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THE CHALLENGE OF ENSURING GOOD PHYSICAL MIXING OF INHIBITOR APPLIED TO A URINE PATCH

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Abstract

Nitrification inhibitors have the potential to reduce nitrogen leaching and nitrous oxide (N_2O) emissions from grazed pastures by slowing the transformation of ammonium (NH_4^+) to nitrate (NO_3^-) in soil, thereby allowing more time for uptake by plants. A review by Di and Cameron (2016) found nitrification inhibitors reduced N_2O emissions from urine patches on average by 57% and NO_3^- leaching by 30%–50%.

It would be desirable to target inhibitor applications to the urine patches only, to reduce the total amount of inhibitor released to the environment and potentially transferred into animal products. However, this necessitates some delay (i.e. 24 to 48 hours) between the grazing event and inhibitor application. During this time the urine could move down the soil profile and therefore not be physically co-located with subsequently applied nitrification inhibitors.

In this study we created 2 L synthetic urine patches by pouring urine onto soil in a manner intended to simulate natural urine deposition. Then, after a delay of 4 to 48 hours, 40 mL of inhibitor solutions was applied using a spray unit. The inhibitors investigated were DCD (dicyandiamide), DMPP (3,4-dimethylpyrazole phosphate), and nitrapyrin (2-Chloro-6-(trichloromethyl) pyridine) applied at two different sites (one in the Massey University Dairy #4 site in Manawatu and one in AgResearch Hamilton site in Waikato) and two different inhibitor loadings. The soils were then sampled from 0–20, 20–50, 50–100, and 100–150 mm and the urine-N and inhibitor amounts in each layer were measured. Pasture samples were also collected to determine the proportion of inhibitors retained by the pasture canopy.

The pasture canopy captured around 30-50% of the inhibitor. Of the inhibitor that reached the soil, c. 80%–90% remained in the top 20 mm. In contrast, between 60% and 80% of the urine-N detected was below 20 mm. This suggests that this application method would be less effective at reducing N_2O emissions due to poor mixing between the inhibitor and the urine. We recommend that future studies include a greater volume of water with the inhibitor application to reduce the amount of inhibitor trapped by the plant canopy and improve the transport of the inhibitor in the soil profile to maximise the effectiveness of inhibitors.

Introduction

Nitrification is a microbial process that transforms ammonium (NH_4^+) to nitrate (NO_3^-) in soils. Nitrification inhibitors (NIs) are compounds that slow down the rate of nitrification. As NO_3^- is more prone to leaching than NH_4^+, slowing the nitrification rate allows more time for plant N uptake, potentially reducing leaching losses. Nitrate may also be denitrified to produce the greenhouse gas nitrous oxide (N_2O). A review by Di and Cameron (2016) found NIs reduced N_2O emissions from urine patches on average by 57% and NO_3^- leaching by 30%–50%. A more

recent review by Adhikari et al. (2021b) found the reductions in N_2O emissions from urine patches using three specific inhibitors: DCD, DMPP, and nitrapyrin were 44 ± 2%, 28 ± 38% and 28 ± 5% (average ± s.e.m.), respectively.

Technologies are available for detecting urine patches (based on electrical conductivity using ground-based Spikey® technology; Bates & Quin (2016)) and mapping urine patches configuration for targeted application of the inhibitor specifically to the patches (Jolly et al. 2021). However, in practice this means there would be a time delay of between a few hours to a couple of days between the urine deposition and the inhibitor application, during which time the urine could travel further down the soil profile. If there is poor physical co-location of the urine and inhibitor, then it is unlikely that any reduction in N_2O emissions will be observed.

In this study, we investigated the distribution of urine-N and inhibitors within the soil profile when the inhibitor was applied to an artificially created urine patch after a delay of 4, 24, or 48 hours. The experiment was conducted at two study sites (Manawatu and Waikato) using three different NIs (DCD, DMPP, and nitrapyrin).

Methodology

Experimental sites and treatments

Field experiments were conducted during spring/summer of 2021 at two typical dairy farms: Massey University No. 4 Dairy Farm (Manawatu) and AgResearch's Ruakura dairy farm (Waikato). The Manawatu site was on a poorly drained Tokomaru silt loam while the Waikato site was on a well-drained Horotiu silt-loam. Both sites were fenced off for 10 weeks prior to the start of the experiment. Table 1 shows some of the soil properties.

Table 1: Basic soil properties of Manawatu and Waikato soils at 0–100 mm depth.

Soil	pH (water)	Total C (%)	Total N (%)	CEC (mEq 100 g^{-1})	Ammonium-N (mg kg^{-1})	Nitrate-N (mg kg^{-1})	Base saturation (%)
Manawatu	5.3	4.5	0.4	14.9	5.6	6.1	82.7
Waikato	5.7	6.8	0.6	23.3	3.5	6.5	41.3

At each site the treatments were: 3 × inhibitor (DCD, DMPP, nitrapyrin); 2 × application rates (approximately 0.5 and 1 g $patch^{-1}$, 0.3 and 0.6 g $patch^{-1}$, and 0.3 and 0.6 g $patch^{-1}$ for DCD, DMPP and nitrapyrin respectively); and 3 × delays between urine deposition and inhibitor application (4, 24, and 48 h). For practicality of sampling, the inhibitors were investigated one at a time. The rest of the treatments were applied in a randomised complete block design, with four replicates.

Individual treatment plots (1 × 1 m), with 0.5 m^2 circular sampling area, were separated by a distance of at least 0.5 m. An additional 2 m buffer zone was established to separate each inhibitor block.

The day before urine application, the grass in the experimental areas was cut to 50 mm height to simulate grazing. Cattle urine deposition was simulated by pouring 2 L of artificial urine (6 g N L^{-1}) onto the centre of the 0.5 m^2 circular sampling area from a height of approximately 1.2 m and allowing it to spread naturally (Fig 1a). The sampling area was kept covered (except during inhibitor application) to avoid any effects from rain on urine movement.

(a)  (b) 

Figure 1. Apparatus used to (a) apply urine patches; (b) apply inhibitor solutions.

Urine was applied in the morning and inhibitors were applied 4, 24, and 48 h after urine application using a Spikey® spot-spray unit, calibrated for delivering 40 mL of inhibitor solution in a 400 mm wide strip (Fig 1b) across the chamber diameter (800 mm). This is equivalent to an application rate of 1307 L ha^{-1} giving dose rates of 1.5 and 3.0 $g.m^{-2}$, 0.9 and 1.8 $g.m^{-2}$, and 1.0 and 1.9 $g.m^{-2}$ for DCD, DMPP, and nitrapyrin respectively.

Pasture and soil sampling

Pasture samples were collected from a 200 mm ring area within 2 h of inhibitor application to determine the proportion of inhibitor captured by the pasture canopy. Samples were transferred to 200 mL plastic containers to avoid any potential loss of inhibitors and kept frozen until lab analysis.

Immediately after pasture sampling, 13 soil cores (150 mm depth) were collected from each patch over a 600 mm diameter area centred on the middle of urine. One sample was taken at the centre of the patch, then samples were taken at 100, 200 and 300mm from the centre along 4 transects (Fig 2).

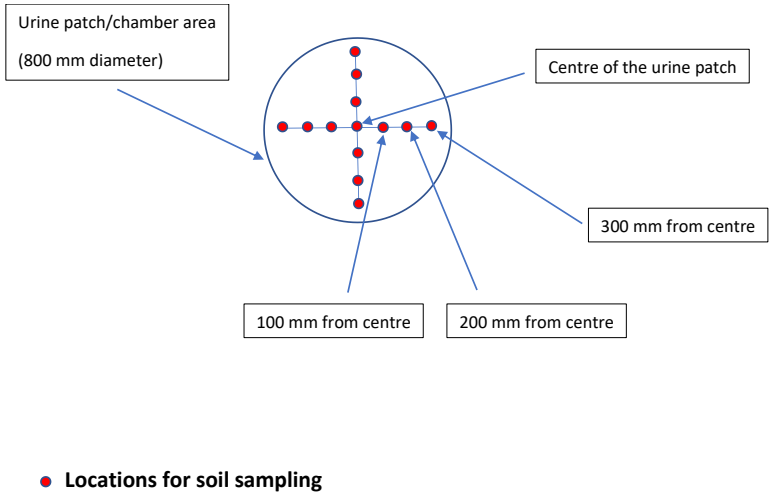


Figure 2. Layout for soil sampling.

Each soil core was cut into 4 parts (0–20, 20–50, 50–100, 100–150 mm), bulked by plot, and sieved to 4 mm, removing plant materials including roots. Sieved samples were mixed thoroughly and subsampled for analysis of inhibitor, urea, mineral-N, and moisture content. Inhibitor analyses were either performed immediately or samples were frozen if this was not feasible.

Laboratory analyses

To determine total N and mineral-N, soil was extracted with 0.5 M K_2SO_4 (1:5, wt/v) and analysis was performed using oxidative combustion-infrared and colourimetric methods, respectively (Mulvaney 1996). Urea as organic N was calculated as the difference between total N from urine and mineral-N from urine.

Measurement of DCD was performed using the methods described in Schwarzer and Haselwandter (1996) and Kim et al. (2012). The concentrations of DCD in the acidified extracts were determined on an UltiMate 3000 HPLC system (ThermoFisher Scientific, NZ) using a ROA-organic acid H^+ column (150 × 4.6 mm) and Diode-Array Detection (DAD) technique. DMPP and nitrapyrin extraction and analysis were carried out following methods as described in Adhikari et al. (2021a), using HPLC-DAD and gas chromatography-mass spectrometry (GC-MS) techniques, respectively. As two of the 13 samples were outside the inhibitor spray zone, results were multiplied by 11/13 to get the inhibitor mass kg^{-1} soil.

Determining the threshold concentration of inhibitor

For the purposes of this study, we assumed that an inhibitor would be effective when its concentration in the soil was equivalent to the concentration that reduced nitrification rates by 40% under laboratory conditions. We defined this concentration as the "threshold concentration". Values for threshold concentrations for each soil and inhibitor were based on a previous laboratory study (Chibuike et al. 2022) and are shown in Table 2.

Table 2. Threshold concentrations by soil and inhibitor type (Chibuike et al. 2022). Note 40% reduction in nitrification was not achieved in the Tokomaru soil at the highest concentration of DMPP tested.

Inhibitor	Threshold concentration ($mg.kg^{-1}$ soil)	
	Manawatu (Tokomaru)	Waikato (Horotiu)
DCD	2.5	3.4
DMPP	-	13
Nitrapyrin	4.95	6.8

Results

Inhibitor capture by pasture canopy

Figures 3–5 show the fraction of the applied DCD, DMPP, and nitrapyrin respectively recovered on the pasture canopy following a 4, 24, and 48 h delay between the urine patch deposition and inhibitor application.

Between 24% and 60% of the applied inhibitor was recovered from the pasture canopy. This means that only 40%–76% of the inhibitor reached the soil. There was a slight trend for the

amount of inhibitor captured to increase with increasing time delay. This could be due to the additional grass growth during this time (Table 3) particularly at Manawatu site.

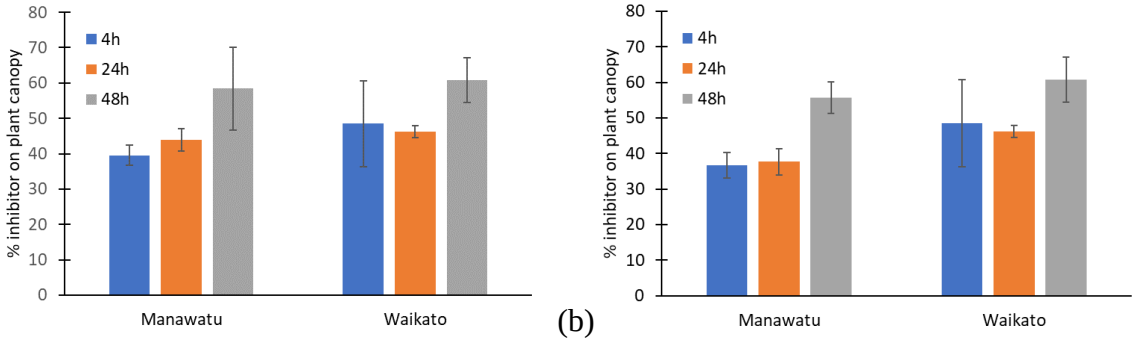


Figure 3. Fraction of DCD recovered in the plant canopy at two different sites with delays between urine and inhibitor application of 4, 24, and 48h. DCD application rate was (a) 0.5 g patch^{-1} ; (b) 1 g patch^{-1}. Error bars represent standard errors of 4 replicates.

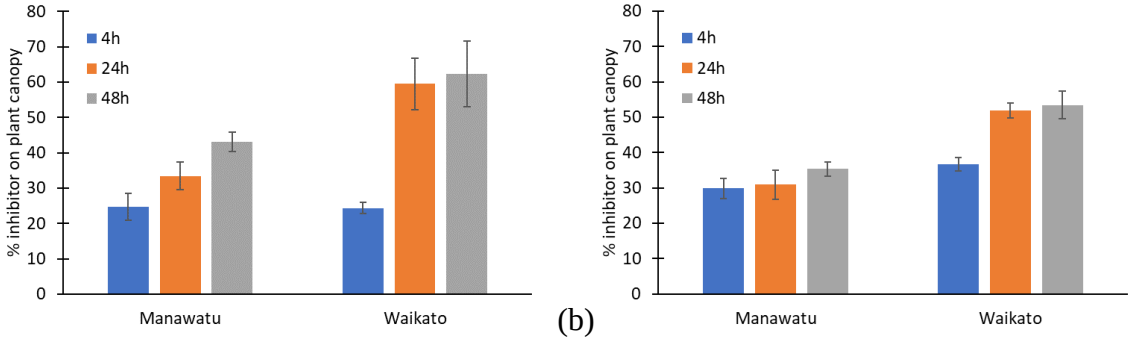


Figure 4. Fraction of DMPP recovered in the plant canopy at two different sites with delays between urine and inhibitor application of 4, 24, and 48 h. DMPP application rate was (a) 0.3 g patch^{-1} and (b) 0.6 g patch^{-1}. Error bars represent standard errors of 4 replicates.

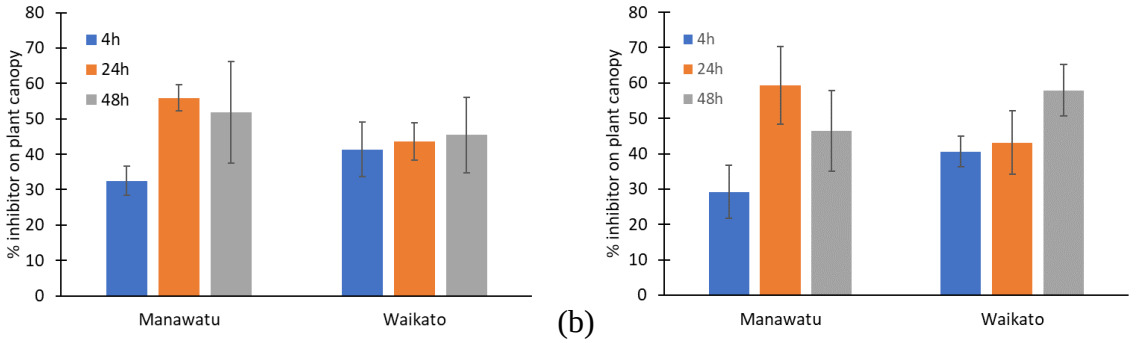
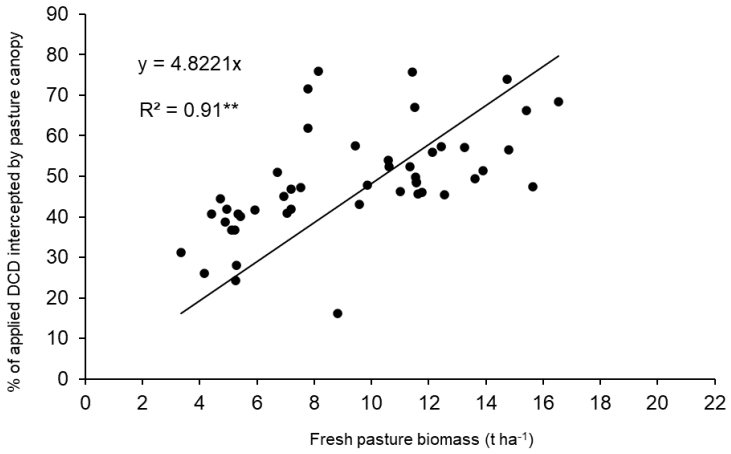
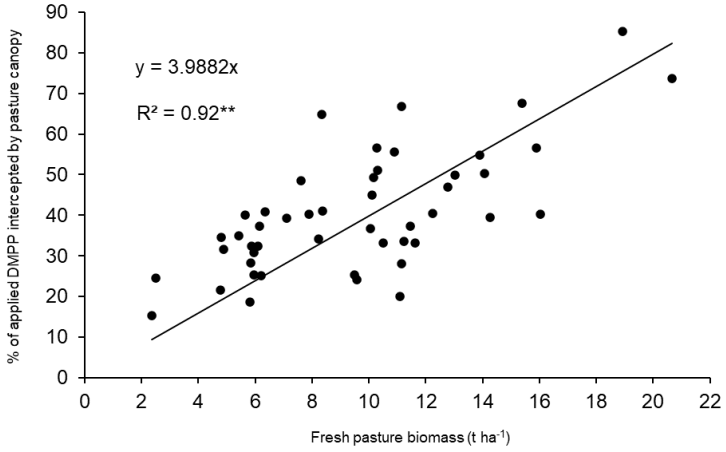


Figure 5. Fraction of nitrapyrin recovered in the plant canopy at two different sites with delays between urine and inhibitor application of 4, 24, and 48 h. Nitrapyrin application rate was (a) 0.3 g patch^{-1} and (b) 0.6 g patch^{-1}. Error bars represent standard errors of 4 replicates.



(a)



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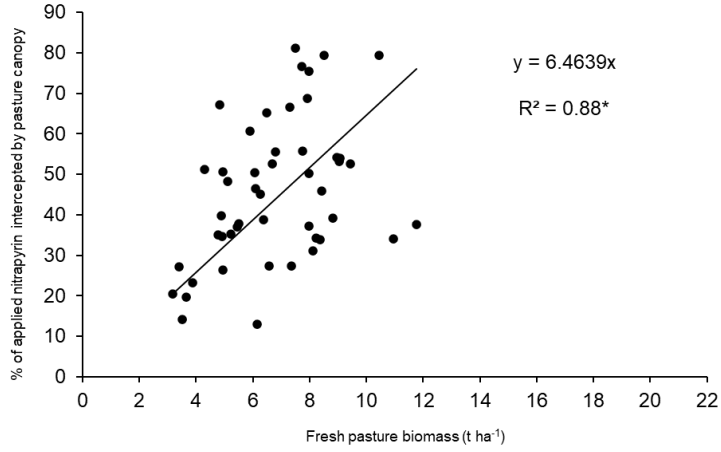

(c)

Figure 6. Relationship between fresh pasture biomass and proportion of applied inhibitor intercepted by pasture canopy: a) DCD treatments; b) DMPP treatments; c) nitrapyrin treatments. * $P < 0.01$, ** $P < 0.001$.

Table 3. Fresh pasture biomass (mean ± s.e.m. combined across the inhibitor rates, *n* = 8) in the Manawatu and Waikato farms. DCD = dicyandiamide; DMPP = 3,4-dimethylpyrazole phosphate; nitrapyrin = 2-Chloro-6-(trichloromethyl) pyridine.

Timing of inhibitor application after urine application	Fresh pasture biomass (kg ha^{-1})		
	DCD treatments	DMPP treatments	Nitrapyrin treatments
Manawatu			
4 h	4875 ± 396^{b}	5112 ± 697^{b}	4902 ± 589^{a}
24 h	6144 ± 351^{b}	7380 ± 715^{ab}	6306 ± 542^{a}
48 h	9346 ± 868^{a}	8678 ± 1189^{a}	5742 ± 456^{a}
Waikato			
4 h	11721 ± 907^{a}	11806 ± 795^{ab}	8339 ± 931^{a}
24 h	11606 ± 1126^{a}	13935 ± 1323^{a}	7601 ± 393^{a}
48 h	10389 ± 862^{a}	10389 ± 1348^{b}	7803 ± 578^{a}

Means followed by different lower-case letters in a column are significantly different (Fisher's LSD test, $P < 0.05$).

The relationship between fresh pasture biomass and the proportion of applied inhibitor intercepted by pasture canopy is presented in Figure 6. There was a positive relationship between fresh pasture biomass standing and the proportion of each inhibitor intercepted by pasture canopy.

Urine distribution in the soil profile

Figures 7 and 8 show the distribution of urine in the soil profiles for the Manawatu and Waikato sites. The distributions have been averaged over the three inhibitors (each inhibitor was investigated at a different time leading to different soil moisture contents) and two inhibitor dose rates. Note that because the area of the urine patch was unknown, we could not estimate the percentage of the total urine-N recovered.

In both soils the amount of urine-N decreases with depth, but there was still about 10% of the recovered N in the lowest measured depth range (100–150 mm). With increasing time since urine deposition, we see the amount of urea-N decrease (due to urea hydrolysis) and the amount of NO_3^- increase (via nitrification).

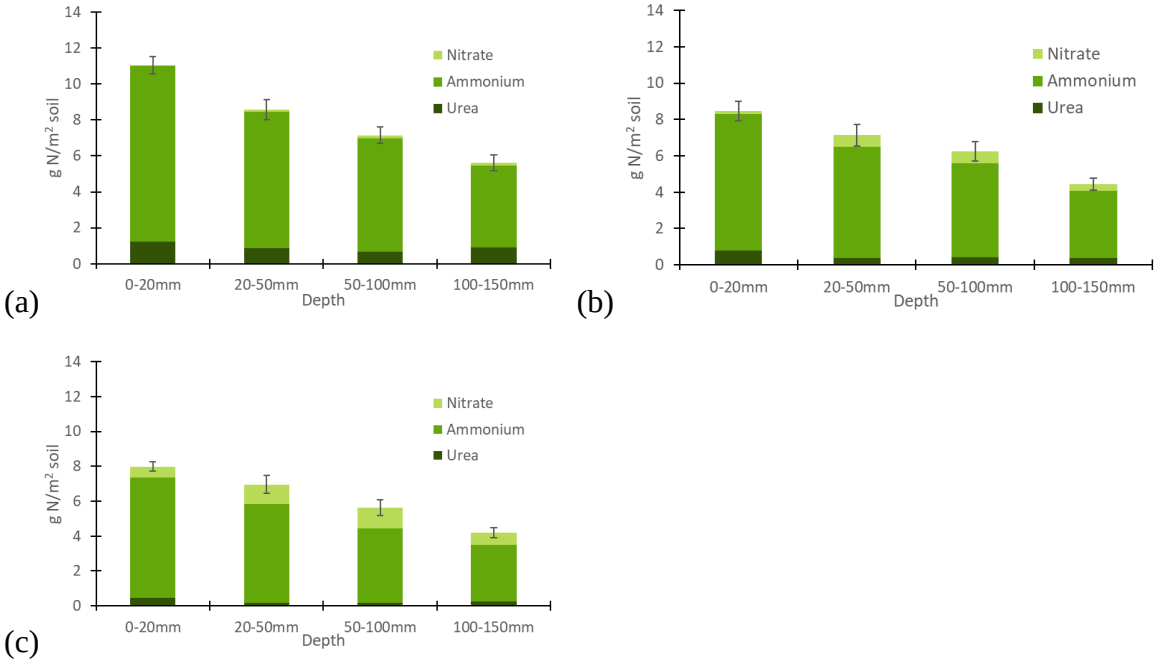

Figure 7. The amount of urine-N (as urea, ammonium, and /nitrate) recovered at different soil depths at the Manawatu site measured at (a) 4 h; (b) 24 h; (c) 48 h after urine deposition. Error bars represent standard errors.

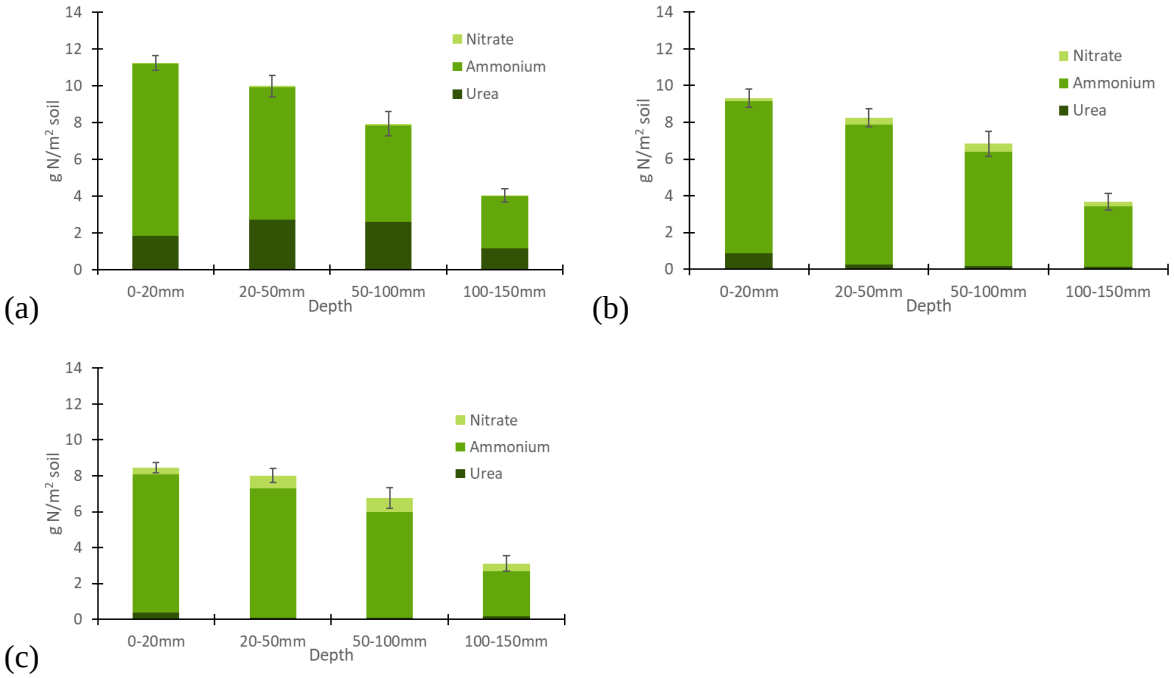

Figure 8. The amount of urine-N (as urea, NH_4^+, and NO_3^-) recovered at different soil depths at the Waikato site measured at (a) 4 h; (b) 24 h; (c) 48h after urine deposition. Error bars represent standard errors.

Inhibitor concentration in the soil

Figures 9–11 show the inhibitor concentration in the soil profile for the DCD, DMPP, and nitrapyrin treatments respectively. The results are averaged across the different inhibitor delay times. Increasing the inhibitor loading resulted in higher concentrations in the top 0–20mm of

the soil, but in general did not result in the threshold concentration being reached in any of the deeper soil layers (the exception being the 1.0 g.patch^{-1} application of DCD at the Waikato site).

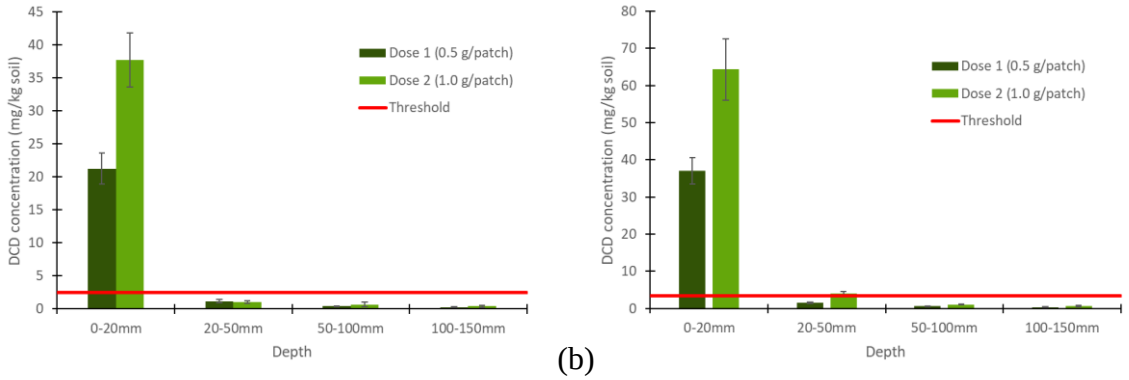

Figure 9. DCD concentration in the soil profile for (a) Manawatu (Tokomaru soil); (b) Waikato (Horotiu soil) sites at two application rates. The horizontal red line shows the threshold concentration for DCD in each soil. Error bars represent standard errors.

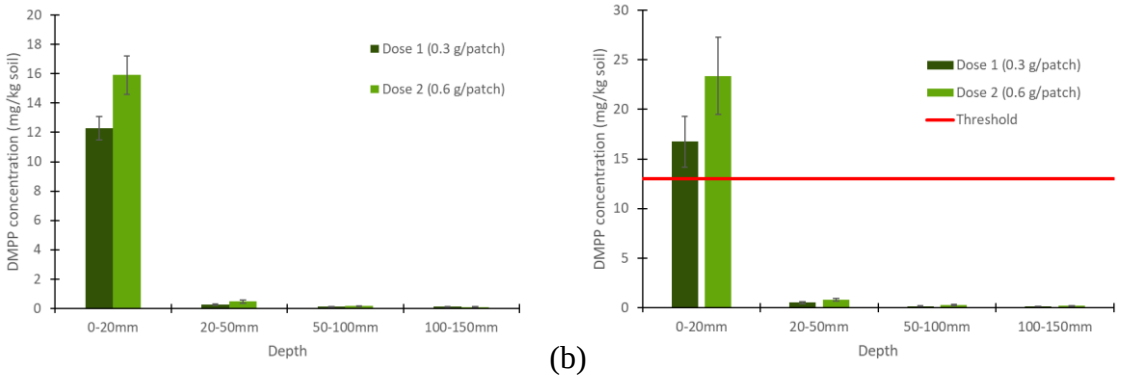

Figure 10. DMPP concentration in the soil profile for (a) Manawatu (Tokomaru soil); (b) Waikato (Horotiu soil) sites at two application rates. The horizontal red line shows the threshold concentration for DMPP (which is unknown for the Tokomaru soil). Error bars represent standard errors.

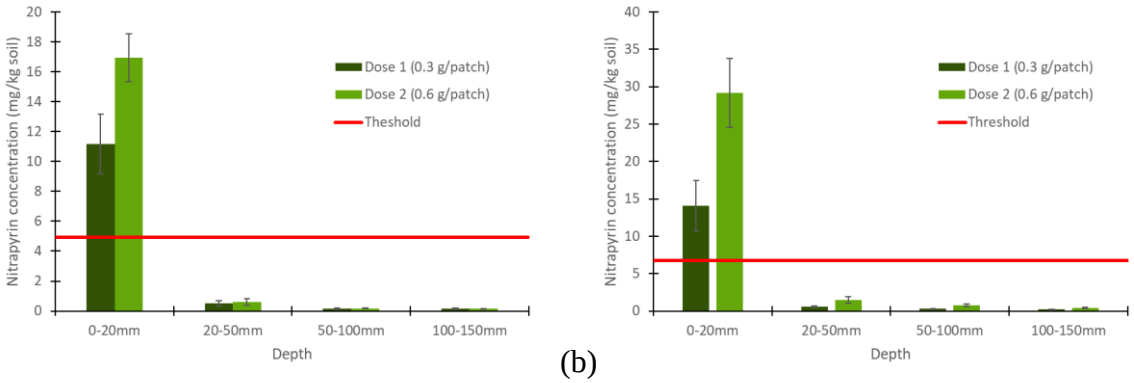

Figure 11. Nitrapyrin concentration in the soil profile for (a) Manawatu (Tokomaru soil); (b) Waikato (Horotiu soil) sites at two application rates. The horizontal red line shows the threshold concentration for nitrapyrin in each soil. Error bars represent standard errors.

Discussion and conclusions

In almost all cases, the inhibitor threshold concentration was only met in the top 0–20 mm of the soil. This corresponded to only ~20-40% of the urine-N being co-located with an effective concentration of the inhibitor. Thus, we would expect the effectiveness of the inhibitor at reducing N_2O emissions to be similarly limited. This could explain why Adhikari et al. (2021c) failed to find an effect of inhibitors on N_2O emissions. In the one case where there was an effective inhibitor concentration down to 50 mm this proportion increased to 68 ± 3%.

We identified two major issues with the physical distribution of the inhibitors. First, a high proportion of each inhibitor was captured on the plant canopy, limiting the amount that makes it to the soil. Second, any inhibitor that did reach the soil was not transported very far down the profile compared to the urine transport. We hypothesise that this is due to the relatively low volume of the inhibitor applied (40 mL) compared to the urine volume (2 L). Current NZAGRC-funded research trials with increased inhibitor : urine volume ratio from 1:50 to 1:10 are indicating higher levels of reduction in N_2O emissions, as reported in reviews of Di and Cameron (2016) and Adhiakari et al. (2021b).

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