4 profoundly (Fig. 4).

Mean CH_4 fluxes of pot experiment were 0.95, 0.75, 0.87 and 0.94 mg CH_4 m^{-2} h^{-1} from Treatment CK_1, DCD-1, DCD-2 and DCD-3, respectively, while those for field experiment were 1.49, 0.95, 1.19 and 1.29 mg CH_4 m^{-2} h^{-1} from Treatment CK_2, HQ/DCD-1, HQ/DCD-2 and HQ/DCD-3 (Table 1). It also indicated that reductions of total N_2O emission by application of DCD together with basal fertilizer, tillering fertilizer and panicle initiation fertilizer were 21, 8 and 1%, respectively, while those for field experiment were 35, 19 and 12% from HQ/DCD with basal fertilizer, tillering fertilizer and panicle initiation fertilize, respectively. Reduction in total CH_4 emission by inhibitor(s) was as following: inhibitor(s) with basal fertilizer > inhibitor(s) with tillering fertilizer > inhibitor(s) with panicle initiation fertilizer. Apparently, the inhibition efficiency of inhibitor(s) with basal fertilizer was significantly higher than that of inhibitor(s) with tillering fertilizer and panicle initiation fertilizer.

GWP and rice yield

Table 2 represents the effect of timing of application of inhibitor(s) on global warming potential (GWP) of N_2O and CH_4 during the whole rice growth season. The GWP of various treatments during experimental period varied between 72.56 and 96.65 mg CO_2-eq m^{-2} h^{-1} in pot experiment, between 57.27 and 99.00 mg CO_2eq m^{-2} h^{-1} in field experiment. The highest GWP was found when no inhibitor(s) added to the soil and the combined use of urea and inhibitor(s) could reduce the GWP of CH_4 and N_2O. Reduction in GWP with the application of inhibitor(s) ranged from 1 to 25% in pot experiment and from 16 to 42% in field experiment. The lowest GWP was observed when the inhibitor(s) were added to the soil together with tillering fertilizer before serious changes of water status during tillering stage. Also the soil with inhibitor(s) applied with basal fertilizer before rice transplanting showed relatively high GWPs. The application of inhibitor(s) with panicle initiation was not sufficient to reduce the GWPs of CH_4 and N_2O considerably.

Rice yields in field experiment were determined by harvesting all the hills excluding those in the three sampling sites of each treatment because they were frequently disturbed by sampling. The yields from treatments with inhibitor(s) were higher than from the CK treatments in pot experiment and field experiment ($P < 0.05$; Table 2). Application of inhibitor(s) with tillering fertilizer was the most effective in increasing rice yield, showing 30 and 18% higher than CK treatments in pot experiment and field experiment, respectively.

Discussion

Effect of timing of application of inhibitor(s) on N_2O emission

Mean N_2O fluxes were lower when urea was combined with nitrification and urease inhibitors than

Table 2 Global warming potential (GWP) of N_2O and CH_4 (E-CO_2) and rice yield during rice growth season in 2005 and 2007

Years	Treatments	N_2O E-CO_2 (mg CO_2-eq m^{-2} h^{-1})	CH_4 E-CO_2 (mg CO_2-eq m^{-2} h^{-1})	GWP (mg CO_2-eq m^{-2} h^{-1})	Rice yields (t ha^{-1})
2005	CK_1	72.90a	23.75a	96.65a	5.46c
	DCD-1	67.08b	18.75c	85.83b	6.37b
	DCD-2	50.81c	21.75b	72.56c	7.10a
	DCD-3	71.76b	23.50a	95.26a	6.23b
	CK_2	61.75a	37.25a	99.00a	7.92c
2007	HQ/DCD-1	47.20bc	23.75bc	70.95c	8.72b
	HQ/DCD-2	27.52d	29.75b	57.27d	9.36a
	HQ/DCD-3	51.29b	32.25a	83.54b	8.40bc

Different letters indicate significantly different means between treatments at $P < 0.05$

when urea alone in our experiment. Mean N_2O flux was lowest from inhibitor(s) together with tillering fertilizer, followed by inhibitor(s) together with basal fertilizer and panicle initiation fertilizer. Also, the combination of inhibitor(s) with tillering fertilizer had affected the highest reduction in total N_2O emission (Table 1). Higher inhibitive effect of urease and nitrification inhibitors occurred if postponing the timing of application in rice paddy field.

Nitrification inhibitor retards the ammonium mono-oxygenase enzyme which is responsible for converting ammonium present in soil to hydroxyl amine which is further oxidized to nitrite and then to nitrate (Prasad and Power 1995). In a drier soil during drainage period, nitrification may play a major role in producing N_2O from accumulated NH_4^+–N and denitrification may also generate N_2O in deeper reduced soil layers and in upper anaerobic microsites simultaneously (Arah and Smith 1989). Two seasonal N_2O peaks occurred during midseason aeration and re-flooded period in our pot experiment. The first N_2O peak was found at the beginning of drainage and the latter occurred during re-flooding period. Drainage of the standing water made the soil system aerobic, which promoted the nitrification process and N_2O emission. When the soil re-flooded again, denitrifying bacteria acted on the nitrate pool released due to nitrification and further N_2O was produced. Some researchers indicated that denitrification of nitrate in soil could be responsible for some N_2O formation whenever the soil was made anaerobic by irrigation (Aulakh et al. 1992; Aulakh and Singh 1997). However, no N_2O flux peak was discovered during re-flooding period in field experiment and water layer depth was the main factor affecting N_2O fluxes. Under submergence, N_2O emission is low even though it's formation in soil may be high as pressure of standing water prevents N_2O from being released into atmosphere and also as it gets denitrified to N_2 within the soil (Granli and Bockman 1994).

Some factors, like varieties of inhibitors, application magnitude and application method of inhibitors, soil varieties and water management of rice paddy field, affect the inhibitive efficiency of HQ and DCD. DCD has been reported to have reduced N_2O emission by 40% in a dry sand loam soil, while in wet conditions no inhibition was noticed (Skiba et al. 1993). Pathak and Nedwell (2001) also indicated that DCD together with urea significantly reduced the N_2O

emission to 63% that of urea alone in field capacity, but no inhibitive effect was found in submergence conditions. Majumdar et al. (2002) studied the effect of DCD at different application rates (5–25% of applied urea–N) on nitrification and N_2O emission, indicating that DCD incorporated at the rate of 5 and 25% of applied urea–N were least and most effective in mitigating total N_2O emission. So, application of HQ and DCD in rice paddy field must be in accordance with the local practice of rice cultivation.

Effect of timing of application of inhibitor(s) on CH_4 emission

CH_4 emissions were always lower in the presence than the absence of inhibitor(s) from our experiments. The application of inhibitor(s) together with basal fertilizer could reduce CH_4 fluxes and hence significantly eliminated total amount of CH_4 emission (Table 1). Saturation of soil creates anaerobic conditions conductive for CH_4 emission, as methanogens are strict anaerobes. When such saturated soils were allowed to drying making them aerobic, emission of CH_4 almost stopped (Pathak et al. 2003). Therefore, lower inhibitive effect of inhibitor(s) on CH_4 emission may be found when the inhibitor(s) applied into soil with tillering fertilizer and panicle initiation fertilizer than with basal fertilizer in our experiments.

Combination of urease and nitrification inhibitors has been considered to be one of potential mitigation strategies in rice paddy field due to the simultaneous reduction of CH_4 and N_2O. However, there are conflicting reports regarding the influence of urease and nitrification inhibitors on CH_4 emission (Cai et al. 1994; Xu et al. 2000a; Malla et al. 2005) and the mechanism of urease inhibitors and nitrification inhibitors on CH_4 emission needs to be considered from three different levels.

1. Microbial community level. Urease and nitrification inhibitors affected CH_4 emission by the influence on methanogenesis. Wang et al. (1991) indicated that urease inhibitors have little influence on the oxidation of ammonium following the hydrolysis of applied urea in soils, but HQ may decrease CH_4 production via inhibition of the methanogenic fermentation of acetate. Lindau et al. (1993) indicated that DCD has an inhibitory effect on CH_4 emission and the

inhibition of methanogenesis by DCD may be the reason for decrease in CH_4 emission.

2. Biochemical level. Application of inhibitor(s) together with urea could effectively regulate the ratio of ammonium to nitrate plus nitrite in soil and ammonium inhibits CH_4 consumption. It has been suggested that nitrification inhibitors have considerable inhibitory effect on CH_4 oxidation in soil due to higher conservation of ammonium in soil, leading to an increase in the population of nitrifiers relative to methanotrophs and thus the overall reduction in CH_4 oxidation, as nitrifiers oxidize CH_4 less efficiently than methanotrophs (Bronson and Mosier 1994; Crill et al. 1994; Wilson et al. 1995). Thus, there could be increase in CH_4 emission with application of inhibitors.

3. Plant/ecosystem level. Urease inhibitors affected CH_4 emission by the positive effect on the redox potential of the rhizosphere and biomass production of rice. DCD, especially in combination with HQ, significantly enhanced CH_4 oxidation in the rice root rhizosphere, particularly between its tillering and booting stages and reduced CH_4 emission profoundly (Xu et al. 2000a). The presence of a more highly oxidized rhizosphere would result in a reduction of CH_4 emission from rice field to the atmosphere (Denier Van der Gon and Neue 1996). Application of ammonium fertilizers and nitrification inhibitor may stimulate CH_4 formation and emission, due to their positive influence on plant growth and the consequent increase of C input to the soil from plant remains and root exudates. High ammonium concentrations may also stimulate the growth of methanogens when C is used as a substrate by microbes and thus it may indirectly increase CH_4 production in the soil. However, some others indicated that ammonia formation following the hydrolysis of applied urea can more comprehensively destroy the growth of plant than the accumulation of urea in the urease inhibitor-treated soil (Krogmeier et al. 1989), thereby influencing the formation and oxidation of CH_4 in rice root rhizosphere.

As we know, CH_4 emission is controlled by production, oxidation and transportation of CH_4 in soil. Therefore, effects of application of urease and nitrification inhibitors on CH_4 emissions from rice fields depend on its integrated effects on CH_4 production, oxidation and transportation. Since effect of application of urease and nitrification inhibitors on CH_4 production, oxidation, and transportation can be negative and positive and take place simultaneously, it is not surprising that the reported effects of application of inhibitor(s) on CH_4 emissions from rice fields are contradictory.

Conclusions

Firstly, application of HQ and DCD with nitrogen fertilizer was effective to simultaneously reduce N_2O and CH_4 emissions from rice paddy soil.

Secondly, application of inhibitor(s) with tillering fertilizer had the highest average inhibitory effect on N_2O emission reaching up to 30% in pot experiment and 56% in field experiment, whereas application of inhibitor(s) with basal fertilizer a relatively high inhibitory effect of N_2O emissions up to 8 and 24% was recorded. The application of inhibitor(s) with panicle initiation fertilizer was not sufficient to reduce the N_2O emissions considerably. However, for CH_4 emissions, application of inhibitor(s) together with basal fertilizer had 21 and 35% reduction of CH_4 emissions in pot experiment and filed experiment. Also, lower inhibitory effect was found if postponing the timing of application of inhibitor(s) during rice growth season.

Thirdly, an increase in rice yield and a reduction of GWP of CH_4 and N_2O could be achieved via application of inhibitor(s) with nitrogen fertilizer in rice paddy field, especially together with tillering fertilizer.

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