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EFFECTIVENESS AND LONGEVITY OF THE UREASE INHIBITORS 2-NPT AND NBTPT IN REDUCING NH_3 EMISSIONS FROM CATTLE URINE APPLIED TO PASTURE SOILS

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Abstract
Our previous laboratory study (Adhikari et al. 2018) suggested that the urease inhibitor N-(2-Nitrophenyl) phosphoric triamide (2-NPT) may reduce ammonia (NH_3) emissions from cattle urine applied up to 58 days after its application to pasture soils, exhibiting greater longevity compared to the N-(n-butyl) thiophosphoric triamide (nBTPT). The objective of this study was to compare the effectiveness and longevity of 2-NPT with nBTPT in reducing NH_3 emissions from cattle urine under field conditions. Two field-plot experiments were conducted during summer and autumn at Massey University No.1 Dairy Farm pasture site in Palmerston North. In the summer experiment, the inhibitors nBTPT and 2-NPT were applied at the start of the experiment and urine was applied at 3 stages; (A) 3 hrs before, (B) 28 days after, and (C) 68 days after inhibitor application. In the autumn experiment, urine was only applied either 3 hrs before or immediately before inhibitor application. Only 2-NPT significantly reduced total NH_3 emissions in summer and only when urine was applied at stage (B). In the autumn experiment, both inhibitors significantly reduced total NH_3 emissions when urine was applied either 3 hrs before or immediately before inhibitor application, however effectiveness was greater when urine was applied immediately before inhibitor application. The reduction was greater with 2-NPT compared to nBTPT when urine was applied immediately before the inhibitors. Overall, 2-NPT showed a greater effectiveness and longevity at reducing NH_3 emissions compared to nBTPT.

Introduction
The background to this field study is given in (Adhikari et al. 2018), which suggested that 2-NPT may reduce emissions from cattle urine applied up to 58 days after its application to pasture soils. However, no field study has evaluated the effectiveness and longevity of 2-NPT and nBTPT in reducing emissions from cattle urine applied to pasture soils. Therefore, the objective of this study was to determine and compare the effectiveness and longevity of 2-NPT with nBTPT in reducing NH_3 emissions from cattle urine applied to pasture soils in field plot experiments.

Materials and methods

Experimental site
Two experiments were conducted at the Massey University No.1 Dairy Farm, Palmerston North, New Zealand. The first experiment was initiated on 23rd of November 2017 and the second experiment was initiated on 1st of May 2018, and hereafter referred as 'summer experiment' and 'autumn experiment' (southern hemisphere) in the subsequent discussion in

this manuscript, respectively. The physio-chemical properties of the soil at the site at the beginning of summer experiment are presented in Table 1.

Table 1 Initial physio-chemical properties of the site soil from 0-5 and 5-10 cm depths (November 2017)

Depth (cm)	pH water	Total C (%)	Total N (%)	CEC (meq 100 g^{-1})	Soil UA (mg kg^{-1} soil hr^{-1})	Field capacity (%)	Bulk density (Mg m^{-3})
0-5	5.5	2.8	0.3	18.5	37	37	1.2
5-10	5.5	2.2	0.3	16.5	21	33	1.4

Experimental design and treatments

Both summer and autumn experiments consisted of a completely randomised block design using four replicates of each treatment. In the summer experiment, the treatments were i) Control (water only), ii) Urine, iii) Urine + nBTPT, and iv) Urine + 2-NPT. Treatments were repeated, using separate plots, for three urine application timings: 3 hrs before inhibitor application (Stage-A), 28 days after inhibitor application (Stage-B), and 68 days after inhibitor application (Stage-C); and NH_3 emissions measurement were performed following each urine application. The application rates of inhibitors to the urine treatments for all three stages were the same and based on the percentage of total urine-N applied at Stage-A. The variability in applied urine N concentration across the all 3 stages resulted in small differences in the application rate of both inhibitors (0.025% vs 0.021%) relative to the amount of N applied for equivalent treatments at different stages (Table 2).

Table 2 The application rates of inhibitors to treatments at different stages of urine application in summer experiment

Stage	Urine application time	Inhibitor rates to treatments (% of urine N applied)			
		Control	Urine	Urine + nBTPT	Urine + 2-NPT
A)	3 hrs before inhibitor application	–	–	0.025	0.025
B)	28 days after inhibitor application	–	–	0.021	0.021
C)	68 days after inhibitor application	–	–	0.021	0.021

In the autumn experiment, the six treatments included were: i) Control (water only), ii) Urine, iii) Urine immediately before nBTPT application (Urine + nBTPT immediate), iv) Urine immediately before 2-NPT application (Urine + 2-NPT immediate), v) Urine 3 hrs before nBTPT application (Urine + nBTPT 3hrs), and vi) Urine 3 hrs before 2-NPT application (Urine + 2-NPT 3 hrs). The inhibitor application rate was 0.025% of urine N applied.

Individual treatment plots were separated by a distance of at least 50 cm. The pasture in experimental area was mown at 3 cm height before inhibitor/urine application to simulate grazing. The solutions of the nBTPT and 2-NPT treatments were prepared by dissolving Agrotain® (liquid form) and 2-NPT salt in deionised water, respectively. A solution volume equivalent to 800 L ha^{-1} was sprayed on to the pasture plot areas using a syringe connected the lid of 50 mL plastic atomizer. The urine was applied to the plot areas using a plastic container.

Meteorological data
Ground level air temperatures inside and outside the chambers were recorded throughout the experimental period using Temperature MicroLogger (G) (Hortplus, New Zealand). Additionally, soil temperatures at 5 cm depth was also recorded in summer experiment but not in the autumn experiment as Temperature MicroLoggers used in autumn was not working. Rainfall data were obtained from a meteorological station located at a distance of about 500 m from the experimental site.

Ammonia emission
Ammonia emissions were measured using the dynamic chamber method as described by Kissel et al. (1977). The PVC chambers (15 cm internal diameter, 4 cm height with a tightly sealed transparent lid to allow photosynthesis) were inserted into the soil at a depth of 1 cm, providing a headspace volume of 500 cm^{-3}. Air from each chamber was sucked at a constant flow rate of 6 L min^{-1} (monitored daily) throughout the experimental period and was then passed through the acid solution to capture NH_3 emitted. The NH_4^+-N concentration in acid solution was measured using a Technicon AutoAnalyzer. The NH_3-N flux (kg ha^{-1} d^{-1}) was calculated by using Eqn. 1.

$$NH_3\text{–}N\ flux = \frac{C \times V}{a \times D \times 100} \qquad Eqn.\ 1$$

where, C = NH_3 concentration in the acid solution (mg L^{-1}); V = the total volume of acid solution at the time of sampling (L); a = total cross-sectional area (m^2) of the chamber inserted into the soil; D = duration (days) of each sampling.

The percentage reduction in total NH_3–N flux with the application of inhibitors relative to the urine only treatment was calculated using Eqn. 2.

$$\%\ reduction\ in\ NH_3 - N\ flux = \frac{C - A}{C} \times 100\% \qquad Eqn.\ 2$$

where, C = total NH_3-N emitted from urine only treatment; A = total NH_3-N emitted from treatment with inhibitor.

Urine analysis
Urine was collected from dairy cattle during milking at the Massey University's Dairy Farm 1 and Dairy Farm 4. The chemical properties and application rates of urine, expressed on a total N in kg ha^{-1}, used in all of the experiments are presented in Table 3.

Table 3 Chemical properties of cattle urine and application rates used in the study

Summer Experiment		pH	Urea-N (g L^{-1})	Total N (g L^{-1})	Application depth (mm)	N applied (kg ha^{-1})
Stage	Urine application time					
A)	3 hrs before inhibitor application	8.1	3.6	6.0	10	597.2
B)	28 days after inhibitor application	8.0	5.2	7.3	10	725.9
C)	68 days after inhibitor application	8.0	5.7	7.2	10	721.4
Autumn Experiment		7.5	4.9	5.9	10	589.7

Statistical methods

The data for NH_3 emissions were analysed using an ANOVA to detect any significant difference and treatment means were compared using Tukey's Studentized Range (HSD) Test. All of the analyses were conducted using the Statistical Analysis System software (SAS 9.4, $P < 0.05$).

Results

Meteorological data

The total rainfall during the summer experiment at stages A, B, and C was 5, 78, and 65 mm, respectively (Fig. 1) and was 28 mm during the autumn experiment (Fig. 2). Most of the rainfall occurred 5 days after urine application during the summer and autumn experiments. Soil water contents varied with rainfall events and was generally higher in autumn compared to summer (Figs. 1 and 2). The soil water contents were similar between the treatments, therefore the mean values for all the treatments are presented.

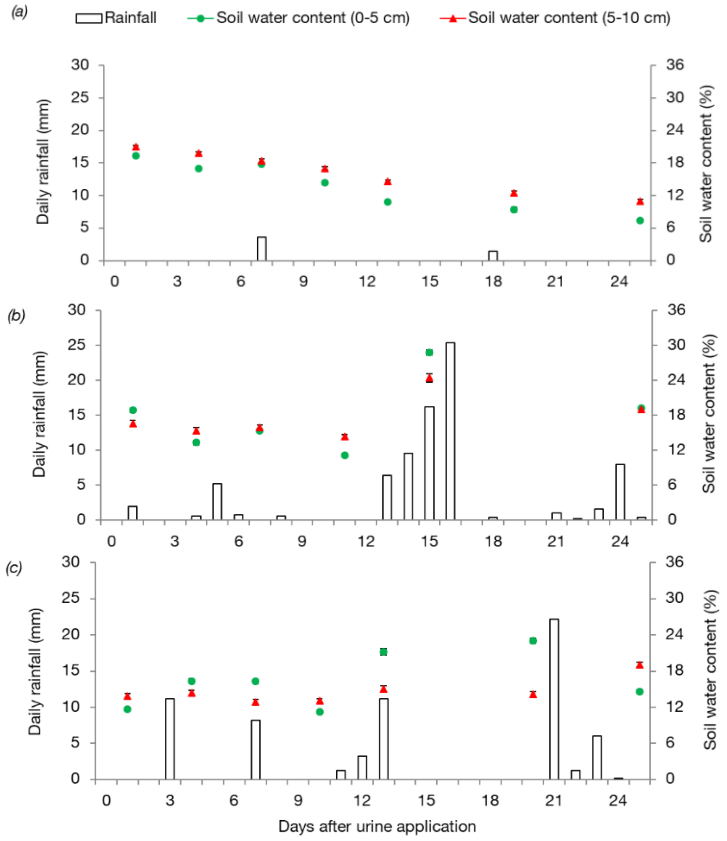

Fig. 1 Rainfall events, and soil water content in different soil depths during the summer experiment: *(a)* urine 3 hrs before the inhibitor *(b)* urine 28 days after the inhibitor, and *(c)* urine 68 days after the inhibitor application, vertical bars on soil water content data indicate standard error values

During the summer experiment, the average daily ground level air temperatures inside the chambers were between 16.3 - 23.6ºC, and outside of the chambers were between 14.8 - 23.4ºC (Fig. 3). During the autumn experiment, the temperatures inside the chambers were between 10.5 - 15.7 ºC, and outside of the chambers were between 7 - 15ºC (Fig. 4). The soil temperatures at the depth of 5 cm were between 16.2 - 25.1ºC during the summer experiment (Fig. 3).

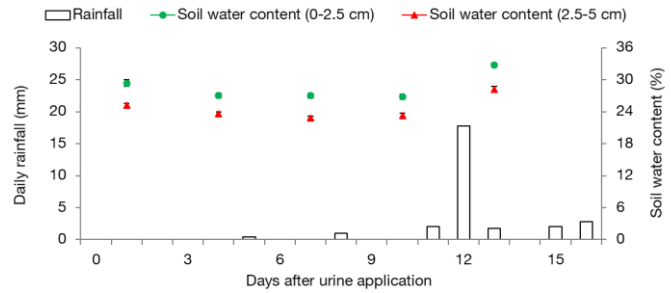

Fig. 2 Rainfall events, and soil water content in different soil depths during the autumn experiment, vertical bars on soil water content data indicate standard error values

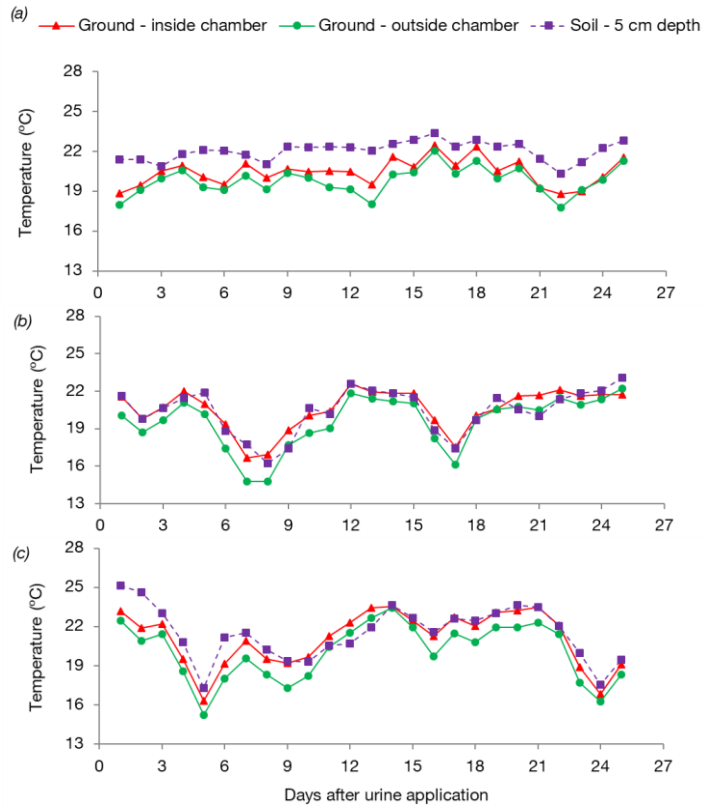

Fig. 3 Ground level air temperatures (inside and outside chamber) and soil temperatures during the summer experiment: *(a)* urine 3 hrs before the inhibitor *(b)* urine 28 days after the inhibitor, and *(c)* urine 68 days after the inhibitor application

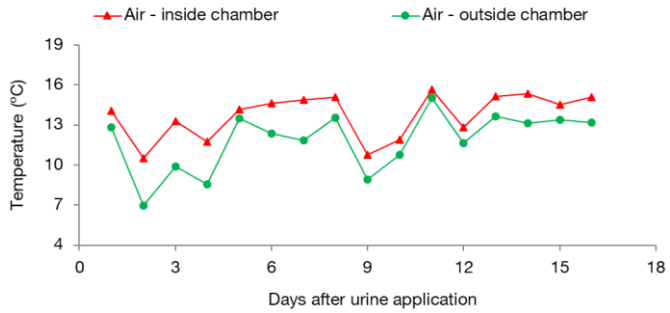

Fig. 4 Ground level air temperatures (inside and outside chamber) during the autumn experiment

Ammonia emission

Total NH_3 emitted from the urine only treatment significantly varied between the two seasons of summer (111 - 142 kg N ha^{-1}) and autumn (27 kg N ha^{-1}), being higher in summer. The NH_3-N emitted in summer was between 15.3 - 23.6 % of the total N applied (Table 4), however, in autumn the emissions were only 4.5 % of the total N applied (Table 5). Most emissions occurred within the first day of urine application. Total emissions from control were significantly lower than treatments with urine with or without inhibitors in both seasons.

Table 4 Effect of inhibitors on NH_3-N emissions from urine-N added to pasture soil at different Stages; A) 3 hrs before, B) 28 days after, and C) 68 days after inhibitor application during the summer experiment [mean (standard error)]

	Stage-A	Stage-B	Stage-C
N added (kg ha^{-1})	597.2	725.9	721.4
Treatments	Cumulative NH_3-N emissions (kg ha^{-1})		
Control	0.8 (0.1) [b]	0.3 (0) [c]	0.7 (0.1) [b]
Urine	141.6 (4.2) [a]	133.2 (5.5) [a]	111.3 (3.3) [a]
Urine + nBTPT	142.3 (6.4) [a]	125.1 (1.9) [a]	113.6 (6.8) [a]
Urine + 2-NPT	144.7 (2.0) [a]	107.2 (3.9) [b]	116.8 (3.0) [a]
	% change in cumulative NH_3-N relative to urine only treatment		
Urine + nBTPT	+0.5	-6.1	+2
Urine + 2-NPT	+2.2	-19.5	+4.9
	% N emitted as NH_3 of the applied N		
Urine	23.6	18.3	15.3
Urine + nBTPT	23.7	17.2	15.6
Urine + 2-NPT	24.1	14.7	16.1

Means followed by different lowercase letters in a column are significantly different ($P < 0.05$)

In the summer, when urine was applied 3 hrs before the inhibitor application, the temporal variations in the daily NH_3 fluxes from soils treated with urine with or without inhibitors were observed. The NH_3 flux from the urine only treatment possessed the highest value on day 1 of 66 kg N ha^{-1} d^{-1}, which was 46% of total N emitted during the experimental period (Fig. 5 *(a)*). The NH_3 emissions decreased sharply to a level of 12 kg N ha^{-1} d^{-1} by day 2. After day 2, emissions decreased further at a slow rate, but remained slightly above those of the control (no urine) treatment. The daily NH_3 emissions from inhibitor treatments followed a similar trend to those of urine only treatment. There was no effect of either of the inhibitors on reducing total emissions compared to the urine only treatment.

Following urine application at 28 days after inhibitor application in summer, the largest peak of NH_3 flux from the urine only treatment was on day 1 and was 62 kg N ha^{-1} d^{-1} (Fig. 5 *(b)*). Compared to the NH_3 fluxes from the urine only treatment for day 1, emissions were only lower for the treatment with 2-NPT, which were 40 kg N ha^{-1} d^{-1}. There was no significant effect of inhibitors on reducing emissions at subsequent sampling times. Over the measurement period 2-NPT significantly reduced total NH_3 emissions by 26 kg N ha^{-1} (19.5%

reduction) compared to total emissions from the urine only treatment that were equivalent to 133 kg N ha^{-1} (Fig. 5 *(b)* and Table 4).

Table 5 Effect of inhibitors on NH_3-N emissions from urine-N added to pasture soil during the autumn experiment [mean (standard error)]

N added (kg ha^{-1})	589.7		
Treatments	Cumulative NH_3-N emissions (kg N ha^{-1})	% change in cumulative NH_3-N relative to urine only treatment	% N emitted as NH_3 of the applied N
Control	0.3 (0.1) [e]	–	–
Urine	27.1 (1.5) [a]	–	4.5
Urine + nBTPT immediate*	12.9 (0.4) [c]	-52.3	2.1
Urine + 2-NPT immediate*	7.4 (0.6) [d]	-72.7	1.2
Urine + nBTPT 3hrs	17.6 (1) [b]	-35	2.9
Urine + 2-NPT 3 hrs	15.9 (1) [bc]	-41.2	2.7

*Means followed by different lower case letters in a column are significantly different ($P < 0.05$), *after treatment indicates n = 3; (Urine + nBTPT immediate = urine immediately before nBTPT application, Urine + 2-NPT immediate = urine immediately before 2-NPT application, Urine + nBTPT 3hrs = urine 3 hrs before nBTPT application, and Urine + 2-NPT 3 hrs = urine 3 hrs before 2-NPT application)*

With the cattle urine applied 68 days after inhibitor application in summer, the NH_3 flux from the urine only treatment exhibited the highest value on day 1 of 47 kg N ha^{-1} d^{-1}. The day 1 NH_3 fluxes for urine with nBTPT or 2-NPT were not significantly different from the urine only treatment. Emissions decreased sharply during the first few days after urine application, and then gradually decreased further over the subsequent three weeks, achieving values close to the control treatment by day 25. Neither inhibitor significantly reduced total emissions over the entire sampling period, compared to the urine only treatment (Fig. 5 *(c)* and Table 4).

In autumn, all urine treatments (with and without inhibitors), except the urine applied immediately before 2-NPT treatment, had their highest emission in the first day after urine application. For urine applied immediately before 2-NPT treatment, the peak emissions occurred on day 2 (1.2 kg NH_3-N ha^{-1} d^{-1}), which was the lowest value of all of the urine treatments. The NH_3 flux from the urine only treatment on day 1 was 15 kg N ha^{-1} d^{-1} (Fig. 6), which was about one third to quarter the peak emissions on day 1 of the summer experiment. At day 1, the average rate of emissions for the first 3 hrs (1.1 kg NH_3-N ha^{-1} hr^{-1}) was higher than remaining 21 hrs (0.5 kg NH_3-N ha^{-1} hr^{-1}). The majority of the emissions from the treatments with urine occurred during the first 3 days, after which the values were lower and steadily decreased over the subsequent week, achieving values close to the control after day 10. The emissions from urine applied immediately before inhibitors were significantly lower than urine applied 3 hrs before inhibitors on day 1. Both inhibitors significantly reduced total NH_3 emissions compared to the urine only treatment, at both timings, but the effectiveness was greater when urine was applied immediately before the inhibitors. The amount of NH_3 emitted from nBTPT and 2-NPT treatments were 13 and 7 kg N ha^{-1}, respectively when urine was applied immediately before the inhibitors with corresponding reductions of 52.3 and

72.7 % compared to urine only treatment, being a significantly greater reduction from using 2-NPT than nBTPT. However, with the urine applied 3 hrs before the inhibitors, the reductions were 35 and 41.2% for nBTPT and 2-NPT, respectively, with corresponding emissions of 18 kg and 16 kg N ha^{-1} but the difference between the inhibitors was not significant (Fig. 6 and Table 5).

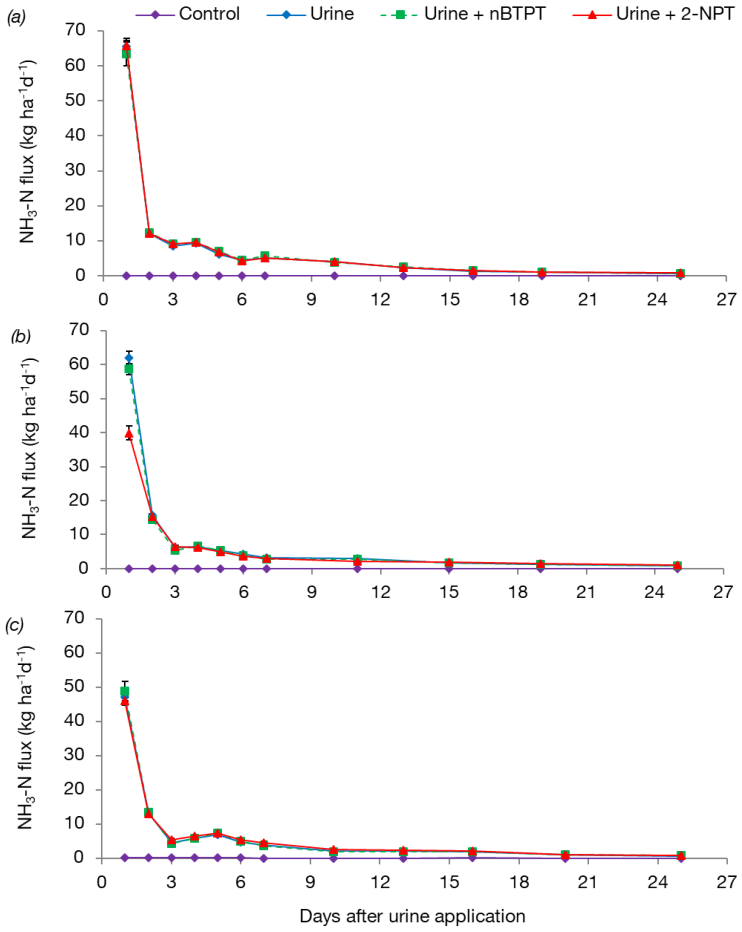

Fig. 5 Effect of applying cattle urine *(a)* 3 hrs before, *(b)* 28 days after, and *(c)* 68 days after the inhibitor application on mean NH_3-N emissions from pasture soils during the summer experiment, vertical bars indicate standard error values

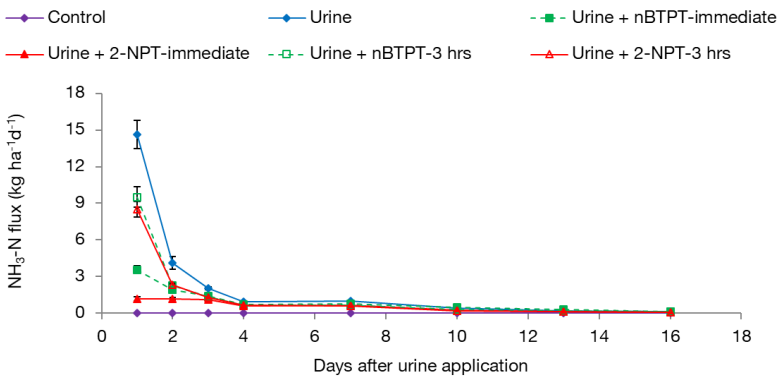

Fig. 6 Effect of applying cattle urine on mean NH_3-N emissions from pasture soils during the autumn experiment, vertical bars indicate standard error values (n= 3 for Urine + nBTPT immediate and Urine + 2-NPT immediate)

Discussion

The total NH_3 emissions from the urine only treatment were higher in summer than in the autumn. Similar losses as a percentage of the applied urine-N have been reported in other NZ field studies, for example, 25.7% in summer (Laubach et al. 2012) and about 5 % in autumn (Zaman and Blennerhassett 2010; Zaman et al. 2013). The higher emissions in summer, in this study, are attributed to higher temperatures (Fig. 3) and drier soil conditions (Fig. 1) than in the autumn (Figs. 6.2 and 6.4).

In this study, the effect of inhibitors at reducing total NH_3 emissions from applied urine was influenced by the season in which the urine was applied, with the inhibitors having an effect in autumn but not in summer, when urine was applied 3 hrs before the inhibitor application. The ineffectiveness of inhibitors in summer could be attributed to the warmer temperatures and drier soil conditions. These conditions would have limited the potential for contact between the inhibitor and the urine urea, and thereby limited the inhibitor's effectiveness.

In contrast, the significant effect of inhibitors in reducing emissions in autumn may be due to potentially slower urea hydrolysis, at lower temperatures, which would have provided better contact and contact time between inhibitor and urine urea. Similarly, higher soil moisture contents in autumn than in the summer would have also enhance the contact between the inhibitor and urine urea. The higher effectiveness of both of the inhibitors when urine was applied immediately before the inhibitors (52.3% from nBTPT and 72.7 % from 2-NPT), compared to urine applied 3 hrs before the inhibitor (35% from nBTPT and 41.2% from 2-NPT) in autumn is probably because of the greater contact between the inhibitor and urine urea when there's a shorter duration between applications.

The inhibitor 2-NPT did reduce NH_3 emissions when urine was applied 28 days after inhibitor application in summer, but not nBTPT. The effect of 2-NPT on reducing emissions was not high (19.5%). The 2-NPT did not reduce emissions from urine applied 68 days after its application in summer. These results indicate that 2-NPT applied to a paddock in summer, following a grazing event, may have an effect at the subsequent grazing when the grazing cycle is short (i.e. $\leq$28 days).

Greater effectiveness of inhibitors in lower temperatures and higher moisture levels, and with the inhibitor applied immediately after urine was observed, compared to warmer and drier soil conditions, and with the inhibitor applied 3 hrs after urine. The inhibitor 2-NPT reduced emissions in the warm summer conditions when inhibitor was present in the soil prior to urine application, showing greater longevity compared to nBTPT, however, overall reductions in N loss via NH_3 emission was low on a whole paddock basis.

Conclusions

The effectiveness of 2-NPT and nBTPT in reducing NH_3 emissions from urine applications was greatly influenced by soil temperature and moisture content at the time of urine application. The timings of inhibitor application in relation to urine application timings also influenced emissions. In summer, only 2-NPT reduced emissions only when urine applied 28 days after inhibitor application. In autumn, both inhibitors were effective in reducing emissions with higher reduction when applied immediately after urine application, compared to 3 hrs after urine.

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