Adhikari, K., Saggar, S., Luo, J., Giltrap, D., Lindsey, S., Berben, P., Palmada, T., Bates, G., 2024. Significance of inhibitor volume in on-farm mitigation of nitrous oxide emission from dairy cattle urine patches. In: *Opportunities for improved farm and catchment outcomes*. (Eds. C.L. Christensen, D.J. Horne and R. Singh). *http://flrc.massey.ac.nz/publications.html*. Occasional Report No, 36. Farmed Landscapes Research Centre, Massey University, Palmerston North, New Zealand. 11 pages

SIGNIFICANCE OF INHIBITOR VOLUME IN ON-FARM MITIGATION OF NITROUS OXIDE EMISSION FROM DAIRY CATTLE URINE PATCHES

Kamal Adhikari[1, 2], Surinder Saggar[1, *], Jiafa Luo[3], Donna Giltrap[1], Stuart Lindsey[3], Peter Berben[1], Thilak Palmada[1], Geoff Bates[4]

[1]Manaaki Whenua – Landcare Research, Palmerston North 4442, New Zealand
[2]Current address: Department of Energy, Environment and Climate Action, Horsham, Victoria Australia 3400
[3]Ruakura Agricultural Centre, AgResearch Ltd, Hamilton 3240, New Zealand
[4]Pastoral Robotics Ltd. 7a Carmont Place, Mt Wellington, Auckland 1060, New Zealand
[*]Email: SaggarS@landcareresearch.co.nz

Abstract

Technologies are being developed for the targeted mitigation of nitrogen (N) losses from livestock urine patches using urease and nitrification inhibitors (UIs and NIs). In our earlier study, we identified a major limitation for inhibitor efficiency, specifically, the application of a 40 mL volume of inhibitor solution to a 2L of urine patch (i.e., 1:50, based on New Zealand recommended dicyandiamide [DCD] application rate of 10 kg DCD dissolved in 800 L water ha^{-1}).This ongoing research evaluates the effect of inhibitor treatments by varying the inhibitor: urine volume ratio from 1:50 to 1:10 (200 mL of inhibitor to the 2L of urine patch) on nitrous oxide (N_2O) mitigation of five nitrification inhibitors: DCD, 3,4-dimethylpyrazole phosphate (DMPP), 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin), and two confidential compounds (named A and C, provided by AgResearch). These inhibitors were applied 24 hours after creating 2L simulated urine patches (within 0.5 m^2 chambers) in two dairy-grazed pasture soils with contrasting drainage (poorly vs well drained). Results showed that the N_2O emissions reduction efficiency from urine patches was the highest (35.8%–46.7%) with DCD followed by inhibitor C (26.9%–27.9%). The reductions in emission from the other inhibitors were not significant (11.0%–23.0% with DMPP and nitrapyrin, respectively; and 1.5%–15.6% with inhibitor A). In this study, diluting the inhibitor solutions resulted in retention of only 3% to 18% of the NIs by the pasture canopy compared with up to 59% (with 1:50) in our previous study. This dilution increases the amount of inhibitor reaching the soil, offering a potential option for effectively reducing N_2O emissions from cattle urine patches. However, dilution may result in concentrations below threshold levels of DMPP, nitrapyrin and inhibitor A, compromising their effectiveness. These results warrant further research to optimise inhibitor application rate and volume and measure inhibitor residues for developing best practice for targeted application of inhibitors to urine patches while addressing unintended food and human health risks.

Introduction

The use of nitrification inhibitors (NIs) has been widely investigated and has shown extensive benefit in reducing nitrous oxide (N_2O) emissions, particularly when used with nitrogen (N) fertiliser and when applied to grazed pasture soils to treat excretal urine-N following grazing events (Qiao et al. 2015; Adhikari et al. 2021). However, reducing N losses from urine patches using N process inhibitors requires close contact between the inhibitor and the urine (Adhikari et al. 2020, 2021b; Rodriguez et al. 2021). The effectiveness of inhibitors depends on the proportion of urine that can be treated by the applied inhibitor (Zaman and Nguyen 2012; Saggar et al. 2013). Various biophysical and biochemical factors, including the characteristics of the inhibitor, soil type, and timing, concentration and volume of inhibitor applied, have a key role in influencing the co-location (physical contact) of inhibitors and

urine, and thereby the inhibitor efficacy at reducing N_2O emissions (Adhikari et al. 2021; Giltrap et al. 2022). However, in on-farm grazing circumstances, where an inhibitor is applied post-grazing, there is a potential time delay between urine deposition and inhibitor application. This time lapse could result in physical separation between the urine and inhibitor, potentially rendering the inhibitor ineffective. Furthermore, interception of NIs by the pasture canopy may also reduce the amounts of NIs reaching the soil.

A major limitation identified in our earlier work in this project (Milestones 03 and 04; evaluating the key inhibitors concentrations applied at 4, 24 and 48 hours of simulated urine patches and estimating optimum inhibitor application rates), was the low volume of inhibitor applied relative to the urine volume (1:50, i.e., 40 mL of inhibitor to the 2L of urine patch). This low inhibitor: urine volume ratio affected the physical distribution of inhibitors applied to urine patches, resulting in large proportions of applied NIs were retained in the pasture canopy (up to 59%). Additionally, the inhibitor threshold concentration was only met in the top 0–20 mm of the soil in most cases. This corresponded to only 16%–40% of the urine-N being co-located with threshold concentration of the inhibitor when NIs were applied at 4, 24 and 48 hours after simulating natural urine patch (Adhikari et al. 2023).

This paper reports the results of two New Zealand (NZ) field trials evaluating the role of increased inhibitor: urine volume ratio from 1:50 to 1:10 on N_2O mitigation efficiency.

Materials and methods

In the current study, field experiments were conducted in Spring 2022 at two typical dairy farms in NZ: Massey University No. 4 Dairy Farm, Palmerston North and AgResearch Ruakura dairy farm. These farms are hereafter referred to as 'Manawatū farm' and 'Waikato farm', respectively, in this report. The pasture consisted predominantly of a mixture of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) at both farms. The poorly drained soil at the Manawatū farm is Tokomaru silt-loam, classified as Argillic-Fragic Perch-Gley Pallic Soil and the well-drained soil of at the Waikato farm is Horotiu silt loam, classified as Typic Orthic Allophanic in the NZ soil classification system (Hewitt 1992), and termed as Alfisol and Andosol, respectively (FAO–UNESCO 1998). The selected areas for the experiments were fenced off for 12–14 weeks (about 3 months) before the start of the experiments to avoid interference from fresh dung and urine inputs and reduce spatial variability from the previous N fertiliser inputs and uneven deposition of dung and urine.

The trial layout for N_2O emission measurements consisted of a randomised block design using 8 replicates of each treatment. The treatments included at each site were: (i) Control, (ii) Urine only, (iii) Urine + A, (iv) Urine + C, (v) Urine + DCD, (vi) Urine + DMPP, and (vii) Urine + nitrapyrin. DCD is, DMPP is 3,4-dimethylpyrazole phosphate, and nitrapyrin is 2-chloro-6-(trichloromethyl) pyridine; the two compounds (named A and C) were provided by AgResearch and remain confidential. Separate sampling chambers were established for determining inhibitor residues in soil and pasture samples from treatments with inhibitors. There was only one replicate for the inhibitor residue sampling plots.

Individual treatment plots (2.5 × 2.5 m) in both sampling areas: 0.5 m^2 gas chambers for N_2O emissions measurements and inhibitor residue sampling plots, which were separated by a distance of at least 0.5 m. The pasture in the experimental areas was mown/cut to 50 mm height on the day before urine application to simulate grazing. For all urine treatments, 2 L of synthetic urine (containing 6 g N L^{-1}) was poured to the midway point of the cylindrical gas chamber base area (of 0.5 m^2) from a height of approximately 1.2 m and allowed to spread naturally (to simulate natural cattle urine deposition). Twenty-four hours after urine application, 200 mL of inhibitor solutions/suspensions prepared in deionised (DI) water was applied within 50 cm diameter ring placed in the middle of the

chamber (0.5 m^2) using a sprayer. The equivalent application rates for NIs were 1 g DCD $patch^{-1}$, 0.6 g DMPP $patch^{-1}$, and 0.6 g nitrapyrin $patch^{-1}$. The application rates for A and C are confidential.

A static chamber technique was used to measure N_2O emissions, and the methodology is based on that of previously published studies on N_2O emissions (e.g., Hoogendoorn et al. 2018; Luo et al. 2019). About a week before the trials began, static chamber bases (800 mm diameter) were inserted 50–100 mm into the soil at each treatment and replicate area. A day before urine application, a pre-treatment gas measurement was taken from each chamber to determine the spatial variability of background N_2O fluxes between the treatment plots and to help interpret patterns of N_2O flux from individual chamber areas post urine application. The flux measurements were carried out at 24, 48, and 72 hours after treatment (i.e., inhibitor application); then twice a week for the first month, and weekly thereafter till the emissions reached the background levels. The flux measurements were also carried out just before each inhibitor's application to determine the amount of N_2O emitted before the inhibitor was applied. During weekly phases of N_2O flux measurements, additional sampling occurred as soon as practical following rainfall events of greater than 10 mm of rain in the previous 24-hour period. The N_2O chamber technology and gas sampling schedule are consistent with recommended guidelines for N_2O chamber methodology (Charteris et al. 2020). Cumulative emissions were calculated via trapezoidal integration of the daily fluxes on measurement dates to estimate the total emissions over the measurement period. Emission factors (N_2O-N emitted as % of urine N applied) were calculated following the IPCC (2006) methodology, using Equation 1:

$$EF_3 = \frac{Total\ treatment\ N_2O - N \quad - \quad Total\ control\ N_2O - N}{Total\ N\ applied} \times 100\% \qquad \text{Eqn 1}$$

where, EF_3 is emission factor; total treatment N_2O and total control N_2O are the cumulative N_2O emissions from the urine treatment and control plots, respectively (mg N urine $patch^{-1}$); and N applied is the rate of treatment N applied (mg N urine $patch^{-1}$).

The data for N_2O EF_3 (N_2O emission factors) and total pasture mass production were analysed using an analysis of variance (ANOVA), and treatment means were compared using Fisher's Least Significant Difference (LSD) Test ($P < 0.1$ and $P < 0.05$). Shapiro-Wilk and Bartlett's tests were used to check the normality and homogeneity of variance of the data, respectively, and appropriate transformations were performed when necessary to meet these requirements. All the analyses were conducted using Genstat statistical software (Genstat 64-bit Release 21.1, VSN International Ltd).

Results and discussion

Site soils properties and environmental conditions

The results for soil physical and chemical properties at the experimental sites are presented in Tables 1 and 2. Soil measurements showed similar values for bulk density, porosity, field capacity and all chemical properties, Except the proportion of sand and soil C was higher in Waikato soil; however, silt content was higher in Manawatū soil.

Table 1. Physical properties of the site soil (0–75 mm depth)

Farm	Bulk density ($Mg\ m^{-3}$)	Total porosity (%)	Field capacity (%)	Sand (%)	Silt (%)	Clay (%)
Manawatū	1.1	56	48	6	70	24
Waikato	0.9	60	48	39	31	30

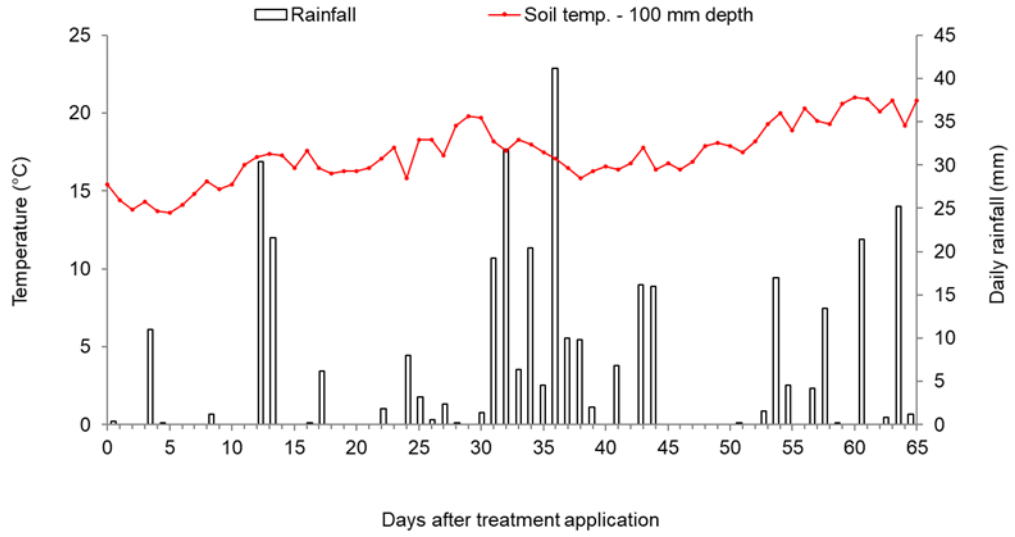
Table 2. Chemical properties of the site soil (0–75 mm depth)

Farm	Total C (%)	Total N (%)	CEC* ($mEq\ 100\ g^{-1}$)	Olsen P ($mg\ L^{-1}$)	NH_4^+-N ($mg\ kg^{-1}$)	NO_3^--N ($mg\ kg^{-1}$)
Manawatū	4.5	0.4	15	39	2.5	3.7
Waikato	5.7	0.6	19	39	4	4

*CEC = Cation exchange capacity

The daily mean rainfall and soil temperature (at 100 mm depth) during the experimental period at both farm sites are presented in Figure 1. The total amount of rainfall throughout the experimental period was about two times higher at Waikato farm compared to Manawatū farm (363 vs 145 mm). The total rainfall at each farm in the first 2 weeks after urine application was 23 and 65 mm, at the Manawatū and Waikato farms, respectively. There were significant rainfall events in weeks 5, and 6 at the Manawatū farm, and in weeks 5, 6, and 9 at the Waikato farm. The daily mean soil temperatures recorded at the Manawatū farm ranged between 15°C and 20°C and were similar to the soil temperatures at the Waikato farm (15ºC –18ºC).

(a)



(b)

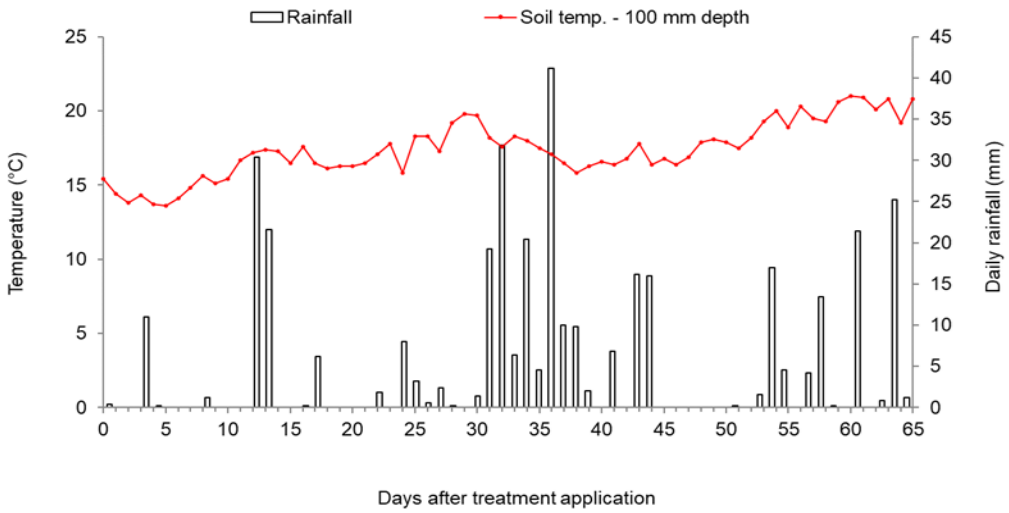
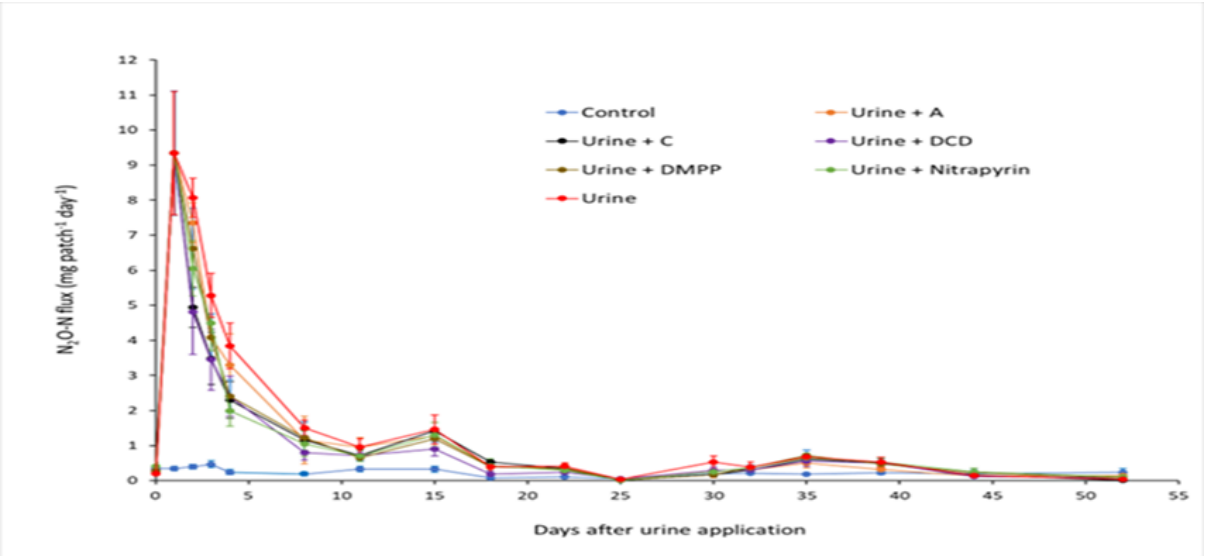


Figure 1. Rainfall events, and soil temperatures at farms during the experimental period: (a) Manawatū farm; (b) Waikato farm.

Nitrous oxide emissions

Temporal variations were observed in the daily N_2O-N fluxes from soils treated with urine (with or without inhibitors) at both farms (Figure 2). At the Manawatū farm, N_2O-N flux from the urine treatments had the highest value (9.3 mg N patch^{-1} day^{-1}) on day 1 after urine application (i.e., before inhibitor application). However, the largest peak of N_2O-N flux from the urine treatments was observed on day 3 after urine application at the Waikato farm (2 mg N patch^{-1} day^{-1} for DCD treatment to 4.1 mg N patch^{-1} day^{-1} for urine only treatment). The emissions from DCD treatment continued to be lower than urine only treatment at both farms for the first two weeks, being more effective among the inhibitor treatments.

(a)Manawatū



(b) Waikato

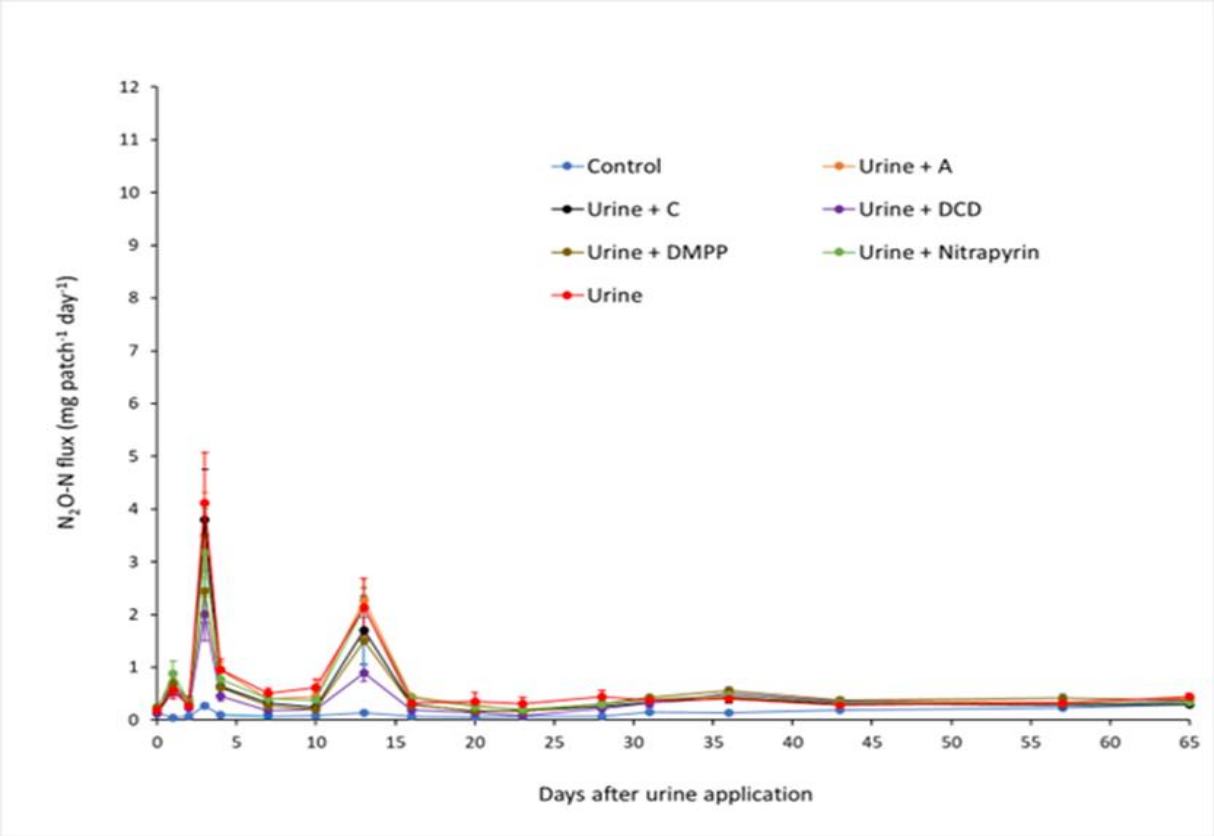
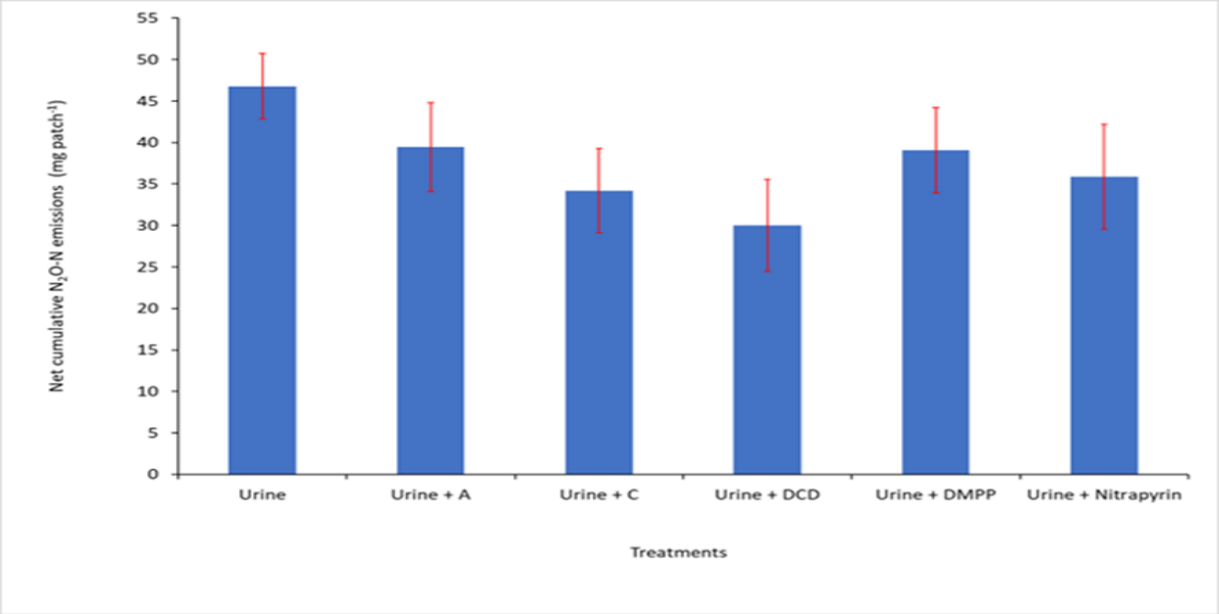


Figure 2. Daily N_2O-N emissions from the urine treatments with and without NIs at the farms: (a) Manawatū farm; (b) Waikato farm. Vertical bars indicate ± standard error values of means, *n* = 8. A and C are confidential NIs supplied by AgResearch; DCD = dicyandiamide; DMPP = 3,4-dimethylpyrazole phosphate; nitrapyrin = 2-chloro-6-(trichloromethyl) pyridine.

The net N_2O-N emissions from the urine treatments with or without inhibitor during the experimental period are presented in Figure 3. Overall, the emissions from the poorly drained soil at the Manawatū farm were higher than those from the well-drained Allophanic soil at the Waikato farm. The average net emissions from the urine only treatment was higher compared to inhibitor treatments (46 vs 30–39 mg N_2O-N patch^{-1} at the Manawatū farm [Figure 3a], and 25 vs 13–24 mg N_2O-N patch^{-1} at the Waikato farm [Figure 3b]). The lower emissions at the Waikato farm were likely to be a result of more leaching losses of N in the free-draining soil in the presence of higher rainfall (363 vs 145 mm, see Figure 1) compared to the Manawatū farm with poorly drained soil. The total emissions in the control treatment during the experimental period were 11 and 10 mg N_2O-N patch^{-1} at the Manawatū and Waikato farms, respectively.

(a)



(b)

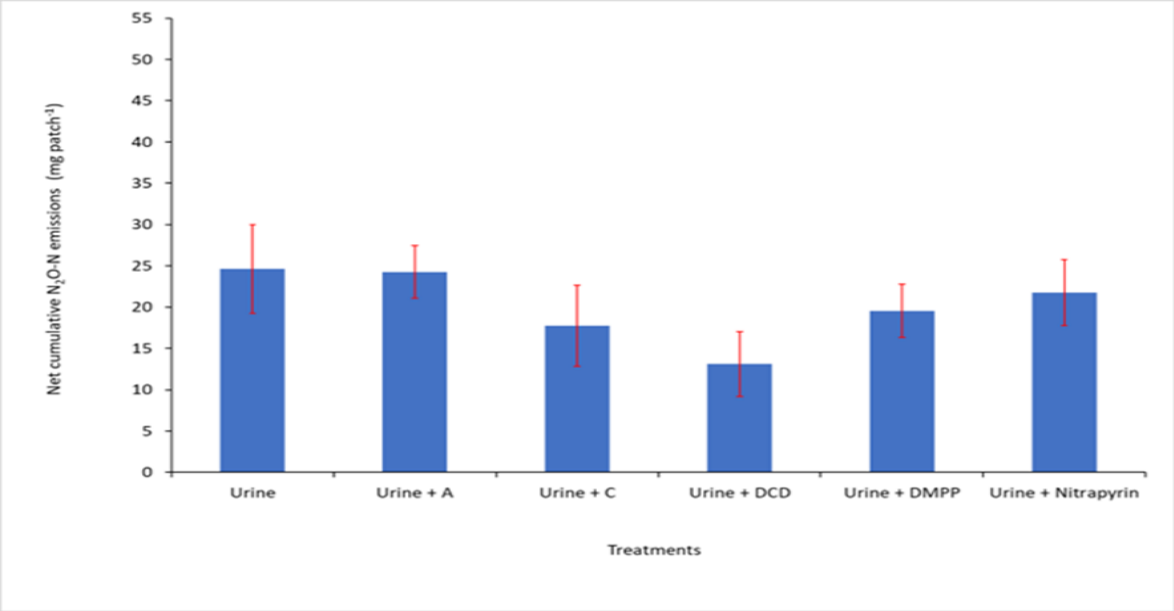
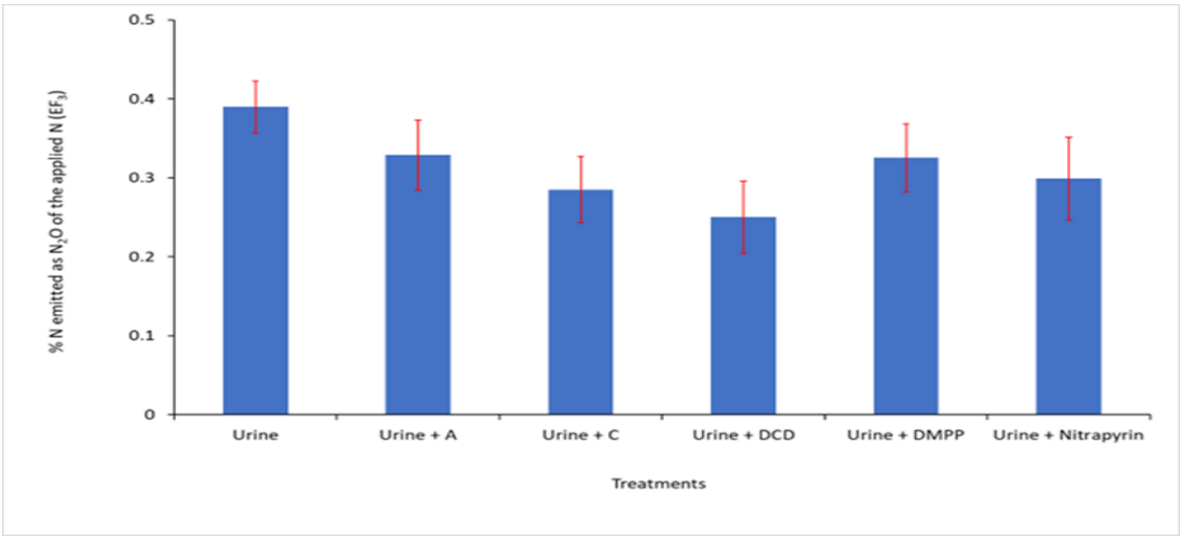


Figure 3. Net total N_2O-N emissions from the urine treatments with and without NIs at the farms: (a) Manawatū farm; (b) Waikato farm. Vertical bars indicate ± standard error values of means, *n* = 8. A and C are the confidential product supplied by AgResearch; DCD = dicyandiamide; DMPP = 3,4-dimethylpyrazole phosphate; nitrapyrin = 2-chloro-6-(trichloromethyl) pyridine.

The mean N_2O EF_3 values for urine only treatment were higher compared to inhibitor treatments (0.39% vs 0.25%–0.33% at the Manawatū farm [Figure 4a], and 0.21% vs 0.11%–0.20% at the Waikato farm [Figure 4]). Emission reduction efficiency in both soils (Table 3) was the highest (between 35.8% and 46.7%) with DCD followed by Cyrene (between 26.9% and 27.9%). However, statistically significant reductions were achieved only with DCD treatments at both farms and with AgResearch inhibitor C at the Manawatū farm. The inhibitor C has a strong effect in this study where

most of the emissions occurred within 2-3 weeks, compared with the other field and lysimeter trials (Saggar and Adhikari 2022; Saggar et al. 2022) where emissions continued beyond 2-3 weeks (data not included). This may be attributed to majority of the N_2O emitted being mitigated within its strong inhibitory effect of period in the first 2–3 weeks (short half-life). These results suggest that there is a potential to consider C derivatives with slower degradation and extended half-life as NI in mitigating emissions from urine patches. Among the other inhibitors, reductions in emission were low with DMPP/nitrapyrin (11.6%–23.3%) and the least with product A (1.5%–15.6%). The large inherent variation in net N_2O-N emissions (from 29% to 68% of the mean) among the inhibitor's treatment replicates at both field sites reduced the statistical significance of some potential inhibitor's emissions reduction efficiency.

(a)



(b)

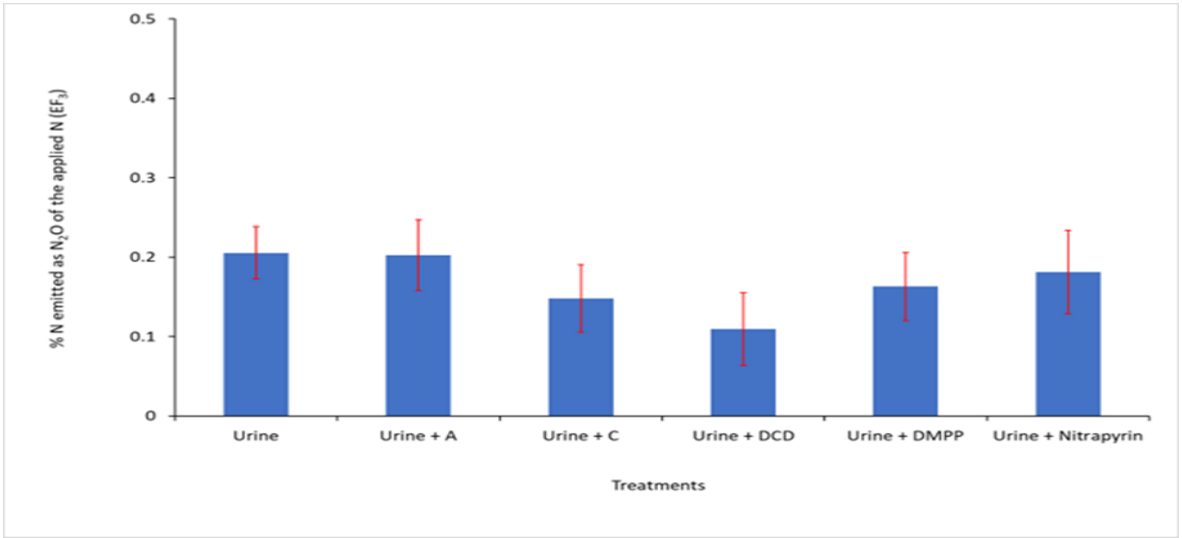


Figure 4. N_2O EF_3 from the urine treatments with and without NIs at the farms: (a) Manawatū farm; (b) Waikato farm. Vertical bars indicate ± standard error values of means, n = 8. A and C = confidential product supplied by AgResearch; DCD = dicyandiamide; DMPP = 3,4-dimethylpyrazole phosphate; nitrapyrin = 2-chloro-6-(trichloromethyl) pyridine.

Table 3. Reduction (%) in N_2O emission factor (EF_3) values by inhibitors at the Manawatū farm, and Waikato farm

Treatment	Reduction in N_2O EF_3 values by inhibitors relative to urine (%)	
	Manawatū farm	Waikato farm
Urine + A	15.6	1.5
Urine + C	26.9*	27.9
Urine + DCD	35.8**	46.7**
Urine + DMPP	16.5	20.7
Urine + nitrapyrin	23.3	11.6

* and ** Following numbers indicates that reduction was significant at $P < 0.1$ and $P < 0.05$, respectively (Fisher's LSD Test)

Conclusions

Our results show that increasing the volume of NIs is a potential option for achieving effective N_2O mitigation from naturally deposited cattle urine patches. The increased volume, rather than the concentration, reduces inhibitor canopy capture, thereby increasing the amount of inhibitor reaching into the soil. This improves downward transport of inhibitors into the urine patch within the soil profile, thus increasing the fraction of urine-N mixed with the threshold concentration of inhibitors for optimal effectiveness. These results warrant further research to optimise inhibitor application rates and volumes for developing best practice for the targeted application of inhibitors to urine patches.

Acknowledgements:

This research was supported by the New Zealand Agricultural Greenhouse Gas Research Centre and in-kind and cash contributions from Pastoral Robotics Limited. Dr Liyin Liang and Helen O'Leary, both from Manaaki Whenua Landcare Research, are acknowledged for reviewing and editing the manuscript, respectively.

References

Adhikari, K.P., Chibuike, G., Saggar, S., Simon, P.L., Luo, J., de Klein, C.A.M., 2021. Management and implications of using nitrification inhibitors to reduce nitrous oxide emissions from urine patches on grazed pasture soils – a review. Science of the Total Environment 791, 148099. https://doi.org/10.1016/j.scitotenv.2021.148099

Adhikari, K.P., Luo, J., Saggar, S., Giltrap, D., 2023. Nitrification inhibitor application time and pasture canopy interception control the proportion of N in the in-situ urien-patch mixing with the inhibitor in grazed pastures. Nutrient Cycling in Agroecosystems *(Submitted).*

Adhikari, K.P., Saggar, S., Hanly, J. A., Guinto, D. F., 2020. Urease inhibitors reduced ammonia emissions from cattle urine applied to pasture soil. Nutrient Cycling in Agroecosystems 117,

317-335. https://doi.org/10.1007/s10705-020-10070-0

Charteris, A.F., Chadwick, D.R., Thorman, R.E., Vallejo, A., de Klein, C.A.M., Rochette, P., Cárdenas, L.M., 2020. Global Research Alliance N_2O chamber methodology guidelines: Recommendations for deployment and accounting for sources of variability. Journal of Environmental Quality 49, 1092–1109. https://doi.org/10.1002/jeq2.20126

FAO–UNESCO, 1998. Soil map of the world. World soil resources report No 84. Rome: FAO.

Giltrap, D., Portegys, N., Saggar, S., Hanly, J.A., 2022. The proportion of deposited urine patch intercepted by a delayed inhibitor application. Environmental Technology 43, 3755–3764. https://doi.org/10.1080/09593330.2021.1934561

Hewit, A., 1992. Soil classification in New Zealand. DSIR Land Resources Scientific Report No. 19. Department of Scientific and Industrial Research, pp 91–120.

Hoogendoorn, C., Saggar, S., Palmada, T., Berben, P., 2018. Do nitrous oxide emissions from urine deposited naturally differ from evenly applied urine? In: Currie LD, Christensen CL eds. Farm environmental planning – Science, policy and practice. Occasional Report No. 31. Palmerston North, New Zealand: Fertilizer and Lime Research Centre, Massey University. Available at http://flrc.massey.ac.nz/publications.html. [Accessed 21 March 2023]

Luo, J., Saggar, S., van der Weerden, T., de Klein, C.A.M., 2019. Quantification of nitrous oxide emissions and emission factors from beef and dairy cattle excreta deposited on grazed pastoral hill lands. Agriculture, Ecosystems and Environment 270, 103–113. http://dx.doi.org/10.1016/j.agee.2018.10.020

Maire, J., Gibson-Poole, S., Cowan, N., Reay, D.S., Richards, K.G., Skiba, U., Rees, R.M., Lanigan, G.J., 2018. Identifying urine patches on intensively managed grassland using aerial imagery captured from remotely piloted aircraft systems. Frontiers in Sustainable Food Systems 2, 10. https://doi.org/10.3389/fsufs.2018.00010

Ministry for Primary Industries, 2023. Food notice: Maximum residue levels for agricultural compounds. Available at https://www.mpi.govt.nz/dmsdocument/19550-maximum-residue-levels-for-agricultural-compounds [Accessed 21 March 2023]

Moir, J.L., Cameron, K.C., Di, H.J., Fertsak, U., 2011. The spatial coverage of dairy cattle urine patches in an intensively grazed pasture system. The Journal of Agricultural Science 149, 473–485. https://doi.org/10.1017/S0021859610001012

Qiao, C.L., Liu, L.L., Hu, S.J., Compton, J.E., Greaver, T.L, Li, Q.L., 2015. How inhibiting nitrification affects nitrogen cycle and reduces environmental impacts of anthropogenic nitrogen input. Global Change Biology 21, 1249–1257. https://doi:10.1111/gcb.12802

Rodriguez, M.J., Saggar, S., Berben, P., Palmada, T, Lopez-Villalobos, N., Pal, P., 2021 Use of a urease inhibitor to mitigate ammonia emissions from urine patches. Environmental Technology 42, 20–31. http://doi.org/10.1080/09593330.2019.1620345

Saggar S., Adhikari K.P., 2022. Efficacy of nitrification inhibitors in reducing nitrous oxide (N_2O) emissions under field conditions. Progress report for New Zealand Agricultural Greenhouse Gas Research Centre. 8 p.

Saggar S., Adhikari K.P., Luo J., Lindsey S., Berben P., Palmada T., 2022. Effect of "Spikey" treated urine patches with inhibitors on N_2O emissions, pasture dry matter yield and N uptake. Progress report for New Zealand Agricultural Greenhouse Gas Research Centre. 13 p.

Saggar, S., Singh, J., Giltrap, D.L., Zaman, M., Luo, J., Rollo, M., Kim, D-G., Rys, G., van der

Weerden, T.J., 2013 Quantification of reductions in ammonia emissions from fertiliser urea and animal urine in grazed pastures with urease inhibitors for agriculture inventory: New Zealand as a case study. Science of the Total Environment 465, 136–146. https://dx.doi.org/10.1016/j.scitotenv.2012.07.088

Zaman, M., Nguyen, M.L., 2012. How application timings of urease and nitrification inhibitors affect N losses from urine patches in pastoral system. Agriculture, Ecosystems and Environment 156, 37–48. https://doi.org/10.1016/j.agee.2012.04.025