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EFFICACY OF SPIKEY®-APPLIED NITROGEN TRANSFORMATION PROCESS INHIBITORS FOR REDUCING NITROGEN LOSSES FROM URINE APPLIED TO WELL-DRAINED DAIRY SOILS IN AUTUMN/WINTER IN NEW ZEALAND

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Abstract

Urine patches deposited by grazing livestock, which account for much of agricultural nitrogen (N) loss in New Zealand, arise mainly through N leaching to ground water and ammonia (NH_3) and nitrous oxide (N_2O) emissions to the atmosphere. We evaluated the N-loss efficacy of three different N transformation process inhibitors when applied to urine-amended soil 4 h after urine deposition in (a) an autumn-initiated field trial in the Manawatū, and (b) a winter-initiated lysimeter trial in the Waikato. The N transformation process inhibitors were: a urease inhibitor (UI; N-(n-butyl) thiophosphoric triamide); a nitrification inhibitor (NI; nitrapyrine); and Orun® (O; a combination UI and plant growth promotor, gibberellic acid). We also compared two N_2O measurement methods, (1) a standard method where urine +/- an inhibitor is applied to the entire surface area of a static chamber base and (2) where only a proportion of the chamber base area received urine +/- an inhibitor and the remaining area was left untreated, enabling accounting for the effect of soil and plants at the periphery of the treated area on N_2O emissions.

Manawatū: Soil conditions were wet at the time of treatment application (50 mm rain in previous week) and remained wet throughout (220 mm rain in the 4 weeks post application). Approximately 7% of urine N applied (660 kg N ha^{-1}) was volatilised as NH_3; applying a UI reduced emissions by 23%, whereas applying a NI had no significant effect on the amount of NH_3 volatilised. N_2O emissions, which were highest from the U and U+UI treatments (average 10.2 kg N ha^{-1}; EF_3 1.5%), were reduced by 30% in the U+NI+UI and U+O treatments and by 60% in the U+NI treatment (4.4 kg N ha^{-1}; EF_3 0.6%). Between-treatment differences in total emissions and EF_3 were less marked, but still significant, when the effect of plant and soil at the periphery was accounted for. Soil inorganic N concentrations over time reflected treatment differences observed in gaseous N emissions. Net herbage DM and N accumulated in the 18 weeks after treatment application were not significantly different between any of the urine and urine + amendment treatments, but were significantly greater than in the control plots (3.4 vs 1.9 t DM ha^{-1}; 59 vs 109 kg N ha^{-1}).

Waikato: Soil conditions were wet at the time of treatment application (73 mm rain in previous 14 days), 28 mm rain fell within 24 h of treatment application and 144 mm in the 4 weeks post application. N_2O emissions were much lower than in the Manawatū field trial, and adding an amendment shortly after urine (425 kg N ha^{-1}) was applied had no significant

effect on total N_2O emissions (range: 0.28 – 0.48 kg N ha^{-1}) or EF_3 (range: 0.06 – 0.08%). Of the three treatments (U, UI and UO) where total emissions measured via the standard measurement method were compared with the edge effects measurement method, only the UO treatment showed a difference between methods. In this case emissions from the edge effect method were lower than the standard method. Measurement of N leaching is ongoing for a full year after treatment application.

Introduction

Urine patches deposited by grazing livestock account for much of agricultural nitrogen (N) loss in New Zealand, mainly through nitrate (NO_3^-) leaching to ground water and ammonia (NH_3) and nitrous oxide (N_2O) emissions to the atmosphere. Nitrous oxide is a potent greenhouse gas (GHG) and NH_3 emissions to air and NO_3^- loss to water bodies are indirect sources. Nationally, N_2O emissions from agricultural soils represent 10.5% (8526 Gg CO_2–equivalents) of all GHG emissions (MfE, 2016) and arise primarily from urine deposited during grazing. In addition, NH_3 and NO_3^- loss from urine patches are a major source of system N loss and an indicator of costly (environmental and financial) system inefficiencies.

Urine contains high concentrations of N, primarily in the organic form as urea. Once urine comes into contact with soil/plants/litter the urea is rapidly hydrolysed to ammonium (NH_4^+) by urease, an enzyme which is ubiquitous in the environment. The conversion of urine N from a relatively stable organic form to a reactive inorganic form (NH_4^+) increases its vulnerability to loss from the soil/plant system. Ammonium is weakly bound to negatively charged clay particles and soil organic matter and therefore loosely retained in the soil system where NH_4^+ can be oxidised by soil microbes to form nitrite (NO_2^-) and then NO_3^-, both highly mobile ions that move with soil water. Ammonium, NO_2^- and NO_3^- ions are sources for N_2O production by autotrophic and heterotrophic soil microbes (bacteria and fungi) via the processes of ammonification, nitrification and denitrification.

Compounds which delay the breakdown of urea to NH_4^+ (urease inhibitors; UI) have been extensively studied as an N loss mitigation option particularly for cropping systems. The compound nBTPT (N-(n-butyl) thiophosphoric triamide) has been shown to be effective in reducing NH_3 losses particularly when used in tandem with fertiliser urea N in cropping situations and has been shown to be most effective shortly before or after urine deposition to pasture soils (Saggar *et al.*, 2013). It's efficacy as an N_2O loss mitigator from urine deposited during grazing is perhaps more variable (Zaman & Nguyen, 2012).

Nitrification inhibitors (NI) retard the oxidation of NH_4^+ to NO_3^- allowing more time for plant uptake of NH_4^+ and therefore less NH_4^+ available for nitrification should conditions be favourable. Research has shown that co-application of the nitrification inhibitor DCD with animal urine significantly reduces both NO_3^- leaching and N_2O emissions (Gillingham *et al.*, 2014; Luo *et al.*, 2015, 2016). However, low residues have been found in milk from dairy cows grazing DCD applied pastures and the product has been removed from the market in New Zealand. There is also concern over the presence of DCD in water bodies from catchments (Otago) draining land where DCD was applied. Apart from being an additional N source, DCD in water ways has been shown to affect the ratio of NH_4^+:NO_3^- and the concentration of dissolved organic N (DON), with consequent impacts on stream water ecology (Smith and Schallenberg, 2013). Nitrapyrin (2-chloro-6-(trichloromethyl) pyridine), used extensively in cropping systems, has been suggested as an alternative NI for grazed pastures. It has a short half-life, and no undesirable crop/soil residues have been detected to date, although studies on pasture/forage plant residues are lacking. There are few studies

where nitrapyrin has been used as an N loss mitigation option for urine deposited during grazing (Ruser & Schulz, 2015).

Increasing herbage DM production, and therefore uptake of N, should reduce the amount of inorganic N in the soil and therefore reduce risk of N moving to air and water. Gibberellins have been shown to increased herbage dry matter production, particularly when applied in early spring or the late summer/early autumn period (Matthew *et al.*, 2009). However, careful consideration must be given to the impact of increased herbage production on whole (farm) system N loss (Whitehead & Edwards, 2015). A product containing both gibberellins and the nBTPT has been proposed as another possible N loss mitigation option for grazing systems (Quin *et al.*, 2016).

In our previous research, animal excreta (dung, urine) and farm effluent are evenly loaded to the entire N_2O flux measurement area (i.e. static chamber base area) (Luo *et al.*, 2015, 2016; Li *et al.*, 2016). There is some suggestion in the literature that plant roots at the edge of the urine treated area are able to utilize the nearby urinary N, even though these roots have not been directly wetted (Moir *et al.*, 2011). However, there is little data available on a potential urine patch edge effect on N_2O emissions and N leaching and the small amount of work that has been done indicates that accounting for an edge effect is important for estimating urine patch N loss (Koops *et al.*, 1997; Marsden *et al.*, 2016).

The objective of our study was to two-fold: Our first objective was to evaluate the N-loss efficacy of three different NTIs when applied to urine-amended soil four hours after urine deposition in (a) an autumn-initiated field trial in the Manawatū, and (b) a winter-initiated lysimeter trial in the Waikato. The NTIs evaluated were: a urease inhibitor (UI; N-(n-butyl) thiophosphoric triamide); a nitrification inhibitor (NI; nitrapyrin); and Orun® (O; a combination UI and plant growth promotor, gibberellic acid). Our second objective was to assess the impact of plants and soil on the periphery of a urine patch (a urine patch edge effect) on N_2O emissions by including an "edge effect" treatment for all or a subset of the treatments in our first objective.

Materials and Methods

Two trials were conducted: Trial 1 was an autumn-initiated field trial situated at Massey University's No. 1 Dairy Farm in the Manawatū (Manawatū fine sandy loam; Typic Fluvial Recent), and Trial 2 a winter-initiated lysimeter trial situated at AgResearch's Ruakuara campus in the Waikato (Otorohanga silt loam; Typic Orthic Allophanic). Both trials were conducted on freely draining soils growing predominantly perennial ryegrass and white clover. The site used for Trial 1 had a history of dairy cattle grazing up to 2008 and a mixture of sheep grazing and cropping since 2008. For Trial 2 the site used to extract soil monoliths a history of rotational grazing by dairy cows. Basic soil physical and chemical characteristics are given in Table 1.

Table 1: Initial soil physical and chemical properties (0–75 mm depth). Soil texture (sand:silt:clay), bulk density (BD) (Mg m^{-3}), pH (1:2 in H_2O), Olsen P (mg kg^{-1}), total N (TN) (%), total C (TC) (%) and C:N ratio

	Sand:silt:clay	BD	pH	Olsen P	TN	TC	C:N
Trial 1 - Manawatū	35:45:20	1.22	6.0	50	0.28	2.53	9.0
Trial 2 - Waikato	24:61:15	0.70	5.9	42	0.69	7.90	11.4

The treatments were: (1) no urine/no inhibitor control (C), (2) urine (U), (3) urine plus nBTPT (UUI), (4) urine plus nitrapyrin (UNI), (5) urine plus nBTPT + nitrapyrin (UUINI), and (6) urine plus ORUN® - (UO). Urine and NTI application rates are given in Table 2 below. An "edge effect" treatment was included for all or a selection of the above treatments as outlined in Table 2 below.

Table 2: Trial dates, duration, treatments, method, NTI application rate and urine N load (kg N ha^{-1})

Trial	Duration	Method	Treatment	NTI app rate	Urine N load
Trial 1 Field	16/05 – 7/09 '16 ~16 weeks	Standard	C	Nil	Nil
			U	Nil	660
			UI	0.5 kg ha^{-1}	660
			UNI	1.0 kg ha^{-1}	660
			UO	0.5 kg ha^{-1}	660
			UUINI	0.5 kg + 1.0 kg ha^{-1}	660
	13/05 – 10/08 '16 ~13 weeks	Edge effects	eC	Nil	Nil
			eU	Nil	660
			eUI	0.5 kg ha^{-1}	660
			eUNI	1.0 kg ha^{-1}	660
			eUO	0.5 kg ha^{-1}	660
			eUUINI	0.5 kg + 1.0 kg ha^{-1}	660
Trial 2 Lysimeter	07/07 – 10/10 '16 ~15 weeks	Standard	C	Nil	Nil
			U	Nil	425
			UI	0.5 kg ha^{-1}	425
			UNI	1.0 kg ha^{-1}	425
			UO	0.5 kg ha^{-1}	425
			UUINI	0.5 kg + 1.0 kg ha^{-1}	425
		Edge effects	eU	Nil	425
			eUI	1.0 kg ha^{-1}	425
			eUO	0.5 kg ha^{-1}	425

Static chambers were used to measure N_2O emissions. For the standard method urine was evenly applied to the entire chamber base area, the base diameter was 240 mm (0.045 m^2) in Trial 1 and in Trial 2 the entire lysimeter surface served as the static chamber base area (500 DIA; 0.196 m^2). For the "edge effect" in Trial 1, a larger sized (800 mm DIA; 0.503 m^2) static chamber was employed. Identical urine N loads but using a patch size of 500 mm DIA (0.196 m^2) were evenly applied to the central area of the larger chambers; thus enabling determination of an "edge effect" for the remaining area (0.306 m^2) of the chamber base (ratio of urine patch to bare area of 0.64). In Trial 2 the "edge effect" treatment was included for U, UUI and UO treatments only. Identical urine N loads but using a patch size of 250 mm diameter (0.049 m^2) were evenly applied to the central area of the 500 mm diameter (0.196 m^2) lysimeter; enabling determination of an "edge effect" for the remaining area (0.147 m^2) of the chamber base (ratio of urine patch to bare area of 0.33).

Trial set-up

In Trial 1 a 15 × 30 m area was fenced off from the main paddock 2 months before the treatments were applied. The area was divided into five blocks, with five 4 × 4 m plots per block. Each plot was divided into four 1 × 1 m quadrants, with a quadrant each to accommodate (1) a standard (i.e. no edge effects) static chamber, (2) an edge effects static chamber, (3) an area for destructive soil sampling, and (4) a chamber (0.15 m DIA, 0.04 m height) for measuring NH_3 volatilisation.

In Trial 2 the 36 monolith lysimeters (500 mm DIA × 650 mm deep) were extracted from a dairy pasture at the AgResearch Tokanui dairy research farm located in the Waikato region, New Zealand in the autumn of 2015; the pasture had been ungrazed for 2 months before monolith extraction. The monoliths were transported to the Ruakura site (~35 km north of Tokanui) and installed in soil at the same level as the surrounding soil and left to equilibrate for 12 months before treatments were applied. The 36 lysimeters were divided into four blocks of nine lysimeters each; the nine treatments (Table 2) were allocated at random to the lysimeters within each block.

NH_3 emissions

Ammonia volatilisation was determined from the C, U, UUI and UNI treatments only in Trial 1. An additional irrigation treatment of 10 mm of irrigation applied 4 h after urine application (i.e. at the same time as the UI was applied) was included for the U (IU) and UUI (IUUI) treatments only. Ammonia emissions were measured using the dynamic chamber method (Kissel *et al.*, 1977) comprising a PVC emission chamber (150 mm DIA, 50 mm height) with a transparent top (to allow photosynthesis), an acid trap to capture the ammonia and a manifold consisting 6 air valves to regulate the flow rate inside the chambers. The chambers were inserted into the soil to a depth of 10 mm to obtain a headspace volume of 0.00053 m^3. The chamber had a vent on the chamber's vertical surface that was connected to an acid trap (250 mL, 0.025 M H_2SO_4) using a tube which was connected to the manifold through to a vacuum cleaner. Air from the chambers was sucked at a constant flow rate (at 6–10 L min^{-1}, monitored daily) and was passed through the acid trap. Samples were collected every day for the first 5 d and then on days 8, 10, 12, 15, 18 and 22 days as the NH_3 reached the background levels in all the treatments. Sub-samples of the H_2SO_4 solution in the acid traps were analysed colorimetrically for NH_4^+ concentrations and total NH_3 emissions calculated.

N_2O emissions

Nitrous oxide emission measurements were conducted using the standard gas sampling protocols and strategy (Luo *et al.*, 2013), i.e. twice a week for the first 4 weeks and once a week for the following 4 weeks and thereafter once every two weeks for the remaining period. During weekly/biweekly phases of N_2O flux measurement, additional sampling occurred as soon as practical following rainfall events of greater than 10 mm of rain in 24 h. Total emissions were calculated via trapezoidal integration of linear flux on measurement days.

In Trial 1 and for the standard method, circular stainless steel chamber bases were inserted into the soil to a depth of 80 mm one week before the urine treatment application. On each sampling day, insulated and non-vented chamber tops were placed onto the bases. For the edge effects method there was no chamber base; rather, a circular ridge of soil placed above the soil surface was employed to act as a seal between the chamber top and soil. In this case the un-vented chamber top was uninsulated, and due to its size contained a fan to circulate air

within the chamber during the cover period. In Trial 2 insulated and non-vented chamber tops were placed over top of the lysimeter casing and sealed down with metal clips.

On each sampling day, chamber tops were placed over their base/soil ridge for 1 h between 1100 and 1300 h. Headspace gas samples were taken at 0, 30, and 60 minutes after cover placement. On each sampling day at each site, two background atmosphere samples were also taken. Nitrous oxide analysis was conducted using Shimadzu GC-17a and Shimadzu GC2010 gas chromatographs (Shimadzu Oceania Pty Ltd, Nelson, New Zealand); both were equipped with a 63Ni-electron capture detector with oxygen-free N as a carrier gas (Saggar *et al.*, 2007).

The hourly N_2O emissions were calculated for each chamber, from the increase in head space N_2O over the sampling time. The hourly N_2O emissions (mg N m^{-2} h^{-1}) were calculated as follows:

$$N_2O\ flux \quad = \quad \frac{\delta N_2O}{\delta T} * \frac{M}{Vm} * \frac{V}{A} \qquad (1)$$

where δN_2O is the increase in head space N_2O over time (µL L^{-1}); δT is the enclosure period (hours); M is the molar weight of N in N_2O; Vm is the molar volume of gas at the sampling temperature (L mol^{-1}); V is the headspace volume (m^3); and A is the area covered (m^2). For each enclosure, these hourly emissions were converted to daily estimates and integrated over time, to estimate the total emission over the measurement period.

Nitrous oxide flux from the edge effects chambers was corrected for the area not directly affected by urine by assuming that the background flux for that block represented flux from the proportion of the chamber area not directly affected by urine.

Soil urea and inorganic N

In Trial 1 only, destructive soil sampling was conducted 24 h after treatment application and then at 3-day intervals for 3 weeks, and then weekly for a further 3 weeks. One core (25 mm × 7.5 mm) per plot was removed, the soil placed in a plastic bag and stored at 4 °C for 24 h. Immediately after removal of the soil core, the hole was back-filled with a sealed PVC tube to minimise any effects on soil aeration. The following day, the soil was thoroughly mixed and approximately 20–30 g fresh soil (5–10 g dry soil equivalent) was extracted for 1 hour in 100 mL 2 M KCl. The supernatant was then filtered using Whatman No 42 filter paper and the filtrate frozen. Samples were subsequently thawed and analysed for NH_4^+-N and (NO_3^- +NO_2^-)-N and concentration using a FIAstar5000 auto analyser (acc. to ISO13395). The remainder of the mixed soil was dried at 105ºC for 24 hours, to determine gravimetric soil water content. Volumetric water content was calculated by multiplying gravimetric water content by bulk density. Water-filled pore space (WFPS; %) was calculated by dividing volumetric water content by total porosity (Linn & Doran, 1984).

In Trail 2 only drainage from below the 650 mm lysimeter depth was collected at monthly intervals and the leachate analysed for NH_4^+-N and (NO_3^-+NO_2^-)-N concentration using a FIAstar5000 auto analyser (acc. to ISO13395). Total amounts of each species leached were multiplied by the volume drained and converted to a kg N leached ha^{-1} basis.

Meteorological information

Daily total rainfall and daily mean 10 cm soil and ambient air temperatures were obtained from a weather station positioned within 500 m of the trial site.

Management of above ground herbage mass
Herbage growing within the gas and soil sampling plots was cut to approximately 50 mm above ground level when a target herbage height of approximately 150 mm was reached. This occurred at approximately 4–5-week intervals over the winter and early spring period. All cut herbage was removed from the gas and soil sampling areas. In Trial 1, and only for the edge effects treatments, the herbage was cut to 50 mm on two occasions: at 8 weeks after treatment application and once again at 18 weeks after the trial start. Cut herbage was removed, weighed and a subsample dried at 60 °C for 24 h to determine DM content. A second subsample was dried and ground for N% determination by TKN digest followed by colorimetric determination using a Technicon II auto analyser (Seal Analytical, Fluidquip Australia Pty).

Statistical analysis
An analysis of variance was performed on total NH_3 and N_2O emitted and on EF_3 data according to a randomised complete block design. In Trial 1 data from the standard chamber treatments was analysed separately from the edge effects treatments, given their start times differed, as did rainfall in the crucial weeks after treatment application and the length of the flux measurement period. In Trial 2 the standard and edge effects treatments were analysed as one dataset. Net herbage accumulation, herbage N%, and net N accumulated data were analysed according to a randomised block design for the edge effects treatment only.

All data sets were checked for normality and homoscedasticity before analysis. In the case of total N_2O emissions data for the edge effects method required logarithmic transformation before analysis and the means presented in Table 4 are back-transformed means. All statistical analyses were conducted using the statistical package GenStat® for Windows® v14 (www.vsni.co.uk).

Results and Discussion

General
Soil conditions were wet at the time of treatment application and remained so for the first 4 – 6 weeks for both trials (Table 3).

Table 3: Rainfall (mm) over days in relation to treatment application day, and 28 day mean 10 cm soil temperature (°C) and water-filled pore space (WFPS; %)

	(-)7– 0	0–1	0–7	0–14	0–28	Soil temp	WFPS
Trial 1 - Standard	61	10	64	157	212	9.6	66
Trial 1 - Edge effects	51	13	25	130	220		
Trial 2 - All treatments	72	28	43	66	144	11.0	n/a

NH_3 volatilisation Trial 1
Daily NH_3 emissions and cumulative losses over the 21 day measurement period were significantly influenced by the application of the UI, nBTPT, and 10 mm irrigation (Figure 1). Almost 41% of the total emission from urine occurred within the first 24 h (19.9 ± 3.9 kg NH_3-N ha^{-1}; Fig. 1a) following application of urine in late autumn/early winter (low temperature and high soil moisture). Approximately 66% of the total NH_3-N emissions from all treatments occurred within the first 5 days. This finding is typical of urine application to a

pastoral soil in New Zealand (Sherlock & Goh 1984; Zaman *et al.* 2009). After this period, NH_3 emissions dropped sharply, reaching background levels by day 21.

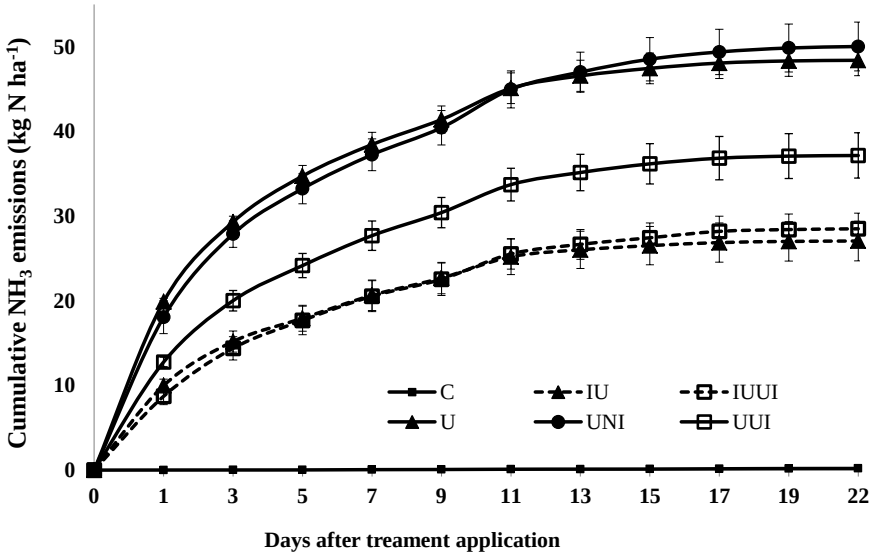

Figure 1: Cumulative NH_3 emissions (kg N ha^{-1}) over 21 days after treatment application. The dashed lines show the treatments U and UI which also received a10 mm irrigation treatment (IUI and IUUI). Error bars are +/- 1 SEM.

Cumulative NH_3 losses for the U treatment averaged 48 kg N ha^{-1} (Fig. 2), giving an average $FracGAS_M$ (% of excreta N applied which is volatilised as NH_3) value of 7.3%. This is lower than a reported 78 kg N ha^{-1} (FracGASM 14.4%) from urine applied at 530 kg N ha^{-1} in autumn when soil temperatures were higher and soil moisture was lower and urea hydrolysis was rapid (Rodriguez *et al.*, 2014). Comparatively lower NH_3 emission from urine in the present study may be attributed partly to the slower rate of urea hydrolysis (lower soil temperature) and also to deeper distribution of applied urine into this already moist and well-drained soil.

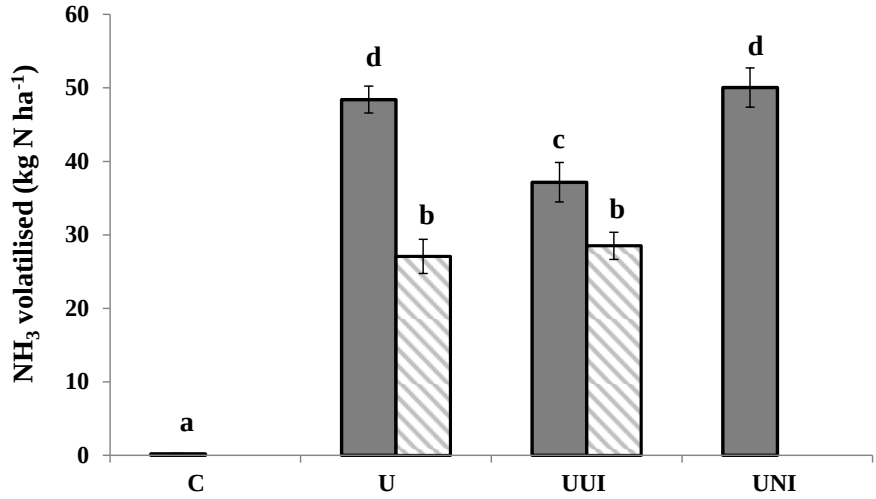

Figure 2: Total NH_3 emissions (kg N ha^{-1}) over 21 days after treatment application. Hatched bars show the irrigation treatments where 10 mm of water was applied to the U and UUI treatments shortly after application. Bars with different letters are significantly different from one another (P<0.001).

The total amount of NH_3-N emitted averaged 37.2 (UUI), 50.0 (UNI) and 27.1 (IU) and 28.5 kg N ha^{-1} (Fig. 2). Compared with urine only, the urease inhibitor nBTPT reduced emissions by 23%, and irrigation applied with or without the inhibitor reduced emissions by just over 40%. However, the application of the nitrification inhibitor nitrapyrin (UNI) had no significant effect on NH_3 emissions. Saggar *et al.* (2013) calculated a 53% reduction in emissions from urine when mixed with UI before being applied to the soil. The lower (23%) emissions reductions obtained in this study would therefore closely resemble field conditions where urine deposited by grazing animals is treated with nBTPT shortly after deposition.

Our results suggest that 10 mm irrigation within 4 hours of urine application (IU and IUUI) may reduce NH_3 emissions from urine by 40%, which is similar to the 50% reduction in emissions reported by Zaman *et al.* (2013), where 5–10 mm of irrigation was applied within 8 hours of a urea fertiliser application. While irrigation can dilute urine N (urea and NH_4^+) concentrations at the soil surface, increasing its dispersion into the soil can retain NH_3 in the soil. In the absence of irrigation or rainfall within 8 h of urine deposition, NH_3 losses associated with a urine patch can be effectively reduced by nBTPT.

Soil urea and inorganic N concentrations Trial 1

Urea was present in the 0 – 2.5, 2.5 – 7.5 and 7.5 – 15.0 cm soil layers within 24 h after treatment application in the U, UUI, IU and UUINI treatments (data not shown). No urea was detected in any of the soil layers soil from the irrigated urine, or urine plus nitrification inhibitor treatments at this time. By comparison, much lower concentrations of urea were present for all treatments in the 0 – 2.5 and 2.5 – 7.0 cm soil layers by 4 days after the trial start and these were reduced to very low concentrations or were absent by day 6.

Soil inorganic N concentrations over time (Fig. 3) reflect treatment differences observed in gaseous N emissions. Soil NH_4^+ concentrations were at their peak 1 day after treatment application and dropped gradually over the following 28 day period. Soil NO_3^- concentrations rose sharply in the first week after treatment application suggesting that nitrification was rapid as the average 10 cm soil temperature for that week was relatively high for the time of year (~13°C). At this time peak NO_3^- concentration were lowest for the NI and UUINI treatments and highest for the urine plus Orun® treatment, which remained elevated until day 28. For all other treatments, concentrations dropped sharply over the following 10 days, then rose to another peak at approximately 30 days after treatment application, and then dropped gradually.

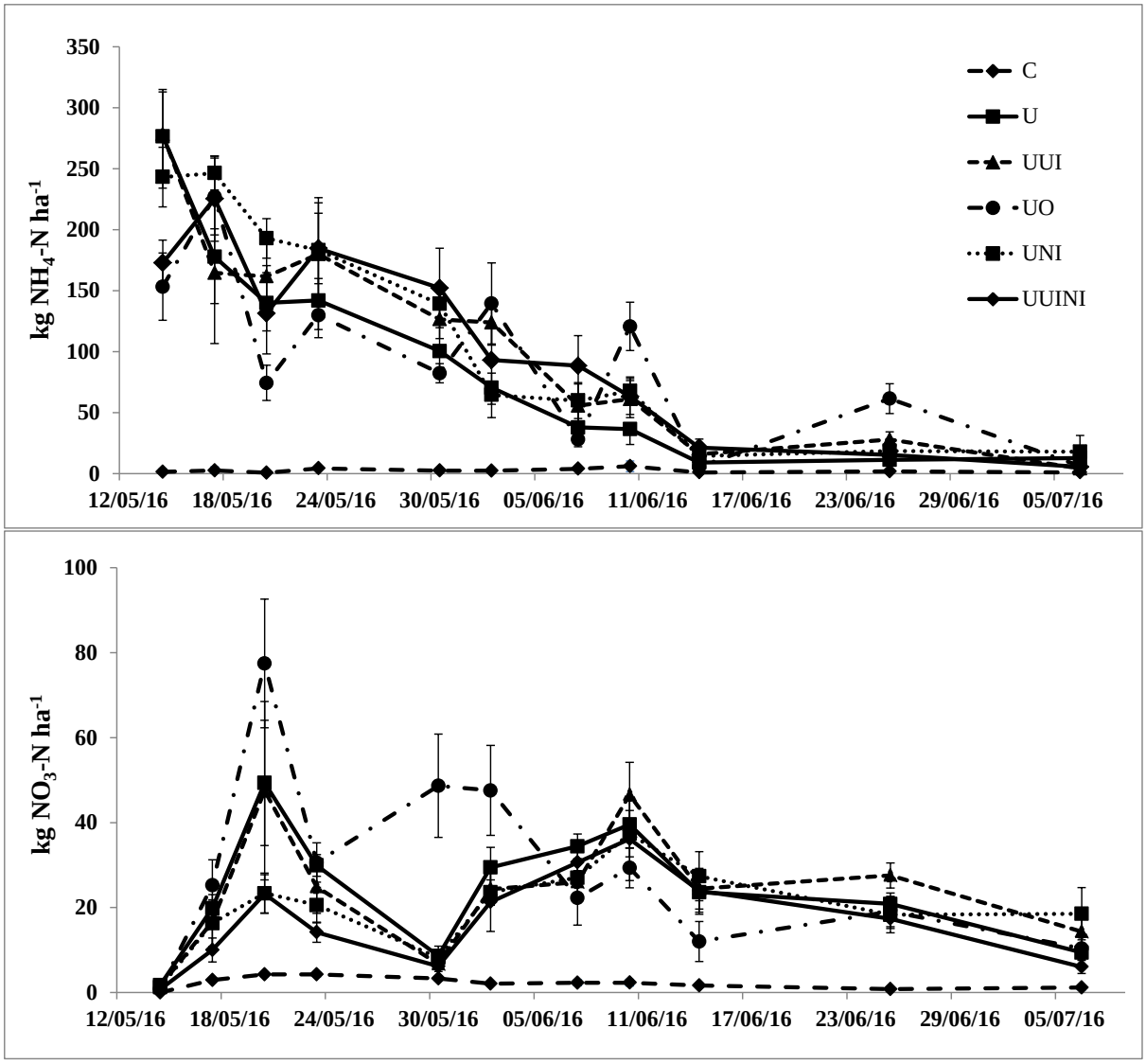

Figure 3: Soil NH_4-N and NO_3-N (kg N ha^{-1}) (0-7.5 cm) from day 1 to day 55 after treatment application.

N_2O emissions Trial 1

Standard method: Peak fluxes (508 g N ha^{-1} $day^{-1}$for the U treatment) were observed 9 days after treatments were applied for all the treatments and dropped sharply after that, but increased again at 21 days and continued to be elevated for up to 50 days before dropping to background levels at (60 days). However, N_2O fluxes did not remain consistently at background level until just over 3 months (100 days) after treatment application. Nitrous oxide flux measured from the urine and urine plus urease inhibitor treatments were generally highest, with flux measured from the urine plus nitrification inhibitor treatment consistently the lowest.

Edge effects method: Peak N_2O fluxes (580 g N ha^{-1} $day^{-1}$for the U treatment) were observed at 13 days after treatment application for all treatments except the urine treated with both the urease and nitrification inhibitors, which peaked at 3 days. Fluxes dropped sharply thereafter for all treatments and reached background levels by 50 days after the trial start. The greatest fluxes occurred from the urine only treatment, followed by the urease inhibitor treated urine, and then the Orun® and combined urease and nitrification inhibitor-treated urine. Lowest fluxes occurred from urine treated with the nitrification inhibitor only.

Although the magnitude of peak flux was relatively similar between the standard and edge effects methods, the edge effects method had consistently lower fluxes over time and therefore total N_2O emissions were much lower, even when corrected for area not directly wetted by the urine +/- NTI's.

While total N_2O emissions from the method designed to account for urine patch edge effects (i.e. the zone of influence) were lower than the emissions measured from the standard method this was confounded by different amounts of rainfall within the week before (51 vs 61 mm) and after treatment application (25 vs 64 mm) (Table 3). Within any one treatment (including the control) total N_2O emissions were 2–3 times greater for the standard compared with the edge effects measurement method; but within a measurement method, the between-treatment ranking was similar – with the highest total emissions coming from the U and UUI treatments followed by the UO and UUINI treatments. Lowest emissions were measured from the UNI treatment (apart from the control) (Table 4). Overall variability in total emissions was comparatively greater for the edge effects compared to standard measurement method (CV 82 vs 37 %).

Table 4: Treatment effects on total N_2O emissions (kg N ha^{-1}) and nitrous oxide emission factor (EF3; %). Statistical analysis was performed for each measurement method separately in Trial 1, and with both measurement methods combined in Trial 2 (refer to text)

Method	Treatment	N_2O emitted			EF_3		
		mean	$LSD_{(95\%)}$	*P*	mean	$LSD_{(95\%)}$	*P*
Trial 1 Standard	C	0.23 a	2.06	<0.001			
	U	10.19 d			1.51 c	0.34	<0.001
	UUI	9.93 d			1.47 c		
	UNI	4.38 b			0.63 a		
	UO	6.85 c			1.00 b		
	UUINI	6.99 c			1.02 b		
Trial 1 Edge effects	C	0.12 a	1.81	<0.001			
	U	3.09 c			0.69	0.41	ns
	UUI	2.57 bc			0.31		(0.409)
	UNI	1.69 b			0.25		
	UO	2.15 bc			0.34		
	UUINI	1.87 bc			0.29		
Trial 2 Standard	C	0.04 a	0.16	<0.001			ns
	U	0.44 bc			0.09	0.04	(0.268)
	UUI	0.39 bc			0.08		
	UNI	0.37 bc			0.08		
	UO	0.48 c			0.10		
	UUINI	0.41 bc			0.09		
Trial 2 Edge effects	eU	0.34 bc			0.07		
	eUUI	0.31 b			0.06		
	eUO	0.28 b			0.06		

For the standard measurement method, applying the nitrification inhibitor nitrapyrin 4 h after urine was applied halved total emissions compared with urine only, and reduced EF3 by nearly a third. When urine patch edge effects were taken into account the effects of the NI inhibitor were somewhat reduced (a 40% reduction in total emissions; EF_3 was lower, but not significantly so).

In general, applying the urease inhibitor nBTPT was not effective in reducing total emissions or EF_3. Applying the combination of urease plus nitrification inhibitors had an intermediate effect on emissions, as did application of the product Orun®, a combination plant growth promotor plus urease inhibitor.

N_2O emissions Trial 2

Nitrous oxide flux measurements are only available from day 7 onward, due to an unfortunate incident with samples taken in the first week after treatments had been applied. Peak flux was measured at 18 days after treatment application, but was much lower than in Trial 1 (24 vs >500 g ha^{-1} day^{-1}). Peak flux is often experienced within 10 days post urine application, so in this case peak flux from all or some of the treatments may have been missed. Total emissions were much lower for all treatments and measurement methods in Trial 2 compared with Trial 1 (Table 4), which can be attributed at least partly to the lower urine N load applied (425 vs 660 kg N ha^{-1}) as well as the missing flux measurements for the first two sampling occasions. There was no significant difference between the urine and any of the urine plus inhibitor treatments within both the standard and edge effects methods. The only significant difference in total emissions between measurement methods was observed for the urine amended with Orun® treatment, where total emissions were greater when measured using the standard method compared the edge effect method.

Trial 2 N leaching

While the soils were wet at the start of the trial and 144 mm of rain fell during the first month, there has not been sufficient drainage for the solute front to have moved below the lysimeter depth (650 mm). Leachate measurements are ongoing and will continue for a full year after treatment application (i.e. to July 2017).

Trial 1 Net herbage DM and N accumulation

In the first 2 months after treatment application net herbage DM accumulation was 30% greater in plots receiving urine +/-NTIs compared to the control (25 vs 17 kg DM ha^{-1} d^{-1}) (Table 5). Herbage N % was similar for all of the urine +/– NTI treatments and was approximately 20% greater than for plots receiving no urine. This resulted in a 40% greater N accumulated when herbage received urine +/– NTI. In the mid-late winter period, 2–4 months after treatment application, NHA from urine +/– NTI treatments was approximately double that of the control (Table 6), but again there was no difference between any of the urine +/– NTI treatments. Herbage N % was similar between control and treated areas, but the urine +/– NTI treatments areas still accumulated 60% more herbage N than controls.

Table 5: Net herbage accumulation (kg DM ha^{-1}), herbage N % and net N accumulated (kg N ha^{-1}) in the first 8 weeks in Trial 1 for the edge effects measurement method

Treatment	Net DM accumulated	Herbage N %	Net N accumulated
C	941 a	3.56 a	33.6 a
U	1390 b	4.16 b	57.7 b
UUI	1298 b	4.01 b	52.3 b
UNI	1352 b	4.03 b	54.5 b
UO	1423 b	3.90 b	55.5 b
UUINI	1309 b	4.09 b	53.2 b
Probability	<0.001	<0.001	<0.001
LSD	179	0.25	7.8

Table 6: Net herbage accumulation (kg DM ha^{-1}), herbage N (%) and net N accumulated (kg N ha^{-1}) between weeks 8 and 18 in Trial 1 for the edge effects measurement method

Treatment	Net DM accumulated	Herbage N %	Net N accumulated
C	951 a	2.36	25.2 a
U	2244 b	2.64	60.2 b
UUI	1856 b	2.73	51.0 b
UNI	2017 b	2.77	56.0 b
UO	1923 b	2.81	54.1 b
UUINI	2027 b	2.55	51.6 b
Probability	<0.001	ns (0.633)	0.004
LSD	500	0.34	16.5

Conclusions

The efficacy of NTIs in reducing system N loss when added to a freshly deposited urine patch varied depending on site/time of year applied and measurement method.

For the autumn-initiated Manawatū field trial, applying the urease inhibitor nBTPT with or without 10 mm irrigation to already wet soils reduced NH_3 volatilisation, but resulted in similar total N_2O emissions and N_2O emission factor compared with applying urine only. Applying the nitrification inhibitor nitrapyrine to a urine patch had no significant impact on NH_3 volatilisation, but reduced total N_2O emissions and the N_2O emission factor greatly. Applying a combination urease plus nitrification inhibitor or a combination urease inhibitor plus gibberellic acid shortly after urine application had an intermediate effect on total emissions and EF_3. Our results affirm that there is potential for reducing NH_3 losses from animal urine deposition in grazed pastures by either applying approximately10 mm irrigation or treating the urine patch with nBTPT within 4 h after deposition.

Urine treated areas of pasture produced more DM and N than non-urine areas for up to four months after deposition over the late-autumn to late winter period. However, applying an NTI to the urine-affected area shortly after deposition had no effect on the amount of DM or N accumulated. There was no difference in net accumulation of herbage DM or N between any of the NTIs tested. Significant rainfall events before and after the treatment applications may have resulted in the movement of much of the urine N below the zone of major N uptake by

plant roots. This may explain the lack of effect of any of the NTIs studied on net DM, and accumulation as has been recently noted by Quin *et al.* (2016) for the product Orun®.

The effect of NTIs on N_2O emissions were most marked when flux measurements were restricted to the wetted area only (standard method). We present some evidence that N_2O emissions from urine deposited during grazing may be overestimated when flux measurements are restricted to just the area directly affected by urine (wetted area). Plant and soil influence on urine N dispersion, transformations and uptake at the periphery of the patch area should be important considerations when estimating N_2O emissions from grazed pastures.

There was no significant effect of any of the NTIs on N_2O emissions from the urine-amended lysimeters in the winter-initiated trial in the Waikato, although in general, trends observed at the Manawatū site were present. Total emissions from the Orun® amended urine treatment measured using the standard method were greater than from the edge effects method. However, in this lysimeter study, measurement method did not have a significant effect on the urine only or urine plus UI treatment.

Overall, the significant rainfall events before and after the treatment applications at both trial sites may have resulted in the movement of much of the urine N below the zone of uptake by plant roots and microbial transformations. This would have reduced emissions as well as reduced the opportunity for the lateral movement of urine N (edge effect) as has measured in other studies (Quin *et al.* 2016). Further study on the spatial distribution of urine deposited during grazing and NTIs applied after urine deposition is needed to clarify best practices for optimal N loss mitigation.

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