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

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Abstract

Nitrous oxide (N\textsubscript{2}O) 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\textsubscript{2}O 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\textsubscript{2}O-N emission. Importantly, the sum of N\textsubscript{2}O-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\textsubscript{2}O 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\textsubscript{2}O emissions from grazing grasslands.

Keywords

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

Introduction

Nitrous oxide (N\textsubscript{2}O) is a greenhouse gas (GHG) with a global warming potential 298 times higher than CO\textsubscript{2} over a 100-year time horizon (IPCC, 2007). In 2011, the atmospheric concentration of N\textsubscript{2}O was 391 ppm, which exceeds pre-industrial levels by approximately 40% (IPCC, 2013). In addition to its role as a GHG, N\textsubscript{2}O can also deplete the stratospheric ozone. The potential of N\textsubscript{2}O 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\textsubscript{2}O to effectively estimate the losses and develop mitigation measures.

Soils are considered to be the dominant source of N\textsubscript{2}O emissions, contributing 65% to the global N\textsubscript{2}O emissions (IPCC, 2001). Agricultural soils are the major source of anthropogenic N\textsubscript{2}O, responsible for about 35% of global emissions (Virkajärvi et al., 2010). Between 30 and 50% of the total N\textsubscript{2}O emissions from agriculture originate from animal production systems (Mosier et al., 1998). Sources of N\textsubscript{2}O include urine and faecal N deposition by livestock, the application of chemical and organic nitrogen (N) fertilisers and, indirectly, from ammonia (NH\textsubscript{3}) volatilisation and leached N (Flechard et al., 2007). Significant uncertainties exist in N\textsubscript{2}O 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\textsubscript{2}O emissions. Fertiliser N application and excretion of animal urine and dung, which are rich in N, create hotspots for N\textsubscript{2}O emission. Urine patches in pastures rank among the highest sources of N\textsubscript{2}O emission from animal production systems (van Groenigen et al., 2005b) and grazing animals have been identified as significant contributors to the global N\textsubscript{2}O budget (Oenema et al., 1997). The effect of urine on N\textsubscript{2}O 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 Baraclough, 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\textsubscript{2}O emissions from grazing systems is less well understood.
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\textsubscript{2}O 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\textsubscript{2}O 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\textsubscript{2}O 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\textsubscript{2}O emissions behave in an additive or a multiplicative manner, when combined with inorganic fertiliser N, will be important for generating accurate estimates of N\textsubscript{2}O 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. 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\textsubscript{2}O 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\textsubscript{2}O measurement collar, either alone or in combination with dung and/or urine at the rates indicated above.

| 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\textsubscript{2}O sampling and analysis**

Over the course of the study, N\textsubscript{2}O 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\textsubscript{2}O and calculation of N\textsubscript{2}O 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\textsubscript{2}O concentrations of 0.2, 0.5, 1.0, 5.0, 10.0 ppm, which were in the expected range of the sample N\textsubscript{2}O concentrations (Argo International, UK).

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Table 1. Nitrogen contents of dung and urine used in N\textsubscript{2}O 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                          |
The chamber N\textsubscript{2}O-N flux was calculated according to Eq. (1):

\[
\text{Chamber N}_2\text{O-N flux} = (\text{the slope of the line between } T_0 \text{ and } T_{60}) \times ((M \times P) / (R \times T)) \times (V/A)
\]

where ambient air samples were used for the time zero \((T_0)\) N\textsubscript{2}O concentration. The N\textsubscript{2}O flux was calculated based on the assumption of a linear increase in the concentration of N\textsubscript{2}O 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\textsubscript{2}O accumulation rate in static chambers is linear and that more than 90% of chambers exhibited linear accumulation of N\textsubscript{2}O in the chamber headspace \((n=1970)\). \(M\) is the molar mass of N\textsubscript{2}O-N \((28 \text{ g/mol})\), \(P\) and \(T\) are the atmospheric pressure \((\text{Pa})\) and temperature \((\text{K})\) measured by a weather station within 1 km of the experimental site, \(R\) the ideal gas constant \((8.314 \text{ J/mol/K})\), \(V\) the headspace volume of the closed chamber \((\text{m}^3)\) and \(A\) the area covered by the base of the gas chamber \((\text{ha})\). The chamber N\textsubscript{2}O-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\textsubscript{2}O-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\textsubscript{2}O 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 Gyrotry shaker) for one hour. The extract was filtered through Whatman No. 2 filter paper (Whatman International Ltd., Maidstone, UK). Nitrate and NH\textsubscript{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\textsubscript{2}O-N flux, soil nitrate (NO\textsubscript{3}^-N) and NH\textsubscript{4}^+-N. Differences in cumulative N\textsubscript{2}O-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\textsubscript{3}^-N and NH\textsubscript{4}^+-N \((P<0.01)\). Initial soil NO\textsubscript{3}^-N and NH\textsubscript{4}^+-N levels measured prior to treatment application were less than 10 kg N/ha (Figure 2 and 3). Soil NH\textsubscript{4}^+-N levels increased rapidly following treatment application, particularly for treatments that included urine. Soil NH\textsubscript{4}^+-N levels for the dung treatments were not significantly different from those of the control. The highest soil NH\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2}O emissions (Figure 4). The majority of the N\textsubscript{2}O 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 N2O 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 N2O-N emission was from the urine & CAN (5.52 kg/ha) and urine & dung & CAN treatments (4.83 kg/ha) (Figure 5). The N2O-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 N2O-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

N2O emissions over time

The largest emissions occurred five days following treatment application and corresponded with high soil NO3--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
potential pool of NO$_3^{-}$-N available for denitrification. Peak N$_2$O 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$_2$O 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$_2$O emissions are time-specific. This interactive effect on N$_2$O emissions indicates that N$_2$O measurements should be taken intensively until emissions approach background levels for all treatments.

**Cumulative N$_2$O emissions: dung, urine and CAN individually**

Net N$_2$O 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$_2$O 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$_2$O emissions.

**Net Cumulative N$_2$O 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$_2$O-N/ha (Figure 5), which was approximately the sum of their individual emissions (0.6 kg N$_2$O-N/ha). When expressed as a percentage of applied N lost as N$_2$O-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$_2$O 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$_2$O emissions (Oenema et al., 1997). Aggregated net individual N$_2$O-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$_2$O-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

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**Figure 5.** Cumulative N$_2$O 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.
or CAN fertiliser may be re-aggregated in the combinations mentioned above to estimate N\textsubscript{2}O-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\textsubscript{2}O-N emissions from urine (0.547 kg/ha) and CAN (1.94 kg/ha) resulted in an emission of 2.49 kg N\textsubscript{2}O-N/ha, less than half of the emission from urine and CAN applied together (net of background), which was 5.52 kg N\textsubscript{2}O-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\textsubscript{2}O-N emission estimates for urine and fertiliser N in a grazing setting. Furthermore, this experiment focuses on CAN, a NO\textsubscript{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\textsubscript{4}+ volatilisation (Forrestal et al., 2015), thus affecting the ratio of direct to indirect N\textsubscript{2}O emissions from fertiliser N. Whether N\textsubscript{2}O 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\textsubscript{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\textsubscript{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\textsubscript{3}--N pool, which forms after the urine application is temporarily isolated from the urine-induced wetting event. It can be seen in Figure 2 that the soil NO\textsubscript{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\textsubscript{3}--N pool from the CAN fertiliser and both a ready carbon source and a wetting event from the urine application.

**Net cumulative N\textsubscript{2}O emissions: dung, urine and CAN in combination**

The cumulative N\textsubscript{2}O 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\textsubscript{2}O to the atmosphere (Granli and Beckman, 1994). The readily available carbon in dung can lead to anaerobic conditions through increased rates of microbial O\textsubscript{2} consumption (van Groenigen et al., 2005b). Anaerobic conditions will decrease nitrification whilst altering the N\textsubscript{2}O/N\textsubscript{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\textsubscript{2}O to N\textsubscript{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\textsubscript{3}--N and NH\textsubscript{4}+-N pools across CAN fertiliser treatments. However, this is not the case and both NO\textsubscript{3}--N and NH\textsubscript{4}+-N pools are significantly lower where dung is added (Figure 3). The lower soil NO\textsubscript{3}--N observed in the urine & dung & CAN treatment is in line with the potentially enhanced denitrification to N\textsubscript{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\textsubscript{4}+-N content. This may be due to significant NH\textsubscript{4}+ 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\textsubscript{2}O emissions were the highest on day 5 after the treatment application (Figure 4). The lower N\textsubscript{2}O 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\textsubscript{2}ON\textsubscript{2} ratio in continuously anaerobic conditions due to the suppression of nitrification by O\textsubscript{2} non-availability. When urine was applied with CAN both mineralisation and denitrification occurred simultaneously as evident in the higher NH\textsubscript{4}+-N and NO\textsubscript{3}--N content of soil with concurrent N\textsubscript{2}O 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 \( \text{N}_2\text{O}-\text{N} \) 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 \( \text{N}_2\text{O} \) 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.

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### 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 \( \text{N}_2\text{O} \) 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., McGough, 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., Kauppenjohann, 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-Brown, J., Raschi, A., Horvath, L., Van Amstel, A., Neftel, A., Jocher, M., Ammann, C., Fuhrer, J., Calanca, P., Thalman, E., Filegaard, 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., Jolivet, 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 Beckman, O.C. 1994. Nitrous oxide from agriculture. *Norwegian Journal of Agricultural Science* 128. Supplement No. 12. pp. 124.

Hatch, D.J., Lovell, R.D., Antl, R.S. and Jarvis, S.C. 2000. Nitrogen mineralization and microbial activity in permanent pastures amended with nitrogen fertilizer or dung. *Biological 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., 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., Khalili, 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 N₂O 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 N₂O 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., Balls, 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 Barracough, 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 N₂O 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: 288-296.

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.

Saaërjärvi, 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., Camo, 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., Janatka, 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 N₂O emissions from urine patches: Effects of urine concentration, soil compaction and dung. Plant and Soil 273: 15-27.

Virkajärvi, P., Malijanen, M., Saaërjärvi, K., Haapala, J. and Martikainen, P.J. 2010. N₂O 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.