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# Effects of inhibitors and slit incorporation on $NH_3$ and $N_2O$ emission processes after urea application

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## ABSTRACT

The use of urea fertilizers in agriculture is associated with many negative environmental impacts and is a source of ammonia ($NH_3$) and nitrous oxide ($N_2O$) emissions. Such losses from urea fertilizer can be avoided by different mitigation techniques. Three different mitigation principles, urease inhibitor (N-(2-Nitrophenyl) phosphoric triamide, 2-NPT) (UI) alone and urease inhibitor in combination with nitrification inhibitors (N-[3(5)-methyl-1 H-pyrazol-1-yl) methyl] acetamide, MPA) (NI) and closed slit incorporation of urea fertilizer into the soil, were compared on a sandy loam soil at a soil water level of 70 % water-holding capacity. An in vitro microcosm approach with open dynamic incubation chambers was used to monitor $NH_3$ emissions over two weeks with $NH_3$ sampling by washing bottles. $N_2O$ emissions were studied over ten weeks in slow throughflow mesocosms with continuous gas chromatographic (GC) measurements. To get insights into $N_2O$ production and consumption processes, gas samples were taken after six weeks and $N_2O$ isotopocules were analyzed by isotope ratio mass spectrometry (IRMS). Slit injection showed the greatest effect on $NH_3$ emission reduction by 79.6 % (40.6 % by UI, and 46.7 % by UINI) compared to surface applied urea. Minor pollution swapping to $N_2O$ was observed at the beginning of the trial due to incorporation but not in the cumulative emissions over the entire incubation time. The reduction effect of UINI on $N_2O$ emissions decreased over time with no cumulative emission reduction at the end of experimentation. $N_2O$ isotopocules confirmed the high contribution of nitrification to $N_2O$ production. In contrast and bacterial denitrification, nitrifier denitrification and fungal denitrification were involved on a much lower level and $N_2O$ reduction to $N_2$ was not pronounced. All $NH_3$ mitigation measurements where effective to decrease $NH_3$ emissions while their effects on $N_2O$ emission varied over time. Factors as crop N uptake and rainfall would further modify the overall effect on $N_2O$ emissions and need to be considered for final pollution swapping assessment. Further research on the impact of NI on non-target microbial communities is warranted to elucidate potential environmental consequences and long-term efficacy of inhibitor compounds.

## 1. Introduction

Due to the increased availability of nitrogen (N) mainly through urea fertilizer production based on the Haber-Bosch process, agricultural productivity increased globally particularly within the decades following the second world war. Compared to other commonly available mineral N fertilizers, urea has the advantages of a high N content of 46 %, a low cost per unit of N due to lower production costs, availability in most markets, low corrosion, compatibility with most fertilizers, and provides prompt N availability to plants (Silva et al., 2017). However, for broadcast urea application, the loss of total applied N through ammonia ($NH_3$) volatilization can reach up to 68 % (Drury et al., 2017; Rochette et al., 2009). Ammonia emissions exert a profound impact on the environment and climate. The deposition of $NH_3$ in terrestrial or aquatic ecosystems can contribute to environmental degradation through processes such as eutrophication (Bergström et al., 2008; Bergström and Jansson, 2006), acidification, and biodiversity loss (Clark and Tilman, 2008). Additionally, $NH_3$ serves as a crucial precursor in the formation of fine particulate matter, characterized by a diameter less than 2.5 μm, which has detrimental effects on both the environment and human health (Griffith et al., 2015; Wang et al., 2016).

In addition, on average 0.62 % (0.43–0.85 %) of urea application is

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lost in the form of nitrous oxide (N2O) emissions from managed European agricultural soils (Mathivanan et al., 2021). Nitrous oxide is one of the three important atmospheric greenhouse gases (GHGs) with about 265 times the global warming potential of carbon dioxide (IPPC, 2014). In alignment with the new National Emission Ceilings Directive (NEC Directive) of Europe 2016/2284, Germany has pledged to reduce national $NH_3$ emissions by 5 % by 2020 and by 29 % by 2030 compared to 2005. This mitigation is mandated to follow a linear path between 2020 and 2030. Since spring 2020 measures have been implemented in the amendment of the German fertilizer ordinance DüV20 [9]. Urea fertilizer must be applied in combination with a urease inhibitor (UI) or, alternatively, has to be incorporated within four hours, aiming to mitigate $NH_3$ emissions associated with the use of mineral N fertilizers.

Fertilizer incorporation or injection, like slit incorporation, provides an increase in the contact area between soil and fertilizer, accelerating the sorption of ammonium ($NH_4^+$) to the soil matrix. Studies demonstrated a mitigation potential in $NH_3$ emissions by up to 91.2 % by incorporation compared to surface application, even lower emissions than a urease inhibitor treatment (Fontoura and Bayer, 2010 Pan et al., 2016 Woodley et al., 2020) Different soil properties e.g. cation exchange capacity (CEC), pH, water content, and texture, can limit the efficiency of incorporation as a mitigation strategy for $NH_3$ emissions (Du Preez and Burger, 1987; Sommer et al., 2004). Additionally, incorporating the urea fertilizer in the soil may increase the N2O emissions by up to 67 % (Götze et al., 2023) since it immediately creates anaerobic conditions favourable for denitrification (Venterea and Coulter, 2015).

Urease inhibitors (UI) added to the fertilizer mitigate $NH_3$ volatilization through the slowdown of urea hydrolysis by binding to the urease enzyme. The delayed hydrolysis of urea results in a lower soil pH increase which allows more time for the adsorption of ammonia by the soil and plants, preventing volatilization (Wang et al., 2020). The efficacy and duration of UI are contingent upon the concentrations of urea and the inhibitor, chemical structure of the inhibitor, as well as the degradation rate of the inhibitor. Presently, only a few urease-inhibiting compounds are applied in commercial agriculture due to cost and toxicity concerns, in particular N-(n-Butyl) thiophosphoric triamide (NBPT). Meta-analyses show that UIs can reduce $NH_3$ emissions by 30–90 % in both laboratory and field experiments compared to surface-applied urea (Klimczyk et al., 2021; Pan et al., 2016; 2018). Although several meta-studies on efficacies of inhibitor compounds exist, actually only NPBT, was studied in great detail. In several agricultural regions other inhibitor compounds are used more widely, in Germany in particular the UI compound 2-NPT (N-(2-Nitrophenyl) phosphoric triamide, 2-NPT) and the nitrification inhibitor (NI) N-[3 (5)-methyl-1 H-pyrazol-1-yl) methyl] acetamide (MPA). There is a strong need to close the research gaps for these compounds. However, since UI primarily affects urea hydrolysis, its use does not directly result in a reduction of $N_2O$ emissions and/or other N losses, such as nitrate ($NO_3^-$) leaching (Sigurdarson et al., 2018). Several studies have shown the risk of "pollution swapping" when UI was used to reduce $NH_3$ losses, because higher soil $NO_3^-$ concentrations resulted in an increase of $N_2O$ emissions by up to 36 % (Hu et al., 2020; Drury et al., 2017; Woodley et al., 2020). Nevertheless, on average there appears to be an emission-reducing effect, although this is still uncertain in terms of the conditions and the extent of its occurrence (Fan et al., 2022). The highlighted risk that under specific soil conditions, incorporation of urea in combination with the addition of UI can increase $N_2O$ emission, is calling for other reduction measures to reduce $NH_3$ emissions in these situations.

Nitrification inhibitors curtail $NO_3$- losses by impeding the production of $NO_3$- through the deceleration of $NH_4^+$-oxidation to $NO_2^-$ by ammonia oxidising bacteria and archaea (Soares et al., 2012). Although several NI compounds have proven to be effective, the risk of higher $NH_3$ emissions, due to the addition of NI should not be neglected in the environmental and economic assessment. The inhibition of nitrification, while reducing N loss through $NO_3^-$ leaching and $N_2O$ emissions, maintained higher soil pH and $NH_4^+$ concentration for an extended period, creating favourable conditions for an increase in $NH_3$ volatilization (Soares et al., 2012).

The combined application of UI and NI (UINI) with urea fertilizer extends the availability of $NH_4^+$ to plants by reducing $NH_3$ volatilization, $N_2O$ emissions, and $NO_3$-leaching losses (Gioacchini et al., 2002). Experiments demonstrated a mitigation potential in $NH_3$ emissions through a combined treatment with UINI by up to 89 % (Hu et al., 2020; Martins et al., 2017) with Fan et al., 2022 showing an average of 38 % (n=77). On the other hand, the combined use of both inhibitors showed the a $N_2O$ emission mitigation potential up to 69 % (Martins et al., 2017; Ni et al., 2018) with an average of 31 % (n=118) (Fan et al., 2022).

In order to better assess the $N_2O$ mitigation potential, it is important to know something about the prevailing $N_2O$ production and consumption processes, because this is the only way the inhibitors can be used in a targeted manner. While a reduction of the nitrification rate can be considered a direct effect of NI on mitigating $N_2O$ emissions, the reduced availability to the former process can also reduce $N_2O$ emissions as an indirect effect of NI application. However, the effect of UI or a combined UINI use on the prevailing $N_2O$ production processes is to our knowledge currently largely unclear. Therefore, site-specific nitrogen isotope ratios of $N_2O$ may be useful, as they provide a more nuanced constraint on biogeochemical cycling in soil than its bulk composition alone (Kelly et al., 2023). Up to now, isotopic analysis of the four most abundant isotopocules of $N_2O$ ($^{14}N^{14}N^{16}O$, $^{14}N^{15}N^{16}O$, $^{15}N^{14}N^{16}O$, $^{14}N^{14}N^{18}O$) (Toyoda et al., 2017) have been widely used to quantify $N_2O$ production and consumption processes in soils (Buchen et al., 2018; Di Wu et al., 2019; Lewicka-Szczebak et al., 2020). This approach allows to study the contributions from the four main $N_2O$ production processes, such as bacterial denitrification (bD), nitrifier denitrification (nD), nitrification (Ni) or fungal denitrification (fD) to the total $N_2O$ emissions (Yu et al., 2020) of different mitigation measures.

This study specifically focused on the effectiveness of incorporation of the rarely investigated UI 2-NPT to mitigate $NH_3$ losses, while taking $N_2O$ emissions and their production and consumption production processes into account. Furthermore, the effectiveness of the combination of UI "2-NPT" and the also rarely studied NI MPA is evaluated considering the mitigation of potential "pollution swapping". Therefore, a laboratory experiment was conducted for optimal understanding of the processes under controlled conditions, investigating the effect of broadcast urea application, urea application with urease inhibitor (urea + UI), urea application combined with urease and nitrification inhibitor (urea + UINI), incorporation of urea by slit injection into the soil (urea - INC), and an untreated control. We hypothesized that (a) all mitigation measures lower $NH_3$ emissions compared to the standard surface broadcast application of urea, with UI showing the greatest effect. Furthermore, we hypothesized that (b) incorporation of urea and urea + UI promote $N_2O$ emissions and (c) urea + UINI has the greatest mitigation effect on $N_2O$ emissions. We further hypothesized that (d) nitrification is the most important $N_2O$ production process with respect to the investigated mitigation measures examined.

## 2. Materials and methods

Two incubation experiments were carried out in the microcosm laboratory at Thünen Institute of Climate−Smart Agriculture from April to June 2022 to investigate $NH_3$ and $N_2O$ emissions following urea application with the varying mitigation techniques: one setup for $NH_3$ (Exp. $NH_3$) and one for $N_2O$ measurements (Exp. $N_2O$) (Fig. 1). Two approaches were necessary, since the two gas species require different air exchange rates.

### 2.1. Experimental design and tested treatments

In both incubation experiments, three different $NH_3$ emission mitigation techniques for urea fertilization were compared with an

**Fig. 1.** Set-up of flow chambers for $N_2O$ quantification.

application rate equivalent to 174 kg urea N ha$^{-1}$ (0.167 g fertilizer, 6 granules) for the Exp. $NH_3$ and to 260 kg N ha$^{-1}$ (0.907 g fertilizer, 19 granules) for the Exp. $N_2O$ according to the soil surface. For the Exp. $N_2O$, a higher fertilizer amount was selected to achieve a sufficiently high measuring signal at a constant flow rate in the chambers. In addition to an unfertilized control, four fertilizer treatments were investigated: standard surface urea application (urea), slit injection (urea - INC), urea with urease inhibitor 2-NPT (urea + UI) and urea with urease inhibitor 2-NPT plus nitrification inhibitor MPA (urea + UINI), with n=3 for the Exp. $NH_3$ and n=4 for the Exp. $N_2O$. In order to simulate closed slit injection of fertilizer, slit injection was mimicked to a DEEPOT 32.1 deep deposit fertilizer machine (Rauch Sinzheim, Germany), placing urea fertilizer in a 5 cm deep slit produced with a plastic spatula. Afterwards, the fertilizer was covered with soil and compacted by a cork rolled over the soil surface. The same number and size of granules (diameter of 3.5 mm) were applied in each replicate container.

The soil for both experiments was sampled in 0–20 cm depth (Ap horizon) from a sandy loam agricultural field in Meine (Germany 2022) (Table 1). In preparation for both experiments, the soil was sieved (<2 mm). Furthermore, the soil water content was determined before each experiment by drying the soil at 105 °C until its weight remained constant. The amount of water required to achieve the soil moisture content was added to the soil by gradual mixing calculated with $\theta_1$ the water-holding capacity [vol%]. A guideline value from the manual of soil mapping (Sponagel, 2005) for soil type Sl3 (27 vol%) with an addition of 5 vol% for medium-humic soils was used to estimate the water-holding capacity ($\theta_1$). This results in 22.4 vol% for 70 % plant available moisture content and 47.49 % water filled pore space (WFPS).

The column was compacted to reach the typical field bulk density of 1.4 g cm$^{-3}$, which is considered as a typical bulk density for sandy soils like Meine. This resulted to 479 g soil per chamber for the Exp. $NH_3$ and 2587 g for the Exp. $N_2O$. To prevent enhanced N transformation and evaporation of water, the soil was continuously covered and stored at 4 °C after sampling.

### 2.2. *Flow chambers for $NH_3$ loss quantification*

The experiments were conducted with flow chambers in a temperature-controlled chamber environment to quantify the $NH_3$ losses from N fertilizers (Götze et al., 2023). Frequent acid trap sampling also enabled comparisons of different incorporation techniques. The chamber airflow ran constantly between sampling periods (to simulate windy conditions) and was humidified with washing bottles. The temperature was set to 15 °C, providing supportive conditions for $NH_3$ volatilization.

Soils with and without urea application were incubated in a climatic chamber (HVST 705 EP, Roller Gerlingen, Germany) at 15 °C. This system was designed to quantify $NH_3$ losses from the different treatments. The design of the experiment was based on findings from previous studies on $NH_3$ volatilization (Gronwald et al., 2018; Roelcke et al., 1996). The basic idea is, that the head space of an incubation container is continuously flushed with air, and the resulting $NH_3$ emissions are absorbed in an acid solution and subsequently analysed.

The dimensions of the round incubation jars were 12 cm in height with a diameter of 7.5 cm deflector plate attached to the jar lid, creating a uniform laminar flow while ensuring sweeping of the entire soil surface (Flura et al., 2013). The volume of the soil column was 300 mL with 150 mL headspace volume and with the deflector protruding 3.5 cm into the jar. One gas wash bottle (114452972, Rettberg GmbH Göttingen, Germany) was filled with water to produce almost complete water saturation of the incoming air stream and one behind the vessel. The exhaust air passed through another wash bottle filled with a sulfuric acid solution (100 mL, 5 mM $H_2SO_4$), absorbing emitted $NH_3$. A fritted glass diffuser was used to obtain complete absorption of $NH_3$ into the solution.

The constant flow of humidified air was led over the soil column surface. The required airflow was supplied by an air compressor with a

**Table 1**
Soil used in the experiments—locations, soil texture, total carbon content (TN), total nitrogen content (TN), cation exchange capacity (CEC) and soil pH.

| Soil | Region of Location | Coordinates North/East | Sand [mass%] | Silt | Clay | Texture (Sponagel, 2005) | TC [mass%] | TN | CEC [cmol kg$^{-1}$] | pH ($CaCl_2$) [mol L$^{-1}$] |
|---|---|---|---|---|---|---|---|---|---|---|
| Sandy | Central Germany Meine | 52.387309/10.562801 | 68.61 | 24.24 | 7.15 | Sl2 | 1.39 | 0.11 | 7.53 | 6.59 |

water separator and controlled by pressure regulators. The airflow of each incubation vessel was monitored and controlled by flow sensors (IDT FS200, Renesas Tokyo, Japan). The flow rate was set to 8 headspace volumes min$^{-1}$. The tightness of all incubation tubes was checked using pressure difference measurement. Preparatory experiments proved a second downstream acid trap and repetitive daily trap changing unnecessary, as the capacities of the 100 mL of 5 mM $H_2SO_4$ were sufficient to capture emissions completely. Furthermore, it was found that emissions after urea application ceased after two weeks, as they showed no difference from emissions from untreated soil.

### 2.3. $NH_3$ analysis flux calculation

The $NH_4^+$ concentration in acid traps was measured using an $NH_3$ selective electrode (Thermo Scientific Orion Versa Star Pro Electrochemistry Meters, Waltham USA, range (ISE) 0.0001–19990 ppm, accuracy (ISE) ±0.2 mV or ±0.05 % of reading). Samples were stored at 4 °C until measurement.

For the calculation of $NH_3$ loss, the daily concentrations ($C_{NH_3}$) in the acid solution were multiplied by the volume of acid ($V_{acid}$) and summed up to the total amount of $NH_3$ ($M_{NH_3cum}$) (Eq. 1).

$$M_{NH_3cum} = \sum\nolimits_{t1}^{ti} C_{NH_3} \times V_{acid} \tag{1}$$

The emissions (kg N ha$^{-1}$) were determined by multiplying the mass loss ($M$) with the fertilized surface area ($A$) in the flask (Eq. 2).

$$F = M \times A \tag{2}$$

### 2.4. Flow chambers for $N_2O$ loss quantification

The microcosm system allows an automated incubation of soil columns (microcosms) under controlled conditions in order to study the formation of greenhouse gases in agricultural soils (Hantschel et al., 1994; Säurich et al., 2019). The used cylindrical acryl glass chambers were 18 cm high and 14.4 cm inner diameter. The headspace volume was 1230 mL, and the soil volume was 1600 mL. The additional amount of water needed to achieve the target soil moisture content was determined. Similar to Exp. $NH_3$, the sieved soil was compacted once to a bulk density of 1.4 g cm$^{-3}$.

### 2.5. $N_2O$ flux calculations

Synthetic air (21 % $O_2$, 78 % $N_2$) was pumped continuously at a flow rate of approximately 15 mL min$^{-1}$ through a chamber containing distilled water with a bubbler into the chambers containing the soil and fertilizer treatments in the chamber at 20 °C. Headspace gas from every incubation chamber was sampled directly every 6 h using steel cannulas connected to a gas chromatograph. Blanks for measuring background concentrations of the synthetic air gas mixture and five standards for calibrations were regularly integrated into the measurement sequence. Gas samples and standards were determined using a gas chromatograph (GC-2014, SHIMADZU Kyōto, Japan) equipped with an electron capture detector. The analytical precision was <1.5 % CV and determined by repeated measurements of standards (0.33, 0.55, 2.01, 6.94, 40.4, and 130 ppm $N_2O$). For flux calculations, the mass concentrations ($C$) were calculated in Eq. 5 via the mass volume ($V_m$) according to the ideal gas law (Eq. 3) from the mole mass in g mol$^{-1}$ ($M$) of $CO_2$ and $N_2O$ provided from the GC measurement.

$$V_m = \frac{R \times T}{p} \tag{3}$$

Taking into the temperature in the microcosm system of 20 °C (288.15 K), the pressure of 101.325 kPa (22.41396954 L/mol), and the general gas constant of 8.314 J mol$^{-1}$ K$^{-1}$ into account, the concentration was corrected according to Eq. 4:

$$C = \frac{(c \quad - \quad c_{blank}) \times M}{V_m} \tag{4}$$

$M$ is the molar mass ($N_2O$ 44 g mol$^{-1}$, $CO_2$ 44 g mol$^{-1}$) and $T$ is the temperature (K). Mass flow ($F$, µg N m$^{-2}$ h$^{-1}$) therefore, was calculated by the mass concentration $C$ and the flow rate ($Q$) provided from the flow meter (Eq. 5).

$$F = \frac{(C \times Q)}{A} \tag{5}$$

$A$ denotes the soil surface of the column in m$^2$, and $Q$ is the flow rate in mL min$^{-1}$. Cumulated fluxes were obtained by integrating the emissions from the whole observation period by linear interpolation.

### 2.6. Soil analysis

For determination of mineral nitrogen content ($N_{min}$ = sum of $NO_3^-$-N and $NH_4^+$-N), all soil samples were frozen at −20 °C until preparation. $N_{min}$ extraction was performed for all samples according to German laboratory standards (VDLUFA, 2002). Mineral N was extracted from 50 g of homogenized soil with 200 mL of 0.0125 M $CaCl_2$ (1:4) and shaken in an overhead shaker for one hour. The mixture was then filtered through nitrogen-free filter paper (MN 614¼) and stored at −20 °C until analysis by a continuous flow analyser (CFA-Analyzer San++, Skalar Analytical B.V.). Soil water content was determined by drying the sample for 24 h at 105 °C.

### 2.7. Recovery of fertilizer N in soil

Soil samples were analysed at the beginning of the experiment and after a period of incubation to evaluate N recovery ($r$). For the calculation, the final $N_{min}$ value was divided by the initial value, which consisted of the $N_{min}$ content at the beginning of the experiment and the fertilized $N$ amount (Eq. 6). The differences that occurred were based, among other things (e.g., N transformation processes), on the loss due to $NH_3$ and $N_2O$ emissions. The mineralization, detected in incubated control chambers without fertilization, was very low and was therefore later neglected.

$$r = \frac{(N_{\min,end} - N_{Mineralisation}) + N_{NH_3} + N_{N_2O}}{(N_{\min,start} + N_{fertilizer})} \tag{6}$$

### 2.8. Isotopocule analyses and calculations

In addition to automatic gas sampling and close to the mid-term N recovery sampling, a manual headspace gas sampling was done six weeks after the beginning (day 43) of the Exp. $N_2O$ in order to get insights into $N_2O$ production and consumption processes during a high emitting period. Thus, one crimped 100 mL vial was connected for 24 h hours to the vents of the soil chamber.

Gas samples were analysed for $N_2O$ isotopcules using a Delta V isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany), which was coupled to an automatic preparation system with PreCon plus Trace GC IsoLink (Thermo Scientific, Bremen, Germany). Briefly, $N_2O$ was pre-concentrated, separated, and purified, and $m/z$ 44, 45, and 46 of the intact $N_2O^+$ ions as well as $m/z$ 30 and 31 of $NO^+$ fragment ions were determined, as described in Lewicka-Szczebak et al. (Lewicka-Szczebak et al., 2014). Results were calculated according to Röckmann et al. (Röckmann et al., 2003) and Toyoda and Yoshida (Toyoda and Yoshida, 1999). The $^{15}$N site preference ($\delta^{15}N^{SP}_{N2O}$) was defined as the difference between $\delta^{15}N\alpha_{N2O}$ and $\delta^{15}N\beta_{N2O}$ values. All isotopic values are given as delta values ($\delta$), expressed in per mille (‰) deviation from the $\delta^{15}N/^{14}N$ and $\delta^{18}O/^{16}O$ ratios of the reference materials (i.e., atmospheric $N_2$ and Vienna Standard Mean Ocean Water (VSMOW), respectively). The analytical precision determined as standard deviation ($1\sigma$) of the internal standards for measurements of $\delta^{18}O_{N2O}$, and $\delta^{15}N^{SP}_{N2O}$ were typically 0.1 ‰ and 0.5 ‰, respectively.

During headspace sampling, the collected $N_2O$ was a mixture of atmospheric and soil-emitted $N_2O$. Thus, δ values of soil-emitted $N_2O$ were calculated using a basic isotopic mixing model (Well et al., 2006). To calculate the contribution of nitrification (Ni), fungal denitrification (fD), nitrifier denitrification (nD) and bacterial denitrification (bD) to $N_2O$ production and the degree of $N_2O$ reduction to $N_2$ (consumption), a dual isotope plot – a so called SP/O MAP based on $\delta^{15}N^{SP}_{N2O}$ and $\delta^{18}O_{N2O}$ values - was applied. A detailed calculation strategy can be found in the supplement (Di Wu et al., 2019). Two possible scenarios in case of $N_2O$ source mixing and reduction were evaluated:

– Case 1 – $N_2O$ produced from bacterial denitrification is first partially reduced to $N_2$, followed by mixing of the residual $N_2O$ with $N_2O$ from other pathways.

– Case 2 – $N_2O$ produced by various pathways is first mixed and afterwards reduced.

For further processing, evaluation of distribution and uncertainty parameters for the contribution of the different $N_2O$ pathways and the residual unreduced fraction of $N_2O$ (r) with respect to the differences scenarios, the recent Monte Carlo modelling tool FRAME for 2D application (Lewicki et al., 2022) was used. Isotopic ranges for each process (endmember areas) and isotopic fractionation factors (ε values) for $N_2O$ reduction processes were derived from literature values (Table S1).

### 2.9. Data and statistics

All the statistical analyses were carried out in R Studio (R Studio, Version 2023.6.0.421, R Studio Inc., Boston, MA USA) with the free statistical software R 4.2.2 (R Core Team, 2022). All graphical representations were created using the appropriate R packages, including [ggplot2]. An ANOVA analysis was used to test for fertilizer treatment effects on cumulative $NH_3$-N losses. Differences between fertilizer treatments were determined using the Sidak post hoc test at the 95 % confidence level. The residual distribution was tested, and the Shapiro–Wilk test for normal distribution was performed. Compact letter displays [multcomp, emmeans] were used to report the results of all pairwise comparisons among treatment means. Means not sharing any letter are significantly different by the Sidak test at the 5 % level of significance.

## 3. Results

### 3.1. $NH_3$ emissions

Overall, all three mitigation measures (urea - INC, urea + UI and urea + UINI), reduced cumulative $NH_3$ losses. The maximum cumulative $NH_3$ loss was 80.37 kg N $ha^{-1}$ (45.98 % of applied N) with urea standard surface broadcast application (Fig. 2a). For the latter treatments, an $NH_3$ emission kinetic for the application techniques could be determined, with a faster increase in emissions at the beginning when urea was applied with standard broadcast application. Urea + UINI had lower emissions in the beginning than urea + UI, but 14 days after the start of the experiment, the same final level was reached.

Slit injection and the two inhibitor treatments showed significantly lower $NH_3$ emissions compared to standard surface application, but the emissions were significantly lowest and the mitigation effect greatest with urea – INC (16.38 kg N $ha^{-1}$, 79.62 %). Slit injection had the lowest losses down to less than 9.41 % loss of the applied N amount. The $NH_3$ losses were 47.72 and 42.82 kg N $ha^{-1}$ for urea + UI and urea + UINI, respectively and in the range of 20.81–31.14 % of applied N (Fig. 2b). There was no significant difference in between the two inhibitor variants urea + UI and urea + UINI. The mitigation effect was 40.62 % and 46.72 % respectively (Fig. 2c). The cumulated $NH_3$ emissions of the experiment are given in Table 2.

### 3.2. $N_2O$ emissions

Nitrous oxide fluxes varied significantly depending on the chosen mitigation strategy (Fig. 3a). The urea incorporated in the soil urea - INC produced one peak with significantly higher hourly $N_2O$ emissions than the other treatments in the first 10 days after fertilization. Emission from urea + UI and urea peaked at day 18 after fertilization at 1.7 and 1.25 g $N_2O$-N $ha^{-1}$ $h^{-1}$ with slow decline afterwards. Urea + UINI showed a slow increase to the highest hourly $N_2O$ of 2.1 g $N_2O$-N $ha^{-1}$ $h^{-1}$ h after 71 days.

Elevated cumulative emissions were observed with urea application via standard surface broadcast application or with the addition of a UI, 0.88 and 0.89 kg N $ha^{-1}$, respectively (Fig. 3b). Notably, urea – INC demonstrated significantly pronounced lower fluxes from day 40 of incubation, with cumulative losses of 0.56 kg N $ha^{-1}$ at the end of the trial after 77 days, corresponding to a 64.31 % reduction in $N_2O$ emissions. The utilization of double-inhibited fertilizer (urea + UINI) initially displayed the most substantial mitigation effect on $N_2O$ losses; however, emissions began to rise after 20 days, increasing exponentially from day 50 until reaching levels similar to those of standard spread fertilizer (Fig. 3b). The emission events prevailed even at the end of the measuring period. The cumulated $N_2O$ emissions of the experiment are shown Table 2.

### 3.3. N balances and recovery of applied fertilizer N

Soil mineral N was determined after 14 days in the $NH_3$ Exp. and

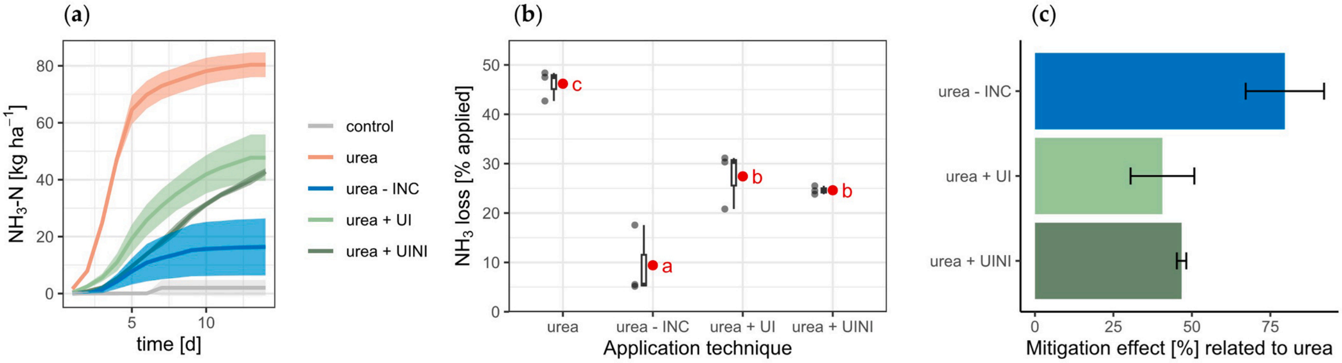

**Fig. 2. a)** Cumulative $NH_3$-N losses from urea applied to the sandy soil from different application techniques, including standard surface application, slit injection, urea + UI and urea + UINI; lines depict mean values ($n = 3$) while shaded bands indicate standard deviation (left diagram), **b)** $NH_3$ losses (% of N applied) from urea fertilizer applied with standard surface application (urea), slit injection technique (urea – INC), urea + UI and urea + UINI; replicates and boxplots are in black, and red indicates the mean values and compact letter display (Means not sharing any letter are significantly different by the Sidak test at the 5 % level of significance with 95 % confidence) (middle diagram) and **c)** and the mitigation effect compared to standard surface application of the mitigation measures (right diagram). n=3.

**Table 2**
N balance in the two experimental setups ($NH_3$ Exp. and $N_2O$ Exp.) with the treatments standard surface urea (urea), urea applied with slit injection technique (urea – INC), urea with urease inhibitor (urea + UI) and urea with urease and nitrification inhibitor (urea + UINI) with start $N_{min}$ and End $N_{min}$ after incubation, N from fertilizer and the emitted N; * are estimated values from the parallel experiment.

| | Treatment | Incubation [d] | Start $N_{min}$ [kg N $ha^{-1}$] | Fertilizer N | End $N_{min}$ | cum. $NH_3$-N | cum. $N_2O$-N | N balance % |
|---|---|---|---|---|---|---|---|---|
| **set-up** | urea | 14 | 6 | 174 | 81±21 | 80±4 | *0.5±0.001 | 90±14 |
| | urea - INC | 14 | 6 | 174 | 161±17 | 16±10 | *0.5±0.06 | 99±15 |
| | urea + UI | 14 | 6 | 174 | 95±13 | 48±8 | *0.5±0.06 | 79±12 |
| | urea + UINI | 14 | 6 | 174 | 127±11 | 43±1 | *0.1±0.001 | 94±7 |
| **$N_2O$ Exp.** | urea | 37 | 14 | 260 | 208±20 | *80±4 | 0.5±0.001 | 105±9 |
| | urea - INC | 37 | 14 | 260 | 208±10 | *16±10 | 0.5±0.06 | 82±7 |
| | urea + UI | 37 | 14 | 260 | 221±15 | *48±8 | 0.5±0.06 | 98±8 |
| | urea + UINI | 37 | 14 | 260 | 187±16 | *43±1 | 0.1±0.001 | 84±6 |

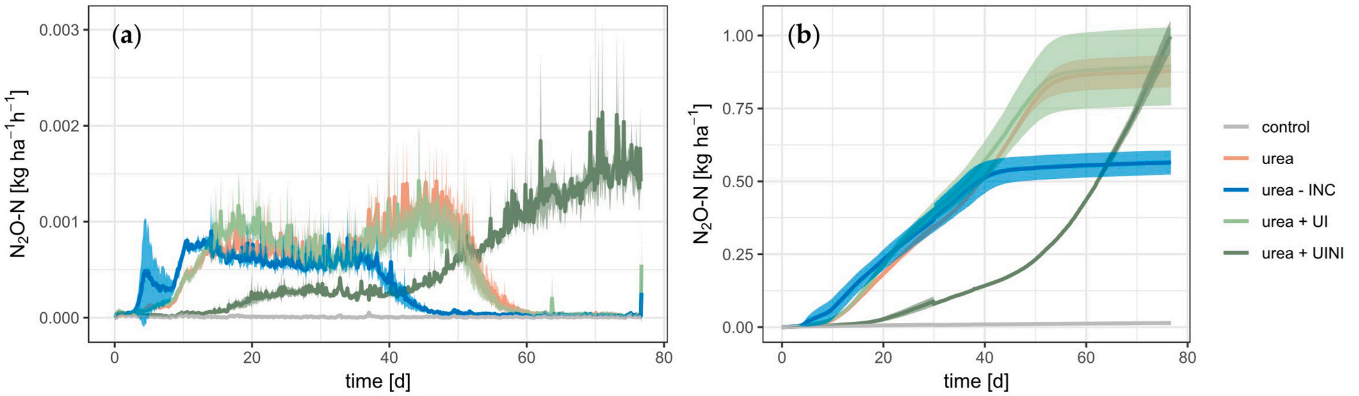

**Fig. 3.** Cumulative losses (left) and fluxes (right) of $N_2O$-N from urea applied with different application techniques including standard surface(urea), slit injection technique (urea – INC), urea + UI and urea + UINI; lines depict mean values (n=3) while shaded bands indicate standard deviation.

after 37 days in the $N_2O$ Exp. (Table 2) details on $NH_4^+$ and $NO_3^-$ composition and dynamics is presented in the supplements (Figure S1). After two weeks of incubation, the $NH_3$ Exp. showed that the $NH_4$-N contents were highest when urea was applied with UINI with 88 kg N $ha^{-1}$. The total mineral N content was highest in the urea - INC treatment with 161 kg N $ha^{-1}$. In the $N_2O$ Exp., the $N_{min}$ values were more similar between the different treatments. After 37 days of incubation, the $NH_4$-N value was also highest when applied urea with UINI, 115 kg N $ha^{-1}$ as in the $NH_3$ Exp. and the highest $N_{min}$ showed urea + UI. The emissions of the respective other experimental design were used for the balances. In the case of $NH_3$ emissions, these measurements are probably erroneous due to the low air exchange rate in the $N_2O$ incubation setup, which can result in very low cumulative $NH_3$ emissions. On the other hand, the soil samples in this experiment were not taken after 14 days but rather 37 days, in contrast to the $NH_3$ Exp., so that the emission process had run longer. The recovery of applied N was high in both experimental set-ups, between 90 % and 99 % in the $NH_3$ Exp.

### 3.4. $N_2O$ isotopocule values

The majority of isotopocule values of soil-emitted $N_2O$, presented in the dual isotope plot (SP/O MAP, Fig. 4) is situated within the area limited by the different $N_2O$ source mixing and the reduction line, which allows the calculation of the two different scenarios and application of the FRAME model. Values clearly scatter between the mixing lines of the three different $N_2O$ production processes, while clustering in the end-member areas of Ni and fD. Graphical evaluation shows nearly no contribution of $N_2O$ reduction to $N_2$ under the prevailing conditions at the sampling day. $\delta^{15}N^{SP}_{N2O}$ values of the urea and urea + UI treatment were with > 28 ‰ slightly higher, when compared to values from the urea - INC and the urea + UINI treatment, while $\delta^{18}O_{N2O}$ ranges were rather similar for all treatments.

### 3.5. $N_2O$ production and consumption processes

Apart from the graphical evaluation, analysis by the FRAME model indicated that $N_2O$ during the high emitting phase (six weeks after beginning the incubation) was mainly produced by nitrification in both scenarios. Modelled (blue dots) and measured data (black dots) were in good agreement (Figure S3-S6, see panel a and d) for both scenarios. The fractional contribution of nitrification (range of the mean value over all treatments) to the total $N_2O$ production was 42–48 % for Case 1 (Red-Mix) and 40–57 % for Case 2 (Mix-Red). The contribution from bD/nD source was rather low in both cases, while a significant contribution of fungal denitrification could not be neglected. The residual unreduced fraction of $N_2O$ (r) was estimated to be 47–70 % in Case 1 and 73–83 % in Case 2 (Table 3), however histograms of model runs were not always optimal for this fraction (Figs. S1–4, panel b and e). When comparing the different treatments, only the urea + UINI treatment was somewhat different, with a higher estimated contribution of bD/nD (> 39 %), as well as a higher residual unreduced fraction (Table 3).

## 4. Discussion

### 4.1. Effect of mitigation technique on $NH_3$

The results of our experiment showed $NH_3$ losses similar to many earlier laboratory and field studies (Rochette et al., 2009; Soares et al., 2012; Wang et al., 2020), but higher than in some experiments (Gioacchini et al., 2002; Hu et al., 2020; Ni et al., 2018). Similar efficacies of the mitigation measures were observed in different studies (Abalos et al., 2012; Soares et al., 2012; Wang et al., 2020), nonetheless also higher $NH_3$ mitigation potential of up to 89 % compared to surface applied urea have been reported for UI and UINI treatments (Gioacchini et al., 2002; Hu et al., 2020; Ni et al., 2018). In comparison, the

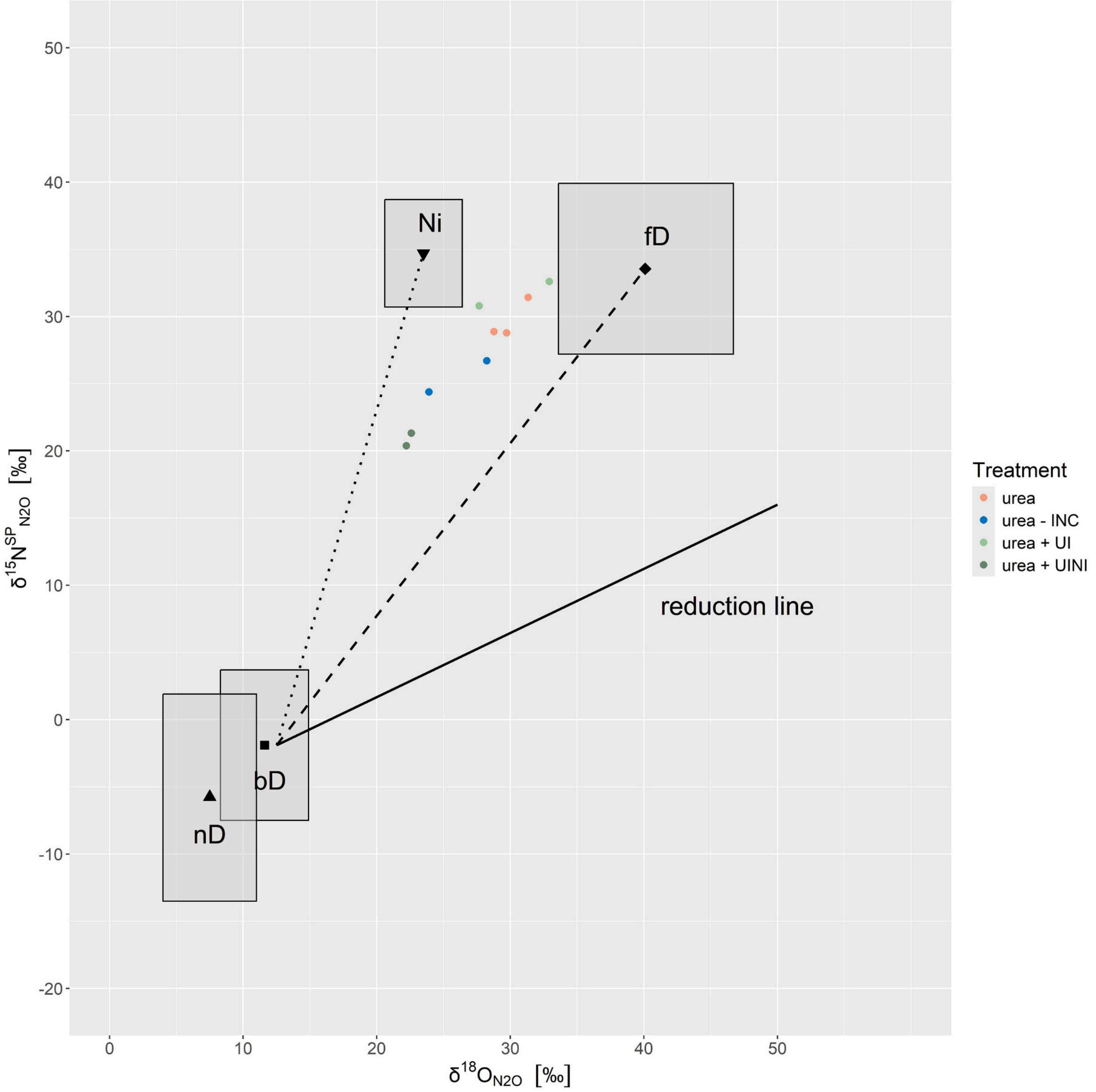

**Fig. 4.** Isotopocule values of soil-emitted $N_2O$ plotted per treatment at day 43 after start of the experiment. The SP/O map is based on $\delta^{15}N^{SP}_{N2O}$ and $\delta^{18}O_{N2O}$ values presented with literature endmember values and theoretical mixing (dotted lines) and reduction (solid line) lines. Mixing lines were drawn between mean values for both $\delta^{15}N^{SP}_{N2O}$ and $\delta^{18}O_{N2O}$ of the respective processes. The reduction line represents the mean route based on $\eta_{red}\ ^{15}N^{SP}_{N2O}/\eta_{red}\ ^{18}O_{N2O}$ ratios. $\delta^{18}O_{N2O}$ values of mixing endmember bacterial denitrification (bD), nitrifier denitrification (nD), and fungal denitrification (fD) are presented in relation to the mean ambient water of −8.4 ‰ (hence present the expected $\delta^{18}O_{N2O}$ originating from a particular pathway under these study conditions). For nitrification (Ni) $\delta^{18}O$ correction was not necessary.

**Table 3**

Simulation results of fractions for $N_2O$ production processes: bacterial denitrification (bD) nitrifier denitrification (nD), fungal denitrification (fD) and nitrification (Ni) and the remaining fraction (r) of $N_2O$ after its reduction by denitrification obtained from FRAME model results (see also Figure S3-S6, panel c and f). Mean values ± standard deviation. n =2, except urea with n=3.

| Treatment | *Case 1: Red-Mix* | | | | *Case 2: Mix-Red* | | | |
|---|---|---|---|---|---|---|---|---|
| | bd/nD | fD | Ni | r | bd/nD | fD | Ni | r |
| urea | 0.13±0.08 | 0.39±0.17 | 0.48±0.16 | 0.47±0.28 | 0.16±0.07 | 0.29±0.17 | 0.54±0.17 | 0.77±0.14 |
| urea - INC | 0.27±0.08 | 0.25±0.14 | 0.48±0.15 | 0.57±0.24 | 0.30±0.08 | 0.22±0.13 | 0.49±0.15 | 0.77±0.15 |
| urea + UI | 0.11±0.07 | 0.41±0.13 | 0.47±0.14 | 0.48±0.29 | 0.15±0.08 | 0.28±0.17 | 0.57±0.17 | 0.73±0.16 |
| urea + UINI | 0.39±0.09 | 0.19±0.13 | 0.42±0.16 | 0.70±0.19 | 0.42±0.09 | 0.18±0.13 | 0.40±0.15 | 0.83±0.12 |

meta-study of Fan et al., (2022) across various UI compounds showed a $NH_3$ mitigation by 51 % (n=76) compared to only 40.62 % in this trial using 2-NPT which, nevertheless, is probably still in the prediction interval of the Fan et al., (2022) analysis. In the group of UINI with various inhibitor components a reduction of 38 % (n=77) was shown in the same meta-study and in this trial using 2-NPT and MPA as inhibitors 46.72 %. Even though $NH_3$ emissions can increase through the addition of NI, depending on the soil properties (Soares et al., 2012; Wang et al., 2020), the meta-analysis of Kim et al. and others reported a decrease when combined with UI (Kim et al., 2012). However, since the measurement period in this experiment was with 14 days not long enough to capture the plateau of the cumulated $NH_3$ emissions of the UINI treatment, the cumulated emissions could have increased significantly to or above the level of the UI treatment. For further experiments on the impact of UINI on $NH_3$ volatilization, we therefore recommend an experimental duration until the end of the emission process. Due to the measurement period, the $NH_3$ emissions of the UINI treatment did not decline as much as the other treatments, with significantly higher emissions of up to 0.002 kg N $ha^{-1}$ $h^{-1}$ at the end of the experiment.

The incorporation of urea into the soil (urea - INC), which simulated the deep depot fertilizer applicator, reduced the cumulated $NH_3$ emissions to 16.38 kg N $ha^{-1}$. This 79.62 % reduction compared to the urea treatment is in line with previously published work. For example Rochette et al. found that urea incorporation reduced cumulated $NH_3$ emissions by 52 % and up to 70 % at a similar depth (Rochette et al., 2009, 2013). Furthermore, an average reduction of $NH_3$ emissions of 63 % at the depth of 5 cm was reported (Rochette et al., 2013). In a recent study from 2023, in which various incorporation techniques were examined on different soil textures and moisture levels, the mitigation potential for urea – INC ranged between 43 % and 87 % (Götze et al., 2023). With a $NH_3$ mitigation of 79.62 % measured in this experiment, results are well in line with 74 % reported for sandy soils and a similar soil moisture (Götze et al., 2023).

The low emissions and the resulting high $N_{min}$ values in urea - INC treatment are reflected in the high recovery rate. This is similar in other treatments where, however, also higher $NH_3$ emissions accounted for the high N recovery. In the urea + UI treatment the N recovery was only 79 % however, did not differ significantly (Figure S2). Causes for the low recovery in the urea + UI treatment could be higher denitrification and $N_2$ production or NO loss.

However, minimal $NH_3$ emissions were measured at a greater incorporation depth than 7.5 cm (Rochette et al., 2013). Overall incorporation was the most effective option in case of $NH_3$ emission mitigation and this trial shows that UINI can achieve the same level of reduction potential as the single-inhibited variant UI. Nevertheless, the implementation of slit injection technique has several limitations in the field, which were investigated in another research project (Mallast et al., 2022). In soils that are too moist and have a high clay content, as well as in higher crops, like wheat and rape, the fertilizer cannot be incorporated using this technology and a traditional spreading method must be used (Mallast et al., 2022). The hypothesis put forward can only be partially accepted, as all mitigation measures showed an effect, but the strongest effect was achieved by incorporation instead of UI.

### 4.2. *Effect of $NH_3$ mitigation technique on $N_2O$ emissions*

All three mitigation measures (INC, UI and UINI) did not lead to a significant increase in cumulative $N_2O$ emissions compared to the reference treatment of surface broadcast urea at the end of the trial. However, the $N_2O$ emission kinetics differ between the urea - INC and urea + UINI treatments. While the emission curve of broadcast urea and urea with UI show a very similar course, the $N_2O$ emissions initially increase with urea addition, while the double-inhibited fertilizer shows almost no emissions for the first 10 days. This is in line, with the well-known dependency of $N_2O$ losses on soil texture and soil moisture, since higher soil moisture in the sandy and clay soils can enhance the denitrification potential, as observed in other studies (Drame et al., 2023; Liu et al., 2022). In previous studies, surface incorporation has shown that pollution swapping from $NH_3$ to $N_2O$ might occur (Mallast et al., 2022), especially on moist sandy soils (Götze et al., 2023). However, in our case the deep incorporation by closed slit injection did not increase cumulative $N_2O$ emissions after 20 days, with a strong decline of emissions after 40 days. The addition of the combined UI and NI (2-NPT and MPA) to urea led to a significant reduction of $N_2O$ emissions by up to 70 % compared to surface applied urea in the measurement period of 50 days. But, reflecting longer dry periods without precipitation or other environmental impacts, $N_2O$ emissions were later increased to the same level as in the single inhibited and broadcast urea treatments. It must be emphasized that the investigated scenario without N uptake by plants and rainfall, which are known to impact $N_2O$ emission kinetics, are not directly transferable to practical conditions in the field. Nevertheless, the application of NI alone without plant N uptake may not be sufficient to reduce $N_2O$ emissions over time and would require further measures.

In this incubation experiment, the recovery was at least 82 %. In contrast to the $NH_3$ Exp., the lowest recovery was detected in the treatment urea - INC, which can be attributed to the more difficult sampling procedure in this trial due to the slit injection and the sampling with soil corer in the soil columns.

Although NI could reduce $N_2O$ emissions and thus significantly reduce the atmospheric concentration of a very climate-damaging greenhouse gas, further research on the non-target microbial communities needs to be conducted to compare the benefits of $N_2O$ emissions reduction with the long-term environmental impacts. Overall, the hypothesis put forward can be partially rejected, since UINI did not show the greatest reduction potential over the whole incubation time. The use of UI did not promote the $N_2O$ emissions and so incorporation except for the first trial days.

### 4.3. *$N_2O$ production and consumption processes*

Even if the $N_2O$ emissions of the different $NH_3$ mitigation measures tested did not lead to an increase in $N_2O$ emissions compared to conventional surface urea application, the isotope analyses of soil-emitted $N_2O$ provided short-term insights into the ongoing production and consumption processes. Results of the SP/O MAP and the FRAME model confirm a high contribution of $N_2O$ production from nitrification for all treatments. A high contribution of nitrification was not surprising, since $NH_4^+$-based fertilizers, like urea provide available $NH_4^+$ immediately. However, a such long lasting effect after six weeks of application was unexpected. After this time, most of the $NH_4^+$ applied with the fertilizer in the soil should have already been converted to $NO_3^-$ (Figure S1) and thus likely promote the process of denitrification. However, in general the contribution of bacterial denitrification or nitrifier denitrification was rather low (Table 3), and the residual unreduced $N_2O$ fraction (r) also showed that the most was emitted as $N_2O$ and that the $N_2O$ reduction played a more minor role under the prevailing soil conditions at the sampling day.

The urea and urea + UI treatments have a very similar $N_2O$ course over time. This can also be confirmed in the proportions of the individual processes, which differed only very slightly for the two treatments. However, over time, the $N_2O$ emissions of the urea + UINI treatment were in the opposite direction and the emissions increase continuously over the experimental period, although they were very low at the beginning. Overall, the urea + UINI emissions were only about half as high as the emissions from the urea and urea + UI treatment, but showed a higher proportion from the bD/nD fraction. This might be due to the delay of urea hydrolysis by the use of UI, which promoted $NH_4^+$ formation. In combination with NI, which might have reduced or delayed the oxidation of $NH_4^+$ to $NO_3^-$ in soil, the $NH_4^+$ was probably more stable and $N_2O$ production was somewhat delayed (Thapa et al., 2016). This assumption can be confirmed by the $N_{min}$ sampling in the $N_2O$ Exp., where 62 % of the mineral nitrogen in the UINI treatment was in the form of $NH_4^+$ (Figure S1). Moreover, available $NH_4^+$ potentially can be rapidly nitrified and then followed by denitrification compared to the use of urea alone (Akiyama, H., Yan, X., and Yagi, K., 2010), which might explain the increased bD/nD fractions.

Surface incorporation (urea - INC) can stimulate $N_2O$ production in sandy soils up to 27 % (Götze et al., 2023). Moreover, fertilizer incorporation is known to stimulate the process of denitrification by the creation of anaerobic hot spots in soils, if soil moisture exceeds 70 % WFPS (Davidson, 1993), $N_2O$ consumption, i.e. reduction to $N_2$ might also occur and might explain a missing N losses in the N recovery (Table 2). However, soil moisture levels were with about 47 % WFPS in our experiment not ideal for complete denitrification, which was further underlined by findings of the SP/O MAP (Fig. 4) and the FRAME model.

### *4.4. Relevance for in situ conditions*

One advantage of combined UI and NI is the reduced use of fertilizer due to the reduced emissions and higher nutrient use efficiency. Thus, saving costs on labour and fertilizer by reducing the frequency of fertilizer application (Abalos et al., 2014; Sanz-Cobena et al., 2012). Currently, however, the price of double-inhibited fertilizers is still higher than that of the single-inhibited variants, motivating a choice for economic over environmental aspects for farmers. To make a full cost-benefit assessment, yield effects also need to be taken into consideration. Additionally, use of nitrification inhibitors may also allow a reduction of the amount and frequency of fertilizer application, therefore saving costs (machine passes, fuel, hourly wages) and allowing more flexible timing of fertilization due to only two fertilizer applications (Abalos et al., 2014). Nonetheless, comparable effects could be attained by adapting the crop irrigation management or applying recommended amounts of fertilizer without the additional costs of the inhibited fertilizers. Reduced N application rates through the use of inhibited fertilizers without yield losses, making the inhibited product more economical. A holistic assessment should also consider the additional benefits of lower N application rates, such as the reduction in soil acidification caused by $NO_3^-$ leaching, which is associated with longer-term management costs (Rose et al., 2018). Moreover, since the emission reduction of inhibitors depends on the physicochemical soil properties and climatic conditions, the economic viability must be evaluated for each situation (Abalos et al., 2014; Klimczyk et al., 2021). Incorporation of urea into the soil is the most economic fertilization option. However, the simulated deep depot fertilizer machine by Rauch is hardly used by farmers, due to the purchase costs of an additional device. More research is required to optimize the combination of UI and NI and to develop practical machines for the incorporation of urea, especially under difficult conditions (wet, clayey or hardened soil) (Mallast et al., 2022), to increase environmental but also economic benefits (Thapa et al., 2016).

This study was conducted under controlled environmental conditions and, therefore, does not account for differences in weather, such as temperature, precipitation, time of day, or season. The experiments were conducted on disturbed soil columns without plant cover and N uptake. For these reasons, this trial should be supplemented by further laboratory tests and confirmed by field trials

## 5. Conclusions

All $NH_3$ mitigation options significantly reduced emissions, with closed-slit incorporation achieving the highest reduction compared to surface-applied urea. However, the $NH_3$ reduction from UI (2-NPT) and UINI (2-NPT+MPA) was lower than expected compared to meta-studies involving mainly the UI NBPT, highlighting the need for individual studies on various inhibitors to better understand their efficacy. In contrast to controlled environment studies, practical implementation of incorporation technology is challenging for many soil properties and conditions and due to limited machinery availability. The study showed that while urea + UINI treatment initially reduced $N_2O$ emissions, $N_2O$ release later on increased over a prolonged dry period without precipitation. Incorporation on the other hand reduced cumulative $N_2O$ emissions in particular considering longer time periods. This highlights the need to differentiate the effect of UI and NI on emissions with and without canopy and crop N uptake. In situations with crops, effects of NI on short term cumulative $N_2O$ emissions are more relevant than from soil without crops. The SP/O MAP approach with the FRAME model was useful for estimating $N_2O$ production and consumption processes, showing nitrification as the main source six weeks post-fertilizer application, with minor contributions from other processes as fungal denitrification. $N_2O$ consumption occurred under experimental conditions, and further isotope sampling is recommended to study $N_2O$ kinetics of UI and UINI in more detail. The presented data contribute to a conclusive assessment of inhibitor substances for which more laboratory and field studies under varying environmental and management conditions are required.

The interest in alternative fertilization methods with innovative injection and placement technologies to reduce the nitrogen problem is steadily increasing. However, many farmers are uncertain as to which regulations and rules will be introduced in the future, which is an obstacle to the willingness to invest. But next to the potential of inhibitor use to greatly improve the sustainability of N-fertilization and achieving globally accepted climate change targets, little is known about their potential to enter the food chain. Further studies with accurate and robust analytical methods for inhibitor detection are required to ensure that these additives do not pose any substantial threat to the environment, food safety and human health.

Supplementary Materials**:** The following supporting information can be downloaded at www.mdpi.com/xxx/s1, Figure S1: Mineral N content in the $NH_3$ Exp. (a) after two weeks and the $N_2O$ Exp. after 37 days (b) of incubation with start $N_{min}$ of the soil (grey line) and applied fertilizer N (orange line). Red letters indicate the compact letter display (Means not sharing any letter are significantly different by the Sidak test at the 5 % level of significance with 95 % confidence). Figure S2: Nitrogen balance (%) from urea fertilizer applied with standard surface application (urea), slit injection technique (urea – INC), urea + UI and urea + UINI; replicates and boxplots are in black, and red indicates the mean values and compact letter display (Means not sharing any letter are significantly different by the Sidak test at the 5 % level of significance with 95 % confidence). n=3. Table S1: Summary of mixing endmember isotopic signatures of particular pathways (bD – bacterial denitrification, nD – nitrifier denitrification, fD – fungal denitrification, Ni – nitrification) and reduction fractionation factors (reduction) with respective references. For the model input, each value is corrected with the respective mean isotopic signature of the substrate: for $\delta^{18}O_{N2O}$ – soil water ($\delta^{18}O_{H2O}$) for bD, nD, and fD. The respective substrate-corrected values were applied as a model input for $\delta^{18}ON_2O$. For $\delta^{15}N^{SP}_{N2O}$ no substrate correction is needed. As the isotopic endmember ranges of nD and bD overlap considerably, a mean value was used for the 2D model. Figure S3: FRAME results of $N_2O$ production/consumption process analyses of the urea treatment. Panels (a) and (d) show the observed $N_2O$ isotopocule ratios (black dots) and the simulated results (blue dots) for both calculation scenarios. Gray squares indicate the endmember ranges of bD (bacterial denitrification) /nD (nitrifier-denitrification), fD (fungal denitrification) and Ni (nitrification). The slope of the grey broken lines shows the ratio of the isotopocule fractionation factors for $N_2O$ reduction by denitrification, along which the isotopocule ratios of the remaining $N_2O$ are expected to increase if $N_2O$ is reduced. Values are based on Table S1. Panel (b) and (e) show variable correlations based on model runs, using Histograms (on the diagonal) calculated from the variables building the Markov chains, contour-plots (top-right) showing the correlation and their correlation coefficients (bottom-left). Panel (c) and (f) summarizes statistics on the simulation results of fractions of the different $N_2O$ production processes and the residual unreduced $N_2O$ fraction (r). The open circles and horizontal lines indicate the mean and median values, respectively. The box encloses the 68 % confidence interval (CI), and the whiskers show 95 % CI. Figure S4: FRAME results of $N_2O$ production/consumption process analyses of the urea - INC treatment. Panels (a) and (d) show the observed $N_2O$ isotopocule ratios (black dots) and the simulated results (blue dots) for both calculation scenarios. Gray squares indicate the endmember ranges of bD (bacterial denitrification) /nD (nitrifier-denitrification), fD (fungal denitrification) and Ni (nitrification). The slope of the grey broken lines shows the ratio of the isotopocule fractionation factors for $N_2O$ reduction by denitrification, along which the isotopocule ratios of the remaining $N_2O$ are expected to increase if $N_2O$ is reduced. Values are based on Table S1. Panel (b) and (e) show variable correlations based on model runs, using Histograms (on the diagonal) calculated from the variables building the Markov chains, contour-plots (top-right) showing the correlation and their correlation coefficients (bottom-left). Panel (c) and (f) summarizes statistics on the simulation results of fractions of the different $N_2O$ production processes and the residual unreduced $N_2O$ fraction (r). The open circles and horizontal lines indicate the mean and median values, respectively. The box encloses the 68 % confidence interval (CI), and the whiskers show 95 % CI. Figure S5: FRAME results of $N_2O$ production/consumption process analyses of the urea + UI treatment. Panels (a) and (d) show the observed $N_2O$ isotopocule ratios (black dots) and the simulated results (blue dots) for both calculation scenarios. Gray squares indicate the endmember ranges of bD (bacterial denitrification) /nD (nitrifier-denitrification), fD (fungal denitrification) and Ni (nitrification). The slope of the grey broken lines shows the ratio of the isotopocule fractionation factors for $N_2O$ reduction by denitrification, along which the isotopocule ratios of the remaining $N_2O$ are expected to increase if $N_2O$ is reduced. Values are based on Table S1. Panel (b) and (e) show variable correlations based on model runs, using Histograms (on the diagonal) calculated from the variables building the Markov chains, contour-plots (top-right) showing the correlation and their correlation coefficients (bottom-left). Panel (c) and (f) summarizes statistics on the simulation results of fractions of the different $N_2O$ production processes and the residual unreduced $N_2O$ fraction (r). The open circles and horizontal lines indicate the mean and median values, respectively. The box encloses the 68 % confidence interval (CI), and the whiskers show 95 % CI. Figure S6: FRAME results of $N_2O$ production/consumption process analyses of the urea + UINI treatment. Panels (a) and (d) show the observed $N_2O$ isotopocule ratios (black dots) and the simulated results (blue dots) for both calculation scenarios. Gray squares indicate the endmember ranges of bD (bacterial denitrification) /nD (nitrifier-denitrification), fD (fungal denitrification) and Ni (nitrification). The slope of the grey broken lines shows the ratio of the isotopocule fractionation factors for $N_2O$ reduction by denitrification, along which the isotopocule ratios of the remaining $N_2O$ are expected to increase if $N_2O$ is reduced. Values are based on Table S1. Panel (b) and (e) show variable correlations based on model runs, using Histograms (on the diagonal) calculated from the variables building the Markov chains, contour-plots (top-right) showing the correlation and their correlation coefficients (bottom-left). Panel (c) and (f) summarizes statistics on the simulation results of fractions of the different $N_2O$ production processes and the residual unreduced $N_2O$ fraction (r). The open circles and horizontal lines indicate the mean and median values, respectively. The box encloses the 68 % confidence interval (CI), and the whiskers show 95 % CI.

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## CRediT authorship contribution statement

**Lea Eder:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Caroline Buchen-Tschiskale:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis. **Hannah Götze:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andreas Pacholski:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

## 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.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2024.109307.

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