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DO NITROUS OXIDE EMISSIONS FROM URINE DEPOSITED NATURALLY DIFFER FROM EVENLY APPLIED URINE?

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Abstract

The majority of research on nitrous oxide (N_2O) emissions from urine deposited during grazing has involved evenly applying urine to the entire N_2O flux measurement area. This approach does not account for the possible effect of heterogeneous horizontal and/or vertical distribution of urine (and hence urine nitrogen) over the patch area, or for possible plant and soil effects at the patch periphery.

We conducted a field trial in mid-spring of 2017 to ascertain the effect of application method on N_2O emissions from urine applied to a dairy soil. Urine was applied (i) evenly over the entire surface area of the measurement chamber (standard chamber) and (ii) to a central point and allowed to spread naturally within a measurement chamber designed to capture any influence of plants and soil at the patch periphery (large chamber). We also superimposed two nitrogen transformation inhibitor treatments over the urine application method treatments: (i) the urease inhibitor N-(n-butyl) thiophosphoric triamide (nBTPT); and (ii) the nitrification inhibitor nitrapyrin, in a randomised complete block design with the 8 treatments: standard chamber: no urine/inhibitor control, evenly applied urine, evenly applied urine + nBTPT, evenly applied urine + nitrapyrin; large chamber: no urine/no inhibitor control , naturally applied urine, naturally applied urine + nBTPT, naturally applied urine + nitrapyrin. The treatments were allocated at random within each of 5 blocks (N=40).

At the end of the 14-week trial, the urine application method/chamber size did not have a significant effect on estimates of the nitrous oxide emission factor (EF_3: % of urine N applied emitted as N_2O-N) for dairy urine (0.86 vs 0.96% for the standard and large chambers, respectively). Under the conditions of the present study neither the urease nor the nitrification inhibitor treatments had a significant effect on EF_3 for dairy urine and this result was consistent for both urine application method/chamber sizes.

Our study is an initial evaluation of the effect of urine application method and chamber size on EF_3 and on assessing the efficacy of nitrogen transformation inhibitors for reducing N_2O emissions. The environmental conditions in the present study were warmer and drier than average for the mid spring – summer period in this locality. A more thorough investigation is planned for the coming late winter/early spring period, and will include detailed soil and plant measurements to aid interpretation of N_2O emissions results.

Introduction

Globally, agricultural soils contribute about 65−70% of N_2O produced by terrestrial ecosystems (Wrage *et al.*, 2004) and animal production accounts for an estimated 1.5 Tg N_2O-N yr^{-1} with 41% of emissions associated urine and dung deposition by grazing animals

(Oenema *et al.*, 2005). Grazed pasture soils exhibit high potential for N_2O emissions from urine deposited by grazing livestock and are the primary source of direct and indirect (emitted from deposition of volatilised NH_3 and leached NO_3^-) N_2O emissions, contributing approximately 80% of New Zealand's 9.07 Mt CO_{2e} agricultural N_2O emissions (MfE, 2016).

An average dairy cow urine patch covers approximately 0.24 m^2 (range: 0.14 to 0.49 m^2) surface area and an estimated effective area of approximately 0.68 m^2 (range: 0.03 to 1.1 m^2) (Selbie *et al.*, 2015), and contains 2−3 times more nitrogen (N) than the affected pasture's requirements for maintenance and growth. This makes the urine patch a hot spot for N losses with potentially deleterious impacts on the environment (Saggar *et al.*, 2005; PCE, 2012).

A number of studies (Doak, 1952; Haynes and Williams, 1993; Koops *et al.*, 1997; Decau *et al.*, 2003; Marsden *et al.*, 2016; Minet *et al.*, 2016) have described urine patch characteristics using soil physical and chemical measurements and characterise three distinct zones within a urine patch: the "wetted area" or area directly affected by urine; the "diffusional area", which incorporates the diffusive edge of urine solutes; and the "pasture response area" or area where plants are able to access the pool of urine derived nutrients via root extension. The spatial distribution and dynamics of reactive N within the urine patches are modified by soil and environment conditions such as micro-topography, soil texture, moisture, compaction, post-deposition rainfall/irrigation, and pasture N uptake (Marsden *et al*., 2016). The majority of research on N_2O emissions from animal urine deposited during grazing has involved applying the urine to the entire N_2O flux measurement area (i.e. static chamber base area) (Luo *et al.*, 2015, 2016; Li *et al.*, 2016). However, there is some suggestion in the literature that plant roots at the periphery of a urine patch are able to utilize the nearby urinary N, even though these roots have not been directly wetted by urine (Moir *et al.*, 2011). Little data are available on the effect of plant N uptake at the edge of a urine patch on patch N_2O emissions although its significance was signalled by Koops *et al.* (1997) several decades ago and two recent studies indicate that, under some conditions, accounting for an edge effect may be critical for estimating N_2O emissions from a urine patch (Marsden *et al.*, 2016; Forrestal *et al.*, 2017). Furthermore, current understanding of the effectiveness of nitrification transformation inhibitors in reducing N_2O emissions is based largely on research using urine applied uniformly within chambers and lysimeters (e.g., Di and Cameron, 2016) and may not reflect their true effectiveness for urine deposited in an "unconstrained" manner during grazing.

Our first objective was to compare the effect of two urine application methods and static chamber sizes on the nitrous oxide emission factor (EF_3: % of urine N applied emitted as N_2O-N) of dairy cow urine applied to a dairy-grazed ryegrass/white clover pasture soil, where urine was applied (i) evenly over the entire surface area of the measurement chamber base (standard chamber) or (ii) to a central point and allowed to spread freely within the base area of a chamber designed to allow any influence of plants and soil at the patch periphery to be expressed (large chamber). Our hypothesis was that EF_3 for dairy cow urine would be lower when estimated by applying urine to the central point of a large-sized chamber compared with urine spread evenly over the entire area of a standard sized chamber. Our second objective was to compare the effect of the two urine application methods/chamber sizes on estimating the efficacy of two nitrogen transformation inhibitors: (i) the urease inhibitor N-(n-butyl) thiophosphoric triamide (nBTPT) and (ii) the nitrification inhibitor, nitrapyrin.

It is important to note that, in the study we report here, the urine application method (evenly spread vs poured to a central point) is confounded with chamber size (and therefore opportunity of plant and soil influence at the patch periphery to be expressed). This confounding was intended, as in this instance our aim was to compare the widely used

"standard" technique for measuring EF_3 of urine deposited during grazing with a technique more applicable to a real situation where urine voided by grazing animals falls to a central point and spreads naturally depending on soil, plant, and micro-topographical conditions at the time of deposition.

Methods

The trial was initiated in mid-spring (3 November 2017) and measurements of N_2O flux continued into late summer (13 February 2018). The trial site was located at Massey University's Dairy 1 farm; the soil was a Manawatū fine sandy loam soil classified as freely draining, although there is some drainage impedance as a result of silt deposits caused by historic flooding events. The 4-year-old ryegrass/white clover pasture had been grazed by dairy cattle throughout the previous autumn/winter and was fenced off from grazing in late August, 8 weeks before the trial start.

A preliminary study to assess static chamber design for the large chambers was conducted in September/October. Practical and logistical issues, i.e. construction material, insulation requirements, number of sampling ports, fan for mixing the gases, assessing linearity of N_2O flux, and suitability for capturing the effective area of an average dairy cow urination event of 2 L. The final design consisted of a PVC chamber, 800 mm diameter and 250 mm height with a base inserted 30 mm into the soil. The top of the chamber is insulated with 20-mm thick polystyrene, and has a port for taking gas samples and a port for inserting a thermometer in order to monitor internal temperature during cover time.

Trial design and treatment application

The field trial was established in a randomised complete block design with 5 blocks and the 8 treatments (Table 1) assigned at random to each block (N = 40). The 8 plots per block were 2 × 2 m in size, with a 1-m guard area between plots within blocks as well as between blocks.

Table 1: Urine application method/chamber type, urine +/– inhibitor treatments and urine application rate/amount

Urine application method/chamber type	Treatment	Urine application rate	Amount of urine applied
Evenly applied/Standard chamber ($0.045\ m^2$)	Control		
	Urine	10 L m^{-2}	0.45 L
	Urine + nBTPT	10 L m^{-2}	0.45 L
	Urine + nitrapyrin	10 L m^{-2}	0.45 L
Naturally spread/Large chamber ($0.503\ m^2$)	Control		
	Urine	4 L m^{-2}	2 L
	Urine + nBTPT	4 L m^{-2}	2 L
	Urine + nitrapyrin	4 L m^{-2}	2 L

Chamber bases were inserted into the soil and herbage within the chamber base was cut to 5 cm above ground level on 23October. Pre-treatment gas flux was taken on 31 October and these flux measurements served as a covariate for statistical analysis of post-treatment emissions. Dairy cow urine was collected from cows grazing a typical ryegrass/white clover pasture at Massey University's Dairy 4 farm and stored for at 4 °C for 4 days before treatments were applied on 3 November. For the standard-sized chambers, urine was evenly applied to the entire chamber base area at the rate of 10 L m^{-2}, and, for the large-sized

chambers, 2 L of urine was poured onto the central point of the chamber base area at a height of approximately 1.2 m and allowed to spread naturally. Note that the urine application rate differed for the two chamber sizes. Inhibitors were applied to the relevant treatments 4 h after urine application.

N_2O emissions

Nitrous oxide flux measurements were conducted 2 and 24 h after urine application, twice weekly for 4 weeks, and then weekly for a further 10 weeks. On each sampling day, chamber tops were placed over their bases for 1 h between 1100 and 1300 h. Headspace gas samples were taken at 0, 30, and 60 minutes after cover placement, and two atmosphere samples were taken at the beginning and end of each cover period. Chamber temperatures were recorded at the beginning and end of the cover period and the average of the two readings considered the chamber temperature for calculating gas flux. Total emissions were calculated via trapezoidal integration of the linear flux on measurement days.

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:

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

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.

Environmental conditions

Daily total rainfall and daily mean 10 cm soil and ambient air temperatures were obtained a meteorological station within 500 m of the trial site (NZ Meteorological Service based at AgResearch Ltd, Palmerston North).

Statistical Analyses

A one-way analysis of variance covariance (Urine application method/chamber size effect) was performed on total N_2O-N emitted for the control treatment data and two-way analysis of variance (Urine application method/chamber size x urine treatment and their interaction) on EF_3 data for the urine treatments, according to a randomised complete block design. The data sets were checked for normality and homoscedasticity before analysis. Both the total N_2O emissions data and EF_3 data showed non-homogeneity of residual variance and non-normality (Shapiro-Wilk test) and so required logarithmic transformation before analysis. All statistical analyses were conducted using the statistical package GenStat® for Windows® v14 (www.vsni.co.uk). Tables in the results section below show raw means, log-transformed means and back-transformed means. Back-transformed means for EF_3 were corrected for bias introduced as an Ln (n+1) transformation was the most appropriate transformation to use.

Results

Soil moisture levels were below field capacity at the time of urine application (VWC=33%) and decreased steadily over a 7-week period thereafter (VWC at week 7=12%) (Fig. 1). Significant rainfall at 8 weeks resulted in an increase in soil moisture over weeks 8 and 9 (VWC 30–21%) and then a gradual decrease in weeks 10 and 11. There were transient increases in soil moisture content between 8 and 14 weeks as rainfall occurred.

N_2O flux was greatest 2 h after urine application and decreased sharply for both the standard and naturally applied treatments/chamber types (Fig. 1). N_2O flux from the urine and urine plus inhibitor treatments were elevated for 2 weeks after treatment application and then decreased sharply, returning to background levels by week 3. Small and transient increases in N_2O flux were measured for all treatments including the controls after episodes of >20 mm rainfall in 24 hr at weeks 7, 9, and 11, when short-lived increases in soil moisture were observed (VWC peaked at ~30%). Gas flux measurements continued for 14 weeks, at which time N_2O flux for all treatments had been at background level for approximately 8 weeks.

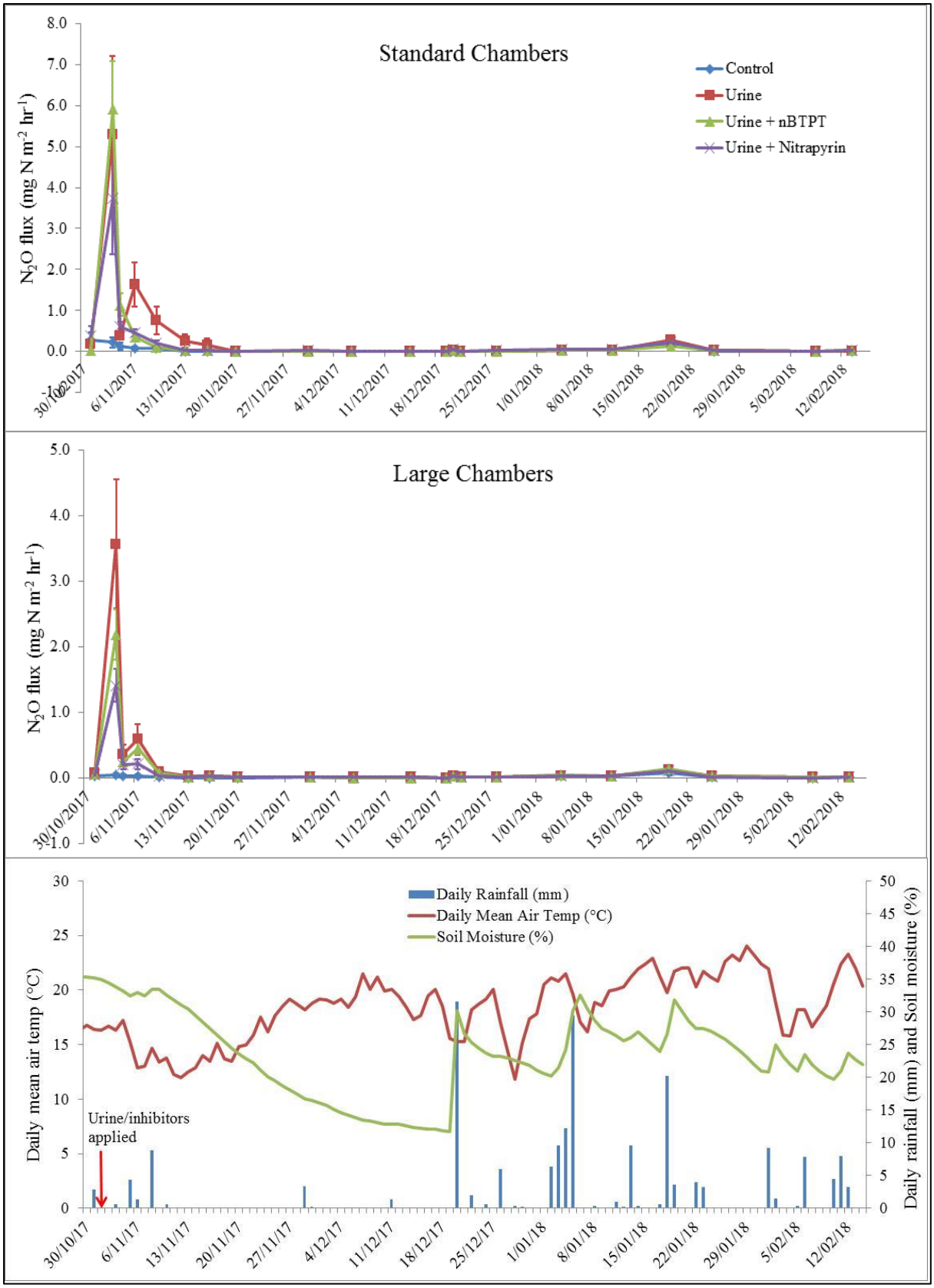

Figure 1: The N_2O fluxes (mg N m^{-2} hr^{-1}) as affected by urine application method/chamber type and urine treatments. The plots show treatment means and the error bars are +/– 1 sem (*n*=5).The bottom graph shows average daily air temperature (°C), soil moisture (VWC; %) and total daily rainfall (mm).

Total N_2O emissions measured from the control treatment were greater and more spatially variable when measured using the standard- compared with the large-sized chamber (1066 vs 444 g N ha^{-1}; CV 85 vs. 64%, respectively) (Table 2).

Table 2: Total N_2O-N emitted (g N ha^{-1}) from the control treatment as affected by chamber type (102 days; 03.11.17–13.02.18)

	Total N_2O emissions (102 days) (g N ha^{-1})				
Chamber	Raw mean	sem	CV (%)	Log transformed mean*	Back-transformed mean
Large	444	128	64	6.07	433
Standard	1066	404	85	6.56	706
Probability	0.016				

*adjusted for covariate (pre-treatment N_2O flux; *P* covariate effect=0.012)

Urine application method/chamber type did not have a significant effect on EF_3 overall (*P*=0.451; Table 3), or for any of the urine +/– inhibitor treatments. As with total emissions from the control treatment, the CV of EF_3 from the urine-only treatment was greater for the standard compared to large chambers (98 vs 43%). In contrast, the CV of EF_3 from the nitrapyrin-treated urine was greater for the large compared to standard chambers (98 vs 60%), but similar for the nBTPT-treated urine (45 vs 48%).

Applying either the nitrification inhibitor nitrapyrin, or the urease inhibitor nBTPT 4 hours after urine application had a marginal but non-significant (*P*=0.081) effect on EF_3.

Table 3: Effect of urine application method /chamber size and nitrogen transformation inhibitors on EF_3 for dairy cow urine: treatment means and results of statistical analyses

		Nitrous oxide emission factor (EF3; %)			
	Raw mean	sem	CV (%)	Log transformed mean*	Back-transformed mean
Large Chamber					
Urine	0.955	0.186	43	0.654	0.916
Urine + nBTPT	0.678	0.137	45	0.507	0.665
Urine + nitrapyrin	0.392	0.172	98	0.301	0.350
Standard Chamber					
Urine	0.862	0.377	98	0.540	0.716
Urine + nBTPT	0.434	0.093	48	0.358	0.433
Urine + nitrapyrin	0.407	0.109	60	0.312	0.363
Probability	Chamber	0.451			
	Urine trt	0.081			
	Interaction	0.815			

*adjusted for covariate (pre-treatment N_2O flux; *P* covariate effect=0.038)

Discussion

Warm temperatures and very low rainfall prevailed during the first 8 weeks of the trial (mean daily average air temperature =16.8 °C; total rainfall =21 mm) leading to very low soil moistures (8 week mean VWC =0.21). Under the environmental conditions of this initial study we reject our hypothesis that EF_3 estimated using the large chambers (with naturally spread urine and allowing for the influence of plants and soil at the patch periphery to be expressed) would be lower than when estimated using the standard chambers (with urine evenly spread over the entire surface area of the chamber and not including plant and soil effects at the patch periphery). It is difficult for us to ascertain why this is so as we did not make any supporting soil and plant measurements in this study. We are conducting a field trial with the two urine application method/chamber sizes but using the same rate of urine-N application, in the upcoming (2018) late-winter/early spring period. This will be a more thorough investigation, for example, we will be setting up contrasting soil moistures and urine volumes and making detailed soil mineral N and plant growth measurements to help interpret our findings.

For the trial we report here we can speculate that as soil moisture decreased plant growth rate also decreased and therefore any opportunity for plant effects at the patch periphery to be expressed was limited. Although we would have expected minimal to no N leaching occurring, volatilisation may have been an immediate source of urine N loss, although we did not measure this. The lack of a significant effect of the two different nitrogen transformation inhibitors on EF_3 is likewise not unexpected, as previous trials where urine patches have been treated with urease or nitrification inhibitors in warm and dry conditions have shown variable results (Di and Cameron, 2016; Cai *et al.*, 2017). However, without supporting soil mineral N and plant growth measurements we are unable to provide further insight on this.

Conclusions

In this mid-spring initiated field trial, where soil moisture levels were below field capacity and decreasing over the first 7 weeks of the trial, urine application method/chamber type had no significant effect on EF_3. We found that spatial variability in total emissions from control areas and in EF_3 from urine-only treated areas may be greater for the standard than for large chambers as illustrated by the greater CV's, although this was not evident for the urine + inhibitor treatments.

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