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Effect of nitrification inhibitor DMPP on nitrogen leaching, nitrifying organisms, and enzyme activities in a rice-oilseed rape cropping system

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Abstract

DMPP (3,4-dimethylpyrazole phosphate) has been used to reduce nitrogen (N) loss from leaching or denitrification and to improve N supply in agricultural land. However, its impact on soil nitrifying organisms and enzyme activities involved in N cycling is largely unknown. Therefore, an on-farm experiment, for two years, has been conducted, to elucidate the effects of DMPP on mineral N (NH_4^+-N and NO_3^--N) leaching, nitrifying organisms, and denitrifying enzymes in a rice-oilseed rape cropping system. Three treatments including urea alone (UA), urea + 1% DMPP (DP), and no fertilizer (CK), have been carried out. The results showed that DP enhanced the mean NH_4^+-N concentrations by 19.1%–24.3%, but reduced the mean NO_3^--N concentrations by 44.9%–56.6% in the leachate, under a two-year rice-rape rotation, compared to the UA treatment. The population of ammonia oxidizing bacteria, the activity of nitrate reductase, and nitrite reductase in the DP treatment decreased about 24.5%–30.9%, 14.9%–43.5%, and 14.7%–31.6%, respectively, as compared to the UA treatment. However, nitrite oxidizing bacteria and hydroxylamine reductase remained almost unaffected by DMPP. It is proposed that DMPP has the potential to either reduce NO_3^--N leaching by inhibiting ammonia oxidization or N losses from denitrification, which is in favor of the N conversations in the rice-oilseed rape cropping system.

Key words: DMPP (3,4-dimethylpyrazole phosphate); nitrification inhibitor; nitrifying organisms; nitrogen leaching; soil enzymes

Introduction

Nitrate (NO_3^-) leaching from agricultural lands and its threat against water quality is one of the important global environmental issues (Di and Cameron, 2002; Babiker *et al*., 2004; Jalali, 2005; Liang *et al*., 2007). High NO_3^--N levels in surface waters result in excessive growth of aquatic plants and cause eutrophication, and high NO_3^--N content in groundwater and drinking water does harm to humans and livestock (Zhu *et al*., 2003; Basso and Ritchie, 2005).

A potential method to reduce NO_3^--N leaching into groundwater is the retardation of biological oxidation of NH_4^+-N to NO_3^--N (Bhupinderpal-Singh *et al*., 1993; Serna *et al*., 2000). One of the proposals currently being considered for inhibiting nitrification is the use of nitrification inhibitors (NIs) (Weiske *et al*., 2001; Maeda *et al*., 2003; Di and Cameron, 2005). DMPP (3,4-dimethylpyrazole phosphate) is a new NI which is very efficient in inhibiting soil nitrification and has no toxicological or ecotoxicological side-effects (Zerulla *et al*., 2001). It can decrease NO_3^--N concentrations in the leachate and reduce the potential accumulative NO_3^--N loss in agricultural land (Weiske *et al*., 2001; Yu *et al*., 2007).

Nitrification is performed by ammonia-oxidizing bacteria (AOB) converting NH_4^+ to NO_2^- and then by nitrite-oxidizing bacteria (NOB) converting the latter to NO_3^- (Abbassi and Adams, 1998). Present researches have concluded that NIs could delay ammonia oxidization through depressing AOB, which will have no effects on NOB population in the soil (Zerulla *et al*., 2001; Irigoyen *et al*., 2003). In addition to nitrification, which produces NO_3^-, the concentrations of NO_3^- also depend on the level of denitrification, that is, the process that consumes NO_3^- in the soil. Nitrate reductase (NaR), nitrite reductase (NiR), and hydroxylamine reductase (HyR) are the major enzymes involved in denitrification (Guan, 1986; Ferguson, 1998). Under flooded conditions, NO_3^- can be reduced to gaseous N oxides through denitrification. It has been reported that NIs can reduce N loss through denitrification (Weiske *et al*., 2001; Zhu *et al*., 2003). However, the information about the relationship between DMPP and nitrifying organisms and denitrifying enzyme activities is far from clear.

The purpose of this experiment was to find out the effects of DMPP on mineral N availability, population of nitrifying organisms, and enzyme activities involved in N cycling under the rice-oilseed rape (referred to simply as

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rape in the following text) cropping system.

1 Materials and methods

1.1 Experimental site conditions

The field experiment is located at the Shuangqiao Farm of Jiaxing City (120°40′ E, 30°50′ N), Zhejiang Province, China, which belongs to the Taihu Lake region. The tested soil is classified as a clayed blue-purple paddy soil (Mollic Endoaquepts). The main characteristics of this soil in the top 10 cm depth are: pH 6.78, total nitrogen (TN) 2.75 g/kg, total phosphorous (TP) 0.47 g/kg, organic matter (OM) 35.01 g/kg, cation exchange capacity (CEC) 8.10 cmol(+)/kg, and bulk density 1.23 g/cm^3.

This field experiment has been carried out in 4 m × 5 m plots for two years with a crop sequence of rice-rape-rice-rape (July to November for rice and November to May in the following year for rape). Each plot was installed with an independent irrigation branch controlled by a hydrant, and water-proof nylon was inserted into the soil down to 40 cm depth at three edges of the plots to prevent water movement across the adjacent plots.

1.2 Fertilizer applications

Taking into account the conventional N application rate for single rice (*Oryza sativa* L.) as 180 kgN/hm^2, the amount of N to rice applied through urea (UA) or urea + DMPP (DP) was 180 kgN/hm^2, and DMPP was added at 1% of the N applied (w/w). Before transplanting of rice in puddled soil, 60% of the total urea or urea combined with DMPP and 40 kg P_2O_5/hm^2 and 150 kg KCl/hm^2 were added. On day 10 and 30 after transplanting, 20% of urea or urea plus DMPP were applied as the first and second top dressings, respectively. All the plots were regularly irrigated to a depth of 80 mm, when water in plots was less than 5 mm until after day 90, except for the soil drying from 22 to 27 d and from 65 to 68 d.

After the rice harvest in November, the soils in the plots were dried until rape (*Brassica Napus*) was sown at the end of November (both in 2004 and 2005). A small trowel was used to loosen the soil, break the aggregates, and mix the litters and stubbles of previous rice in the plots. For fertilizer application, 60% of the total urea amount (the same as the amount under rice growth) was applied before the rape sowing, either with or without DMPP. The first and second top dressings were applied on day 40 and 90 after sowing with 20% and 20% of total fertilizer amount, respectively. Other nutrients (phosphorus and potassium as described for rice) were applied uniformly along with urea or urea plus DMPP at the time of sowing.

The whole experiment was conducted following a completely randomized design with four replications for each treatment (CK, UA, and DP).

1.3 Sampling and analysis

The wick lysimeter, which has a 40-cm by 40-cm top surface fixed with an inversely bounded frame, was installed 60 cm below the soil surface of the plots. In the lysimeter, the upper part includes three 2-cm layers, namely, quartz sand layer, gravel layer, and pebble layer. The left bottom part is a buffer space for the leachate discharged by a tube. The other tube is laterally inserted into the pebble layer for connecting to the air outside.

Leachate samples were elicited from the tubes and collected at an interval of 10 d in the first 60 d and at an interval of 20 d from day 60 to day 120, after rice transplanting or rape sowing, in the two years of experimentation. All the samples were stored in a refrigerator (4°C) before analysis. NO_3^--N and NH_4^+-N concentrations in the leachate were determined by using a continuous-flow analyzer (AA3, BRAN+LUEBBE, Germany).

Soil samples (0–20 cm) were collected on days 10, 20, 40, 80, and 120 after rice transplanting and on days 10, 30, 50, 100, and 120 after rape sowing. The sampling schedule allowed the authors to investigate the variation in nitrifying bacteria and denitrifying enzymes at day 10 after each fertilizer application. NO_3^--N and NH_4^+-N concentrations in soil were determined by using the continuous-flow analyzer (AA3, BRAN+LUEBBE, Germany) after extraction, with 2 mol/L KCl.

The most probable number (MPN) of AOB and NOB was enumerated using the media of Stephenson with six-fold dilutions from 10^{-2} to 10^{-7} and incubation at 28°C for 14 d. The presence of AOB and NOB was revealed by the presence of NO_2^--N and NO_3^--N and by using the Griess's reagent, respectively (Xu and Zheng, 1986). The MPN of nitrifying microorganisms was estimated by using the Cochran's method (Cochran, 1950).

For the assay of NaR, NiR, and HyR activities, 1 ml of 1% KNO_3 solution, 0.5% $NaNO_2$ solution, and 0.5% NH_2OH was added to 1.0 g soil, respectively. The mixture was incubated at 30°C for 24 h and the amount of reduced NO_3^--N, NO_2^--N, and NH_2OH was estimated to represent the activities of NaR, NiR, and HyR, respectively (Guan, 1986).

1.4 Statistic analysis

One-way ANOVA and Duncan's test for comparison of means were performed using SPSS 11 window version. Unless otherwise stated, the level of significance referred to in the results was $P < 0.05$.

2 Results

2.1 NO_3^--N and NH_4^+-N concentrations in leachate

The application of urea or urea + DMPP increased NO_3^--N concentrations in the leachate compared to the CK treatment, and significantly lower ($P < 0.05$) concentrations were found in DP treatments under rice-rape crops in two years (Fig.1). On days 10, 20, and 40 (10 d after urea application), under rice growth, NO_3^--N concentrations in UA plots reached high values of 4.1, 4.3, and 3.7 mg/L in 2004, and 4.3, 4.4, and 4.2 mg/L in 2005, respectively. The respective figures achieved in urea + DMPP-treated plots were 1.9, 2.1, and 1.4 mg/L in 2004, and 2.0, 2.2,

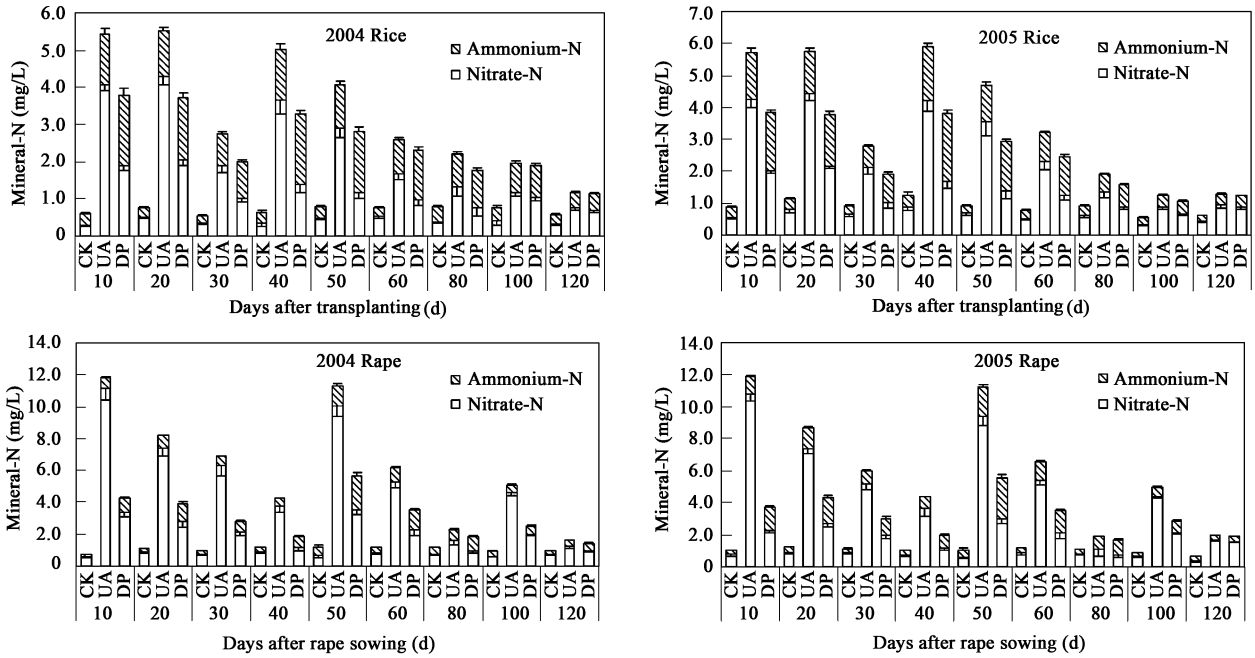

Fig. 1 Mineral-N (NH_4^+-N and NO_3^--N) concentrations in leachate during rice and oilseed rape growth. Bars above the gray portions and down the white portions indicate standard errors of means (n = 4). CK: control; UA: urea treatment; DP: urea+DMPP.

and 1.7 mg/L in 2005, respectively. DMPP reduced the mean NO_3^--N concentrations in the leachate by 44.9% and 47.3% through a four-month investigation, in the years 2004 and 2005, respectively, compared to the plots with urea alone. Under rape growth, NO_3^--N concentrations in the leachate also were much lower in DP plots than UA ones. Ten days after each application (i.e., day 10, 50, 100), the concentrations of NO_3^--N in UA plots were 11.2, 10.0, and 4.6 mg/L in 2004 and were 10.8, 9.4, and 4.4 mg/L in 2005, respectively. The corresponding values in DMPP-treated plots were 3.4, 3.5, and 2.0 mg/L in 2004 and were 2.3, 3.0, and 2.1 mg/L in 2005, respectively. The average concentrations of NO_3^--N during the experiment were 56.6% and 55.1% lower in DP plots compared with UA plots in years 2004 and 2005, respectively.

NH_4^+-N concentrations in the leachate also increased with urea or urea + DMPP application and were slightly higher ($P < 0.05$) in the DP treatment than in the UA treatment (Fig.1). Under rice growth, NH_4^+-N concentrations were 0.9–1.4 mg/L and 1.0–1.9 mg/L in UA and DP treatments in 2004, respectively, and were 0.7–1.7 mg/L and 0.9–2.1 mg/L in 2005, respectively. The average concentrations of NH_4^+-N were 20.5% and 19.1% higher in DP plots than in the UA ones in 2004 and 2005, respectively. Similar variations of NH_4^+-N concentrations in leachate were observed during rape growth. The concentrations of NH_4^+-N were lower than 2.0 mg/L in UA plots and DMPP increased NH_4^+-N concentrations by 24.3% and 22.0% in 2004 and 2005, respectively.

For total mineral-N (NO_3^--N and NH_4^+-N), DMPP reduced the concentrations by 0.9, 3.3, 1.2, and 3.2 mg/L during the four-month investigation in 2004 rice, 2004 rape, 2005 rice, and 2005 rape, respectively (Fig.1). NH_4^+-N concentrations were much lower than NO_3^--N, implying NO_3^--N was the predominant form in the leachate.

2.2 AOB, NOB population, and NO_3^--N, NH_4^+-N concentrations in soil

DMPP significantly reduced the MPN values of AOB ($P < 0.05$), although AOB population increased obviously in UA and DP treatments after each application of urea or urea plus DMPP (Fig.2). In rice soil, DMPP inhibited

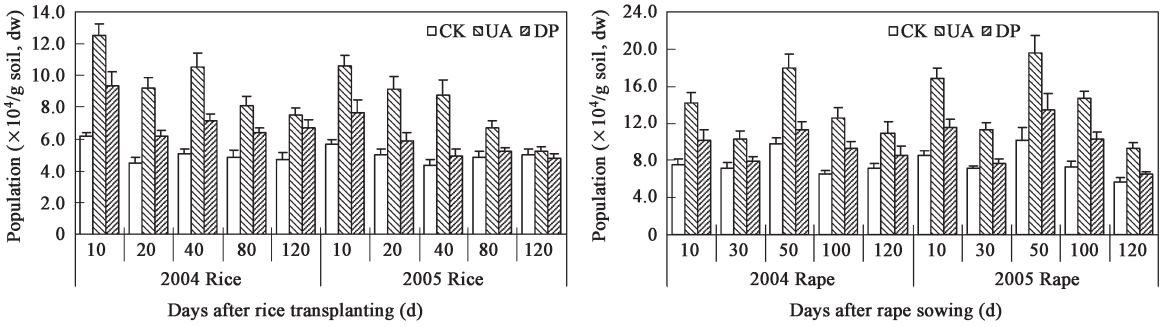

Fig. 2 MPN of ammonia oxidizing bacteria (AOB) in soils during rice and oilseed rape growth. Bars above the columns represent the standard errors of means (n = 4). CK, UA, and DP are the same meaning as that in Fig.1.

the mean AOB population by 24.5% and 27.6% compared to UA plots in 2004 and 2005, respectively. Comparison of means under rice growth revealed obviously higher values in the UA treatment ((7.5–12.5) × 10^4/g dry soil in 2004, (5.2–10.6) × 10^4/g dry soil in 2005) than in the DP treatments ((6.2–9.4) × 10^4/g dry soil in 2004, (4.8–7.7) × 10^4/g dry soil in 2005). The lowest MPN values of AOB at given times ((4.5–6.2) × 10^4/g dry soil in 2004, (4.3–5.7) × 10^4/g dry soil in 2005) were recorded in the CK treatment. In soils under rape crop, the AOB population varied from (5.7–19.6) × 10^4/g dry soil, and much higher MPN values of AOB ((9.3–19.6) × 10^4/g dry soil) were observed in the plot with urea only. The application of DMPP reduced the mean AOB population by 27.5% and 30.9% in 2004 and 2005, respectively, as compared to UA plots.

The density of NOB in soil under the rice crop was slightly higher ($P < 0.05$) in UA and DP treatments after each application. However, no significant differences among UA and DP treatments were recorded in a two-year study of rape soil (Fig.3).

The application of urea or urea + DMPP increased NO_3^--N concentrations obviously, and significantly lower ($P < 0.05$) concentrations were found in DP treatment compared to UA treatment (Tables 1 and 2). Ten days after three applications of urea, the combined application of DMPP reduced soil NO_3^--N concentrations by 26.2%–39.4% under rice growth, and by 40.4%–59.0% under rape growth, compared to UA plots.

Soil NH_4^+-N concentrations also increased with urea or urea + DMPP application and were slightly higher ($P < 0.05$) in DP plots than UA ones (Tables 1 and 2). Ten days after each application, NH_4^+-N concentrations in DP plots were 12.4%–24.1% higher in rice soil, and 19.1%–27.5% higher in rape soil, compared to the plots with only urea. DMPP decreased soil NO_3^--N concentrations, but increased NH_4^+-N concentrations through inhibiting AOB in the soil.

2.3 Denitrifying enzymatic activities

The application of DMPP effectively ($P < 0.05$) reduced NaR activity under rice and rape crops (Fig.4). In rice soil, NaR activity increased significantly after each application of urea or urea + DMPP. The lowest values in a range of 30.6–51.2 mg NO_3^--N/(g·d) and 32.1–68.8 mg NO_3^--N/(g·d) were observed in the CK in 2004 and 2005, respectively, and the maximum at given times were found in the UA, in a range of 67.8–255.8 mg NO_3^--N/(g·d) and a range of 59.8–219.6 mg NO_3^--N/(g·d) in 2004 and 2005, respectively. 43.5% and 42.5% of NaR activity were inhibited by DMPP application in 2004 and 2005, respectively. Similarly, in rape soil, urea application stimulated NaR activity and the highest NaR activity was as much as 47.6 and 55.6 mg NO_3^--N/(g·d) in the UA on day 10 after basal fertilization in 2004 and 2005, respectively. About 14.9%–33.0% and 17.5%–30.0% of NaR activity were inhibited by DMPP in 2004 and 2005, respectively. The lowest NaR

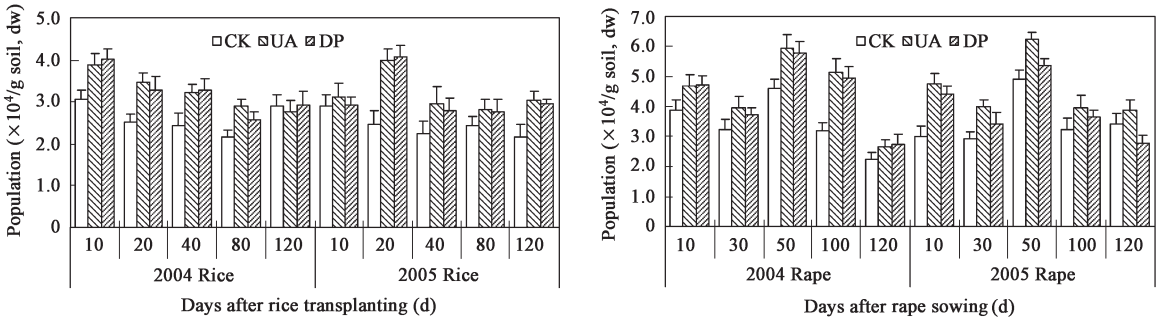

Fig. 3 MPN of nitrite oxidizing bacteria (NOB) in soils during rice and oilseed rape growth. Bars above the columns represent the standard errors of means ($n = 4$). CA, UA, and DP are the same meaning as that in Fig.1.

Table 1 Effect of DMPP on mineral-N concentrations in soils during rice growth

Treatment		Days after rice transplanting (d)				
		10	20	40	80	120
NO_3^--N (mg/L)						
2004	CK	7.3±0.8c	5.6±0.5c	8.2±0.8c	6.6±0.4b	4.9±0.4a
	UA	16.5±1.6a	18.2±1.4a	15.7±1.3a	9.5±0.7a	5.2±0.3a
	DP	10.0±0.8b	11.4±1.8b	10.8±1.1b	7.4±0.6b	4.5±0.4a
2005	CK	10.4±1.1c	8.6±1.0c	11.2±1.1c	9.7±0.4b	7.9±0.4a
	UA	18.4±1.4a	21.2±1.5a	18.7±1.3a	12.5±0.4a	6.5±0.5b
	DP	13.0±1.5b	14.4±1.5b	13.8±0.9b	10.4±0.6b	7.2±0.4ab
NH_4^+-N (mg/L)						
2004	CK	33.0±1.5c	46.5±2.0c	25.5±1.3c	27.6±1.5ab	22.3±1.2b
	UA	65.6±2.3b	76.6±2.1b	47.3±2.2b	29.3±1.3a	24.8±1.6ab
	DP	81.7±2.6a	88.5±2.7a	62.3±2.5a	24.6±1.1b	25.7±1.1a
2005	CK	43.8±1.4c	55.7±2.3c	33.6±1.1c	37.5±1.3b	31.2±1.4ab
	UA	73.6±2.4b	86.3±2.9b	55.6±2.3b	39.7±1.4ab	29.7±1.5b
	DP	87.9±1.5a	98.5±2.7a	72.9±3.1a	41.3±1.2a	33.7±1.4a

Values ± symbol are standard errors of means of four replicates. Treatments comparing soil mineral-N concentrations in the same column at a given year with the same letter are not significantly different at $P < 0.05$.

Table 2 Effect of DMPP on mineral-N concentrations in soils during rape growth

Treatment		Days after rice transplanting (d)				
		10	30	50	100	120
NO_3^--N (mg/L)						
2004	CK	11.3±0.8c	12.1±0.6b	14.5±1.1c	13.9±1.0c	8.2±0.7b
	UA	56.4±2.2a	19.7±1.3a	41.1±2.4a	36.4±1.9a	10.6±1.2a
	DP	23.1±1.1b	13.2±0.7b	18.2±1.2b	19.5±1.2b	9.8±1.0ab
2005	CK	17.6±0.6c	15.6±0.8c	19.9±1.4c	20.6±1.1b	16.3±1.0a
	UA	59.9±3.4a	25.2±1.5a	48.8±2.5a	40.2±2.8a	17.5±1.6a
	DP	35.7±1.6b	17.8±0.9b	24.4±1.8b	22.8±2.2b	16.8±1.2a
NH_4^+-N (mg/L)						
2004	CK	7.5±0.5c	5.2±0.5c	6.3±0.5c	4.4±0.4c	5.1±0.4b
	UA	12.3±1.3b	7.6±0.8b	12.2±1.0b	10.3±0.7b	5.8±0.6ab
	DP	16.5±1.1a	9.5±0.6a	15.9±1.1a	14.2±1.1a	6.5±0.5a
2005	CK	8.5±0.7c	7.3±0.4c	7.9±0.7c	4.6±0.3c	5.2±0.4c
	UA	14.6±1.6b	9.3±0.7b	15.7±1.2b	9.6±0.6b	7.9±0.6ab
	DP	18.8±1.4a	12.0±0.9a	19.4±1.2a	12.9±0.6a	8.5±0.4a

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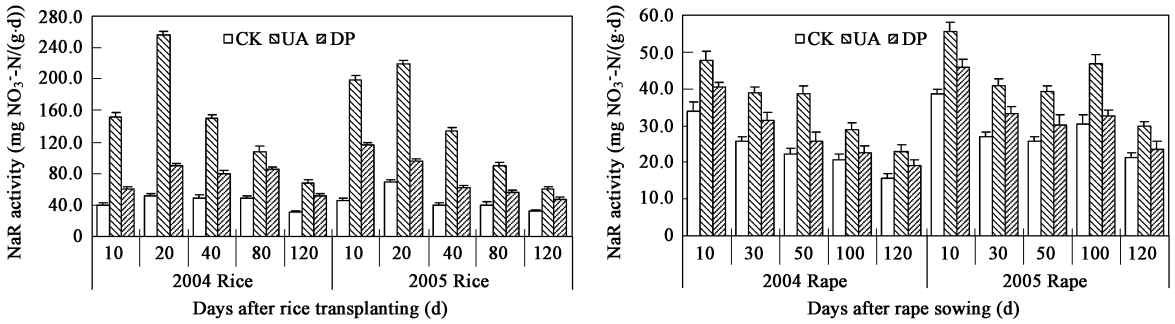

Fig. 4 NaR activity in soils during rice and oilseed rape growth. Bars above the columns represent the standard errors of means (n = 4).

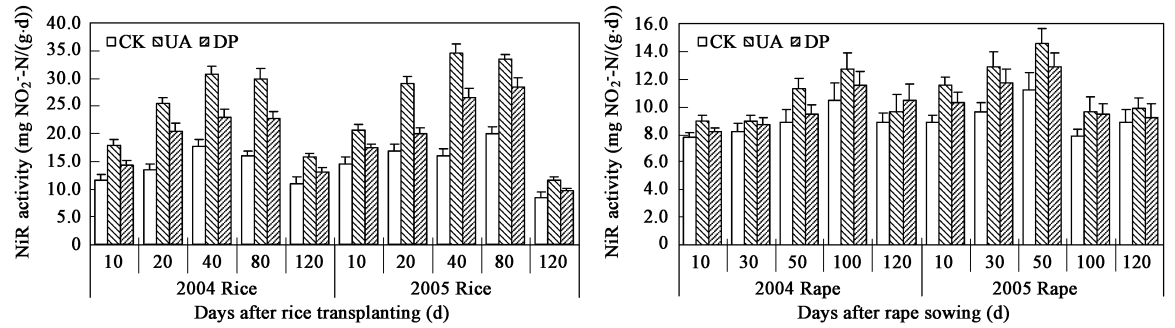

Fig. 5 NiR activity in soils during rice and oilseed rape growth. Bars above the columns represent the standard errors of means (n = 4).

activity was observed in unfertilized plots.

There was a similar trend of NiR activity variation with NaR activity under rice cropping (Fig.5). The highest values of NiR activity existed in the plots with urea alone, and 17.1%–25.6% and 14.7%–31.6% of NiR activities were inhibited by DMPP application in 2004 and 2005, respectively. However, there was no significant difference in NiR activity between DP and UA treatments under rape soil in the two-year investigation.

In case of HyR, there was no significant difference among three treatments under rice and rape soils in two years (data not shown).

3 Discussion

An application rate of 1.8 kg/hm^2 for DMPP (1% of urea amount) was sufficient under these field conditions to significantly inhibit nitrification, which resulted in lower NO_3^--N concentrations in the leachate from DP plots. NO_3^--N concentrations in leachate under rape growth were much higher than under rice soil, which was induced by stronger nitrification in the rape season compared to the flooded rice season. Even when NO_3^--N concentrations in the leachate were higher than 10 mg/L under rape growth, DMPP application decreased the concentrations to less than 3.6 mg/L, which largely reduced NO_3^--N harm to groundwater. NO_3^--N concentrations in the surface soil were also much lower in the DP plots compared to the UA plots. As one of the new NIs, DMPP reduced NO_3^--N concentrations significantly in clayey loam soil (Weiske *et al.*, 2001) and vegetable soil (Xu *et al.*, 2005) under field conditions, and reduced NO_3^--N concentrations in

the leachate with the same soil type as in soil columns (Yu *et al*., 2007). Besides, DMPP was effective at very low rates. An application of 0.5–1.5 kg/hm^2 (depending on the amount of applied N), was sufficient under field conditions to securely inhibit nitrification (Zerulla *et al*., 2001). Compared to other NIs, such as, DCD, less than one-tenth of the application rate was enough for DMPP to get a better inhibition effect (Zerulla *et al*., 2001). The benefit of DMPP in this experiment was also reflected in the yields of rice and rape (data not presented). Average rice grain yields were 7.36 and 7.52 mg/hm^2, and rape grain yields were 2.65 and 2.88 mg/hm^2 in UA plots in 2004 and 2005, respectively. DMPP application had a positive impact on yields, although it only increased 4.2%–4.7% and 6.6%–7.5% of rice and rape grain yields, respectively, compared to the plots with urea alone. Slightly higher NH_4^+-N contents in NI plots might have played an important role in enhancing N availability in soil (Patra *et al*., 2006), which promoted crop growth. For minimizing nitrate leaching, chemical fertilizer cost, and positive effect on crop grain yields, regular urea application, with additions of 1.0% DMPP might be a good alternative, to be used as a large-scale chemical fertilizer, especially in the rape season.

NIs could theoretically reduce nitrate leaching by retaining nitrogen in a low mobility form (e.g. NH_4^+-N) (Kurtz, 1980; Shen *et al*., 2003). There were significant effects of DMPP on NH_4^+-N concentrations in the leachate and surface soil, compared to the treatment with urea alone (UA), which was approved by most researchers (Serna *et al*., 2000; Linzmeier *et al*., 2001; Zerulla *et al*., 2001). However, NH_4^+-N concentrations in the leachate may be unaffected by DMPP because of the strong adsorption character of soil colloid for soil NH_4^+-N (Yu *et al*., 2007) or enhanced uptake of NH_4^+ by the crop (Weiske *et al*., 2001). NH_3 volatilization should be noticed when higher NH_4^+-N concentrations were observed in surface soil or floodwater of paddy fields. In fact, the addition of NIs had no effect on NH_4^+-N concentrations in the soil (Weiske *et al*., 2001) and did not lead to higher NH_3 losses when compared to the control (Linzmeier *et al*., 2001). The results might depend on soil properties, such as soil pH and N uptake by crops (Zerulla *et al*., 2001; Weiske *et al*., 2001). Therefore, the effect of DMPP on NH_3 volatilization from the agricultural land in the Taihu Lake region deserved further research, to evaluate the role of DMPP in total N loss from agricultural lands.

For nitrifying organisms that regulate nitrification, DMPP decreased AOB population, whereas, it did not decrease NOB population. Ammonia oxidation was the first and rate-limiting step in nitrification, where ammonia was oxidized to nitrite by AOB (Prosser, 1989). NIs could delay the bacterial oxidation of NH_4^+ to NO_2^- in the soil (Hauck, 1980) by depressing the activities of *Nitrosomas* bacteria in the soil (Zerulla *et al*., 2001; Irigoyen *et al*., 2003). However, DMPP and DCD did not affect the oxidation of NO_2^- to NO_3^- in clayey loam soils (Weiske *et al*., 2001). Patra *et al*. (2006) reported that the application of one NI (ECC) could reduce AOB population, whereas, it could have no significant effect on NOB density in the soil. The second step of nitrification was normally not influenced (Zerulla *et al*., 2001). Therefore, the major mechanism of nitrification inhibition by DMPP was the inhibition of ammonia oxidation rather than nitrite oxidation.

In addition to decreasing NO_3^--N concentrations in the leachate and runoff (Fettweis *et al*., 2001), NIs could also reduce N loss through denitrification (Delgado *et al*., 1996; Weiske *et al*., 2001; Zhu *et al*., 2003). As the major denitrifying enzymes, NaR and NiR activities were inhibited, whereas, HyR was not affected by DMPP in this study. For a productive agricultural practice, reduction of NaR and NiR was desirable, because it would lead to conservation of N by reducing denitrification loss, particularly in flooded rice soils, where it was a major pathway of N loss (Buresh and De Datta, 1990). Furthermore, it was beneficial to increase N uptake by crops at the basal or topdressing stage (Patra *et al*., 2006). However, in Müller *et al*.'s (2002) research, DMPP did not affect NaR or N_2O reductase capacity in a silty clay soil even at a concentration, 14 times more than that of the recommended practice concentration. The impacts of DMPP on denitrifying enzymatic activities was dependent on soil characteristics (Barth *et al*., 2001; Pasda *et al*., 2001), climatic conditions (Pasda *et al*., 2001) and probably the cultivated crops (Zerulla *et al*., 2001).

4 Conclusions

DMPP enhanced NH_4^+-N concentrations, but reduced NO_3^--N concentrations in the leachate and soil under a two-year observation compared to the plots with urea alone. The population of the ammonia oxidizing bacteria, and the activity of nitrate reductase and nitrite reductase in the DMPP treatment decreased obviously as compared to the urea treatment. However, nitrite oxidizing bacteria and hydroxylamine reductase remained almost unaffected by DMPP. DMPP application could significantly decrease NO_3^- leaching to groundwater through inhibiting the major microorganisms involved in nitrification and denitrification.

As N losses include a few other methods in addition to leaching, further studies on N runoff and gaseous N emission, when affected by DMPP, should be carried out, to provide a clear pattern of the ecological benefits, from the adoption of DMPP combined with urea. Furthermore, more research is required to elucidate the effect of DMPP on the N cycle in a range of soil types, cultivated vegetation types, and climatic conditions.

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