The interactive effects of various nitrogen fertiliser formulations applied to urine patches on nitrous oxide emissions in grassland

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Abstract

Pasture-based livestock agriculture is a major source of greenhouse gas (GHG) nitrous oxide (N_2O). Although a body of research is available on the effect of urine patch N or fertiliser N on N_2O emissions, limited data is available on the effect of fertiliser N applied to patches of urinary N, which can cover up to a fifth of the yearly grazed area. This study investigated whether the sum of N_2O emissions from urine and a range of N fertilisers, calcium ammonium nitrate (CAN) or urea ± urease inhibitor ± nitrification inhibitor, applied alone (disaggregated and re-aggregated) approximated the N_2O emission of urine and fertiliser N applied together (aggregated). Application of fertiliser to urine patches did not significantly increase either the cumulative yearly N_2O emissions or the N_2O emission factor in comparison to urine and fertiliser applied separately with the emissions re-aggregated. However, there was a consistent trend for approximately 20% underestimation of N_2O loss generated from fertiliser and urine applied separately when compared to figures generated when urine and fertiliser were applied together. N_2O emission factors from fertilisers were 0.02%, 0.06%, 0.17% and 0.25% from urea ± dicyandiamide (DCD), urea + N-(n-butyl) thiophosphoric triamide (NBPT) + DCD, urea + NBPT and urea, respectively, while the emission factor for urine alone was 0.33%. Calcium ammonium nitrate and urea did not interact differently with urine even when the urea included DCD. N_2O losses could be reduced by switching from CAN to urea-based fertilisers.

Keywords

nitrification inhibitor • nitrogen fertiliser • nitrous oxide emission factors • urease inhibitor • urine

Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas with a global warming potential 298 times higher than carbon dioxide (Intergovernmental Panel on Climate Change [IPCC], 2013). Moreover, N_2O transformation processes in the stratosphere lead to the destruction of the ozone layer (Ravishankara *et al*., 2009). N_2O is a long-lived compound and its atmospheric concentration had increased by approximately 40% by 2011 compared with the pre-industrial levels (IPCC, 2013). Consequently, it is vital to accurately account for N_2O sources from human activities as well as sinks in order to develop effective mitigation strategies (Oenema *et al*., 2005).

Agricultural soils are a substantial source of N_2O, accounting for 35% of the global annual emission of N_2O (Virkajärvi *et al*., 2010). Pasture-based livestock agriculture in particular is considered a nitrogen (N) 'leaky' system, with less than 30% of applied N recovered in final products (Goulding *et al*., 2008). This is primarily due to low N utilisation efficiency by the ruminant livestock, which deposit 70–95% of their N intake onto pastures as dung and urine (Saggar *et al*., 2013; Dijkstra *et al*., 2013). Emissions of N_2O arising from

these pasture, range and paddock returns comprise > 40% of the N_2O associated with animal production systems (Oenema *et al*., 2005). N_2O is mainly produced during nitrification and denitrification processes (Wrage *et al*., 2001) although N can also be lost as dinitrogen (N_2) through co-denitrification and complete denitrification (Selbie *et al*., 2015), ammonia (NH_3) volatilisation (Fischer *et al*., 2016), nitrate leaching (NO_3^- N) and dissolved organic nitrogen (DON) (Flechard *et al*., 2007). The IPCC (2006) has estimated that 2% of animal excreta N is lost as N_2O. This is the default value emission factor (EF) for pasture, range and paddock (EF_3) used in Tier 1 methodology for reporting national N_2O emissions. However, there is large uncertainty around this value and the IPCC encourages the use of country-specific (Tier 2) values where possible. Countries such as New Zealand have already refined the methodology (de Klein *et al*., 2003; van der Weerden *et al*., 2011), while the UK is currently in the process of obtaining higher-Tier EFs (Bell *et al*., 2015).

Animal excreta N is not the only source of N_2O in grass-based pastoral agriculture. In intensively managed grazing systems,

mineral fertiliser is typically spread shortly after the grassland has been grazed to promote regrowth between rotational grazing cycles. Consequently, a portion of this fertiliser is applied to urine patches that cover between 14% and 21% of pasture annually (Dennis *et al*., 2011). Typical grassland fertiliser application rates do not exceed 200 kg N/ha, split among multiple applications over the year. The IPCC default EF for the application of synthetic fertiliser (EF$_1$) is 1% (IPCC, 2006); however, similarly to EF$_3$, there is a large uncertainly around this figure. Current scientific efforts have focussed on establishing disaggregated EFs (van der Weerden *et al*., 2011), which means separate EF values for each source of N$_2$O, i.e. animal excreta EF can be disaggregated (separated) between urine and dung. In a similar manner, the EF for fertiliser N applied to urine patches would consist of separate EF$_1$ and EF$_3$ added together (re-aggregated). However, there is a knowledge gap in terms of N$_2$O emission from urine and fertiliser N applied simultaneously. There have been few studies investigating the effect of fertiliser application onto urine patches on N$_2$O emissions to date. The study of Hyde *et al*. (2016) found a multiplicative effect of calcium ammonium nitrate (CAN) application to urine patches in grazed grassland, i.e. the N$_2$O emission from urine and CAN applied together exceeded the sum of the N$_2$O emission for urine and CAN applied separately. This would suggest that re-aggregating disaggregated EF$_1$ and EF$_3$ values in order to calculate the N$_2$O emission associated with N fertilisation of urine patches could be inaccurate. Therefore, there is a need to improve the understanding of the interaction of fertiliser N with urine N in grazed grassland environments. There has been little research to date on the implications for N$_2$O losses of applying other fertiliser formulations, such as urea or urea stabilised with urease [e.g. *N*-(*n*-butyl) thiophosphoric triamide (NBPT)], or nitrification (e.g. dicyandiamide, DCD) inhibitors to urine patches. While NBPT inhibits hydrolysis of urea to ammonium (NH$_4^+$-N), hence mitigating NH$_3$ loss from urea fertiliser (Forrestal *et al*., 2016), DCD delays the bacterial oxidation of NH$_4^+$-N into NO$_3^-$ N, which reduces denitrification and leaching losses (Halvorson *et al*., 2014). We hypothesise that fertiliser N will interact with urine, leading to enhanced N$_2$O emissions, and that emissions will vary depending on fertiliser formulation.

The objectives of this study were to: a) determine whether the sum of N$_2$O emissions from urine and a range of N fertilisers applied alone (disaggregated and then re-aggregated) approximated the N$_2$O emission of urine and fertiliser N applied together (aggregated), i.e. was the effect additive or multiplicative (as observed by Hyde *et al*. 2016 for CAN); b) establish whether the relationship varied among N fertilisers.

Materials and methods

Experimental site

The experiment was conducted between May and August 2015 at the Teagasc Johnstown Castle Research Centre, Co. Wexford, Ireland (52°18′N; 6°30′W). The field site was a moderately drained soil with a sandy loam surface (0–10 cm) soil texture (51.7% sand, 33.9% silt, 14.4% clay; pH 5.7; 0.3% N; 2.8% C), classified as Eutric Cambisol (Food and Agriculture Organization of the United Nations [FAO]–United Nations Educational, Scientific, and Cultural Organization [UNESCO], 1988). The pasture sward consisted of mainly perennial ryegrass (*Lolium perenne* L.) and was reseeded in 2013. Animals were excluded from the experimental site for > 1 yr, and no fertiliser was applied for 8 mo prior to the experiment. The climate is temperate maritime, with mean annual air temperature of 10.4°C and mean cumulative rainfall of 1,037 mm (1981–2010, 30 yr average). Rainfall and the air/soil temperature were recorded at the meteorological station 200 m from the experimental site. Modelled soil moisture deficit (SMD) values were calculated using a modified Penman–Monteith equation (Allen *et al*., 1998; Schulte *et al*., 2005). Water-filled pore space (WFPS) was calculated based on volumetric soil moisture content determined using a Theta probe (type ML2; Delta-T Devices, Cambridge, UK) and the gravimetric moisture content of soil samples.

Treatments

The experimental treatments (urine ± CAN and urine ± urea ± urease inhibitor ± nitrification inhibitor) and rates of application are displayed in Table 1. The same source of urea was used for all urea formulations. The nitrification inhibitor DCD was incorporated into the urea melt at a rate of 1.6% on a urea weight basis. The source of the urease inhibitor NBPT was Agrotain® (Koch Agronomic Services, Wichita, Kansas, USA), which was coated onto the urea granules at 660 mg/kg NBPT. Urine was collected from lactating dairy cows grazing at pasture and this urine was stored at 4°C prior to analysis and application. The urinary-N content was 6.5 g N/L. The experimental design was a randomised complete block with five replicates. Each experimental unit contained a 40 cm × 40 cm area within the chamber collars where the experimental treatment was applied. This area was used for gas sampling. In three out of five blocks, the experimental units also contained soil-sampling plots (i.e. three replicates per treatment for soil sampling). For treatment application to the soil sampling plots, a template collar of 40 × 40 cm was used. In order to obtain enough soil sampling area for the whole length of the experiment, three patches were installed per replicate. Urine was applied at a rate of 2 L/collar on 5 May 2015 and the

Table 1. List of treatments together with application rates, as well as cumulative N_2O emissions and fractions of N applied lost as N_2O as determined by the calculation method (disaggregated and re-aggregated losses *vs.* aggregated losses)

Treatment	Application rate (kg N/ha)		N_2O emission (N_2O-N kg/ha)		Fraction of N applied lost as N_2O (%)		Re-aggregated N_2O from disag-gregated urine and fertiliser		Relative N_2O loss from re-aggregated urine and fertiliser compared to ag-gregated urine and fertiliser
	Fertiliser	Urine							
Control	0	0	0.11	B	-	-			
Urea	40	0	0.21	B	0.25	b			
Urea + DCD	40	0	0.12	B	0.02	b			
Urea + NBPT	40	0	0.18	B	0.17	b			
Urea + NBPT + DCD	40	0	0.14	B	0.06	b			
CAN	40	0	1.07	A	2.39	a			
LSD			0.2		0.01				
Control	0	0	0.11	b	-	-			
Urine	0	861	2.93	a	0.33	a			
Urine + Urea	40	861	3.73	a A	0.4	a	3.14	A	0.84
Urine + Urea + DCD	40	861	3.68	a A	0.4	a	3.05	A	0.83
Urine + Urea + NBPT	40	861	4.77	a A	0.52	a	3.11	A	0.65
Urine + Urea + NBPT + DCD	40	861	3.77	a A	0.41	a	3.07	A	0.81
Urine + CAN	40	861	5.07	a A	0.55	a	4.00	A	0.84
LSD			0.65		0.003				

Mean values within columns followed by same letter (lower-case lettering) in each section are not significantly different at *P*<0.05. Separate statistical analyses performed for the top and bottom parts of the table.
Mean values within rows followed by same letter (upper-case lettering) are not significantly different at *P*<0.05.
CAN = calcium ammonium nitrate; DCD = dicyandiamide; LSD = least significant difference; NBPT = *N*-(*n*-butyl) thiophosphoric triamide.

fertiliser formulation treatments were applied the following day at 40 kg N/ha, simulating typical grazing management practice.

N_2O sampling and analysis

N_2O fluxes were measured on 23 occasions between May and August 2015 using the closed static chamber technique (Mosier, 1989; de Klein and Harvey, 2012). N_2O was sampled daily for the first week, every second day for the next 3 wk, every third day in the following week and then once a week for the remaining experimental period. Square stainless steel collars inserted at a minimum of 5 cm depth into the soil and 10 cm high covers, both with dimensions of 40 cm × 40 cm, were used for N_2O sampling. Collars were covered with a neoprene strip, and a 10 kg weight was placed on top of the cover, compressing the neoprene in order to ensure airtight sealing of the headspace at sampling. Following 40 min of chamber deployment, a 10 mL air sample was removed through a rubber septum (Becton Dickinson, Oxford, UK) using a 10 mL polypropylene syringe (BD Plastipak; Becton Dickinson) fitted with a hypodermic needle (BD Microlance 3; Becton Dickinson). Air samples were injected into a pre-evacuated (to –1,000 mbar) 7 mL screw-cap septum glass vials (Labco, High Wycombe, UK). Eight samples of ambient air collected on each gas sampling occasion were used as t_0 N_2O concentration for the flux calculations. Linearity of accumulation of N_2O in the chamber headspace was checked on each sampling day by sub-sampling five various treatments throughout the 60 min chamber enclosure period, collecting five headspace samples per chamber (Chadwick *et al.*, 2014). Samples were returned to ambient pressure immediately before analysis and fed into the system by a Combi-PAL automatic sampler (CTC Analytics, Zwingen, Switzerland). N_2O concentrations were analysed using a gas chromatograph (GC) (Varian CP 3800 GC; Varian, Walnut Creek, CA, USA) fitted with a [63]Ni electron capture detector (ECD) with high-purity helium as a carrier gas. Areas under N_2O peaks were integrated using Star Chromatography Workstation (Varian). Hourly N_2O emissions were calculated based on the rate of change in N_2O concentration within the chamber during the measurement period. Gas sampling was undertaken between 10:00 and 13:00 for an enclosure time of 40 or 60 min for treatment and linearity samples, respectively, to obtain measurements representative of the average hourly flux of the day, and these were used to calculate daily emissions (Blackmer *et al.*, 1982; de Klein *et al.*, 2003).

Cumulative emissions were obtained by integration of daily fluxes and linear interpolation between measurement points (de Klein and Harvey, 2012).

Soil mineral N content and analysis

Soil samples were collected on seven occasions following treatment application. Samples were collected once a week for the first 4 wk, then once 2 wk later and finally twice at monthly intervals. Each fertilised 40 cm x 40 cm patch was sampled a maximum of three times during the study; therefore, three patches per replicate were created. On each sampling occasion, five soil cores were taken from across the patch to a depth of 10 cm. The cores were bulked in plastic sample bags and kept in cool conditions during the transport to the laboratory. Samples were sieved using a 4 mm sieve and extracted in 2 M KCl (5:1 ratio, shaken for 1 h). The extracts were analysed for NH_4^+-N (Standing Committee of Analysts, 1981) and total oxidised N (NO_2^- N + NO_3^- N) (Askew, 2012) by colourimetric analysis using an Aquakem 600 discrete analyser (Thermo Electron OY, Vantaa, Finland). Sub-samples were analysed for gravimetric moisture content by drying the soils for 24 h at 105°C in order to express mineral N concentrations in dry soil.

Statistical analysis

The proc GLIMMIX procedure of SAS 9.3 (2002–2010; SAS Institute Inc., Cary, NC, USA) was used to output lsmeans by treatment and time for daily N_2O fluxes and soil mineral N concentrations. The terms in the model were treatment, time and the interaction of these two factors. Differences in cumulative N_2O and fraction of N applied lost as N_2O (%) between treatments over the study period were determined using the proc GLIMMIX procedure of SAS using the F-protected least significant difference (LSD) test (Table 1). Data on N_2O and the percentage of applied N lost as N_2O was checked for normality of distribution and log-transformed prior to analysis. The terms in the model were treatment as a fixed effect and block as a random effect. Statistical analysis was performed separately for fertiliser-only treatments and for urine and urine + fertiliser treatments. The difference between the sum of N_2O emissions from urine and a range of N fertilisers applied alone (disaggregated and then re-aggregated) and the N_2O emission of urine and fertiliser N applied together (aggregated) was examined using statistical (proc GLIMMIX procedure in SAS) and relative approaches. N_2O emissions are highly variable (Mathieu *et al*., 2006), making statistical difference between treatments difficult to detect; hence, the conundrum of Type 1 and Type 2 statistical errors (Edmeades and McBride, 2012) is particularly pertinent to studies testing for difference in N_2O emissions between treatments. Consequently, the present study also uses what is a common approach in agronomy, i.e. the relative performance of treatments compared with a chosen reference treatment (e.g. Watson *et al*., 1990; Forrestal *et al*., 2012) as a tool to draw insights into trends between treatments.

Results

Environmental variables

The field site received 274 mm of rainfall during the 95 d of the experiment, which is approximately 39% more than the 30 yr average for the same period (Figure 1). There were seven events with the level of rainfall exceeding 10 mm. Within 10 d following urine application, 68.4 mm rainfall was recorded, which is just greater than the 30 yr average for the month of May. Air temperature first increased between May and July and decreased thereafter, with daily averages recorded between 8.3°C and 18.5°C. Soil temperature followed a similar pattern, recording a daily average of between 9.6°C and 20.6°C (Figure 1). Mean air temperature throughout the experiment was approximately 7% lower than the 30 yr average. In the first 10 d of the experiment, soil moisture deficit was often at 0%, indicating soil saturation. High moisture content of the soil was also reflected in the WFPS, which averaged 72% for the experimental period and ranged between 32% and 100% (Figure 1), with 64 out of the total 92 d recording WFPS > 65%.

Soil mineral N content

Soil NH_4^+-N varied significantly over time following N application ($P < 0.0001$). Treatment was a significant source of variation ($P < 0.05$) in the case of fertiliser treatments applied to urine patches. Mean soil NH_4^+-N concentrations in the control plots ranged between 0.8 and 2.6 mg N/kg soil. Soil NH_4^+-N levels increased rapidly following treatment application, particularly for treatments that included urine (Figure 2a, d), with concentrations ranging between 17.8 and 232.6 mg N/kg for the urea and urine + urea + NBTPT treatments, respectively on Day 0. There were two well-defined peaks in soil NH_4^+-N within the urine treatment, namely 124.8 and 143.1 mg N/kg observed 2 d and 16 d after urine deposition, respectively (Figure 2d).

While the NH_4^+-N concentration in the soil in the fertiliser-only and urine + fertiliser treatments followed a similar pattern, soil NO_3^- N response varied between these two groups of treatments (Figure 2b, e). A significant interaction between time and treatment was detected for soil NO_3^- N ($P < 0.01$) in the fertiliser-only treatments. Soil NO_3^- N in the fertiliser treatments increased only in the case of CAN treatment, while in the urine + fertiliser treatments, an increase in NO_3^- N was observed in all cases. However, the NO_3^- N peak of 50 mg/kg (urine + fertiliser treatments) was relatively small compared to the NH_4^+-N peaks and coincided with a decrease in WFPS.

Soil mineral N was elevated only during the first sampling in the fertiliser treatments, whereas in the urine + fertiliser treatments, NH_4^+-N and NO_3^- N returned to background levels within 1 mo and 2 mo of application, respectively.

N_2O emissions

A significant time-by-treatment interaction was detected for N_2O emissions ($P < 0.0001$), indicating that treatment effect varied over time. The majority of N_2O was emitted shortly post-application, within between 2 wk and 4 wk for the fertiliser (CAN) and urine + fertiliser treatments, respectively (Figure 2c, f). The largest mean daily N_2O flux was 992 g N_2O-N/ha per day observed from urine + CAN 12 d post-application. Emissions returned to background levels within 5 wk following treatment
application. The highest cumulative N_2O emission was observed from the urine + CAN treatment (5.1 kg N_2O-N/ha) (Table 1). Cumulative N_2O emissions from urea-based fertilisers were not different from those of the control, whereas emission from the CAN treatment was significantly higher than that from the control and other fertilisers. All treatments including urine showed significantly higher values than the control and all the fertiliser treatments. However, there were no significant differences between urine and urine + various fertiliser groups in terms of N_2O emissions. When N loss was expressed as a percentage of N applied, the CAN treatment showed significantly higher values than all other treatments (at 2.39%), with no significant difference observed for percentage N lost across all other treatments (Table 1). There was also no significant effect of urease or nitrification inhibitors on N_2O emissions or percentage N emitted from either the urea-only or the fertiliser + urine treatments. There was no statistical difference between the sum of disaggregated N_2O emissions (re-aggregated) and aggregated N_2O from urine and fertiliser N applied simultaneously (Table 1); however, there was a trend for 20% underestimation of N_2O from re-aggregated values compared with aggregated losses.

Discussion

N_2O emissions and soil mineral N patterns

N_2O emissions in this study were clearly separated into two groupings between fertiliser-only treatments and treatments that combined urine and synthetic fertiliser (Figure 2c, f). This is primarily attributed to a) the application rate, with fertilisers applied at 40 kg N/ha, while treatments including urine provided between 860 and 900 kg N/ha and b) stimulation
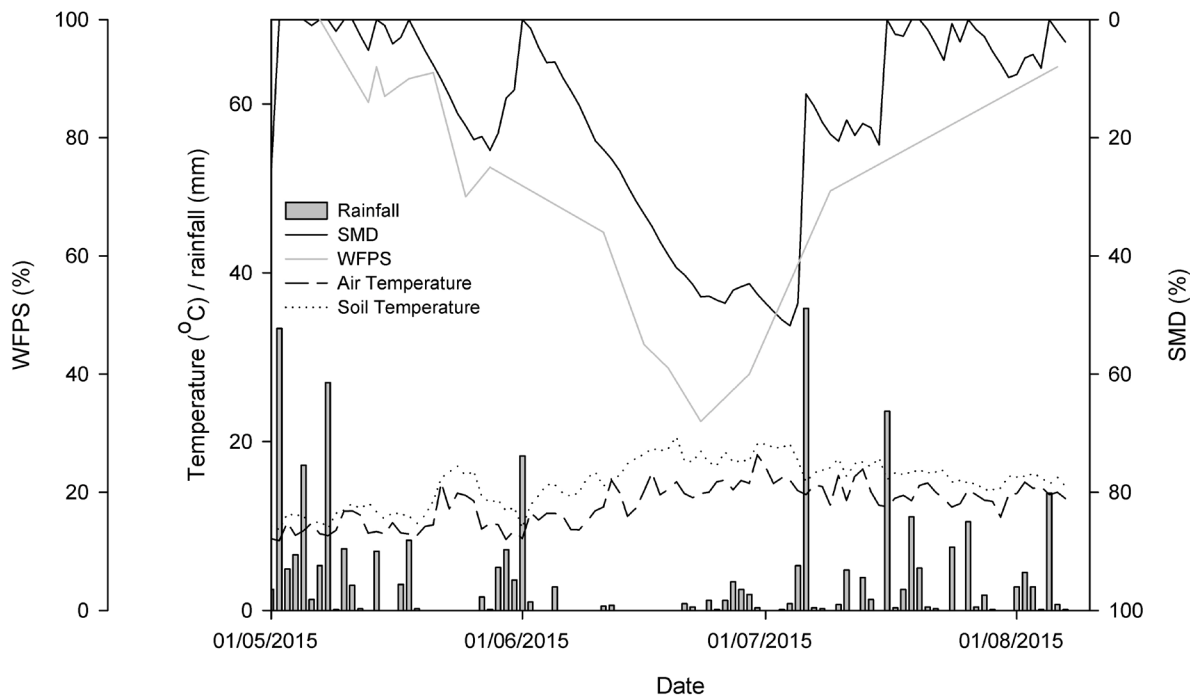

Figure 1. Soil and air temperature, soil WFPS, SMD and rainfall over the experimental period. SMD = soil moisture deficit; WFPS = water-filled pore space.
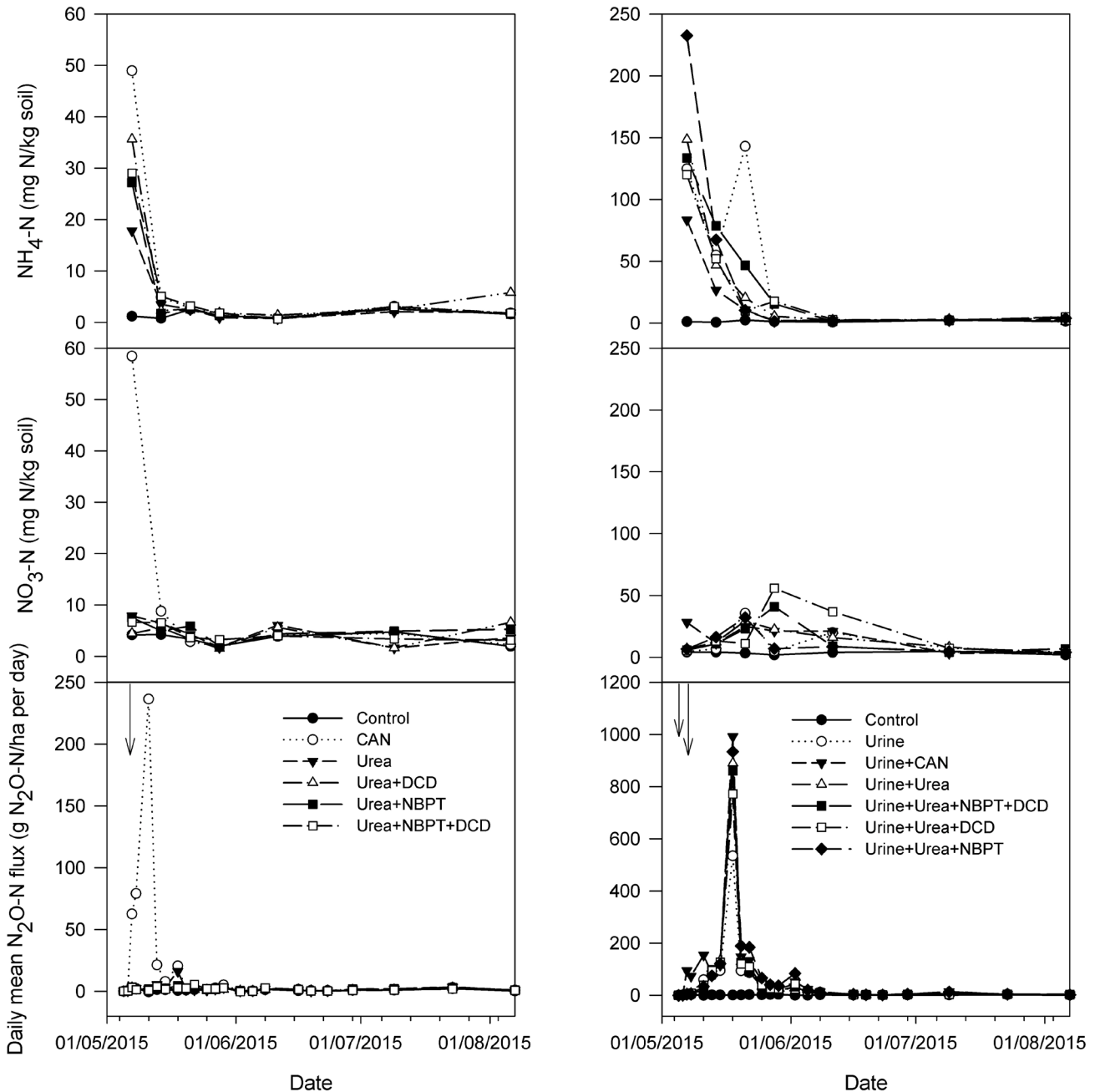
Figure 2. Mean temporal trace of soil NH_4^+-N and NO_3^- N (0–10 cm) and N_2O from control and fertiliser-only treatments (a, b and c) and from control, urine, and urine + fertiliser treatments (d, e and f). Arrow in (c) indicates fertiliser application. Arrows in (f) indicate application of urine (first arrow) and fertilisers (second arrow). CAN = calcium ammonium nitrate; DCD = dicyandiamide; NBPT = *N*-(*n*-butyl) thiophosphoric triamide.

of microbial activity by urine as well as the denitrification of indigenous soil N pool (Lambie *et al*., 2012). In fact, Wachendorf *et al*. (2008) found that 75% of urine-induced N_2O emission originated from the indigenous soil mineral N pool

in a German soil. In the case of fertiliser treatments, there was a clear N_2O peak observed only in the CAN treatment (Figure 2c). The largest N_2O flux was observed 5 d after the application, following an increase in soil NH_4^+-N and NO_3^- N

coming directly from the CAN fertiliser; however, soil mineral N and N_2O were close to background levels by the next soil sampling. Where urea-based fertilisers were applied, little N_2O emission was observed (Figure 2c). A peak in soil NH_4^+-N quickly declined. These results are in agreement with those of Harty et al. (2016b), who found the largest N_2O emissions following CAN fertiliser application, as opposed to urea-based fertilisers, particularly under wet conditions promoting denitrification of the NO_3^--N pool from the applied fertiliser. Similar environmental conditions were observed in this experiment, where 139% of the long-term average (LTA) rainfall was recorded, sustaining high WFPS favourable to denitrification activity. In fact, WFPS remained > 80% during the first 2 wk post-application. In these conditions, N_2O is expected to be produced through denitrification (Weier et al., 1993; Mathieu et al., 2006).

Treatments that combined urine and fertiliser exhibited a substantial peak in soil NH_4^+-N, which was depleted within 1 mo of application. A decrease in soil NH_4^+-N corresponded with a slower, smaller increase in soil NO_3^--N pool, suggesting the occurrence of other processes that consumed NH_4^+-N before it was nitrified to NO_3^--N. Application of urine causes the soil pH to rise in the urine patch (Clough et al., 2004). High pH shifts the $NH_3 <=> NH_4^+$-N equilibrium from hydrolysed urea (of the urine) in the soil towards the formation of NH_3, which subsequently can inhibit the conversion of NO_2^--N to NO_3^--N (Monaghan and Barraclough, 1993; Ali et al., 2013). If this urine-N is present in the soil in NO_2^--N form, it may be quickly lost via leaching (Dennis et al., 2012) and/or co-denitrification (Selbie et al., 2015). Low concentrations of soil NO_3^--N were observed in the current study, with a relatively small peak in NO_3^--N observed when the soil started to dry following approximately 2 wk of rainfall and as the soil pH decreased (data not shown). Dennis et al. (2012) observed large leaching losses from urine applied to grassland soil in the same location in the study using lysimeters. Plant uptake was a less-important N pathway as the study by Dennis et al. (2012) was conducted in the autumn; moreover, the authors also noted that weed ingress–reducing ryegrass cover could have reduced the uptake. The current study was conducted in spring/summer, when it was observed that perennial ryegrass was growing vigorously. The study of Selbie et al. (2015) observed large losses of N from urine patches in the form of N_2 from co-denitrification. Urine application to grazed grasslands might provide optimal conditions for co-denitrification through high N application rate, high supply and turnover of organic N and C as well as elevated pH (Clough et al., 2003; Selbie et al., 2015), which could explain the low soil NO_3^--N observed in the current study. Since the soil pH favours the creation of NH_3, its volatilisation is another possible N loss pathway from urine (Fischer et al., 2016) or fertiliser (Forrestal et al., 2016). However, volatilisation is believed to be of minor importance in this study due to high ambient and soil moisture conditions at application.

Temporal N_2O pattern

The temporal pattern of N_2O loss following urine deposition was comparable with that found in literature. The length of the N_2O peak was also comparable with the 30–70 d result reported by Yamulki et al. (1998), van Groenigen et al. (2005), van der Weerden et al. (2011) and Bell et al. (2015). A study by Krol et al. (2015) on the same research farm observed N_2O peak 11 d post-application, returning to background level by Day 44, peaking at approximately 700 g N/ha per day, in comparison to the 550 g N/ha per day in the current experiment. Hyde et al. (2016) observed a slightly earlier N_2O peak between Days 5 and 16, as well as relatively low emissions from urine patches alone. However, the highest N_2O peaks for CAN and urine + CAN treatments were very similar in both the current experiment and in the study by Hyde et al. (2016). CAN peaked at 240 g N/ha per day in the current experiment and in Hyde et al. (2016), while urine + CAN peaked at 670 g N/ha per day in Hyde et al. (2016) and at 1,000 g N/ha per day in the current study (Figure 2). The largest daily urine N_2O peak was higher in this study in comparison with previous work (Selbie et al., 2014; Hyde et al., 2016) despite similar application rates and significant plant demand. These high values may reflect the wet soil conditions, and consequently, the high WFPS, which would be favourable to denitrification.

Cumulative disaggregated emissions

Cumulative N_2O ranged from 0.1 to 5.1 kg N_2O-N/ha over 3 mo and was lowest in the absence of urine (Table 1). While all urea-based fertilisers had cumulative N_2O emissions that did not differ statistically from the control, emissions from CAN were significantly greater (P<0.05). The percentage N lost for urea was relatively low (at 0.25%), and inclusion of DCD reduced emissions to 0.02–0.06%. In contrast, CAN treatment showed significantly higher value (at 2.39%), similar to the 2.15% reported by Hyde et al. (2016). Harty et al. (2016b) also reported that fertiliser-only treatments that included DCD had the numerically lowest N_2O emissions, whereas CAN treatment showed significantly higher rates. However, although the fraction of N_2O loss from applied N was measured over 94 d, it could still be usefully compared to the IPCC Tier 1 methodology (Harty et al., 2016a) because this period captured most of the N input-associated N_2O emission. The percentage N_2O-N loss from urine patches was 0.33% in comparison with the 2% IPCC default value and could have been low due to alternative N loss pathways such as complete denitrification or co-denitrification to N_2. Urea-based fertilisers also exhibited low percentage N_2O-N loss, between 0.02% and 0.25%, which is well below the 1% IPCC default value.

It is believed that low N_2O losses from urea-based fertilisers are a result of the N form applied, which needs to undergo a series of processes to convert urea through ammonium to the mobile and susceptible-to-loss NO_3^- N. As a result, N can be efficiently utilised by the pasture before it becomes available for loss. This argument is further supported by the high N_2O and EF values from the CAN fertiliser group, where half of N is already in the NO_3^- N form.

Comparison of disaggregated and re-aggregated emissions with aggregated cumulative emissions

Fertiliser is commonly applied to pastoral soils shortly after grazing, meaning some of it is applied to fresh urine patches. In intensive and semi-intensive grazing systems with a stocking rate of between 2.0 and 2.94 cows/ha, these urine patches cover between 14% and 21% of the yearly grazed area (Dennis *et al*., 2011), and as these urine patches are zones of high N loading exceeding plant demand, they are susceptible to N loss. Urine patches yielded 2.9 kg N_2O-N/ha and had EF of 0.33%, which is comparable with previous results of 0.3%–0.9% (van Groenigen *et al*., 2005), 0.29% (van der Weerden *et al*., 2011), 0.4% (Selbie *et al*., 2014) and 0.4% (Buckthought *et al*., 2015). The application of CAN fertiliser to urine patches led to a rise in both N_2O emissions (to 5.1 kg N_2O-N/ha) and the EF (from 0.33% to 0.55%; Table 1). The application of urea-based fertilisers to urine patches yielded similar results but with a smaller increase in N_2O flux. Lower values may reflect either more efficient utilisation of urea N by plants as it takes longer for denitrification to occur or that a proportion of N has been volatilised following application. There was a trend towards higher emissions for the urea-only samples compared to the urea + NBPT (which inhibited volatilisation) samples. Therefore, the lower N_2O emissions associated with urea under these conditions were most likely due to the fact that urea had to undergo ammonification and nitrification processes prior to denitrification, allowing more time for plant uptake.

It was hypothesised that addition of urea-based fertilisers to urine patches would result in an increase in N_2O equal to the sum of N_2O from urine patches and from fertiliser separately applied to the pasture. A more complicated relationship between urine and CAN – as observed by Hyde *et al*. (2016) – was anticipated. Hyde *et al*. (2016) found that the combined emission was more than double the sum of the emission from urine and CAN fertiliser applied individually, i.e. application of urine and CAN applied together had a multiplicative effect on N_2O emissions. The rationale proposed by the authors was that CAN delivers half of N in NO_3^- N form with high denitrification loss potential on top of the urine patch, which provides a source of readily available carbon (Lambie *et al*., 2012), enhances soil carbon mobilisation and denitrification of N (Weier *et al*., 1993) and increases WFPS (Linn and Doran,

1984). However, in the present study, the relative emission of the re-aggregated separately derived emissions was typically in the range 81%–84% of the emission from fertiliser and urine applied together (Table 1), an underestimate – but less than that observed by Hyde *et al*. (2016). There was no evidence that the relationship between most of the urea fertilisers and urine was different than for CAN, the exception being urine and urea + NBPT, for which the aggregated emission was only 65% of the emission from both applied together. In all cases, although not statistically different at the 5% probability level, the N_2O loss relative to that from urine and fertiliser applied separately was consistently lower than when they were applied together, which is consistent with the findings of Hyde *et al*. (2016) that the N_2O emission from urine and fertilisers applied separately may underestimate the emission in a farm system where they are applied together. This has important implications for greenhouse gas inventories as the area affected by urine and fertiliser accounts for 14%–21% of the farm area annually. The lack of sensitivity of the experiment to detect a significant effect may be due to the relatively small addition of N applied to the urine patch. While the urine patch delivered a loading of 861 kg N/ha, synthetic fertiliser provided an additional 40 kg N/ha, which increased N loading by only 5%. Buckthought *et al*. (2015) observed similar results, where addition of either 200 or 400 kg urea-N/ha (in eight even splits of 25 and 50 kg N/ha, respectively) to urine patches did not significantly increase N_2O emissions or the fraction of applied N lost through N_2O. Buckthought *et al*. (2015) suggested either better utilisation of fertiliser N by pasture or loss through leaching or N_2. Similar environmental conditions in the current study and in the Buckthought *et al*. (2015) study, with high rainfall influencing soil moisture content, could have favoured loss through N_2 in both experiments. Hyde *et al*. (2016) found a larger discrepancy when re-aggregating emissions than was noted in the relative losses in the present study; however, Hyde *et al*. (2016) used a N rate of 90 kg N/ha applied on top of the urine patch, which would be representative of a ground grazed and fertilised for silage, whereas the current study used a lower N rate of 40 kg N/ha, which is more typical of a grazing rotation.

Conclusions

When the N_2O loss measured from fertiliser and urine applied separately (disaggregated) was re-aggregated, the calculated N_2O emissions consistently underestimated N_2O loss by 20% compared to that measured from urine and fertiliser applied together (aggregated). Although statistical difference was not detected, most probably due to the inherent variability in N_2O emissions data, the trend observed is important. These findings are significant for the re-aggregation of disaggregated

emissions at a national inventory or farm scale because areas of fertiliser and urine overlap can account for 14%–21% of grazed paddocks annually. Urine-N remained the main driver of losses, due to large N loading in urine in comparison with that of fertiliser. CAN and all urea-based fertilisers interacted with urine in the same manner. The short-term EFs generated in this study were lower than the IPCC default values in the case of urine and all urea-based fertilisers, while CAN treatment substantially exceeded the default. Therefore, farmers can reduce N_2O emissions in grazed temperate maritime pastures by switching from CAN to urea-based fertilisers without reducing the N fertiliser application rate.

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