Article

Not peer-reviewed version

Short-Term Nitrous Oxide Emissions from Cattle Slurry for Silage Maize: Effects of Placement and the Nitrification Inhibitor 3,4-Dimethylpyrazole Phosphate (DMPP)

Arezoo Taghizadeh-Toosi * , Khagendra Raj Baral , Peter Sørensen , Søren O. Petersen

Posted Date: 12 September 2023

doi: 10.20944/preprints202309.0739.v1

Keywords: Slurry application method; silage maize; nitrous oxide emissions; mineral nitrogen; nitrification inhibitor

Article

Short-Term Nitrous Oxide Emissions from Cattle Slurry for Silage Maize: Effects of Placement and the Nitrification Inhibitor 3,4-dimethylpyrazole Phosphate (DMPP)

Arezoo Taghizadeh-Toosi [1,2,3,*], Khagendra Raj Baral [1,4], Peter Sørensen [1] and Søren Ole Petersen [1]

1 Department of Agroecology, Aarhus University, Tjele, Denmark
2 Danish Technological Institute, DTI, Agro Food Park 15, Skejby, 8200 Aarhus N, Denmark
3 Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Lancaster LA1 4AP, UK
4 Agri-Environment Branch, Agri-Food and Biosciences Institute, Belfast, Northern Ireland, UK
* Correspondence: aretag@ceh.ac.uk

Abstract: Cattle slurry is an important nitrogen source for maize on dairy farms. Slurry injection is an effective measure to reduce ammonia emissions after field application, but with higher risk of nitrous oxide emission than surface application. This study compared soil mineral nitrogen dynamics and nitrous oxide emissions with two ways of application. First, traditional injection at 25 cm spacing between rows followed by ploughing (called "non-placed slurry"), and second, injection using a new so-called goosefoot slurry injector that placed the slurry in ploughed soil as a c. 30 cm broad band at 10 cm depth below maize crop rows with 75 cm spacing (named "placed slurry"). Furthermore, the effect of treating slurry with the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in Vizura® was tested with both application methods. The field experiment was conducted on a sandy loam soil in a temperate climate. Both nitrous oxide emissions, and the dynamics of soil mineral nitrogen, were monitored for eight weeks after slurry application and seeding of maize using static chambers. The level of nitrous oxide emissions was higher with non-placed compared to placed slurry, mainly due to higher emissions during the first four weeks. That might be due to higher rates of nitrification rate and in turn stimulation of denitrification process. In both placed and non-placed slurry treatments, Vizura® caused higher soil ammonium concentrations and lower nitrate concentrations, particularly from 3 to 8 weeks after slurry application. The final level of soil nitrate was similar with and without the nitrification inhibitor, but higher with placed compared to non-placed slurry. Adding Vizura® to non-placed slurry reduced nitrous oxide emissions by 70 % when compared to placed slurry. Surprisingly, there was a non-significant trend towards higher cumulative emissions from placed slurry with the nitrification inhibitor compared to untreated slurry, which were due to higher emissions in the last part of the monitoring period (5-7 weeks after slurry application). Possibly degradation of the nitrification inhibitor, and nitrification activity inside the slurry band as the soil dried, promoted nitrous oxide emissions by this time. In summary, placement of untreated slurry in a broad band under maize seeds reduced nitrous oxide emissions compared to non-placed slurry with more soil contact. A comparable reduction was achieved by adding a nitrification inhibitor to non-placed slurry. The pattern of nitrous oxide emissions from placed slurry treated with the inhibitor was complex and requires more investigation.

Keywords: slurry application method; silage maize; nitrous oxide emissions; mineral nitrogen; nitrification inhibitor

1. Introduction

In agroecosystems, nitrogen (N) is an essential nutrient and a key limiting factor for the growth and develop¬ment of crops (Spiertz, 2010). Animal manure has been used as a source of N for centuries. Generally, the application of organic fertilizers increases the risk for environmental losses via ammonia volatilization, leaching, or emissions of other N gases including nitrous oxide (N_2O) emissions. Intensive dairy production is dominated by liquid manure management (Leip et al., 2009),

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 September 2023 doi:10.20944/preprints202309.0739.v1

and field application of liquid manure (slurry) involves risks for environmental impacts via ammonia volatilization, leaching, and N_2O emissions. Ammonia volatilization from slurry is effectively reduced by injection or immediate incorporation (Webb et al., 2010; Hafner et al., 2018). When cattle slurry is applied before seeding of spring crops such as maize, nitrate (NO_3^-) leaching is a risk depending mainly on soil type and rainfall during spring. For example, in a lysimeter study with maize on a coarse sandy soil, Nair et al. (2020) observed a 3- to 4-fold increase in NO_3^- leaching due to cattle slurry application when simulating high rainfall during spring. Nitrous oxide has a high global warming potential (GWP) of 265 with a 100-year time horizon and plays a central role in the depletion of stratospheric ozone (Ravishankara et al., 2009; Myhre et al., 2013). Both nitrification and denitrification can be potential sources of N_2O from agricultural soil, however, studies under controlled laboratory conditions (e.g., Zhu et al., 2013; Duan et al., 2017) as well as field conditions (Ibraim et al., 2020) have found that denitrification and nitrifier denitrification are the main sources of N_2O emissions.

Following field application, manure-saturated soil volumes result in the development of organic hotspots supporting nitrification and denitrification for several weeks (Petersen et al., 1992; Nielsen and Revsbech, 1994). With a high content of ammoniacal N, water, and easily degradable carbon (C), manure hotspots can become anoxic because of diffusional constraints and elevated oxygen (O_2) consumption rates for microbial respiration (Weir et al., 1993), and in anoxic volumes electrons then are available for denitrification (Akiyama et al., 2010; VanderZaag et al., 2011).

The conversion of ammonia to nitrite and nitrate in the soil around manure hotspots is often the limiting factor in coupled nitrification-denitrification. Preventing nitrification using a nitrification inhibitor (Zerulla et al., 2001) can be an effective strategy to mitigate N_2O emissions from manure-treated soil (Peixoto and Petersen, 2023). Nitrification inhibitors can desynchronize C and N turnover after slurry application by limiting the availability of NO3- as electron acceptor for denitrification. Synthetic nitrification inhibitors have been widely shown to suppress the activity of ammonia-oxidizing bacteria by inhibition of ammonia-monooxygenase (AMO), the enzyme responsible for the first step in the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-), thereby reducing the nitrification rate and the risk for subsequent losses through NO_3^- leaching or denitrification and N_2O emissions (Ruser and Schulz, 2015; Beeckman et al., 2018; Zhou et al., 2020). Nitrification inhibitors are sensitive to temperature, and at 20 C the inhibitory effect is lost within 5-6 weeks (Subbarao et al., 2006).

Silage maize as feed for dairy cattle is a main crop in Denmark, especially on sandy soils, and approximately 7% of the total agricultural area is under maize (Danmarks Statistik, 2020). Cattle slurry is being widely used as a fertilizer for maize. Traditionally cattle slurry is injected before soil ploughing and seed bed preparation to reduce N losses. Another strategy to decrease environmental N losses, including N_2O emissions, after slurry application is to improve the ability of living roots to take up nutrients such as N. This can be achieved by placing the slurry close to crop seeds for better root access (Pedersen et al., 2022). The infiltration of slurry liquid into the soil is impeded by sealing effects (Miller et al., 1985) and water retention by organic dry matter in the manure (Petersen et al., 2003). Therefore, after placement of slurry under the seed, suspended and dissolved slurry organic matter gets lodged inside pores of the soil surrounding the slurry-saturated soil (Barrington and Madramootoo, 1989), increasing the retention of N in the root zone. Maize seedlings prefer uptake of NH_4^+ over NO_3^- (Zhang et al., 2019). In addition, placement of slurry close to maize seeds can also increase the initial phosphorus uptake of the young plants (Pedersen et al., 2022).

The main objective of this study was to investigate effects of cattle slurry application method (traditional direct injection and ploughing vs. placement as a broad band under maize), and use of the nitrification inhibitor Vizura® with the active compound 3,4-dimethylpyrazole phosphate (DMPP), on soil mineral nitrogen and N_2O emission dynamics during the initial growth phase with signficant soil N dynamics. It was hypothesized that DMPP would reduce N_2O emissions independent of slurry distribution, and that placement of slurry would reduce N_2O emissions compared to non-placed slurry by enhancing the plant removal of soil mineral N from the soil solution.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 September 2023 doi:10.20944/preprints202309.0739.v1

2. Materials and Methods

2.1. Field experiment

The field experiment was conducted in 2020 at Foulumgaard (56°49′ N; 9°56′ E); an experimental farm at the Viborg campus of Aarhus University; Denmark. The 3 × 18 m2 experimental plots; established to evaluate N and P utilization of maize (Pedersen et al.; 2022); were organized as a randomized block design with three replications (Table 2); except that non-fertilized sampling positions had to be placed in a separate plot. The soil is a sandy loam based on ground morainic deposits from the last glaciation classified as a Mollic Luvisol according to the WRB (FAO) system (Krogh and Greve; 1999). Selected soil properties are shown in Table 1. Figure 1 (in results section) shows temperature and rain events during experimental period

Table 1. Soil properties (0–25 cm) at experimental site.

Texture	Clay (%)	Silt (%)	Fine Sand (%)	Coarse Sand (%)	pH	Total soil organic carbon (%)
Sandy loam	9	9.3	42.8	38.8	5.3	1.5

Note: Clay: < 2 µm; Silt: 2-20 µm; Fine sand: 20-200 µm: Coarse sand: 200-2000 µm; pH: 0.01 M $CaCl_2$.

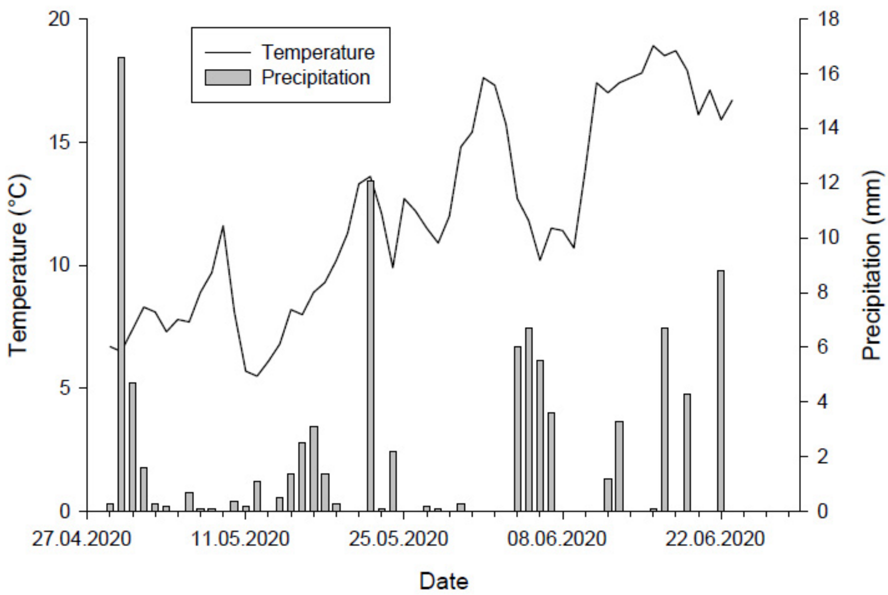

Figure 1. Daily average air temperature and precipitation during experimental period.

The cattle slurry was obtained from a local farm. It contained 5.6 % dry matter, 3.4 kg total N Mg^{-1} and 1.9 kg NH_4^+-N Mg^{-1}. All fertilized plots received cattle slurry at a rate of 110 kg NH_4^+-N ha^{-1}, equivalent to 57 Mg ha^{-1}. Where required, the nitrification inhibitor Vizura® (BASF, 97 Ludwigshafen, Germany) with the active compound 3,4-dimethylpyrazole phosphate (DMPP) was admixed in the slurry tanker at a rate corresponding to 2 L ha^{-1} before field application. In treatments without placement (called non-placed slurry), cattle slurry was injected using a traditional closed-slot injector with narrow tines at 10 cm depth at a band width of 25 cm, and this was immediately followed by ploughing (0-25 cm depth) before seeding. In treatments with slurry placement, cattle slurry was injected into ploughed soil in a broad band (about 30 cm broad) with the upper edge of the slurry band at 10 cm soil depth. Here, slurry injection was performed with a 26 cm wide goosefoot tine and a roller to ensure an accurate injection depth. The distance between tines was 75 cm, corresponding to the distance between maize rows in this trial. Maize (Zea mays) was sown at 5 cm depth immediately over the injection bands with placed slurry. All fertilized treatments also received 27 kg N ha^{-1} in ammonium sulphate as a starter fertilizer placed 5 cm beside the seeds (Pedersen et al., 2022).

2.2. N_2O measurements

Monitoring of N_2O took place between 29 April and 22 June 2020 with a total of 12 sampling days, i.e., three times during the first week and then at weekly intervals. Flux measurements were conducted using 37 cm × 27 cm × 22 cm static chambers placed over 35 cm × 25 cm × 15 cm support frames of stainless steel that were permanently installed within or between maize crop rows. The chambers were made of PVC and covered with an outer layer of Aluthermo Quattro (Adflexion Aps, Odense, Denmark) for insulation and reflection of solar radiation; they had a butyl rubber septum for gas sampling and a rubber seal at the bottom; together with an elastic strap fixed to the ground on either side of the chamber, this ensured a tight seal during measurements. The support frames were placed with the long side perpendicular to crop rows, i.e., row and inter-row positions together covered 70 of the 75 cm row and inter-row area. The support frames were installed immediately after slurry application, and only temporarily removed for seeding after 5-6 days.

Gas sampling was generally completed between mid-morning and noon. Ten ml gas samples were collected using a 10 mL plastic syringe with hypodermic needle immediately after chamber deployment, and at three additional time points; each time the syringe was purged several times with headspace air before withdrawing the gas sample. Gas samples were stored in pre-evacuated 6 mL exetainer vials (Labco Ltd., Ceredigion, UK) until analysis for N_2O using an Agilent 7890 (Agilent, Nærum, Denmark) gas chromatograph configured as previously described by Petersen et al. (2012).

2.3. Soil sampling

Soil samples were collected in the five main treatments (Table 2) on the same days as gas sampling. Six individual subsamples were randomly taken from each treatment and block using an auger (2 cm diameter, 0–20 cm depth), and from each plot were pooled and transferred to zip-lock plastic bags and stored at 2 °C until analyzed. Soil samples were mixed and sieved (< 6 mm mesh size), and subsamples extracted for analysis of NH_4^+-N and NO_3^--N, within two days of soil sampling. Approximately 10 g of soil was extracted in 40 ml of 1 M KCl for 30 min (end-over-end) and then allowed to settle. The supernatant was filtered (1.6 µm glass microfiber filters; VWR, Sweden) and frozen at −20 °C for later analysis on a AA500 Autoanalyzer (SEAL analytical GmbH, Norderstedt, Germany). Gravimetric soil water content was determined by drying c. 10 g of soil for 24 h at 105 °C.

Table 2. An overview of treatments in the experiment.

Treatment	Definition
Plac-Unt	Untreated slurry placed under maize seeds
Plac-Viz	Vizura treated slurry placed under maize seeds
Inje-Unt	Non-placed injected untreated slurry
Inje-Viz	Non-placed injected Vizura treated slurry
Control	No slurry application

2.4. Distribution of mineral N after placement

In a separate activity, additional soil samples were taken on day 2, 10 and 18 of the monitoring periods to map the horizontal and vertical distribution of mineral N after placement of cattle slurry with or without Vizura. Soil samples (2 cm diam., 0-20 cm depth) were taken at the center of the slurry band, and at 10 and 20 cm distance on either side; these samples were taken in three different positions along the length of the slurry band 10 cm apart. Each soil core was sub-divided into three depth intervals (0-5, 5-10, and 10-20 cm depth), and subsamples from each distance and depth were then pooled for analysis, i.e., for each depth three subsamples were pooled in the in-row position, and six subsamples per depth at 10 and 20 cm distance from the crop row. Hence, there were in total

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 September 2023 doi:10.20944/preprints202309.0739.v1

nine pooled samples from each of the two treatments. With three replicate field plots and three sampling days, there were162 individual soil samples for analysis of moisture content and mineral N (NH_4^+-N and NO_3^--N) as explained above.

2.5. Data analyses

Nitrous oxide fluxes were calculated by the HMR package available as a package in R-programming language (Pedersen et al., 2010). Parameter settings were as explained in Meng et al. (2023). Table 3 shows an overview of treatments and chamber placements.

Table 3. An overview of treatments and chamber placements for N_2O emissions in the experiment.

Treatment	Application method	Slurry treatment	Sampling position
Plac-Unt-Rw	Placed	Untreated	Row
Plac-Unt-IR	Placed	Untreated	Inter-row
Plac-Viz-Rw	Placed	Vizura	Row
Plac-Viz-IR	Placed	Vizura	Inter-row
Non placed-Unt-Rw	Non-placed	Untreated	Row
Non placed-Unt-IR	Non-placed	Untreated	Inter-row
Non placed-Viz-Rw	Non-placed	Vizura	Row
Non placed-Viz-IR	Non-placed	Vizura	Inter-row
Control	NA	NA	Row and Inter-row

NA – Not applicable.

Statistical analyses of the data were performed using R programming language version 4.1.2. Temporal N_2O fluxes and cumulative N_2O were analyzed for the inter-row (IR) and within-row (Row) positions separately, as well as together, and in both cases excluding control treatments (0N). Mineral N data for all main treatments were analyzed as well. Main and interacting effects of slurry application method and use of Vizura were investigated with block as a random factor in the analyses. All data were analyzed with the linear mixed effect (lme) function of the nlme package using the restricted maximum likelihood (REML) method. Model assumptions, i.e., normality and homogeneity of variance, were assessed using diagnostic plots of residuals. In order to satisfy model assumptions, the daily and cumulative N_2O emissions and mineral N (from both main treatments and the separate samplings around placed slurry) were log-transformed. For time series of N_2O emissions and mineral N data, auto-correlation between sampling positions were accounted with the corAR1 function. Pairwise comparisons between treatments were performed using the estimated marginal means (emmeans) function. The p-values were adjusted by Tukey's HSD method, and the hypothesis rejection threshold was 0.05.

3. Results

3.1. Environmental conditions

Figure 1 shows daily average air temperature and precipitation during the monitoring period, and the daily mean air temperature ranged from 5.5 to 18.9 °C. Daily rainfall ranged from 0 to a maximum of 17 mm, the latter occurring one day after placement of slurry, and the cumulative rainfall was 97 mm during the period of measurement.

3.2. Soil moisture and mineral N dynamics

Soil gravimetric water content on the sampling days varied between 18 and 21 % during the experimental period; assuming a bulk density of 1.37 g cm^{-3}, the bulk denisty recorded in a neighboring field under similar management (ploughed soil, crop residues removed). The calculated range in water content corresponded to 48-56 % water-filled pore space (WFPS). The application of 110 kg NH_4^+-N corresponded to an average of 34 mg NH_4^+-N kg^{-1} dry wt. soil. This was close to the values observed on Day 1 except for a higher value in the treatment with non-placed cattle slurry

 doi:10.20944/preprints202309.0739.v1

amended with Vizura (Figure 2, top). With application of non-placed slurry, soil NH_4^+-N concentrations showed a declining trend during May irrespective of the use of Vizura and then stabilized, but at a higher level in the treatment with Vizura. A different pattern was seen after placement of the slurry (Figure 2, bottom). Here, both treatments showed an increase to around 50 mg NH_4^+-N kg^{-1} in early (unamended slurry) or late May (Vizura-amended slurry). Then followed a period with rapid disappearance in both treatments, but with a 10-14 d delay in the presence of Vizura. The statistical analyses showed significant effects of date and treatment with Vizura on NH_4^+-N content, but no overall difference between application methods was observed (Table 4).

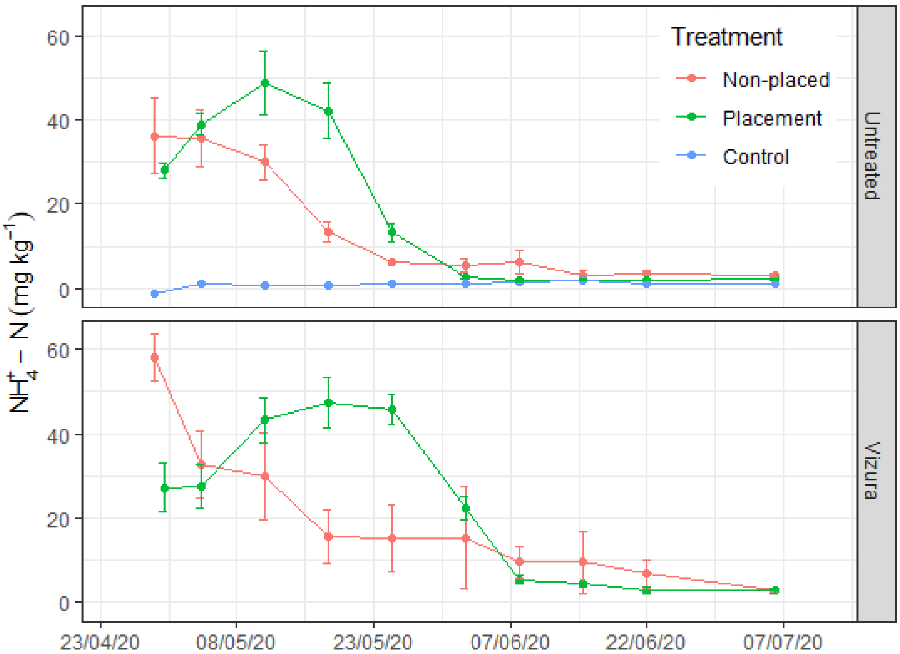

Figure 2. Soil NH4+-N at 0-20 cm depth in all treatments during experimental period (error bars represent s.e.m.; n = 3) shown for different treatments, which include slurry treatments (Untreated and Vizura-treated) and slurry application methods (non-placed and placement).

Table 4. Analysis of variance (ANOVA) for NH_4^+-N and NO_3^--N contents in soil, excluding the Control. Significance of *p* values: *** < 0.001, ** < 0.01, * < 0.05, non-significance (ns) > 0.05.

Treatments	**Num DF**	**Den DF**	***F***	***p***
Ammonium-N				
Intercept	1	93	643.74	***
Application method	1	93	3.26	ns
Slurry treatment	1	93	16.61	***
Date	10	93	25.88	***
Slurry application * Slurry treatment	1	93	0.18	ns
Slurry treatment * Date	10	93	1.85	ns
Nitrate-N				
Intercept	1	94	7685.17	***
Application method	1	94	2.40	ns
Slurry treatment	1	94	17.54	***
Date	10	94	100.39	***
Application method * Slurry treatment	11	94	0.29	ns
Slurry treatment * Date	10	94	3.16	**

Following the application of slurry, irrespective of treatment and application method, the NO_3^--N concentration increased gradually during the monitoring period, but with different temporal dynamics. With untreated cattle slurry, the increase was faster during May with placed compared to non-placed slurry, and the final level reached during June was higher, 70-80 mg NO_3^--N kg^{-1}, with placement of the slurry, compared to 50 mg NO_3^--N kg^{-1} with injection (Figure 3). When treated with Vizura, the development of NO_3^--N concentrations were more similar between injected and placed slurry until late May, where the NO_3^--N level plateaued at around 40 mg NO_3^--N kg^{-1} with injection but continued to increase to reach 70 mg NO_3^--N kg^{-1} with placement of the slurry. There was no overall difference between application methods, but a highly significant effect of treating the slurry with Vizura, and significantly different temporal dynamics with and without Vizura (Table 4).

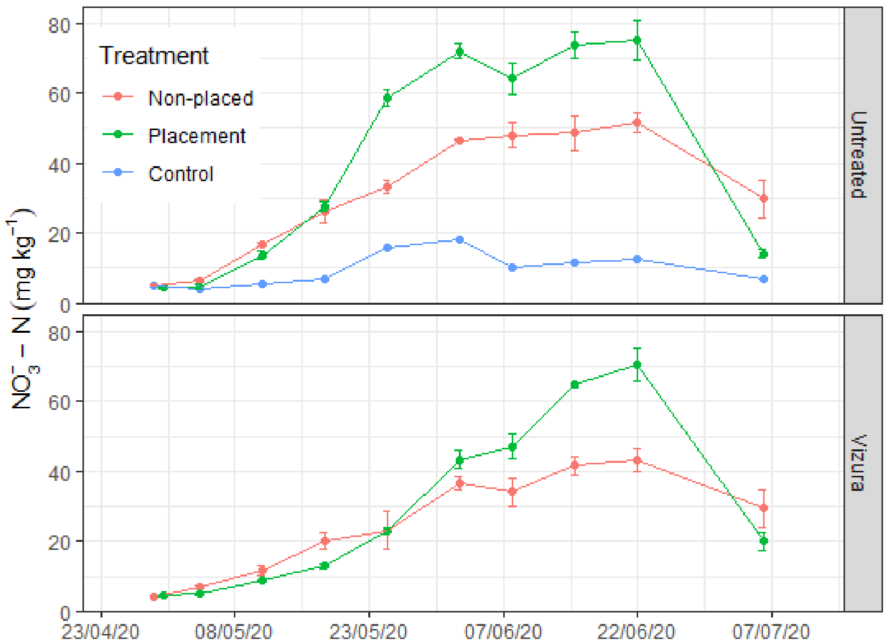

Figure 3. Soil NO_3^--N at 0-20 cm depth in all treatments during the experimental period (error bars represent s.e.m.; n = 3) shown for different treatments, which include slurry treatments (Untreated and Vizura-treated) and slurry application methods (Injection and placement).

3.3. Mineral N distribution after placement of slurry

The distribution of NH_4^+-N and NO_3^--N in the soil profile with placed slurry was determined after 2, 10 and 18 days (Figure 4); the results for untreated and Vizura-treated slurry are summarized by depth interval in Figure 5, and by distance from the row in Figure 6. There was some variability in NH_4^+-N concentrations that probably reflected heterogeneity in how the soil structure broke and voids filled with slurry during placement. Ammonium was concentrated at 10-20 cm depth, as expected, and remained at the same level during the 18-d period. Some accumulation of NO_3^--N was seen at all depths, but a reduction of NO_3^--N accumulation with Vizura was only recorded in the 5-20 cm layers and not at 0-5 cm depth receiving little or no slurry with placement (Figure 5). The width of the injection tine used for placement of slurry was 26 cm, and in accordance with this the NH_4^+-N concentrations at 20 cm distance from the row were low and changed little between day 2 and day 18 (Figure 6). By day 18, there was a trend of NO_3^--N accumulation where untreated cattle slurry had been placed, whereas in the treatment with Vizura the accumulation was similar at all distances.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 September 2023 doi:10.20944/preprints202309.0739.v1

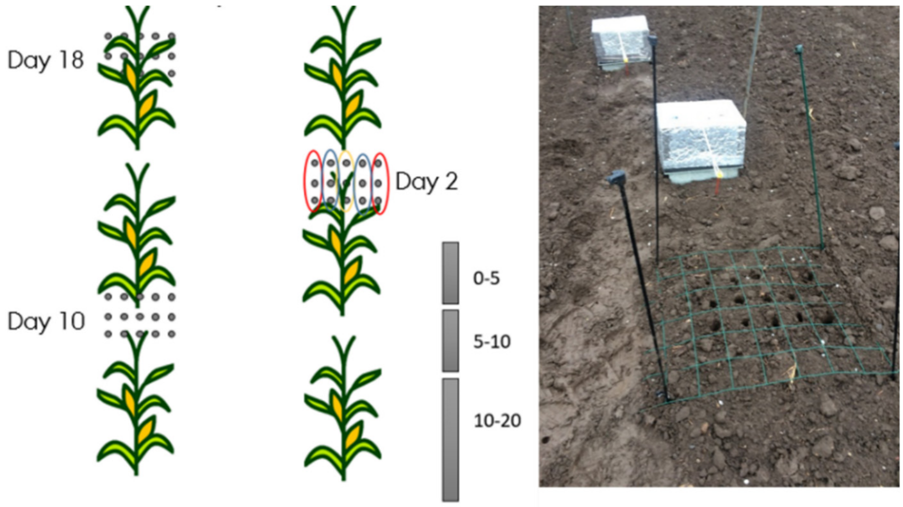

Figure 4. Separate soil samplings took place in placed slurry treatments after 2, 10 and 18 d, with three separate 2 cm diam. soil cores taken at 0, 10 and 20 cm distance from the crop row in each block as indicated in the schematic (left; see text for additional details). A picture of soil sampling on Day 2 after slurry application is also shown (right).

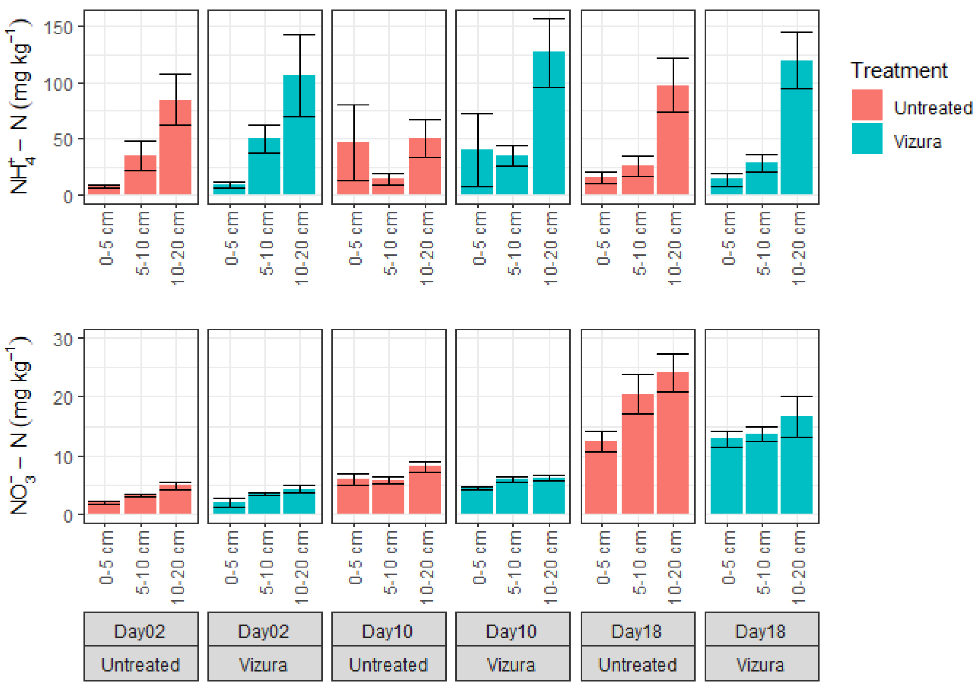

Figure 5. Soil NH_4^+-N and NO_3^--N at different soil depths on the days of separate soil samplings which show the vertical distribution of slurry N (error bars represent s.e.m.; n = 3).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 September 2023 doi:10.20944/preprints202309.0739.v1

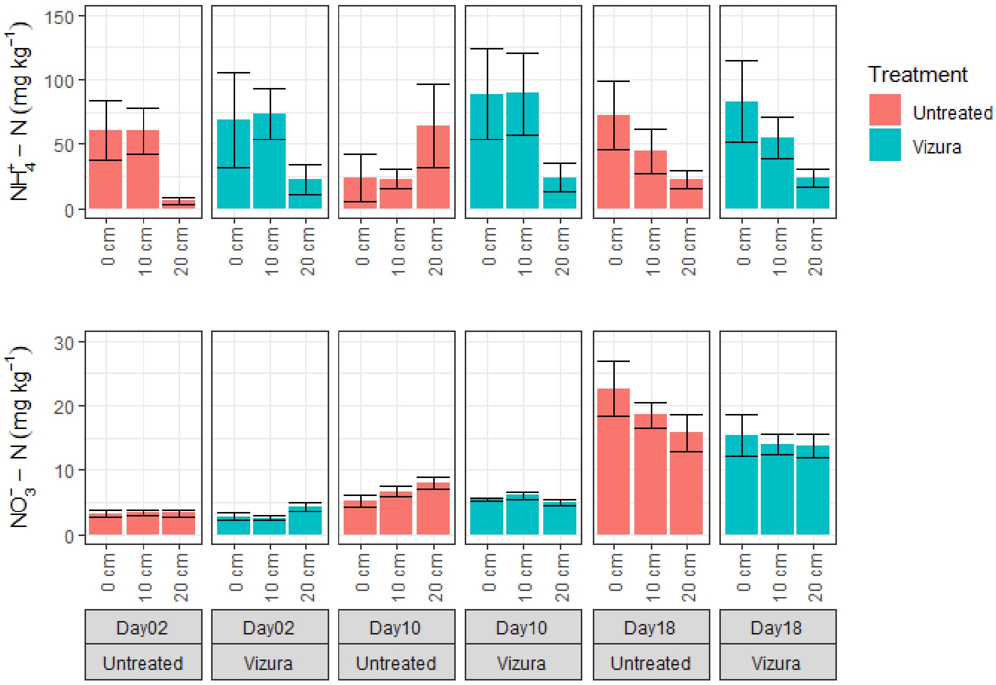

Figure 6. Soil NH_4^+-N and NO_3^--N at 0-20 cm depth at different distances from the plant row on the days of separate soil samplings showing the horizontal distribution of slurry N (error bars represent s.e.m.; n = 3).

3.4. N_2O emissions

The temporal dynamics of N_2O emissions are shown in Figure 7, with separate plots for within-row (Row) and inter-row (IR) positions, and results of an analysis of variance are shown in Table 5. Trends were similar but with a tendency for higher emissions from with-in row sampling positions. During May there was a gradual increase in N_2O emissions after injection both with and without placement of slurry without Vizura, but from end of May/early June this trend was reversed, and N_2O emissions declined during June and were close to zero at the last sampling.

Table 5. Analysis of variance (ANOVA) for temporal N_2O-N emissions, excluding the untreated control. Significance of *p* values: *** < 0.001, ** < 0.01, * < 0.05, non-significance (ns) > 0.05.

	Num DF	Den DF	*F*	*p*
Within-row				
Intercept	1	101	976.07	***
Application method	1	101	1.66	ns
Slurry treatment	1	101	0.33	ns
Date	11	101	4.77	***
Application method * Slurry treatment	1	101	20.44	ns
Slurry treatment * Date	11	101	2.18	*
Inter-row				
Intercept	1	225	2701.87	***
Application method	1	225	5.75	*
Slurry treatment	1	225	0.07	ns
Date	11	225	10.83	***
Application method * Slurry treatment	1	225	25.98	***
Slurry treatment * Date	11	225	2.47	ns

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 September 2023 doi:10.20944/preprints202309.0739.v1

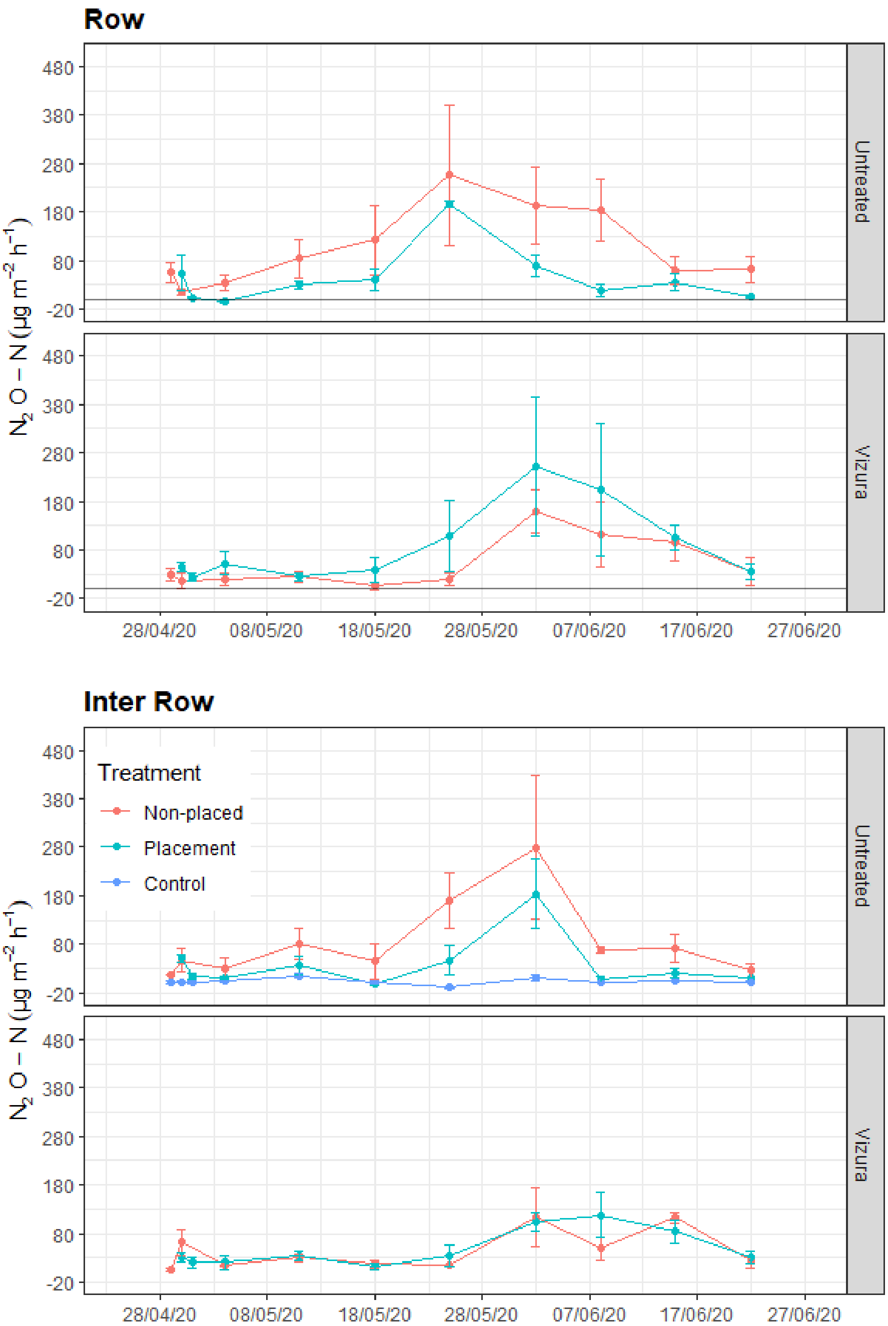

Figure 7. Nitrous oxide (N_2O) emissions from the different treatments; untreated and Vizura-treated cattle slurry applied with non-placed injection or placement) (error bars represent s.e.m.; n = 3). Asterisk show statistically significant pair-wise differences (*p* = 0.05).

The N_2O emissions after application of Vizura-treated slurry remained low during most of May, and then followed by a period increasing emissions from both within-row and inter-row positions. This increase was greater with placed compared to non-placed slurry (Figure 7). The N_2O emissions from Vizura-treated slurry also declined and were close to zero at the last sampling. During June, N_2O emissions were significantly higher from treatments with Vizura-amended slurry compared to untreated slurry, as indicated by the analysis of variance and pair-wise comparisons (Table 5). For inter-row positions there was a significant interaction ($p < 0.001$) between slurry application method and treatment with Vizura (Table 5).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 September 2023 doi:10.20944/preprints202309.0739.v1

The cumulative N_2O emissions during the monitoring period are shown in Figure 8, and the associated analysis of variance in Table 6. This analysis was based on average emissions from inter-row and within-row positions. Nitrous oxide emissions differed significantly between application methods and was higher from non-placed compared to placed cattle slurry. However, there was a strong interaction ($p < 0.001$) with slurry treatment, for untreated slurry the N_2O emission was lower from placed slurry. But the opposite pattern was true for slurry treated with Vizura where placed slurry showed higher cumulative N_2O emission during the monitoring period (Figure 8). All treatments except the Control received 221 kg N ha^{-1} in cattle slurry plus mineral N fertilizers, and cumulative N_2O emissions constituted 2.8 and 0.3 % of slurry N in non-placed slurry without and with Vizura®, respectively. The N_2O emissions constituted 0.3 and 0.4 % of slurry N without and with the inhibitor in placed slurry.

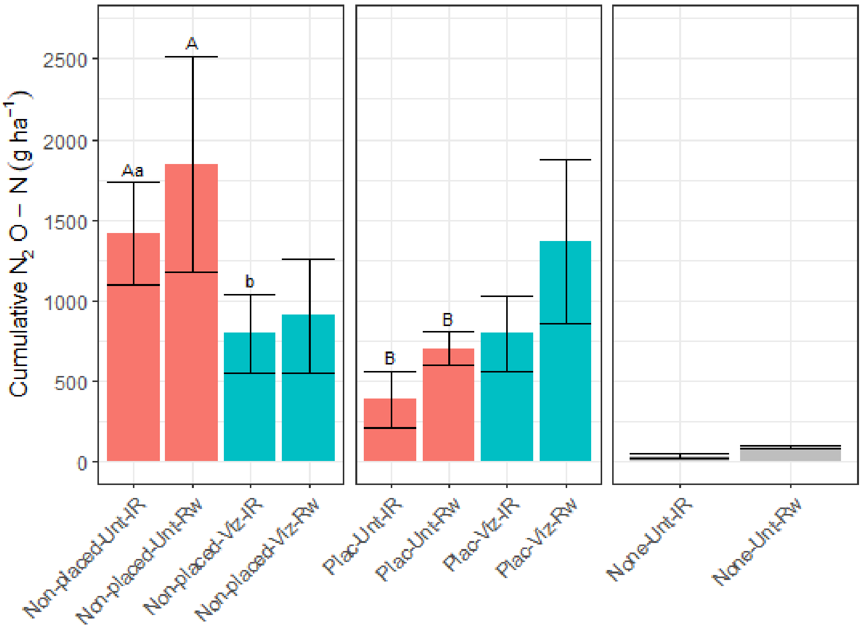

Figure 8. Cumulative N2O emissions from row (RW) and inter-row soil (IR) with placed and non-placed cattle slurry that was untreated (Unt) or treated with Vizura (Viz), and compared with soil without N application (error bars represent s.e.m.; n = 3). Lower-case letters indicate pairwise differences between slurry treatments (Untreated and Vizura-treated), while capital letters indicate pairwise differences between slurry application methods (Non-placed vs Placed).

Table 6. Analysis of variance (ANOVA) for cumulative N_2O-N emissions, excluding the untreated control. Significance levels: *** $p< 0.001$; ** $p< 0.01$; * $p< 0.05$; ns – non-significant.

	Num DF	**Den DF**	***F***	***p***
Intercept	1	123	705.57	***
Application method	1	123	6.93	**
Slurry treatment	1	123	0.08	ns
Date	1	123	0.11	ns
Application method * Slurry treatment	1	123	3.64	***
Slurry treatment * Date	1	123	1.98	ns

4. Discussion

Application technique can influence the potential for gaseous losses from liquid manure in the form of ammonia (NH_3) and N_2O emissions, which are, respectively, lower, and higher with incorporation or injection compared to surface application (Hou et al., 2015). The enhanced N_2O

emissions from incorporation as opposed to surface application of slurry are likely due to the decomposition of labile organic C in compounds such as volatile fatty acids (Guenzi and Beard, 1981). If the oxygen (O_2) demand by the slurry exceeds the supply, denitrification is enhanced if NO3- is present (Van Groenigen et al., 2014; Rochette et al., 2000). Elevated concentrations of water-soluble C were measured in the slurry injection zone for at least 40 d after application under field conditions (Comfort et al., 1988), and throughout a 20-day incubation experiment with simulated injection (Petersen et al., 1991). The labile C in this environment is probably protected by a higher water content in manure-saturated soil (Petersen et al., 2003), which impedes diffusive transport of O_2 and helps maintain anaerobic conditions. Hence, liquid manure incorporation leads to the development of organic hotspots with a potential for N_2O emissions in the weeks following incorporation (Wagner-Riddle et al., 2020; Velthof and Mosquera, 2011).

Injection (or acidification) of manure is mandatory when applied to bare soil according to local legislation in Denmark (Ministry of Food, Agriculture and Fisheries, 2022). In this study, slurry injection followed by ploughing (non-placement) resulted in higher cumulative N_2O emissions than placement of slurry in a band under crop rows (Figure 8 and Table 6), mainly because of higher emissions from untreated slurry during May. With 25 cm spacing between injection tines and subsequent ploughing, the contact between slurry and soil was probably greater for non-placed slurry compared to the placed slurry applied at 75 cm spacing and with no further disturbance. A larger surface area would promote the exchange of O_2, NH_4^+ and NO_3^- between manure and soil, and this in turn will stimulate the decomposition of labile C (Sørensen and Jensen, 1995) and support the growth of microbial populations, including nitrifiers and denitrifiers, around manure-soil interfaces (Petersen et al., 1991;1992). The faster onset of NO_3^--N accumulation with untreated non-placed slurry (Figure 3) could thus be due to a higher NH_4^+ availability for nitrifiers, and more coupled nitrification-denitrification during May enhancing N_2O emissions compared to placed slurry (Figure 7). This interpretation is consistent with the lower initial N_2O emission and effect of the nitrification inhibitor with placed slurry (Figure 7).

Variation in soil temperature or moisture can shift the balance between O_2 supply and demand and result in N_2O emission spikes or multi-day peaks on top of the emission driven by the manure applied (Molodovskaya et al., 2012). In accordance with this, an increase in N_2O emissions was observed with untreated slurry after 20th May that followed a temperature increase of nearly 10 ◦C and a 12 mm rain event during the previous week (Figure 1). Similarly, there was an increase in N_2O emissions from placed slurry after this change in temperature and rainfall.

Nitrification inhibitors are chemical compounds that reduce primarily bacterial oxidation of ammonia (NH_3) to nitrite (NO_2^-) in fertilized soil (Lei et al., 2022). The addition of nitrification inhibitors has been frequently reported to reduce both N_2O and NO emissions from agricultural soils, although their efficiency depends on soil conditions (Ruser and Schulz, 2015; Guardia et al., 2023). Nitrification inhibitors can reduce N_2O emissions from slurry by desynchronizing C and NO_3^- availability for denitrifiers after field application, but they may be less effective in compacted and/or wet soil with low nitrification activity and a higher potential for complete reduction of NO_3^- to N_2 (Recio et al., 2018; Peixoto and Petersen, 2023). In the present study, treating cattle slurry with DMPP reduced N_2O emissions for non-placed slurry, as hypothesized, and the reduction of 70 % was in line with or higher than previous studies (Misselbrook et al., 2014; Dittert et al., 2001; Menendez et al., 2006). Ammonia oxidation was clearly delayed, but there were higher N_2O emissions from placed slurry treated with DMPP in the last part of the monitoring period (Figure 7), and overall, a non-significant tendency for higher cumulative emissions from this treatment (Figure 8). Therefore, the effect of DMPP on N_2O emissions did not extend into June. The air temperature had increased prior to this period and reached 18 ◦C in mid-June. The rate of nitrification increases with temperature to a maximum at 30 °C in most soils (Subbarao et al., 2006), and whereas DMPP is effective at 5 °C where the inhibitory effect on ammonia oxidation may last for several months (Zerulla et al., 2001). Therefore, inhibitor effectiveness probably declined with increasing temperature. A recent study with pasture soil reported a half-life at 15 ◦C of 12–17 days for DMPP (Chibuike et al., 2022). The increasing rates of NO_3^- accumulation observed may thus have been due to DMPP degradation.

During June the emission of N_2O from placed slurry treated with DMPP was significantly higher than from untreated slurry (Figure 7). Denitrification has been found to be the main source of N_2O emissions from soil amended with cattle slurry (Meng et al., 2022), but the distribution and average concentrations of NO_3^- were similar in placed slurry with and without Vizura®. We propose that the higher N_2O emissions were due to nitrification activity taking place in close association with the slurry treated with Vizura®. By the time the inhibition by DMPP was relieved in June, some O_2 reaching interior parts of the placed slurry allowed nitrification to proceed under O_2 limited conditions, which are known to enhance N_2O emission via ammonia oxidation (Bollmann and Conrad, 1998) or, possibly, nitrifier denitrification (Wrage et al., 2001). It is, however, also possible that nitrifier activity throughout the slurry layer enhanced denitrification activity in nearby anoxic microsites via coupled nitrification-denitrification. A higher NO_3^- availability tends to increase the N_2O:N_2 product ratio of denitrification (Wu et al., 2019), and furthermore a stimulation of N_2O reductase activity (Friedl et al., 2020) could have ceased with disappearance of DMPP; both mechanisms would tend to increase N_2O emissions.

Nitrous oxide emissions approached the background level around the time of the last sampling, but a risk for environmental losses would continue to exist until N uptake was complete (Figure 3). A possible reason for the decline in N_2O emissions is that by this time the pool of reactive C sustaining denitrification had become depleted, and the manure-soil mixture therefore dominated by aerobic decomposition (Petersen et al., 1996).

Placement in a broad band reduced cumulative N_2O emissions compared to non-placed injection. However, the results showed an interaction with respect to the effect of Vizura® on cumulative N_2O emissions, with a reduction when slurry was injected without placement and the opposite trend with placed slurry (Figure 8). The discussion above pointed to a loss of nitrification inhibitor efficiency over time, and to nitrifier activity under oxygen-limited conditions in the manure layer of placed slurry. Unfortunately, the last measurement of soil mineral N distribution took place in mid-May, and the spatial resolution was also insufficient to evaluate this suggestion.

The placement of slurry was expected to improve nutrient availability for the crop. In a separate part of the field study which investigated N and P use efficiency, Pedersen et al. (2022) found that placement of cattle slurry increased the dry matter yield and N uptake of maize at harvest compared to injected slurry, but only in the presence of the nitrification inhibitor. It usually takes several weeks after seeding before the N uptake by maize significantly affects soil mineral N content, and in accordance with this the NO_3^--N concentrations remained constant during the last two weeks of the monitoring period where NH_4^+ pools had become depleted (Figure 3). The final plateau was higher with placed compared to non-placed slurry irrespective of treatment with the nitrification inhibitor, indicating that environmental N losses had been reduced by placement. However, soil NO_3^- in mid-June was very similar in slurry with and without DMPP and did not suggest a difference in N availability for the crop at this time. Presumably NO_3^- was lost through leaching or gaseous emissions after the monitoring ended, but before crop N uptake was complete, and the potential for loss was higher with non-placed compared to placed slurry, and with untreated compared to Vizura treated slurry.

5. Conclusions

This short-term study investigated effects of cattle slurry distribution in the soil, and effects of a nitrification inhibitor, on soil mineral N dynamics and N_2O emissions. Placement of slurry in a broad band under maize rows reduced N_2O emissions from untreated slurry, possibly because this distribution had less coupled nitrification-denitrification. A nitrification inhibitor, DMPP, reduced N_2O emissions for several weeks with traditional, non-placed slurry application. However, unexpectedly, no effect of DMPP was observed on cumulative N_2O emission after placement of the slurry, where N_2O production from slurry with the inhibitor increased after several weeks. Understanding the interactions between slurry distribution and nitrification inhibitor efficacy will be crucial for recommendations to mitigate N_2O emissions.

Author Contributions: Arezoo Taghizadeh-Toosi: Conceptualization, Investigation, Formal analysis, Writing - original draft. Khagendra R. Baral: Formal analysis, Writing - review & editing. Søren O. Petersen: Conceptualization, supervision, Writing – review & editing. Peter Sørensen: Project administration, Review & editing, Funding acquisition.

Data Availability Statement: The data can be available by request.

Acknowledgments: We would like to thank Maja Hørning Skjødtfor gas sampling, Bodil Stensgaard and Karin Dyrberg for gas analysis and Marianne Ahrenfeldt M Stevenson for mineral nitrogen analysis. The authors thank technical support at Department of Agroecology, Aarhus University, Henrik Nørgaard and Jens Bonderup Kjeldsen for field operations and sampling, and Peter Storegård, Department of Biological and Chemical Engineering, Aarhus University for constructing the injection machine for slurry placement. We would also like to thank GUDP for providing financial support for the study performed as part of the Gyllemajs project.

Conflicts of Interest: The authors declare no conflicts of interest or state.

References

1. Akiyama, H., Yan, X., and Yagi, K. 2010. Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N_2O and NO emissions from agricultural soils: meta-analysis. Global Change Biology 16: 1837-46.
2. Barrett, M., Khalil, M. I., Jahangir, M. M. R., Lee, C., Cardenás, L. M., Collins, G., Richards, K. G., and O'Flaherty, V. 2016. Carbon amendment and soil depth affect the distribution and abundance of denitrifiers in agricultural soils. Environmental Science and Pollution Research 23: 7899-7910.
3. Beeckman, F., Motte, H., and Beeckman, T. 2018. Nitrification in agricultural soils: impact, actors and mitigation. Current Opinion in Biotechnology 50: 166-173.
4. Barrington, S.F., and Madramootoo, C. A. 1989. Investigating seal formation from manure infiltration into soils. Transactions of the ASAE 32:851-856.
5. Bhandral, R., Saggar, S., Bolan, N. S., and Hedley, M. J. 2007. Transformation of nitrogen and nitrous oxide emission from grassland soils as affected by compaction. Soil & Tillage Research 94: 482-492.
6. Bollmann, A., Conrad, R., 1998. Influence of O_2 availability on NO and N_2O release by nitrification and denitrification in soils. Global Change Biology 4: 387-396.
7. Chadwick, D., Sommer, S., Thorman, R., Fangueiro, D., Cardenas, L., Amon, B., Misselbrook, T. 2011. Manure management: Implications for greenhouse gas emissions. Animal Feed Science and Technology 166: 514-531.
8. Chibuike, G., Saggar, S., Palmada, T., Luo, J. 2022. The persistence and efficacy of nitrification inhibitors to mitigate nitrous oxide emissions from New Zealand pasture soils amended with urine. Geoderma Regional 30: e00541.
9. Comfort, S. D., Kelling, K. A., Keeney, D. R., Converse, J. C. 1988. The fate of nitrogen from injected liquid manure in a silt loam soil. Journal of Environmental Quality 17:317-322.
10. Danmarks-Statistik, 2020.Landbrugsstatistik: https://www.dst.dk/.
11. Dittert, K., Bol, R., King, R., Chadwick, D. and Hatch, D. 2001. Use of a novel nitrification inhibitor to reduce nitrous oxide emission from N-15-labelled dairy slurry injected into soil. Rapid Communications in Mass Spectrometry 15: 1291–1296.
12. Duan, Y.-F., Kong, X.-W., Schramm, A., Labouriau, R., Eriksen, J. and Petersen, S.O. 2017. Microbial N transformations and N_2O emission after simulated grassland cultivation: Effects of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP). Applied and Environmental Microbiology 83 doi:10.1128/AEM.02019-16.
13. Friedl, J., Scheer, C., Rowlings, D. W., Deltedsco, E., Gorfer, M., De Rosa, D., Grace, P. R., Müller, C., and Keiblinger, K. M. 2020. Effect of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on N-turnover, the N_2O reductase-gene *nosZ* and N_2O:N_2 partitioning from agricultural soils. Scientific Reports: 2399.
14. Guardia, G., Abalos, D., Mateo-Marín, N., Nair, D., Petersen, S. O. 2023. Using DMPP with cattle manure can mitigate yield-scaled global warming potential under low rainfall conditions. Environmental Pollution 316: 120679.
15. Guenzi, W. D., Beard, W. E. 1981. Volatile fatty acids in a redox controlled cattle manure slurry. Journal of. Environmental Quality 10: 479-482.
16. Hafner, S.D., Pacholski, A., Bittman, S., Burchill, W., Bussink, W., Chantigny, M., Carozzi, M., Génermont, S., Häni, C., Hansen, M. N., Huijsmans, J., Hunt, D., Kupper, T., Lanigan. G., Loubet, B., Misselbrook, T., Meisinge, J. J., Neftel, A., Nyord, T., Pedersen, S. V., Sintermann, J., Thompson, R. B., Vermeulen, B., Vestergaard, A. V., Voylokov, P., Williams, J. R., and Sommer, S. G. 2018. The ALFAM2 database on

ammonia emission from field-applied manure: description and illustrative analysis. Agric. For. Meteorol. 258, 66-79.
17. Hou, Y., Velthof, G.L. and Oenema, O. 2015. Mitigation of ammonia, nitrous oxide and methane emissions from manure management chains: a meta-analysis and integrated assessment. Global Change Biology 21: 1293-1312.
18. Ibraim, E., Denk, T., Wolf, B., Barthel, M., Gasche, R., Wanek, W., et al. (2020). Denitrification is the main nitrous oxide source process in grassland soils
19. according to quasi-continuous isotopocule analysis and biogeochemical modeling. Global Biogeochemical Cycles, 33, e2019GB006505. https://doi.org/10.1029/2019GB006505
20. IPCC, 2019. 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories, Switzerland.
21. Kirchmann, H. and Lundvall. A. 1993. Relationship between N immobilization and volatile fatty acids in soil after application of pig and cattle slurry. Biology and Fertility of Soils 15:161-164.
22. Krogh, L. and Greve, M. H. 1999. Evaluation of world reference base for soil resources and FAO soil map of the world using nationwide grid soil data from Denmark. Soil Use & Management 15:157-166.
23. Lei, J., Fan, Q., Yu, J., Ma, Y., Yin, J. and Liu, R. 2022. A meta-analysis to examine whether nitrification inhibitors work through selectively inhibiting ammonia-oxidizing bacteria. Frontiers in Microbiology 13: 962146.
24. Leip, A., Weiss, F., Wassenaar, T., Perez, I., Fellmann, T., Loudjani, P., Tubiello, F., Grandgirard, D., Monni, S., Biala, K. 2010. Evaluation of the livestock sector's contribution to the EU greenhouse gas emissions (GGELS) – final report. European Commission, Joint Research Centre.
25. Linzmeier, W., Gutser, R., Schmidhalter, U. 2001. Nitrous oxide emission from soil and from a nitrogen-^{15}-labelled fertilizer with the new nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP). Biology and Fertility of Soils 34: 103-108.
26. Menéndez, S., Barrena, I., Setién, I., González-Murua, C., Estavillo, J. M. 2012. Efficiency of nitrification inhibitor DMPP to reduce nitrous oxide emissions under different temperature and moisture conditions. Soil Biology & Biochemistry 53: 82-89.
27. Meng, X., Sørensen, P., Møller, H.B., Petersen, S.O. 2023. Greenhouse gas balances and yield-scaled emissions for storage and field application of organic fertilizers derived from cattle manure. Agriculture, Ecosystems and Environment 345: 108327.
28. Meng, X., Ma, C., Petersen, S.O., 2022. Sensitive control of N_2O emissions and microbial community dynamics by organic fertilizer and soil interactions. Biology and Fertility of Soils 58: 771-788.
29. Menéndez, S., Merino, P., Pinto, M., Gonzalez-Murua, C. and Estavillo, J. M. 2006. 3,4-dimethylpyrazol phosphate effect on nitrous oxide, nitric oxide, ammonia, and carbon dioxide emissions from grasslands. Journal of Environmental Quality 35: 973-981.
30. Miller, M. H., Robinson, J. B., and Gillham, R. W. 1985. Self-Sealing of earthen liquid manure storage ponds: I. A Case study1. Journal of environmental quality 14: 533-538.
31. Misselbrook, T., Smith, K., Johnson, R. A., Pain, B. 2002. Slurry application techniques to reduce ammonia emissions: Results of some UK field-scale experiments. Biosystems Engineering 81: 313-321.
32. Molodovskaya, M., Singurindy, O., Richards, B.K., Warland, J., Johnson, M.S., Steenhuis, T.S. 2012. Temporal variability of nitrous oxide from fertilized croplands: Hot moment analysis. Soil Science Society of Am. J. 2012, 76, 1728–1740.
33. Nair, D., Baral, K. R., Abalos, D., Strobel, B. W., and Petersen, S. O. 2020. Nitrate leaching and nitrous oxide emissions from maize after grass-clover on a coarse sandy soil: Mitigation potentials of 3, 4-dimethylpyrazole phosphate (DMPP). Journal of environmental management 260: 110165.
34. Nielsen, N.E., Jensen, H.E. 1986. The course of nitrogen uptake by spring barley from soil and fertilizer nitrogen. Plant and Soil 91: 391-395.
35. Nielsen, T.H., Revsbech, N.P. 1994. Diffusion Chamber for Nitrogen-15 Determination of Coupled Nitrification-Denitrification around Soil-Manure Interfaces. Soil Science Society of American Journal 58:795-800.
36. Pedersen, A. R., Petersen, S. O., and Schelde, K., 2010. A comprehensive approach to soil atmosphere trace-gas flux estimation with static chambers. European Journal of Soil Science 61: 888-902.
37. Pedersen, I. F., Nyord, T., and Sørensen, P. 2022. Tine tip width and placement depth by row-injection of cattle slurry influence initial leaf N and P concentrations and final yield of silage maize. European Journal of Agronomy 133: 126418.
38. Petersen, S.O., Henriksen, K. and Blackburn, T.H. (1991) Coupled nitrification¬-denitrification associated with liquid manure in a gel-stabilized model system. Biology and Fertility of Soils 12: 19-27.
39. Petersen, S. O., Hoffmann, C. C., Schäfer, C. -M., Blicher-Mathiesen, G., Elsgaard, L., Kristensen, K., Larsen, S. E., Torp, S. B., and Greve, M. H. 2012. Annual emissions of CH_4 and N_2O, and ecosystem respiration, from eight organic soils in Western Denmark managed by agriculture. Biogeosciences 9: 403-422.

40. Ravishankara, A. R., Daniel, J. S., Portman, R.W. 2009. Nitrous oxide (N_2O): The dominant ozone-depleting substance emitted in the 21st century. Science 326: 123-125.
41. Rochette, P., van Bochove, E., Prévost, D., Angers, D., Côté, D., Bertrand, N. 2000. Soil carbon and nitrogen dynamics following application of pig slurry for the 19th consecutive year. II. Nitrous oxide fluxes and mineral nitrogen. Soil Science Society of American Journals 64: 1396-1403.
42. Ruser, R., and Schulz, R. 2015. The effect of nitrification inhibitors on the nitrous oxide (N_2O) release from agricultural soils - A review. Journal of Plant Nutrition and Soil Science 178: 171-188.
43. Severin, M., Fuß, R., Well, R., Garlipp, F., Van denWeghe, H. 2016. Soil, slurry and application effects on greenhouse gas emissions. Plant, Soil and Environment 61: 344-351.
44. Schröder, J. J., Vermeulen, G. D., van der Schoot, J. R., van Dijk, W., Huijsmans, J. F. M., Meuffels, G. J. H. M., van der Schans, D. A., 2015. Maize yields benefit from injected manure positioned in bands. European Journal of Agronomy 64: 29-36.
45. Spiertz, J. H. J. 2010. Nitrogen, sustainable agriculture, and food security. A review. Agronomy for Sustainable Development 30: 43-55.
46. Subbarao, G. V., Ito, O., Sahrawat, K. L., Berry, W. L., Nakahara, K., Ishikawa, T., Watanabe, T., Suenaga, K., Rondon, M., Rao, I. 2006. Scope and strategies for regulation of nitrification in agricultural systems – Challenges and opportunities. Critical Reviews in Plant Sciences 25: 303-335.
47. Sørensen, P., and Jensen, E.S 1995. Mineralization-immobilization and plant uptake of nitrogen as influe-nced by the spatial distribution of cattle slurry in soils of different texture. Plant and Soil 173: 283-291.
48. Ruser, R., and Schulz, R. 2015. The effect of nitrification inhibitors on the nitrous oxide (N_2O) release from agricultural soils—a review. Journal of Plant Nutrition and soil science 178: 171-188.
49. VanderZaag, A. C., Jayasundara, S., and Wagner-Riddle, C. 2011. Strategies to mitigate nitrous oxide emissions from land applied manure. Animal Feed Science and Technology 166–167: 464-479.
50. Van Groenigen, J.W., Kasper, G.J., Velthof, G.L., Van den Pol van Dasselaar, A., Kuikman, P.J. 2004. Nitrous oxide emissions from silage maize fields under different mineral nitrogen fertilizer and slurry applications. Plant and Soil 263: 101-111.
51. Velthof, G. L., and Mosquera, J. 2011. The impact of slurry application technique on nitrous oxide emission from agricultural soils. Agriculture, Ecosystems and Environment 140: 298–308.
52. Webb, J., Pain, B., Bittman, S., Morgan, J. 2010. The impacts of manure application methods on emissions of ammonia, nitrous oxide and on crop response—A review. Agriculture, Ecosystems & Environment 137: 39-46.
53. Webb, J., Pain, B., Bittman, S., Morgan, J. 2010. The impacts of manure application methods on emissions of ammonia, nitrous oxide and on crop response—a review. Agriculture, Ecosystems and Environment 137: 39-46.
54. Wrage, N., Velthof, G.L., van Beusichem, M.L., Oenema, O. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology & Biochemistry 33: 1723-1732
55. Wu, D., Cárdenas, L. M., Calvet, S., Brüggemann, N., Loick, N., Liu, Sh., Bol, R. 2017. The effect of nitrification inhibitor on N_2O, NO and N_2 emissions under different soil moisture levels in a permanent grassland soil. Soil Biology & Biochemistry 113: 153e160.
56. Wu, D., Well, R., Cárdenas, L. M., Fuβ, R., Lewicka-Szczebak, D., Köster, J. R., Brüggemann, N., and Bol, R. 2019. Quantifying N_2O reduction to N_2 during denitrification in soils via isotopic mapping approach: Model evaluation and uncertainty analysis. Environmental Research 179: 108806.
57. Zerulla, W., Barth, T., Dressel, J., Erhardt, K., Horchler von Locquenghien, K., Pasda, G., Rädle, M., Wissemeier, A. 2001. 3,4-Dimethylpyrazole phosphate (DMPP) - a new nitrification inhibitor for agriculture and horticulture. Biology and Fertility of Soils 34: 79e84.
58. Zhang, H.Q., Zhao, X.Q., Chen, Y.L., Zhang, L. Y., Shen, R. F. 2019. Case of a stronger capability of maize seedlings to use ammonium being responsible for the higher ^{15}N recovery efficiency of ammonium compared with nitrate. Plant and Soil 440: 293–309 (2019).
59. Zhou, X., Wang, S., Ma, S., Zheng, X., Wang, Z., and Lu, C. 2020. Effects of commonly used nitrification inhibitors dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP), and nitrapyrin—on soil nitrogen dynamics and nitrifiers in three typical paddy soils. Geoderma 380:114637.
60. Zhu, X., Burger, M., Doane, T. A., Horwath, W. R. 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. Proceedings of the National Academy of Sciences 110: 6328-33.