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Effects of nitrification inhibitors (DCD and DMPP) on nitrous oxide emission, crop yield and nitrogen uptake in a wheat–maize cropping system

C. Liu, K. Wang, and X. Zheng

State Key Laboratory of Atmospheric Boundary Layer Physics and Atmospheric Chemistry (LAPC), Institute of Atmospheric Physics, Chinese Academy of Sciences (IAP-CAS), Beijing 100029, China

Correspondence to: X. Zheng (xunhua.zheng@post.iap.ac.cn)

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Abstract. The application of nitrification inhibitors together with ammonium-based fertilizers is proposed as a potent method to decrease nitrous oxide (N_2O) emission while promoting crop yield and nitrogen use efficiency in fertilized agricultural fields. To evaluate the effects of nitrification inhibitors, we conducted year-round measurements of N_2O fluxes, yield, aboveground biomass, plant carbon and nitrogen contents, soil inorganic nitrogen and dissolved organic carbon contents and the main environmental factors for urea (U), urea + dicyandiamide (DCD) and urea + 3,4-dimethylpyrazol phosphate (DMPP) treatments in a wheat–maize rotation field. The cumulative N_2O emissions were calculated to be 4.49 ± 0.21, 2.93 ± 0.06 and 2.78 ± 0.16 kg N ha^{-1} yr^{-1} for the U, DCD and DMPP treatments, respectively. Therefore, the DCD and DMPP treatments significantly decreased the annual emissions by 35 % and 38 %, respectively ($p < 0.01$). The variations of soil temperature, moisture and inorganic nitrogen content regulated the seasonal fluctuation of N_2O emissions. When the emissions presented clearly temporal variations, high-frequency measurements or optimized sampling schedule for intermittent measurements would likely provide more accurate estimations of annual cumulative emission and treatment effect. The application of nitrification inhibitors significantly increased the soil inorganic nitrogen content ($p < 0.01$); shifted the main soil inorganic nitrogen form from nitrate to ammonium; and tended to increase the dissolved organic carbon content, crop yield, aboveground biomass and nitrogen uptake by aboveground plant. The results demonstrate the roles the nitrification inhibitors play in enhancing yield and nitrogen use efficiency and reducing N_2O emission from the wheat–maize cropping system.

1 Introduction

Nitrogen (N) is an essential nutrient for all crops. In the past several decades, the global growth of crop yield has mainly been dependent on the increasing application rates of synthetic fertilizer. Further increases in fertilizer rates are unlikely to be effective at increasing crop yields, as the use efficiency of fertilizer N sharply declines at higher application rates (Tilman et al., 2002). A significant percentage of fertilizer N flows to aquatic systems and the atmosphere via runoff of ammonium (NH_4^+), nitrate (NO_3^-) leaching and gaseous N emissions (Ju et al., 2009). Attention to N fertilizer application has shifted from the role of promoting crop production to environmental pollution. There are a variety of new management practices and technologies that can promote N use efficiency and alleviate environmental pollution. One of the mitigation technologies that has proved to be highly effective in reducing fertilizer N losses and increasing N use efficiency and yield in a few cropping systems is the application of nitrification inhibitors (Majumdar et al., 2002; Zaman et al., 2009; Cui et al., 2011; Moir et al., 2012).

Nitrification inhibitors can delay the microbial oxidation of NH_4^+ to nitrite (NO_2^-) for a certain period (several weeks or months) and are therefore very effective at blocking microbial nitrification and subsequent denitrification (Weiske et al., 2001; Zerulla et al., 2001). Hundreds of nitrification

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inhibitors are known, but only a few so far have gained commercial importance for practical use, such as dicyandiamide (DCD) and 3,4-dimethylpyrazol phosphate (DMPP). The application of DCD and DMPP together with NH_4^+-based fertilizers, cow urine or cattle slurry has demonstrated efficiency in reducing the N losses in forms of nitrous oxide (N_2O) emission and NO_3^- leaching while increasing the yield and use efficiency of fertilizer N in croplands and grasslands (Weiske et al., 2001; Majumdar et al., 2002; Zaman et al., 2009; Cui et al., 2011; Di and Cameron, 2012; Moir et al., 2012; Pfab et al., 2012). Compared with DCD, a comparable or even better inhibition effect on N_2O emission and NO_3^- leaching can be achieved with approximately 1/25 to 1/2 of the application rate for DMPP (Weiske et al., 2001; Belastegui-Macadam et al., 2003; Di and Cameron, 2012). The extent to which DCD and DMPP inhibit N_2O emission and NO_3^- leaching is primarily dependent on factors such as the application rate, time and method of nitrification inhibitors (Barth et al., 2008; Verma et al., 2008; Zaman and Blennerhassett, 2010; Zaman and Nguyen, 2012); field management (irrigation, type, geometry and application method of NH_4^+-based fertilizers, Sanz-Cobena et al., 2012); climate (precipitation and temperature, Shepherd et al., 2012); and soil properties (moisture, pH, texture, organic carbon and mineral N, Barth et al., 2001; Shepherd et al., 2012).

The roles DCD and DMPP application play in yield, plant N uptake, soil inorganic N ($NH_4^+ + NO_3^-$) stock and N_2O emission need to be further evaluated in different cropping systems and climate zones. Furthermore, high-frequency and year-round measurements are recommended to lower uncertainty in the evaluation of the inhibition effects of DCD and DMPP on N_2O emissions. Therefore, we carried year-round measurements of N_2O fluxes using an automated chamber system, crop yield, plant carbon (C) and N contents, soil inorganic N and dissolved organic carbon (DOC) contents, and the main environmental factors in a typical wheat–maize rotation field in northern China. The aims of the study were to quantify the effects of the use of DCD and DMPP coated on urea on N_2O emission, soil inorganic N and DOC stocks, crop yield and plant N uptake in the wheat–maize rotation system, which is the most popular double-cropping system in China.

2 Materials and methods

2.1 Experimental site

The experimental site (34°55.51′ N, 110°42.59′ E) is situated within the Dong Cun Farm in Yongji county, Shanxi province, northern China. The wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) were sown on 21 October 2009 and 17 June 2010, and were harvested on 15 June and 14 October 2010, respectively. The stubble was mechanically chopped into pieces of 5–10 cm after harvest and ploughed into the soil (0–20 cm) just before seeding. A manually movable sprinkler irrigation system was used to irrigate the crops using underground water (depth: 130–140 m). Nitrogen fertilizer in the form of urea was applied three times per year at sowing time and the turning-green stage of wheat and the 18- to 19-leaf stage of maize. Phosphate (P) and potassium (K) fertilizers in the forms of calcium superphosphate and potassium sulfate were applied at the wheat sowing time together with urea. The annual fertilizer rates were 430-60-30 kg N-P-K ha^{-1}. Detailed information regarding the field management can be found in Table 1. In addition, the meteorological data, main soil properties and management history of the experimental field are provided in Liu et al. (2011, 2012).

Three treatments (urea, urea + DCD, urea + DMPP, hereafter refer as U, DCD and DMPP, respectively) were set up on 16 October 2009. Twelve experimental plots (6×6 m each) with four replicates of each treatment were established by a randomized design. The nitrification inhibitors were coated on the granules of urea with mass ratios of 1.4 % (DCD : urea) and 0.464 % (DMPP : urea). The application rates were 6 and 2 kg ha^{-1} yr^{-1} for the DCD and DMPP treatments, respectively.

2.2 Measurement of nitrous oxide flux

Nitrous oxide fluxes were continuously measured for the U, DCD and DMPP treatments between 20 October 2009 and 15 October 2010, using an automated chamber system, as described by Liu et al. (2010, 2011). Twelve static translucent chambers (length × width × height = 70 × 70 × 90 cm) were attached to the system, and therefore each treatment had four replicated chambers. The translucent chambers were made of polycarbonate with thickness of 1 mm and stainless steel. All chambers were fixed on stainless steel frames, which were inserted 10 cm into the soil in the center of each plot. To minimize chamber effects, two frames were installed for each chamber so that each chamber could be swapped between two positions on a bi-weekly basis. Whenever a difference of plant growth between inside and outside of chambers was visible, the base frames were moved to new locations. Rubber seals ensured the gas-tightness of the joints of the chamber and frame when the chambers were closed. A simple ventilation tube (inner diameter: 3.95 mm; length: 40 cm) was installed on the top of each chamber. The chambers could cover the wheat and the maize seedlings. When the maize height was above 90 cm, we moved the chambers and frames to the space between rows and measured the emissions from the soil. Five gas samples were sampled during the chamber closure time of 38–44 min and were transported (flow rate: 500 ml min^{-1}) to the analysis system. The latter consisted of a gas chromatograph (GC, Agilent 4890D, Agilent Technologies Inc., USA) that was equipped with an electron capture detector. The gas chromatograph configurations described by Zheng et al. (2008) were adopted for the N_2O con-

Table 1. Information regarding main field management.

Date	Field management
17 Oct 2009	Chopping of stubble
21 Oct 2009	Straw tillage, fertilization (60-60-30 kg N-P-K ha^{-1}, tillage for 20 cm after surface broadcast), and wheat sowing (20–23 cm row spacing)
9 Jan 2010	Irrigation (89.4 mm)
17 Mar 2010	Herbicide spraying (atrazine)
18 Mar 2010	Fertilization (120 kg N ha^{-1} soil covering for 0–5 cm after band application)
23 Mar 2010 (19:00)–24 Mar 2010 (14:00)	Irrigation (86.7 mm)
4 May 2010 (07:00)–5 May 2010 (19:00)	Irrigation (77.6 mm)
15 Jun 2010	Wheat harvest
16 Jun 2010	Chopping of stubble
17 Jun 2010	Straw tillage and maize sowing (60 cm row spacing and 20–22 cm plant spacing)
28 Jun 2010 (23:00)–19 Jun 2010 (10:00)	Irrigation (60.5 mm)
29 Jun 2010	Herbicide spraying (atrazine)
13 Jul 2010	Fertilization (250 kg N ha^{-1} soil covering for 0–5 cm after band application)
21 Jul 2010	Insecticide spraying (mixture of emamectin benzoate and chlorpyrifos)
7 Aug 2010 (08:00)–9 Aug 2010 (14:00)	Irrigation (93.5 mm)
14 Oct 2010	Maize harvest

centration analysis. Each flux was calculated from five N_2O concentrations of the chamber headspace air using a first-order differential or linear equation (Liu et al., 2010; Wang et al., 2013). Every hour, only one of the four chambers for each treatment was closed. Thus, 24 fluxes were obtained daily from the four replicate chambers (six fluxes per replicate) for each treatment if the automated system ran properly. When the Spearman correlation coefficients of linear and nonlinear fittings between N_2O concentrations and sampling time differences for the five samples were higher than 0.88, the fluxes were regarded as valid. The daily mean fluxes were calculated as the arithmetic average values of valid flux. The missing daily fluxes due to power failure and system maintenance were replaced by means of daily fluxes of adjacent four days to calculate the cumulative emissions. The detection limit of N_2O flux was estimated to be 5.4 µg N m^{-2} h^{-1} for a chamber height of 90 cm, a chamber closure time of 44 min and a gas chromatograph precision of ±3.5 ppb. The fluxes that were less than the detection limit were still used for the calculation of daily mean fluxes and were regarded as the random values between 0 and 5.4 µg N m^{-2} h^{-1}.

2.3 Auxiliary measurements

In addition to flux measurements, we also measured crop yield, aboveground biomass, C and N contents of crop straw and grain, air temperature (height: 1.5 m), atmospheric pressure, precipitation, irrigation amount, soil temperature (5 cm), soil volumetric water content (0–6 cm), soil NH_4^+, NO_3^- and DOC contents (0–10 cm). Due to the effects of chamber closure on plant growth, plant samples were taken outside of the chambers. At harvest, three replicates (0.36 m^2 each for wheat and 2 m^2 each for maize) for each treatment were harvested to measure the crop yield and aboveground biomass by oven drying at 105 °C for 30 min and then 80 °C for two days. The C and N contents of harvested crop straw and grain were measured by the potassium dichromate-volumetric method and the semi-micro Kjeldahl method, respectively. The observed volumetric water content was converted into water-filled pore space (WFPS) using the determined bulk density of 1.17 g cm^{-3} and a theoretical particle density of 2.65 g cm^{-3}. Details of the methods for auxiliary measurement can be found in Liu et al. (2010, 2011, 2012).

2.4 Statistical analysis

The software packages SPSS Statistics Client 19.0 (SPSS China, Beijing, China) and Origin 8.0 (OriginLab Ltd., Guangzhou, China) were used for the statistical data analysis. A general linear model for repeated measures (soil moisture, NO_3^-, NH_4^+ and DOC contents), a nonparametric test of two related samples (N_2O flux and soil temperature) and two independent samples (crop yield, C and N contents of crop straw and grain) were used to analyze the significance of differences between treatments for the wheat season, maize season and annual scale. Linear and nonlinear regressions were

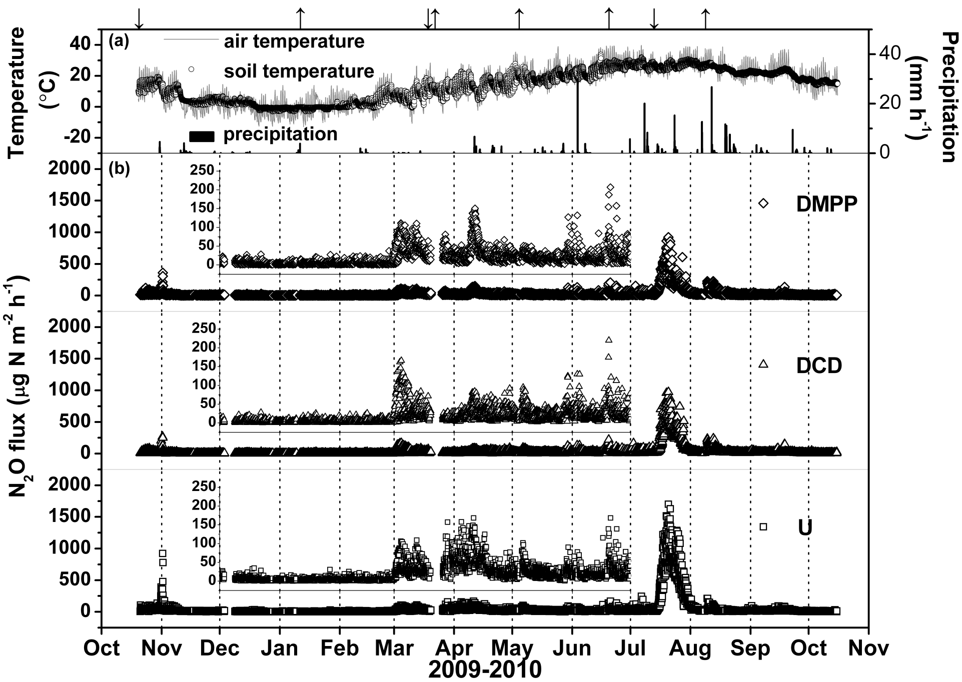

Fig. 1. Temporal courses of hourly **(a)** air and soil temperatures, precipitation, and **(b)** N_2O flux for urea (U), urea + DCD (DCD) and urea + DMPP (DMPP) treatments. The upward (↑) and downward (↓) arrows indicate the dates of irrigation and fertilization, respectively.

applied to describe the relationships among soil moisture (WFPS), temperature, inorganic N content and N_2O flux. The significance of linear and nonlinear regressions was determined using an F test.

3 Results

3.1 Nitrous oxide flux

We obtained 7018, 6896 and 6994 valid fluxes for the U, DCD and DMPP treatments, respectively, approximately 14–19 % of which were below the estimated detection limit ($5.4\,\mu g\,N\,m^{-2}\,h^{-1}$). The low fluxes ($< 5.4\,\mu g\,N\,m^{-2}\,h^{-1}$) were mainly observed between November 2009 and February 2010 (Fig. 1). The cumulative emissions during the period only accounted for 5–7 % of the annual total emissions. The field management of fertilization, irrigation, straw application and the following rain events after fertilization can significantly enhance N_2O emissions (Fig. 1a and b; Table 1). The fertilization on 13 July 2010, and the following frequent rain events resulted in extremely high emissions (Fig. 1a and b). The high emissions (daily mean $> 6\,mg\,N\,m^{-2}\,d^{-1}$ or $250\,\mu g\,N\,m^{-2}\,h^{-1}$) were continuously observed from 16–30 July 2010, and contributed 36–55 % of the annual emissions.

The calculated mean (± s.e.) fluxes were 53.0 ± 7.9, 34.6 ± 4.2 and $32.5 \pm 3.3\,\mu g\,N\,m^{-2}\,h^{-1}$ for the U, DCD and DMPP treatments, respectively. The cumulative emissions were estimated to be 4.49 ± 0.21, 2.93 ± 0.06 and $2.78 \pm 0.16\,kg\,N\,ha^{-1}\,yr^{-1}$ for the U, DCD and DMPP treatments, respectively (Table 2). The application of the DCD and DMPP nitrification inhibitors decreased the cumulative emissions by 1.56 and $1.71\,kg\,N\,ha^{-1}\,yr^{-1}$ (equal to 35 % and 38 % of the annual emission of the U treatment), respectively. The significant inhibition effects of the nitrification inhibitors on N_2O emissions were immediately detected after the application on 21 October 2009, and 18 March and 13 July 2010, and lasted for 44, 24 and 23 days, respectively. The application of nitrification inhibitors on 13 July 2010 significantly decreased the cumulative N_2O emissions of 1.25 and $1.53\,kg\,N\,ha^{-1}$ (equal to 80 % and 89 % of the annual inhibition effects, $p < 0.01$) within the 23 days for the DCD and DMPP treatments, respectively, compared with the U treatment.

3.2 Environment, soil inorganic nitrogen and dissolved organic carbon

The annual mean (± s.e.) air temperature was 13.8 ± 0.6 °C. The soil temperatures ranged from −1.9 to 29.0 °C, with annual means of 13.4 ± 0.5, 13.5 ± 0.5 and 13.6 ± 0.5 °C for the U, DCD and DMPP treatments, respectively (Fig. 2a; Table 2). The annual total precipitation and irrigation amount were 666.2 mm and 407.7 mm, respectively. The soil WFPS

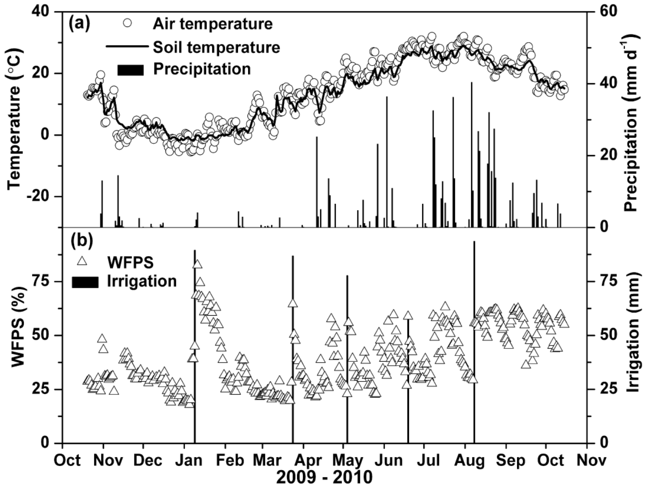

Fig. 2. Temporal courses of **(a)** daily averaged air and soil temperatures, daily precipitation, and **(b)** soil water-filled pore space (WFPS) and irrigation amount at the experimental field (urea treatment).

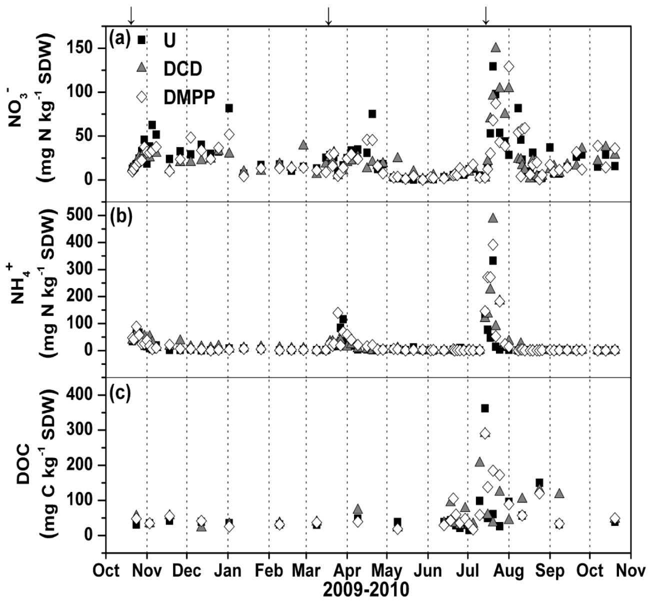

Fig. 3. Temporal courses of soil **(a)** nitrate (NO_3^-), **(b)** ammonium (NH_4^+) and **(c)** dissolved organic carbon (DOC) contents for the urea (U), urea + DCD (DCD) and urea + DMPP (DMPP) treatments. SDW: soil dry weight. The downward (↓) arrows indicate the fertilization dates.

ranged from 17.2 % to 82.8 %, with annual means of 39.2 ± 0.7 %, 39.4 ± 0.8 % and 39.1 ± 0.7 % for the U, DCD and DMPP treatments, respectively (Fig. 2b; Table 2).

The soil NO_3^- contents varied between 0.3 and 149.5 mg N kg^{-1} soil dry weight (SDW), with annual means of 24.5 ± 2.6, 22.7 ± 2.8 and 22.4 ± 2.4 mg N kg^{-1} SDW for the U, DCD and DMPP treatments, respectively. The soil NH_4^+ contents ranged from 0.1 to 486.3 mg N kg^{-1} SDW, with annual means of 17.5 ± 4.9, 28.0 ± 7.4 and 29.0 ± 7.5 mg N kg^{-1} SDW for the U, DCD and DMPP treatments, respectively (Table 2). The fertilization events considerably promoted the soil inorganic N ($NO_3^- + NH_4^+$) contents. The maximum values were observed on the seventh day after fertilization on 13 July 2010, for all treatments (Fig. 3a and b). The application of nitrification inhibitors slightly decreased the annual means of the soil NO_3^- content and significantly increased the soil NH_4^+ contents ($p < 0.01$, Table 2). Therefore, the annual means of the total inorganic N content were 21 % and 22 % higher for the DCD and DMPP treatments, respectively, than for the U treatment ($p < 0.01$). Furthermore, the application of nitrification inhibitors shifted the main form of soil inorganic N from NO_3^- to NH_4^+ (Fig. 3a and b; Table 2).

The soil DOC contents varied between 15.6 and 362.1 mg C kg^{-1} SDW, with annual means of 57.0 ± 12.9, 70.3 ± 11.8 and 70.1 ± 12.1 mg C kg^{-1} SDW for the U, DCD and DMPP treatments, respectively (Fig. 3c; Table 2). The maximum values were obtained on the second day after fertilization on 13 July 2010, for all treatments (Fig. 3c). The application of nitrification inhibitors tended to increase the soil DOC contents. However, the trend was not statistically significant due to the high spatial heterogeneity for the DOC measurements.

3.3 Yield, aboveground biomass, crop carbon and nitrogen uptakes

The crop yields for the U, DCD and DMPP treatments were 6.7 ± 0.6, 7.1 ± 0.2 and 7.1 ± 0.6 ton ha^{-1} for the wheat season and 6.3 ± 0.4, 7.0 ± 0.8 and 7.1 ± 0.4 ton ha^{-1} for the maize season, respectively. The aboveground biomass for the U, DCD and DMPP treatments was 15.1 ± 1.0, 15.8 ± 0.5 and 15.6 ± 1.4 ton ha^{-1} for the wheat season and 18.1 ± 0.8, 20.5 ± 1.2 and 20.5 ± 0.8 ton ha^{-1} for the maize season, respectively (Table 2). The annual crop yield and aboveground biomass increased by 8.5–9.1 % (1.1–1.2 ton ha^{-1} yr^{-1}) and 8.6–9.7 % (2.8–3.2 ton ha^{-1} yr^{-1}) for the DCD and DMPP treatments, respectively, compared with the U treatment. The application of nitrification inhibitors also tended to increase the N contents of grain, especially for maize ($p < 0.05$). Due to the increases in crop yield, aboveground biomass and N content of grain, the N uptakes of grain and aboveground plant were 12.8–15.8 % (29.4–36.1 kg N ha^{-1} yr^{-1}) and 10.9–13.2 % (44.0–53.5 kg N ha^{-1} yr^{-1}) higher for the DCD and DMPP treatments than with the U treatment. The C fixations by grain and aboveground plant also increased by 9.5–9.6 % (0.5–0.6 ton C ha^{-1} yr^{-1}) and 8.9–9.6 % (1.3–1.4 ton C ha^{-1} yr^{-1}) for the DCD and DMPP treatments compared with the U treatment (Table 2).

Table 2. Average (AF, µg N m^{-2} h^{-1}), median (MF, µg N m^{-2} h^{-1}) and cumulative fluxes of N_2O (CF, kg N ha^{-1}), averaged soil temperature (ST, °C), water-filled pore space (WFPS, %), nitrate (NO_3^-, mg N kg^{-1} SDW), ammonium (NH_4^+, mg N kg^{-1} SDW) and dissolved organic carbon contents (DOC, mg C kg^{-1} SDW), grain yield (ton ha^{-1}), aboveground biomass (AB, ton ha^{-1}), carbon (GC and APC, ton C ha^{-1}) and nitrogen uptakes (GN and APN, kg N ha^{-1}) by grain and aboveground plants for the urea (U), urea + DCD (DCD) and urea + DMPP (DMPP) treatments.

	Wheat season			Maize season			Annual scale		
	U	DCD	DMPP	U	DCD	DMPP	U	DCD	DMPP
AF	22.9^a	16.0^b	18.2^c	109.0^a	69.1^a	59.2^b	53.0^a	34.6^b	32.5^b
	(1.6)	(0.9)	(1.1)	(21.5)	(11.4)	(8.8)	(7.9)	(4.2)	(3.3)
MF	16.7	11.7	14.1	25.0	24.1	24.4	21.0	19.7	18.6
CF	1.32^a	0.92^b	1.05^b	3.18^a	2.02^b	1.73^b	4.49^a	2.93^b	2.78^b
	(0.06)	(0.02)	(0.06)	(0.24)	(0.05)	(0.12)	(0.21)	(0.06)	(0.16)
ST	8.3^a	8.5^a	8.4^a	23.5^a	23.5^a	23.8^a	13.4^a	13.5^a	13.6^a
	(0.5)	(0.4)	(0.5)	(0.3)	(0.3)	(0.3)	(0.5)	(0.5)	(0.5)
WFPS	33.4^a	33.5^a	33.3^a	49.0^a	49.2^b	48.7^c	39.2^a	39.4^a	39.1^a
	(0.8)	(0.8)	(0.8)	(1.0)	(1.0)	(1.0)	(0.7)	(0.8)	(0.7)
NO_3^-	22.8^a	17.4^b	18.8^c	25.9^a	28.9^b	26.2$^{a,\,b}$	24.5^a	22.7^b	22.4^b
	(2.8)	(1.5)	(2.1)	(5.0)	(6.2)	(4.8)	(2.6)	(2.8)	(2.4)
NH_4^+	15.5^a	18.7^b	19.5^b	19.4^a	39.6^b	40.8^b	17.5^a	28.0^b	29.0^b
	(3.6)	(2.8)	(4.1)	(10.5)	(16.6)	(16.4)	(4.9)	(7.4)	(7.5)
DOC	36.4^a	39.7^a	35.6^a	68.8^a	87.9^a	90.1^a	57.0^a	70.3^a	70.1^a
	(1.8)	(5.1)	(3.4)	(20.2)	(17.2)	(17.4)	(12.9)	(11.8)	(12.1)
Yield	6.7^a	7.1^a	7.1^a	6.3^a	7.0^a	7.1^a	13.0^a	14.1^a	14.2^a
	(0.6)	(0.3)	(0.6)	(0.4)	(0.8)	(0.4)	(0.7)	(0.8)	(0.7)
GC	2.9^a	3.1^a	3.1^a	2.8^a	3.2^a	3.2^a	5.8^a	6.3^a	6.3^a
	(0.3)	(0.1)	(0.3)	(0.2)	(0.4)	(0.2)	(0.3)	(0.4)	(0.3)
GN	141.5^a	152.4^a	156.0^a	87.1^a	105.5^a	108.8^a	228.6^a	258.0^a	264.7^a
	(13.3)	(5.3)	(13.3)	(6.1)	(12.1)	(7.4)	(14.6)	(13.2)	(15.3)
AB	15.1^a	15.8^a	15.6^a	18.1^a	20.5^a	20.5^a	33.2^a	36.4^a	36.0^a
	(1.0)	(0.5)	(1.4)	(0.8)	(1.2)	(0.8)	(1.3)	(1.2)	(1.6)
APC	6.7^a	7.0^a	7.0^a	8.2^a	9.3^a	9.2^a	14.9^a	16.3^a	16.2^a
	(0.3)	(0.4)	(0.4)	(0.2)	(0.4)	(0.3)	(0.4)	(0.6)	(0.5)
APN	188.7^a	199.9^a	203.6^a	215.7^a	258.0^a	244.8^a	404.4^a	457.9^a	448.4^a
	(13.6)	(7.6)	(14.1)	(7.9)	(14.0)	(11.7)	(15.7)	(15.9)	(18.4)

Different superscripts of small letters (a, b and c) indicate significant differences at the $p < 0.05$ level between treatments for the wheat season, maize season and annual scale, respectively. Values in parentheses indicate standard error of the seasonal and annual averages and the means of spatial replicates. SDW: soil dry weight.

3.4 Effects of soil temperature, moisture and inorganic nitrogen content on nitrous oxide emission

We defined "inhibited N_2O fluxes" as the differences of daily averaged N_2O flux between the treatments with and without nitrification inhibitors. Soil temperature, moisture, and inorganic N content significantly affected daily averaged N_2O fluxes and inhibited N_2O fluxes (Table 3). The daily averaged N_2O fluxes and inhibited N_2O fluxes were exponentially or linearly correlated with soil temperatures, WFPS and inorganic N contents ($p < 0.01$, Figs. 4, 5; Table 3). Both N_2O emissions and inhibited N_2O fluxes were facilitated by an appropriate range of soil WFPS (37–63 %) and high soil temperatures (> 25 °C).

Compared with the single factors, the correlation coefficients of regressions between multi-factors and N_2O flux were obviously higher (Table 3). As indicated by the correlation coefficients of multiple regression (0.55, 0.88 and 0.83), the combined effects of soil temperature, moisture and inorganic N content well explained the seasonal fluctuations of the daily averaged N_2O fluxes for the U, DCD and DMPP treatments.

4 Discussion

4.1 Optimized sampling schedule for intermittent measurement

The manual chamber measurements were extensively used for the estimation of inhibition effects of nitrification inhibitors on N_2O emissions (Table 4). The inhibition effects may be over- or underestimated, as the low-frequency measurements may randomly pick up or miss the main

Table 3. Correlations between soil temperature (T, °C), water-filled pore space (W, %), nitrate (NO_3^-), ammonium (NH_4^+) and inorganic nitrogen contents ($IN = NO_3^- + NH_4^+$, mg N kg^{-1} SDW), and daily averaged N_2O flux (F, µg N m^{-2} h^{-1}) or inhibited N_2O flux (F_i, µg N m^{-2} h^{-1}) for the urea (U), urea + DCD (DCD) and urea + DMPP (DMPP) treatments.

RA	Factor	Equation	n	r^2	p	T
SR	T and F (Fig. 4a)	$F = \exp(0.19 \cdot T)$	346	0.14	< 0.01	U
		$F = \exp(0.17 \cdot T)$	346	0.16	< 0.01	DCD
		$F = \exp(0.17 \cdot T)$	346	0.15	< 0.01	DMPP
	W and F (Fig. 4b)	$F = \exp(0.17 \cdot W - 0.002 \cdot W^2)$	335	0.04	< 0.01	U
		$F = \exp(0.14 \cdot W - 0.001 \cdot W^2)$	334	0.06	< 0.01	DCD
		$F = \exp(0.14 \cdot W - 0.001 \cdot W^2)$	335	0.05	< 0.01	DMPP
	NO_3^- and F (Fig. 4c)	$F = 4.32 \cdot NO_3^-$	73	0.45	< 0.01	U
		$F = 2.74 \cdot NO_3^-$	73	0.59	< 0.01	DCD
		$F = 1.83 \cdot NO_3^-$	73	0.32	< 0.01	DMPP
	NH_4^+ and F (Fig. 4d)	$F = 2.71 \cdot NH_4^+$	73	0.34	< 0.01	U
		$F = 1.48 \cdot NH_4^+$	73	0.78	< 0.01	DCD
		$F = 1.19 \cdot NH_4^+$	73	0.76	< 0.01	DMPP
	T and F_i (Fig. 5a)	$F_i = \exp(0.15 \cdot T)$	346	0.10	< 0.01	DCD
		$F_i = \exp(0.16 \cdot T)$	346	0.11	< 0.01	DMPP
	W and F_i (Fig. 5b)	$F_i = \exp(0.13 \cdot W - 0.001 \cdot W^2)$	335	0.02	< 0.01	DCD
		$F_i = \exp(0.13 \cdot W - 0.001 \cdot W^2)$	335	0.02	< 0.01	DMPP
SMR	IN, T and F	$F = -113.55 + 2.20 \cdot IN + 6.39 \cdot T$	73	0.50	< 0.01	U
	IN, W, T and F	$F = -79.90 + 1.13 \cdot IN + 1.13 \cdot W + 1.73 \cdot T$	72	0.87	< 0.01	DCD
	IN, W, T and F	$F = -54.89 + 0.95 \cdot IN + 0.86 \cdot W + 1.25 \cdot T$	72	0.80	< 0.01	DMPP
MR	T, IN and F	$F = IN \cdot \exp(0.04 \cdot T)$	73	0.51	< 0.01	U
		$F = IN \cdot \exp(0.01 \cdot T)$	73	0.83	< 0.01	DCD
		$F = IN \cdot \exp(0.002 \cdot T)$	73	0.76	< 0.01	DMPP
	W, IN and F	$F = IN \cdot \exp(-0.01 \cdot W + 0.0005 \cdot W^2)$	72	0.51	< 0.01	U
		$F = IN \cdot \exp(-0.03 \cdot W + 0.0006 \cdot W^2)$	72	0.87	< 0.01	DCD
		$F = IN \cdot \exp(-0.04 \cdot W + 0.0008 \cdot W^2)$	72	0.83	< 0.01	DMPP
	T, W and F	$F = \exp(0.18 \cdot T - 0.02 \cdot W + 0.0006 \cdot W^2)$	335	0.20	< 0.01	U
		$F = \exp(0.17 \cdot T - 0.04 \cdot W + 0.0008 \cdot W^2)$	334	0.26	< 0.01	DCD
		$F = \exp(0.19 \cdot T - 0.08 \cdot W + 0.001 \cdot W^2)$	335	0.28	< 0.01	DMPP
	T, W, IN and F	$F = IN \cdot \exp(0.17 \cdot T - 0.15 \cdot W + 0.002 \cdot W^2)$	72	0.55	< 0.01	U
		$F = IN \cdot \exp(0.05 \cdot T - 0.09 \cdot W + 0.001 \cdot W^2)$	72	0.88	< 0.01	DCD
		$F = IN \cdot \exp(0.03 \cdot T - 0.07 \cdot W + 0.001 \cdot W^2)$	72	0.83	< 0.01	DMPP

SDW: soil dry weight; n: sample number; r: correlation coefficient; p: probability value; T: treatment; RA: regression analysis; SR: simple regression; SMR: stepwise multiple regression (criteria: probability of F test to enter ≦ 0.05, probability of F test to remove ≧ 0.10); MR: multiple regression.

emission events. Based on the flux data obtained by high-frequency measurement in this study, we preliminarily assessed the possible deviation induced by low-frequency measurements. We assumed the flux measurements were conducted daily and only once per day for each spatial replicate, which meant that we randomly chose 4 of the 24 fluxes observed by the automated chamber system for each day to calculate the daily means and annual cumulative emissions. The calculated emissions varied from 3.68 to 5.57 kg N ha^{-1} yr^{-1}, from 2.41 to 3.57 kg N ha^{-1} yr^{-1} and from 2.28 to 3.41 kg N ha^{-1} yr^{-1} for the U, DCD and DMPP treatments, respectively. The estimated inhibition effects of N_2O ranged from 1.22 to 2.07 kg N ha^{-1} yr^{-1} and from 1.39 to 2.20 kg N ha^{-1} yr^{-1} for the DCD and DMPP treatments, respectively, which imply that the virtual inhibition effects (1.56 and 1.71 kg N ha^{-1} yr^{-1}) may be over- or underestimated by 33 % or 22 %, respectively. If the intermittent flux measurements were conducted once per several days, the deviation range would increase rapidly with the increase in days of the sampling interval. For instance, the

Table 4. The (inhibited: ↓; enhanced: ↑; no: ×; and not measured: –) effects of DCD and DMPP on N_2O emission (N_2O), soil nitrate (NO_3^-) and ammonium (NH_4^+) contents, NO_3^- leaching, yield and plant nitrogen uptake (PNU).

Ecosystem	FT	NI	AR	AM	Period	N_2O	N_2O%	NO_3^-	NH_4^+	Leaching	Yield	PNU	Literature
Cropland	ASN	DMPP	2.9	C	24	↓	40 %, 45 %	×	↑, –	–	×	×	Pfab et al. (2012)
	ASN	DMPP	1.1, 1.8, 2.0	C	28	↓	49 %	↓	×	–	×	–	Weiske et al. (2001)
	ASN	DCD	10, 18, 19	C	28	↓	26 %	↓	×	–	×	–	Weiske et al. (2001)
	Urea	DCD	10	L	8	↓	62–68 %	×	×	↓	↑	↑	Cui et al. (2011)
	Urea	DCD	20	C	4	↓	39 %	×	×	–	↑	–	Ding et al. (2011)
	Urea	DCD	9	C	3	↓	56 %	↓	×	–	–	–	Jumadi et al. (2008)
	Urea	DCD	18	C	3	↓	49 %	↓	↑	–	↑	↑	Majumdar et al. (2002)
	Urea, AN	DCD	12.5	L	3	↓, ×	40 %, 0 %	–	–	–	–	–	McTaggart et al. (1997)
Grassland	ASN, CAN, CS	DMPP	0.5, 0.7, 1.0	MS, C	3	↓	58 %, 61 %	–	↑	–	–	–	Belastegui et al. (2003)
	CS	DMPP	1	MS	1, 3	↓	48 %, 69 %	↓	↑	–	×	×	Merino et al. (2005)
	CU	DMPP	1, 5	L	3	↓	62–66 %	↓	↑	↓	–	–	Di and Cameron (2012)
	CU	DCD	10	L	3	↓	62–66 %	↓	↑	↓	–	–	Di and Cameron (2012)
	Urea, AS	DCD	12.5	L	24	↓	58 %, 56 %	↓	×	–	–	–	McTaggart et al. (1997)
	CU	DCD	20, 30	L	6	↓	17–68 %	↓, ×	×	–	–	–	de Klein et al. (2011)
	CU	DCD	7	L	12	↓	17–52 %	↓	↑	–	↑	↑	Zaman et al. (2009)
	Urea	DCD	10	L	2	↓	53 %, 64 %	↓	↑	–	–	–	Ball et al. (2012)
	Urea, CU	DCD	10	FPS	6–10	–	–	↓	↑	–	↑	↑	Moir et al. (2012)
	ASN, CAN, CS	DCD	25	MS, C	3	↓	43 %, 60 %	–	↑	–	↓	↑	Belastegui et al. (2003)

FT: fertilizer type; NI: nitrification inhibitor; AR: application rate of nitrification inhibitor (unit: $kg\,ha^{-1}$); AM: application method of nitrification inhibitor; Period: measuring period (unit: months); N_2O %: inhibition effect of N_2O in percentage; ASN: ammonium sulfate nitrate; AN: ammonium nitrate; CAN: calcium ammonium nitrate; AS: ammonium sulfate; CS: cattle slurry; CU: cow urine; C: coated on fertilizer granules; L: liquid application; MS: mixed with slurry; FPS: fine particle suspension.

flux measurements were made once per week for each spatial replicate. The calculated inhibition effects of N_2O ranged from 0.86 to 2.49 $kg\,N\,ha^{-1}\,yr^{-1}$ and from 1.20 to 2.44 $kg\,N\,ha^{-1}\,yr^{-1}$ for the DCD and DMPP treatments, which means the virtual inhibition effects may be over- or underestimated by 60 % or 45 %, respectively. However, the deviations could be slashed by improving manual sampling schedules (Smith and Dobbie, 2001; Liu et al., 2010). In this study if the intermittent sampling was made daily for each spatial replicate at the times when daily mean air temperature appeared (local standard time 08:00–9:00 or 19:00–20:00), the deviations of inhibition effect were less than 8 %. If the intermittent sampling was conducted daily after the main field managements and once per week during the remaining period at the times when daily mean air temperature appeared, the estimated deviations were less than 12 %. Here the main field managements included fertilization, irrigation and straw application. The daily sampling was continued for 5–19 days until the peak N_2O emissions decreased to the initial values before the field managements. Therefore, when the N_2O fluxes showed clearly diurnal and seasonal variations as in most fertilized fields, the manual sampling schedule needed to be optimized to accurately quantify the inhibition effects of nitrification inhibitors on N_2O emission.

4.2 Positive effects of DCD and DMPP application

Microbial nitrification is the oxidation process of NH_4^+ to NO_2^-, which is further oxidized to NO_3^- under aerobic conditions. Nitrification inhibitors can delay the microbial oxidation of NH_4^+ to NO_2^- and, therefore, can stabilize the NH_4^+ content and decrease the oxidation rate of the NO_2^- to NO_3^- and NO_3^- content in the soil (Weiske et al., 2001; Zerulla et al., 2001). In our study, we observed that the application of DCD and DMPP slightly decreased the soil NO_3^- contents and significantly increased the soil NH_4^+ contents. Due to the substantial increase in soil NH_4^+ contents, the total inorganic N contents were significantly higher for the DCD and DMPP treatments than for the U treatment ($p < 0.01$). Under normal conditions, free NH_4^+ does not exist and most of the inorganic N occurs as NO_3^- in the soil. However, the application of DCD and DMPP shifted the primary form of soil inorganic N from NO_3^- to NH_4^+ in our field. Because NO_3^- is more easily lost by leaching, the change in major form of soil inorganic N should benefit the reduction of N loss by leaching. The continuously higher soil inorganic N contents for the DCD and DMPP treatments were also beneficial for the growth and N assimilation of the crops. Therefore, we observed higher aboveground biomass, grain yield and N content of grain for the DCD and DMPP treatments. The N uptake by aboveground plant increased 13.2 % and 10.9 % for the DCD and DMPP treatments, respectively, compared with the U treatment. The higher values of plant N uptake imply higher N use efficiencies for the DCD and DMPP treatments than with the U treatment.

Nitrous oxide is the product of denitrification and the byproduct of nitrification. The application of nitrification inhibitors together with NH_4^+-based fertilizers can inhibit microbial nitrification and subsequent denitrification and, therefore, decrease N_2O production and emissions (Weiske et al., 2001; Zerulla et al., 2001). We observed that the soil inorganic N contents linearly correlated with the N_2O emissions in the wheat–maize rotation field ($p < 0.01$). The slopes for

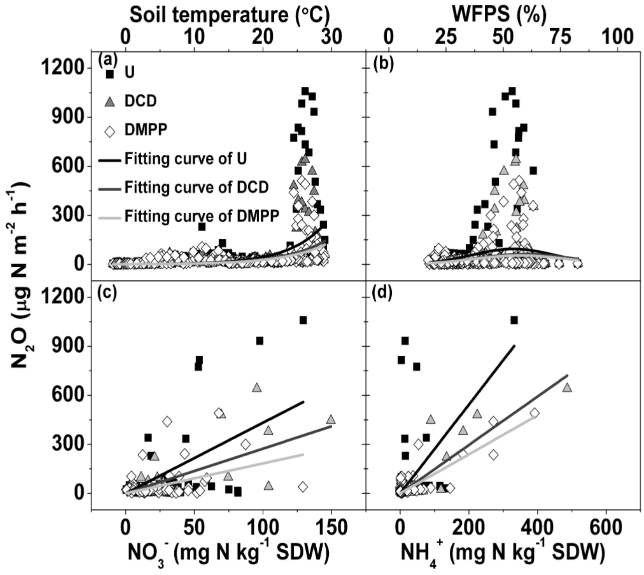

Fig. 4. Relationship between daily averaged soil temperatures, water-filled pore space (WFPS), nitrate (NO_3^-) content, ammonium (NH_4^+) content and daily means of N_2O flux. SDW: soil dry weight. U, DCD and DMPP: urea, urea + DCD and urea + DMPP treatments.

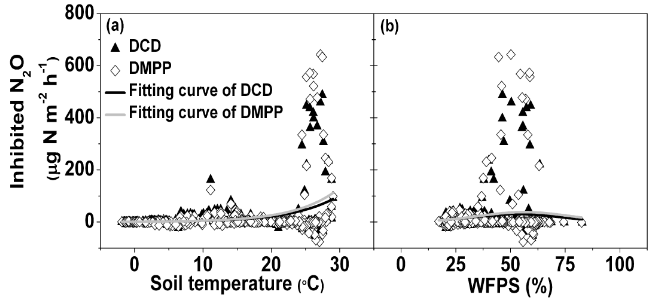

Fig. 5. Relationship between daily averaged soil temperatures, soil water-filled pore space (WFPS) and inhibited N_2O flux. DCD and DMPP: urea + DCD and urea + DMPP treatments.

the DCD and DMPP treatments were obviously lower than for the U treatment. After fertilization, the main substrates NH_4^+ and NO_3^- for the DCD and DMPP treatments were abundant for microbial nitrification and denitrification in the soil. However, the high soil inorganic N contents for the DCD and DMPP treatments did not result in similar high N_2O emissions compared with the U treatment. This phenomenon proves that the conversion processes between NH_4^+ and NO_3^- (nitrification and denitrification) were inhibited by the nitrification inhibitors; therefore, the products and byproducts of nitrification and denitrification, including N_2O, were reduced. The cumulative N_2O emissions were reduced by 1.56 and 1.71 kg N ha^{-1} yr^{-1} due to the application of DCD and DMPP coated on the urea granules in the wheat–maize rotation field. The most efficient period for the inhibition of N_2O emissions was the maize season. The application of DCD and DMPP in the maize season decreased the cumulative N_2O emissions by 1.25 and 1.53 kg N ha^{-1} within the 23 days after fertilization, accounting for 80 % and 89 % of the annual total inhibition effects (1.56 and 1.71 kg N ha^{-1} yr^{-1}), respectively. Therefore, to decrease the cost and workload of nitrification inhibitor application, DCD and DMPP should be coated on urea granules and applied once in the maize season for wheat–maize rotation fields.

Through a review of the literature (Table 4), we can see that the application rates of DCD and DMPP normally ranged from 7 to 30 kg ha^{-1} and from 0.5 to 5 kg ha^{-1}, respectively. The very low application rates of DMPP resulted in comparable or even better inhibition effects of N_2O emission compared with DCD. Both nitrification inhibitors were very effective at reducing N_2O emissions induced by NH_4^+-based fertilizers (e.g., urea, cow urine, cattle slurry, ammonium sulfate nitrate, calcium ammonium nitrate and ammonium sulfate). Even when no effect was often observed, the use of DCD and DMPP generally tended to increase the soil NH_4^+ content, crop yield, aboveground biomass, plant N uptake and N use efficiency and to decrease the soil NO_3^- content and NO_3^- leaching (Table 4). If DCD application rates are too high (e.g., 50 kg ha^{-1} in grassland), phytotoxic effects and yield reduction may occur (Belastegui-Macadam et al., 2003). The application rates of DCD and DMPP (6 and 2 kg ha^{-1} yr^{-1}) in our study were in the low and middle ranges of the reported values, respectively. For the current application rates, both DCD and DMPP well inhibited the N_2O emissions and increased soil inorganic N availability, yield, plant N uptake and use efficiency of fertilizer N in the wheat–maize rotation field. The one-third application rate for DMPP had similar effects on these factors compared with DCD. No phytotoxic effect was observed for the current application rate of DCD. Mahmood et al. (2011) evaluated the effects of DCD on the fate of ^{15}N-labelled urea applied to an alkaline calcareous soil under greenhouse conditions. The results showed that the application of DCD increased the fertilizer N losses and decreased the N uptakes for cotton, maize and wheat; therefore, the authors suggested that the use of DCD may not be beneficial in alkaline calcareous soils. Their conclusion contrasts with our data, which show that the use of DCD increased fertilizer N uptake and soil inorganic N stock and decreased N_2O emission and, probably, NO_3^- leaching. The use of DCD in this alkaline calcareous soil should be recommended. However, further studies are needed to evaluate the effects of nitrification inhibitors on another important fertilizer N loss pathway, namely, ammonia volatilization, as a few studies have reported that nitrification inhibitors may enhance ammonia volatilization from soils with high pH (Kim et al., 2012).

5 Conclusions

We conducted year-round measurements of N_2O fluxes, crop yield, C and N contents of crop, soil NH_4^+, NO_3^- and DOC contents and environmental factors for treatments with and without nitrification inhibitor (DCD and DMPP) application in a typical wheat–maize rotation field in northern China. The soil temperatures, moisture and inorganic N contents significantly regulated the N_2O emissions. The emissions showed clearly daily and seasonal fluctuations, and therefore high-frequency measurements or optimized sampling schedules for low-frequency measurements were necessary to accurately quantify the effects of nitrification inhibitors on N_2O emissions. The application of nitrification inhibitors significantly decreased the annual cumulative N_2O emissions ($p < 0.01$); increased the soil inorganic N availability ($p < 0.01$); shifted the main form of soil inorganic N from NO_3^- to NH_4^+; and tended to increase the soil DOC availability, crop yield, aboveground biomass, plant C and N uptakes. The one-third application rate for DMPP obtained similar inhibition effects on N_2O emission as DCD. The study demonstrates the effectiveness of DCD and DMPP in reducing N losses to the environment, enhancing yield and N use efficiency. The method of coating DCD and DMPP on urea granules and applying them once in the maize season is recommended for wheat–maize rotation fields in northern China.

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