Research Article • DOI: 10.1515/ijafr-2016-0001 IJAFR • 55(1) • 2016 • 1-9

Irish Journal of Agricultural and Food Research

The interactive effects of fertiliser nitrogen with dung and urine on nitrous oxide emissions in grassland

B.P. Hyde[1], P.J. Forrestal[2†], M.M.R. Jahangir[2,3], M. Ryan[2], A.F. Fanning[2], O.T. Carton[2], G.J. Lanigan[2], K.G. Richards[2]

[1]*Office of Climate, Licensing and Resource Use, Environmental Protection Agency, Regional Inspectorate, Monaghan, Ireland*
[2]*Department of Crops, Environment, & Land Use, Teagasc, Johnstown Castle, Wexford, Ireland*
[3]*Department of Civil, Structural & Environmental Engineering, Trinity College Dublin, Dublin 2, Ireland*

Abstract

Nitrous oxide (N_2O) is an important and potent greenhouse gas (GHG). Although application of nitrogen (N) fertiliser is a feature of many grazing systems, limited data is available on N_2O emissions in grassland as a result of the interaction between urine, dung and fertiliser N. A small plot study was conducted to identify the individual and interactive effects of calcium ammonium nitrate (CAN) fertiliser, dung and urine. Application of CAN with dung and urine significantly increased the mass of N_2O-N emission. Importantly, the sum of N_2O-N emitted from dung and CAN applied individually approximated the emission from dung and CAN fertiliser applied together, that is, an additive effect. However, in the case of urine and CAN applied together, the emission was more than double the sum of the emission from urine and CAN fertiliser applied individually, that is, a multiplicative effect. Nitrous oxide emissions from dung, urine and fertiliser N are typically derived individually and these individual emission estimates are aggregated to produce estimates of N_2O emission. The presented findings have important implications for how individual emission factors are aggregated; they suggest that the multiplicative effect of the addition of CAN fertiliser to urine patches needs to be taken into account to refine the estimation of N_2O emissions from grazing grasslands.

Keywords

calcium ammonium nitrate fertiliser • disaggregated emission factors • dung • nitrous oxide • urine

Introduction

Nitrous oxide (N_2O) is a greenhouse gas (GHG) with a global warming potential 298 times higher than CO_2 over a 100-year time horizon (IPCC, 2007). In 2011, the atmospheric concentration of N_2O was 391 ppm, which exceeds pre-industrial levels by approximately 40% (IPCC, 2013). In addition to its role as a GHG, N_2O can also deplete the stratospheric ozone. The potential of N_2O to influence global warming and stratospheric ozone depletion, in combination with its increasing concentration and long lifetime in the atmosphere, makes it crucial to understand the sources and sinks of N_2O to effectively estimate the losses and develop mitigation measures.

Soils are considered to be the dominant source of N_2O emissions, contributing 65% to the global N_2O emissions (IPCC, 2001). Agricultural soils are the major source of anthropogenic N_2O, responsible for about 35% of global emissions (Virkajärvi *et al*., 2010). Between 30 and 50% of the total N_2O emissions from agriculture originate from animal production systems (Mosier *et al*., 1998). Sources of N_2O include urine and faecal N deposition by livestock, the application of chemical and organic nitrogen (N) fertilisers and, indirectly, from ammonia (NH_3) volatilisation and leached N (Flechard *et al*., 2007). Significant uncertainties exist in N_2O estimates from grazed pasture because of the spatial distribution of urine and dung deposition (Watson and Foy, 2001), the heterogeneity of these deposits and the episodic nature of N_2O emissions. Fertiliser N application and excretion of animal urine and dung, which are rich in N, create hotspots for N_2O emission. Urine patches in pastures rank among the highest sources of N_2O emission from animal production systems (van Groenigen *et al*., 2005b) and grazing animals have been identified as significant contributors to the global N_2O budget (Oenema *et al*., 1997). The effect of urine on N_2O emissions has been investigated using artificial urine (Anger *et al*., 2003; de Klein and van Logtestijn, 1994; Clough *et al*., 1996), in controlled laboratory conditions (Monaghan and Barraclough, 1993; van Groenigen *et al*., 2005a), on lysimeters (Selbie *et al*., 2014) and in field studies with real urine (Krol *et al*., 2015; Sordi *et al*., 2013; de Klein *et al*., 2003). The contribution of dung patches to N_2O

†*Corresponding author: Patrick J. Forrestal*
E-mail: patrick.forrestal@teagasc.ie

emissions have also been investigated (Flessa *et al*., 1996; Allen *et al*., 1996; Yamulki *et al*., 1998; van der Weerden *et al*., 2011; Sordi *et al*., 2013). Recent approaches have focused on generating 'disaggregated' emission factors for dung and urine (van der Weerden *et al*., 2011). However, there is a significant gap in our understanding of the interaction between fertiliser N, dung and urine in terms of N_2O emission. Fertiliser N application is a feature of intensive grazing systems whereby the fertiliser is typically spread shortly after the grassland has been grazed to promote regrowth between rotational grazing. Consequently, we need to understand how dung or urine patch N_2O emissions behave in combination with fertiliser N, which reflects reality in rotationally grazed grasslands. The objectives of the present research were to determine the effects on N_2O emission of dung, urine and calcium ammonium nitrate (CAN) applied alone and to then determine how the sum of individual emissions for these treatments compared to their application in combination. The goal was to establish if the effects are additive or if aggregating individual emission factors is more complex. Understanding if dung and urine N_2O emissions behave in an additive or a multiplicative manner, when combined with inorganic fertiliser N, will be important for generating accurate estimates of N_2O emissions in fertilised systems.

Materials and methods

Experimental site

The experiment was undertaken between May and November 2003 on an imperfectly drained clay loam soil site at the Teagasc Environment Research Centre, Johnstown Castle, Wexford, Ireland. The plots chosen for the experiment had not received N for two years but herbage was cut and removed during this period, thus background soil inorganic N levels were expected to be relatively homogeneous across the site compared with a site with a history of grazing. The sward was predominantly perennial ryegrass (*Lolium perenne* L.).

Treatments

The experimental treatments were: zero N (control), dung, urine, CAN fertiliser N, urine & dung, dung & CAN, urine & CAN and urine & dung & CAN. A completely randomised experimental design with three replications per treatment was used. Urine was collected directly from dairy cows and stored at 4°C prior to analysis and application. Dung was collected immediately after defecation at pasture and stored, as above, prior to analysis and application. Representative sub-samples of both dung and urine were analysed for N content (Table 1). Based on the results of N content analysis (Table 1), 0.75 kg of dung and 1.25 L of urine was applied to a 15-cm diameter area within the larger 30-cm diameter N_2O measurement collars on 9 May (day 0). This approach was taken to allow for the area of soil affected by the excreta, which is approximately twice the area of the initial excreta (Lantinga *et al*., 1987). Each measurement collar was placed in the centre of a 0.83 × 1.5 m plot that had no treatment applied. The chosen application rates are representative of typical cattle excreta deposition rates (Lantinga *et al*., 1987). CAN fertiliser was applied at a rate equivalent to 90 kg N/ha to the full 30-cm diameter N_2O measurement collar, either alone or in combination with dung and/or urine at the rates indicated above.

Table 1. Nitrogen contents of dung and urine used in N_2O emissions measurements.

Excreta	N content (g N/kg)	Moisture content (%)	Mean N loading/collar (kg N/ha)
Dung	4.1	87	435
Urine	6.75	100	1194

N_2O sampling and analysis

Over the course of the study, N_2O emissions were measured on 31 occasions between May and November. Emission measurements were conducted on a daily basis for the first two weeks after treatment application, subsequently reduced to twice weekly, and thereafter to once weekly. Nitrous oxide emissions were measured using the static chamber technique. Permanent steel collars (30 cm diameter) were inserted to a minimum depth of 3 cm into the soil two weeks prior to the treatment application. Steel chambers (30 cm diameter, 33 cm high) were attached to steel collars during measurement periods using rubber seal to ensure an airtight seal. Following 60 minutes of chamber deployment, an air sample was taken. In the current experiment, air samples were collected through rubber septum (BD vacutainers, Becton Dickinson, Spain) using 10 mL polypropylene syringes (BD Plastipak, Becton Dickinson, Spain). The headspace air sample was transferred to pre-evacuated 7 mL screw-cap septum vials (Perbio Science, UK) fitted with Tuf-Bond (Teflon-Silicone) septa (Perbio Science, UK) for storage and analysis within six hours. The injection of 11 mL over-pressurised the sample vials, thus preventing any back-diffusion of ambient air.

Analysis of N_2O and calculation of N_2O flux

Nitrous oxide concentration was analysed using a Varian 3800 gas chromatograph (Agilent Inc., UK) coupled to a 63Ni electron capture detector (ECD) and Combi-Pal auto-sampler (CTC Analysis, Switzerland) and Porapack Q 80/100 mesh packed column (Sigma Aldrich, UK). For each sample run, a calibration curve was used. There were five calibration standards included with N_2O concentrations of 0.2, 0.5, 1.0, 5.0, 10.0 ppm, which were in the expected range of the sample N_2O concentrations (Argo International, UK).

The chamber N_2O-N flux was calculated according to Eq. (1): Chamber N_2O-N flux = (the slope of the line between T_0 and T_{60}) × ((M × P) / (R × T)) × (V/A)
where ambient air samples were used for the time zero (T_0) N_2O concentration. The N_2O flux was calculated based on the assumption of a linear increase in the concentration of N_2O in the chamber during 60 minutes (T_{60}) of deployment. Although this assumption was not verified in the current study, it has been verified at this location by Krol *et al*. (2015). Furthermore, Chadwick *et al*. (2014) investigated the assumption that N_2O accumulation rate in static chambers is linear and that more than 90% of chambers exhibited linear accumulation of N_2O in the chamber headspace (n=1970). *M* is the molar mass of N_2O-N (28 g/mol), *P* and *T* are the atmospheric pressure (Pa) and temperature (K) measured by a weather station within 1 km of the experimental site, *R* the ideal gas constant (8.314 J/mol/K), *V* the headspace volume of the closed chamber (m^3) and *A* the area covered by the base of the gas chamber (ha). The chamber N_2O-N flux was used to calculate the emission per ha for the day of measurement. The trapezoidal integration method (de Klein and Harvey, 2012) was used to interpolate between measurement days and to determine the cumulative N_2O-N loss for the experimental period.

Measurement of soil mineral N content
Soil samples were collected on five occasions during the study period. Samples were collected to 10-cm depth from three positions within the 30-cm diameter N_2O measurement collar one from under the centre of the excreta patch, one from the edge of the patch and one from within the area described by Saarijärvi and Virkajärvi (2009) as the non-initially wetted zone of influence. Dung was placed on Netlon™ windbreak with 7 mm aperture size (Tenstar International, Blackburn, UK) to allow the dung patch to be removed to sample beneath its centre. The three soil samples from each patch were bulked and soil mineral N was determined by extraction using 2 *M* KCl at a ratio of 5:1 and shaking with an automated shaker (New Brunswick Scientific Model G-10 Gyrotory shaker) for one hour. The extract was filtered through Whatman No. 2 filter paper (Whatman International Ltd., Maidstone, UK). Nitrate and NH_4^+-N in extractant was determined by colorimetric analysis using a Chemlab System 4 (3 channel) auto analyser (Chemlab Instruments, Essex, England).

Rainfall, soil temperature and moisture measurements
Environmental parameters were measured by the meteorological station at Johnstown Castle. Soil temperature was recorded by a Model 107 temperature probe (Campbell Scientific, UK). Three CS 615 water content sensors (Campbell Scientific, UK) were inserted into the soil within the experimental area of each plot at an angle of 45° to monitor the volumetric soil moisture content of the surface 15 cm.

Statistical analysis
The proc GLIMMIX procedure of SAS 9.3 (© 2002-2010, SAS Institute Inc., Cary, NC, USA) was used to test for treatment effects. The terms in the model were treatment, day of measurement and the interaction of these two factors. The response variables were daily N_2O-N flux, soil nitrate (NO_3^--N) and NH_4^+-N. Differences in cumulative N_2O-N flux between treatments over the study period were determined using the proc GLIMMIX procedure of SAS using the *F*-protected LSD test.

Results

Environmental variables
Soil temperature increased as the summer progressed and declined during autumn and winter (Figure 1). Following the treatment application, a period of sustained rainfall occurred and the volumetric moisture content exceeded 50% for the initial 40 days of the experiment. The elevated moisture content led to elevated water filled pore space (WFPS) levels, which were >80% during this initial 40 days of the experiment (Figure 1).

Soil mineral N content
A significant interaction between the day of sampling and treatment was detected for soil NO_3^--N and NH_4^+-N (P<0.01). Initial soil NO_3^--N and NH_4^+-N levels measured prior to treatment application were less than 10 kg N/ha (Figure 2 and 3). Soil NH_4^+-N levels increased rapidly following treatment application, particularly for treatments that included urine. Soil NH_4^+-N levels for the dung treatments were not significantly different from those of the control. The highest soil NH_4^+-N levels of 51, 48 and 46 kg N/ha, were observed in the urine & CAN, CAN only and the urine only treatments, respectively (Figure 3).
Soil NO_3^--N levels increased significantly compared with the control for treatments that included either urine alone or CAN (Figure 2); nitrogen levels were highest for the urine & CAN and the CAN only treatments at 40 and 38 kg N/ha, respectively on day three. Soil NO_3^--N for the dung treatment was not significantly different from control (Figure 2).

Nitrous oxide emissions
A highly significant (P < 0.001) treatment by measurement-day interaction was observed for N_2O emissions (Figure 4). The majority of the N_2O emissions during the 180-day

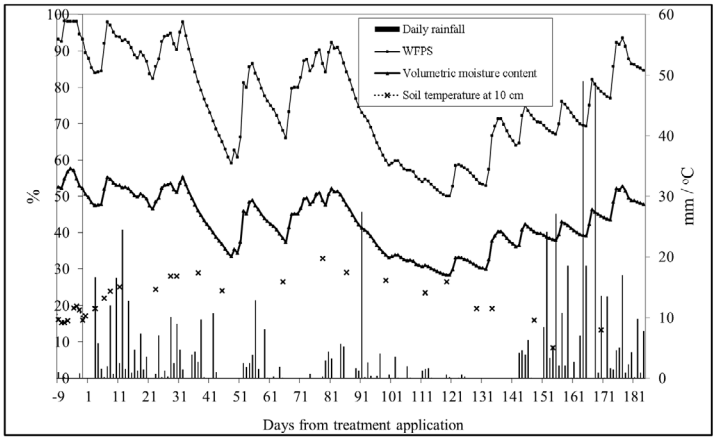

Figure 1. Soil temperature, moisture, water-filled pore space (WFPS) and rainfall over the experimental period.

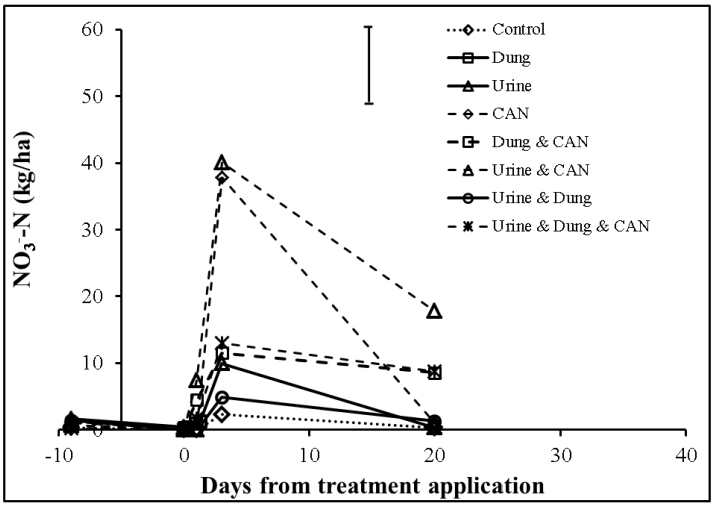

Figure 2. Soil NO_3^--N (0–10 cm) over the initial weeks following treatment application; error bar indicates the pooled standard error of the mean.

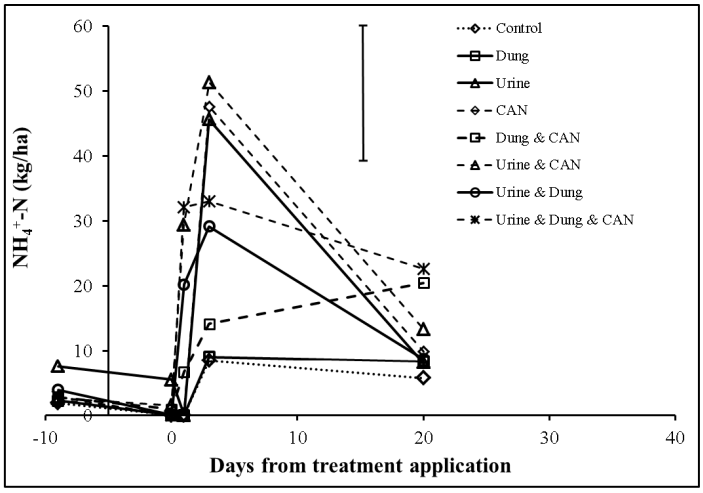

Figure 3. Soil NH_4^+-N (0–10 cm) over the initial weeks following treatment application; error bar indicates the pooled standard error of the mean.

measurement period following treatment application occurred during the 20 days following the application. Figure 4 focuses on this active period. The largest N_2O emissions occurred within 10 days of the treatment application (Figure 4). By day 20, emissions for all treatments had returned to background levels (Figure 4) and remained at this level throughout the remainder of the measurement period of 180 days (data not shown). The highest net cumulative N_2O-N emission was from the urine & CAN (5.52 kg/ha) and urine & dung & CAN treatments (4.83 kg/ha) (Figure 5). The N_2O-N emissions from CAN alone and dung & CAN were not significantly different. When applied individually, emissions followed a trend CAN > Urine > dung. However, while the sum of the individual N_2O-N emissions from dung and CAN (2 kg/ha) approximated the emission from dung + CAN (2.12 kg/ha), the sum of the emission from urine + CAN applied individually (2.49 kg/ha) was less than 50% of the emission from urine + CAN applied together (5.52 kg/ha).

Discussion

N_2O emissions over time

The largest emissions occurred five days following treatment application and corresponded with high soil NO_3^--N levels (Figure 2) and a precipitation event (Figure 1). For many treatments, soil mineral N levels had declined to levels approaching the control 20 days following application (Figures 2 and 3). The decline in soil mineral N is attributed to vigorous uptake of applied N by grass, which reduced the

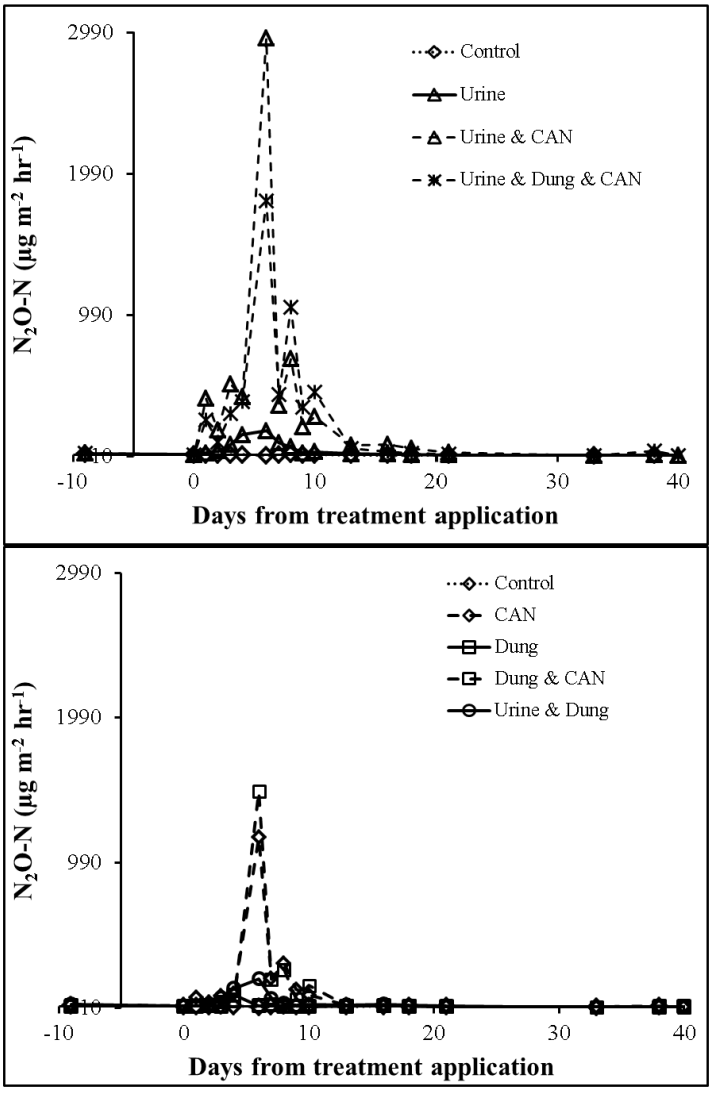

Figure 4. Temporal flux of N_2O-N emission; presentation confined to the active 40-day period following treatment application on 9 May (day zero) and treatments split across two graphs to aid visual interpretation.

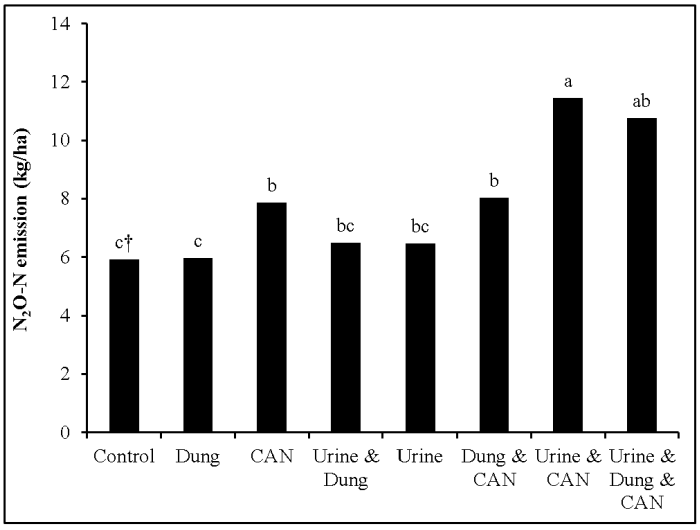

Figure 5. Cumulative N_2O emissions (kg/N/ha) for experimental treatments over the experimental period. †Means with different letter indicate significant differences according to *F*-protected L.S.D. test.

potential pool of NO_3^--N available for denitrification. Peak N_2O emission from similar animal excreta experiments returned to background levels by day 10 (Flessa *et al*., 1996), day 35 (van Groenigen *et al*., 2005b), day 40 (Yamulki *et al*., 1998) and day 36 (Krol *et al*., 2015). Similar to the current study, Allen *et al*. (1996) attributed the highly contrasting occurrences of peak dung-derived N_2O emissions and their timing to application timing, weather conditions and soil type.

The significant (P<0.01) interaction between treatment and measurement day indicates that the effects of each treatment on N_2O emissions are time-specific. This interactive effect on N_2O emissions indicates that N_2O measurements should be taken intensively until emissions approach background levels for all treatments.

Cumulative N_2O emissions: dung, urine and CAN individually

Net N_2O emissions from dung alone were low. Although the ammonification of water-soluble organic N compounds in dung is rapid, the remaining N is resistant to mineralisation (Hoekstra *et al*., 2011). Consequently, mineralisation of the organic N in dung may take months to years (Ball and Ryden, 1984; Hoekstra *et al*., 2011). Net cumulative N_2O emissions followed the trend dung < urine < CAN, although cumulative emissions did not differ significantly across these treatments (Figure 5). This trend in emission is close to the trend of soil NO_3^--N on day 3 both in order and in relative magnitude (Figure 2), emphasising the strong link between the size of the soil NO_3^--N pool and N_2O emissions.

Net Cumulative N_2O emissions: dung and urine in combination each other and with CAN

Dung and urine, when applied together, produced a net cumulative emission of 0.56 kg N_2O-N/ ha (Figure 5), which was approximately the sum of their individual emissions (0.6 kg N_2O-N/ ha). When expressed as a percentage of applied N lost as N_2O-N (emission factor), the individual emissions for dung (0.0027%) was less than 0.04%, as reported by van der Weerden *et al*. (2011). The urine emission factor of 0.115% was lower than 0.29%, as reported by van der Weerden *et al*. (2011) or by Krol *et al*. (2015) who reported 0.9–1.3%. Differing emission factors in other studies could be due to soil type and climatic conditions, which can be important factors affecting N_2O emissions (Rochette *et al*., 2008) and its conversion to N_2, resulting in a lower emission factor (Jahangir *et al*., 2011; Jahangir *et al*., 2012). Different fodders, feed additives and grazing regimes may affect N concentrations in urine and dung, which can have a significant effect on N_2O emissions (Oenema *et al*., 1997). Aggregated net individual N_2O-N emissions (2.0 kg/ha) from dung (0.05 kg/ha) and CAN (1.94 kg/ha) were approximately equal to the emission from these two N sources applied together, which was 2.12 kg N_2O-N/ha. The emission factor for CAN of 2.15% is within the range of 1.0% (0.3–3.0%) used in the IPCC guidelines (IPCC, 2006) and is similar to that found in other studies (e.g. mean 0.75% and range 0.01–3.56%; Flechard *et al*., 2007). Data from the current experiments indicate that the effects of applying dung and urine together or dung and CAN fertiliser N together are additive. Consequently, disaggregated emissions derived individually for dung, urine

or CAN fertiliser may be re-aggregated in the combinations mentioned above to estimate N_2O-N emissions at pasture. This is important because the presence of CAN and dung together or dung and urine together both spatially and temporally is a feature of intensive and semi-intensive grazing systems.

By contrast, the effects of aggregating urine and CAN emissions are more complex. Cumulative emissions from urine applied with CAN were significantly greater than either urine applied alone or CAN fertiliser applied alone (Figure 4). Furthermore aggregation of the individual net N_2O-N emissions from urine (0.547 kg/ha) and CAN (1.94 kg/ha) resulted in an emission of 2.49 kg N_2O-N/ha, less than half of the emission from urine and CAN applied together (net of background), which was 5.52 kg N_2O-N/ha. The more than doubling of the emission when urine and CAN are applied together compared with the sum of their separate emissions is a complicating factor in the aggregation of separately derived N_2O-N emission estimates for urine and fertiliser N in a grazing setting. Furthermore, this experiment focuses on CAN, a NO_3^--N based fertiliser commonly used in Ireland as the N source, whereas grazing systems globally use other N fertiliser sources including urea and ammonium sulphate. In addition, fertiliser formulations including urease and/or nitrification inhibitors such as dicyandiamide and/or 3,4-dimethylpyrazole phosphate (Goos, 2013; Soares *et al*., 2014; Halvorson *et al*., 2014) are becoming more widely used in commercial agriculture. These inhibitors result in differential effects on NH_3 volatilisation (Forrestal *et al*., 2015), thus affecting the ratio of direct to indirect N_2O emissions from fertiliser N. Whether N_2O emissions from these other N fertiliser formulations are additive or multiplicative when combined with urine is unknown. CAN fertiliser will provide 50% of its N as NO_3^--N, which has high denitrification loss potential. Urine provides a source of readily available carbon compounds, enhances soil C solubilisation (Lambie *et al*., 2012) and these urine-related carbon additions in the presence of NO_3^--N increases denitrification loss (Weier *et al*., 1993). Furthermore urine application will shift the soil matrix moisture levels higher compared with CAN applied alone, this is important because moisture is a major driver of denitrification (Linn and Doran, 1984). In the case of urine applied alone, soil moisture would also be expected to be elevated by the application of urine. However, the N in urine is in the form of urea, which takes time to hydrolyse and nitrify, thus the NO_3^--N pool, which forms after the urine application is temporally isolated from the urine-induced wetting event. It can be seen in Figure 2 that the soil NO_3^--N levels for the urine treatment are more similar to the control than the CAN or urine & CAN treatments. In summary, the multiplicative effects observed are thought to be the result the combination of a NO_3^--N pool from the CAN fertiliser and both a ready carbon source and a wetting event from the urine application.

Net cumulative N_2O emissions: dung, urine and CAN in combination

The cumulative N_2O emission from urine, dung and CAN applied in a three way combination was significantly greater than urine or dung alone or urine and dung in combination (Figure 5). Although the three-way combination did not differ from urine and CAN applied together, the emission was numerically lower, even though the addition of dung increased the pool of total N and the carbon available as well as adding additional moisture. This suggests that the addition of dung may have a moderate net effect of suppressing emissions.

It has been reported that the presence of dung on the soil surface may reduce diffusion of N_2O to the atmosphere (Granli and Bøckman, 1994). The readily available carbon in dung can lead to anaerobic conditions through increased rates of microbial O_2 consumption (van Groenigen *et al*., 2005b). Anaerobic conditions will decrease nitrification whilst altering the N_2O/N_2 ratio during denitrification. It is also possible that the higher C content added in the dung and the wet soil conditions may have promoted a more complete reduction of N_2O to N_2 (Jahangir *et al*., 2012). In the case of the three-way combination treatment, urine & dung & CAN, the addition of CAN might be expected to result in similar soil NO_3^--N and NH_4^+-N pools across CAN fertiliser treatments. However, this is not the case and both NO_3^--N and NH_4^+-N pools are significantly lower where dung is added (Figure 3). The lower soil NO_3^--N observed in the urine & dung & CAN treatment is in line with the potentially enhanced denitrification to N_2. There is also potential that the applied dung may have increased soil N immobilisation through the addition of large quantities of carbon (Hatch *et al*., 2000). Very little difference was found between the urine only, the CAN only and the urine & CAN treatments in terms of soil NH_4^+-N content. This may be due to significant NH_3 volatilisation in this experiment. Ammonia volatilisation from urine is a feature of Irish temperate grassland systems (Fischer *et al*., 2015).

Following the highest peak of mineral N (Figures 2 and 3), N_2O emissions were the highest on day 5 after the treatment application (Figure 4). The lower N_2O emissions resulting from the urine only treatment highlights the potential occurrence of a coupling between nitrification and denitrification from urine applied to soils. This coupling is thought to have decreased the N_2O/N_2 ratio in continuously anaerobic conditions due to the suppression of nitrification by O_2 non-availability. When urine was applied with CAN both mineralisation and denitrification occurred simultaneously as evident in the higher NH_4^+-N and NO_3^--N content of soil with concurrent N_2O peaks.

Conclusions

Emissions from dung and urine or dung and CAN fertiliser N applied together are well approximated by the addition

of emissions measured from dung, urine and CAN applied separately. Thus the effect of their combination is additive. However, in the case of combining urine with CAN the effect on N_2O-N emission is multiplicative with the sum of the individually applied emission amounting to less than half the emission of these N sources applied together. This work points to the importance of considering interactive effects for aggregating N_2O loss estimates based on estimates derived from use of disaggregated emission factors when estimating national loss inventories. This work also highlights the need to examine the effects of combining urine with other commonly used mineral fertiliser N sources.

Acknowledgements

The authors wish to thank the Environmental Protection Agency for funding support under the Environmental Research Technological Development and Innovation programme and the Irish Department of Agriculture, Food and the Marine for funding support (Grant numbers RSF 13S430 and 11S138).

References

Allen, A.G., Jarvis, S.C. and Headon, D.M. 1996. Nitrous oxide emissions from soils due to inputs of nitrogen from excreta return by livestock on grazed grassland in the U.K. *Soil Biology and Biochemistry* **28**: 597-607.

Anger, M., Hoffmann, C. and Kuhbauch, W. 2003. Nitrous oxide emissions from artificial urine patches applied to different N-fertilized swards and estimated annual N_2O emissions for differently fertilized pastures in an upland location in Germany. *Soil Use and Management* **19**: 104-111.

Ball, P.R. and Ryden, J.C. 1984. Nitrogen relationships in intensively managed temperate grasslands. *Plant and Soil* **76**: 23-33.

Chadwick, D.R., Cardenas, L., Misselbrook, T.H., Smith, K.A., Rees, R.M., Watson, C.J., McGeough, K.L., Williams, J.R., Cloy, J.M., Thorman, R.E. and Dhanoa, M.S. 2014. Optimizing chamber methods for measuring nitrous oxide emissions from plot-based agricultural experiments. *European Journal of Soil Science* **65**: 295-307.

Clough, T.J., Sherlock, R.R., Cameron, K.C. and Ledgard, S.F. 1996. Fate of urine nitrogen on mineral and peat soils in New Zealand. *Plant and Soil* **178**: 141-152.

de Klein, C.A.M. and van Logtestijn, R.S.P. 1994. Denitrification in the top soil of managed grasslands in The Netherlands in relation to soil type and fertilizer level. *Plant and Soil* **163**: 33-44.

de Klein, C.A.M., Barton, L., Sherlock, R.R., Li, Z. and Littlejohn, R.P. 2003. Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. *Australian Journal of Soil Research* **41**, 381-399.

de Klein, C.A.M. and Harvey, M. 2012. Nitrous oxide chamber methodology guidelines. Retrieved 23 November 2015, from http://globalresearchalliance.org/wp-content/uploads/2015/11/Chamber_Methodology_Guidelines_Final-V1.1-2015.pdf

Fischer, K., Burchill, W., Lanigan, G.J., Kaupenjohann, M., Chambers, B., Richards, K.G. and Forrestal, P.J. Ammonia emissions from cattle dung, urine and urine with dicyandiamide. *Soil Use and Management* doi: 10.1111/sum.12203.

Flechard, C.R., Ambus, P., Skiba, U.M., Rees, R.M., Hensen, A., Van den Pol A., Soussana, J.F., Jones, M., Clifton-Brwon, J., Raschi, A., Horvath, L., Van Amstel, A., Neftel, A., Jocher, M., Ammann, C., Fuhrer, J., Calanca, P., Thalman, E., Pilegaard, K., Di Marco, C., Campbell, C., Nemitz, E., Hargreaves, K.J., Levy, P., Ball, B., Jones, S., Van de Bulk, W.C.M., Groot, T., Blom, M., Gunnink, H., Kasper, G., Allard, V., Cellier, P., Laville, P., Henault, C., Bizouard, F., Jolivot, D., Abdalla, M., Williams, M., Baronti, S., Berretti, F., Grosz, B. and Dominques, R. 2007. Effects of climate and management intensity on nitrous oxide emissions in grassland systems across Europe. *Agriculture, Ecosystems and Environment* **121**: 135-152.

Flessa, H., Dörsh, P., Besse, F., König, H. and Bouwman, A.F. 1996. Influence of cattle wastes on nitrous oxide and methane fluxes in pasture land. *Journal of Environmental Quality* **25**: 1366-1370.

Forrestal, P.J., Harty, M., Carolan, R., Lanigan, G.J., Watson, C.J., Laughlin, R.J., McNeill, G., Chambers, B., and Richards, K.G. Ammonia emissions from urea, stabilised urea and calcium ammonium nitrate: insights into loss abatement in temperate grassland. *Soil Use and Management* doi: 10.1111/sum.12203.

Goos, R.J., 2013. A comparison of a maleic-itaconic polymer and *N*-(*n*-butyl) thiophosphoric triamide as urease inhibitors. *Soil Science Society of America Journal* **77**: 1418-1423.

Granli, T. and Bøckman, O.C. 1994. Nitrous oxide from agriculture. *Norwegian Journal of Agricultural Science* **128**: Supplement No. 12. pp. 124.

Hatch, D.J., Lovell, R.D., Antil, R.S. and Jarvis, S.C. 2000. Nitrogen mineralization and microbial activity in permanent pastures amended with nitrogen fertilizer or dung. *Biology and Fertility of Soils* **30**: 288-293.

Halvorson, A.D., Snyder, C.S., Blaylock, A.D., and Del Grosso, S.J. 2014. Enhanced-efficiency nitrogen fertilizers: potential role in nitrous oxide emission mitigation. *Agronomy Journal* **106**: 715-722.

Hoekstra, N.J., Lalor, S., Richards, K.G., O'Hea, N., Dungait, J., Schulte, R.P.O. and Schmidt, O. 2011. The fate of slurry N fractions in herbage and soil during two growing seasons following application. *Plant and Soil* **342**: 83-96.

IPCC. 2001. Climate change 2001: The scientific basis. Contribution of Working Group 1 to the Third Assessment Report of the Inter-

governmental Panel on Climate Change. Cambridge: Cambridge University Press.

IPCC. 2006. Guidelines for national greenhouse gas inventories. Prepared by the National Greenhouse Gas Inventories Programme. Eggleston, H.S., Miwa, K., Ngara, T. and Tanabe, K. Hayama, IGES, Japan.

IPCC. 2007. IPCC, 2007: Climate change 2007: Synthesis report. Contribution of working groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri, R.K and Reisinger, A. (eds.)]. IPCC, Geneva, Switzerland, pp. 104.

IPCC. 2013. Summary for Policymakers. In: "Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth assessment Report of the Intergovernmental Panel on Climate Change", (eds. Stocker,T.F., D.Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley), Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jahangir, M.M.R., Khalil, M.I., Johnston, P.M., Cardenas, L.M., Butler, M., Hatch, D., Barrett, M., O'Flaherty, V. and Richards, K.G. 2012. Denitrification potential in subsoils: A mechanism to reduce nitrate leaching to groundwater. *Agriculture, Ecosystems and Environment* **147**: 13-23.

Jahangir, M.M.R., Roobroeck, D., Van Cleemput, O., Boeckx, P. 2011. Spatial variability and biophysicochemical controls on N_2O emissions from differently tilled arable soils. *Biology and Fertility of Soils* **47**: 753-766.

Krol, D.J., Forrestal, P.J., Lanigan, G.J. and Richards, K.G. 2015. In situ N_2O emissions are not mitigated by hippuric and benzoic acids under denitrifying conditions. *Science of the Total Environment* **511**: 362-368.

Lambie, S.M., Schipper, L.A., Balks, M.R. and Basden, W.T. 2012. Solubilisation of soil carbon following treatment with cow urine under laboratory conditions. *Soil Research* **50**: 50-57.

Lantinga, E.A., Keuning, J.A., Groenwold, J., Deenen, P.J.A.G. 1987. Distribution of excreted nitrogen by grazing cattle and its effects on sward quality, herbage production and utilization. *Developments in Plant and Soil Sciences* **30**: 103-117.

Linn, D.M. and Doran, J.W. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. *Soil Science Society of America Journal* **48**: 1267-1272.

Monaghan, R.M. and Barraclough, D. 1993. Nitrous oxide and dinitrogen emissions from urine-affected soil under controlled conditions. *Plant and Soil* **151**: 127-138.

Mosier, A.R., Kroeze, C., Nevison, C., Oenema, O., Seitzinger, S.P. and Van Cleemput, O. 1998. Closing the global N_2O budget: nitrous oxide emissions through the agricultural nitrogen cycle. OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology. *Nutrient Cycling in Agroecosystems* **52**: 225-248.

Oenema, O., Velthof, G.L., Yamulki, S. and Jarvis, S.C. 1997. Nitrous oxide emissions from grazed grassland. *Soil Use and Management* **13**: 288-295.

Rochette, P., Angers D.A., Chantigny, M.H. and Bertrand, N. 2008. Nitrous oxide emissions respond differently to no-till in a loam and a heavy clay soil. *Soil Science Society of America Journal* **72**: 1363-1369.

Saarijärvi, K. and Virkajärvi, P. 2009. Nitrogen dynamics of cattle dung and urine patches on intensively managed boreal pasture. *Journal of Agricultural Science*. **147**: 479–491.

Selbie, D.R., Cameron, K.C., Di, H.J., Moir, J.L., Lanigan, G.J. and Richards, K.G. 2014. The effect of urinary nitrogen loading rate and nitrification inhibitor on nitrous oxide emissions from a temperate grassland soil. *Journal of Agricultural Science*. **152**: 159-171.

Soares, J.R., Cantarella, H., Vargas, V.P., Carmo, J.B., Martins, A.A., Sousa, R.M. and Andrade, C.A. 2014. Enhanced-efficiency fertilisers in nitrous oxide emissions from urea applied to sugarcane. *Journal of Environmental Quality* 44: 423-430.

Sordi, A., Dieckow, J., Bayer, C., Alburquerque, M.A., Piva, J.T., Zanatta, J.A., Tomazi, M., da Rosa, C.M. and de Moraes, A. 2013. Nitrous oxide emission factors for urine and dung patches in a subtropical Brazilian pastureland. *Agriculture, Ecosystems and Environment* **190**: 94-103.

van der Weerden, T.J., Luo, J.F., de Klein, C.A.M., Hoogendoorn, C.J., Littlejohn, R.P. and Rys, G.J. 2011. Disaggregating nitrous oxide emission factors for ruminant urine and dung deposited onto pastoral soils. *Agriculture Ecosystems and Environment* **141**: 426-436.

van Groenigen, J.W., Kuikman, P.J., de Groot, W.J.M. and Velthof, G.L. 2005a. Nitrous oxide emission from urine-treated soil as influenced by urine composition and soil physical conditions. *Soil Biology and Biochemistry* **37**: 463-473.

van Groenigen, J.W., Velthof, G.L., van der Bolt, F.J.E., Vos, A., Kuikman, P.J. 2005b. Seasonal variation in N_2O emissions from urine patches: Effects of urine concentration, soil compaction and dung. *Plant and Soil* **273**: 15-27.

Virkajärvi, P., Maljanen, M., Saarijärvi, K., Haapala, J. and Martikainen, P.J. 2010. N_2O emissions from boreal grass and grass - clover pasture soils. *Agriculture Ecosystems and Environment* **137**: 59–67.

Watson, C.J. and Foy, R.H. 2001. Environmental impacts of nitrogen and phosphorus cycling in grassland systems. *Outlook on Agriculture* **30**: 117–127.

Weier, K.L., Doran, J.W., Power, J.F. and Walters, D.T. 1993. Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Science Society of America Journal* **57**: 66-72.

Yamulki, S., Jarvis, S.C. and Owen, P. 1998. Nitrous oxide emissions from excreta applied in a simulated grazing pattern. *Soil Biology and Biochemistry* **30**: 491-500.