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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

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BGD

10, 711–737, 2013

**Effects of
nitrification inhibitors
on N₂O emission**

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Abstract

The application of nitrification inhibitors together with ammonium-based fertilizers is proposed as a potent method to decrease nitrous oxide (N₂O) emission while promoting yield and nitrogen use efficiency in fertilized agricultural fields. To evaluate the effects of nitrification inhibitors, we conducted year-round measurements of N₂O 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 N₂O 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 decreased the annual emissions by 35% and 38%, respectively. The variations of soil temperature, moisture and inorganic nitrogen content regulated the seasonal fluctuation of N₂O emissions. When the emissions presented clearly temporal variations, year-round and high-frequency measurements should be adopted to estimate annual cumulative emissions and treatment effects. The application of nitrification inhibitors increased the soil inorganic nitrogen and dissolved organic carbon availability and shifted the main soil inorganic nitrogen form from nitrate to ammonium. The annual yield, aboveground biomass and nitrogen uptake by aboveground plants increased by 8.5–9.1%, 8.6–9.7% and 10.9–13.2%, respectively, for the DCD and DMPP treatments compared with the U treatment. The results demonstrate the roles the nitrification inhibitors play in enhancing yield and nitrogen use efficiency and reducing N₂O 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

BGD

10, 711–737, 2013

Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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) by depressing the activities of *Nitrosomonas* sp. in soil and are therefore very effective at blocking microbial nitrification and subsequent denitrification (Weiske et al., 2001; Zerulla et al., 2001). Hundreds of nitrification 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,

BGD

10, 711–737, 2013

**Effects of
nitrification inhibitors
on N_2O emission**

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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).

5 The roles DCD and DMPP application play in yield, plant N uptake, soil inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) 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

20 The experimental site ($34^\circ 55.51' \text{N}$, $110^\circ 42.59' \text{E}$) 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

Effects of nitrification inhibitors on N_2O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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 250–60–30 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. 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. 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

BGD

10, 711–737, 2013

Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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 N₂O concentration analysis. Each flux was calculated from five N₂O concentrations of the chamber headspace air using a first-order differential or linear equation (Liu et al., 2010; Wang et al., 2012). Every hour, only one of the four chambers for each treatment was closed. Thus, twenty-four fluxes were obtained daily from the four replicate chambers (six fluxes per replicate) for each treatment. When the Spearman correlation coefficients of linear and nonlinear fittings between N₂O concentrations and sampling time differences for the five samples were higher than 0.88, the fluxes were regarded as valid. The detection limit of N₂O 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 annual cumulative emissions 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 NH₄⁺, NO₃⁻ and DOC contents (0–10 cm). 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

BGD

10, 711–737, 2013

Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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 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 ($5.4 \mu\text{gN m}^{-2} \text{h}^{-1}$). The low fluxes ($< 5.4 \mu\text{gN m}^{-2} \text{h}^{-1}$) 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, b; Table 1). The fertilization on 13 July 2010, and the following frequent rain events resulted in extremely high emissions (Fig. 1a, b). The high emissions (daily mean $> 6 \text{mgN m}^{-2} \text{d}^{-1}$ or $250 \mu\text{gN m}^{-2} \text{h}^{-1}$) were continuously observed from 16 July to 30 July 2010, and contributed to 36–55% of the annual emissions.

The calculated mean (\pm s.e.) fluxes were 53.0 ± 7.9 , 34.6 ± 4.2 and $32.5 \pm 3.3 \mu\text{gN m}^{-2} \text{h}^{-1}$ for the U, DCD and DMPP treatments, respectively. The cumulative emissions were estimated to be 4.49 ± 0.21 , 2.93 ± 0.06 and $2.78 \pm 0.16 \text{kgN ha}^{-1} \text{yr}^{-1}$ for the U, DCD and DMPP treatments, respectively (Table 2). The application of the DCD and DMPP nitrification inhibitors decreased the

BGD

10, 711–737, 2013

Effects of nitrification inhibitors on N_2O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



cumulative emissions by 1.56 and 1.71 kgNha⁻¹ yr⁻¹ (equal to 35 % and 38 % of the annual emissions of the U treatment), respectively. The significant inhibition effects of the nitrification inhibitors on N₂O emissions were immediately detected after the application on October 21, 2009, and March 18 and July 13, 2010, and lasted for 44, 24 and 23 days, respectively. The application of nitrification inhibitors on July 13, 2010, significantly decreased the cumulative N₂O emissions of 1.25 and 1.53 kgNha⁻¹ (equal to 80 % and 89 % of the annual inhibition effects) 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 (\pm s.e.) air temperature was 13.8 \pm 0.6 $^{\circ}$ C. The soil temperatures ranged from -1.9 to 29.0 $^{\circ}$ C, with annual means of 13.4 \pm 0.5, 13.5 \pm 0.5 and 13.6 \pm 0.5 $^{\circ}$ 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 ranged from 17.2 % to 82.8 %, with annual means of 39.2 \pm 0.7 %, 39.4 \pm 0.8 % and 39.1 \pm 0.7 % for the U, DCD and DMPP treatments, respectively (Fig. 2b; Table 2).

The soil NO₃⁻ contents varied between 0.3 and 149.5 mgNkg⁻¹ soil dry weight (SDW), with annual means of 24.5 \pm 2.6, 22.7 \pm 2.8 and 22.4 \pm 2.4 mgNkg⁻¹ SDW for the U, DCD and DMPP treatments, respectively. The soil NH₄⁺ contents ranged from 0.1 to 486.3 mgNkg⁻¹ SDW, with annual means of 17.5 \pm 4.9, 28.0 \pm 7.4 and 29.0 \pm 7.5 mgNkg⁻¹ SDW for the U, DCD and DMPP treatments, respectively (Table 2). The fertilization events considerably promoted the soil inorganic N (NO₃⁻ + NH₄⁺) contents. The maximum values were observed at the 7th day after fertilization on 13 July 2010, for all treatments (Fig. 3a, b). The application of nitrification inhibitors slightly decreased the annual means of the soil NO₃⁻ content and significantly increased the soil NH₄⁺ 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,

BGD

10, 711–737, 2013

Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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^+ (Figs. 3a and 3b; 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 at the 2nd 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 $\text{ton ha}^{-1} \text{yr}^{-1}$) and 8.6–9.7% (2.9 – 3.2 $\text{ton ha}^{-1} \text{yr}^{-1}$) 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% (29.3 – 36.1 $\text{kg N ha}^{-1} \text{yr}^{-1}$) and 10.9–13.2% (44.0 – 53.5 $\text{kg N ha}^{-1} \text{yr}^{-1}$) higher for the DCD and DMPP treatments compared with the U treatment. The C fixations by grain and aboveground plant also increased by 9.5–9.6% (0.5 – 0.6 $\text{ton C ha}^{-1} \text{yr}^{-1}$) and 8.9–9.6% (1.3 – 1.4 $\text{ton C ha}^{-1} \text{yr}^{-1}$) for the DCD and DMPP treatments compared with the U treatment (Table 2).

BGD

10, 711–737, 2013

Effects of nitrification inhibitors on N_2O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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

We defined “inhibited N₂O fluxes” as the differences of daily averaged N₂O flux between the treatments with and without nitrification inhibitors. Soil temperature, moisture, and inorganic N content significantly affected daily averaged N₂O fluxes and inhibited N₂O fluxes (Table 3). The daily averaged N₂O fluxes and inhibited N₂O fluxes were exponentially or linearly correlated with soil temperatures, WFPS and inorganic N contents ($p < 0.01$, Fig. 4; Fig. 5; Table 3). Both N₂O emissions and inhibited N₂O fluxes were facilitated by an appropriate range of soil WFPS (37–63 %) and high soil temperatures ($> 25^{\circ}\text{C}$).

Compared with the single factors, the correlation coefficients of regressions between multi-factors and N₂O flux were obviously higher, which proved the synergetic effects of soil temperature, moisture and inorganic N content on N₂O emission (Table 3). As indicated by the correlation coefficients of the regressions (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 N₂O fluxes for the U, DCD and DMPP treatments, respectively.

4 Discussion

4.1 Importance of high-frequency measurement

Based on low-frequency measurements of flux, the former studies evaluated the inhibition effects of nitrification inhibitors on N₂O emissions (Table 4). The effects may be over- or underestimated, as the low-frequency measurements may randomly pick up or miss the main 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

BGD

10, 711–737, 2013

Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



daily and only once per day for each spatial replicate, which meant that we chose four of the twenty-four 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 kgNha⁻¹yr⁻¹, from 2.41 to 3.52 kgNha⁻¹yr⁻¹ and from 2.28 to 3.41 kgNha⁻¹yr⁻¹ for the U, DCD and DMPP treatments, respectively. The estimated inhibition effects of N₂O ranged from 1.27 to 2.04 kgNha⁻¹yr⁻¹ and from 1.39 to 2.15 kgNha⁻¹yr⁻¹ for the DCD and DMPP treatments, respectively, which imply that the virtual inhibition effects (1.56 and 1.71 kgNha⁻¹yr⁻¹) may be over- or underestimated by 31 % or 19 %, 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 flux measurements were made once per week for each spatial replicate. The calculated inhibition effects of N₂O ranged from 1.07 to 2.49 kgNha⁻¹yr⁻¹ and from 1.21 to 2.41 kgNha⁻¹yr⁻¹ for the DCD and DMPP treatments, which means the virtual inhibition effects may be over- or underestimated by 60 % or 31 %, respectively. Therefore, when the N₂O fluxes showed clearly diurnal and seasonal variations as in most fertilized fields, high-frequency and year-round measurements were necessary to accurately quantify the inhibition effects of nitrification inhibitors on N₂O emission.

4.2 Positive effects of DCD and DMPP application

Microbial nitrification is the oxidation process of NH₄⁺ to NO₂⁻, which is further oxidized to NO₃⁻ under aerobic conditions. Nitrification inhibitors can delay the microbial oxidation of NH₄⁺ to NO₂⁻ and, therefore, can stabilize the NH₄⁺ content and decrease the oxidation rate of the NO₂⁻ to NO₃⁻ and NO₃⁻ 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 NO₃⁻ contents and significantly increased the soil NH₄⁺ contents. Due to the substantial increase in soil NH₄⁺ contents, the total inorganic N contents were significantly higher for the DCD and DMPP treatments than for the U treatment

BGD

10, 711–737, 2013

Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



($p < 0.01$). 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 compared 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 (1.14 and 0.99) for the DCD and DMPP treatments were obviously lower than for the U treatment (2.18). 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 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ 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

Effects of nitrification inhibitors on N_2O emission

C. Liu et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

23 days after fertilization, accounting for 80 % and 89 % (1.56 and 1.71 kgNha⁻¹ yr⁻¹) of the annual total inhibition effects, 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 literatures (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 N₂O emission compared with DCD. Both nitrification inhibitors were very effective at reducing N₂O emissions induced by NH₄⁺-based fertilizers (e.g. urea, cow urine, cattle slurry, ammonium sulfate nitrate, calcium ammonium nitrate and ammonium sulfate). Even when no effect was observed, the use of DCD and DMPP generally tended to increase the soil NH₄⁺ content, crop yield, aboveground biomass, plant N uptake and N use efficiency and to decrease the soil NO₃⁻ content and NO₃⁻ 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 N₂O 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 ¹⁵N-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 N₂O emission and, probably, NO₃⁻ leaching. The use of DCD in this alkaline calcareous soil should be recommended.

Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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 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; increased the soil inorganic N availability; 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 the wheat-maize rotation fields in northern China.

BGD

10, 711–737, 2013

Effects of nitrification inhibitors on N_2O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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BGD

10, 711–737, 2013

**Effects of
nitrification inhibitors
on N₂O emission**

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Table 1. Information regarding main field management.

Date	Field management
17 Oct 2009	Stubbling
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	Stubbling
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 14	Maize harvest

Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Table 2. Average (AF, $\mu\text{gNm}^{-2}\text{h}^{-1}$) and cumulative N₂O fluxes (CF, kgNha^{-1}), averaged soil temperature (ST, °C), water-filled pore space (WFPS, %), nitrate (NO_3^- , mgNkg^{-1} SDW), ammonium (NH_4^+ , mgNkg^{-1} SDW) and dissolved organic carbon contents (DOC, mgCkg^{-1} SDW), grain yield (tonha^{-1}), aboveground biomass (AB, tonha^{-1}), carbon (GC and APC, tonCha^{-1}) and nitrogen uptakes (GN and APN, kgNha^{-1}) by grain and aboveground plants for the U, DCD and DMPP treatments.

	Wheat season			Maize season			Annual scale		
	U	DCD	DMPP	U	DCD	DMPP	U	DCD	DMPP
AF	22.9 ^a (1.6)	16.0 ^b (0.9)	18.2 ^c (1.1)	109.0 ^a (21.5)	69.1 ^a (11.4)	59.2 ^b (8.8)	53.0 ^a (7.9)	34.6 ^b (4.2)	32.5 ^b (3.3)
CF	1.32 ^a (0.06)	0.92 ^b (0.02)	1.05 ^b (0.06)	3.18 ^a (0.24)	2.02 ^b (0.05)	1.73 ^b (0.12)	4.49 ^a (0.21)	2.93 ^b (0.06)	2.78 ^b (0.16)
ST	8.3 ^a (0.5)	8.5 ^a (0.4)	8.4 ^a (0.5)	23.5 ^a (0.3)	23.5 ^a (0.3)	23.8 ^a (0.3)	13.4 ^a (0.5)	13.5 ^a (0.5)	13.6 ^a (0.5)
WFPS	33.4 ^a (0.8)	33.5 ^a (0.8)	33.3 ^a (0.8)	49.0 ^a (1.0)	49.2 ^b (1.0)	48.7 ^c (1.0)	39.2 ^a (0.7)	39.4 ^a (0.8)	39.1 ^a (0.7)
NO_3^-	22.8 ^a (2.8)	17.4 ^b (1.5)	18.8 ^c (2.1)	25.9 ^a (5.0)	28.9 ^b (6.2)	26.2 ^{a,b} (4.8)	24.5 ^a (2.6)	22.7 ^b (2.8)	22.4 ^b (2.4)
NH_4^+	15.5 ^a (3.6)	18.7 ^b (2.8)	19.5 ^b (4.1)	19.4 ^a (10.5)	39.6 ^b (16.6)	40.8 ^b (16.4)	17.5 ^a (4.9)	28.0 ^b (7.4)	29.0 ^b (7.5)
DOC	36.4 ^a (1.8)	39.7 ^a (5.1)	35.6 ^a (3.4)	68.8 ^a (20.2)	87.9 ^a (17.2)	90.1 ^a (17.4)	57.0 ^a (12.9)	70.3 ^a (11.8)	70.1 ^a (12.1)
Yield	6.7 ^a (0.6)	6.8 ^a (0.3)	7.1 ^a (0.6)	6.3 ^a (0.4)	7.0 ^a (0.8)	7.1 ^a (0.4)	13.0 ^a (0.7)	13.8 ^a (0.9)	14.2 ^a (0.7)
GC	2.9 ^a (0.3)	3.1 ^a (0.1)	3.1 ^a (0.3)	2.8 ^a (0.2)	3.2 ^a (0.4)	3.2 ^a (0.2)	5.8 ^a (0.3)	6.3 ^a (0.4)	6.3 ^a (0.3)
GN	141.5 ^a (13.3)	152.4 ^a (5.3)	156.0 ^a (13.3)	87.1 ^a (6.1)	105.5 ^a (12.1)	108.8 ^a (7.4)	228.6 ^a (14.6)	258.0 ^a (13.2)	264.7 ^a (15.3)
AB	15.1 ^a (1.0)	15.8 ^a (0.5)	15.6 ^a (1.4)	18.1 ^a (1.4)	20.5 ^a (0.8)	20.5 ^a (0.8)	33.2 ^a (1.3)	36.4 ^a (1.2)	36.0 ^a (1.6)
APC	6.7 ^a (0.3)	7.0 ^a (0.4)	7.0 ^a (0.4)	8.2 ^a (0.2)	9.3 ^a (0.4)	9.2 ^a (0.3)	14.9 ^a (0.4)	16.3 ^a (0.6)	16.2 ^a (0.5)
APN	188.7 ^a (13.6)	199.9 ^a (7.6)	203.6 ^a (14.1)	215.7 ^a (7.9)	258.0 ^a (14.0)	244.8 ^a (11.7)	404.4 ^a (15.7)	457.9 ^a (15.9)	448.4 ^a (18.4)

Different superscripts of capital letters 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.

Table 3. Correlations between soil temperature (T , °C), soil water-filled pore space (W , %), soil inorganic nitrogen content (IN, mg N kg⁻¹ SDW) and daily averaged N₂O flux (F , μg N⁻² h⁻¹) or inhibited N₂O flux (F_i , μg N m⁻² h⁻¹) for the U, DCD and DMPP treatments.

Factor	Equation	n	r^2	p	T
T and F (Fig. 4a)	$F = \exp(0.190 \cdot T)$	346	0.14	< 0.01	U
	$F = \exp(0.171 \cdot T)$	346	0.16	< 0.01	DCD
	$F = \exp(0.165 \cdot T)$	346	0.15	< 0.01	DMPP
W and F (Fig. 4b)	$F = \exp(0.167 \cdot W - 0.002 \cdot W^2)$	335	0.04	< 0.01	U
	$F = \exp(0.139 \cdot W - 0.001 \cdot W^2)$	334	0.06	< 0.01	DCD
	$F = \exp(0.139 \cdot W - 0.001 \cdot W^2)$	335	0.05	< 0.01	DMPP
IN and F (Fig. 4c)	$F = 2.183 \cdot \text{IN}$	73	0.42	< 0.01	U
	$F = 1.141 \cdot \text{IN}$	73	0.81	< 0.01	DCD
	$F = 0.992 \cdot \text{IN}$	73	0.75	< 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)	$F_i = \exp(0.129 \cdot W - 0.001 \cdot W^2)$	335	0.02	< 0.01	DCD
	$F_i = \exp(0.129 \cdot W - 0.001 \cdot W^2)$	335	0.02	< 0.01	DMPP
T , IN and F	$F = \text{IN} \cdot \exp(0.038 \cdot T)$	73	0.51	< 0.01	U
	$F = \text{IN} \cdot \exp(0.007 \cdot T)$	73	0.83	< 0.01	DCD
	$F = \text{IN} \cdot \exp(0.002 \cdot T)$	73	0.76	< 0.01	DMPP
W , IN and F	$F = \text{IN} \cdot \exp(-0.010 \cdot W + 0.0005 \cdot W^2)$	72	0.51	< 0.01	U
	$F = \text{IN} \cdot \exp(-0.032 \cdot W + 0.0006 \cdot W^2)$	72	0.87	< 0.01	DCD
	$F = \text{IN} \cdot \exp(-0.043 \cdot W + 0.0008 \cdot W^2)$	72	0.83	< 0.01	DMPP
T , W and F	$F = \exp(0.181 \cdot T - 0.022 \cdot W + 0.0006 \cdot W^2)$	335	0.20	< 0.01	U
	$F = \exp(0.170 \cdot T - 0.038 \cdot W + 0.0008 \cdot W^2)$	334	0.26	< 0.01	DCD
	$F = \exp(0.190 \cdot T - 0.077 \cdot W + 0.001 \cdot W^2)$	335	0.28	< 0.01	DMPP
T , W , IN and F	$F = \text{IN} \cdot \exp(0.173 \cdot T - 0.148 \cdot W + 0.002 \cdot W^2)$	72	0.55	< 0.01	U
	$F = \text{IN} \cdot \exp(0.054 \cdot T - 0.086 \cdot W + 0.001 \cdot W^2)$	72	0.88	< 0.01	DCD
	$F = \text{IN} \cdot \exp(0.028 \cdot T - 0.068 \cdot W + 0.001 \cdot W^2)$	72	0.83	< 0.01	DMPP

SDW: soil dry weight; n : sample number; r : correlation coefficient; p : probability value; T : treatment.

Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Table 4. The (inhibited: ↓; enhanced: ↑; no: ×; and not measured: –) effects of DCD and DMPP on N₂O emission (N₂O), soil nitrate (NO₃⁻) and ammonium (NH₄⁺) contents, NO₃⁻ leaching, yield and plant nitrogen uptake (PNU).

Ecosystem	FT	NI	AR	AM	Period	N ₂ O	N ₂ O%	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); N₂O%: inhibition effect of N₂O in percentage; ASN: ammonium sulfate nitrate; AN: ammonium nitrate; CAN: calcium ammonium nitrate; AS: ammonium sulphate; CS: cattle slurry; CU: cow urine; C: coated on fertilizer granules; L: liquid application; MS: mixed with slurry; FPS: fine particle suspension.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

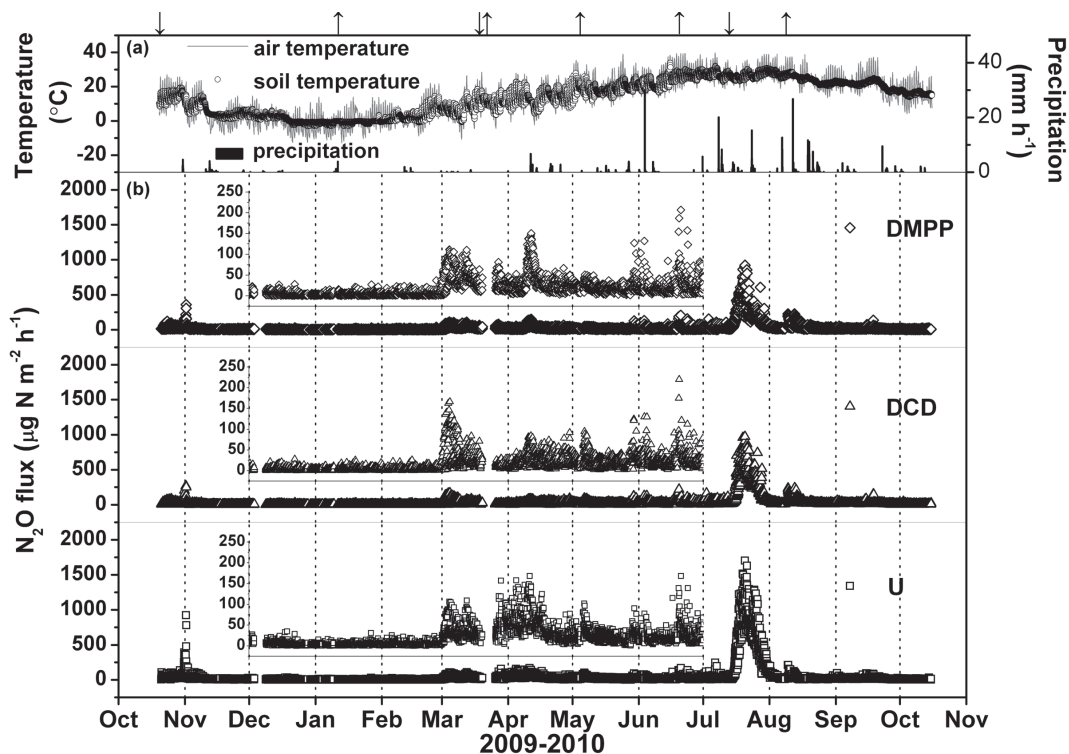


Fig. 1. Temporal courses of hourly (a) air and soil temperatures, precipitation, and (b) N₂O flux for U, DCD and DMPP treatments. The upward (↑) and downward (↓) arrows indicate the dates of irrigation and fertilization, respectively.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



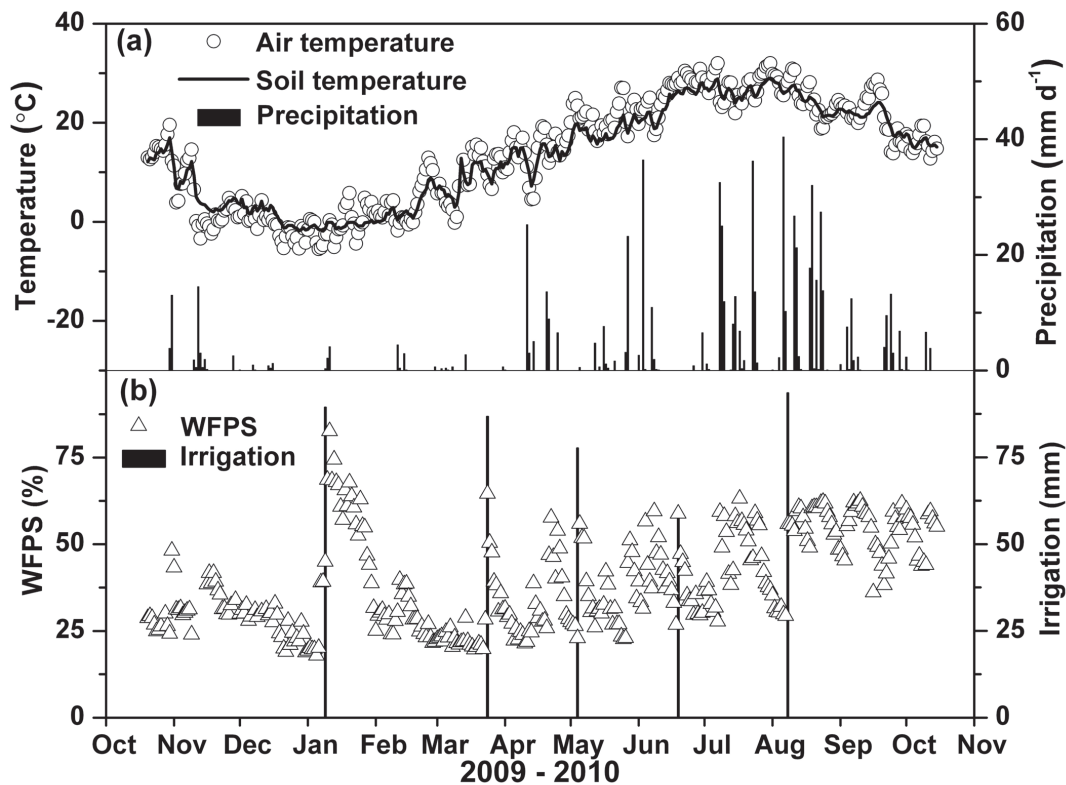


Fig. 2. Temporal courses of (a) daily averaged air and soil temperatures, daily precipitation, and (b) soil water-filled pore space (WFPS) and irrigation amount at the experimental field (U treatment).

Effects of nitrification inhibitors on N₂O emission

C. Liu et al.

Title Page

Abstract Introduction

Conclusions References

Tables Figures

◀ ▶

◀ ▶

Back Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



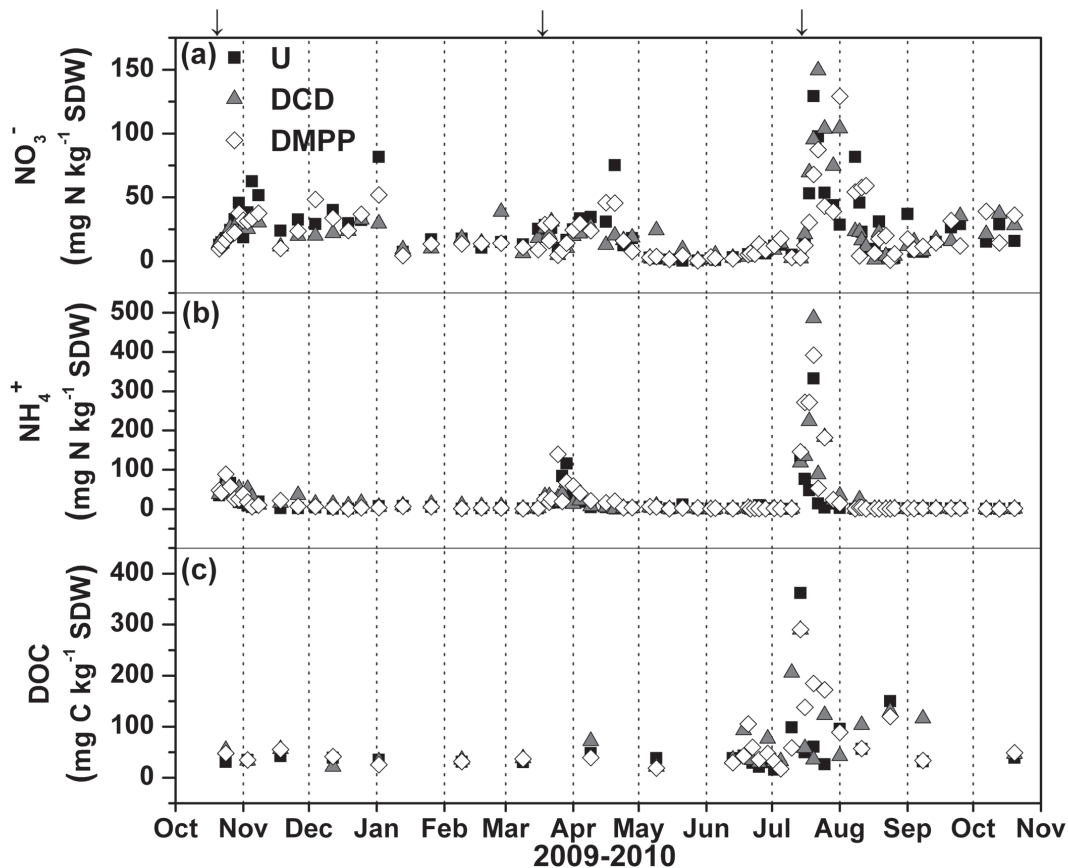


Fig. 3. Temporal courses of soil **(a)** nitrate (NO_3^-), **(b)** ammonium (NH_4^+) and **(c)** dissolved organic carbon (DOC) contents for the U, DCD and DMPP treatments. SDW: soil dry weight. The downward (↓) arrows indicate the fertilization dates.

Effects of nitrification inhibitors on N_2O emission

C. Liu et al.

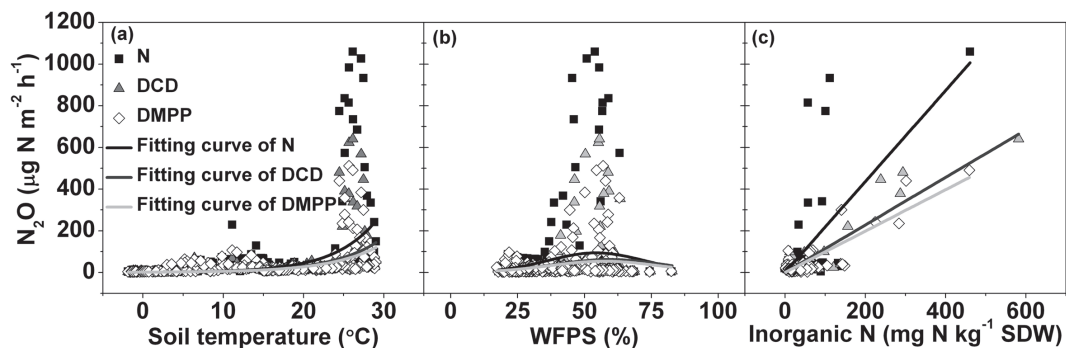


Fig. 4. Relationship between daily averaged soil temperatures, soil water-filled pore space (WFPS), soil inorganic nitrogen (N) contents and daily means of N_2O flux. SDW: soil dry weight.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Effects of
nitrification inhibitors
on N₂O emission**

C. Liu et al.

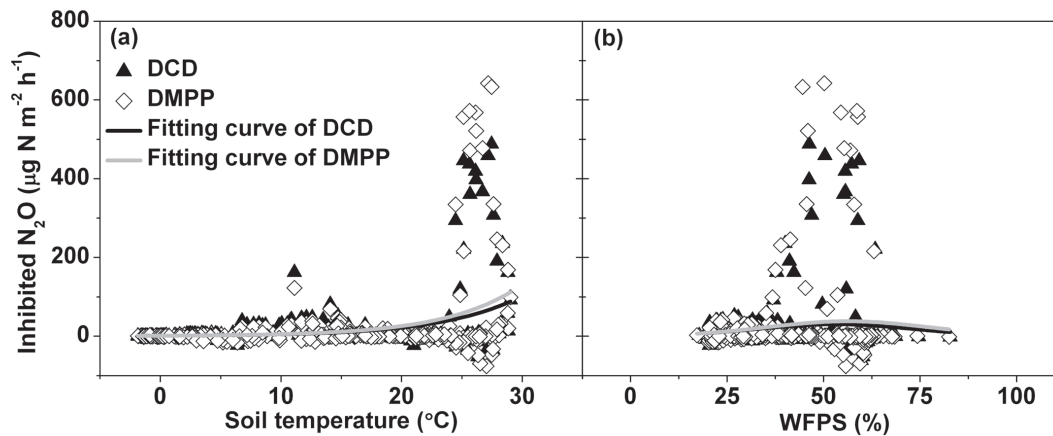


Fig. 5. Relationship between daily averaged soil temperatures, soil water-filled pore space (WFPS) and inhibited N₂O flux.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

