



Targeting yield and reducing nitrous oxide emission by use of single and double inhibitor treated urea during winter wheat season in Northern Germany

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ARTICLE INFO

Keywords:

N₂O emission
Crop yield
Urea
Nitrification inhibitor
Urease inhibitor
Winter wheat

ABSTRACT

Nitrous oxide (N₂O) is a powerful greenhouse gas and has an adverse effect on stratospheric ozone. Field application of synthetic N fertilizers is the largest source of global N₂O emission and different N forms (nitrate vs. ammoniacal N) may play a significant role. In addition, the use of nitrification inhibitor (NI) is considered as a reliable way to mitigate agricultural N₂O emission, whereas this effect is still debated for urease inhibitors (UI). However, the efficacy of NI or UI is still variable among different inhibitor products and environmental conditions. This study was conducted to test the efficacy of N form (calcium ammonium nitrate CAN vs. urea) and the almost unstudied UI, N-(2-nitrophenyl) phosphoric triamide (2-NPT), as well as an NI, mixture of dicyandiamide and 1H-1,2,4-triazol (DCD/TZ) and the combination of both inhibitors on N₂O emission and crop yield. The measurements were carried out in winter wheat growth season in the subsequent years of 2012–2013 in the North of Germany. No difference in cumulated N₂O emissions were observed between urea and CAN. The results confirmed the positive effect of NI (DCD/TZ) on reducing N₂O emission. Compared with untreated urea, NI addition caused ~75 % reduction of fertilizer derived N₂O emissions within the vegetation period. The combination of UI and NI did not result in a further reduction of relative or yield-scaled N₂O emission, although it resulted in higher grain yield and nitrogen recovery. Addition of UI showed no consistent effect on N₂O emission compared to untreated urea, however in year 2013 a significant reduction of fertilizer derived emissions by ~50 % was observed. Higher yields were observed for CAN fertilization compared to urea, though not significant. For both treatments including UI the yield effects, in particular N use efficiency, were stronger than for untreated urea and urea solely treated with NI. Therefore, the combined treatment with UI and NI was the most advantageous fertilizer solution for concomitantly achieving high yield, high nitrogen utilization efficiency and N₂O emission reduction.

1. Introduction

Nitrous oxide (N₂O) is the most powerful long-lived greenhouse gas (GHG) with ~268 times higher global warming potential (GWP) than carbon dioxide (CO₂) on a 100-year perspective (IPCC, 2019). Beside global warming, nitrous oxide also has a severe effect in depleting stratospheric ozone (Ravishankara et al., 2009). Agriculture is the most important source of anthropogenic emission of N₂O, mostly by N fertilizer application (Davidson, 2009). Nevertheless, sufficient synthetic N fertilizer should be used to meet the global food demand with expected

population increase over time (Tilman et al., 2011). Thus, decreasing the N₂O emission with adapted N application rates and timings, and the use of enhanced efficiency fertilizers with urease and nitrification inhibitors, have great potential for improving future agricultural management practices.

Since N fertilizer derived N₂O is mostly produced by microbial driven nitrification and denitrification processes, reducing the corresponding substrate concentration or retarding the related microbial activity in the soils is a reliable path to mitigate soil N₂O emission (Wrage et al., 2001). Using nitrification inhibitors (NI) to slow down the

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<https://doi.org/10.1016/j.agee.2023.108391>

Received 24 October 2022; Received in revised form 19 January 2023; Accepted 27 January 2023

Available online 3 February 2023

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transformation of ammonium to nitrate has been considered as an efficient way to reduce N₂O emission or N leaching in many studies (Di et al., 2016; Meng et al., 2021). Nevertheless, single use of NI may increase the ammonium availability in soil and subsequent ammonia emissions, especially in calcareous soil with high pH, which was substantiated in a review study by Lam et al. (2017). Fan et al. (2018) reported that use of a nitrification inhibitor decreased soil N₂O emissions by 1.8–61.0 %, but promoted NH₃ volatilization by 3.2–44.6 % in vegetable production systems in China. Thus, the combined use of NI with urease inhibitor (UI) has been promoted to concomitantly control the emission of ammonia and N₂O (Weiske et al., 2001; Tao et al., 2018).

There are three commonly used NI, i.e. dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP) and Nitrapyrin. Generally, DMPP showed the strongest effect (per unit weight of compound) on nitrification inhibition since it has a lower mineralization rate and can remain longer in soil than DCD (Zhou et al., 2020). Moreover, the efficacy of NI seems also to depend on crop type, field management, soil and climatic conditions (Behnke et al., 2018; Essich et al., 2020; Ottaiano et al., 2020). Thus, in addition to laboratory testing, the efficacy and performance of a new developed inhibitor should be assessed under field conditions,

A new nitrification inhibitor, DCD/TZ (mixture of dicyandiamide and 1H-1,2,4-triazole) was introduced in Germany in the early 2000 s. The positive effect of DCD/TZ alone on retarding N₂O emission after urea application were found in both lab incubation and field studies (Ni et al., 2018; Hu et al., 2020). Like NI, urease inhibitor (UI) is another nitrogen fertilizer synergist which slows down the speed of transformation of urea to ammonium. UI is beneficial for decreasing nitrogen loss by ammonia volatilization. But the knowledge on the effect of UIs on N₂O emission is scarce.

The UI N-(2-Nitrophenyl) phosphoric triamide, shortly as 2-NPT, was also introduced in early 2000s, and its efficacy to reduce NH₃ emission has been assessed (Ni et al., 2014; Schraml et al., 2016; Adhikari et al., 2020), but its efficiency on decreasing N₂O emission has not yet been tested. It was reported that additional 2-NPT increased the N₂O emission compared with DCD/TZ addition alone (Hu et al., 2020). The decrease of efficiency of NI by UI addition implies the key role of climate and soil conditions on N₂O emissions.

In addition to the effect of inhibitors on N₂O losses, the specific N form (e.g. urea vs. calcium ammonium nitrate) can play a role. Cowan et al. (2019) showed that under the conditions of Ireland (clayey soils, high rainfall) N₂O emissions from the nitrate-based fertilizer CAN could be considerably higher than from urea, due to faster and stronger denitrification. Overall, there is a strong need to evaluate the N₂O emission behavior of fertilizer N forms and the influence of inhibitors on this behavior under various agroecological conditions. This is in particular of importance for regionalized N₂O emission assessment which can support optimized allocation of N₂O emission reduction measures and policies (Mathivanan et al., 2021).

Another important aspect of N form and use of inhibitors is their agronomic effect on crop yield and nutrient efficiency with relevant repercussions for fertilizer economy and additional environmental benefits by allowing the reduction of application rates and of concomitant environmental risks of excess N supply including reduction of indirect N₂O emissions. On a global scale, recent meta studies found general agronomic benefits (Li et al., 2018; Kanter and Searchinger, 2018) combined with emission reduction. But effects also depend on inhibitor active ingredients and agronomic environment, with smaller effects e.g. in grain crops. For a full assessment of a potential benefit of an inhibitor active ingredient both agronomic outcomes and emissions need to be accounted for and can be combined in the variable yield scaled emissions.

In this study, a field experiment was conducted to evaluate the effect of urea and CAN and the efficacy of 2-NPT, DCD/TZ and their combination on N₂O emission reduction, crop yield and nitrogen use efficiency in winter wheat in northern Germany. The main objective was to

quantify the response of crop yield and N₂O emission to fertilizer type and different inhibitor treatments of urea. We hypothesized that (1) CAN has no higher relative N₂O emission than urea under typical central European climate and arable soil conditions, (2) UI or NI addition alone have positive effect on reducing N₂O emission, but combined use of UI and NI has no additive effect as the NI dominates the urea turnover process, (3) single use of UI or NI can increase yield due to reduced N losses, and a combined use of UI and NI has a synergistic effect.

2. Materials and methods

2.1. Site description

The field experiment was carried out on two different areas of a field (10 ha) in the Experimental Farm “Hohenschulen”, an affiliate of Christian-Albrechts-University of Kiel in Northern Germany (54°18'N, 9°58'E). The soil is sandy loam in texture (sand 58 %, silt 29 % and clay 13 %) and classified as Luvisol with the following properties: bulk density 1.37 g cm⁻³, pH 6.5, total organic C 1.5 %, total N 0.1 %, water holding capacity (WHC) 37 %.

The climate in this region is maritime, with the mean annual temperature and precipitation 8.5 °C and 750 mm respectively. An automatic weather station (CR200, Campbell Scientific, USA) was installed in the field center to monitor air temperature and rainfall. During the experiment, the mean air temperature was 11.5 °C and 9.5 °C during the wheat growth season in 2012 and 2013, respectively (Fig. 1). In March 2013, the monthly mean temperature was -1.2 °C, and lowest temperature was -4.7 °C, which was quite lower than those in 2012 with 5.8 °C in monthly mean temperature (Fig. S1). The wheat growth season was also drier in 2013, since the total rain from March to early August was 387 mm and 321 mm in 2012 and 2013, respectively, with particularly lower precipitation in early spring. The rainfall events were also more frequent in 2012, since there were 79 rainy days in 2012, more than 58 rainy days in 2013 (Fig. 1).

2.2. Field experiment design

The field study was conducted during two successive winter wheat seasons from 2011 to 2013 on two neighboring field sites. It included 6 different treatments, i.e. control with no N supply (CK), calcium ammonium nitrate (CAN), common granular urea (U), granular urea mixed with urease inhibitor (U+UI), granular urea mixed with nitrification inhibitor (U+NI), and granular urea mixed with urease- and nitrification inhibitors (U+UI+NI). Each treatment was replicated 4 times and was randomly distributed in 24 square-shape plots (9 m × 9 m).

The urease and nitrification inhibitors were N-(2-Nitrophenyl) phosphoric triamide (2-NPT), and a mixture of dicyandiamide and 1 H-1,2,4-Triazol (DCD/TZ), respectively. The concentrations of urease and nitrification inhibitors were 0.04 % and 2 % of urea N content (w/w), respectively. The two inhibitors were mixed into the granule during granulation. All used fertilizers were commercial products and obtained from SKW fertilizer company (Stickstoffwerke Piesteritz GmbH, Germany).

The total nitrogen rate was 200 kg N ha⁻¹ in all treatments except CK (no fertilizer N), according to regional optimum N levels. To meet the typical application for the regional N management, total N was divided into 3 doses at different growth stages (EC stages, BBCH Monograph 2001) in CAN, U, and U+UI treatments, i.e. 70 kg N ha⁻¹ at EC 21, 70 kg N ha⁻¹ at EC 32 and 60 kg N ha⁻¹ at EC51, respectively. With respect to treatments with NI addition, U+NI and U+UI+NI were applied in 2 doses, with 110 kg N ha⁻¹ at EC 21 and 90 kg N ha⁻¹ at EC37, respectively, to reduce the machine and labor cost without yield loss (Hu et al., 2014). This follows the common practice and the recommendation of fertilizer producing companies to take advantage of the lower mobility of stabilized ammonium N in soil to reduce fertilizer

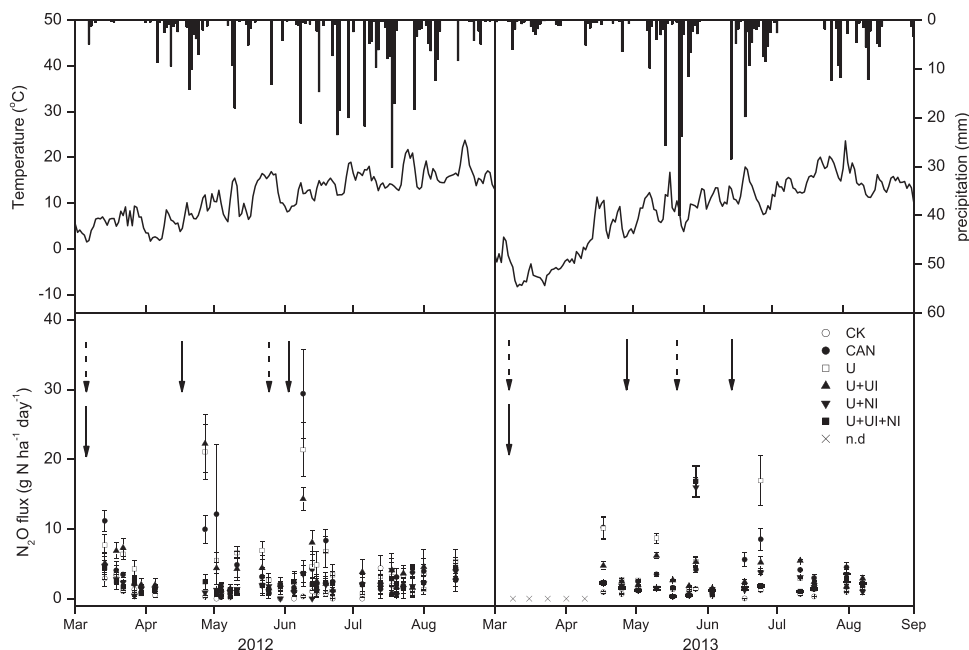


Fig. 1. Air temperature (1 m height), rainfall and soil N_2O fluxes during the winter wheat growth season in 2012 (left panel) and 2013 (right panel). The arrow lines in bottom panel represented the fertilization event in treatments with 3 split applications (CAN, U and U+UI, solid line) and 2 split applications (U+NI and U+UI+NI, dashed line). n.d. = no fluxes detectable.

application field work. The study was designed to reflect common agronomic practices in the use of fertilizer N forms and inhibitors.

All N fertilizers were evenly applied on the surface by special tractor-driven fertilizing machinery. Fertilization dates in treatments with 3 split-applications (CAN, U, and U+UI) were March 6, April 16, June 4 in year 2012 while March 7, April 29, June 17 in year of 2013. The fertilization dates in treatments with 2 split-applications (U+NI, and U+UI+NI) were March 6 and May 21 in the year 2012, while March 7 and May 24 in the year 2013.

Other management practices were the same in all treatments according to the local farm practice. The P and K rates were 35 kg P ha^{-1} and 100 kg K ha^{-1} , respectively. All plots were ploughed before plant establishment and sowed in September with ~ 300 kernels m^{-2} .

2.3. Crop yield and biomass

At harvest, the aboveground biomass was collected in 2 randomly selected subplots ($0.5 \text{ m} \times 0.5 \text{ m}$) in each plot. The plant samples were then taken to the laboratory and separated into grain and straw parts. The fresh samples were oven dried at 100°C and weighed to obtain dry matter. The samples were used for determining N concentration by near-infrared-spectroscopy (FOSS NIR Systems Modell 5000).

2.4. Soil sampling and soil mineral N

At beginning of the vegetation period (mid-February) and after harvest (August) soil samples (0–0.9 m) were taken with a soil corer, to account for N supply at the beginning and fertilizer specific residual soil mineral N after harvest. Six soil cores were taken per plot and mixed (for layers 0–30 cm, 30–60 cm, 60–90 cm, separately). Soil mineral nitrogen (sum of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ contents) was determined with a spectrophotometer after extraction of 50 g soil with 400 mL of a 125 mM CaCl_2 solution and 45 min of mechanical shaking followed by centrifugation. A subsample of each soil sample was used for determination of gravimetric water content (dried at 105°C), which was converted to volumetric water content via multiplication with bulk densities of the three soil layers.

2.5. N_2O flux measurement

Soil N_2O flux was measured by the static chamber method (Hutchinson and Mosier, 1981). Before fertilization, a chamber base (PVC ring, 60 cm in diameter) was inserted into the soil (inserted to 5 cm depth) in each plot. Two types of cylindrical chambers with different heights (25 and 90 cm) were used in this study for different crop height periods. The weekly gas sampling started after the first fertilization and ended one week after harvest (28. Aug. 2012 and 07. Aug. 2013). Considering the fertilizer application dates, this resulted in measurement periods of 175 days in 2012 and 153 days in 2013.

Before chamber settlement, the vent on the chamber was opened to allow air pressure equilibrium between the chamber and ambient air. Then, the static chamber was fitted on the PVC ring and sealed with a tight rubber belt. After that, the vent on the chamber was sealed by a rubber stopper. With 20 min interval, 30 mL of gas was sampled into a pre-evacuated glass vial (Labco, High Wycombe, UK) in each plot at 0, 20, 40 and 60 min after sealing, respectively. Nitrous oxide concentration was analyzed by ECD gas chromatography (Model 3400 CX, Varian Inc., Palo Alto, CA, USA). Operating conditions for the GC were as follows: injector temperature 95°C , column temperature 85°C , and detector temperature 320°C . Samples were introduced using a Gilson 222 XL autosampler (Gilson Inc., Middleton, USA).

2.6. Calculation and statistical analysis

The flux rate of N_2O (F , $\text{g N ha}^{-1} \text{ d}^{-1}$) was calculated based on the N_2O concentration gradient with closure time:

$$F = \rho \times \frac{V}{A} \times \frac{\Delta c}{\Delta t} \times \frac{273}{273 + T} \times 10000 \times 3600 \quad (1)$$

where F is the N_2O flux ($\text{g N ha}^{-1} \text{ d}^{-1}$); ρ is the density of N_2O -N (g m^{-3}); V is the volume of the chamber (m^3); A is the base area of the chamber (m^2); $\Delta c/\Delta t$ is the slope of concentration gradient (ppmv min^{-1}); T is the temperature ($^\circ\text{C}$) in the chamber; 10,000 is the transfer coefficient between square meter and hectare; and 3600 is the conversion factor between minutes and day.

Cumulative N_2O emission (E , g N ha^{-1}) was calculated using

integration:

$$E = \sum_{i=1}^{N-1} \frac{F_i + F_{i+1}}{2} \times (t_{i+1} - t_i) \quad (2)$$

where F is the N_2O flux ($g\ N\ ha^{-1}\ d^{-1}$); t is the days after first fertilization; i is the sequence of gas sampling (from 1 to N).

The N_2O relative emission (RE, %) was calculated as:

$$RE = \frac{E_N - E_{CK}}{\text{applied N}} \times 100 \quad (3)$$

where E_N or E_{CK} was cumulative N_2O emission ($kg\ N\ ha^{-1}$) in plots with or without N fertilization; applied N was the annual N rate ($kg\ N\ ha^{-1}$) in each plot. RE should not be mixed up with IPCC emission factors as the latter need to account for whole year emissions.

The N_2O reduction efficiency was calculated as the relative decrease of relative emission, compared to reference U:

$$\text{Reduction}(\%) = \frac{RE_i - RE_U}{RE_U} \times 100\% \quad (4)$$

Where RE_i represents the relative N_2O emission (RE) in treatments with different N types except U, while RE_U represents the RE in U.

Four different yield-scaled N_2O indices of emissions were calculated by dividing the cumulative N_2O emissions by (i) grain yield, (ii) aboveground biomass, (iii) grain N uptake and (iv) aboveground N uptake (Niu et al., 2017).

Nitrogen utilization efficiency (NUE), was calculated as apparent nitrogen recovery by dividing the quantity of total N absorbed by amount of total N applied:

$$NUE(\%) = \frac{NU_f - NU_{uf}}{N\ \text{application rate}} \times 100\% \quad (5)$$

Where NU_f and NU_{uf} were the amount of recovered N (NU) in fertilized (f) and unfertilized (uf) plot, respectively.

NU was calculated as the sum of the dry matter of grain and straw multiplied by the respective N concentration (Baligar and Fageria, 2015):

$$NU = \text{Biomass}_{\text{grain}} \times N_{\text{grain}} + \text{Biomass}_{\text{straw}} \times N_{\text{straw}} \quad (6)$$

Where NU ($kg\ N\ ha^{-1}$) was the amount of recovered N, biomass ($kg\ ha^{-1}$) was the dry matter of grain or straw, while N (%) was the concentration of nitrogen in the grain or straw.

One-way analysis of variance (ANOVA) was conducted to compare the effect of inhibitor application on cumulative N_2O emissions, dry matter, recovered N and yielded-scaled N_2O emissions for each year. The Tukey test (HSD) was used for post hoc pairwise comparisons between different fertilization treatments.

In case of no interaction effect of year and treatment, two-way analysis of variance (ANOVA) was conducted across both experimental years to compare the effect of year and fertilization treatment with the effect size represented by partial eta-squared (η^2p):

$$\text{Partial eta squared} = SS_{\text{effect}} / (SS_{\text{effect}} + SS_{\text{error}})$$

$$\eta^2p = \frac{SS_{\text{effect}}}{SS_{\text{effect}} + SS_{\text{error}}} \quad (7)$$

where SS_{effect} and SS_{error} represented the sum of squares of a specific factor effect and model residuals in the ANOVA. The Tukey test (HSD) was used for post hoc pairwise comparisons between different fertilization treatments.

All statistical analyses were performed using the statistical package R (R-4.1.0, CRAN, 2021).

3. Results

3.1. Grain yield, N concentration and NUE

Grain yield was higher in 2012, and N fertilization significantly yielded 2 times higher grain or straw biomass than CK (Fig. 2). CAN resulted in very high grain yields in both years while U+UI+NI showed the highest grain yield across years among urea treatments, 8 % higher than U (Table 1). No significant differences in grain yield between fertilizer treatments were observed across years.

Compared to untreated U, neither single or combined use of inhibitors showed significant effects on straw or total aboveground biomass (Table 1). There was no significant difference in total biomass among the fertilization treatments (Table 1). Both year (Y) and treatment (T), showed significant effects on biomass (grain and straw), but fertilizer type (T) exhibited a larger effect size (higher partial-eta values) than annual variation (T).

In contrast to dry matter, grain or straw N concentration was only affected by the treatment, and N fertilization significantly increased the grain N concentration. However, treatment factor (T) had a smaller effect on straw N concentration than in grain, since the partial eta-squared value was 0.3, which was much lower than 0.87 in grain (Table 1).

Grain N concentrations varied significantly between 1.38 % and 1.95 %, while straw N concentration varied between 0.48 % and 0.60 % (Table 1). CAN gave slightly higher N concentrations than untreated urea. Compared with U (1.83 %) and CAN (1.85 %), addition of NI alone significantly decreased the grain N concentration to 1.69 %, while UI addition increased the value to 1.95 %. Combined use of UI and NI showed similar grain N concentration as U, but still lower as UI addition alone. A similar pattern was found in straw N concentration.

Most N was stored in grain, as dry matter and N concentration were both higher in grain compared to straw (Table 1, Fig. S2). CAN gained the highest recovered N, whereas U+NI gained the least N in all N treatments. Both year and treatments showed significant effects on N recovery. The harvested grain recovered 140–192 $kg\ N\ ha^{-1}$ in 2012, while the amount was smaller in 2013 with 121–165 $kg\ N\ ha^{-1}$, which accounted for 60–83 % of applied N (Fig. S2).

In the studied site, the NUE in winter wheat was 60–96 % during the 2 years (Table 1, Fig. S3). There was a significant year effect, with a higher NUE in 2012, but with no significant year x fertilizer interaction across years. Among all fertilized treatments, U+UI and CAN obtained the highest NUE with 83 % and 87 %, respectively, while U+NI gained the lowest NUE (65 %). Compared with U, UI non-significantly increased (+9 %), but NI significantly decreased (−9 %) the NUE. U+UI+NI gained a tradeoff, which caused a NUE non-significantly higher (+7 %) than U and 16 % significantly higher than U+NI.

3.2. Soil N_2O flux

In 2012, soil N_2O flux peaks in CAN and U were always observed immediately after N fertilization, while there was a lag of several days for peaks in treatments with inhibitor addition (Fig. 1). After first application in March, the largest N_2O flux was 11.16 $g\ N\ ha^{-1}\ d^{-1}$ in CAN on March 14, while U+UI and U showed significantly smaller maximum fluxes with 5.08 $g\ N\ ha^{-1}\ d^{-1}$ to 7.69 $g\ N\ ha^{-1}\ d^{-1}$, respectively. But after the second application, U and U+UI showed similar flux peaks with 21.07 $g\ N\ ha^{-1}\ d^{-1}$ and 22.06 $g\ N\ ha^{-1}\ d^{-1}$, respectively, which were higher than that in CAN. The fluxes in June were similar to those in March, with the flux peaks ranked as CAN>U>U+UI. Significantly, treatments with NI addition showed much lower fluxes than other treatments although they received 60 $kg\ ha^{-1}$ more N in the second application.

Due to snow and frost in March of 2013, soil N_2O flux was negligible at the beginning of measurements, since no N_2O concentration gradient was built-up during 1-hour chamber closure (3 samplings in March and 2 samplings in earlier April). The fluxes recovered to 5–10 $g\ N\ ha^{-1}\ d^{-1}$



Fig. 2. Effect of different synthetic fertilizers on grain yield (dry matter, ton ha⁻¹) in the experimental years 2012 and 2013. Upper case letters indicate significance levels between treatments in year 2012, lower case in year 2013 (HSD Tukey, $p < 0.05$). CK = 0 N added, CAN = calcium ammonium nitrate, U = urea, U+NI = urea + nitrification inhibitor (DCD/TZ), U+UI = urea + urease inhibitor (2-NPT), U+UI+NI = urea + urease inhibitor + nitrification inhibitor.

Table 1

Harvested dry matter, N concentration and recovered N (NUE) in wheat season of 2012 and 2013.

Treatment†	Dry matter (t h ⁻¹)			N concentration (%)		Recovered N (kg N ha ⁻¹)			NUE (%)
	Grain	Straw	Total	Grain	Straw	Grain	Straw	Total	
CK	4.93b	3.05b	7.98b	1.38d	0.54abc	68.3c	16.4d	84.7c	
CAN	11.6a	7.5a	19.1a	1.85b	0.6a	214.8a	44.6a	259.5a	87.4a
U	10.7a	6.95a	17.7a	1.83b	0.51 bc	197.4ab	35.9bc	233.2ab	74.3ab
U+UI	10.7a	7.03a	17.8a	1.95a	0.57ab	209.8a	40.7ab	250.6a	83a
U+NI	10.8a	6.6a	17.4a	1.69c	0.48c	183.1b	31.8c	214.9b	65.1b
U+UI+NI	11.6a	7.05a	18.6a	1.82b	0.51 bc	210.6a	36.1bc	246.7ab	81a
Year									
2012	10.9a	7.15a	18a	1.76	0.55	195.5a	39.4a	234.9a	87a
2013	9.26b	5.57b	14.8b	1.75	0.52	165.8b	29.1b	194.9b	69.4b
ANOVA‡									
Year (Y)	0.49***	0.71***	0.6***	0	0.04	0.42***	0.5***	0.49***	0.4***
Treatment (T)	0.89***	0.9***	0.89***	0.87***	0.3*	0.9***	0.75***	0.9***	0.35*
Y×T	0.17	0.15	0.16	0	0.22	0.16	0.25	0.18	0.11

† Values were marginal means, and different lowercase letters indicate the significant difference at $P < 0.05$.

‡ Symbols *, ** and *** represents significant effect at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; values were the partial eta-squared represent effect size.

when air temperature increased to ~ 5 °C at the middle of April (April 17th). After N fertilization in June, the flux pattern was $U > CAN > U+UI$. Although U+NI and U+UI+NI included NI, significant flux peaks of $16.0 \text{ g N ha}^{-1} \text{ d}^{-1}$ and $16.8 \text{ g N ha}^{-1} \text{ d}^{-1}$ were observed in May, 15 days after the N fertilization, which could be due to heavy rainfall during that period.

3.3. Cumulative N₂O emission and yield-scaled emission

The cumulative N₂O emission in 2013 was smaller than that in 2012 (Fig. 3). Nitrogen addition significantly increased cumulative N₂O emission during the winter wheat growing season in 2012 and 2013. Compared with U, CAN showed similar, while inhibitor treated urea showed significantly smaller N₂O emissions, except for U+UI in 2012. Nitrification inhibitor addition showed the least N₂O emission and caused ~ 50 % reduction compared to U.

Year, treatment, and their interaction showed significant influences on both cumulative N₂O emissions and relative emissions, while the effect size (partial squared eta) of year was smaller (Table 2). The relative emission (RE) for the vegetation period in treatments with 3 split-applications (CAN, U and U+UI) was 0.15–0.31 % N applied (Fig. 4). The RE values decreased to 0.07 % in treatments with

nitrification inhibitor and 2 split-applications (U+NI and U+UI+NI). Nitrification inhibitor addition significantly reduced the RE in both years, while UI only showed a significant reduction effect in 2013. Averaged over both years, U+NI or U+UI+NI reduced the RE to 0.07 %, which was only 25 % of RE in U (Table 2).

U and CAN caused the highest N₂O emission per unit of grain yield during the winter wheat growing season, in 2012 and 2013 (Fig. S4). Compared with U, U+NI or U+UI+NI resulted in a significant reduction by 45–75 % of cumulative N₂O emission per unit of grain yield. The same pattern was also found in N₂O emission per unit of aboveground biomass, grain recovered N, and aboveground biomass recovered N (Table 2).

4. Discussion

4.1. Nitrous oxide emissions

Averaged over all fertilizer types, N₂O emissions were higher in 2012 than in 2013. Initial soil mineral N contents (0–0.3 m) were much higher in this year (on average 25 kg N/ha, Fig. S5) compared to 2013 (15 kg N/ha). In addition, higher temperature and much more frequent rainfall in 2012 were probably favorable for N₂O production, and would

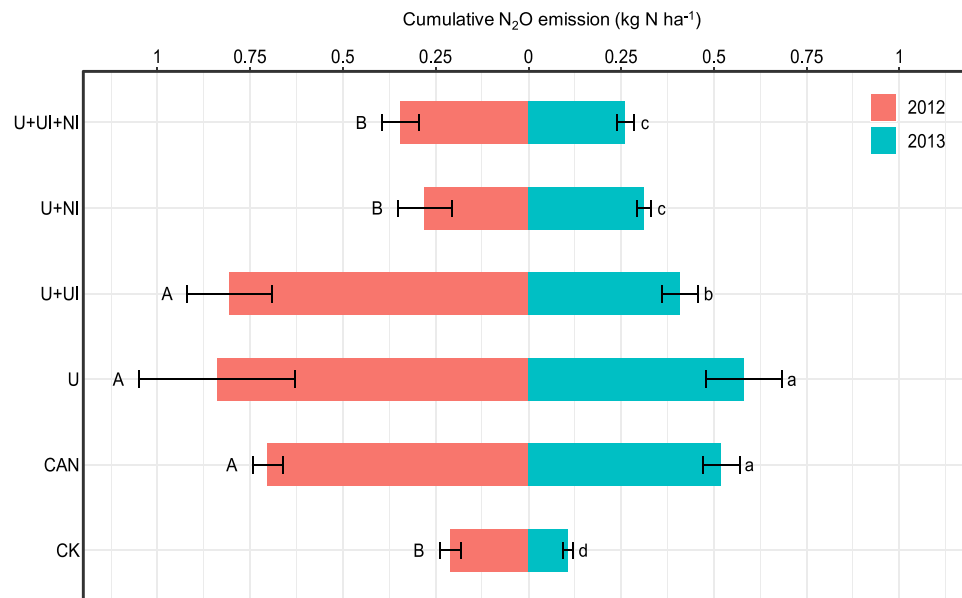


Fig. 3. Effect of different synthetic fertilizers on cumulative N₂O emissions (kg N h⁻¹) in the experimental years 2012 and 2013. Upper- and lower-case letters indicate significance difference (HSD Tukey, $p < 0.05$) between treatments in year 2012 and 2013, respectively. CK = 0 N added, CAN = calcium ammonium nitrate, U = urea, U+NI = urea + nitrification inhibitor (DCD/TZ), U+UI = urea + urease inhibitor (2-NPT), U+UI+NI = urea + urease inhibitor + nitrification inhibitor.

Table 2

Effects of different fertilization regimes on N₂O emission during the winter wheat season.

Treatment	Cumulative N ₂ O emission (g N ha ⁻¹)	RE (%)	Yield-scaled N ₂ O emission			
			g N t ⁻¹ grain	g N t ⁻¹ biomass	g N kg ⁻¹ grain N	g N kg ⁻¹ biomass N
<i>Treatment</i> †						
CK	159	-	32c	20c	2.37 bc	1.9 bc
CAN	611	0.23	53b	32b	2.83b	2.35b
U	710	0.28	67a	41a	3.72a	3.15a
U+UI	607	0.22	56ab	34ab	2.87b	2.4b
U+NI	295	0.07	28c	17c	1.63 cd	1.39c
U+UI+NI	303	0.07	26c	16c	1.45d	1.23c
<i>Year</i>						
2012	594.4	0.19	49a	29.4a	2.79a	2.3a
2013	416.3	0.16	38.4b	23.9b	2.17b	1.84b
<i>ANOVA</i> ‡						
Year (Y)	0.57***	0.16*	0.23**	0.18**	0.19**	0.17*
Treatment (T)	0.89***	0.89***	0.72***	0.72***	0.6***	0.6***
Y×T	0.47***	0.47***	0.239	0.225	0.207	0.182

† Values were marginal means, and different lowercase letters indicate the significant difference at $P < 0.05$. Multi-comparisons of each factor were not carried out in cumulative and relative N₂O emission due to the significant interaction between year (Y) and treatment (T).

‡ Symbols *, ** and *** represents significant effect at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; values were the partial eta-squared represent effect size.

have promoted denitrification which is the predominant source of soil N₂O emission under central European conditions (Wrage et al., 2001). This difference could be observed for the second split of 3 split application treatments, which were strongly affected by the dry early spring in 2013 compared to treatments including NI with a later second application.

The two year mean RE in U was 0.28 % (Table 2), which was much lower than the general IPCC default values of 1 % (IPCC, 2006) and 1.6 % for synthetic fertilizers (IPCC, 2019). The low RE in this study could be partly attributed to the lack of sampling during inter-growth season. According to the comparison between growth season and annual measurement, the cumulative N₂O emission during the wheat growth season (from March to August) accounted for 67 % of the annual cumulative N₂O emission in a study by Lebender et al. (2014). In a recent assessment by Shang et al. (2020) emissions outside the vegetation period added on average > 10 % emissions to vegetation period emissions. This may be even higher under central European climate conditions with frost thaw cycles (Mathivanan et al., 2021) to which, however, the study region

does not belong. The implied new regional emission factor for the study region – Atlantic North - in this German meta-study which should be mainly considered as the reference for this study, is 0.49 % or about 1.8 times the emissions from CAN and U measured in this study. Nevertheless, the emission was similar to the 0.1 % at N rate of 220 kg N ha⁻¹ CAN in Kiel (Lebender et al., 2014) and 0.31 % in the corn field of North China Plain (Dong et al., 2018). Furthermore, N₂O emission stems mainly from fertilizer-N not taken up by the plant. Considering 10 % of applied N lost through ammonia volatilization (Ni et al., 2014), and 82 % of applied N recovered in aboveground biomass (Table 2), there was not much excess N available for other losses. Meta-analysis also showed that significant N₂O emission increased strongly when the N rate was higher than 200 kg N ha⁻¹ (Groenigen et al., 2010). Due to the comparatively light soil conditions and moderate rainfall at the study site, no differences of N₂O emissions between the N forms urea and CAN were observed, confirming the first hypothesis. This agrees with a regionalized assessment for arable soils in the UK and Ireland (Cowan et al., 2020), CAN even showing slightly lower losses.

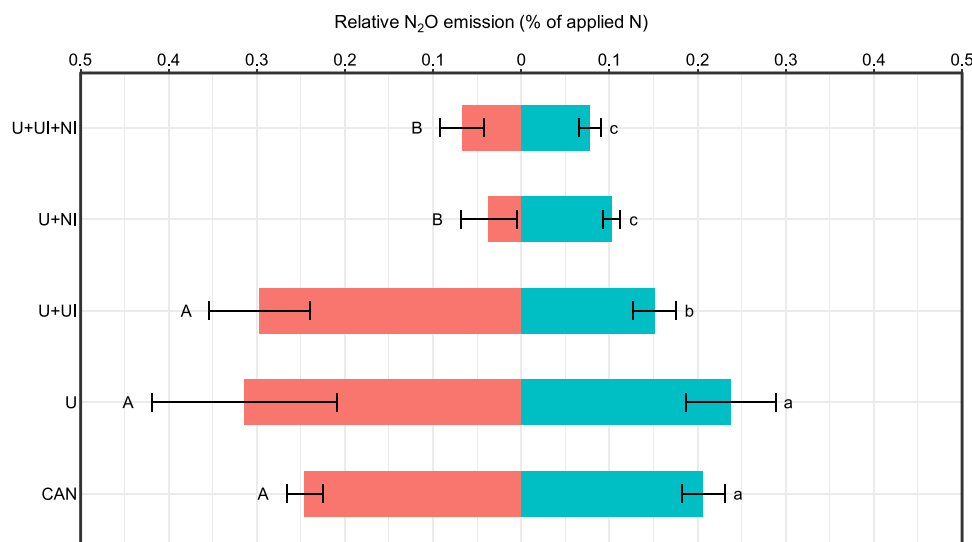


Fig. 4. Effect of different synthetic fertilizers on relative N₂O emissions (%) in the experimental years 2012 and 2013. Upper- and lower-case letters indicate significance difference (HSD Tukey, $p < 0.05$) between treatments in year 2012 and 2013, respectively. CAN = calcium ammonium nitrate, U = urea, U+NI = urea + nitrification inhibitor (DCD/TZ), U+UI = urea + urease inhibitor (2-NPT), U+UI+NI= urea + urease inhibitor + nitrification inhibitor.

4.2. Effects of NI on nitrous oxide emission

This study confirmed the positive effect of nitrification inhibitor (NI) addition on reducing N₂O emission from urea fertilizer. Averaged across years, DCD/TZ addition alone (U+NI) reduced the relative N₂O emission from 0.275 % in U to 0.06 %, with a relative reduction by 74 %. It was reported that efficiency of DCD and DMPP on reducing N₂O emission was 48.3 % and 32.9 %, respectively in a recent meta-analysis (Li et al., 2021). Thus, the 74 % reduction of RE from U to U+NI in this study could indicate a stronger emission mitigation of DCD/TZ compared to average values for DMPP or DCD alone. However, assuming about 80 % higher total emissions of uninhibited fertilizers for the whole year as discussed above and a reduction effect of inhibitors on emissions only in the vegetation period, this would decrease the emission reduction effect to about 44 % which is quite close to above cited meta studies. The latter assumption is supported by the observation that the reduction effect of NI mainly occurred shortly after fertilizer application while no differences of N₂O emissions between fertilizer treatment were observed later in the vegetation period (Fig. 1). In addition, no increase of post-harvest soil mineral nitrogen contents (Fig. S6) were observed for inhibitor treated fertilizers which could stimulate increased inhibitor induced N₂O emissions in the following autumn and winter period.

Besides the difference of N₂O emission caused by NI addition between pairwise U and U+NI or pairwise U+UI and U+UI+NI, it should be noticed that the split N rate was also different for fertilizers with and without NI. Since the N input dose and environmental condition were different during each split application, the soil N concentration and soil nitrification-denitrification rates could also be different (Ni et al., 2018). Thus, the reduction of N₂O emission between U and U+NI was the combined effect of fertilization system rather than the fertilizer type alone. Nevertheless, because more splits of the same N rate could reduce cumulative N₂O emission, we can deduce that the treatments U+NI or U+UI+NI could result in even less N₂O emission if they were applied with 3 doses like in U or U+UI. Therefore, the difference of N-split between U and U+NI or between U+UI and U+UI+NI may not affect their efficacy in reducing N₂O emission.

4.3. Effects of single and combination use of UI on nitrous oxide emission

Although the effect of urease inhibitor (UI) on N₂O emission was equivocal in this study, the combination of UI with NI showed a strong

effect since U+UI+NI showed much smaller N₂O emission than U+UI (Table 2). This additional effect of NI addition to UI was only shown in 2013, where U+UI+NI showed smaller N₂O emission than U+UI or U+NI. However, additional NI applied to U+UI caused higher N₂O emissions compared to U+UI+NI in 2012, since the cumulative N₂O emission was higher than that in U+NI, but in both years the differences were statistically insignificant.

Several studies reported that UI addition to urea caused higher N₂O emission (Khalil et al., 2009; Hu et al., 2020). However, in our study 2-NPT had no or a reducing effect on N₂O emissions, which contrasted with the result of Hu et al. (2020), but were consistent with the results by Ni et al. (2018), Krol et al. (2020) and Souza et al. (2021). Since N₂O was probably produced by nitrification and denitrification UI could retard the ammonium supply, which reduces substrate availability for both nitrification and subsequent denitrification (Bateman and Baggs, 2005).

Although UI and NI treatment had a different N-split strategy in this study (3 splits in U+UI vs 2 splits in U+NI), we propose that the difference in reducing N₂O between UI and NI with the same amount and application techniques would yield similar results, since NI always performed better than UI in reducing N₂O emission for its effect on retarding nitrification. Nevertheless, for the comparison of sole inhibitor type effects additional investigations with same fertilizer splits would be desirable.

With respect to the effect of UI on N₂O emissions, the second hypothesis is therefore only partly supported by the data, while its second aspect, the dominance of the NI in N₂O emission reduction is corroborated by the measurements. There is still more research needed to understand the variable effect of UI on N₂O emissions.

In contrast to UI reducing the emissions of the indirect greenhouse gas ammonia (NH₃) from urea, NI treatment of urea can lead to higher NH₃ emissions compared to untreated urea (Wu et al., 2021) which, however, was not observed under conditions of this study site (Ni et al., 2014). Nevertheless, as urea treated with both inhibitors gives both ammonia and N₂O emissions reduction (Ni et al., 2014) with beneficial yield effects, this inhibitor treatment seems to be favorable for both greenhouse emission reduction purposes and improving overall N uptake by crops.

4.4. Effects of UI and NI on yield and yield-scaled N₂O emission

In this study, N fertilizer significantly increased the grain yield, while

the effects of UI and NI on grain yield were highly variable among years. The popular UI, NBPT showed yield increase by -0.8% – 10.2% depending on crop species (Cantarella et al., 2018). Meta-analysis also showed that UI and NI caused about 7.5% yield increase (Abalos et al., 2014). But in our study, UI and NI addition only increased grain yield by 2% , which was similar to results from grasslands (Krol et al., 2020), and potatoes (Souza et al., 2021). However, yields were significantly increased by 14% in year 2012 with double inhibited urea compared to untreated urea.

Addition of NI alone significantly decreased grain yield (2012) and N uptake (2012 and 2013) compared to urea alone. During the early spring periods (middle of March to middle of May) rainfall amounts were not high enough (Fig. 1, Fig S1) to cause leaching losses of nitrate which might had been reduced by stabilization of the ammonium form of fertilizer N by NI. In contrast, dryer and cooler soil conditions, as particularly in spring 2013, may have been detrimental to N uptake in the ammonium form in that time period. In addition, the omission of a specific N dosage at flowering and grain filling by NI application and connected 2 split fertilization strategy had probably reduced N uptake of the grain compared to the 3 split treatments. High ammonia emissions in the U+NI treatment (Ni et al., 2014) connected with the specific disadvantage of the U+NI fertilization strategy resulted in lower yields and N uptake compared to the U+UI+NI. The ammonia emission reduction effect of the latter treatment, i.e. the effect of the UI, overcompensated the negative agronomic effects of the NI application at the conditions of the study site.

According to the NUE concept by the EU Nitrogen Expert Panel (2015), the biomass recovered N were all higher than 200 kg ha^{-1} in fertilized treatments in 2012, which implies no N surplus, high NUE and the risk of soil N mining. However, it needs to be considered that soil mineral nitrogen which amounted to about 80 kg and 40 kg N/ha in year 2012 and 2013, respectively, would reduce this effect. The lower NUE in 2013 could indicate higher N loss after the dynamic N uptake phase of the crop from April-May. However, ammonia emission was lower in 2013 due to low temperatures and snow after the first application and higher rainfall (Ni et al., 2014) in later applications. Low temperatures and low water supply at early growth stages in 2013 may also have hampered crop development and, in particular, development of generative plant organs leading to overall lower N uptake of the crop. There was no effect of fertilizer application on post-harvest soil mineral nitrogen in this year (Fig. S6). Relative to U, the NUE was not affected by any inhibitor (Table 1). This could be explained by the fact that the N fertilization rate chosen in this study was close to the local optimum N rates (Groenigen et al., 2010).

Yield-scaled N_2O emission indices were all lower in treatments with UI or NI addition (Table 2), but the values were much lower than those in other sites (Lebender et al., 2014) with yield-scaled N_2O emission by $\sim 200\text{ g N}_2\text{O t}^{-1}$ grain yield. Addition of nitrification inhibitor alone (U+NI) also gave a higher efficiency in yield-scaled N_2O emission than U (Table 2), but can be considered critical, due to lower grain quality with lower N concentration (Table 1) in this treatment.

On average across years, U+UI+NI showed the best performance, when compared to urea alone (Table 2). A meta-analysis revealed that yield-scaled N_2O emissions per aboveground N uptake was $8.4\text{ g N}_2\text{O-N kg}^{-1}\text{ N}$ in annual crops at N rates of approximately $180\text{--}190\text{ kg N ha}^{-1}$ (Groenigen et al., 2010). However, the yield scaled N_2O emissions per aboveground N uptake were $1.2\text{--}3.5\text{ g N}_2\text{O-N kg}^{-1}\text{ N}$ in our study, due to the low N_2O emission levels with no annual sampling. This can eventually also be explained by the low N rates in this study, as in case of a N surplus of 90 kg N ha^{-1} , yield-scaled emissions could increase threefold (Groenigen et al., 2010; Lebender et al., 2014). With respect to U+UI+NI the third hypothesis is supported by the data, while yield results for single inhibition (either UI or NI) were partly contradictory to it as some treatments showed no or even negative yield effects.

5. Conclusion

No difference between CAN and urea fertilizer on N_2O emissions were observed under light soil and moderate rainfall conditions. As CAN fertilization resulted in higher crop yields than urea, yield scaled N_2O emissions were lower for CAN. Nitrification inhibitor DCD/TZ addition to urea showed a beneficial effect on reducing soil N_2O emission compared to both urea and CAN, but urea treated with NI alone showed a lower N recovery rate. Furthermore, combination of UI 2-NPT and NI on urea did not show a significant synergistic effect on reducing N_2O emission or yield-scaled N_2O emission compared to single NI addition. Nevertheless, considering the requirement of concomitantly reducing N_2O and NH_3 emissions from urea fertilization, this study and connected other investigations give evidence that the combined use of UI and NI on urea can be recommended to increase nitrogen utilization efficiency in crop production system.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We want to express our grateful thanks to Jun Yang, Gunda Schnack and the field technicians' team at Hohenschulen experimental farm for their technical support. This study was funded by the German fertilizer producer SKW Stickstoffwerke Piesteritz GmbH (SKWP). But SKWP has no influence and decision on the results and publication.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2023.108391.

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