Direct and Indirect Effects of Urease and Nitrification Inhibitors on N₂O-N Losses from Urea Fertilization to Winter Wheat in Southern Germany

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Abstract: Urea (U) is the most important nitrogen (N) fertilizer in agriculture worldwide, and as N fertilizer can result in large gaseous losses of NH_3 and N_2O. Thus, urease inhibitors (UIs) and nitrification inhibitors (NIs) have been coupled with U fertilizers to mitigate NH_3 and N_2O emissions. However, it is still unclear whether adding NIs and/or UIs to U stimulates other pollutants, while reducing one pollutant. Furthermore, part of the NH_3 deposition to earth is converted to N_2O, leading to indirect N_2O emission. To estimate direct and indirect effect of UIs and NIs on the N_2O-N and NH_3-N losses from U; therefore, we analyzed multi-year field experiments from the same site during 2004 to 2005 and 2011 to 2013. The field experiments with U fertilization with or without UI (IPAT, N-isopropoxycarbonyl phosphoric acid triamide) and NI (DCD/TZ, Dicyandiamide/1H-1, 2, 4-Triazol) in winter wheat and with calcium ammonium nitrate (CAN) were conducted in southern Germany. Fluxes of NH_3 or N_2O emissions were determined following each split N fertilization in separate experiments on the same site. Our results showed that U with NIs considerably reduced N_2O emissions, and adding UIs decreased NH_3 emissions. However, the effect on N_2O emissions exerted by (U + UIs) or (U + UIs + NIs) was inconsistent. In contrast to the treatment of (U + UIs + NIs), the addition of NIs alone to U stimulated NH_3 emission compared to treatment with U. When 1% indirect N_2O emission from NH_3 (IPCC emission factor (EF4)) was considered to estimate the indirect N_2O emission, total N_2O emissions from (U + NIs) were approximately 29% compared to that from U alone and 36% compared to that from (U + UI), indicating that indirect N_2O emission from NH_3 induced by NIs may be negligible.

Keywords: ammonia emissions; nitrification inhibitor; N_2O emissions; urea; urease inhibitor; winter wheat

1. Introduction

Over the past century, a large quantity of mineral nitrogen (N) fertilizer was produced using the Haber–Bosch process that converts atmospheric N_2 to ammonia (NH_3) in order to meet the rapid increase in population for food production [1]. In mineral N fertilizer production, the use of urea (U) as an N fertilizer has dramatically increased during the past decades due to its advantages of a high N content, low cost, and safe and easy transportation. Urea is now the most important N fertilizer in the world, accounting for 55% of mineral N fertilizer production in 2018, and U is expected to increase to

70% during the next five years [2]. However, there are problems resulting from large gaseous losses using U as an N fertilizer, which are due to the increase in pH resulting from U hydrolysis.

In Germany, U is currently the second most used N fertilizer after calcium ammonium nitrate (CAN) with a market share of 25% [3]. Although there is consensus regarding the higher ammonia emissions resulting from U fertilization compared to those of CAN, there are contrasting reports regarding N_2O losses of these fertilizers [4]. Thus, there is a need to further compare N_2O emissions from U to those of CAN.

To mitigate NH_3 and N_2O emissions from U fertilization, urease and nitrification inhibitors have been coupled with U fertilizers. Urease inhibitors (UIs) slow the hydrolysis of U into NH_3 or NH_4^+. In addition to incorporating U into soil, the addition of UIs is another best practice to avoid NH_3 volatilization, and had a significant effect on NH_3 emissions and reduced NH_3 losses by 26–83% and 24–32%, respectively, of the applied urea-N on average [5,6]. Starting from 2020, the new German fertilizer ordinance requires the application of either UIs or the immediate incorporation of U into soils. The main working principle of UIs is slowing down the hydrolysis of U, and thus giving more time for the U to increase its dispersion into the soil [6].

Nitrification is a key process of N transformation with two steps: the ammonia oxidizing bacteria such as *Nitrosomonas* or *Nitrosospira* convert NH_4^+ to nitrite (NO_2^-), and then the nitrite oxidizing bacteria such as *Nitrobacter* or *Nitrospira* convert NO_2^- to NO_3^- [7]. NIs act by depressing the activity of the principal exponent of this first step (*Nitrosomonas* spp.); thereby, delaying the oxidation process. Depending on the soil conditions and seasons, UIs or NIs can delay hydrolysis of U or nitrification of NH_4^+ from week to months.

The Intergovernmental Panel on Climate Change IPCC [8] has recommended nitrification inhibitors (NIs) as a potential mitigation option for agricultural N_2O emission. Recent meta-analyses have indicated that NI decreased N_2O emissions by 31–48% across diverse agricultural ecosystems, including upland, grassland, and paddy fields [9,10]. The use of NIs, however, prolongs the retention of NH_4^+ in soil, which could increase NH_3 emission [11]. The application of the NI dicyandiamide (DCD) has been found to increase NH_3 emissions by 18–29% [12,13] and by up to 38% [14]. However, Ni et al. [5,15] reported that there was no effect of NIs on NH_3 emissions from U fertilization. Therefore, there is now a concern regarding if (U + UIs) or (U + NIs) results in a reduction in the loss of one pollutant but leads to an increase in another. In contrast to (U + NI), several recent studies have indicated the existence of inconsistencies in the impact of (U + UIs) on N_2O emission [16]. For instance, after a meta-analysis of 113 field experiments, Akiyama et al. [9] found that UI tended to reduce N_2O emissions by 10% on average, though this was not significant. In soils from New Zealand, van der Weerden et al. [17] found that NBPT (N-(n-butyl) thiophosphoric triamide) had no effect on the overall N_2O emissions from U. For reduction of both NH_3 and N_2O emissions, a combination of UIs and NIs for U, i.e., (U + UIs + NIs), has been recommended [18]. Beyond reducing N losses via inhibitors, recent studies have found that NI uses can also simplify fertilization in intensive crop production to save labour and machinery costs, mainly by allowing a reduction in split N application by three to two times, while without affecting the agricultural crop yield [19].

According to the IPCC guidelines [8,20], approximately 1% (a range of 0.2–5%) of the emitted NH_3 is converted to N_2O after its deposition (IPCC emission factor EF4, (kg N_2O-N [kg NH_3-N + NO_x-N volatilized]$^{-1}$)). Furthermore, although the studies on the effects of NIs on NH_3 and N_2O emissions are available in literature [16,20], there is still lack of information about their effects in cereal crops grown under temperate field conditions. To evaluate the potential of NIs to mitigate agricultural greenhouse gas emissions, this indirect N_2O emission should therefore also be included.

Therefore, the main objectives of this study were to investigate the effect of NI and UI on N_2O and NH_3 emissions from winter wheat with U in southern Germany, to evaluate whether adding NIs and UIs to U stimulates NH_3 or N_2O emissions, while they reduce one pollutant, and to estimate indirect effect of urease and nitrification inhibitors on the N_2O-N losses of uU based on the IPCC emission factor EF4.

2. Experiments

2.1. Experimental Site and Design

Five-year field experiments with winter wheat, from 2004 to 2005 and from 2011 to 2013, which was sown at the beginning of October with a seed density of 350 seeds m^{-2} and was harvested at the end of July, were conducted at the experimental station of the Technical University of Munich at Dürnast in Germany (11°41′60″ E, 48°23′60″ N). The experimental site is characterized by a temperate climate with a mean annual temperature of 7.8 °C and an average annual precipitation of 821 mm (14.2 °C and 557 mm from March to September, respectively). Daily meteorological data were obtained from a weather station from Deutscher Wetterdienst (DWD), which is approximately 500 m from the experimental site.

The soil is a mostly homogeneous Cambisol of a silty clay loam texture (IUSS Working Group WRB, 2007). Based on the guideline of VDLUFA (Association of German Agricultural Analytic and Research Institutes) for soil sampling and analysis [21], 16 representative soil samples are required for an experimental site, and were collected from the experimental site (0–0.3 m) and analyzed (Table 1). The sampling area was homogenous. As a rule in Germany, there is no requirement to have standard deviation for soil analysis, since the samples are already composited. Soil pH was determined following Schofield and Taylor [22] (0.01 M $CaCl_2$). Soil textural analysis was conducted in compliance with ISO (2002). Organic carbon (C_{org}) and total-nitrogen (N_{total}) were analyzed using the Dumas procedure (combustion at 1100 °C) [21].

Table 1. Soil characteristics of the experimental site from 2004 to 2005 and 2011 to 2013.

Year	Soil Type	pH (CaCl$_2$)	Clay	Silt	Sand	N$_{total}$	C$_{org}$
			%			% DM *	
2004–2005	Cambisol	5.7	24.7	54.9	20.4	0.19	1.43
2011–2013		6.5	23	61	16	0.14	1.17

* DM = Dry matter.

At the beginning of spring, all plots received a compound fertilizer consisting of 25 kg P ha^{-1}, 100 kg K ha^{-1}, 10 kg Mg ha^{-1}, and 40 kg S ha^{-1}, respectively. The fertilizer was surface-applied using a plot fertilizer distributor. The nitrogen (N) fertilizer treatments and the strategies for fertilizer application are listed in Table 2. Before the first N split application, soil NO_3-N in the top layer (0–60 cm) was determined and was around 20 kg N ha^{-1}. The UI, IPAT (N-isopropoxycarbonyl phosphoric acid triamide), at 0.4% *w/w* (SKW Stickstoffwerke Piesteritz GmbH, Lutherstadt-Wittenberg, Germany) and the NI, DCD/TZ (DCD/TZ: Dicyandiamide/1H-1,2,4-Triazol), at 2% *w/w* (SKW Stickstoffwerke Piesteritz GmbH, Lutherstadt-Wittenberg, Germany) were added to the urea. According to the fertilizer recommendation, the total N supplied was 180 kg N ha^{-1}, and based on the common fertilization practices, there were 3× split applications for fertilizer treatments without NI during the growing season and 2× split applications for treatments with NI since NI is able to maintain soil NH_4^+ for a longer time (Table 2).

For the simultaneous comparison of the effect of the fertilization treatments on N_2O emission under field conditions, the five fertilization treatments were randomly assigned to 10 plots (2 × 10 m) in 2004 and 2005. In 2012, since the treatment (U + UI) was omitted, only four treatments were randomly assigned to 12 plots. For studying the effect of fertilization treatments on NH_3 emission, 15 plots (2 × 10 m) in 2011 and 2013 were randomized with three replicates, and additional 3 plots (2 × 10 m) were as control treatment with zero N fertilizer in 2011 and 2013. The distance between the neighboring plots was 2 m.

Table 2. Nitrogen fertilization strategies (CAN: calcium ammonium nitrate; U: urea; NI: nitrification inhibitor; and UI: urease inhibitor) for field experiments in 2004, 2005, and 2012 for N_2O measurements and in 2011 and 2013 for NH_3 measurements, respectively.

Fertilization Treatments		3-Year Study for N_2O Measurements						2-Year Study for NH_3 Measurements					
		2004 and 2005			2012			2011			2013		
N Fertilizers	Nitrification or Urease Inhibitors	Vegetation Beginning	Stem Elongation	Booting	Vegetation Beginning	Stem Elongation	Anthesis	Vegetation Beginning	Stem Elongation	Anthesis	Vegetation Beginning	Stem Elongation	Anthesis
		1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
		N Supplied (kg N ha^{-1} $time^{-1}$)											
CAN	–	80	50	50	80	60	40	80	70	40	80	60	40
U	–	80	50	50	80	60	40	80	70	40	80	60	40
U	UI (IPAT)	80	50	50	-	-	-	80	70	40	80	60	40
U	NI (DCD/TZ)	120	-	60	100	-	80	110	-	80	100	-	80
U	NI + UI (IPAT + DCD/TZ)	120	-	60	100	-	80	110	-	80	100	-	80

In this study, the measurements of N_2O and NH_3 emission were not parallel. This is because, in our long-term study on N_2O and NH_3 emission, fertilization treatments with UI or NI varied with years. The comparable fertilization treatments including U, U + NI, U + UI, and U + NI + UI altogether were present only in the years from 2004 to 2005, and 2010 to 2012 (Table 2). Furthermore, our long-term studies on N_2O emissions have shown that averaged N_2O emissions over many years were consistent (back to the 1990s, e.g., [23–26]). In particular, NH_3 emission from U from 2001 to 2010 were much less variable with years and were limited to a small range of 1–3.4% of urea-N applied during the growing seasons of winter wheat [6,25–29]. A simulation study on modeling NH_3 emissions across Germany [30] showed that NH_3 emissions (% urea N applied) were in a range of 0–10% in strong contrast to the averaged 24% for European arable soils assumed by EMEP/EEA [31].

2.2. N_2O Flux Measurements

The measurements of N_2O fluxes were carried out using the closed-chamber method according to Hutchinson and Mosier [32] (Figure 1) and are described briefly as follows. Before the first-time sampling, four metal rings with an inner diameter of 0.3 m and a height of 0.15 m were installed in a depth of 0.08 m in the middle of each plot (Figure 1a). In order to sample the same area, the rings within the plots remained until the last measurements were completed. Nitrous flux rates were determined by placing a closed chamber over the permanently installed rings in each plot and taking three gas samples from the chamber's atmosphere with sampling intervals of 20 min, i.e., at 0, 20, and 40 min after opening the each chamber were collected between 11:00 a.m. and 12:00 p.m. An overview of the N_2O measurements in a 3-year study in 2004, 2005, and 2012 is summarized in Table 3.

| (a) | (b) |

Figure 1. Metal ring permanently installed on the field (**a**) and closed-chamber, air syringe with needle, and vacuum test tube (**b**).

Table 3. Overview of N_2O measurements in a 3-year study in 2004, 2005, and 2012.

	2004	2005	2012
Number of measurement points per plot		4	
Position of measurement points in plots		Plot center	
Period of measurements	30 March to 25 June	4 April to 30 June	2 April to 23 July
Measurement frequency	Every other day for the first two weeks and then twice a week	Every other day	Weekly

At sampling time, a volume of 20 mL of gas was collected using an air-tight syringe from the headspace (Figure 1b) and injected into a vacuum serum tube fitted with butyl rubber stopper to keep the gas under pressure. Gas samples were analyzed for N_2O within a week using an automated gas chromatograph (Varian Star 3400, Varian, Walnut Creek, California, USA) equipped with electron capture and thermal-conductivity detectors. Computer software Star Chemstation (Varian, Walnut Creek, California, USA) was used to calculate N_2O concentrations. N_2O content was determined using a gas chromatography system (Varian Star 3400, Varian, Walnut Creek, California, USA). Two calibration curves, i.e., for both low and high concentrations, were used [33].

For each measurement day, the N_2O emission flux rate (F_N) (μg N_2O-N m^{-2} h^{-1}) was calculated for each chamber based on a linear model as reported by Flessa et al. [34]:

$$F_N = [V/A \cdot \Delta C/\Delta t)] \times \varrho_N$$

where V is the volume of the chamber in L; ΔC is the difference of the N_2O concentration (μL L^{-1}) before closure (t_0) and at the end of closure (t_1 or t_2); A is the ground area enclosed by the chamber in m^2; Δt is the time interval between t_0 and t_1 or between t_1 and t_2 in h; and ϱ_N is the mass percentage of N in N_2O (1.25 μg N μL^{-1}).

The N_2O emission flux rate at a given day was averaged from those at t_1 and at t_2. To avoid the high spatial heterogeneity within a plot, four measurements of N_2O fluxes per plot were averaged at each time. The area of the closed-chamber covered 0.01131 m^2, and the volume was 1.787 dm^{-3} (Figure 1b). Thus, the measured area (4×0.01131 m^2) in each plot accounted for approximately 0.23% of the area of a plot (20 m^2).

Flux rates of each plot were integrated over three or two periods of N_2O-N emissions to calculate cumulative N_2O-N emissions (g N_2O-N ha^{-1}), i.e., the amount of N_2O-N emissions between two subsequent split N fertilizations was estimated by "averaged N_2O-N emission rate (g ha^{-1} d^{-1}) $\times$ duration (days)".

2.3. NH_3 Measurements

Ammonia emissions were measured by a combination of the Dräger Tube Method (DTM) with modified passive samplers [35–38] (Figure 2a,b). Briefly, the passive sampler gives semi-quantitative results of NH_3 emission (NH_3-N ppm, sum), which properly reflects the relative difference between treatments. For the absolute flux values (kg NH_3-N ha^{-1}) from passive sampler measurements (Figure 2b), a transfer coefficient is required. The DTM (Figure 2a), a calibrated dynamic chamber method, is employed to obtain a transfer coefficient for converting the semi-quantitative NH_3 emission of the passive sampler to quantitative units (kg N ha^{-1}) [37,38]. For N fertilizer treatments, a control treatment without N fertilizers should be included as well. The DTM is a variant of dynamic chamber technique and adjusted to actual meteorological conditions by a calibration equation [36]. The principle is firstly obtaining primary flux during a short measurement interval (~1 min) in the chamber with a fast reading indicator of NH_3 concentration, and then scaling the primary flux into actual ambient flux using temperature and wind speed outside of the chamber through an empirical calibration equation. This method has been shown to be well-suited for NH_3 measurements in multi-plot filed trials [35–38].

| (a) | (b) |

Figure 2. Passive-flux-collector from a square bottle with a modified acid trap for NH_3 (**a**) and Dräger-Tuber method (DTM) with Teflon tubes for hand pumping (**b**).

To measure NH_3 emission in the field, a passive sampler with 20 mL of absorption solution (0.05 M H_2SO_4) was installed in the center of each plot at 0.15 m above plant canopy [35–38]. The gaseous NH_3 in the air was absorbed by H_2SO_4 solution in the passive samplers to form $(NH_4)_2SO_4$. The absorption solution from the passive samplers was exchanged daily for all treatments, except during rainy days. On rainy days, the solution in samplers was replaced at the day after the rain stopped. An overview of NH_3 measurements in a 2-year study in 2011 and 2013 is shown in Table 4.

Table 4. Overview of NH$_3$ measurements in a 2-year study in 2011 and 2013.

		2011	2013
Number of Measurement	**Passive Sampler**	1	
Points Per Plot	**Dräger Tube Method**	4	
Position of Measurement Points in Plots		Plot center	
Period of Measurements		8 April to 12 July	15 April to 7 July
Measurement Frequency		Daily except during rainy days *	

* (i) On rainy days, the passive solution samplers were replaced at the day after the rain stopped. (ii) Between two subsequent N fertilizer applications, the measurements were stopped after NH$_3$ emission was zero for three days.

The NH$_4^+$ concentration from each passive ampler was subsequently analyzed in the laboratory using the standard distillation method. Briefly, the samples collected from passive samplers were firstly mixed with 10 mL 10 M NaOH solution. During distillation, NH$_3$ was released and, as the gas cooled down, NH$_4$OH formed was collected in an Erlenmeyer flask. After the distillation was completed, titration with 0.005 M H$_2$SO$_4$ was done to determine the amount of NH$_3$ in the distillate. 1 mL 0.005 M H$_2$SO$_4$ corresponded to 0.14 mg N.

The DTM requires much fewer frequent measurements than the passive sampler measurements. However, a recent study [37] suggested that the range of valid calibration conditions has to be kept in mind when applying the DTM. To obtain reliable transfer coefficients for the same treatment at given time, the measurements for the transfer coefficients by the DTM were carried out simultaneously with the passive sampling for all treatments during the growing seasons. Ambient air was sucked through four soil chambers (height of 0.105 m, diameter of 0.115 m, and surface area of 0.0415 m^2) using a manual pump (Drägerwerk AG & Co., KGaA, Lübeck, Germany). NH$_3$ concentrations in the air passing through the chamber system of the DTM were instantaneously measured with indicator tubes (Drägerwerk AG & Co., KGaA, Lübeck, Germany). Due to the low air-exchange rate in the chambers, the NH$_3$-flux values need to be corrected by means of a calibration approach proposed by Pacholski et al. [37]. For more details on the DTM method and the calculation of transfer coefficients see Gericke et al. [37] and Pacholski [38].

The cumulative NH$_3$-N emissions (kg NH$_3$-N ha^{-1}) were the sum of daily NH$_3$-N emissions from the beginning up to the end of the NH$_3$ measurements.

2.4. Estimation for Considering NH$_3$ as an Indirect N$_2$O Emission

Estimation for considering NH$_3$ as an indirect N$_2$O emission was based on the IPCC guidelines [8,19], i.e., 1% of the emitted NH$_3$ (N$_2$O-N + 1% × NH$_3$-N) is converted to N$_2$O after its deposition.

2.5. Statistical Analysis

Depending on the number of replicates of treatments, Tukey's (HSD) post hoc (>2 replicates) and the nonparametric (Kruskal–Wallis-H-test) (2 replicates) statistical tests were used to test for differences between the fertilization treatments in terms of N$_2$O and NH$_3$ emissions using SPSS software (Proc. Mixed, SPSS ver. 26, IBM). The statistical significance of all tests was evaluated at the 0.05 level.

3. Results and Discussion

3.1. N$_2$O Flux Pattern and Cumulative N$_2$O-N during Growth Periods under Different Fertilization Treatments

The spatial and temporal variability of N$_2$O fluxes with weather conditions was notably high after the N applications (Figures 3–5). For all three years, our results showed a general pattern of N$_2$O fluxes, such as an increase in the N$_2$O flux rate followed both N fertilization and rainfall events (Figures 3–5). For example, the first peak of N$_2$O flux rate was 100–600 g N$_2$O-N ha^{-1} d^{-1} on

9 April 2004, 10–50 g N_2O-N ha^{-1} d^{-1} on 11 April 2005, and 15–60 g N_2O-N ha^{-1} d^{-1} on 9 April 2012, respectively. In particular, the first highest peak of N_2O in 2005 appeared two weeks after the first N application following a heavy rainfall. Similar patterns of N_2O emissions from arable soils with increased flux rates following N fertilization and rainfall have been reported, e.g., for winter wheat [39] and for oilseed rape [40,41]. This type of pattern is induced by the main drivers of N_2O emission from N fertilization and precipitation, and thus could be explained by enhanced denitrification due to (i) an increase in the availability of nitrate as a substrate for N_2O production [33]; (ii) formation of anaerobic conditions as a result of lower gas diffusivity in soil water, and thus reduced O_2 diffusion into the soil combined with O_2 consumption by soil microbes [42–44]; and (iii) soil drying and rewetting. As the content of available organic matter in soils may increase with soil drying, organic substrates for soil microorganisms, which may promote denitrification and thus N_2O release, may be highly available when the soil is rewetted [39]. A meta-analysis study [45] showed that rewetting can increase N_2O fluxes by nearly five times in cropland following a drying period. Figure 3a shows that, although N_2O emission in (U + NI) and (U + UI + NI) treatments was lower at most measurement dates compared with the treatment of U alone, N_2O emission on 9 April 2004 was higher in (U + NI) and (U + UI + NI) treatments than that of U alone, which could still not be explained. Furthermore, it was unusual to observe the negative N_2O flux on 15 May 2005. This may have been due to a longer dry period, resulting in no N_2O emissions on the day when the measurement was taken.
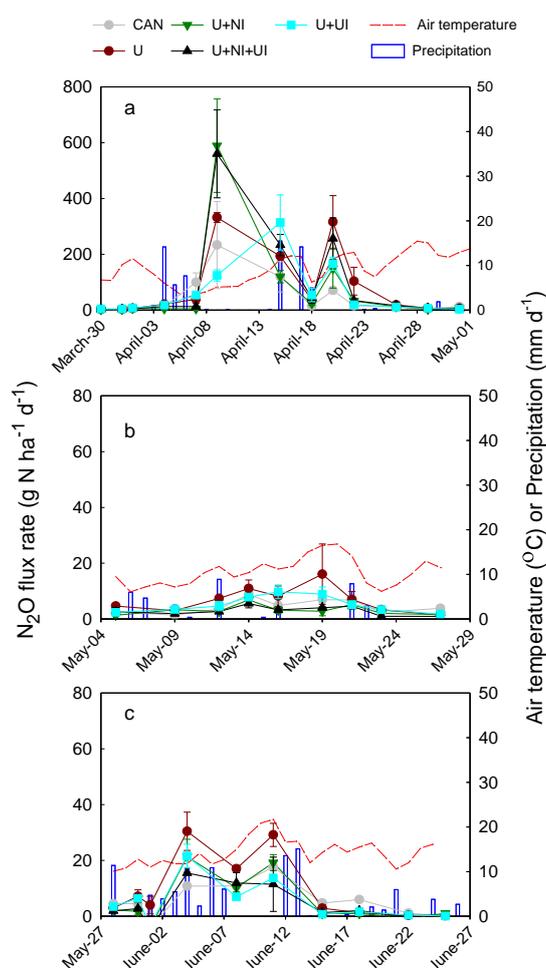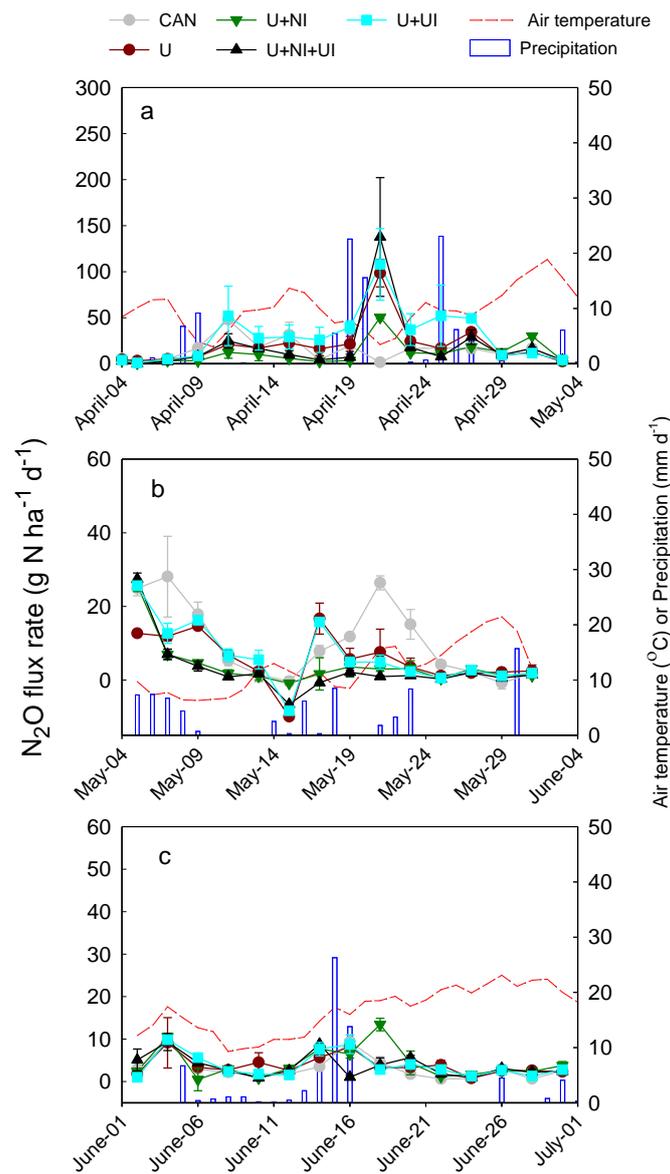

Figure 3. N_2O flux rate (left vertical axis) and air temperature or precipitation (right vertical axis) for the fertilization treatments (CAN: calcium ammonium nitrate; U: urea; NI: nitrification inhibitor; and UI: urease inhibitor) after the 1st (**a**), 2nd (**b**), and 3rd (**c**) split N application in 2004. The vertical bars indicate the standard deviation of the mean (n = 2).
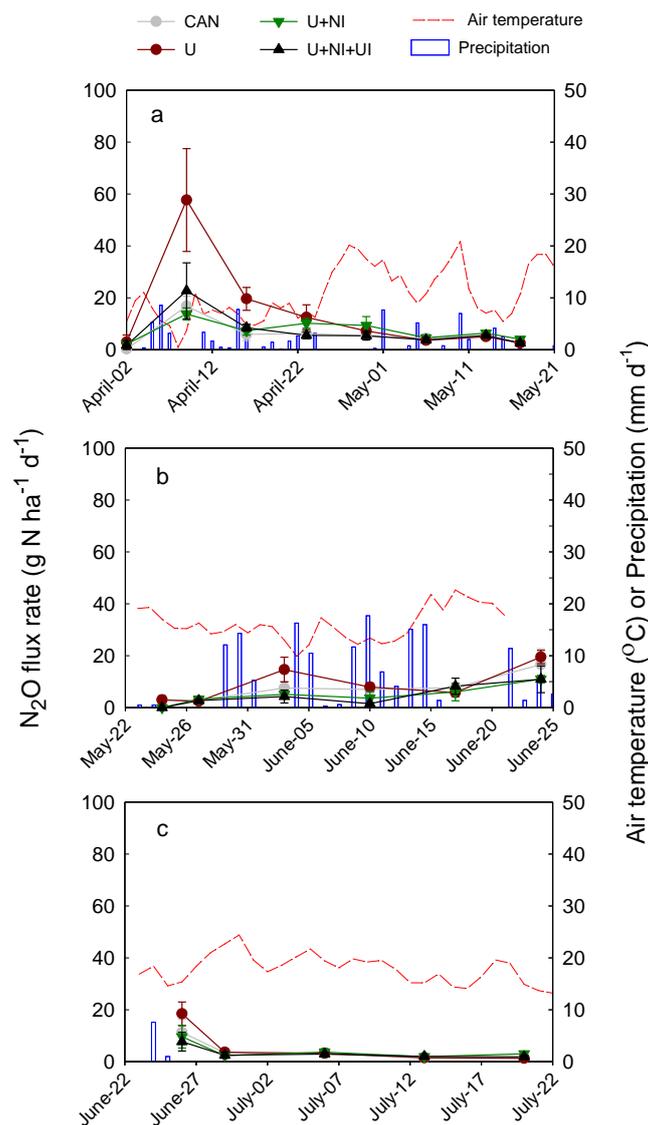
Figure 4. N_2O flux rate (left vertical axis) and air temperature or precipitation (right vertical axis) for the fertilization treatments (CAN: calcium ammonium nitrate; U: urea; NI: nitrification inhibitor; and UI: urease inhibitor) after the 1st (**a**), 2nd (**b**), and 3rd (**c**) split N application in 2005. The vertical bars indicate the standard deviation of the mean (n = 2).

Figure 5. N_2O flux rate (left vertical axis) and air temperature or precipitation (right vertical axis) for the fertilization treatments (CAN: calcium ammonium nitrate; U: urea; NI: nitrification inhibitor; and UI: urease inhibitor) after the 1st (**a**), 2nd (**b**), and 3rd (**c**) split N application in 2012. The vertical bars indicate the standard deviation of the mean (n = 3).

In this study, a rapid drop in air temperature followed a peak of N_2O flux on 9 April 2004 and 8 April 2012 (Figures 3 and 6). Since the temperature drop in April lasted only a very short time and the temperate was still above 0 °C, effects of frozen-thawing cycles on N_2O flux probably did not occur. A number of studies on the effect of frozen-thawing cycles on N_2O flux in arable soils in Germany have shown that the distinct frozen-thawing cycles occur mainly during winter season, i.e., from January to February or March (e.g., [39–43,46]). As described earlier, the N_2O flux pattern in this study was therefore probably induced by the main drivers from N_2O emission resulting from N fertilization and wet–dry fluctuation, i.e., precipitation.

The cumulative N_2O emission during the wheat growth period ranged from 2133 to 3614 g ha^{-1} in 2004, from 596 to 1217 g ha^{-1} in 2005, and from 622 to 1179 g ha^{-1} in 2012, corresponding to an emission factor of the applied N (%): 2.1–3.7% in 2004, 0.3–1.2% in 2005, and 0.4–0.9% in 2012, (Figure 6 and Table 5). Early reports for different winter cereal fields in Germany showed that annual N_2O emissions ranged from 1700 g to 4000 g N_2O-N ha^{-1} a^{-1} [39,47,48]. However, N_2O emission post-harvest or during winter may account for approximately 50% of the total annual N_2O emission [47]. Thus, N_2O emissions

during the winter wheat growth season from our experiment in southern Germany were of the same order of magnitude as previously reported.
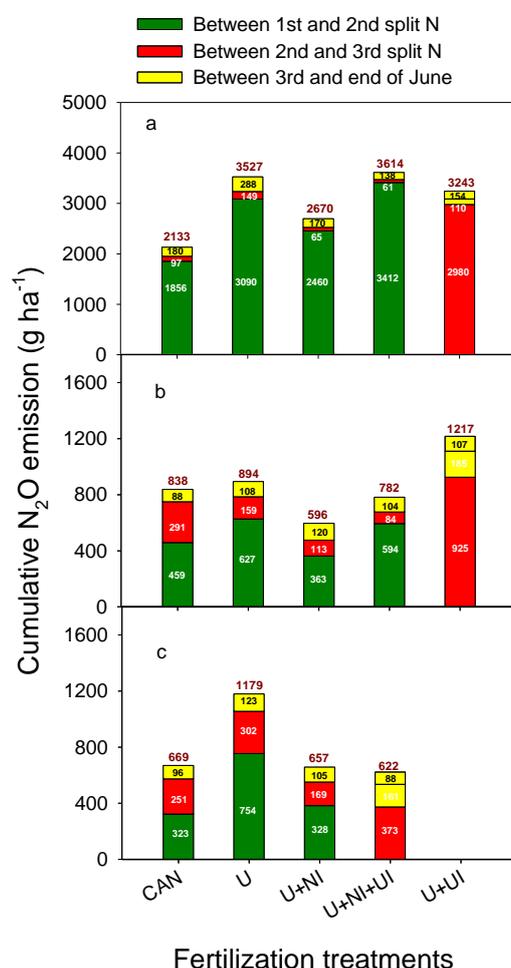

Figure 6. Cumulative N_2O emission of different fertilization treatments of winter wheat (CAN: calcium ammonium nitrate; U: urea; NI: nitrification inhibitor; and UI: urease inhibitor) with and without an NI in 2004 (**a**), 2005 (**b**), and 2012 (**c**). Kruskal–Wallis-H test indicates no significant difference between fertilization treatments in 2004 (**a**), 2005 (**b**), and 2012 (**c**) at *p* < 0.05.

Table 5. Relative cumulative N_2O-N losses (% of applied N; Mean ± SD) of different fertilization treatments (CAN: calcium ammonium nitrate; U: urea; NI: nitrification inhibitor; and UI: urease inhibitor) in 2004, 2005, and 2012. Kruskal–Wallis-H test indicates no significant difference between fertilization treatments in 2004, 2005, and 2012 at *p* < 0.05.

Fertilization Treatments	Relative Cumulative N_2O-N Losses (% of Applied N)						
	2004		**2005**		**2012**		**3-Year Average**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
CAN	2.3	1.3	0.6	0.2	0.4	0.0	1.1
U	3.9	0.1	0.8	0.1	0.9	0.3	1.9
U + NI	2.1	0.4	0.3	0.0	0.4	0.1	0.9
U + NI + UI	2.8	0.9	0.5	0.2	0.4	0.1	1.2
U + UI	3.7	1.4	1.2	0.6			2.4

Accumulative N_2O emission during the growth season varied considerably according to the tested year (Figure 6). For example, N_2O emission from fertilization with U alone in 2004 was nearly three times higher than that in 2005 and 2012, and that from U with NIs in 2004 was nearly four times

higher than that in 2005 and 2012. A consistently higher emission was observed between the first and second applications compared to that during the later growth period (Figure 6). For example, the N_2O emissions after the first two N applications were approximately 87–94% in 2004, 55–76% in 2005, and 48–64% in 2012, respectively, of the total emission during the growth period of winter wheat. A high variation in N_2O fluxes with growth season and year has often been reported in field studies with N_2O measurements [40,49], as well as in modelling approaches from sites with different climatic conditions [50,51]. Despite a uniform management approach (N fertilization and crop type), annual N_2O emission varied by up to a factor of seven between single experimental years. Different rainfall frequencies, i.e., the interval of drying and rewetting periods, could explain the difference in N_2O emission between 2004 and 2012 (Figures 3 and 5). Although a similar rainfall frequency, particularly between the first and second split N applications (Figures 3 and 4), the difference in N_2O emissions between 2004 and 2005 was still considerable.

Between the fertilization treatments with U alone and CAN, N_2O losses during the growth period of winter wheat were 1394 g N_2O-N ha^{-1} and 510 g N_2O-N ha^{-1} higher for U alone than for CAN in 2004 and 2012, respectively, whereas there was no difference in N_2O emissions between these two N forms in 2005 (Figure 6). Lebender et al. [52] reported there was a difference in N_2O emissions between U and CAN during the growth period from March to the end of July for winter wheat. The higher N_2O-N losses from U might be due to an increase in pH from U hydrolysis, leading to a higher N_2O emission. Bremner and Blackmer [53] reported that nitrification, which produces N_2O as a by-product, generally increases under higher soil pH (>6) conditions. Denitrification has been reported to occur naturally over a wide range of soil pH (5.0–8.0) [54]. As an alkaline-hydrolyzing N fertilizer, U influences nitrification through a transient rise in pH with subsequent denitrification leading to the formation and release of larger amounts of N_2O [45,55,56]. A recent study by Wrage-Monnig et al. [57] showed that high pH may favor nitrifier denitrification that accounts for up to 100% of nitrous oxide emissions from ammonium (NH_4^+) in soils.

A decrease in N_2O fluxes via the use of NIs has already been confirmed across climates, soil types, and soil characteristics [9,24,33,39,44]. Recent meta-analyses indicated that NIs decreased N_2O emissions by 31–48% across diverse agricultural ecosystems, including upland, grassland, and paddy fields [9,10]. Weiske et al. [58] reported a reduction of 49% during the cropping season for measurements in southern Germany. Our study confirmed these previous findings. For example, compared to U alone, the cumulative N_2O emission of U with NIs during the winter wheat growth season was reduced by 24% in 2004, 30% in 2005, and 44% in 2012. Because the number of split N application was reduced from 3 to 2 times, the results of this study further suggest that beyond reducing N_2O-N losses by NIs, the use of NIs can also simplify fertilization in intensive crop production to save labour and machinery costs, which is in keeping with the results of our previous report [19].

In contrast, the effect of (U + UIs) and/or the interactive effect of (U + UIs) and (U + NIs) on N_2O emissions from U fertilization was inconsistent. A meta-analysis of the effect of UIs on N_2O emissions from U fertilization by Akiyama et al. [9] showed that (U + UIs) did not reduce N_2O emissions. Figure 6 shows that N_2O emissions from (U + UIs) or (U + UIs + NIs) decreased, increased, or did not change compared to those of U alone. Compared to U alone, the cumulative N_2O emission of (U+ UIs) remained nearly unchanged in 2004 and increased by 36%, while that for (U + UIs + NIs) decreased by 11% in 2004, 13% in 2005, and 47% in 2012. This result is probably observed because unlike the nitrification process, U hydrolysis is not directly related to N_2O emissions. The use of UIs only delays U hydrolysis, and all U will eventually be hydrolyzed to become NH_4^+. With the use of NIs, if plant uptake of N in the form of NH_4^+ does not significantly increase, a similar amount of NH_4^+ will eventually undergo nitrification and subsequent denitrification compared to U alone [9].

3.2. Cumulative NH₃ Emissions during Growth Periods under Different Fertilization Treatments and Added Inhibitor Effect on NH₃ Emissions

The cumulative NH_3 emissions from a two-year study in 2011 and 2013 are shown in Figure 7. The cumulative NH_3 emissions from U alone were 12.7 kg N ha^{-1} in 2011 and 7.1 kg N ha^{-1} in 2013, which corresponded to an emission factor of 11.5% and 3.9% of the applied N during 2011 and 2013, respectively (Table 6). On average, during the two-year study, NH_3-N losses amounted to 7.7% of the applied urea N, which was similar to recent findings (i.e., 8%) reported by Ni et al. [5]. Generally, the emissions observed in this study were relatively low compared to the NH_3 losses of up to 64% reported in the literature [13] and of 16% assumed for the calculation of the national emissions inventory for Germany [31]. Among many factors affecting soil NH_3 emissions, air temperature, precipitation, and soil moisture and pH are the key factors. However, the major reasons leading to low NH_3 emission in southern Germany may be due to low pH values of the investigated soils together with increased cation exchange capacity, and low temperature prevailing in April and frequent precipitation events after N application. However, the 7.7% of the applied urea N from NH_3-N losses was higher than the 0.1–2.7% in southern Germany reported by Schraml et al. [6]. This discrepancy may be attributable to the higher pH at the experimental site of this study (pH = 6.7).
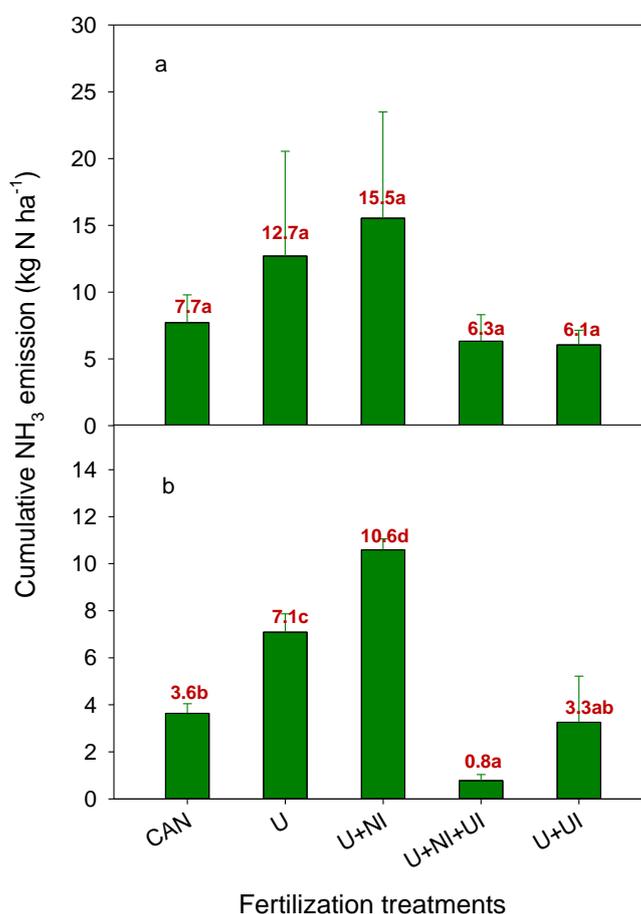

Figure 7. Cumulative NH_3 emissions of different fertilization treatments (CAN: calcium ammonium nitrate; U: urea; NI: nitrification inhibitor; and UI: urease inhibitor) of winter wheat during 2011 (**a**) and 2013 (**b**). The vertical bars indicate the standard deviation of the mean (n = 3). A mean comparison between fertilization treatments from Tukey´s HSD-test indicates a significant difference at $p < 0.05$. The same letters indicate groups that were not significantly different from one another.

Table 6. Relative cumulative NH_3-N losses (% of applied N; Mean ± SD) of different fertilization treatments (CAN: calcium ammonium nitrate; U: urea; NI: nitrification inhibitor; and UI: urease inhibitor) during 2011 and 2013. A mean comparison between fertilization treatments from Tukey´s HSD-test with different letters indicates a significant difference at $p < 0.05$. The same letters in the same column indicate groups that were not significantly different from one another.

Fertilization Treatments	Relative Cumulative NH_3 Loss (% of Applied N)							2-Year Average
	2011				2013			
	Mean	±	SD		Mean ±		SD	
CAN	7.0	±	1.9	a	2.0	±	0.2	b	4.5
U	11.5	±	7.1	a	3.9	±	0.4	c	7.7
U + NI	14.1	±	7.2	a	5.9	±	0.3	d	10.0
U + NI + UI	5.8	±	1.8	a	0.4	±	0.1	a	3.1
U + UI	5.5	±	1.0	a	1.8	±	1.1	ab	3.7

Compared to the NH_3 loss associated with U treatment, NH_3 loss from CAN has been less intensively studied in the past [4]. The results in this study showed an emission factor of 4.5% of the applied N based on the average of a two-year study (Table 6), which was lower than the NH_3 emission from U. Ni et al. [5] reported that the average NH_3 losses from CAN were approximately 2.1% of the applied N, which was near the EEA emission factor of 2%. The lower NH_3 emissions of CAN could be explained by the lower NH_4^+ concentration in CAN and its considerably smaller effect on soil pH.

Addition of the UIs to U caused a significant reduction in NH_3 emissions and reduced NH_3 losses by 26–83% and 24–32%, respectively, of the applied urea-N [5,6]. Our study showed that (U + UIs) reduced NH_3 losses by 52% in 2011 and 54% in 2013, respectively, compared to those observed with U treatment alone. Similarly, (U + UIs + NIs) reduced NH_3 losses by 50% in 2011 compared to those of U alone, but there was a considerably greater reduction in 2013, i.e., 89%, compared to that of the NH_3 losses from U alone. This finding may suggest that (U + UIs + NIs) might be a potential approach to minimizing the negative environmental effects of U application under agro-ecological conditions.

In contrast to (U + UIs + NIs), the addition of (U + NIs) in this study stimulated NH_3 emissions compared to treatment with U alone (Figure 6 and Table 6). The cumulative NH_3 emission from (U + NIs) was highest in both 2011 and 2013 among all N fertilization treatments, which corresponded to an emission factor of 14.1% and 5.9% of the applied N, respectively (Table 6). This result may be due to the retention of NH_4^+ in the soil during the use of NIs, which could increase NH_3 emissions [11]. The application of an NI (DCD) has been found to increase NH_3 emissions by 18–29% [11,13], and by up to 38% [14]. However, previous studies have found inconsistent results, e.g., stimulating [13], neutral [5,15], and retarding [59] effects of NIs on NH_3 emissions, which may also depend on soil properties. For example, the study [45] showed that the effect of NIs was positively correlated with soil pH and negatively correlated with CEC.

3.3. Estimation of NH_3 as an Indirect N_2O Emission Based on the Default Value of EF4

According to the IPCC guidelines [8], approximately 1% of the emitted NH_3 is converted to N_2O through nitrification and denitrification processes [20], which is referred to as an indirect N_2O emission from NH_3 deposition (IPCC emission factor EF4, (kg N_2O-N (kg NH_3-N + NO_x-N volatilized)$^{-1}$)). Direct N_2O emissions and the estimation of indirect N_2O emissions under treatments of U with inhibitors and U alone are shown in Table 7. Although the current studies on N_2O and NH_3 emission were not parallel, and the indirect N_2O emission derived from NH_3 was not measured, further evidences that may support the assumption of estimation above are that the experiments for NH_3 measurements were conducted in the same sites, and that the results of NH_3 were in close agreement with long term experiments done on these sites [6,24,29,60,61].

The results in Table 7 demonstrate that the indirect N_2O emission from NH_3 was lowest from CAN and (U + UI). Although (U + NI) caused a higher indirect N_2O emission, NIs decreased overall

N_2O emissions, i.e., totally 1.4 kg N_2O-N ha^{-1} from direct and indirect N_2O emissions, compared to 3.9 kg N_2O-N ha^{-1} from (U + UI) and 4.9 kg N_2O-N ha^{-1} from U alone (Table 7), which was only locally deposited NH_3 on the same agricultural field and did not include further deposition. Lam et al. [16] suggested that the beneficial effect of NIs in decreasing direct N_2O emissions can be outweighed by an increase in NH_3 volatilization. In contrast, our study may indicate that considering indirect N_2O emissions from NH_3 induced by NIs may be negligible for winter wheat in southern Germany.

Table 7. Combined effect of N_2O-N and NH_3-N losses on N_2O emissions for urea (U) with nitrification inhibitors (NIs) and urease inhibitors (UIs) during the growth season of winter wheat estimated according to IPCC emission factor EF$_4$.

Fertilization Treatments	3-Year Average	2-Year Average	EF$_4$: 1% HN$_3$-N	Total N_2O-N
	N_2O-N kg ha^{-1}	NH_3-N kg ha^{-1}	N_2O-N kg kg^{-1} NH_3-N	kg ha^{-1}
CAN	1.21	5.65	0.06	1.3
U	4.81	9.90	0.10	4.9
U + NI	1.31	13.05	0.13	1.4
U + NI + UI	1.67	3.55	0.04	1.7
U + UI	3.85	4.70	0.05	3.9

4. Conclusions

This field study on winter wheat in southern Germany confirms that U added with NIs greatly reduced N_2O emissions, and additive UIs decreased NH_3 emissions. Compared to U alone, the cumulative N_2O emissions of U with NIs during the winter wheat growth season decreased by 24–44%, and UIs added to U reduced NH_3 losses by as much as 52–54%. The difference in the effect of UIs or (UIs + NIs) on N_2O emissions from U fertilization may suggest that the combined use of UIs and NIs might be a potential approach to minimizing negative environmental effects of U application under agro-ecological conditions. Furthermore, in contrast to the treatment of (U + UIs + NIs), (U + NIs) stimulated NH_3 emissions compared to the treatment with U alone. According to the IPCC guidelines for emission factor EF4 [8,20], approximately 1% (a range of 0.2–5%) of the emitted NH_3 is converted to N_2O after its deposition. The results of the indirect N_2O emissions from NH_3 estimated based on the emission factor EF4 by IPCC showed that total N_2O emissions from (U + NIs) were approximately 2.8–3.5 times lower than those from U and (U + UI), which may suggest that indirect N_2O emission from NH_3 induced by NIs may be negligible.

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