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# Nitrous oxide emission and nitrogen use efficiency in response to nitrophosphate, N-(n-butyl) thiophosphoric triamide and dicyandiamide of a wheat cultivated soil under sub-humid monsoon conditions

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## Abstract

A field experiment was designed to study the effects of nitrogen (N) source and urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) or nitrification inhibitor dicyandiamide (DCD) on nitrous oxide (N<sub>2</sub>O) emission and N use efficiency (NUE) in a sandy loam soil. Six treatments including no N fertilizer (control), N fertilizer urea alone (U), urea plus NBPT (NBPT), urea plus DCD (DCD), urea plus NBPT and DCD (NBPT + DCD), and nitrate-based fertilizer nitrophosphate (NP) were designed and implemented separately during the wheat growth period. Seasonal cumulative N<sub>2</sub>O emissions with urea alone amounted to  $0.49 \pm 0.12$  and were significantly ( $P < 0.05$ ) reduced to  $0.28 \pm 0.03$ ,  $0.31 \pm 0.01$  and  $0.26 \pm 0.01$  kg N<sub>2</sub>O-N ha<sup>-1</sup> by application of DCD, NBPT and NBPT + DCD, respectively. Cumulative N<sub>2</sub>O emissions from NP were  $0.28 \pm 0.01$  kg N<sub>2</sub>O-N ha<sup>-1</sup>. A single N<sub>2</sub>O flux peak was identified following basal fertilization, and DCD and/or NBPT inhibition effects mainly occurred during the peak emission period. The NP application significantly ( $P < 0.05$ ) increased wheat yield by 12.3% and NUE from 28.8% (urea alone) to 35.9%, while urease and/or nitrification inhibitors showed a slight increase effect. Our results clearly indicated that the application of urea as basal fertilizer, but not as supplemental fertilizer, together with DCD and NBPT is an effective practice to reduce N<sub>2</sub>O emissions. The application of NP instead of urea would be an optimum agricultural strategy for reducing N<sub>2</sub>O emissions and increasing crop yield and NUE for wheat cultivation in soils of the North China Plain.

## 1 Introduction

Nitrous oxide (N<sub>2</sub>O) is a potent and long-lived atmospheric greenhouse gas, with an annual increasing rate of 0.26% over the past decades and a contribution of 7% to the annual increase in radiative forcing (IPCC, 2007). Agricultural soils are identified as the major source of atmospheric N<sub>2</sub>O, contributing 4.1 Tg N yr<sup>-1</sup> (IPCC, 2013) to the global atmospheric N<sub>2</sub>O budget of ~ 14 Tg N yr<sup>-1</sup> (Fowler et al., 2009). Field management

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practices and soil and climatic factors are recognized as being determinants of N<sub>2</sub>O emissions from agricultural soils (Stehfest and Bouwman, 2006; Gagnon et al., 2011). Among management practices, the large inputs of industrially fixed N in agriculture are a major perturbation to terrestrial N cycling and a major contribution to accelerating N<sub>2</sub>O emissions (Galloway et al., 2008). During the period 1990–2005, agricultural N<sub>2</sub>O emissions were globally estimated to have increased by 17 % (USEPA, 2006), and are projected to increase by 35–60 % by 2030 due to the continuous increase of global N fertilizer consumption and animal manure production (FAO, 2003).

China is a major agricultural producer (West et al., 2014) and the amount of applied N fertilizer has increased from 7.07 to 26.21 Tg N yr<sup>-1</sup> over the period from 1977 to 2005 (Ju et al., 2009). The North China Plain, primarily containing low organic carbon (C) calcareous soils (6.40 vs. 9.60 g C kg<sup>-1</sup> for national upland soils) (Xie et al., 2007), is an intensive agricultural region. It covers ~ 300 000 km<sup>2</sup> and produces up to one-fourth of the total annual grain yield in China (Liu et al., 2001). A winter wheat and summer maize rotation is a commonly used cropping system, and the annual application rates of synthetic N fertilizers have amounted to 600 kg N ha<sup>-1</sup> or more (Zhao et al., 2006; Ju et al., 2009). However, a low proportion of fertilizer N is taken up by crops (< 30 %) and it is estimated that up to 41 % of N applied during the growth season is subject to losses via leaching, nitrification, denitrification and ammonia volatilization (Cai et al., 2002). At present, up to 33 % of fertilizer N was over-applied to the fields in China, resulting in China's contribution to 28 % of the global annual N<sub>2</sub>O emissions from croplands (West et al., 2014). Hence, it is urgent to develop optimum methods for enhancing the recycling of N in the agricultural ecosystem and reducing the fertilizer N-induced N<sub>2</sub>O emissions.

In the past decade, a number of field measurements of N<sub>2</sub>O emissions have been conducted in the North China Plain (Meng et al., 2005; Ding et al., 2007; Ju et al., 2011). A 3 year field measurement showed that the direct N<sub>2</sub>O emission factors of synthetic N applied to the wheat-maize cropping systems was 0.82 % (Cai et al., 2013), which was higher than the 0.6 % reported from fertilizer N-treated upland soils in China

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(Xing, 1998). Nitrification is found to be the main process for the N<sub>2</sub>O emission because low availability of easily degradable organic C limits denitrification in this region (Ding et al., 2007; Ju et al., 2011). Nitrification inhibitors such as DCD help to retard the oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> by inhibiting the activities of *Nitrosomonas* bacteria in soil (Prasad and Power, 1995), resulting in the reduction of N<sub>2</sub>O emissions directly by decreasing nitrification or indirectly by reducing the availability of NO<sub>3</sub><sup>-</sup> for denitrification and leaching. As a consequence, DCD can increase NUE by increasing plant growth and N uptake (Asing et al., 2008). Similarly, a urease inhibitor like NBPT can slow the conversion of urea to NH<sub>4</sub><sup>+</sup>, thereby reducing N losses by NH<sub>3</sub> volatilization (Manunza et al., 1999; Zaman et al., 2009) and potentially reducing nitrification and subsequent denitrification rates. So the use of NBPT with urea-based fertilizers may be a potential management strategy to mitigate N<sub>2</sub>O emissions (Menéndez et al., 2009). A combined application of nitrification inhibitor and urease inhibitor with urea can maintain N as NH<sub>4</sub><sup>+</sup> for a longer time with more chance of the fertilizer-derived N being taken up by the crops or immobilized by the organic or mineral component of the soil, thereby reducing the gaseous loss (Xu et al., 2002). Though application of inhibitors to reduce N<sub>2</sub>O emissions has attracted more attention recently and has already been investigated in many areas (Menéndez et al., 2009; Zaman et al., 2009), their effect on N<sub>2</sub>O emissions in the North China Plain has not been fully investigated.

Soil N<sub>2</sub>O emissions are also influenced by the source of fertilizer N. Gagnon et al. (2011) found that N<sub>2</sub>O emissions from urea ammonium nitrate were drastically than those from anhydrous ammonia during the maize growth season in a poorly drained clay soil of Canada. In contrast, Venterea et al. (2005, 2010) reported N<sub>2</sub>O emissions from soils amended with anhydrous NH<sub>3</sub> to be 2- to 4-fold greater than that from soils receiving urea ammonium nitrate in a silt loam of the United States. Based on the analysis of published data in the literature, Stehfest and Bouwman (2006) concluded that the N<sub>2</sub>O emissions from nitrate-based fertilizers were on average lower than those from ammonium-based fertilizers. During the winter wheat growth season in the North China Plain, limited precipitation occurs. Therefore, it is likely that apply-

ing nitrate-based fertilizer instead of urea will not accelerate the leaching of  $\text{NO}_3^-$  but reduce  $\text{N}_2\text{O}$  emissions and increase NUE.

In this study, we hypothesize that application of urease inhibitor and/or nitrification inhibitor with urea will lower  $\text{N}_2\text{O}$  emission and increase wheat yield by suppressing the nitrification rate and increasing NUE in the North China Plain. We also hypothesize that use of a nitrate-based fertilizer nitrophosphate by replacing urea will have similar effects. The objectives of this study were: (1) to evaluate the influence of application of urea with NBPT, DCD and NBPT + DCD on  $\text{N}_2\text{O}$  emissions and (2) to investigate whether the use of nitrophosphate instead of urea reduces  $\text{N}_2\text{O}$  emissions from an intensively cultivated calcareous soil during the wheat growth season.

## 2 Materials and methods

### 2.1 Experimental site and soil characteristics

The field experiment was conducted at the Fengqiu State Key Agro-ecological Experimental Station, Chinese Academy of Sciences, Henan Province, China (35°00' N, 114°24' E), a typical region of the North China Plain. The region has a sub-humid temperate continental monsoon climate with dry cold winters and wet hot summers. A winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.) rotation is selected as an intensively managed double-cropping system. The 30 year mean annual temperature was 13.9°C, with a range varying from -1.0°C in January to 27.2°C in July. The mean annual precipitation is 615 mm, two thirds of which falls between June and September. The soil is derived from alluvial sediments of the Yellow River and is classified as aquic inceptisol. The physicochemical properties of the soil are summarized in Table 1.

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## 2.2 Treatment and crop management

The field experiment was carried out during the winter wheat growth season and included six fertilization treatments: (1) no N fertilizer (control), (2) N fertilizer urea alone (U), (3) urea plus N-(n-butyl) thiophosphoric triamide (NBPT), (4) urea plus dicyandiamide (DCD), (5) urea plus NBPT and DCD (NBPT + DCD), and (6) nitrate-based fertilizer nitrophosphate (NP). The plots were arranged in a randomized complete block with three replicates and the plot size was 5 m × 5 m. Urea and nitrophosphate (Jinkai chemical, Kaifeng, China), totaling 200 kg N ha<sup>-1</sup>, were added in two applications: 120 kg N ha<sup>-1</sup> as basal fertilizer and 80 kg N ha<sup>-1</sup> as supplemental fertilizer. Calcium superphosphate was applied as basal fertilizer at a rate of 125 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> for all treatments. For the NP treatment, calcium superphosphate was added as the basal fertilizer to ensure the same application rate of phosphate between the treatments. The NBPT (Hengshuo Chemical, Wuhan, China) and DCD (Sunnyfield Chemicals, Ningxia, China) were applied at a rate of 0.2 % and 10 % of the applied N (w/w), respectively. Inhibitor(s) and urea were thoroughly mixed. All basal fertilizers were evenly spread onto the soil surface by hand and immediately incorporated into the surface soil (0–20 cm) by plowing before sowing on 15 October 2009. The supplemental urea and inhibitor(s) or nitrophosphate were surface applied by hand, then integrated into the plowed layer with irrigation water (40 mm) on 6 March 2010. The mature wheat was harvested on 10 June 2010.

## 2.3 N<sub>2</sub>O flux measurement

In situ soil-surface fluxes of N<sub>2</sub>O were measured using the static chamber-gas chromatograph (GC) method. Flux measurements were taken over the period from 16 October 2009 to 8 June 2010 (235 days) during the wheat growth season. Immediately after sowing, a PVC chamber base (30 cm × 30 cm × 10 cm) was inserted into the soil about 5 cm deep between wheat rows in the center of each plot. The PVC chamber (30 cm × 30 cm × 15 cm) was tightly fitted to the top of the base by inserting the flange

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of the chamber into the water trough at the upper end of the chamber base. The chamber was equipped with two ports: a small, silicon-sealed vent for sampling and a second port for measuring chamber temperature. Gas samples were initially taken twice a week and later reduced weekly then fortnightly over the winter. Sampling was done in the morning between 09:00 LT and 12:00 LT in order to minimize diurnal variation in flux patterns. Each time, four samples of the chamber air were manually pulled into 50 mL syringes at 0, 10, 20 and 30 min after closure, injected into 20 mL pre-evacuated vials fitted with butyl rubber stoppers and taken to our laboratory for analysis. The air temperature inside the chamber was simultaneously measured with a mercury thermometer.

N<sub>2</sub>O concentrations were analyzed on a gas chromatograph (Agilent 7890, Santa Clara, CA, USA) equipped with an electron capture detector. The interfering oxygen contained in the injected gas sample (1.0 mL) was separated by a pre-column (1 m) in combination with an analytical column (3 m). Both columns, packed with Porapak Q (80/100 mesh), were attached directly to the 6-port valve to control the backflush. The temperatures of column oven, injector and detector were 40 °C, 100 °C and 300 °C, respectively. The flow rate of carrier gas (95 % Argon + 5 % CH<sub>4</sub>) was 40 mL min<sup>-1</sup>. The standard N<sub>2</sub>O gas was provided by the National Institute for Agro-Environmental Sciences, Japan. The N<sub>2</sub>O fluxes were calculated using the following equation:

$$F = \rho \times (P/760) \times (V/A) \times (\Delta C/\Delta t) \times [273/(273 + T)] \quad (1)$$

where  $F$  is the N<sub>2</sub>O flux ( $\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ ),  $\rho$  is the density of N<sub>2</sub>O at 0 °C and 760 mm Hg ( $\text{kg m}^{-3}$ ),  $V$  is the chamber volume ( $\text{m}^3$ ),  $A$  is the area from which N<sub>2</sub>O was emitted into the chamber ( $\text{m}^2$ ),  $\Delta C/\Delta t$  is the rate of N<sub>2</sub>O accumulation in the chamber ( $\text{ppbv N}_2\text{O-N h}^{-1}$ ),  $T$  is the chamber air temperature in Celsius, and  $P$  is the air pressure of the experimental site (mm Hg). The altitude of the experimental site for this study is very close to sea level, so  $P/760 \approx 1$ . Few sample sets were discarded when they yielded a linear regression value of  $R^2$  greater than 0.90.

## 2.4 Grain yield and aboveground N uptake

After crops reached physiological maturity (10 June 2011), grain and straw were manually harvested from each plot. Grain and straw were air-dried, then further dried for 3 days at 65 °C and weighed to obtain dry matter yields. Subsamples were ground with a ball mill and analyzed for N concentration with an elemental N analyzer (VarioMax, Elementar, Hanau, Germany). Total N content in aboveground biomass was calculated from the sum of N masses harvested in grain and straw from each plot.

## 2.5 Auxiliary variables

Soil temperatures were measured, simultaneously with gas sampling, at vertical depths of 5, 10 and 15 cm with a digital thermometer (Model 2455, Yokogawa, Japan). Soil moisture was measured at 5 cm depth at three different positions in the vicinity of each chamber using time domain reflectometry probes and was expressed as water-filled pore space (WFPS) by the equation:

$$\text{WFPS [\%]} = (\text{volumetric water content [\%]} / \text{total soil porosity [\%]}) \times 100 \quad (2)$$

where total soil porosity =  $1 - (\text{soil bulk density} / 2.65)$ , with 2.65 [g cm<sup>-3</sup>] being the assumed particle density of the soil. The precipitation and air temperature were monitored at a neighboring meteorological station 100 m away from the experimental field.

During the growth season, six soil samples were taken from the 0–20 cm soil layer at different positions in each plot just after flux measurement using a 5 cm diameter stainless steel soil sampler and then all samples from each plot were thoroughly mixed to form a composite. After visible roots and litter materials were removed, soil samples were passed through a 2 mm sieve and then extracted with 2 M KCl (soil/KCl suspension of 30 : 100 ratio) for 1 h on a rotary shaker. The extracted solutions were filtered and stored in a deep freezer (–18 °C) until analysis. The NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations were measured using a colorimetric method on a Skalar segmented flow analyzer (SAN<sup>++</sup>, the Netherlands).



## 2.6 Data analysis and statistics

Average fluxes and standard errors of the N<sub>2</sub>O fluxes were calculated from triplicate plots. Seasonal cumulative N<sub>2</sub>O emissions were calculated using the following equation:

$$\text{Cumulative N}_2\text{O emission} = \sum_{i=1}^n (F_i + F_{i+1})/2 \times (t_{i+1} - t_i) \times 24 \quad (3)$$

where  $F$  is the N<sub>2</sub>O flux ( $\mu\text{g N}_2\text{O-Nm}^{-2} \text{h}^{-1}$ ),  $i$  is the  $i$ th measurement, the term of  $(t_{i+1} - t_i)$  is the number of days between two measurements, and  $n$  is the total number of the measurements. The N<sub>2</sub>O direct emission factor (%) of fertilizer N applied to the soil with background adjustment was calculated as follows:

$$\text{Emission factor} = ((\text{N}_2\text{O-N}_{\text{fertilizer}} - \text{N}_2\text{O-N}_{\text{control}})/\text{N}_{\text{fertilizer}}) \times 100 \quad (4)$$

where  $\text{N}_2\text{O-N}_{\text{fertilizer}}$  and  $\text{N}_2\text{O-N}_{\text{control}}$  are the cumulative N<sub>2</sub>O emissions ( $\text{kg N}_2\text{O-N ha}^{-1}$ ) in the N-fertilized treatment and the control treatment, respectively, and  $\text{N}_{\text{fertilizer}}$  is the amount of fertilizer N applied ( $\text{kg N ha}^{-1}$ ). Yield-scaled N<sub>2</sub>O emissions were calculated by dividing cumulative N<sub>2</sub>O emission by grain yield for each plot. NUE was calculated by dividing differences of the N amount in the aboveground biomass between N-fertilized plots and control plots within the same block by the N application rate ( $200 \text{ kg N ha}^{-1}$ ). Soil inorganic N intensities were calculated separately for  $\text{NH}_4^+$  ( $\text{NH}_4\text{I}$ ),  $\text{NO}_3^-$  ( $\text{NO}_3\text{I}$ ) and the sum of  $\text{NO}_3^- + \text{NH}_4^+$  ( $\text{IONI}$ ) as the summation of daily  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N or  $(\text{NO}_3^- + \text{NH}_4^+)\text{-N}$  concentrations in the 0–20 cm layer over the same period as for cumulative N<sub>2</sub>O emissions using linear interpolation between sampling dates, and presented in units of  $\text{g d kg}^{-1}$ , the index being what is commonly reported (Zebarth et al., 2008; Engel et al., 2010).

All data were statistically analyzed using the SPSS software package for Windows (Version 13.0, SPSS inc, Chicago, IL, USA). The effects of fertilization management

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on  $N_2O$  emissions, emission factor, and grain yields were evaluated using one-way ANOVA, followed by the least significant difference (LSD) test at  $P < 0.05$ . All dependent variables were evaluated for normality and were log-transformed to normalize the distributions if necessary prior to statistical analysis. Correlation and nonlinear regression analyses were used to test relationships between  $N_2O$  fluxes and other factors.

### 3 Results

#### 3.1 Wheat yield and nitrogen use efficiency

Grain yield in the urea alone treatment was  $4652 \text{ kg ha}^{-1}$  and this was increased by 1.3%, 1.8% or 1.8% when NBPT, DCD or both were added with urea fertilizer (Table 2). Compared with the urea alone, the increase in the grain yield in the NP treatment was 12.3% (Table 2). The N process inhibitors, NBPT, DCD or the combination of both, slightly increased the amount of N uptake by wheat plants. However, this increase was not statistically significant ( $P > 0.05$ ). A significant increase in the plant N uptake was observed in the NP treatment compared with urea only treatment ( $P < 0.05$ ). The NUE was calculated at 28.8% for the urea alone treatment and this was slightly increased to 29.2–31.2% when urea was applied with NBPT, DCD or the combination of NBPT and DCD. However, the NUE was increased to 35.9% for the NP treatment, which was significantly higher than that for all the urea treatments ( $P < 0.05$ ).

#### 3.2 Soil temperature and moisture

Temporal variations of air temperature, precipitation, and soil WFPS and soil temperature at 5 cm depth over the experimental period are presented in Fig. 1. The cumulative rainfall over the wheat growth season was 97.6 mm which was lower than the long-term average. Soil moisture levels were highly variable, with WFPS values varying from 10.7% to 80.4%. Periods with high soil moisture ( $> 75\%$  WFPS) were observed

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the emissions from the urea alone treatment, significantly lower N<sub>2</sub>O emissions were also observed from the NP treatment ( $P < 0.05$ ) (42.9% less than those from the urea alone treatment). The direct N<sub>2</sub>O emission factor for urea application alone was 0.17%, and the addition of NBPT, DCD or the combination of both reduced the emission factor for urea to 0.05–0.08%. These reductions were statistically significant ( $P < 0.05$ ). The direct N<sub>2</sub>O emission factor for NP was 0.06%, which was also significantly lower than that for urea application alone ( $P < 0.05$ ).

The grain yield-scaled N<sub>2</sub>O emission from the NP treatment was significantly lower than that from the urea alone treatment ( $P < 0.05$ ), but not different from those from the NBPT, DCD or NBPT + DCD treatments during the wheat growth season (Table 4).

### 3.4 Soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations

Soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentration drastically increased after application of basal N fertilizers compared with the control. The levels of NO<sub>3</sub><sup>-</sup> in the NBPT and NBPT + DCD treatments were relatively low for one week after basal fertilizer application compared with those in the other treatments. However, the levels of NO<sub>3</sub><sup>-</sup> in the NBPT and NBPT + DCD treatments gradually increased, this was probably due to degradation of NBPT and its subsequent loss of effectiveness. Following application of supplemental fertilizer urea, no apparent increase in soil NO<sub>3</sub><sup>-</sup> levels was observed, and NO<sub>3</sub><sup>-</sup> concentration kept at a relatively constant level. In contrast, soil NO<sub>3</sub><sup>-</sup> concentration following application of supplemental fertilizer NP showed a rapidly decreasing trend. In all urea-added treatments, soil NO<sub>3</sub><sup>-</sup> concentration sharply decreased to less than 10 mg N kg<sup>-1</sup> from 15 April onwards.

Soil NH<sub>4</sub><sup>+</sup> concentration increased from 2 to 10 mg N kg<sup>-1</sup> after application of basal fertilizer; however it sharply decreased soon afterwards. Application of DCD or NBPT + DCD sustained soil NH<sub>4</sub><sup>+</sup> concentrations at higher levels compared with urea application alone. In the NP treatment, soil NH<sub>4</sub><sup>+</sup> concentrations were always at low levels. The natural logarithms of the N<sub>2</sub>O fluxes were more correlated with NH<sub>4</sub><sup>+</sup> con-

centrations than with  $\text{NO}_3^-$  concentrations in the soil, despite the fact that a significant relationship was only observed in the urea alone treatment.

Mean soil  $\text{NH}_4\text{I}$  levels in the NP treatment were the lowest among all N-added treatments and tended to be higher in the DCD and NBPT + DCD treatments compared with urea alone (Table 5). Mean soil  $\text{NO}_3\text{I}$  levels showed a similar trend among the treatments and were ranked in the order of NBPT, NBPT + DCD > DCD > urea alone, NP > control. Mean soil IONI levels were also similar among the treatments and were ranked as NBPT + DCD > NBPT, DCD > urea alone, NP > control.

## 4 Discussion

### 4.1 Nitrous oxide emissions as affected by nitrogen sources

Compared with the urea alone, application of NP significantly reduced  $\text{N}_2\text{O}$  emissions by 42.9% during the wheat growth season (Table 4), and increased wheat yield by 12.3% and NUE by 24.7% (Table 2). Ju et al. (2011) obtained a similar result in the North China Plain, finding that emissions of  $\text{N}_2\text{O}$  derived from  $\text{Ca}(\text{NO}_3)_2$  were lower than those from  $\text{NH}_4(\text{SO}_4)_2$  during the maize growth season (0.38–0.81 vs. 1.31–3.52  $\text{kg N}_2\text{O-N ha}^{-1}$ ). A lower  $\text{N}_2\text{O}$  emission for urea ammonium nitrate than for anhydrous ammonia was also reported in a silt loam of the United States (Venterea et al., 2005). In contrast, Gagnon et al. (2011) measured a significantly higher  $\text{N}_2\text{O}$  emission following application of urea ammonium nitrate or calcium ammonium nitrate compared with anhydrous ammonia in a poorly drained clay soil of Canada. In a German grassland ecosystem, Müller and Sherlock (2004) found that the emissions for ammonia-based fertilizer were lower than those for nitrate-based fertilizer. These researchers suggested that higher emissions from nitrate-based fertilizers were because of the propensity of the fine-textured clay soil to become anaerobic following rainfall and a strong fixation of  $\text{NH}_4^+$  in clay lattices reducing  $\text{NH}_4^+$  available for  $\text{N}_2\text{O}$  production (Chantigny et al., 2004). In this study, mean soil  $\text{NO}_3^-$  intensities ( $\text{NO}_3\text{I}$ ) were not signif-

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icantly different between the NP and urea alone treatments (Table 5), and mean NO<sub>3</sub><sup>-</sup> concentrations (Fig. 3) were higher than the suggested threshold value for denitrification of 5 mg N kg<sup>-1</sup> (Dobbie and Smith, 2003) during the growth season except for the period from 24 April to 10 June. These results imply that soil NO<sub>3</sub><sup>-</sup> concentration was not the only limiting factor affecting denitrification and N<sub>2</sub>O emission in the test soil.

The notable difference in the seasonal N<sub>2</sub>O emissions between the NP and urea alone treatments occurred mainly during the 18 day peak emission period following the basal fertilizer application and concurrent irrigation from 16 October to 3 November. It has been reported that application of ammonium-based fertilizers emitted more N<sub>2</sub>O than nitrate-based fertilizers under aerobic soil conditions, while application of nitrate-based fertilizers induced a greater increase in N<sub>2</sub>O production when soil conditions were anoxic (Pathak and Nedwell, 2001; Tenuta and Beauchamp, 2003). For cultivated soils, the primary mechanism of N<sub>2</sub>O production is generally believed to be the nitrification process when soil WFPS levels are between 30 % and 70 % and the denitrification process when soil WFPS levels were between 70 % and 90 % (Granli and Bøckman, 1994). Some other studies also suggest that denitrification in general could produce more N<sub>2</sub>O compared with nitrification (eg. Dobbie et al., 1999). According to the studies of Ding et al. (2007) and Wan et al. (2009), N<sub>2</sub>O in sandy loam soils of the North China Plain was primarily produced by nitrification unless soil WFPS reached 75 % or more. Pihlatie et al. (2004) reported that even at 100 % WFPS in a loamy sand soil with 24 g organic C kg<sup>-1</sup>, nitrification was still the dominant N<sub>2</sub>O production process. In this study, the highest soil WFPS measured during the peak emission period was ~ 65 %; thus we suggest that low soil moisture limited denitrification and N<sub>2</sub>O production from the nitrate-based fertilizer in the test soil.

In the North China Plain, the addition of starch to soil treated with nitrate-based fertilizers in the field stimulated N<sub>2</sub>O production through denitrification, but wheat straw amendment did not do so (Wan et al., 2009; Ju et al., 2011). Previous studies demonstrated that denitrification was not only controlled by soil moisture and nitrate, but also by organic C supply, and increasing organic C availability could reduce the minimum

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soil moisture threshold for denitrification (van Groenigen et al., 2004; Chantigny et al., 2013). Yu et al. (2012) found that the mass proportion of macroaggregates in a NPK-treated soil with 6.0 g organic C kg<sup>-1</sup> only accounted for 8.8 %, while this proportion amounted to 30.8 % in an 18 year compost-added soil with 10.0 g organic C kg<sup>-1</sup> in the North China Plain. This change significantly increased the proportion of pores with a neck diameter < 4 μm by reducing the proportion of pores with a neck diameter of 15–60 μm, which in turn lowered the effective diffusion coefficient of oxygen in the soils and the ratio of monounsaturated to branched phospholipid fatty acids (PLFAs) i.e. aerobic to anaerobic microorganisms (Zhang et al., 2014). According to results found by Myrold and Tiedje (1984), only large aggregates have anaerobic microsites. Thus, it is likely that the relatively low organic C concentration in the test soil retards macroaggregation and slows formation of anaerobic microsites, which in turn results in rise of the minimum moisture threshold required for denitrification. Consequently, the denitrification process is of much less importance than nitrification for N<sub>2</sub>O production and emissions in soils of the North China Plain.

In a German silt loam soil, similar to that tested in this study, Rover et al. (1998) reported that winter was a key period for N<sub>2</sub>O emissions from arable crops in the temperate climate zone, contributing ~ 70 % of the annual N<sub>2</sub>O losses during the thawing from December to February. Wolf et al. (2010) also verified that N<sub>2</sub>O pulses due to spring thaw dominated total annual N<sub>2</sub>O emission in a steppe grassland of Inner Mongolia, China. At our site, spring thawing of the soil at the fertilized plots only caused minor N<sub>2</sub>O emission pulses, which were considerably lower than those reported earlier for other arable soils (Syväsalo et al., 2004; Teepe et al., 2000). It is suggested that reduced oxygen supply through alteration of pore structure during thawing, and high soil water contents in the winter, would promote microbial denitrification (Edwards and Killham, 1986; Mørkved et al., 2006). Our present study, together with previous measurements (Ding et al., 2007; Ju et al., 2010; Cai et al., 2012; Cui et al., 2012), showed that the highest soil WFPS was no more than 70 % during the spring thawing period, a value that was lower than the threshold value of 80 % for thawing N<sub>2</sub>O pulses in a silt

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loam found by Rover et al. (1998). The cumulative rainfall during the winter period from December 2009 to February 2010 was only 4.4 mm and no apparent snow cover was observed at our study site. So the warm temperate monsoon zone, with cold and dry winter in the North China Plain, which is distinctly different from other climatic zones such as western Europe (Dobbie and Smith, 2003) and inner Mongolia of China (Wolf et al., 2010), would not induce thawing N<sub>2</sub>O pulses from arable soils, as found in our study. Our results confirm that N fertilizer sources influence soil N<sub>2</sub>O emissions, but that this effect probably depends on soil properties and especially climate conditions. Our study also suggests that, compared with urea or ammonium-based fertilizer, applying nitrate-based fertilizer is an effective management strategy to mitigate N<sub>2</sub>O emissions and to increase NUE and wheat yield in the North China Plain.

### 4.2 Nitrous oxide emissions as affected by inhibitors

The application of basal fertilizer urea followed by flooding irrigation resulted in N<sub>2</sub>O emission pulses for 18 days. This finding is in agreement with those of other studies for arable fields (Bouwman et al., 2002; Ding et al., 2007; Cui et al., 2012). The presence of inhibitors NBPT and/or DCD significantly lowered N<sub>2</sub>O peak fluxes, and cumulative N<sub>2</sub>O emissions during the 18 day peak emission period were reduced by 50.0 % by NBPT, 78.6 % by DCD and 67.9 % by NBPT + DCD, compared with application of urea alone. Our results indicate that the addition of DCD alone or in combination with NBPT effectively reduced N<sub>2</sub>O emissions from application of urea. In other sites of the North China Plain, Liu et al. (2013) also reported that nitrification inhibitors DCD and DMPP (3,4-dimethylpyrazole phosphate) could reduce N<sub>2</sub>O emissions from application of N fertilizers by 30 % and 21 %, respectively, during the wheat growth season. Ju et al. (2011) observed no apparent differences in cumulative N<sub>2</sub>O emissions between zero N control and urea with DMPP during the maize growth season, suggesting strong nitrification inhibition effectiveness of DMPP.

N<sub>2</sub>O emission is directly related to the amount of mineral N available in the soil and application of inhibitors with urea can effectively regulate the NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concen-



trations (Li et al., 2009; Zaman et al., 2009). Recently, Maharjan and Venterea (2013) demonstrated that N<sub>2</sub>O emissions were more correlated with soil NO<sub>2</sub><sup>-</sup> intensity rather than NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup> intensity, and that inhibitors controlled N<sub>2</sub>O production by adjusting soil NO<sub>2</sub><sup>-</sup> intensity. In this study, soil NH<sub>4</sub><sup>+</sup> concentration slightly increased in the presence of DCD and, in contrast, relatively low NH<sub>4</sub><sup>+</sup> concentration was found after NBPT application following application of basal fertilizer. NBPT delays urea hydrolysis, thereby lowering soil pH elevation and NH<sub>4</sub><sup>+</sup> production, which can in turn reduce NH<sub>3</sub> toxicity effects on nitrite-oxidizing bacteria (NOB). DCD slows oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>2</sub><sup>-</sup> mainly by inhibiting activities of ammonia-oxidizing bacteria (AOB), which allows NOB to use NO<sub>2</sub><sup>-</sup> at the rate closely matched to its production rate (Zaman et al., 2008; Maharjan and Venterea, 2013). Both NBPT and DCD could additively attenuate formation of N<sub>2</sub>O from urea in the soil. Thus, the reduction of N<sub>2</sub>O emissions by inhibitors is probably due to both low oxidation rate of NH<sub>4</sub><sup>+</sup> and low NO<sub>2</sub><sup>-</sup> concentration, thereby reducing N<sub>2</sub>O “leaking” as a by-product of nitrification (Firestone and Davidson, 1989).

Following supplemental fertilization with or without inhibitors, no distinct N<sub>2</sub>O flux peaks were found in our study. This may be attributable to no significant increase of soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations after urea top-dressing. Cui et al. (2012) ascribed low increases in mineral N concentrations to large losses of urea-derived ammonia via volatilization. However, a field measurement at our study site showed that < 1 % of the N applied was lost via volatilization following urea top-dressing in March (Ni et al., 2009); so a large amount of NH<sub>3</sub> loss would not occur at our site. Milchunas et al. (1988) suggested that urea hydrolysis is primarily affected by soil moisture. An incubation at 13 °C demonstrated that lowering soil moisture level from 60 % to below 40 % water holding capacity produced a longer lag before ammonia evolution and considerably retarded urea hydrolysis (Foster et al., 1980). The range of soil WFPS between 40 and 60 % during the period following urea top-dressing with subsequent irrigation suggested that soil moisture could partly have affected N<sub>2</sub>O production. Suter et al. (2011) observed that lowering incubation temperature from 25 °C to 5 °C greatly retarded the hydrolysis of urea when WFPS was below 60 %, especially for an alka-

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treatments following the supplemental fertilizer in this study supports this speculation. Based on this study, it is not necessary to apply DCD with supplemental fertilizer urea and a combination of urease and nitrification inhibitors would be a better approach to reduce N<sub>2</sub>O emission than urease or nitrification inhibitor application alone with basal fertilizer urea for wheat cultivation.

## 5 Conclusions

The present field study provided an insight into N<sub>2</sub>O emissions from a calcareous soil during the wheat growth season in the North China Plain, as affected by application of urease or nitrification inhibitors and nitrate-based fertilizer nitrophosphate. A single N<sub>2</sub>O flux peak was found following basal fertilization during the wheat growth period. Application of urea with NBPT, DCD or NBPT + DCD significantly reduced N<sub>2</sub>O emissions from urea by 36.7 %, 42.9 % or 46.9 %, respectively. Application of nitrophosphate also resulted in reduction of total N<sub>2</sub>O emissions by 42.9 %, compared with application of urea alone. NBPT and/or DCD were effective in reducing N<sub>2</sub>O emissions following basal fertilization. Compared with urea application alone, application of inhibitors with urea, either individually or combined together, slightly increased wheat yield and NUE, while nitrophosphate significantly increased wheat yield by 12.3 % and increased NUE from 28.8 % (urea alone) to 35.9 %. N<sub>2</sub>O flux was primarily affected by soil temperature and low temperature at the study site minimized fertilizer N-induced N<sub>2</sub>O peaks following application of supplemental fertilizer. Based on our findings, the combination of NBPT and DCD with basal fertilizer urea would be an effective practice for reducing N<sub>2</sub>O emission. As well, this study suggests that application of nitrophosphate, instead of urea, is an optimum agricultural strategy for reducing N<sub>2</sub>O emission and for increasing crop yield and NUE for wheat cultivation in the soils of the North China Plain.

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**Table 1.** Soil properties.

Soil depth (cm)	pH (H <sub>2</sub> O)	Bulk density (Mg m <sup>-3</sup> )	Organic C (g C kg <sup>-1</sup> )	Total N (g N kg <sup>-1</sup> )	C/N	NO <sub>3</sub> <sup>-</sup> -N (mg N kg <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> -N (mg N kg <sup>-1</sup> )	Particle size distribution (%)		
								Sand	Silt	Clay
0–20	8.60	1.40	12.0	1.50	8.0	14.70	2.48	17.0	72.0	11.0

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**Table 2.** Effects of urease and/or nitrification inhibitors and nitrophosphate on wheat biomass, amount of N uptake by crops and N use efficiency.

Treatment	Biomass (kg ha <sup>-1</sup> )			Amount of N uptake (kg N ha <sup>-1</sup> )			N use efficiency (%)
	Grain	Straw	Total	Grain	Straw	Total	
Control	2297 ± 150 c	2215 ± 134 b	4513 ± 283 c	24.4 ± 1.6 c	11.9 ± 0.7 b	36.4 ± 2.3 c	–
U	4652 ± 11 b	4075 ± 81 a	8727 ± 85 b	59.6 ± 0.1 b	34.2 ± 0.7 a	93.9 ± 0.7 b	28.8 ± 0.8 b
NBPT	4711 ± 126 b	4098 ± 356 a	8809 ± 472 b	60.4 ± 1.6 b	34.4 ± 3.0 a	94.8 ± 4.5 b	29.2 ± 1.0 b
DCD	4736 ± 103 ab	4080 ± 52 a	8816 ± 86 b	60.7 ± 1.3 b	34.3 ± 0.4 a	95.0 ± 1.1 b	29.3 ± 0.9 b
NBPT + DCD	4735 ± 290 ab	4535 ± 503 a	9271 ± 764 ab	60.7 ± 3.7 b	38.1 ± 4.2 a	98.8 ± 7.6 ab	31.2 ± 1.2 b
NP	5225 ± 142 a	4906 ± 251 a	10 131 ± 370 a	67.0 ± 1.8 a	41.2 ± 2.1 a	108.2 ± 3.7 a	35.9 ± 1.1 a

Mean ± standard error ( $n = 3$ ).

Different letters within the column indicate significantly difference between treatments at  $P < 0.05$ .

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**Table 3.** Correlation between  $\ln[\text{N}_2\text{O flux} + 1]$  and soil WFPS, soil temperature at depths of 5 ( $T_{5\text{cm}}$ ), 10 ( $T_{10\text{cm}}$ ) and 15 cm ( $T_{15\text{cm}}$ ), ammonium ( $\text{NH}_4^+\text{-N}$ ), nitrate ( $\text{NO}_3^-\text{-N}$ ) or inorganic nitrogen ( $\text{NH}_4^+\text{-N}$  plus  $\text{NO}_3^-\text{-N}$ ) concentration.

Treatment	WFPS	$T_{5\text{cm}}$	$T_{10\text{cm}}$	$T_{15\text{cm}}$	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	Inorganic N
Control	0.095	0.413**	0.376*	0.392*	-0.153	0.140	0.109
U	0.023	0.381**	0.340*	0.346*	0.274*	0.365**	0.380**
NBPT	0.118	0.275*	0.264	0.274	0.215	0.206	0.222
DCD	0.323*	0.282*	0.180	0.189	0.104	-0.127	-0.092
NBPT + DCD	0.021	0.216	0.252	0.272	0.074	0.155	0.156
NP	0.084	0.301*	0.370*	0.403**	0.105	-0.056	-0.037

\*  $P < 0.05$ , \*\*  $P < 0.01$ .

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**Table 4.** Effects of urease and/or nitrification inhibitors and nitrophosphate on cumulative N<sub>2</sub>O emissions, fertilizer N-induced N<sub>2</sub>O emission factors and yield-scaled N<sub>2</sub>O emissions.

Treatment	Cumulative N <sub>2</sub> O emission (kg N <sub>2</sub> O-N ha <sup>-1</sup> )		Ratio of peak to total emissions (%)	Emission factor (% of applied N)	Yield-scaled N <sub>2</sub> O emission (g N <sub>2</sub> O-N kg <sup>-1</sup> grain)
	Total	Peak			
Control	0.16 ± 0.02 c	0.03 ± 0.00 d	18.8 ± 2.3 d	–	0.068 ± 0.006 b
U	0.49 ± 0.12 a	0.28 ± 0.10 a	57.1 ± 4.2 a	0.17 ± 0.05 a	0.105 ± 0.026 a
NBPT	0.31 ± 0.01 b	0.14 ± 0.01 b	45.2 ± 3.9 b	0.08 ± 0.00 b	0.065 ± 0.003 b
DCD	0.28 ± 0.01 b	0.06 ± 0.01 c	21.4 ± 2.1 d	0.06 ± 0.00 b	0.060 ± 0.004 b
NBPT + DCD	0.26 ± 0.01 b	0.09 ± 0.00 bc	34.6 ± 3.2 c	0.05 ± 0.00 b	0.056 ± 0.003 b
NP	0.28 ± 0.03 b	0.11 ± 0.03 bc	39.3 ± 3.7 c	0.06 ± 0.01 b	0.053 ± 0.008 b

Mean ± standard error ( $n = 3$ ).

Peak emission denotes cumulative emissions during the 18 days' period following the basal fertilizer application from 16 October to 3 November. Different letters within the column indicate significant difference between treatments at  $P < 0.05$ .

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**Table 5.** Effects of urease and/or nitrification inhibitors and nitrophosphate on soil ammonium (NH<sub>4</sub>), nitrate (NO<sub>3</sub>) and inorganic N (IONI) intensities.

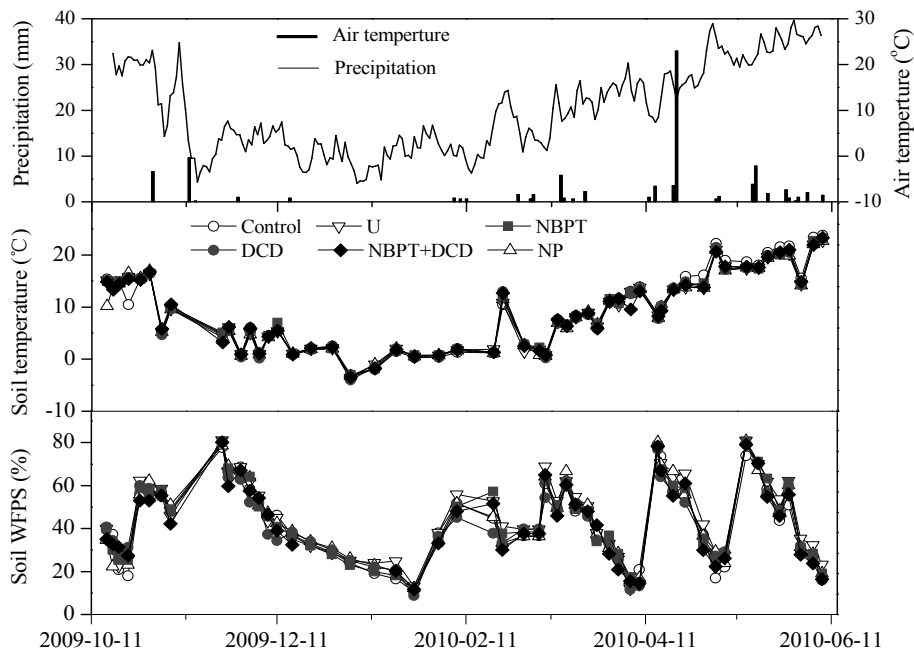
Treatment	NH <sub>4</sub> I (g N d kg <sup>-1</sup> )	NO <sub>3</sub> I (g N d kg <sup>-1</sup> )	IONI (g N d kg <sup>-1</sup> )
Control	0.24 ± 0.01 e	2.58 ± 0.01 d	2.82 ± 0.00 d
U	0.40 ± 0.03 d	4.75 ± 0.13 c	5.15 ± 0.16 c
NBPT	0.61 ± 0.02 c	6.18 ± 0.08 a	6.79 ± 0.08 b
DCD	0.96 ± 0.01 b	5.74 ± 0.01 b	6.70 ± 0.01 b
NBPT + DCD	1.07 ± 0.01 a	6.11 ± 0.16 a	7.17 ± 0.16 a
NP	0.36 ± 0.02 d	4.69 ± 0.09 c	5.05 ± 0.07 c

Mean ± standard error ( $n = 3$ ).

Different letters within the column indicate significantly difference between treatments at  $P < 0.05$ .

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**Figure 1.** Temporal variation of daily precipitation and air temperature, and mean soil moisture and water-filled pore space (WFPS) at time of N<sub>2</sub>O sampling in the control, urea alone (U), urea with NBPT (NBPT), urea with DCD (DCD), urea with NBPT + DCD (NBPT + DCD) and nitrophosphate (NP) treatments during the wheat growth season. The standard errors of soil temperature and moisture were not shown for figure clarity.

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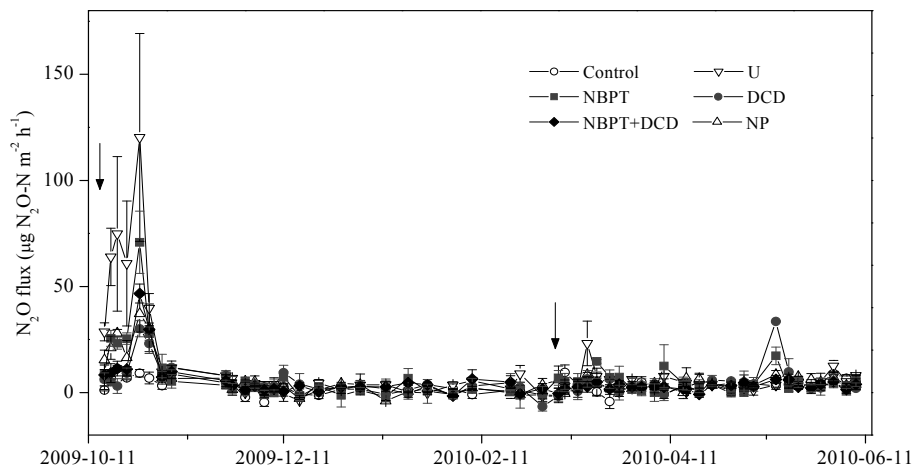
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**Figure 2.** Temporal variation of nitrous oxide fluxes in the control, urea alone (U), urea with NBPT (NBPT), urea with DCD (DCD), urea with NBPT + DCD (NBPT + DCD) and nitrophosphate (NP) treatments during the wheat growth season. Flux values are mean values  $\pm$  standard errors for three replicates. Arrows indicate date of fertilizer application.

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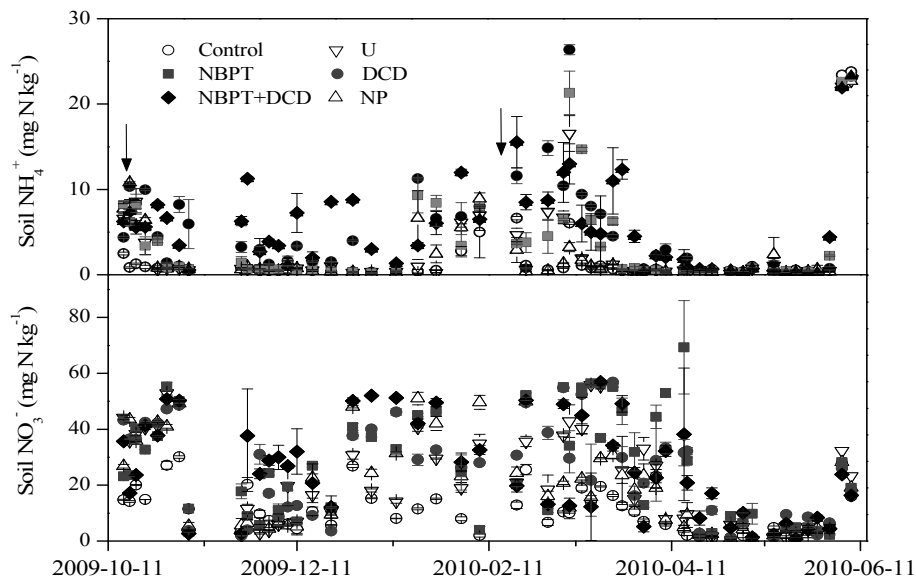
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**Figure 3.** Temporal variation of ammonium and nitrate concentrations in samples from the 0–20 cm depth in the control, urea alone (U), urea with NBPT (NBPT), urea with DCD (DCD), urea with NBPT + DCD (NBPT + DCD) and nitrophosphate (NP) treatments during the wheat growth season. Vertical bars denote the standard error of the means ( $n = 3$ ).

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