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Effect of Nitrification Inhibitors on N$_2$O Emissions after Cattle Slurry Application

Christina Herr [1], Thomas Mannheim [2], Torsten Müller [1] and Reiner Ruser [1,*

[1] Institute of Crop Science, Fertilization and Soil Matter Dynamics, University of Hohenheim (340i), 70593 Stuttgart, Germany; christinaherr@live.de (C.H.); torsten.mueller@uni-hohenheim.de (T.M.)

[2] EuroChem Agro GmbH, 68165 Mannheim, Germany; t.mannheim@web.de

* Correspondence: reiner.ruser@uni-hohenheim.de

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Abstract: Cattle slurry injection (INJ) has shown to be an efficient measure to reduce ammonia (NH$_3$) losses from soils but it might also significantly increase nitrous oxide (N$_2$O) emissions, which can dominate the total greenhouse gas (GHG) release in silage maize production (*Zea mays* L.). Nitrification inhibitors (NIs) are known for their potential to mitigate N$_2$O. Therefore, we tested the effect of NIs added to cattle slurry before INJ on N$_2$O fluxes from a Haplic Luvisol under silage maize in southwest Germany. We determined N$_2$O fluxes at least weekly, with the closed chamber method over two full years. NIs differ in their chemical and physical behavior and we therefore tested a range of commercially available NIs: 3,4-dimethylpyrazole phosphate, 3,4-dimethylpyrazol succinic acid, a mixture of both, nitrapyrin, dicyandiamide, and 1,2,4 triazol and 3-methylpyrazol. Although not significant, INJ treatments with NI showed lower mean annual N$_2$O emissions than the INJ treatment without NI in the 1st year. The emission reduction by NI of 46% in the 2nd year was statistically significant. In both years, we did not find any difference in N$_2$O release, crop yield, or nitrogen removal between the different NI treatments. In the 1st year, which was extraordinary dry and warm, emission factors (EFs) for all INJ treatments were 4 to 8-fold higher than default EF from the IPCC. Even in the 2nd year, only three NI treatments reached EFs within the range provided by the IPCC. Direct N$_2$O accounted for between 81 and 91% of the total GHG emission. Area- and yield-related GHG emission of the broadcast application with subsequent incorporation was in both years in the statistical class with lowest emission. In contrast, INJ with NIs showed similar GHG emissions in only one year, and consequently, incorporation was found to be the optimum management practice for livestock farmers in our study region.

Keywords: 3,4-dimethylpyrazole phosphate; 3,4-dimethylpyrazol succinic acid; nitrapyrin; dicyandiamide; 1,2,3 triazole; 3-methylpyrazole; 3-methylpyrazole; N$_2$O emission factor; greenhouse gas emission

1. Introduction

Application techniques of organic fertilizers can have an immense impact on direct nitrous oxide (N$_2$O) and ammonia (NH$_3$) emissions from agricultural soils [1–3]. N$_2$O losses contribute to global warming and stratospheric ozone depletion [4,5]. NH$_3$ losses are harmful to the environment in terms of acidification, eutrophication, and, since they are precursors for secondary aerosols, they can also have adverse effects on human health [6]. Gaseous nitrogen (N) losses also decrease nitrogen use efficiency (NUE) of cropping systems [7]. Apart from the objective to reduce gaseous N losses for improving the internal N cycle and avoid extra cost for N-fertilizers [8], the reduction of N losses as sources for the release of greenhouse gases (GHG) from cropping systems was declared as a national and international political goal to protect the earth's climate [9,10].

Among the NH_3 abatement strategies, slurry injection (INJ) has shown to reduce NH_3 emissions from agricultural soils very efficiently [11,12]. Slurry incorporation (INC) after surface application has also shown to be efficient in NH_3 reduction but it might hold the risk for increased NH_3 volatilization as long as the slurry is not incorporated [12,13]. In this context, NH_3 losses have shown to be particularly high in periods with elevated air temperature, wind velocity, and solar radiation [14,15]. Contrary to slurry injection where nutrients are banded closer to the plant roots, broadcast incorporation (INC) can result in a lower N use efficiency and lower yield response. This is because part of the nutrients in INC are placed distant from the plant roots, thus decreasing ammonium (NH_4^+) availability near the roots [16].

In contrast to the beneficial reduction of NH_3 losses, slurry INJ can promote anaerobic conditions in the injection slot and thus stimulates N_2O release from denitrification [17,18]. The high N_2O emission after cattle slurry INJ can even exceed the NH_3 loss following slurry INC with a disc harrow two hours after broadcast application [19].

Consequently, a fertilization strategy that reduces both NH_3 and N_2O losses efficiently and thus reduces the atmospheric burden during silage maize (*Zea mays* L.) production with organic fertilization is still required. The use of nitrification inhibitors (NIs) has often been shown to decrease the N_2O emission after application of N fertilizers rich in NH_4^+ [20,21].

The ammonia monooxygenase (AMO) is the first enzyme involved in the oxidation of NH_4^+ to nitrate (NO_3^-) [22]. The inhibition of the AMO by NIs directly decreases the nitrification rate and it reduces the NO_3^- concentration, which serves as a substrate for denitrification [23]. Hence, the two main pathways of N_2O production [24] in soils are blocked or their source strength is at least decreased or delayed. Due to the additional supply of easily available carbon (C), organic fertilizers bear the risk of strongly promoting denitrifying conditions because it can result in enhanced oxygen (O_2) consumption through microbial respiration [25,26] and it provides electrons essential for C heterotrophic NO_3^- reduction [27]. Thus, NIs seem to be an appropriate tool for N_2O reduction since they desynchronize C and NO_3^- availability after slurry application. Therefore, the reduction of direct N_2O emission through NIs could be interesting for management systems with surface application and subsequent INC and also in particular for systems with slurry INJ.

Up to now, several studies have confirmed a significant reduction of N_2O emissions through the addition of NIs to liquid manure [28–33]. However, annual data sets on the effect of NIs in liquid manures on N_2O release are still limited. Specifically, for the climatic conditions in South Germany, it was shown that annual N_2O measurements are necessary for comprehensive quantification of N_2O emissions in study years with intense frost/thaw cycles [34–36]. Moreover, most of the studies on N_2O emissions from organic fertilizers with NI investigated just one or two NIs. However, NIs differ in their chemical and physical characteristics, like water solubility, volatility, sorption properties, and decomposition rates in soils. Consequently, the effectiveness and persistence of the particular active compound is influenced differently by varying environmental and soil conditions [23]. Thus, a comparison of the effectiveness from different NIs between studies is difficult.

In this context, our present study aimed to determine the effectiveness of NIs currently approved in the German market for N_2O emission reduction following cattle slurry application and consequently their potential for GHG abatement in maize production. For this purpose, we tested 3,4-dimethylpyrazole phosphate (DMPP) (trade name: ENTEC© FL), nitrapyrin (trade name: N-LOCK©), dicyandiamide (DCD) (trade name: ALZON©), mixture of 1,2,4-triazol and 3-methylpyrazole (trade name: PIADIN©), as well as a new NI currently not available on the market: 3,4-dimethylpyrazole succinic acid (DMPSA).

The following hypotheses were postulated: (i) NIs delay the availability of NO_3^- after slurry application and therefore reduce N_2O emissions, (ii) different NIs have different N_2O reduction potentials due to their varying chemical and physical characteristics after slurry INJ, and (iii) slurry application with NIs strongly reduces field-related GHG release compared to slurry application without NI.

2. Materials and Methods

2.1. Study Site

The experiment was carried out at the University of Hohenheim's research station "Heidfeldhof" (48°43′0.30″ N; 9°11′30.64″ E; 404 m a.s.l.). The soil had a silty loamy texture (2% sand, 74% silt, and 24% clay) and was classified as Haplic Luvisol. The study site is characterized with a mean precipitation of 663 mm year^{-1} and an annual mean air temperature of 10.1 °C (means 2007–2019; [37]). The pH in the top soil layer (0−30 cm depth) was 6.8 (0.01 *M* CaCl$_2$). C$_{org}$ and N$_t$ content of the A$_p$ horizon was 0.72% and 0.10%. At the beginning of the experimental period, the mean initial mineral N (N$_{min}$ = ammonium-N + nitrate-N) content (0–60 cm soil depth) over the whole experimental area was 44 and 21 kg N ha^{-1} in the 1st and 2nd year, respectively.

2.2. Experimental Design and Crop Management

In the 1st year (28 April 2015–19 April 2016) and the 2nd experimental year (28 April 2016 and 3 May 2017) data collections were conducted on two adjacent subareas of a field that had been uniformly managed before. In both years, preceding crop was winter wheat (*Triticum aestivum* L.) followed by fallow until maize seeding. We established a fully randomized block experiment in both years with four replicates and a plot size of 10 m × 3 m. Treatments investigated are listed in Table 1.

Table 1. Abbreviation of the different treatments with N fertilizer form, application technique, nitrification inhibitor added (active substances), and sampling period.

Treatment		N Fertilizer	Application Technique	Nitrification Inhibitor	Sampling Period	
					1st Year	2nd Year
CON *		unfertilized	-	-	X	X
INC *		cattle slurry	trail hose + incorp.	-	X	X
INC	+DMPP&DMPSA	cattle slurry	trail hose + incorp.	3,4-dimethylpyrazole phosphate & 3,4-dimethylpyrazole succinic acid		X
INJ *		cattle slurry	injection	-	X	X
INJ	+DMPP	cattle slurry	injection	3,4-dimethylpyrazole phosphate	X	X
INJ	+DMPSA	cattle slurry	injection	3,4-dimethylpyrazole succinic acid	X	X
INJ	+DMPP&DMPSA	cattle slurry	injection	3,4-dimethylpyrazole phosphate & 3,4-dimethylpyrazole succinic acid	X	X
INJ	+nitrapyrin	cattle slurry	injection	Nitrapyrin	X	X
INJ	+DCD	cattle slurry	injection	dicyandiamide	X	X
INJ	+TZ&MP	cattle slurry	injection	1,2,4 triazole & 3-methylpyrazole	X	

* data from Herr et al. [19].

N$_2$O emissions from treatments INC and INJ have already been published by Herr et al. [19]. Except for the treatment INJ + TZ&MP (tested only in the 1st experimental year) and the treatment INC + DMPP&DMPSA (tested only in the 2nd experimental year), all remaining treatments were tested in both experimental years.

In both years, slurry stemmed from the same slurry pit from a nearby dairy farm. Storage in this pit lasted for approximately two months. The slurry pit was stirred for 1 h prior to removal for our experiment. Each slurry was analyzed for total N, NH_4^+-N, dry matter, and pH using common laboratory methods (Table S1). Total N of the slurry varied between 2.8 and 3.1 kg N m^{-3} and ammonium varied between 1.3 and 2.2 kg NH_4^+-N m^{-3}. Depending on treatment, slurry was surface applied with trail hose applicator for experimental plots with 3 m working width and incorporated with a disk harrow after 2 h (0.15 m depth) (INC) or directly injected with a self-constructed INJ cultivator placing the slurry in 0.15 m soil depth (share width 0.05 m) (INJ). The space between trail hoses or injector tines was 0.75 m.

Before fertilization, a homogenous mixture of the particular NI and 0.8 m^3 slurry was ensured by mixing both components for 15 min with a rotary piston pump with cutter (Vogelsang, R116-60S, pump capacity 0.63 m^3 min^{-1}).

Based on measured total N, we applied 170 kg N ha^{-1} in all fertilized treatments in the 1st year. This corresponded to the maximum amount of organic N-fertilizer permitted by the German legislation on N-fertilization [38]. To ensure a better compatibility between the two experimental years, we applied 190 kg total N ha^{-1} due to the lower initial N_{min} content in soil in the 2nd year.

In the 1st experimental year, cattle slurry was applied in two doses. At the 1st fertilization (10 May), approximately 80% of the total N was applied. The 2nd cattle slurry dose (18 June) was surface applied by trail hose instead of INJ or INC in order to avoid damages of the growing maize plants in 2015. NIs were only applied with the 1st N slurry dose. In the 2nd experimental year, slurry was completely applied in one dose (3 May).

Maize (variety "Amadeo") was sown in both years (100.000 plants ha^{-1}) with a sowing depth of 0.05 m and a row distance of 0.75 m. In 2015, maize was sown on 13 May and harvested on 26 August as silage maize. In 2016, maize was sown on 4 May and harvested for silage biomass on 13 September. In both years, the seedbed was prepared one week before sowing with a cultivator. After harvests, the remaining maize stubbles were mulched and subsequently incorporated with a cultivator. During the rest of the study period, the field lay fallow without any further soil cultivation activity and crop management practice.

2.3. Nitrification Inhibitor Application Rates

According to the manufacturer specification, we applied 6 L ha^{-1} ENTEC® FL [39]. Despite that the manufacturer recommends a lower rate (4 L ha^{-1}) for slurry INJ compared to broadcast INC (6 L ha^{-1}), we used the same amount for both application techniques in order to ensure directly comparable rates of DMP active ingredient applied to both treatments. This was justified by Ruser and Schulz [21] indicating that increasing active inhibiting compound concentrations above half of the recommended concentration did not affect cumulative N_2O emission. For the treatment with DMPSA, we applied equal rates of DMP as in the treatment with 3,4-DMPP. For the treatment with combined application of DMPP and DMPSA, half of the recommended rate of each NI was added to the slurry. As recommended by the manufacturers, we applied 2.5 L ha^{-1} of N-LOCK™ [40], 6 L ha^{-1} of PIADIN® [41], and 102.3 g m^{-3} slurry of DCD [42].

2.4. Trace Gas Sampling and Flux Calculation

During the two experimental years, N_2O, carbon dioxide (CO_2), and methane (CH_4) fluxes were measured at least weekly using the closed chamber method [43]. In total, 114 samplings were conducted within the two experimental years. One base frame (0.15 m height, 0.3 m inner diameter) per plot was inserted 0.1 m deep in the soil between the maize rows capturing an injection band area-representative; 7% of the base area covered the injection slot and 93% covered noninjection slot area [44]. We used dark, vented PVC chambers. During sampling, which was usually conducted in the morning, we periodically took four gas samples from each chamber's atmosphere using a syringe and transferred

the sample into evacuated glass vials (Exetainer, Labco Limited, Lampeter, UK). For more details of the gas sampling procedure see Pfab et al. [45].

Trace gas concentrations in the gas samples were measured with a gas chromatograph equipped with an electron capture detector (Ni^{63} ECD) for the determination of the N_2O and CO_2 and with a flame ionization detector for the measurement of the CH_4 (GC 450 Greenhouse Gas Analyzer, Bruker Daltonic, Bremen, Germany). Trace gas fluxes were calculated using the package "gasfluxes" [46] for R software [47]. Based on the Akaike information criterion (AIC) and the kappa value, the package selects the most suitable model for calculating trace gas fluxes (linear model, nonlinear HMR model [48], or robust linear regression model [49]). More details for the calculation of the fluxes was given by Ruser et al. [50]. Results from the CH_4 fluxes will be presented in a separate publication, but we here included the cumulative flux for the calculation of the GHG balance. Cumulative N_2O emissions were calculated stepwise assuming constant N_2O fluxes between two sampling dates.

The N_2O emission factor (EF) related to total N applied was calculated as described by Velthof and Mosquera [51]:

$$EF = (N_2O\text{-}N_{fertilized} - N_2O\text{-}N_{CON}) \text{ total } N_{fert}^{-1} \times 100 \tag{1}$$

with EF: emission factor of total N applied [%]; N_2O-N emission is the mean cumulative direct N_2O-N emission of the particular experimental year either from the treatments fertilized (N_2O-$N_{fertilized}$) or from the unfertilized control (N_2O-N_{CON}) [kg N_2O-N ha^{-1} year^{-1}], and total N_{fert} is the amount of total N-fertilizer applied as cattle slurry [kg N ha^{-1} year^{-1}].

2.5. Weather Data, Soil and Plant Sampling, and Laboratory Analyses

Weather data were provided from the University's meteorological station, which was located 500 m from the study site. Soil temperature in a depth of 0.05, 0.1, and 0.15 m was measured during the whole observation period in each block separately ($n = 4$) using data loggers (Logtag, TRIX-8, CIK solutions, Karlsruhe, Germany).

For the calculation of the fertilizer demand, we sampled soil in every single plot. We took six samples per plot, homogenized them, and determined N_{min} in 0–0.3, 0.3–0.6, and 0.6–0.9 m depth before sowing.

Simultaneously to each gas sampling, soil sampling in the A_p horizon (0–0.3 m) was conducted. We took three samples in every plot and pooled these samples over the four replicates. During the two cropping seasons, we increased spatial sampling in the plots with slurry INJ. We took samples directly over the INJ slots (representative for 7% of the whole field) and separate samples between the INJ slots (representative for 93% of the whole field). Because data from the INJ slots correlated considerably better with the N_2O flux rates than the data from the area between the slots, we show the NO_3^- and NH_4^+-contents from the slot area in Figures 1 and 2.

All samples were stored frozen and gently thawed at 4 °C in a refrigerator before analysis. We extracted 20 g of field moist soil with 80 mL of 0.5 M K_2SO_4 solution. NO_3^- and NH_4^+ concentrations in the filtrates were measured using flow injection analysis (3 QUAAtro, SEAL Analytical, Norderstedt, Germany). Another aliquot of the soil was used to determine gravimetrical soil moisture after drying at 105 °C. Water-filled pore space (WFPS) was calculated assuming a mean bulk density of 1.3 Mg m^{-3} in the A_p horizon (0–0.3 m depth) according to Equation (2).

$$WFPS = \text{gravimetric soil moisture} \times \text{soil bulk density} \times \text{total porosity}^{-1} \tag{2}$$

with soil porosity calculated as

$$\text{Soil porosity} = 1 - \text{soil bulk density} \times 2.65^{-1} \tag{3}$$

where 2.65 Mg m^{-3} (particle density of quartz) was the assumed particle density of the soil.

In both years, fresh matter (FM) yield was measured in the two central maize rows of each plot with a maize chopper during harvest. An aliquot amount of each plot was dried and used for DM yield calculation. Additionally, C and N content of plant samples was measured with a C/N analyzer (Vario MAX CN, Elementar Analysensysteme, Hanau, Germany) to calculate biomass N removal.

2.6. Calculation of CO_2 Equivalents

To enable a comparable evaluation between different cattle slurry application techniques (INC, INJ near Stuttgart, Germany) with and without NI as well as the NI effectiveness in reducing atmospheric burden, we summarized gaseous losses (N_2O, CH_4, and NH_3), NO_3^- leaching, and fuel consumption after conversion into CO_2 equivalents (CO_{2e}) on field base. NH_3 losses were taken from Herr et al. [19] for the INC and INJ treatments and based on model calculations in the 1st year and on measurements in the 2nd year. Usage of modeled data for the 1st year was justified by a high statistically significant correlation between modeled and measured data in the 2nd year ($r^2 = 0.91$, data not shown). We assumed that NH_3 fluxes were not affected by NI addition due to injection.

It was assumed that soil organic carbon stocks remained unchanged and therefore the SOC component was not taken into consideration for the GHG balance. Emissions related to other agricultural inputs, such as pesticides and seeds, were not included in the analysis as these were considered to be negligible [52]. Based on the low amounts of active compound applied, this was also assumed for the NIs. The calculation of CO_2 equivalents according to the IPCC [53] approach is outlined in depth by Herr et al. [19]. In contrast, we used the default values of the IPCC [54] refinement, and therefore the following values have changed: EF_5 (emission factor for N_2O emissions from leaching): 0.011, and $Frac_{LEACH}$ (fraction of N added that is lost through leaching): 0.24. All other values remained the same.

To evaluate the contribution of NH_3 emissions on total GHG release, we used the same NH_3 emissions as already previously reported by Herr et al. [19] for the INC and INJ treatment.

Because NIs may also decrease NO_3^- leaching after the application of organic fertilizers we tried to consider this effect by modifying the IPCC values. In accordance with the results of Quemada et al. [55], we calculated 24% lower NO_3^- leaching for the treatments with NI.

2.7. Statistical Analyses

Statistical analyses were carried out using the Statistical Software package SigmaStat 3.5. Data were tested for variance homogeneity. Normal distribution of residuals was tested with the Shapiro Wilk Test. The dataset from total and yield-related CO_2 equivalents from the 1st experimental year did not follow a normal distribution, thus a Kruskal-Wallis One Way ANOVA on Ranks was chosen for statistical analysis. The annual N_2O emission from the 1st experimental year were log_{10}-transformed to fulfill variance homogeneity. Remaining data showed a variance homogeneity without any transformation. A two-factorial analysis of variance (ANOVA) was run with the factors block and treatment, to test the block effect in each experimental year.

Because of the differences between the two experimental years (e.g., precipitation in the period after fertilization and abundance of fertilization), a one factorial ANOVA was performed separately for each experimental year, to detect differences between the treatments concerning yield, N removal, and cumulative emissions. Significant differences were determined using a pairwise multiple comparison procedure (Student-Newman-Keuls, $p < 0.05$). The data are presented as arithmetic means with standard errors.

To explore relationships between trace gas fluxes and soil variables we calculated Pearson Rank Sum correlations over all treatments for each year separately.

3. Results

3.1. Weather Conditions

Annual precipitation in the 1st experimental year (May 2015 to April 2016) was 518 mm. This was 22% lower than the long-term mean rainfall. During the cropping season (10 May until 26 August), rainfall was low (170 mm; Figure 1f). More specifically, precipitation was minimal immediately after the first N application between May and July. This dry period was interrupted by a heavy rainfall event with 27 mm d^{-1} on 8 June. The air temperature in the 1st year was 0.8 °C higher than the mean temperature. The mean air temperature during the first four weeks after slurry application (10 May to 28 May) was 21.8 °C. In contrast to earlier years when frost can be experienced during 74 days, only eight frost days occurred over winter of the 1st experimental year.

Annual precipitation in the 2nd year (May 2016 to April 2017) was 557 mm, which was 16% lower than the long-term mean. Precipitation during the cropping season (4 May until 13 September) was 262 mm. In the two months following slurry application (May and June), precipitation was 190 mm. Cumulative rainfall was 33% higher in the 2nd year than in the 1st year. Rainfall events after slurry application in the 2nd year also occurred more frequently. With 9.9 °C, air temperature was only slightly (0.2 °C) below the mean air temperature of this site. With 14.9 °C, mean air temperature during the first four weeks after slurry application (4 May to 31 May) was 6.9 °C lower than in the corresponding period of the 1st year. In the winter of the 2nd year, 41 frost days occurred with only one longer permanent frost period between 15th and 29th of January.

3.2. N_2O Fluxes and Drivers

Over both two experimental years, N_2O flux rates showed large emission pulses after slurry application (Figures 1a and 2a). High N_2O fluxes occurred in conjunction with rainfall events and a high NO_3^- availability.

In the 1st year, mean N_2O flux rates increased already one day after the first application of the slurry. The fluxes in this period varied between 1800 and 3800 µg N_2O-N m^{-2} h^{-1} without any statistically significant difference between all treatments. After the rewetting of the dry soil in June, highest mean flux rate of the entire data set was measured with 6720 µg N_2O-N m^{-2} h^{-1} in the INJ treatment without NI. The corresponding mean flux rates of the INJ treatments with NIs were lower at this sampling date. When compared to the first slurry application, the response of the N_2O flux rates to the second N dose was lower. Throughout the rest of the 1st experimental year, N_2O flux rates remained low.

Similarly to the 1st year, N_2O fluxes in the 2nd year strongly increased after slurry application and rainfall events during approximately four weeks after N-fertilization. Again, the addition of NIs reduced the magnitude of the corresponding flux rates. With approximately 10 µg N_2O-N m^{-2} h^{-1}, postharvest N_2O fluxes remained mainly low. Only one frost/thaw event in January 2017 resulted in slightly increased flux rates of up to 155 µg N_2O-N m^{-2} h^{-1} (INJ).

In both years, soil CO_2 flux showed the highest correlation coefficient with the N_2O fluxes (Spearman Rank Order coefficient, $p < 0.001$; 0.79 in the 1st and 0.66 in the 2nd year, Table S2). Soil temperature (0.51 and 0.47), NO_3^- concentrations (0.49 and 0.25), NH_4^+ concentrations (0.44 and 0.21), and soil moisture in the 1st year (0.13) were also significantly correlated with the N_2O flux rates. In both years, we further found a relationship between N_2O flux rates and the amount of precipitation between two sampling dates (0.15 and 0.19). We also calculated Spearman Rank Order coefficients for each single treatment in every year. This did not change the results above since the differences between the single treatments were only marginal.
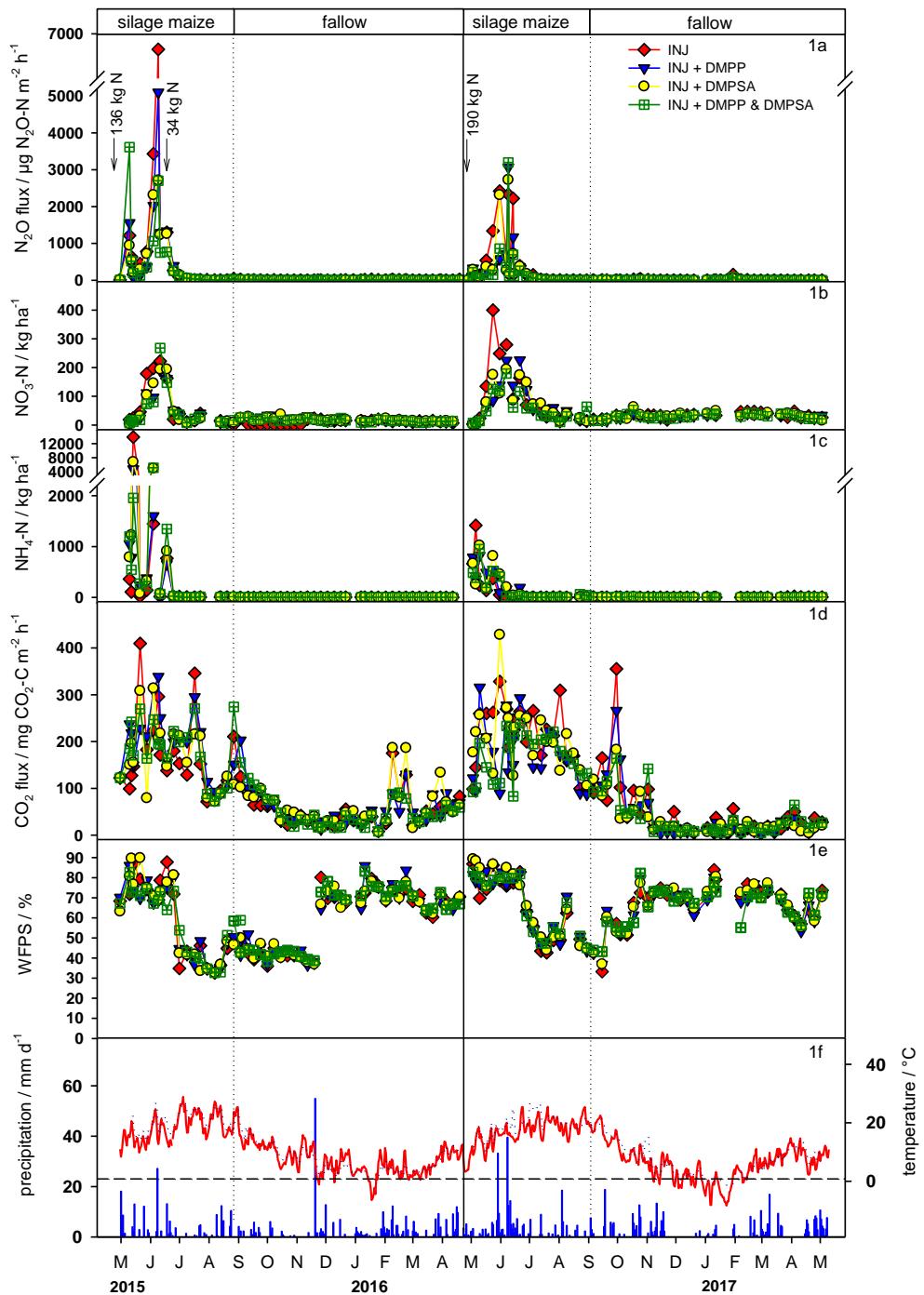
Figure 1. Mean N_2O flux rates ($n = 4$) (**a**), NO_3^--N contents (0−30 cm) (**b**), NH_4^+-N contents (0−30 cm) (**c**), mean CO_2 soil flux rates ($n = 4$) (**d**), and water-filled pore space (WFPS, 0−30 cm) (**e**) in the slurry injection treatment without NI (INJ), and in the treatments with DMPP (INJ + DMPP), with DMPSA (INJ + DMPSA), and with the combination of DMPP&DMPSA (INJ + DMPP&DMPSA). Mean air temperature (solid line), soil temperature in 0.1 m depth (dotted line), and daily precipitation (bars) (**f**). Arrows indicate fertilization measures. Error bars were omitted for clarity. Data from the INJ treatment were taken from Herr et al. [19]. During the cropping seasons, NO_3^--N and NH_4^+-N contents are shown for the slot area.
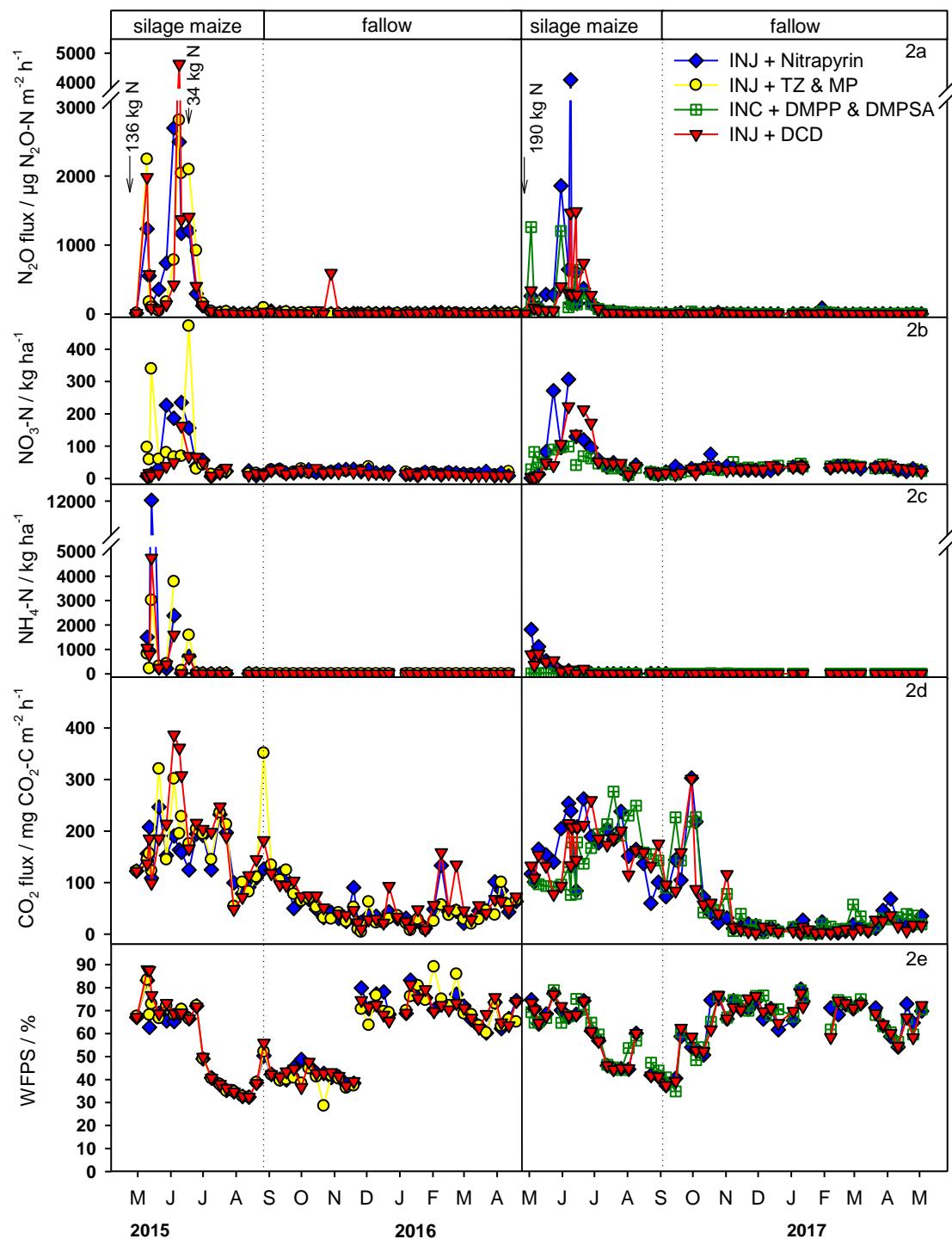
Figure 2. Mean N_2O flux rates (*n* = 4) (**a**), NO_3^--N contents (0−30 cm) (**b**), NH_4^+-N contents (0−30 cm) (**c**), mean CO_2 soil flux rates (*n* = 4) (**d**), and water-filled pore space (WFPS, 0−30 cm) (**e**) in the slurry injection treatments with nitrapyrin (INJ + nitrapyrin), DCD (INJ + DCD), TZ&MP (INJ + TZ&MP), and the slurry incorporation treatment (INC) with the combination of DMPP&DMPSA (INC + DMPP&DMPSA). Arrows indicate fertilization measures treatment. Error bars were omitted for clarity. During the cropping seasons, NO_3^--N and NH_4^+-N contents of the treatments with injection are shown for the slot area.

Immediately after slurry applications, the NH_4^+ contents, in particular in the INJ slot area, were strongly increased (Figures 1c and 2c). NH_4^+ oxidation increased noticeable shortly after slurry

application and resulted in a higher NO_3^- availability for a period between 45 days (1st year) and 61 days (2nd year) (Figures 1b and 2b). Treatments with NIs showed lower NO_3^- contents and a retarded increase of the NO_3^- availability when compared to the treatments without NI (INJ and INC). Following four weeks after slurry application in the 2nd year, the NO_3^- contents in the INJ treatments with NIs (mean over all NI treatments) was 23% lower when compared to INJ without NI.

3.3. Annual N_2O Emission and EFs

As shown in Table 2, the mean annual N_2O emission in the unfertilized control was in both years in the statistical group with the lowest cumulative emissions.

In the 1st year, emissions from the treatments with INJ ranged between 9.6 kg N_2O-N ha^{-1} year^{-1} (INJ + DMPP&DMPSA) and 16.2 kg N_2O-N ha^{-1} year^{-1} (INJ). Although not statistically significant, the mean cumulative N_2O emission from slurry INJ in the 1st year was lower when a NI was applied. Within three weeks between 28 May and 18 June, cumulative N_2O emissions in the INJ treatments accounted for between 41.7 and 66.4% of the total annual N_2O emissions. The mean NO_3^- concentrations in this period were all above 10 mg NO_3^--N kg^{-1} (not shown).

Furthermore, in the 2nd year, highest N_2O emission was measured in the INJ treatment without NI. All tested NIs reduced the annual N_2O emission of the INJ treatment. The reduction ranged between 27% and 62% without significant differences between the NIs. In contrast to the INJ treatments, application of DMPP&DMPSA did not reduce the N_2O emission from the INC treatment.

EF varied between 0.0 and 8.4% with higher EFs in the 1st year (Table 2). In the 2nd year, application of NIs decreased the EFs by more than 50% when compared to the EF in the INJ treatment without NI.

EFs for all treatments with incorporation (INC 1st and 2nd year and INC + DMPP&DMPSA 2nd year) and for the injection treatments INJ + DMPP, INJ + DMPP&DMPSA, and INJ + DCD in the 2nd year were within the range of uncertainty (0.1–1.8%) provided by the IPCC [54], whereas all other treatments had EFs above this range.

Table 2. Mean cumulative direct N_2O emissions (*n* = 4) as affected by NI addition and application technique (injection (INJ), incorporation (INC)). The emission factors (EF) value indicates mean direct N_2O emission. Different letters indicate statistically significant differences between groups within the same year (*Student-Newman-Keuls test*, $p < 0.05$). Direct N_2O emission from unfertilized control (CON) indicate background emissions. The data from CON, INJ, and INC treatment were taken from Herr et al. [19]. For the treatment abbreviations we refer to Table 1.

Treatment	N_2O Emission [kg N_2O-N ha^{-1} yr^{-1}]			EF [% of N Applied]		
	1st Year	2nd Year	Mean	1st Year	2nd Year	Mean
CON	2.3 [b]	3.3 [c]	2.8			
INC	2.1 [b]	6.7 [b,c]	4.4	0.0	1.8	0.9
INC + DMPP&DMPSA	n.d.	5.4 [b,c]	5.4	n.d.	1.1	1.1
INJ	16.2 [a]	11.5 [a]	13.9	8.4	4.4	6.4
INJ + DMPP	12.8 [a]	5.5 [b,c]	9.2	6.3	1.2	3.8
INJ + DMPSA	12.4 [a]	8.4 [b]	10.4	6.1	2.7	4.4
INJ + DMPP&DMPSA	9.6 [a]	4.9 [b,c]	7.3	4.4	0.9	2.7
INJ + nitrapyrin	12.8 [a]	7.9 [b]	10.4	6.3	2.4	4.4
INJ + DCD	11.0 [a]	4.4 [b,c]	7.7	5.2	0.6	2.9
INJ + TZ&MP	13.4 [a]	n.d.	13.4	6.7	n.d.	6.7

n.d. not determined.

3.4. Maize Yield and N Removal

The maize yield ranged between 33.9 and 49.8 Mg FM ha^{-1} year^{-1} (not shown). These yields are well in accordance with the mean silage maize yields in German agriculture, which was 42.6 Mg FM ha^{-1} year^{-1} between 2010 and 2019 (range: 35.3 to 47.6 Mg FM ha^{-1} year^{-1}; [56]).

Dry matter contents and N removal did not differ between the years (Table S3). Among all N-fertilized treatments, they were neither significantly affected by application technique nor by the use of a NI. However, in both years, mean dry matter and mean N removal in the INC treatments tended to be lower than in the INJ treatments.

3.5. CO$_2$ Footprint

Area-related greenhouse gas (GHG) release varied between 1298 kg CO$_2$ equivalents (CO$_2$e) ha^{-1} year^{-1} (unfertilized control, 1st year) and 8151 kg CO$_2$e ha^{-1} year^{-1} (INJ, 1st year) (Table 3). In both experimental years, the INJ treatment without NI had the highest GHG release. Cumulative direct annual N$_2$O emissions accounted for 78% to 93% of the total GHG emission. Due to this high share of the direct N$_2$O emission, area-related GHG emissions followed the same order as the mean direct N$_2$O emission: INJ > INJ + NI > INC > INC + NI.

Table 3. Mean greenhouse gas (GHG) emissions as affected by treatment (slurry injection (INJ) without NI (nitrification inhibitor) and with DMPP (INJ + DMPP), DMPSA (INJ + DMPSA), with the combination of DMPP&DMPSA (INJ + DMPP&DMPSA), with nitrapyrin (INJ + nitrapyrin), with DCD (INJ + DCD), with TZ&MP (INJ + TZ&MP) and the slurry incorporation treatment (INC) with the combination of DMPP&DMPSA (INC + DMPP&DMPSA)) and experimental year as CO_2 equivalents (CO_2e) calculated from field-related direct N_2O, CH_4, and NH_3 emissions, NO_3^- leaching, and fuel consumption. Different superscript letters indicate statistically significant differences between treatments within the same year and column (Kruskal-Wallis ANOVA on Ranks for dataset from 1st and Student-Newman-Keuls test for dataset from 2nd year, $p < 0.05$, $n = 4$).

Year	Treatment	Percentage of the Total CO_2 Equivalents					Area-Related CO_2e	Yield-Related CO_2e
		N_2O Emissions	NH_3 Emissions	CH_4 Emissions	NO_3^- Leaching	Fuel Consumption	[kg ha^{-1} year^{-1}]	[kg Mg^{-1} DM^{-1}]
1st	INJ	90.9	0.5	2.1	3.1	3.5	8362 [a]	585 [a]
	INJ + DMPP	89.8	0.7	1.4	3.8	4.3	6668 [a]	574 [a]
	INJ + DMPSA	89.6	0.7	1.3	4.0	4.5	6481 [a]	459 [a]
	INJ + DMPP&DMPSA	86.8	0.9	1.8	4.9	5.6	5194 [a]	348 [a]
	INJ + nitrapyrin	89.9	0.7	1.2	3.9	4.3	6680 [a]	436 [a]
	INJ + DCD	88.5	0.8	1.3	4.4	5.0	5814 [a]	394 [a]
	INJ + TZ&MP	90.3	0.7	1.2	3.7	4.2	6955 [a]	514 [a]
2nd	INJ	89.4	1.0	0.3	4.7	4.6	6005 [A]	395 [A]
	INJ + DMPP	82.0	1.9	0.5	6.8	8.9	3138 [B]	206 [B]
	INJ + DMPSA	87.4	1.3	0.3	4.8	6.2	4491 [AB]	283 [AB]
	INJ + DMPP&DMPSA	80.5	2.1	0.2	7.5	9.8	2851 [B]	182 [B]
	INJ + nitrapyrin	86.8	1.4	0.2	5.0	6.6	4235 [AB]	279 [AB]
	INJ + DCD	80.7	2.0	0.4	7.3	9.5	2919 [B]	198 [B]
	INC + DMPP&DMPSA	83.7	1.9	−0.9	6.8	8.4	3029 [B]	239 [B]

Fuel consumption, NO_3^- leaching, and CH_4 as well as NH_3 losses contributed much smaller proportions to the atmospheric burden. The mean contribution of these components to the total area-related GHG emission was 6.9%, 5.9%, 0.6%, and 1.4%, respectively (mean over both years and all treatments).

Yield-related GHG emission varied between 107 kg CO_2e Mg^{-1} DM^{-1} (unfertilized control, 1st year) and 570 kg CO_2e Mg^{-1} DM^{-1} (INJ, 1st year) (Table 3). In agreement with the area-related GHG emission, yield-related emission was in both years highest in the INJ treatment without NI. Lowest yield-related emission within every experimental year was found for the unfertilized control treatment.

In the 1st year, the use of NIs did not significantly improve the CO_2 footprint for silage maize production (Table 3). In the 2nd year, the GHG emission from all NI treatments efficiently reduced GHG release when compared to the INJ treatment without NI. The same GHG reduction was observed for the yield-related emissions in the 2nd year, although the differences between the mean emissions of some NI treatments (INJ + DMPSA and INJ + nitrapyrin) was not significant when compared to the INJ treatment without NI.

4. Discussion

4.1. Effect of Environmental Condition and NIs on N_2O Flux Rates

High N_2O flux rates in our study were measured for approximately two months following slurry application. Many studies also reported high N_2O flux rates after the application of slurry (e.g., [51,57]). Microbial respiration of the easily available C provided with the slurry and intense oxidation of the NH_4^+ to NO_3^- during nitrification result in a high O_2 consumption, which enhances O_2 depletion and thus the development of anaerobic conditions [18,58]. These conditions were more pronounced in the INJ treatment and they promoted stronger denitrifying conditions and N_2O release than in the broadcast incorporated slurry treatment [13,19,51].

The positive correlation of the N_2O flux rates with the CO_2 flux rates, NO_3^- concentrations and increasing rainfall amounts between two sampling dates clearly hint on denitrification as the main N_2O source. This also explains the increase of the N_2O flux rates after rainfall in the fertilization periods. O_2 diffusion is approximately factor 10^4 lower in soil water than in soil air [59] and therefore increases in soil moisture fuel the development of anaerobic conditions in periods with high microbial O_2 consumption through constraining atmospheric O_2 diffusion into the soil, thus enhancing N_2O production and release from denitrification.

NH_4^+ concentrations were also significantly correlated with the N_2O flux rates and consequently nitrification might also have contributed quantitatively to N_2O fluxes but high N_2O pulses also occurred in times were NH_4^+ contents were already back on baseline level.

The highest N_2O flux rates of the entire study were measured on 8 June in the 1st year one day after heavy rainfall rewetted dry soil. N_2O pulses after rewetting of dry soil have been often reported e.g., [60–62]. Easily available C enriches in soils during drying thus also serving as substrate for C-heterotrophic microorganisms after rewetting [63]. As summarized by Lundquist et al. [64], several processes may contribute to increased C availability after drying–rewetting: (i) reduced microbial decomposition in dry periods, (ii) enhanced turnover of microbial biomass, and (iii) release of available C by the disruption of soil aggregates. The high C availability (from slurry and air-dry soil) was indicated through the strong increase in soil CO_2 flux at this date. Concurrent high C and NO_3^- contents at this date again suggest denitrification as main N_2O source during rewetting of dry soil. As shown by Ruser et al. [62], rewetting resulted in a strong shift towards denitrification as main N_2O source when compared to constant soil moisture, even under dry conditions. The high WFPS at this day (>70% WFPS) also suggests denitrification as main N_2O source after rewetting [65].

In the 1st year, N_2O fluxes from the treatments with INJ did not differ immediately after slurry application. In agreement with Comfort et al. [17], we assume that the first N_2O peak occurring one day after slurry application in the 1st year can be attributed to a reduction of soil derived

NO_3^- due to increased soil moisture through slurry amendment and intense O_2 depletion. Nitrate contents at this date ranged between 40 and 50 kg N ha^{-1}, indicating high substrate availability for denitrification. Similarly, high moisture of up to 85% WFPS fueled the onset of anaerobic conditions favoring denitrification. Because NO_3^- content was the same in all treatments, differences between the injection treatments could not be expected. The lower N_2O peaks in the 2nd year with lower initial N_{min} level underline this assumption.

As reported by Dittert et al. [33] and by Vallejo et al. [32], the addition of NIs delay NH_4^+ conversion and result in lower NO_3^- concentrations and thus in lower N_2O fluxes over a certain period after fertilization. In our study, the duration of this period of inhibition differed between the two experimental years, it lasted approximately 45 days in the 1st and 60 days in the 2nd year.

4.2. Effect of Environmental Conditions and NIs on Nnnual N_2O Emission and Corresponding EFs

When compared to the 2nd year, annual N_2O emissions in the INJ treatments of the 1st year were distinctively higher. Because all other statistically significant soil parameters, e.g., NO_3^-, NH_4^+, or soil moisture did not vary between the two experimental years in this crucial period, we assume that the higher soil temperature after slurry application in the 1st year was the main reason for the higher N_2O emission.

Though not statistically significant, application of NIs reduced the mean annual N_2O emission from the INJ treatment by 26% in the 1st year (calculated as mean over all NIs). In the 2nd year, the use of NIs resulted in a significant reduction of the emission after INJ by 46%. Reduction in the same order was frequently reported for the application of NIs together with mineral N-fertilizers or urine [20,21] and for application together with organic fertilizers such as slurry in field studies [66,67] or in laboratory investigations [68].

In the three weeks between 21 May and 11 June of the 1st year, 66% of the annual N_2O emission in the INJ treatment was released, indicating the importance of this period for the whole annual N_2O budget. Although the NO_3^- contents in the INJ treatments with NI were slightly lower, the difference was obviously too small to have a statistically significant effect. The corresponding NO_3^- concentrations (>10 mg NO_3^--N kg^{-1}) were in the upper third of the range of NO_3^- concentrations that yield in maximum denitrification rates in agricultural soils (summarized by Granli and Bøckman [69]), proving that in none of the NI treatments, NO_3^- availability was limiting for denitrification in this period. Consequently, high NO_3^- and C availability even in the treatments with NI might have been the main reason for nonsignificant differences in N_2O emission when compared to INJ without NI in the 1st year.

In contrast, NIs significantly reduced N_2O emissions in the INJ treatments in the 2nd year. As indicated by the lower NO_3^- concentration in the NI treatments, NIs reduced substrate availability for denitrification because the first step of nitrification (oxidation of NH_4^+ to NO_3^-) was inhibited.

When compared to the 2nd year, the shorter inhibitory effect of all NIs in the 1st year could be explained by a faster degradation of the active compounds due to a higher mean soil temperature. This observation is in agreement with numerous studies, which detected a decreasing persistence of NIs with increasing soil temperatures [70–76].

In contrast to the INJ treatments, NI application in the treatment with INC did not reduce N_2O emission in the 2nd year. Since the N_2O emission from the treatment INC did not differ from the emission from the unfertilized control, the effect of NIs could not be expected. A high soil slurry interaction of this application technique may result in a high N immobilization through broadcast slurry fertilization [77]. The low N availability of the INC treatment in the 1st year was underlined through the low DM yield in this experimental year (not different from unfertilized control treatment; Table S3). Moreover, harrowing increased soil aeration thus reducing anaerobic conditions for denitrification. Further, when compared to injection, incorporation with a harrow distributed N more evenly decreased locally high substrate concentrations for N_2O hot spots.

Winter emissions did not significantly contribute to the annual N_2O release. Pfab et al. [45] and Seiz et al. [78] reported a portion of the winter emissions to the total annual emission between 20 and 95% from vegetable fields, and Guzman-Bustamante et al. [79] between 12 and 21% for winter wheat fields. All these measurements were conducted earlier at our study site (in maximum 750 m away from our plot experiment). In contrast to the conditions of these studies, both winters during our experiment were mild. It has been often shown that frost/thaw induced N_2O pulses increase with increasing duration of frost periods and with intensity of the soil freezing [80–82]. In the winter of the 1st year, only eight frost days were recorded. Obviously the conditions were too mild for the induction of frost/thaw related N_2O emissions. Although 41 frost days occurred in the 2nd year, many of these frost days were not continuous. The only small increased thaw related N_2O flux peak was measured after the longest continuous frost period in the second half of January with 14 days of frost.

In both experimental years, there were no differences between N_2O emissions from all NIs tested. The reason for the similar N_2O emissions in the DMPP, DMPSA, and the mixture of both inhibitors (DMPP&DMPSA) may be that they all consist of the same active inhibiting compound (DMP). It has been previously shown for mineral N fertilizers, that the inhibition after DMPSA application was delayed when compared to DMPP because the succinic acid group must be degraded prior to the activation of inhibition [71,83]. For the combination of DMPP and DMPSA (INJ+DMPP&DMPSA), we therefore expected a longer period of combined inhibition. Although NO_3^- contents in the INJ+DMPP&DMPSA tended to increase with a slight delay, this did not affect N_2O emission.

Comparing DCD with DMPP, Weiske et al. [84] reported a lower N_2O reduction efficiency of DCD. In agreement with Zerulla et al. [76], they explained this phenomenon with the higher water solubility of DCD. In contrast, Marsden et al. [85] reported an even higher sorption of DCD when compared to DMPP. The low amount of rainfall preventing leaching loss as well as a similar adsorption behavior might explain the comparable efficiency of DCD and DMPP in our study. However, the DCD treatment showed the highest variability of N_2O emissions of all treatments in the 1st year (not shown), suggesting less consistency in inhibiting nitrification under the warm conditions.

Zerulla et al. [76] mentioned the high vapor pressure and the resulting volatility of nitrapyrin as a disadvantage for NIs. Capsulation of nitrapyrin in N-Lock® reduces volatilization of the active compound. The same inhibitory efficiency as for the other NIs proved that the approach with capsulation was successful.

Although several studies confirmed the reduction of N_2O emissions after application of urea or biogas digestate with Piadin® [86,87], we did not find any reference where N_2O emissions after application of this product were tested against another NI. 3-methylpyrazole (3-MP) is one of the both active compounds and it seems that the effect of 3-MP is similar to the effect of 3,4-dimethylpyrazol in DMPP.

All N_2O EFs in the treatments with injection were considerably high. With 8.4% (1st year) and 4.4% (2nd year), the EF for slurry INJ was 8- and 4-times higher, respectively, than the IPCC default value (EF$_1$ for N additions from synthetic fertilizers, organic amendments and crop residues) of 0.01 kg N_2O-N (kg N)$^{-1}$ [54]. The IPCC [54] default does not account for different application techniques for organic fertilizers. The mean EF of the INC treatment without NI over both years was 0.9 and thus in good agreement with the IPCC default. The difference between the EFs of the INJ and the INC treatment clearly shows, that the high EF in the INJ treatment was a result of the injection technique and not a general problem of organic fertilization. It seems undoubted, that the high N_2O emissions after slurry injection are mainly the result of enhanced denitrification (e.g., [18,88]), and consequently, slurry injection may be a suitable technique for NH_3 mitigation at production sites with a low denitrification potential. Low EFs for organic fertilizers have been reported for the Mediterranean region with low precipitation [89], and consequently, Louro et al. [90] did not find any effect of slurry injection on N_2O emissions. On well aerated sandy soils, slurry injection might also help to improve NH_3 reduction with only a small increase in N_2O emission, but other factors like high humus contents may counteract N_2O mitigation [91]. Experiments at study sites with higher risk for intense denitrification came to the result

that NH_3 mitigation through injection must accept the trade-off of increased N_2O emissions [51,92] and thus also increased EFs. Charles et al. [93] ran a meta-analysis ending up in an EF of 1.21% for organic fertilizers from the group they called "high-risk group" including animal slurries. They also reported, that the EF from fine-textured soils was 2.8 times higher than from sandy soils and therefore, broadcast application with subsequent incorporation seems to be the environmentally friendlier technique for our soil with a finer soil texture [19].

Addition of NIs reduced N_2O EFs by approximately one-third (mean over all treatments and years). Thus, the enzymatic inhibition of NH_4^+ oxidation can be confirmed as an appropriate approach to mitigate N_2O release, particularly for slurry INJ in the 2nd year. Under the weather conditions in this year, which were more representative for our study site, the inhibitors DMPP, DMPP&DMPSA, and DCD performed a significant reduction of N_2O release after INJ with EFs near or below the implied IPCC [54] default. However, the differences in EFs between our two experimental years as well as results from previous studies [32,51,94,95] showed that the EFs for N_2O release after slurry application is not well quantified and illustrate the relevance of considering the use of NIs as well as application technique and environmental conditions to enable a reliable calculation of N_2O releases with higher accuracy. Consequently, an adaption of the currently used IPCC methodology would be desirable.

4.3. Impact of Different NIs on N Removal and Silage Maize Yield

Previous studies noted an improved yield and/or NUE through the addition of DMPP, DCD [96], nitrapyrin [97,98], or TZ&MP [99] to liquid manure due to reduced N losses. However, McCormick et al. [97] and Schmitt et al. [100] reported a variable yield response of maize plants after manure injection with NIs. They attributed the missing beneficial impact of NIs on yield response and N offtake to high levels of plant available N in soil or to high N application rate and environmental conditions unfavorable for N losses. In the present study, the addition of NIs did not increase DM yield or N removal in the slurry INJ treatments. Even an increased N fertilization rate by 50% from the INJ treatment (data not shown) did not increase yield response or N removal significantly in both years. Thus, in agreement with the results from McCormick et al. [97], it can be assumed that soil N was not the limiting factor for yield and N offtake and following an additional and prolonged N supply through NIs did not lead to an improvement. Additionally, it can be assumed that the risk of NO_3^- leaching losses on the medium-textured soil from our study site was low, and consequently, the beneficial effect of NIs through NH_4^+ conservation also remained small [101].

Only the broadcast INC treatment with the combination of DMPP&DMPSA tended to be inferior to all INJ treatments. Lower yield and N removal of slurry incorporation in comparison to injection was reported in several studies [99,100] and mainly explained with nutrient distribution less concentrated to maize roots and with a higher N immobilization rate [77].

4.4. Atmospheric Burden

Independent of the treatment, N_2O had always the highest share to the total GHG emission (between 80.5 and 90.9%). Such high portions of N_2O to the carbon footprint have also been reported from Huang et al. [102] for upland soils in China, where direct N_2O emissions accounted for 62% to 98% of the total carbon footprint of crop rotations including maize. Calculations on GHG release from maize fields that received mineral N fertilizer resulted in a share of total N_2O emission (direct and indirect emissions) around 50% [103]. Börjesson et al. [103] used the IPCC [53] default EF of 0.01 kg N_2O-N (kg N)$^{-1}$. Assuming higher factors as measured in our study for the calculation of the contribution of direct N_2O emissions to GHG would have resulted in N_2O portions in the same order of magnitudes.

Evaluating the use of NIs regarding atmospheric burden, total GHG release from slurry INJ could be reduced by 24% and 33% in the 1st and 2nd experimental year, respectively (mean over all treatments). In agreement with Smeets et al. [104], the use of NIs could improve the greenhouse gas balance by reducing N_2O emissions by 36% (mean over all treatments and years).

Neither N_2O nor total GHG was significantly reduced after slurry injection with NI in the 1st year with unusual weather conditions for our study site. In this year, broadcast application with subsequent INC of slurry was the method of choice. Under the more typical climate conditions in the 2nd year, slurry injection with NIs turned out to be a likewise appropriate fertilization strategy regarding atmospheric burden under climate conditions typical for our experimental region. Moreover, slurry injection has a lower risk of NH_3 losses [13], needs less manpower [105], and tends to have higher N removal as well as increased yields when compared to harrowing after broadcast application. Hence, this fertilization strategy allows a combination of protecting earths' climate by efficiently reducing N_2O and NH_3 losses and guarantees beneficial effects for farmers by avoiding extra costs for N fertilizers [7] and reducing working time requirement [105].

5. Conclusions

The mean N_2O emission in the INJ treatment without NIs was 8-fold higher than the emission calculated with the IPCC [54] default. Therefore, further annual N_2O measurements on the effect of slurry injection in soils with different texture and different climatic conditions are urgently needed to verify our results, particularly given the fact, that European Union countries have included slurry injection into the environmental law and regulation in order to meet the goals of the National Emission Ceilings Directive [106]. This also concerns the effect of NIs on the reduction of N_2O emissions, because even in the treatments with INJ + NI, N_2O emission was still substantially high. These additional measurements are of essential importance for the assessment of silage maize production because N_2O emission contributed more than 80% of the total GHG emission. Further measurements would also help to understand the different response of the N_2O emissions to NIs across years. However, all the assessed NIs behaved in general in the same way, and they are therefore all suitable for the reduction of N_2O losses from slurry application.

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