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## Nitrous oxide and methane emissions during rice growth and through rice plants: effect of dicyandiamide and hydroquinone

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**Abstract** There is growing interest in  $N_2O$  and  $CH_4$  transport through rice plants, but very little information is available on the effects of inhibitors on these gaseous emissions during rice growth and through rice plants. The closed chamber technique was used to study the effect of the urease inhibitor hydroquinone (HQ) and the nitrification inhibitor dicyandiamide (DCD) on  $N_2O$  and  $CH_4$  emissions. As rice plants grew, the  $N_2O$  emission through rice plants was significantly reduced in all treatments;  $N_2O$  emissions were always lower in the presence than in the absence of inhibitor(s). These variations paralleled those in  $NO_3^-$ -N content of fresh rice plants. During the rice growth period, increasing  $NO_3^-$ -N content in rice plants paralleled the increase in the  $N_2O$  emission through rice plants. Hence,  $NO_3^-$ -N in young rice plants can substantially contribute to the plant-mediated  $N_2O$  flux. A substantial  $CH_4$  emission through rice plants occurred at their vigorous growth stage;  $CH_4$  emissions were always lower in the presence than in the absence of inhibitor(s). Under the experimental conditions, application of DCD, especially of DCD+HQ, could significantly improve the growth of rice, and reduce the emissions of  $N_2O$  and  $CH_4$  during rice growth.

**Keywords** Dicyandiamide · Hydroquinone · Methane · Nitrous oxide · Urea

### Introduction

Rice fields have been considered as one of the important sources of atmospheric  $CH_4$  (Khalil and Rasmussen 1983; Bartlett and Harriss 1993). For permanently flooded rice fields, >80% of  $CH_4$  is emitted from rice plants (Nouchi et al. 1990). The pattern and amount of aerenchyma in rice plants can substantially contribute to the wide variation in the  $CH_4$  transport capacity of rice plants during different growth stages (Aulakh et al. 2000; Wassmann and Aulakh 2000). Khalil et al. (1998) showed a large  $N_2O$  emission from rice fields using N-based fertilizers. By comparing  $N_2O$  emissions in chambers with and without rice plants, Mosier et al. (1990) showed that young rice plants facilitated the emission of  $N_2O$ . In recent years there has been growing interest in  $N_2O$  and  $CH_4$  emissions through rice plants (Yu et al. 1997; Yan et al. 2000). When soils were flooded,  $N_2O$  emissions through rice plants were substantial. The reduction of  $NO_3^-$ -N in fresh plants can influence  $N_2O$  and  $NO_x$  fluxes within the plants and contribute to total  $N_2O$  through the plants (Chen et al. 1990; Klepper 1990; Rockel et al. 1996).

Under flooded conditions, dicyandiamide (DCD), a nitrification inhibitor, alone or in combination with hydroquinone (HQ), a urease inhibitor, can substantially reduce  $CH_4$  emission during rice growth (Xu et al. 2000a) and effectively regulate the behaviour of applied urea (U)-N in a soil-plant system (Xu et al. 2000b, 2001). This could possibly affect the N loss as  $N_2O$  from rice fields after application of U, and the status of N in the rice plants.

Majumdar et al. (2000) and Kumar et al. (2000) showed that in field experiments, application of DCD together with U significantly reduced  $N_2O$  emission. In the presence of nitrification inhibitors such as DCD (Lindau et al. 1993; Xu et al. 2000a), wax-coated  $CaC_2$  (Lindau et al. 1993; Keerthisinghe et al. 1993) and nitrapyrin (Keerthisinghe et al. 1993) and Nimin (Rath et al. 1999),  $CH_4$  emission was significantly reduced from rice fields fertilized with U. Even so, very little information is

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available on the effects of both inhibitors on  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions during rice growth and through rice plants.

This work reports the emissions of  $\text{N}_2\text{O}$  and  $\text{CH}_4$  over the rice growing period. The effects of HQ and DCD were studied in pot experiments. The objective of this work is to present an effective method for the mitigation of both  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions during rice growth. Of additional interest was also the contribution of the  $\text{NO}_3^-$ -N content of rice plants to the  $\text{N}_2\text{O}$  emission through rice plants.

## Materials and methods

### Soil characteristics

Samples (0–20 cm depth) of a sandy loam soil in Belgium were collected. Some properties of the soil, classified as luvisol (FAO soil classification), were measured using the methods of Kim (1995) and Keeney and Nelson (1982). The amounts of clay, silt and sand in the soil were 14.9%, 28.9% and 56.2%, respectively. Concentrations of soil total N, available N and total C were  $1.5 \text{ g N kg}^{-1}$ ,  $16.7 \text{ mg N kg}^{-1}$  and  $17.5 \text{ g C kg}^{-1}$ , respectively. The soil pH was 7.6 (1:5, soil:water ratio). The soil samples were slightly air-dried and passed through a 2-mm-mesh sieve before use.

### Experimental description

Pot experiments were conducted in a greenhouse. Two kilograms of soil was thoroughly mixed with  $1.0 \text{ g K}_2\text{HPO}_4$  and  $2.0 \text{ g U}$  and placed into each pot. According to the experimental treatments, corresponding amounts of inhibitor(s) were mixed with the soil before flooding. Application rates of HQ and DCD were 0.3% and 5% of the applied U (w/w), respectively (Zhao et al. 1993; Xu et al. 2000a). Seven 20-day-old healthy rice seedlings were transplanted into the pot after 1 day of flooding. The experimental design contained four treatments with nine replicates per treatment. These treatments included U, U+HQ, U+DCD and U+HQ+DCD. During the entire experimental period a floodwater layer on the surface of the soil was 2 cm deep.

### Sampling and analysis

Within an 80-day period of rice cultivation, the pots were, at regular intervals, sealed air-tight with a PVC lid (height×diameter=85 cm×30 cm) (Xu et al. 2000a) from 10:00 to 12:00 am o'clock. During this period, gas samples were taken using 10-ml vacutainers. Concentrations of  $\text{CH}_4$  and  $\text{N}_2\text{O}$  in the gas samples were measured using a Chrompack 437 A with an ECD and a chrompack cp 9000 with a FID, respectively (De Groot et al. 1994; Boeckx et al. 1996). The emissions of  $\text{CH}_4$  and  $\text{N}_2\text{O}$  from pots after cutting plants were also measured on days 20, 60 and 80 after fertilization, respectively, corresponding to early tiller, stem elongation and booting stages of rice plants. Fresh weight of the above-ground biomass of rice was recorded, and  $\text{NO}_3^-$ -N content of the fresh rice plants was measured according to Singh (1988).  $\text{NH}_4^+$ -N and ( $\text{NO}_3^-$ + $\text{NO}_2^-$ )-N concentrations in the standing floodwater were measured by the MgO-Devarda's alloy procedure (Keeney and Nelson 1982).

### Calculation and statistical analysis

Gas emission was determined by collecting four headspace samples at 30-min intervals, during a 1.5-h period of closure. Results showed that the increase in the headspace gas concentration could

be assumed to be time-linear. The rates of gas emissions were derived from linear regressions of concentration changes over time in the PVC cover, by the methods of Granberg et al. (1997). Only measurement sets with a  $R^2$  value  $>0.90$  were accepted. The  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions were calculated as follows:  $F=V/W \times dC/dt$ , where  $F$  represents the  $\text{N}_2\text{O}$  emission rate ( $\mu\text{g N}_2\text{O-N kg}^{-1}$  air-dried soil  $\text{h}^{-1}$ ) or the  $\text{CH}_4$  emission rate ( $\mu\text{g CH}_4 \text{ kg}^{-1}$  air-dried soil  $\text{h}^{-1}$ );  $W$  is the weight of air-dried soil in each pot (kg);  $V$  is the available headspace volume (ml) and  $dC/dt$  is the increase in the  $\text{N}_2\text{O}$  or  $\text{CH}_4$  concentration during  $dt$  ( $\mu\text{g N}_2\text{O-N}$  or  $\text{CH}_4 \text{ ml}^{-1} \text{ h}^{-1}$ ).

All samplings were replicated 3 times. Total emissions of  $\text{CH}_4$  and  $\text{N}_2\text{O}$  during the study period were calculated by integrating the emissions on sampling days and cumulative emissions in between the sampling days. Cumulative emissions were quantified by multiplication of the average emissions of 2 successive sampling days by the number of non-sampling days in between. The mean values and SEs were calculated. Significant differences between means were analysed using SPSS (version 9.0), with a confidence interval of 95%. Regression analysis was used to fit the independent variables in the model, which related the contribution of the  $\text{NO}_3^-$ -N content of fresh rice plants to the  $\text{N}_2\text{O}$  emission.

## Results and discussion

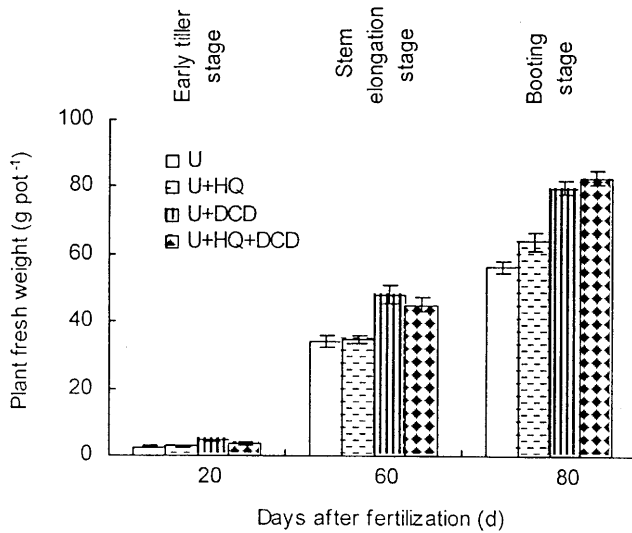
### Growth of rice plants

Fresh weight of the above-ground biomass of rice plants was much higher in the U+DCD and U+HQ+DCD treatments than in the other treatments throughout the experimental period (Fig. 1). However, no significant difference was found between the U+DCD and U+HQ+DCD treatments. The influence of HQ on U hydrolysis is of short duration under waterlogged conditions (Zhao et al. 1993). For this reason, it was assumed that the positive effect of the U+HQ+DCD treatment on rice growth was mainly due to DCD.

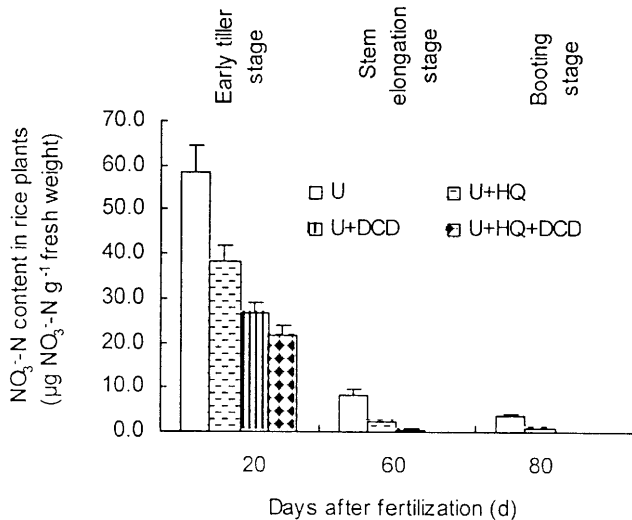
### $\text{NO}_3^-$ -N content of fresh rice plants

For the treatments with inhibitor(s), especially with DCD and with DCD+HQ, the content of  $\text{NO}_3^-$ -N in the plant tops of fresh rice was much lower than that in the U treatment (Fig. 2). This result confirmed those by Grylls (1988), who reported that application of nitrification inhibitors together with  $\text{NH}_4^+$ -based fertilizers effectively reduced  $\text{NO}_3^-$ -N content in lettuces. Kallio et al. (1982) also showed that the nitrification inhibitor, N-serve, could reduce the  $\text{NO}_3^-$ -N content in the roots and leaves of red beet when fertilized with U or  $\text{NH}_4\text{NO}_3$ . As the rice plants grew, the  $\text{NO}_3^-$ -N content of the fresh plants decreased significantly (Fig. 2), suggesting that an accumulation of  $\text{NO}_3^-$ -N in rice plants occurred only during the early growth period.

Application of DCD and of DCD+HQ can effectively enhance the concentration ratio of  $\text{NH}_4^+$ -N to ( $\text{NO}_3^-$ + $\text{NO}_2^-$ )-N in U-treated soil, and regulate the N status in plants and grains (Xu et al. 2000b, 2001). These effects can, to some extent, explain the small  $\text{NO}_3^-$ -N content in the DCD-treated rice plants at the early growth stage.



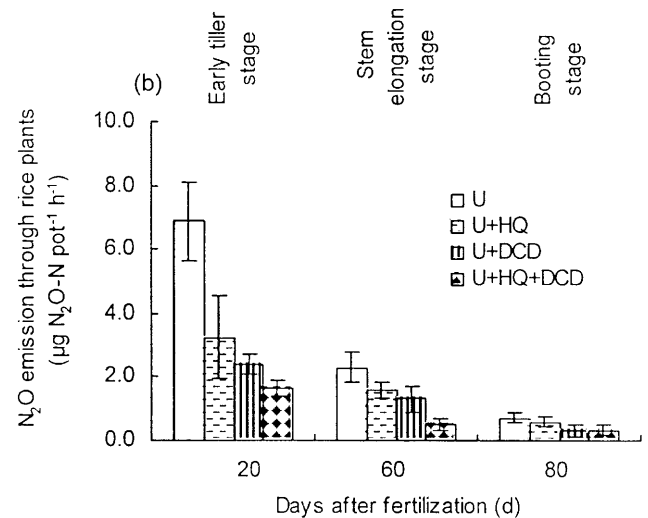
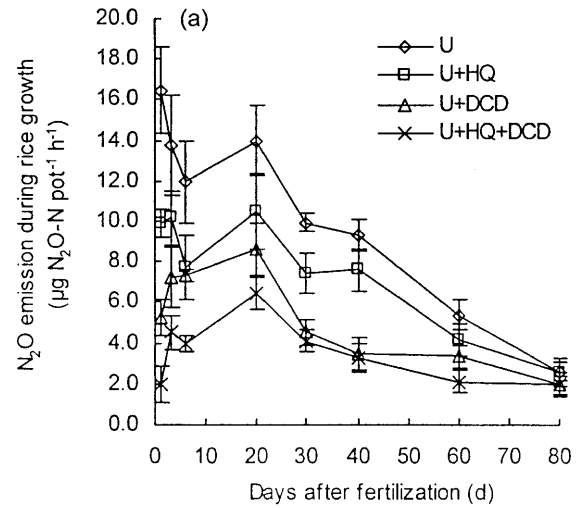
**Fig. 1** Fresh weight of the above-ground parts of rice plants during different growth stages; mean $\pm$ SE ( $n=3$ ). *U* Urea, *U+HQ* U+hydroquinone, *U+DCD* U+dicyandiamide



**Fig. 2**  $\text{NO}_3^-$  content in the above-ground parts of rice plants during different growth stages; mean $\pm$ SE ( $n=3$ ). For abbreviations, see Fig. 1

### $\text{N}_2\text{O}$ emissions during rice growth and through rice plants

$\text{N}_2\text{O}$  emissions during rice growth and through rice plants are shown in Fig. 3a, b. The  $\text{N}_2\text{O}$  emission during rice growth in any treatment decreased with time (Fig. 3a). All treatments with inhibitor(s), especially with HQ+DCD, presented smaller  $\text{N}_2\text{O}$  emissions than the *U* treatment during the entire experimental period, thus resulting in a substantial reduction of total  $\text{N}_2\text{O}$  emission during rice growth (Table 1). A combined application of DCD and HQ gave the smallest  $\text{N}_2\text{O}$  emission during rice growth within a 20-day period after fertilization (Fig. 3a). The total  $\text{N}_2\text{O}$  emission in the *U+HQ+DCD* treatment was about one-third of that in the



**Fig. 3** **a** Dynamics of  $\text{N}_2\text{O}$  emission during rice growth; **b** dynamics of  $\text{N}_2\text{O}$  emission through rice plants; mean $\pm$ SE ( $n=3$ ).  $\text{N}_2\text{O}$  emission through rice plants= $\text{N}_2\text{O}$  emission from pots- $\text{N}_2\text{O}$  emission from pots after cutting plants. For abbreviations, see Fig. 1

**Table 1** Total amount of the  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions during rice growth. Values are the means and SEs (in parentheses) of three replicates. Within each column, means followed by different letters are significantly different at  $P<0.05$ . *U* Urea, *U+HQ* U+hydroquinone, *U+DCD* U+dicyandiamide

Treatments	$\text{CH}_4$ emission (mg $\text{CH}_4$ pot $^{-1}$ )	$\text{N}_2\text{O}$ emission (mg $\text{N}_2\text{O-N}$ pot $^{-1}$ )
<i>U</i>	190.3 (15.9) c	17.3 (2.1) b
<i>U+HQ</i>	133.0 (10.7) b	13.2 (1.1) b
<i>U+DCD</i>	89.2 (7.9) a	9.1 (0.8) a
<i>U+HQ+DCD</i>	79.5 (10.1) a	6.5 (0.6) a

*U* treatment (Table 1). The synergistic effect of the two inhibitors on the  $\text{N}_2\text{O}$  emission in wetland rice cultivation occurred immediately after application of *U*. A similar synergistic effect was also observed under well-drained conditions (Xu et al. 2000b). Inhibition of the

$\text{N}_2\text{O}$  emission during rice growth by addition of DCD was in accordance with that shown by Majumdar et al. (2000) and by Kumar et al. (2000).

In flooded rice fields oxic-anoxic interfaces are found at the floodwater-soil interface and in the rhizosphere of rice plants, where  $\text{N}_2\text{O}$  can be produced after application of U, via nitrification of  $\text{NH}_4^+$  and the following denitrification. Addition of DCD and of DCD+HQ can effectively inhibit the oxidation of  $\text{NH}_4^+$ -N produced in the flooded surface soil and in the floodwater immediately following the hydrolysis of U, with a consequent reduction in the  $(\text{NO}_3^- + \text{NO}_2^-)$ -N concentration (data not shown). The inhibition of nitrification may reduce  $\text{N}_2\text{O}$  emission immediately after fertilization.

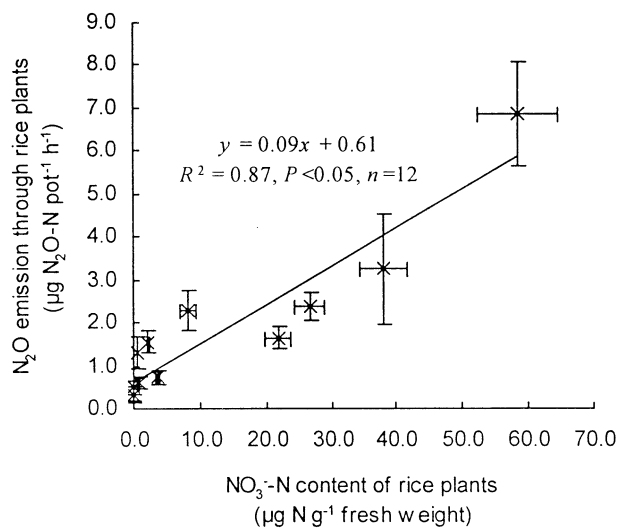
In the presence of DCD and of DCD+HQ, the fresh weight of rice plants was much higher than in the absence of inhibitor(s) (Fig. 1), indicating probably that the amount of available soil N absorbed by plants was higher during the rice growing period. This can extensively reduce gaseous N losses (e.g.  $\text{N}_2\text{O}$ ) from rice fields amended with U. Williams et al. (1992) discussed that soil NO or  $\text{N}_2\text{O}$  emission rates depended on the soil N content and water availability.

$\text{N}_2\text{O}$  emissions through rice plants are calculated as the differences between emissions from pots and those from pots after cutting plants. On days 20 and 60 after fertilization, the rice plants entered their early tiller and stem elongation stages, respectively. During these periods, the treatments with inhibitor(s), especially with HQ+DCD resulted in a small  $\text{N}_2\text{O}$  emission through rice plants (Fig. 3b). No significant differences in  $\text{N}_2\text{O}$  emissions among all treatments were observed on day 80.

As rice plants grew, the  $\text{N}_2\text{O}$  emission through rice plants was significantly reduced in all treatments (Fig. 3b);  $\text{N}_2\text{O}$  emission was always lower in the presence than in the absence of inhibitor(s) (Fig. 3a). These changes paralleled those in the  $\text{NO}_3^-$ -N content of fresh rice plants (Fig. 2). Regression analysis showed a significant and positive correlation between the  $\text{N}_2\text{O}$  emission through rice plants and the  $\text{NO}_3^-$ -N content of fresh rice plants (Fig. 4). Such a relation also occurred in young upland plants (Chen et al. 1997). By comparing  $\text{N}_2\text{O}$  emission in chambers with and without rice plants, Mosier et al. (1990) demonstrated that young rice plants facilitated the emission of  $\text{N}_2\text{O}$ . At low light density, the  $\text{NO}_3^-$ -N content of fresh plant organs can be reduced probably due to  $\text{N}_2\text{O}$  and NO emissions (Klepper 1990; Rockel et al. 1996). Chen et al. (1990) also reported that plants might emit  $\text{N}_2\text{O}$  when stressed. Accordingly, under the experimental conditions,  $\text{NO}_3^-$ -N of fresh rice plants can substantially contribute to the plant-mediated  $\text{N}_2\text{O}$  emission.

#### $\text{CH}_4$ emissions during rice growth and through rice plants

Figure 5a shows the  $\text{CH}_4$  emission during rice growth. In contrast to the  $\text{N}_2\text{O}$  emission (Fig. 3a), the  $\text{CH}_4$  emission increased with time in all treatments, and reached its

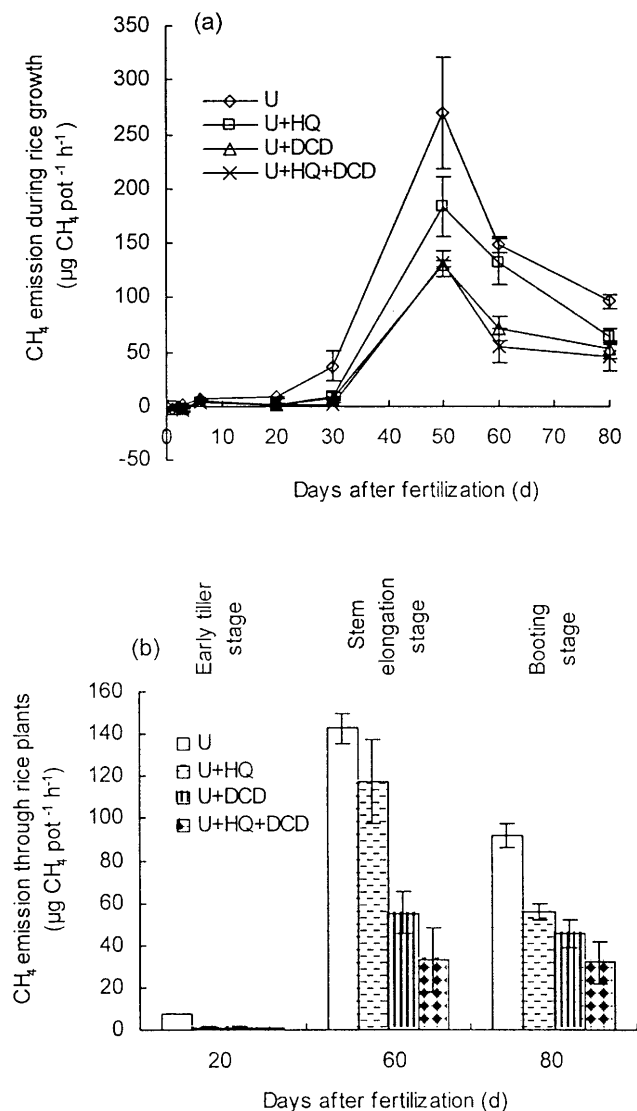


**Fig. 4** Relation between  $\text{N}_2\text{O}$  emission through rice plants and  $\text{NO}_3^-$ -N content of rice plants at three sampling periods. The solid line is the linear regression of  $\text{N}_2\text{O}$  emission through rice plants,  $y$ , against  $\text{NO}_3^-$ -N content of rice plants,  $x$ . The horizontal and vertical bars represent SEs of the  $\text{NO}_3^-$ -N content and the  $\text{N}_2\text{O}$  emission ( $n=3$ ), respectively.  $\text{N}_2\text{O}$  emission through rice plants =  $\text{N}_2\text{O}$  emission from pots -  $\text{N}_2\text{O}$  emission from pots after cutting plants

maximum value on day 50 after fertilization (Fig. 5a), when the rice plants entered their vigorous vegetation growth stage. This peak of  $\text{CH}_4$  emission probably resulted from some substances released by rice roots (Kimura et al. 1991; Thomas et al. 1995; Wassmann and Aulakh 2000).

All treatments with inhibitors, especially with DCD and with DCD+HQ, could substantially reduce  $\text{CH}_4$  emission during the entire experimental period, and consequently the total  $\text{CH}_4$  emission (Table 1). Lindau et al. (1993) found that  $\text{CH}_4$  emissions from rice fields decreased by 35% and 14% after application of encapsulated  $\text{CaC}_2$  and DCD, respectively. Rath et al. (1999) suggested that the use of nitrification inhibitors such as Nimin could be considered as a suitable option for mitigating  $\text{CH}_4$  emissions from rice fields.

$\text{CH}_4$  emissions from pots after cutting plants were much higher in the presence than in the absence of inhibitor(s) (Xu et al. 2000a). This is apparently contrary to the  $\text{CH}_4$  emissions during rice growth (Fig. 5a). Addition of DCD could significantly improve rice growth (Fig. 1), and the total amount of root exudate C was closely related to the root dry weight ( $r^2=0.92$ ) and to the above-ground biomass ( $r^2=0.95$ ) (Wassmann and Aulakh 2000). Aulakh et al. (2001) showed that in the anoxic rice soils, the proportion of root exudate C converted to  $\text{CH}_4$  ranged from 61% to 83%. These results indicated that the  $\text{CH}_4$  production in the rhizosphere of rice plants was higher in the presence than in the absence of DCD. Since the  $\text{CH}_4$  efflux is the net result of production and oxidation of  $\text{CH}_4$ , the reduction of  $\text{CH}_4$  emission in the wetland rice cultivation probably resulted from the inhibition of the conversion of root exudates to  $\text{CH}_4$ . This phe-



**Fig. 5** **a** Dynamics of  $\text{CH}_4$  emission during rice growth; **b** dynamics of  $\text{CH}_4$  emission through rice plants; mean $\pm$ SE ( $n=3$ ).  $\text{CH}_4$  emission through rice plants= $\text{CH}_4$  emission from pots- $\text{CH}_4$  emission from pots after cutting plants. For abbreviations, see Fig. 1

nomenon was possibly related to the variation of the Eh potential in the root zone. According to our previous rhizosphere experiments, the Eh value in the rhizosphere of rice plants was significantly higher in the presence than in the absence of DCD, particularly when the rice plants entered their vigorous growth stage (data not shown). The quantity and quality of root exudates can be affected by many factors such as N status and presence of toxic substances in the rhizosphere (Aulakh et al. 2001). Hence, it is necessary to study further the variations in the root exudates and in the aerenchyma of the roots after application of U and inhibitor(s).

$\text{CH}_4$  emission through rice plants can be calculated as the difference between emissions from pots and those from pots after cutting plants (Fig. 5b). At the early tiller stage, a small  $\text{CH}_4$  emission through rice plants occurred

in each pot. However, when the rice plants entered their vigorous growth stage (e.g. stem elongation stage), substantial emission of  $\text{CH}_4$  occurred through rice plants (Fig. 5b), probably as the result of the development of aerenchyma tissue (Wassmann and Aulakh 2000). The pattern of the plant-mediated  $\text{CH}_4$  emission during rice growth was different from that of the plant-mediated  $\text{N}_2\text{O}$  emission (Fig. 5a). Indeed, these emissions were not significantly correlated ( $r=-0.28$ ,  $P>0.05$ ).

In the presence of DCD, 60.4–86.7% of  $\text{CH}_4$  emission occurred through rice plants, and was smaller than the percentage (94.2–96.3%) of the U treatment (Fig. 4a, b). Hence, the combined application of U and DCD caused a small  $\text{CH}_4$  emission through rice plants.

In conclusion, in the presence of floodwater, the  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions through rice plants presented an apparent difference during rice growth. As rice plants grew, the  $\text{N}_2\text{O}$  emission through rice plants decreased significantly. However, a substantial  $\text{CH}_4$  emission through rice plants occurred at their vigorous growth stage. Both emissions from the flooded soil were related to the conduit of rice plants, and the  $\text{NO}_3^-$ -N content of fresh rice plants could substantially contribute to the plant-mediated  $\text{N}_2\text{O}$  emission. DCD and DCD+HQ together with U appeared to be effective tools in reducing emissions of  $\text{N}_2\text{O}$  and  $\text{CH}_4$  during rice growth. This paper provides interesting results on how to reduce  $\text{CH}_4$  and  $\text{N}_2\text{O}$  emissions in wetland rice cultivation, but further field experiments will be needed in order to attain the objective of an effective method for mitigating both  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions.

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