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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_2O) 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), (4) 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_2O emissions with urea alone amounted to 0.49 ± 0.12 kg N_2O-N ha^{-1} and were significantly ($P < 0.05$) reduced to 0.28 ± 0.03, 0.31 ± 0.01 and 0.26 ± 0.01 kg N_2O-N ha^{-1} by application of DCD, NBPT and NBPT + DCD, respectively.

Cumulative N_2O emissions from NP were 0.28 ± 0.01 kg N_2O-N ha^{-1}. A single N_2O 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_2O emissions. The application of NP instead of urea would be an optimum agricultural strategy for reducing N_2O emissions and increasing crop yield and NUE for wheat cultivation in soils of the North China Plain.

Key words: Ammonium-based fertilizer; Inhibitors; Nitrate-based fertilizer; Nitrogen use efficiency; Nitrous oxide; Wheat yield

1 Introduction

Nitrous oxide (N_2O) 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_2O, contributing 4.1 Tg N yr^{-1} (IPCC, 2013) to the global atmospheric N_2O budget of ~ 14 Tg N yr^{-1} (Fowler et al., 2009). Field management practices along with soil and climatic factors are recognized as being determinants of N_2O 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_2O emissions (Galloway et al., 2008). During the period 1990–2005, agricultural N_2O 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^{-1} 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^{-1} for national upland soils) (Xie et al., 2007), is an intensive agricultural region. It covers ~ 300 000 km^2 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^{-1} 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_2O 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_2O emissions.

In the past decade, a number of field measurements of N_2O 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_2O 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 (Xing, 1998). Nitrification is found to be the main process for the N_2O 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_4^+ to NO_3^- by inhibiting the activities of *Nitrosomonas* bacteria in soil (Prasad and Power, 1995), resulting in the reduction of N_2O emissions directly by decreasing nitrification or indirectly by reducing the availability of NO_3^- 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_4^+, thereby reducing N losses by NH_3 volatilization (Manunza et al., 1999; Zaman et al., 2009) and potentially reducing nitrification and subsequent denitrification rates. As such the use of NBPT with urea-based fertilizers may be a potential management strategy to mitigate N_2O emissions (Menéndez et al., 2009). A combined application of nitrification inhibitor and urease inhibitor with urea can maintain N as NH_4^+ 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_2O

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_2O emissions in the North China Plain has not been fully investigated.

Soil N_2O emissions are also influenced by the source of fertilizer N. Gagnon et al. (2011) found that N_2O emissions from urea ammonium nitrate were drastically greater 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_2O emissions from soils amended with anhydrous NH_3 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_2O 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 applying nitrate-based fertilizer instead of urea will not accelerate the leaching of NO_3^- but reduce N_2O emissions and increase NUE.

In this study, we hypothesize that application of urease inhibitor and/or nitrification inhibitor with urea will lower N_2O 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 N_2O emissions and (2) to investigate whether the use of nitrophosphate instead of urea

111 reduces N_2O emissions from an intensively cultivated calcareous soil during the wheat
112 growth season.
113
114 **2 Materials and methods**
115 **2.1 Experimental site and soil characteristics**
116 The field experiment was conducted at the Fengqiu State Key Agro-ecological
117 Experimental Station, Chinese Academy of Sciences, Henan Province, China
118 (35º00'N, 114º24'E), a typical region of the North China Plain. The region has a
119 sub-humid temperate continental monsoon climate with dry cold winters and wet hot
120 summers. A winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.)
121 rotation is selected as an intensively managed double-cropping system. The 30 year
122 mean annual temperature was 13.9 °C, with a range varying from –1.0 °C in January
123 to 27.2 °C in July. The mean annual precipitation is 615 mm, two thirds of which falls
124 between June and September. The soil is derived from alluvial sediments of the
125 Yellow River and is classified as aquic inceptisol (Soil Survey Staff, 1994). The
126 physicochemical properties of the soil are summarized in Table 1.
127
128 **2.2 Treatment and crop management**
129 The field experiment was carried out during the winter wheat growth season and
130 included six fertilization treatments: (1) no N fertilizer (control), (2) N fertilizer urea
131 alone (U), (3) urea plus N-(n-butyl) thiophosphoric triamide (NBPT), (4) urea plus
132 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^{-1}, were added in two applications: 120 kg N ha^{-1} as basal fertilizer and 80 kg N ha^{-1} as supplemental fertilizer. Calcium superphosphate was applied as basal fertilizer at a rate of 125 kg P_2O_5 ha^{-1} 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_2O flux measurement

In situ soil-surface fluxes of N_2O 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 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_2O 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_4) was 40 mL min^{-1}. The standard N_2O gas was provided by the National Institute for Agro-Environmental Sciences, Japan. The N_2O 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_2O flux (μg N_2O-N m^{-2} h^{-1}), ρ is the density of N_2O at 0 ºC and 760

177 mm Hg (kg m^{-3}), V is the chamber volume (m^3), A is the area from which N_2O was
178 emitted into the chamber (m^2), $\Delta C/\Delta t$ is the rate of N_2O accumulation in the chamber
179 (ppbv N_2O-N h^{-1}), T is the chamber air temperature in Celsius, and P is the air
180 pressure of the experimental site (mm Hg). The altitude of the experimental site for
181 this study is very close to sea level, so $P/760 \approx 1$. Few sample sets were discarded
182 when they yielded a linear regression value of R^2 greater than 0.90.
183
184 **2.4 Grain yield and aboveground N uptake**
185 After crops reached physiological maturity (10 June 2011), grain and straw were
186 manually harvested from each plot. Grain and straw were air-dried, then further dried
187 for 3 days at 65 °C and weighed to obtain dry matter yields. Subsamples were ground
188 with a ball mill and analyzed for N concentration with an elemental N analyzer
189 (VarioMax, Elementar, Hanau, Germany). Total N content in aboveground biomass
190 was calculated from the sum of N masses harvested in grain and straw from each plot.
191
192 **2.5 Auxiliary variables**
193 Soil temperatures were measured, simultaneously with gas sampling, at vertical
194 depths of 5, 10 and 15 cm with a digital thermometer (Model 2455, Yokogawa, Japan).
195 Soil moisture was measured at 5 cm depth at three different positions in the vicinity of
196 each chamber using time domain reflectometry probes and was expressed as
197 water-filled pore space (WFPS) by the equation:
198 WFPS [%] = (volumetric water content [%]/total soil porosity [%]) × 100 (2)

where total soil porosity = 1 – (soil bulk density/2.65), with 2.65 [g cm^{-3}] 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_4^+-N and NO_3^--N concentrations were measured using a colorimetric method on a Skalar segmented flow analyzer (SAN^{++}, the Netherlands).

2.6 Data analysis and statistics

Average fluxes and standard errors of the N_2O fluxes were calculated from triplicate plots. Seasonal cumulative N_2O emissions were calculated using the following equation:

$$\text{Cumulative } N_2O \text{ emission} = \sum_{i=1}^{n} (F_i + F_{i+1})/2 \times (t_{i+1} - t_i) \times 24 \quad (3)$$

where F is the N_2O flux (μg N_2O-N m^{-2} h^{-1}), i is the ith 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_2O direct emission factor (%) of fertilizer N applied to the

soil with background adjustment was calculated as follows:

Emission factor = $((N_2O\text{-}N_{fertilizer} - N_2O\text{-}N_{control})/N_{fertilizer}) \times 100$ (4)

where $N_2O\text{-}N_{fertilizer}$ and $N_2O\text{-}N_{control}$ are the cumulative N_2O emissions (kg N_2O-N ha^{-1}) in the N-fertilized treatment and the control treatment, respectively, and $N_{fertilizer}$ is the amount of fertilizer N applied (kg N ha^{-1}). Yield-scaled N_2O emissions were calculated by dividing cumulative N_2O 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 kg N ha^{-1}). Soil inorganic N intensities were calculated separately for NH_4^+ (NH_4I), NO_3^- (NO_3I) and the sum of NO_3^- + NH_4^+ (IONI) as the summation of daily NH_4^+-N, NO_3^--N or (NO_3^- + NH_4^+)-N concentrations in the 0–20 cm layer over the same period as for cumulative N_2O emissions using linear interpolation between sampling dates, and presented in units of 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 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 using the Kolmogorov-Smirnov test 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 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 following heavy rainfall or irrigation events. Soil temperature at 5 cm depth was below zero in early January and increased to 23 °C in early June.

3.3 N_2O emissions

Variations of the N_2O fluxes over the wheat growth season are illustrated in Fig. 2. N_2O fluxes from all fertilizer-incorporated treatments showed almost the same pattern with peak N_2O fluxes being observed soon after application of basal fertilizers. The fluxes from all N fertilizer treatments were generally low on most of the other sampling dates and these fluxes were not significantly different from those from the control. On several occasions in the winter the fluxes were negative. No significant increases in the N_2O fluxes were found following the supplemental fertilization coupled with irrigation. N_2O fluxes did not increase after heavy rainfall events either.

The peak N_2O flux was 120.4 μg N_2O-N m^{-2} h^{-1} in the urea alone treatment. Compared with the urea only, application of NBPT, DCD or the combination of both reduced the peak fluxes by 41.1 %, 75.0 % and 61.2 %, respectively. Application of NP reduced peak fluxes by 69.1 % compared with application of urea alone. Analysis showed that the natural logarithms of the N_2O fluxes were weakly, but not significantly, correlated with soil WFPS in all treatments except the DCD treatment, but significantly ($P < 0.05$) correlated with soil temperature in all treatments except the NBPT + DCD treatment (Table 3).

Cumulative N_2O emissions from the different treatments are listed in Table 4. Total N_2O emissions from the control, urea alone, urea + NBPT, urea + DCD, urea +

NBPT + DCD and NP treatments were 0.16 ± 0.02, 0.49 ± 0.12, 0.31 ± 0.01, 0.28 ± 0.01, 0.26 ± 0.01 and 0.28 ± 0.03 kg N_2O-N ha^{-1}, respectively, over the wheat growth season. The highest total N_2O emission was found from the plot which only received urea. These emissions mainly occurred during the 18 day peak emission period following basal fertilizer application from 16 October to 3 November. Application of NBPT, DCD or the combination of both significantly reduced the seasonal N_2O emissions from urea by 36.7 %, 42.9 % or 46.9 %, respectively ($P < 0.05$). Compared with the emissions from the urea alone treatment, significantly lower N_2O emissions were also observed from the NP treatment ($P < 0.05$) (42.9 % less than those from the urea alone treatment). The direct N_2O 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_2O 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_2O 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_4^+ and NO_3^- concentrations

Soil NH_4^+ and NO_3^- concentrations drastically increased after application of basal N fertilizers compared with the control. The levels of NO_3^- in the NBPT and NBPT +

309 DCD treatments were relatively low for one week after basal fertilizer application
310 compared with those in the other treatments. However, the levels of NO_3^- in the
311 NBPT and NBPT + DCD treatments gradually increased, this was probably due to
312 degradation of NBPT and its subsequent loss of effectiveness. Following application
313 of supplemental fertilizer urea, no apparent increase in soil NO_3^- levels was observed,
314 and NO_3^- concentration kept at a relatively constant level. In contrast, soil NO_3^-
315 concentration following application of supplemental fertilizer NP showed a rapidly
316 decreasing trend. In all urea-added treatments, soil NO_3^- concentration sharply
317 decreased to less than 10 mg N kg^{-1} from 15 April onwards.

318 Soil NH_4^+ concentration increased from 2 to 10 mg N kg^{-1} after application of
319 basal fertilizer; however it sharply decreased soon afterwards. Application of DCD or
320 NBPT + DCD sustained soil NH_4^+ concentrations at higher levels compared with urea
321 application alone. In the NP treatment, soil NH_4^+ concentrations were always at low
322 levels. The natural logarithms of the N_2O fluxes were more correlated with NH_4^+
323 concentrations than with NO_3^- concentrations in the soil, despite the fact that a
324 significant relationship was only observed in the urea alone treatment.

325 Mean soil NH_4I levels in the NP treatment were the lowest among all N-added
326 treatments and tended to be higher in the DCD and NBPT + DCD treatments
327 compared with urea alone (Table 5). Mean soil NO_3I levels showed a similar trend
328 among the treatments and were ranked in the order of NBPT, NBPT + DCD > DCD >
329 urea alone, NP > control. Mean soil IONI levels were also similar among the
330 treatments and were ranked as NBPT + DCD > NBPT, DCD > urea alone, NP >

331 control.

332

333 **4 Discussion**

334 **4.1 Nitrous oxide emissions as affected by nitrogen sources**

335 Compared with the urea alone, application of NP significantly reduced N_2O emissions
336 by 42.9 % during the wheat growth season (Table 4), and increased wheat yield by
337 12.3 % and NUE by 24.7 % (Table 2). Ju et al. (2011) obtained a similar result in the
338 North China Plain, finding that emissions of N_2O derived from $Ca(NO_3)_2$ were lower
339 than those from $NH_4(SO_4)_2$ during the maize growth season (0.38–0.81 vs. 1.31–3.52
340 kg N_2O-N ha^{-1}). A lower N_2O emission for urea ammonium nitrate than for
341 anhydrous ammonia was also reported in a silt loam of the United States (Venterea et
342 al., 2005). In contrast, Gagnon et al. (2011) measured a significantly higher N_2O
343 emission following application of urea ammonium nitrate or calcium ammonium
344 nitrate compared with anhydrous ammonia in a poorly drained clay soil of Canada. In
345 a German grassland ecosystem, Müller and Sherlock (2004) found that the emissions
346 for ammonium-based fertilizer were lower than those for nitrate-based fertilizer.
347 These researchers suggested that higher emissions from nitrate-based fertilizers were
348 because of the propensity of the fine-textured clay soil to become anaerobic following
349 rainfall and a strong fixation of NH_4^+ in clay lattices reducing NH_4^+ available for N_2O
350 production (Chantigny et al., 2004). In this study, mean soil NO_3^- intensities (NO_3I)
351 were not significantly different between the NP and urea alone treatments (Table 5),
352 and mean NO_3^- concentrations (Fig. 3) were higher than the suggested threshold value

353 for denitrification of 5 mg N kg^{-1} (Dobbie and Smith, 2003) during the growth season
354 except for the period from 24 April to 10 June. These results imply that soil NO_3^-
355 concentration was not the only limiting factor affecting denitrification and N_2O
356 emission in the test soil.

357 The notable difference in the seasonal N_2O emissions between the NP and urea
358 alone treatments occurred mainly during the 18 day peak emission period following
359 the basal fertilizer application and concurrent irrigation from 16 October to 3
360 November. It has been reported that application of ammonium-based fertilizers
361 emitted more N_2O than nitrate-based fertilizers under aerobic soil conditions, while
362 application of nitrate-based fertilizers induced a greater increase in N_2O production
363 when soil conditions were anoxic (Pathak and Nedwell, 2001; Tenuta and Beauchamp,
364 2003). For cultivated soils, the primary mechanism of N_2O production is generally
365 believed to be the nitrification process when soil WFPS levels are between 30 % and
366 70 % and the denitrification process when soil WFPS levels were between 70 % and
367 90 % (Granli and Bøckman, 1994). Some other studies also suggest that
368 denitrification could in general produce more N_2O compared with nitrification (eg.
369 Dobbie et al., 1999). According to the studies of Ding et al. (2007) and Wan et al.
370 (2009), N_2O in sandy loam soils of the North China Plain was primarily produced by
371 nitrification unless soil WFPS reached 75 % or more. Pihlatie et al. (2004) reported
372 that even at 100 % WFPS in a loamy sand soil with 24 g organic C kg^{-1}, nitrification
373 was still the dominant N_2O production process. In this study, the highest soil WFPS
374 measured during the peak emission period was ~65 %; thus we suggest that low soil

moisture limited denitrification and N_2O 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_2O 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 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^{-1} 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^{-1} 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., 2014a). 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_2O

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_2O emissions from arable crops in the temperate climate zone, contributing ~ 70 % of the annual N_2O losses during the thawing from December to February. Wolf et al. (2010) also verified that N_2O pulses due to spring thaw dominated total annual N_2O 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_2O 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; Cui et al., 2012; Cai et al., 2013), 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_2O pulses in a silt 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_2O pulses from arable soils, as found in our study. However, Zhang

et al. (2014b) found that the North China Plain is a large agricultural N_2O source in China, contributing 36.3 % of the total annual N_2O emission from the China's croplands. To make a global comparison, we compiled the literature data of N_2O emissions from the temperate uplands under inorganic N fertilizer application in some countries of Asia, Europe and North America with similar latitudes to the studied region (Table 6). The emission factors of N applied in the North China Plain are generally lower than in the other countries, indicating a lower capacity of N applied being converted into N_2O in the test soil. This is probably because N_2O is predominantly produced from nitrification and denitrification is organic C-limited as discussed above. In contrast, the total N_2O emissions from the studied region are obviously higher due to the greater N fertilizer loading (Ju et al., 2009), suggesting that agricultural practices for reducing N_2O emission are urgently required. Our results confirm that N fertilizer sources influence soil N_2O 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_2O 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_2O 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_2O peak fluxes, and

442 cumulative N_2O emissions during the 18 day peak emission period were reduced by
443 50.0 % by NBPT, 78.6 % by DCD and 67.9 % by NBPT + DCD, compared with
444 application of urea alone. Our results indicate that the addition of DCD alone or in
445 combination with NBTP effectively reduced N_2O emissions from application of urea.
446 In other sites of the North China Plain, Liu et al. (2013) also reported that nitrification
447 inhibitors DCD and DMPP (3,4-dimethylpyrazole phosphate) could reduce N_2O
448 emissions from application of N fertilizers by 30 % and 21 %, respectively, during the
449 wheat growth season. Ju et al. (2011) observed no apparent differences in cumulative
450 N_2O emissions between zero N control and urea with DMPP during the maize growth
451 season, suggesting strong nitrification inhibition effectiveness of DMPP.

452 N_2O emission is directly related to the amount of mineral N available in the soil
453 and application of inhibitors with urea can effectively regulate the NO_3^- and NH_4^+
454 concentrations (Li et al., 2009; Zaman et al., 2009). Recently, Maharjan and Venterea
455 (2013) demonstrated that N_2O emissions were more correlated with soil NO_2^-
456 intensity rather than NO_3^- or NH_4^+ intensity, and that inhibitors controlled N_2O
457 production by adjusting soil NO_2^- intensity. In this study, soil NH_4^+ concentration
458 slightly increased in the presence of DCD and, in contrast, relatively low NH_4^+
459 concentration was found after NBPT application following application of basal
460 fertilizer. NBPT delays urea hydrolysis, thereby lowering soil pH elevation and NH_4^+
461 production, which can in turn reduce NH_3 toxicity effects on nitrite-oxidizing bacteria
462 (NOB). DCD slows oxidation of NH_4^+ to NO_2^- mainly by inhibiting activities of
463 ammonia-oxidizing bacteria (AOB), which allows NOB to use NO_2^- 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_2O from urea in the soil. Thus, the reduction of N_2O emissions by inhibitors is probably due to both low oxidation rate of NH_4^+ and low NO_2^- concentration, thereby reducing N_2O "leaking" as a by-product of nitrification (Firestone and Davidson, 1989).

Following supplemental fertilization with or without inhibitors, no distinct N_2O flux peaks were found in our study. This may be attributable to no significant increase of soil NH_4^+ and NO_3^- 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_3 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_2O 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 alkaline soil with low urease activity. In contrast, the temperature decrease increased the inhibitory effectiveness of NBPT on urea hydrolysis. In this

study, soil temperature measured in the field after urea top-dressing varied from 2 °C to 9 °C, close to or just above the thresholds for nitrification (above 5.0 °C) (Anderson et al., 1971) and urea hydrolysis (~ 2 °C) (Xu et al., 1993; Yadav et al., 1987). It is obvious that low soil temperature led to the absence of fertilizer N-induced N_2O flux peaks following the supplemental fertilization and urease or nitrification inhibitors should not necessarily be applied with supplemental fertilizers during the wheat growth season.

Application of urea with NBPT and/or DCD compared with urea alone slightly increased wheat yields, which differs from application of NP. Similar results were also obtained by Ju et al. (2011) and Liu et al. (2013) in the North China Plain. A meta-analysis of data measured in Germany showed that N fertilizers with nitrification inhibitors did not significantly influence the yields of all investigated crops (Hu et al., 2014). The absence of inhibitor effects on crop yields might have been ascribed to the following three reasons. Firstly, low precipitation during the wheat growth season reduced the risk of N leaching and resulted in low N losses. This is evidenced by the significant stimulation of NP on wheat yields. Secondly, it is well known that the application rate of N fertilizers is far above optimum for crops (West et al., 2014). The overloading of N fertilizer might mask the influence on crop yields of increased mineral N in soils caused by inhibitors. The result of Sharma and Prasad (1996) supported the hypothesis that application of DCD significantly increased maize yield when the application rate of fertilizer N was as low as 60 kg N ha^{-1}. It should be noted that the increase in NH_4^+ concentration in the test soil due to DCD

application alone following the basal fertilization may stimulate NH_3 volatilization, resulting in higher N losses compared with urea alone, since NH_3 volatilization accounted for ~ 13 % of N applied (Ni et al., 2009). Mahmood et al. (2011) demonstrated that application of DCD to an alkaline calcareous soil increased fertilizer N losses. Finally, also more importantly, application of DCD with supplemental fertilizer slowed the nitrification rate and then lowered NO_3^- supply for wheat growth when it was at the rapid growth stage. A lower soil NO_3^- concentration in the NP treatment than in the urea-added 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_2O 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_2O 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_2O flux peak was found following basal fertilization during the wheat growth period. Application of urea with NBPT, DCD or NBPT + DCD significantly reduced N_2O emissions from urea by 36.7 %, 42.9 % or 46.9 %, respectively. Application of nitrophosphate also resulted in reduction of total N_2O emissions by 42.9 %, compared

with application of urea alone. NBPT and/or DCD were effective in reducing N_2O 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_2O flux was primarily affected by soil temperature and low temperature at the study site minimized fertilizer N-induced N_2O 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_2O emission. As well, this study suggests that application of nitrophosphate, instead of urea, is an optimum agricultural strategy for reducing N_2O emission and for increasing crop yield and NUE for wheat cultivation in the soils of the North China Plain.

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785 **Table 1.** Soil properties.

Soil depth	pH	Bulk density	Organic C	Total N	C/N	NO_3^--N	NH_4^+-N	Particle size distrubution (%)		
(cm)	(H_2O)	(Mg m^{-3})	(g C kg^{-1})	(g N kg^{-1})		(mg N kg^{-1})	(mg N kg^{-1})	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

786

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

788 efficiency.

Treatment	Biomass (kg ha^{-1})			Amount of N uptake (kg N ha^{-1})			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	10131±370 a	67.0±1.8 a	41.2±2.1 a	108.2±3.7 a	35.9 ±1.1 a

789 Mean ± standard error (n = 3).

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

Table 3. Correlation between ln [N_2O flux + 1] and soil WFPS, soil temperature at depths of 5 (T_{5cm}), 10 (T_{10cm}) and 15 cm (T_{15cm}), ammonium (NH_4^+-N), nitrate (NO_3^--N) or inorganic nitrogen (NH_4^+-N plus NO_3^--N) concentration.

Treatment	WFPS	T_{5cm}	T_{10cm}	T_{15cm}	NH_4^+-N	NO_3^--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

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

Table 4. Effects of urease and/or nitrification inhibitors and nitrophosphate on cumulative N_2O emissions, fertilizer N-induced N_2O emission factors and yield-scaled N_2O emissions.

Treatment	Cumulative N_2O emission (kg N_2O-N ha^{-1})		Ratio of peak to total emissions (%)	Emission factor (% of applied N)	Yield-scaled N_2O emission (g N_2O-N kg^{-1} 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 significantly difference between treatments at $P < 0.05$.

800 **Table 5.** Effects of urease and/or nitrification inhibitors and nitrophosphate on soil

801 ammonium (NH_4I), nitrate (NO_3I) and inorganic N (IONI) intensities.

Treatment	NH_4I (g N d kg^{-1})	NO_3I (g N d kg^{-1})	IONI (g N d kg^{-1})
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

802 Mean±standard error (n = 3). Different letters within the column indicate significantly

803 difference between treatments at $P < 0.05$.

804 **Table 6.** Summary of N_2O emissions from uplands under inorganic fertilizer application in the countries with temperate climate.

Site	MAT (°C)	MAP (mm)	SOC (g C kg^{-1})	pH	Warm season				Cold season				Whole year			Reference
					Crop	Applied N (kg N ha^{-1})	N_2O emission (kg N ha^{-1})	N_2O EF (%)	Crop	Applied N (kg N ha^{-1})	N_2O emission (kg N ha^{-1})	N_2O EF (%)	Applied N (kg N ha^{-1})	N_2O emission (kg N ha^{-1})	N_2O EF (%)	
Fengqiu, China	14	615	7	8.7	Maize	250	3.8	1.3	Wheat	250	0.6	0.3	500	4.5	0.8	Ding et al. (2007)
Huantai, China	13	586	10	8.3	Maize	330	1.6	0.4	Wheat	270	2.4	0.8	600	4.0	0.6	Cui et al. (2012)
Baoding, China	12	555	9	8.1	Maize	173	4.5	2.2	Wheat	165	3.3	1.3	338	7.7	1.8	Zhang et al. (2014c)
Tsukuba, Japan	16	1460	19	5.7	Soybean	20	2.7	13	Wheat	100	0.5	0.5	120	3.2	2.7	Nishimura et al. (2005)
Fukushima, Japan	14	1207	14	7.4	Barley	150	3.2	2.0	–	–	–	–	–	–	–	Shoji et al. (2001)
Madrid, Spain	13	430	8	7.3	Onion	110	0.8	0.6	Fallow	0	0.25	–	110	1.2	0.7	Meijide et al. (2009)
Lavesum, Germany	10	887	18	5.3	Wheat	220	0.6	0.2	Fallow	0	1.0	–	220	1.9	0.5	Lebender et al. (2014)
Turin, Italy	12	734	10	8.1	Maize	130	0.0	0.0	Fallow	0	2.9	–	130	2.9	3.4	Alluvione et al. (2010)
Boone, USA	9	825	33	7.2	Maize	168	2.9	1.0	–	–	–	–	–	–	–	Parkin and Hatfield (2014)
Michigan, USA	8	628	20	7.0	Maize	225	3.9	1.4	–	–	–	–	–	–	–	Hoben et al. (2011)
Michigan, USA	8	628	20	7.0	Maize	180	2.5	1.2	–	–	–	–	–	–	–	Hoben et al. (2011)
Michigan, USA	8	628	20	7.0	Maize	135	1.7	0.9	–	–	–	–	–	–	–	Hoben et al. (2011)
Michigan, USA	8	628	20	7.0	Maize	90	1.1	0.7	–	–	–	–	–	–	–	Hoben et al. (2011)
Michigan, USA	8	628	20	7.0	Maize	45	0.9	1.1	–	–	–	–	–	–	–	Hoben et al. (2011)
Morris, USA	6	645	32	7.2	Maize	78	–	–	Fallow	0	–	–	78	5.2	3.0	Johnson et al. (2012)
Morris, USA	6	645	32	7.2	Wheat	78	–	–	Fallow	0	–	–	78	4.2	2.8	Johnson et al. (2012)

805 MAT, mean annual temperature; MAP, mean annual precipitation; EF, the N_2O emission factor of applied N.

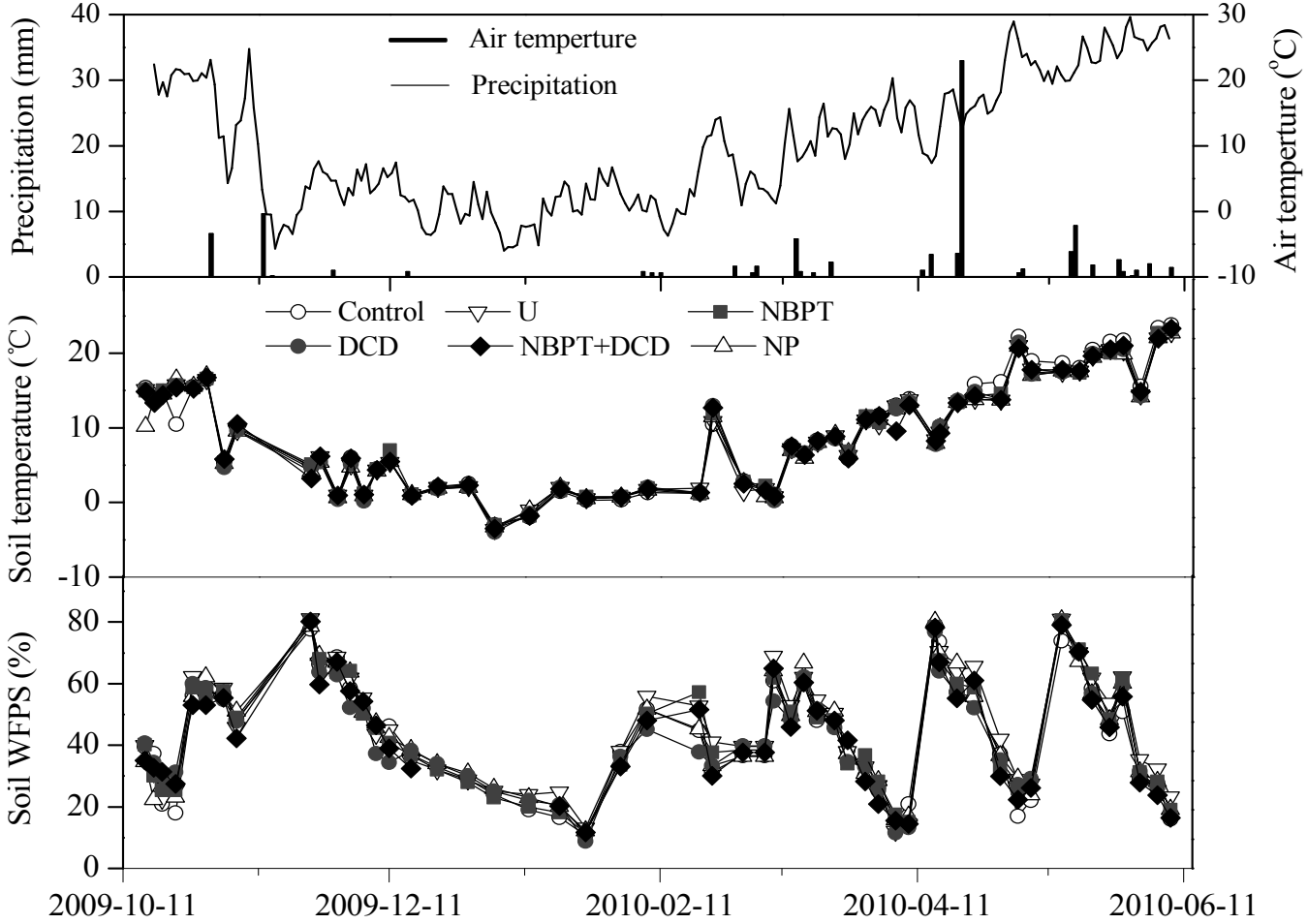
Figure caption

Figure 1. Temporal variation of daily precipitation and air temperature, and mean soil moisture and water-filled pore space (WFPS) at time of N_2O 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.

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 ± standard errors for three replicates. Arrows indicate date of fertilizer application.

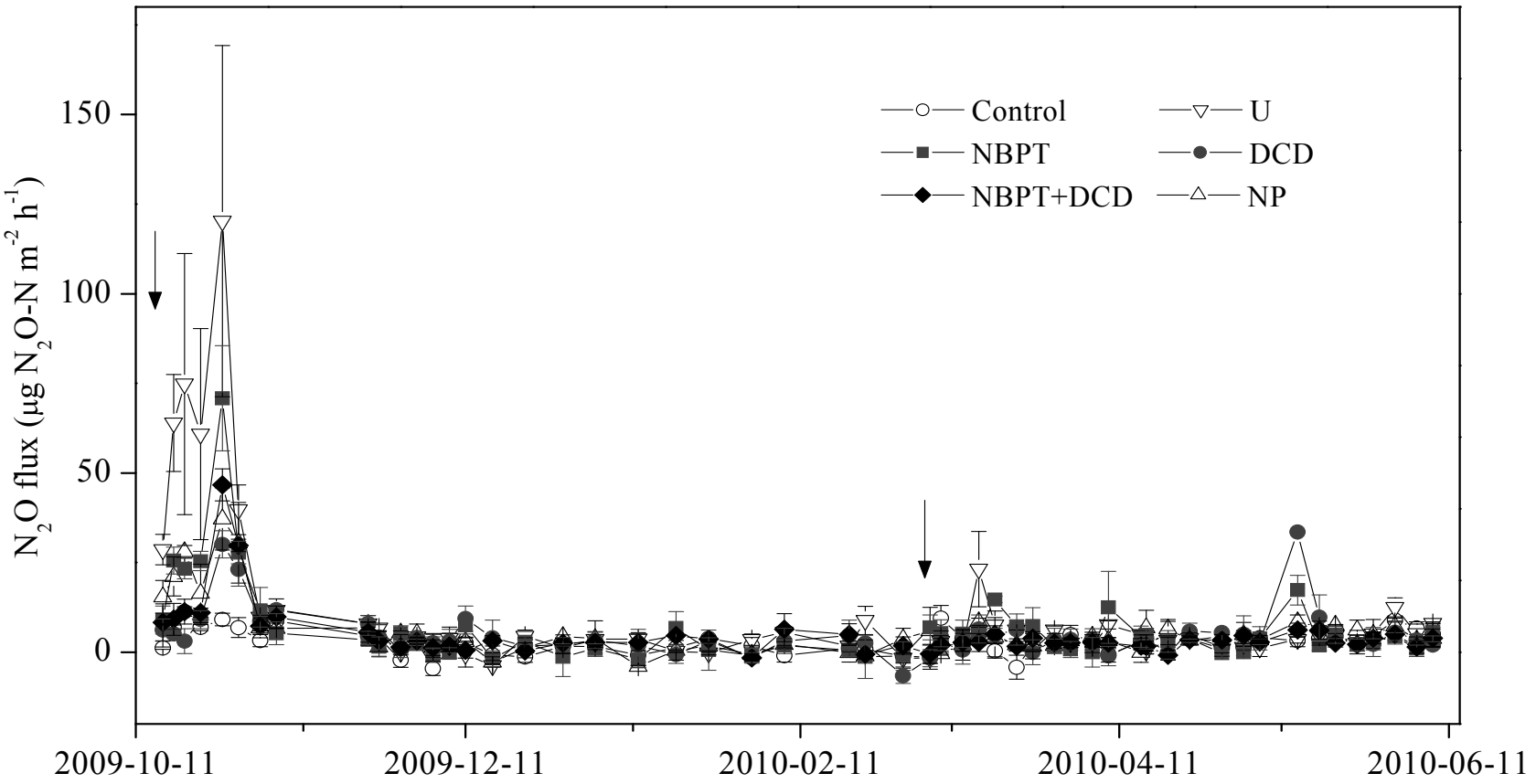
Figure 3. Temporal variation of ammonium and nitrate concentrations in samples from the 0–20 cm depth in the control, urea alone (U), urea with NBPT (NBPT), urea with DCD (DCD), urea with NBPT + DCD (NBPT + DCD) and nitrophosphate (NP) treatments during the wheat growth season. Vertical bars denote the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

825 **Figure 1**



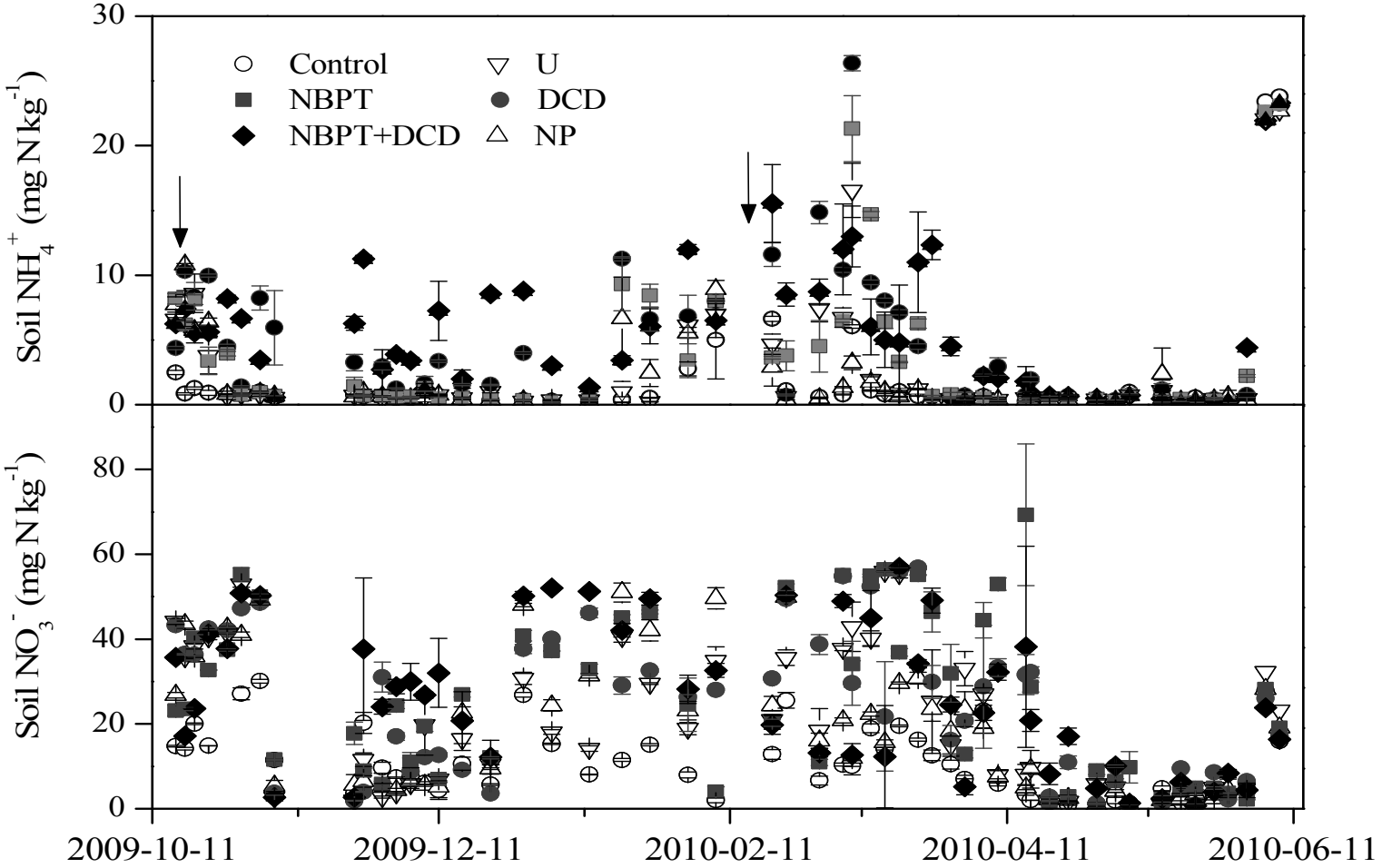
826

827 **Figure 2**



828

829 **Figure 3**



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