TITLE Improving and disaggregating N₂O emission factors for ruminant excreta on temperate pasture soils

AUTHORS D.J. Krol, R. Carolan, E. Minet, K.L. McGeough, C.J. Watson, P.J. Forrester, G.J. Lanigan, K.G. Richards

This article is provided by the author(s) and Teagasc T-Stór in accordance with publisher policies.

Please cite the published version.

The correct citation is available in the T-Stór record for this article.

NOTICE: **This is the author's preprint version** of a work that was accepted for publication in *Science of the Total Environment*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *Science of The Total Environment*, Volume 568, 15 October 2016, Pages 327-338, ISSN 0048-9697, <http://dx.doi.org/10.1016/j.scitotenv.2016.06.016>

This item is made available to you under the Creative Commons Attribution-Non-commercial-No Derivatives 3.0 License.

Improving and disaggregating N₂O emission factors for ruminant excreta on temperate pasture soils

D.J. Krol^{a*}, R. Carolan^b, E. Minet^a, K.L. McGeough^b, C. J. Watson^b, P.J. Forrester^a, G.J. Lanigan^{a*}, K.G. Richards^a

^a Teagasc, Crops, Land Use and Environment, Johnstown Castle, Co.Wexford, Ireland

^b Agri-Food and Biosciences Institute (AFBI), Belfast, BT9 5PX, Northern Ireland

*Corresponding authors: kroldj@tcd.ie (D.J. Krol); gary.lanigan@teagasc.ie (G.J. Lanigan);

Abstract

Cattle excreta deposited on grazed grasslands are a major source of the greenhouse gas (GHG) nitrous oxide (N₂O). Currently, many countries use the IPCC default emission factor (EF) of 2% to estimate excreta-derived N₂O emissions. However, emissions can vary greatly depending on the type of excreta (dung or urine), soil type and timing of application. Therefore three experiments were conducted to quantify excreta-derived N₂O emissions and their associated EFs, and to assess the effect of soil type, season of application and type of excreta on the magnitude of losses. Cattle dung, urine and artificial urine treatments were applied in spring, summer and autumn to three temperate grassland sites with varying soil and weather conditions. Nitrous oxide emissions were measured from the three experiments over 12 months to generate annual N₂O emission factors. The EFs from urine treated soil was greater (0.30 – 4.81% for real urine and 0.13 – 3.82% for synthetic urine) when compared with dung (-0.02 – 1.48%) treatments. Nitrous oxide emissions were driven by environmental conditions and could be predicted by rainfall and temperature before, and soil moisture deficit after application; highlighting the potential for a decision support tool to reduce

N₂O emissions by modifying grazing management based on these parameters. Emission factors varied seasonally with the highest EFs in autumn and were also dependent on soil type, with the lowest EFs observed from well-drained and the highest from imperfectly drained soil. The EFs averaged 0.31 and 1.18% for cattle dung and urine, respectively, both of which were considerably lower than the IPCC default value of 2%. These results support both lowering and disaggregating EFs by excreta type.

Key words: nitrous oxide; emission factor; grazed pasture; cattle excreta; dung; urine;

1. Introduction

Cattle excreta deposited on grazed grasslands is a major source of direct and indirect emissions of the greenhouse gas (GHG) nitrous oxide (N₂O), which has a global warming potential 298 times greater than CO₂ over a 100 year time horizon (IPCC, 2013). Transformations of N₂O in the stratosphere also lead to the destruction of the ozone layer (Ravishankara et al., 2009). Agriculture contributes to over 40% of global N₂O emissions (Denman et al., 2007), with managed grazed grasslands producing higher N₂O emissions compared with either un-grazed grasslands or arable cropland (van Groenigen et al., 2005). Grasslands occupy 25% of the earth's surface and provide feed for an estimated 1800 million livestock units (Saggar et al., 2009). However, due to low nitrogen (N) utilisation efficiency, 70-95% of N ingested by ruminant livestock is returned onto pasture as dung and urine (Oenema et al., 2005; Saggar et al., 2013). Emissions of N₂O arising from these returns comprise over 40% of the N₂O associated with animal production systems (Oenema et al., 2005). Therefore, N intake is a principal driver of N losses from ruminant livestock returns (Dijkstra et al., 2013).

Agriculture is the single largest contributor to Ireland's national GHG profile, producing 32% of overall emissions (22.5 Mt CO₂—eq yr⁻¹; CSO, 2014; EPA, 2014). As the predominant agricultural system is pastoral-based ruminant livestock production (Breen et al., 2010), grazing related N₂O emissions have a proportionately large impact on sectoral emissions. Indeed, 90% of agricultural land is devoted to improved grassland, or rough grazing, and supports 6.2 million cattle, including 1.3 million dairy cows (CSO, 2014; Duffy et al., 2014). However, there are large uncertainties around the currently used estimate of 8.46 kt N₂O for urine and dung returned onto pasture, range, and paddock (PRP) systems by grazing animals (Duffy et al., 2014). The current approach for quantifying and reporting national N₂O emissions from PRP grazing returns is to use the default Tier 1 emission factor (EF_{PRP}) of 2% from the current Intergovernmental Panel on Climate Change (IPCC) guidelines (IPCC, 2006). This single EF_{PRP} is based upon a limited number of studies, some of them laboratory based, and may not necessarily reflect country-specific conditions (Bell et al., 2015). The reported EFs from urinary N vary widely, between 0.3 % (van der Weerden et al., 2011) and 13.3% (Kool et al., 2006a). Additionally, the default EF_{PRP} does not take into account soil type, climatic conditions, timing of deposition, or excreta form, all of which can influence the magnitude and duration of N₂O emissions. Furthermore, the IPCC encourages the development of country-specific (Tier 2) factors, especially for key N emission sources (de Klein et al., 2003). New Zealand developed and adopted a country-specific EF_{PRP} over a decade ago (de Klein et al., 2003), later disaggregated by animal excreta type, and the same process is currently underway in the UK, supported by the recently published work of Bell et al. (2015) who produced the disaggregated EFs for Scottish grassland.

The main drivers of N₂O production in soils are mineral N content, soil water-filled pore space (WFPS), and temperature (Conen et al., 2000). As a direct result of grazing returns, patches with very high rates of N loading between 600 and 1000 kg N ha⁻¹ are created (Welten et al., 2013; Selbie et al., 2014). Between 50 and 90% of this excreted urinary-N is in the form of urea (Doak, 1952; Bristow et al., 1992), which undergoes a rapid transformation to mineral N in soil. Nitrogen supply in excreta patches exceeds the potential for assimilation and retention by plants, microorganisms and soil.

Therefore excess N is lost from the system through leaching of nitrate (NO_3^- -N) and gaseous emissions (e.g. N_2O) (Saggar et al., 2011). In conditions where the N substrate is not a limiting factor, N_2O production is primarily affected by soil WFPS (Linn and Doran, 1984; van der Weerden et al., 2011; ME, 2015). North-West Europe has a temperate, humid climate with high rainfall in autumn and winter, leading to high soil WFPS, which could potentially result in higher denitrification rates and subsequent N_2O emissions from excreta deposited onto pasture at those times of year (Bell et al., 2015). Denitrification rates in Irish pasture soils have also been shown to be highly sensitive to temperature (Abdalla et al., 2009).

A rise in ruminant numbers and associated GHG emissions is anticipated as a consequence of both a rising demand for meat and dairy produce and the recent removal of EU milk quotas. Therefore accurate accounting and reporting of national N_2O emissions stemming from excreta, as well as mitigation strategies, are urgently needed (Oenema et al., 2005). Existing and anticipated mitigation options include improved soil and fertiliser management (such as the addition of nitrification inhibitors), effluent management, reducing wet season grazing and animal interventions, such as altered diet, feed additives and selective animal breeding (de Klein and Eckard, 2008; Luo et al, 2010). However, in order to plan and implement suitable N_2O mitigation strategies, a better handle on quantifying emissions and identifying the processes responsible for N_2O production is of paramount importance (Bell et al., 2015).

The aim of this work was to reduce the uncertainty around the quantity of N_2O emitted from agricultural animal excreta deposited onto pasture. Specific objectives of the study were to: 1) investigate the timing of cattle dung and cattle urine deposition and soil type on N_2O emissions from temperate pasture, range, and paddock; and 2) to elucidate the drivers of N_2O emissions from dung and urine returns to temperate pasture, range, and paddock.

2. Materials and Methods

2.1 Soil and site description and experimental design

The experiment was carried out across three seasons, at three experimental field sites on contrasting soils across Ireland. The soils were a well drained sandy loam located at Teagasc Moorepark, in Fermoy (52°9'N, 8°14'W), a moderately drained sandy loam located at Teagasc Johnstown Castle in Wexford (52°17'N, 6°30'W), and an imperfectly drained clay loam located at the Agri-Food and Biosciences Institute (AFBI) in Hillsborough (54°45'N, 6°08'W). The climate on all sites is temperate. Moorepark (MP) has an annual rainfall of 1202 mm and a mean annual temperature of 11.3°C (1981-2010, 30 year average), Johnstown Castle (JC) has an annual rainfall of 1037 mm and a mean annual temperature of 10.4°C (1981-2010, 30 year average), and Hillsborough (HB) has an annual rainfall of 944 mm and a mean annual temperature of 9.9°C (1981-2010, 30 year average). Further soil and site details are listed in Table 1. Experimental period rainfall, ambient air and soil temperature information were recorded at meteorological stations ca. 500 m from the experimental sites.

The pasture at all sites was dominated by perennial ryegrass (*Lolium perenne* L.). Animals were excluded from the sites for a minimum of six months prior to commencement of the experiment.

The study design consisted of three separate 365 day measurement periods in order to fully assess seasonal effects on N₂O emissions from applied urine and dung. A split plot experimental design was employed with site as the main plot factor, application season as the main split plot factor and excreta treatment as the split-split plot factor. Five replicates of real urine, synthetic urine, dung, and an untreated control were applied in April ('spring'), July ('summer'), and September 2014 ('autumn'). For each season N₂O measurements were made from a single urine/dung patch in each of the five replicates. Soil samples were taken from three replicates (blocks 1, 3, 5) where there were five additional urine/dung patches dedicated to soil sampling.

2.2 Treatments

Urine and dung were collected at JC and HB prior to each application, and the material from JC was also applied at MP. Urine and dung were collected a maximum of five days prior to application from lactating Holstein–Friesian dairy cows grazing at pasture, homogenised, and stored at 4°C for a maximum of five days, until application. Synthetic urine was prepared in the laboratory according to method R2 in Kool et al. (2006b). Samples of urine and dung were taken at application to determine total C and N, NH_4^+ -N and NO_3^- -N contents and dry matter (dung only). These data and calculated rates of N application for all treatments are shown in Table 2. At Hillsborough, sub-samples of bulked fresh and artificial urine were collected at application to determine urea, hippuric acid, allantoin, uric acid and creatinine content (Table 3). Briefly, sub-samples were diluted 1:3 with HPLC grade de-ionised water before addition of either 1 M sulphuric acid (to pH 3) or 9 μl chloroform to prevent sample degradation before analysis. Preserved samples were analysed using HPLC-UV (Phenomex Luna C 18 (2), 250 mm x 4.6 mm; pH 4, flow rate 1.0 ml min⁻¹) with the diode ray detector set at 218 nm.

Urine was applied inside the stainless steel static chamber bases (40 cm x 40 cm) to avoid seepage and run off from the patch at a loading rate of $2 \text{ L} / (0.4 \text{ m})^2 = 12.5 \text{ L m}^{-2}$. The chamber base was left in place until the complete infiltration of the applied urine. Individual dung patches were 28 cm in diameter (0.062 m^2), with 2 kg of fresh dung evenly applied at a loading rate of 33.3 kg m^{-2} . As the dung patch or the soluble nutrients from the patch spread over time as they would naturally in the field, thereby influencing the whole chamber base, application rate was calculated to be 12 kg m^{-2} .

2.3 N₂O sampling and analysis

The same N₂O sampling protocol was used at each site and season to enable direct comparison of results. The sampling protocol of Chadwick et al. (2014) was followed due to the high number of chambers deployed in this experiment (180). Most intensive measurements were taken for the first month after each application, with three sampling measurements per week, including sampling

immediately after treatment application and then one and three days later, before sampling frequency was reduced to 2, 1, 1 times a week in the following weeks, then once every two weeks until week 24, and thereafter once a month until the end of the experiment, with the final measurement taken on 365 days post application. Nitrous oxide was sampled on 33 occasions during the 12-month period using the closed static chamber technique (Mosier, 1989; de Klein and Harvey, 2012). Stainless steel 40 cm x 40 cm chambers were inserted at a minimum soil depth of 5 cm a minimum three days prior to treatment application. Chamber lids were 10 cm high creating an approximately 16 L headspace. Upon sampling, chambers were closed for 40 mins before withdrawing a 10 mL (20 mL in HB) gas sample through a rubber septum using a 10 mL (20 mL in HB) syringe fitted with a hypodermic needle. The sample was injected into a pre-evacuated 7 mL (12 mL in HB) screw-cap septum glass vial. The average of ten samples of ambient air, collected at each sampling, was used to determine T_0 for the N_2O flux calculation. Linearity of headspace N_2O concentrations was tested on each sampling occasion by collecting five samples at periodic intervals throughout a 60 minute period from five randomly selected chambers (Chadwick et al., 2014). Nitrous oxide concentrations were analysed using a gas chromatograph (GC) (Varian CP 3800 GC, Varian, USA at JC and MP; Bruker 450 GC, Bremen, Germany at HB) fitted with an electron capture detector (ECD). Gas sampling was performed between 10:00 and 13:00, according to good IPCC practice guidelines (IPCC, 2006). Hourly N_2O flux was calculated by linear regression of changes in N_2O concentration over time (Eq. 1).

$$F(hourly) = \left(\frac{\partial c}{\partial t} \right) \times \frac{M \times P}{R \times T} \times \left(\frac{V}{A} \right) \quad (1)$$

Where ∂c is the change in head space N_2O concentrations during the enclosure period in ppbv or $\mu L L^{-1}$, ∂t is the enclosure period expressed in hours, M is the molar weight of N in N_2O (28 g mol^{-1}), P and T the atmospheric pressure (Pa) and temperature (K) at the time of sampling, R the ideal gas constant (8.314 J $K^{-1} mol^{-1}$), V the headspace volume of the closed chamber (m^3) and A the area covered by the collar of the gas chamber (ha).

Calculated hourly N₂O flux was assumed to be representative of the average flux of the day and was subsequently used to calculate daily N₂O flux (Blackmer et al., 1982; de Klein et al., 2003). Cumulative N₂O emissions were calculated by linear interpolation of daily fluxes (de Klein and Harvey, 2012). The N₂O emission factor (EF₃-PRP; N₂O-N emitted as % of dung or urine N applied) for each treatment was calculated using cumulative N₂O emission following Eq. (2):

$$EF_3 = \frac{N_2O(Treatment) - N_2O(Control)}{N\ applied} \times 100\% \quad (2)$$

Where N₂O (Treatment) is cumulative N₂O emission (kg N₂O-N ha⁻¹ yr⁻¹) for the dung or urine treatment, N₂O (Control) is cumulative N₂O emission (kg N₂O-N ha⁻¹ yr⁻¹) for the control, and N applied is annual N application rate in kg ha⁻¹ yr⁻¹.

2.4 Soil sampling and analysis

Soil was sampled on 14 occasions during each 12-month measurement period with most intensive measurements (weekly) within the first month following application, after which sampling intensity was reduced. Soil samples (0-10 cm) were collected with a soil auger from dedicated patches in blocks 1, 3, and 5 and analysed for NH₄⁺-N, NO₃⁻-N, and gravimetric moisture content. Fresh soil samples were sieved (< 4 mm), extracted with 2 M KCl and mineral N concentration in the extract determined using an Aquakem 600 discrete analyser at JC/MP and a SKALAR automated continuous flow wet chemistry analysis (San++ System, Breda, The Netherlands) at HB. Soil samples were dried at 105°C until constant weight for gravimetric moisture content. Soil bulk density (0-10 cm) was measured once for each season by a core method (USDA, 1999) in order to calculate volumetric moisture content and water-filled pore space (WFPS). Separate soil cores (0-5 cm) were used for measurement of soil field capacity (Šimůnek and Nimmo, 2005).

2.5 Statistical analysis

Statistical analysis of variance was performed using GenStat 16.2.0 (VSN International Ltd., Oxford, UK). Initially, all variables were analysed as a split-split plot ANOVA with Site as the main plot factor, Season as the split-plot factor and Treatment as the split-split plot factor. Nitrous oxide was the response variable. The treatment factors as described were used to analyse all variables in a full factorial arrangement. Fisher's Least Significant Difference (LSD) test was used to assess pairwise differences between individual treatments. In each case, the adequacy of the model was assessed by examination of the appropriate residual plots. Nitrous oxide data were log transformed where necessary prior to analysis in order to ensure normal distribution of residuals.

Cumulative N₂O emissions were modelled with stepwise multiple regression analysis using parameters measured in the field using the GLMSELECT procedure in SAS 9.3 (© 2002–2010, SAS Institute Inc., Cary, NC, USA, 2011). The explanatory variables were fitted as polynomial effects allowing main effects, interactions and quadratic terms. Stepwise selection was used to assess explanatory variables. The entry criterion was set to 0.15 to allow flexible entry with a retention threshold of $p=0.05$. Robustness of the model was assessed by the Akaike Information Criterion (AIC). Selected models were fitted with the MIXED procedure to allow residual checks to ensure that the assumptions of the analysis were met. Nitrous oxide emissions were modelled based on the real urine and synthetic urine treatments. Dung was not used in this analysis, despite the emissions associated with this treatment often being significantly greater than those of the control treatment, because measured emissions were low and relatively unresponsive in comparison to those of urine. Modelled soil moisture deficit (SMD) was included in this multiple regression analysis (Schulte et al., 2005). Soil moisture deficit (SMD) is a parameter describing the amount of rainfall needed to bring the soil to field capacity. The model of Schulte et al. (2005) is used by the Irish Meteorological Service to calculate SMD in Irish soils belonging to different drainage classes. Soil moisture deficit values were calculated using a modified Penman–Monteith equation (Allen et al., 1998; Schulte et al., 2005).

3. Results

3.1 Environmental and soil conditions

3.1.1 Temperature and Rainfall

Climatic conditions during the first 60 days of the experiment are presented in Figures 1-3, a-c. Departures from long-term averages are provided in supplementary material (Fig. 4). Rainfall was above the LTA in autumn in MP, in summer and autumn at JC and at all three seasons in HB, but below in spring at JC and in spring and summer in MP. Temperature was above the LTA in spring and summer in MP and in spring and autumn in JC, but below in autumn in MP, and at all three seasons in HB.

3.1.2 Soil WFPS

Temporal patterns of WFPS during the first 60 days of the experiment are presented in Figures 1-3, a-c. Overall, there was a significant site*season interaction on mean WFPS over the whole experimental period ($P<0.05$). In summer JC and MP had the lowest WFPS, while in autumn HB had the highest. Following a significant site*season interaction, simple effects were analysed. Average WFPS in the spring for the first 60 days of the experiment was 60%, 82% and 71% in MP, JC and HB, respectively. Site was significant in the spring experiment ($P<0.05$) with WFPS following the trend $MP<JC=HB$. In the summer experiment, average WFPS was 44%, 48% and 57% at MP, JC and HB, respectively (site significant at $P<0.05$, $MP=JC<HB$), while in the autumn experiment WFPS averaged 53%, 55% and 74% at MP, JC and HB, respectively (site significant at $P<0.05$, $MP=JC<HB$).

3.1.3 Soil Mineral N

Temporal patterns of soil mineral N ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$) concentrations in the 0-10 cm soil depth over the first 60 days post-application are presented in Figures 1-3, d-f. Profiles of soil mineral N were typical. Applications of cow excreta (urine or dung) and synthetic urine increased mineral N in all seasons and at all sites. Soil $\text{NH}_4^+\text{-N}$ increased immediately following each treatment application and generally returned to background levels within one month (spring MP and JC, summer JC and HB), up to a maximum of three months (autumn HB). In general, the largest and most prolonged $\text{NH}_4^+\text{-N}$ concentrations occurred following the autumn application, however high concentrations were also measured at HB after the spring application.

Concentrations of soil $\text{NO}_3^-\text{-N}$ returned to background levels within the same timeframe as those of $\text{NH}_4^+\text{-N}$. Maximum $\text{NO}_3^-\text{-N}$ concentrations occurred later than those of $\text{NH}_4^+\text{-N}$ and were considerably lower. Nitrate concentrations were highest after the application of synthetic urine, followed by the urine treatment and then the dung treatment, which reflected the N application rates.

In 8 out of 9 cases of the site*season interaction soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations were significantly higher ($P<0.05$) from synthetic urine compared to urine treatment on at least one sampling date. Significant differences occurred at times of peak concentrations, therefore soil $\text{NH}_4^+\text{-N}$ levels differed significantly shortly after treatment application, whereas this effect was delayed for soil $\text{NO}_3^-\text{-N}$.

3.2 Temporal N_2O emissions

Temporal fluxes of N_2O are shown in Fig. 1-3, g-i. While N_2O from control plots ranged between - 1.13 and 38.3 g $\text{N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ the application of cow excreta to grassland soil resulted in an immediate large increase in N_2O emissions. Nitrous oxide followed a typical spike pattern of emissions at all sites in the three experimental periods, however, the magnitude of the fluxes varied. Peak mean daily fluxes recorded in spring, summer and autumn were 1458, 1105 and 2993 g $\text{N}_2\text{O-N}$

ha⁻¹ d⁻¹ respectively, which were all from the urine treatment at the imperfectly drained HB site. Lowest daily fluxes were consistently observed from the well-drained MP soil. Peak emissions from urine were between four and eight times lower from the JC (summer and autumn) and MP (spring) soils, respectively, compared to the HB soil, in the respective seasons.

Peak daily N₂O emissions were highest in autumn, being 456, 767 and 2993 g N₂O-N ha⁻¹ d⁻¹ from MP (urine), JC (synthetic urine) and HB (urine), respectively. Large N₂O fluxes were recorded on the day of treatment application in all seasons at the HB site and in autumn from the MP soil, whereas on other occasions fluxes on the day of application were low and increased in the following days. Large fluxes on the day of application occurred at a range of soil moisture conditions, with WFPS ranging between 38% and 72%. However, WFPS was comparable (ranging from 36% to 79%) in instances where there was no response to excreta application on the day of application (i.e. JC site, all seasons). In general, the largest N₂O fluxes following treatment application occurred in the presence of rainfall. For example, large, sharp N₂O peaks from urine and synthetic urine at JC in summer coincided with substantial rainfall events up to 30 mm over the first two weeks post-application. Similar occurrences were observed at MP in autumn. In the absence of rainfall in the first 10 days post-application the magnitude of the daily fluxes was lower, however, emissions were more prolonged (e.g. at JC in spring or MP in summer). Assessment of linearity samples revealed that non-linear accumulation of gas within the chamber headspace was <5% at all sites and seasons. Linear equation was used in the N₂O flux calculations.

3.3 Cumulative N₂O emissions

All cumulative losses of N₂O are shown in Table 4. Statistical analysis revealed a significant treatment, site, and season interaction (P<0.001), therefore simple effects were analysed (by season and by site).

Cumulative N₂O emissions from the dung treatment were low throughout all experimental periods and in spring emissions ranged from 1.05 to 1.40 kg N₂O-N ha⁻¹ yr⁻¹, which were not significantly

different from those of the control plots ($0.65 - 0.76 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$). Cumulative emissions in summer ranged from 0.94 to $3.89 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$ and in autumn they ranged from 1.25 to $7.01 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$, which were generally significantly larger than the control ($P < 0.05$), apart from MP in summer. Cumulative N_2O emissions from dung were significantly higher at the HB site in the summer and autumn compared to the other sites and there was no significant seasonal effect elsewhere.

Application of urine universally produced significantly larger N_2O losses ($2.77 - 43.1 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$) in comparison with the control and dung ($P < 0.05$). No clear seasonal effect was observed at the MP or JC sites with only N_2O from the autumn application at JC significantly larger than the summer application. However, a large seasonal effect was observed at the HB site, where emissions followed the order $\text{spring} < \text{summer} < \text{autumn}$, which is in line with the N application rate for this treatment. Cumulative emissions from urine at the MP site were consistently lower than at the HB site. Similarly to real urine, synthetic urine produced large cumulative N_2O emissions ranging from 2.26 to $44.4 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$. Seasonal effects varied with site. There was no significant difference between seasons at JC. At MP cumulative N_2O emissions post autumn application were lower than in spring and summer ($P < 0.05$), whereas at HB all seasons differed significantly with $\text{summer} < \text{spring} < \text{autumn}$.

3.4 N_2O emission factors

The EFs from dung ranged from -0.02% to 1.48% and were consistently lower and less variable (SEM 0.16%) than urine (SEM 0.48%) or synthetic urine (SEM 0.39%). The lowest EF calculated for the dung treatment was negative indicating that the N_2O emitted from the dung plots was numerically lower than that from control plots, however, this was not statistically significant (Table 5). There was a significant ($P < 0.05$) seasonal effect with EFs from dung overall sites in autumn being higher (0.52%) than in summer (0.21%) or spring (0.09%) (Table 6).

The EFs from urine ranged from 0.30% to 4.81%. Similarly to dung the EFs from urine, overall sites, were significantly higher in autumn (1.56%) compared with summer (0.71%) or spring (0.67%). The EFs from synthetic urine ranged from 0.13% to 3.82% and when compared at each site, EFs did not differ significantly from urine, despite the difference in composition (Table 3). There was a significant site x treatment interaction ($P<0.01$) with EFs for dung, urine and synthetic urine being significantly higher at the HB site compared to the other two sites. Overall, only two of the 27 measured EFs were higher than the IPCC default value of 2% for cattle grazing returns. These were both recorded at HB in the autumn, where the EFs for synthetic urine and real urine were 3.82% and 4.81%, respectively.

3.5 Drivers of N₂O emissions

Drivers of N₂O emission were assessed using a stepwise multiple regression analysis of the two urine treatments (real urine and synthetic urine) and are shown in Table 7 a and b. The multiple regression analysis including weather and soil parameters showed that 72% of the variation in N₂O emissions ($P<0.005$) could be explained by cumulative rainfall five days prior to treatment application, soil clay content and N application rate (Table 7a). Second run of the multiple regression analysis using only weather data predicted cumulative rainfall for the five days prior to application, mean soil temperature in the seven days prior to application, and mean soil moisture deficit for the five days post-application (Table 7 b and supplementary information Table 8) to be responsible for 72% of the variation ($P<0.005$). The relationship with rainfall was described by a squared term rather than a linear fit. Including squared terms in the statistical model allowed for deviations from the simplest model with just the main effects included.

4. Discussion

4.1 N₂O emissions

Similarly to previous studies (Maljanen et al., 2007; Luo et al., 2008; van der Weerden et al., 2011; Krol et al., 2015), N₂O emissions increased rapidly in response to urine application. The initial peak of N₂O is reported to be driven by denitrification of the indigenous soil nitrate pool, stimulated by an increase in water soluble carbon released as an effect of increased pH during urea hydrolysis, lysing of microbial cell membranes or slaking of soil aggregates (Monaghan and Barraclough, 1993; Lambie et al., 2012, Burchill et al. 2014). In fact, Wachendorf et al. (2008) established that 75% of urine-induced N₂O originated from the indigenous soil mineral N pool rather than applied urinary N. This rapid N₂O emission on the day of urine application was particularly noticeable at the HB and MP sites.

In most cases N₂O remained elevated for approximately 60 days, however in autumn on the imperfectly drained HB soil, emissions from the urine and synthetic urine treatments remained higher than the control for approximately 130 and 200 days, respectively. Cumulative N₂O emissions on both the synthetic urine and real urine treatments were substantially higher at the HB site in spring, summer and autumn, compared to the same time period at the other two sites. This reflected the higher WFPS of the imperfectly drained HB soil, particularly following the autumn application (Fig. 3 a-c). At HB the higher N content of real urine in autumn compared to spring and summer could be attributed to seasonal differences in the dairy cow diet. Quality of pasture is subject to seasonal changes, with grass digestibility decreasing in autumn and crude protein content (CP) increasing after a drop in the summer months (McCarthy et al., 2012). This leads to lower utilisation of the ingested N by grazing animals and higher N excretion (O'Donovan et al., 2011; Dijkstra et al., 2013). Furthermore, grass growth and as a consequence plant uptake of applied urine N declines in autumn (Corré et al., 2014) leaving more mineral N susceptible to N₂O loss.

Selbie et al. (2014) reported a non-linear relationship between N₂O flux and increasing N application rate. Despite the N application rate from synthetic urine being considerably higher than from real urine, cumulative N₂O emissions were only significantly higher on two occasions (following

spring application and JC and HB). The urea-N content of the synthetic urine was consistently higher than in real urine, which may have led to greater ammonia volatilisation. High urea-N content can also lead to N_2 production as urine patches create optimal conditions for co-denitrification (Selbie et al. 2015).

The period of enhanced N_2O emissions corresponds well with other studies. Van der Weerden et al. (2011) reported enhanced emissions over 12-173 days from a variety of well and poorly drained soils, and Bell et al. (2015) observed emissions returning to background levels within 2-3 months after application on a free draining soil. However, de Klein et al. (2003) reported elevated emissions lasting between four and 18 months depending on region, soil type and soil drainage properties.

Cattle dung deposition also resulted in enhanced N_2O emissions that were significantly different from those of the control in five out of nine cases. However, cumulative emissions were lower than those of urine, and showed a slower response following application. Urine patches were characterised by very high N application rates of up to $1240 \text{ kg N ha}^{-1}$ which had a large impact on soil mineral N. Moreover, the majority of N in urine converted quickly to the mineral form, which is readily available for microbial processes leading to N_2O formation. Levels of soil mineral N were significantly lower beneath dung patches, which resulted in lower N_2O emissions. This was due to a lower N application rate as this form of excreta is characterised by a relatively low N content in comparison to urine. The vast majority of N in dung is in an organic form (Haynes and Williams, 1993) which, combined with high levels of organic C in dung, may lead to temporary N immobilisation during C decomposition upon application. Furthermore, the high dry matter content of dung can cause drying and crusting of material in dry weather conditions, which may affect the rate of N infiltration into the soil (van der Weerden et al., 2011).

In all seasons and at all sites, N_2O fluxes followed a large increase in the soil NH_4^+ -N pool. Peaks in soil NO_3^- -N concentrations occurred later than those of NH_4^+ -N and were considerably lower, probably due to plant uptake, N immobilisation and high N_2 emissions. Nitrification is reported to be an important source of N_2O on occasions when the increased flux precedes changes in the soil NO_3^- -

N pool Bell et al., 2015). However, in high WFPS conditions such as at JC in spring or HB in autumn (79% and 72%, respectively) denitrification is considered to be the main N₂O loss pathway. This is supported by the secondary N₂O peaks occurring after the peak in the soil NO₃⁻-N pool.

4.2 N₂O emission factors

The average EF over all sites for real urine was 1.18% (ranging from 0.30% to 4.81%) and for synthetic urine was 1.01% (ranging from 0.13% to 3.82%), which were in line with previous studies. Allen et al. (1996) reported EFs of between 0.0 and 2.3% for a grassland site in the south-west of the UK, while studies in New Zealand found EFs between 0.5 % from a well-drained stony silt loam and 3.7% from a moderately-drained silt loam (de Klein et al., 2003). Van der Weerden et al. (2011) reported EFs of 0.05 and 0.94% from a well-drained silt loam in Hawkes Bay and a poorly drained silt loam in Waikato in spring, respectively, while Clough et al. (1996) reported the EF below 1% from peat soil and 3% from mineral soil, and later below found EFs below 2% from various soil types (Clough et al., 1998). The recent study of Bell et al. (2015) found N₂O EFs from urine to be 0.20 and 1.07% from a sandy to sandy-loam soil in Scotland in spring and summer, respectively. In the current study, soil type had an effect on both real and synthetic urine EFs, with EFs from well-draining soils significantly lower than those on imperfectly drained soils. Synthetic urine, being made to a standard recipe, was used to assess both soil and seasonal effects. There was a large seasonal effect on N₂O emission from synthetic urine where EFs were overall lowest in summer (0.51%), significantly higher in spring (0.91%), and highest in autumn (1.09%). Synthetic urine does not fully mimic real urine composition, especially with respect to minor constituents (Table 3), which vary considerably with diet and for this reason van Groenigen et al., (2005) did not recommend using it to determine emission factors. However, in the current study, EFs from synthetic urine were similar to urine, when compared at each site and therefore it appears to be a reasonable proxy for real urine.

Dung EFs were consistently lower than urine, in agreement with other studies (van der Weerden et al., 2011; Dijkstra et al., 2013; Lessa et al., 2014, and Bell et al., 2015). The average EF for dung

overall sites was 0.31% ($\pm$ 0.16%). These EFs are comparable to those reported in the literature of 0.02 to 0.40% for grazed hill land in New Zealand (Luo et al., 2013a), 0.10 to 0.20% for grassland in Scotland (Bell et al., 2015), or 0.00 to 0.16% in Brazilian pastures (Lessa et al., 2014) and are in line with the EF_{3DUNG} of 0.25% used in New Zealand for calculating national greenhouse gas inventories (Saggar et al., 2015). The dung EF of 1.48% at the HB site in autumn had a large effect as the average of the remaining EFs was only 0.16%. However, as previously described, conditions in autumn in this imperfectly drained soil were favourable towards denitrification, leading to high N₂O losses. There was a large seasonal effect on N₂O emissions from dung where EFs were significantly higher in autumn than in spring or summer. Dung EFs were also higher on the imperfectly-drained soil compared to the better drained soils. As dung EFs are considerably lower than urine, there is potential to reduce N₂O emissions through manipulating N excretion towards the dung fraction. Partitioning of excretal N between urine and dung can be affected by diet, mainly by manipulating N intake. Therefore dietary manipulation such as supplementing pasture forage with condensed tannins and/or maize or cereal silage, or sucrose can lower the N concentration in urine (Jarvis et al., 1996; Oenema et al., 1997) and increase the proportion of excretal N in the form of dung (Valk, 1994; Howard et al., 2007).

Bell et al. (2015) found lower EFs from animal excreta in comparison with the IPCC default value of 2% and suggested that this should be reduced for countries with temperate climates, such as the UK. Results of the current study, with an average EF of 0.31% from dung, 1.18% from urine and 1.01% from synthetic urine, and an overall average of 0.87%, support this view. This has practical implications for the national N₂O inventory and any future mitigation strategies. Variation in EFs between type of excreta, soil type and season indicate that use of the IPCC default EF is not appropriate for temperate grasslands and therefore the study of Bell et al. (2015) and the current experiment support recommendations to adopt country-specific N₂O EFs for grazing returns and support the disaggregation of EFs by excretal type.

Two of the EFs calculated from the current study were above the IPCC default EF of 2% and both of these occurred from urine treatments on the imperfectly drained soil under high denitrifying conditions in autumn. In contrast, EFs from well-drained soils were all below 2%. This highlights practical concerns in terms of grazing management on different soil types. These results suggest that N₂O emissions can be reduced by avoiding grazing imperfectly drained soils in wet spring and autumn conditions. Improving soil drainage of imperfectly drained soils may extend grazing season and mitigate N₂O (Touhy et al., 2016).

4.3 Drivers of N₂O emissions

The results of this study suggest that the extent of N₂O emissions was strongly influenced by soil and weather conditions shortly before and after deposition of excreta ($r^2=72\%$) (Table 7 a). Nitrous oxide increased with increasing level of precipitation during the five days prior to application of excreta. Rainfall is a proxy of soil moisture which is known to be one of the main parameters responsible for N₂O emission (Dobbie and Smith., 2001; del Prado et al., 2006; Schaufler et al., 2010; Luo et al., 2013b). Soil moisture is influenced by soil texture and drainage properties and soil clay content was another parameter that influenced N₂O fluxes. Following similar amounts of precipitation, soils with higher clay content respond to rainfall more slowly and the soil moisture remains higher for longer, whereas moisture in light-textured, free draining soils closely follow rainfall patterns, quickly increasing and decreasing afterwards. The imperfectly drained soil in HB consistently received more rainfall in the first 60 days following each seasonal application than the LTA during the experimental periods, while the other two soils received mixed levels of rainfall in relation to the LTA (drier springs and wetter autumns). This was reflected in WFPS which remained high for most of the experimental periods at HB, and was less variable in comparison with the other two soils. In fact, the WFPS at HB ranged between 60% and 80 % for at least 50 days post-application in both spring and autumn. High WFPS restricted oxygen concentration in soil leading to anaerobic conditions stimulating denitrification which is believed to be a major source of large N₂O losses in

the HB soil. These results relate well to the study of de Klein et al. (2003), who found highest N₂O emissions generated from poorly drained silt loam soil amended with urine, even under rainfall conditions lower than on the other sites. However large N₂O emissions also occurred shortly after application, even in low WFPS conditions, following a rainfall event, which is in line with findings of Bell et al. (2015) who found the highest N₂O emissions in summer (low WFPS). According to Carter et al. (2007) high N₂O at low WFPS (at approximately 45%) could be due to equal contributions of nitrification and denitrification to emissions. The maximum N₂O emissions throughout the experiment were seen at WFPS 70-80%, mainly in spring and autumn when soils were at their wettest which is similar to findings of Luo et al. (2008) and Di et al. (2014). However, cumulative N₂O emissions showed a weak correlation with WFPS on its own, possibly due to the large variability in the data and rainfall was a more suitable proxy of emissions in this case. The quadratic term used in the model in relation to rainfall indicates a curved relationship, possibly a flattening increase or a decrease in N₂O emission above a certain level of precipitation, similar to the bell-shaped relationship between N₂O and WFPS described by Bouwman (1998).

As expected, the magnitude of the N₂O loss also depends on the rate of N applied, which varied markedly between sites and seasons. Higher N application rate translated into more substrate in the soil (Figs 1-3, d-f) available for nitrification and denitrification and was subsequently responsible for higher N₂O emissions. This effect has been widely reported in the literature with regard to synthetic fertiliser (Bouwman et al., 2002), synthetic urine (de Klein et al., 2014), real urine (Selbie et al., 2014) and organic manure (Meade et al., 2011).

A suite of parameters tested in the stepwise multiple regression analysis was then narrowed down strictly to weather data available through weather forecasts (Table 6 b). The analysis used weather data ranging from ten days prior, to ten days post-treatment; and resulted in a statistical model potentially useful for predicting conditions of high N₂O loss and guiding management decisions. Similarly to above, N₂O flux was mostly controlled by cumulative rainfall for the five days prior to application, but also mean soil temperature in the seven days prior to application, and mean

soil moisture deficit for the five days post-application ($r^2=72\%$) (Table 7 b and 8). The effect of temperature on N_2O emissions has been extensively described in the literature (Mosier et al., 1998; Dobbie and Smith, 2001; Luo et al., 2013b). A study of Abdalla et al. (2009) found a very high sensitivity of denitrification to temperature and low activation energy for the process in Irish sandy loam pasture soil. Inclusion of SMD in the statistical model is complementary to precipitation, with low N_2O emissions at low levels of precipitation and high SMD, and N_2O increasing with higher rainfall and lower SMD. The highest cumulative N_2O coincided with the zero SMD indicating saturation of the soil. As a means of N_2O mitigation soil moisture deficit could be used as a decision support tool for farmers to indicate times that grazing should be avoided through a more dynamic decision process for housing of animals. Improving soil drainage may also reduce N_2O emissions through increasing SMD, however care is needed due to the potential of enhancing CO_2 respiration.

5. Conclusions

The application of cattle urine, and to a smaller extent cattle dung, increased N_2O emissions from pasture soils, with peak losses occurring within the first 60 days of application. The average N_2O emission factor was 0.31% and 1.18% for cattle dung and urine respectively, which were both considerably lower than the IPCC default value of 2%. These results support the disaggregation of EFs by excreta type. Consequently, calculation of N_2O emissions from grazing returns should be weighted accordingly to the partitioning of forms of excreta and this will require better activity data to account for variable animal types and diets. N_2O emissions can be predicted by rainfall and temperature before and soil moisture deficit after excreta application. Greatest N_2O losses and highest EFs in this study were associated with high rainfall and high soil moisture conditions, conducive to denitrification, on an imperfectly drained site. There is potential for a decision support tool to reduce N_2O emissions to be developed by modifying grazing management based on these parameters. High variability of N_2O emissions with soil type and season suggest that using a

566 universal EF in national GHG inventories is inappropriate and application of country, or if possible
567 soil and season specific values, should be used.

568

569

570 Acknowledgements

571 The authors would like to thank the laboratory and field staff at both Teagasc Johnstown Castle and
572 the Agri-Food and Biosciences Institute with their assistance on this project. This research was
573 financially supported under the National Development Plan, through the Research Stimulus Fund,
574 administered by the Department of Agriculture, Food and the Marine (Grant numbers
575 RSF10/RD/SC/716 and 11S138).

576

577 References:

- 578 Abdalla, M., M. Jones, P. Smith, M. Williams, 2009. Nitrous oxide fluxes and denitrification sensitivity
579 to temperature in Irish pasture soils. *Soil Use and Management* 25 (4): 376-388.
- 580 Allen, A. G., S. C. Jarvis and D. M. Headon, 1996. Nitrous oxide emissions from soils due to inputs of
581 nitrogen from excreta return by livestock on grazed grassland in the UK. *Soil Biology and*
582 *Biochemistry* 28 (4-5): 597-607.
- 583 Allen, R.G., Pereira, L.S., Raes, D. and Smith, M. 1998. Crop evapotranspiration. Guidelines for
584 computing crop water requirements. FAO irrigation and drainage paper 56.
- 585 Bell, M. J., R. M. Rees, J. M. Cloy, C. F. E. Topp, A. Bagnall, D. R. Chadwick, 2015. Nitrous oxide
586 emissions from cattle excreta applied to a Scottish grassland: Effects of soil and climatic conditions
587 and a nitrification inhibitor. *Science of the Total Environment* 508: 343-353.
- 588 Blackmer, A.M., Robbins, S.G., Bremner, J.M., 1982. Diurnal variability in rate of emission of nitrous-
589 oxide from soils. *Soil Science Society of America Journal* 46: 937-942.
- 590 Bouwman A. F., 1998. Environmental science: Nitrogen oxides and tropical agriculture. *Nature* 392:
591 866-867.
- 592 Bouwman, A. F., L. J. M. Boumans, N. H. Batjes, 2002. Emissions of N₂O and NO from fertilized fields:
593 Summary of available measurement data. *Global Biogeochemical Cycles* 16 (4): 13.
- 594 Breen, J.P., Donnellan, T., Westhoff, P., 2010. Food for thought: EU climate change policy presents
595 new challenges for agriculture. *EuroChoices* 9 (3): 24–29.
- 596 Bristow, A.W., Whitehead, D.C., Cockburn, J.E., 1992. Nitrogenous constituents in the urine of cattle,
597 sheep and goats. *Journal of the Science of Food and Agriculture* 59 (3): 387–394.

598 Burchill, W., Li, D., Lanigan, G.J., Williams, M., Humphreys, J., 2014. Inter-annual variation in nitrous
599 oxide emissions from perennial ryegrass/white clover grassland used for dairy production. *Global*
600 *Change Biology* 20 (10): 3137–3146.

601 Carter, M.S., 2007. Contribution of nitrification and denitrification to N₂O emissions from urine
602 patches. *Soil Biology and Biochemistry* 39: 2091-2102.

603 Chadwick, D. R., L. Cardenas, T. H. Misselbrook, K. A. Smith, R. M. Rees, C. J. Watson, K. L.
604 McGeough, J. R. Williams, J. M. Cloy, R. E. Thorman and M. S. Dhanoa , 2014. Optimizing chamber
605 methods for measuring nitrous oxide emissions from plot-based agricultural experiments. *European*
606 *Journal of Soil Science* 65 (2): 295-307.

607 Clough, T.J., Sherlock, R.R., Cameron, K.C., Ledgard, S.F., 1996. Fate of urine nitrogen on mineral and
608 peat soils in New Zealand. *Plant and Soil* 178 (1): 141–152.

609 Clough, T.J., Ledgard, S.F., Sprosen, M.S., Kear, M.J., 1998. Fate of N-15 labelled urine on four soil
610 types. *Plant and Soil* 199 (2): 195–203.

611 Conen, F., Dobbie, K.E., Smith, K.A., 2000. Predicting N₂O emissions from agricultural land through
612 related soil parameters. *Global Change Biology* 6: 417–426.

613 Corré, W.J., Van Beek, C.L., Van Groenigen, J.W., 2014. Nitrate leaching and apparent recovery of
614 urine-N in grassland on sandy soils in the Netherlands. *NJAS – Wageningen Journal of Life Sciences*,
615 70-71: 25-32.

616 CSO, 2014. Livestock Survey December 2014. Retrieved 05.10.2015, 2015, from
617 <http://www.cso.ie/en/releasesandpublications/er/lld/livestocksurveydecember2014/>

618 de Klein, C.A.M., Barton, L., Sherlock, R.R., Li, Z., Littlejohn, R.P., 2003. Estimating a nitrous oxide
619 emission factor for animal urine from some New Zealand pastoral soils. *Australian Journal of Soil*
620 *Research* 41 (3): 381–399.

621 de Klein, C.A.M., Eckard, R.J., 2008. Targeted Technologies for nitrous oxide abatement from Animal
622 Agriculture. *Australian Journal of Experimental Agriculture* 48 (2): 14–20.

623 de Klein, C.A.M., Harvey, M., 2012. Nitrous Oxide Chamber Methodology Guidelines. Retrieved
624 02.06.2016, from: [http://globalresearchalliance.org/wp-](http://globalresearchalliance.org/wp-content/uploads/2015/11/Chamber_Methodology_Guidelines_Final-V1.1-2015.pdf)
625 [content/uploads/2015/11/Chamber_Methodology_Guidelines_Final-V1.1-2015.pdf](http://globalresearchalliance.org/wp-content/uploads/2015/11/Chamber_Methodology_Guidelines_Final-V1.1-2015.pdf)

626 de Klein, C. A. M., J. F. Luo, K. B. Woodward, T. Styles, B. Wise, S. Lindsey and N. Cox, 2014. The
627 effect of nitrogen concentration in synthetic cattle urine on nitrous oxide emissions. *Agriculture*
628 *Ecosystems and Environment* 188: 85-92.

629 del Prado, A., P. Merino, J. M. Estavillo, M. Pinto, C. Gonzalez-Murua, 2006. N₂O and NO emissions
630 from different N sources and under a range of soil water contents. *Nutrient Cycling in*
631 *Agroecosystems* 74 (3): 229-243.

632 Denman, K. L., G. Brasseur, A. Chidthaisan, P. Ciais, P. M. Cox, R. E. Dickinson, D. Hauglustaine, C.
633 Heinze, E. Holland, D. Jacob, U. Lohmann, S. Ramachandran, P. L. da Silva Dias, S. C. Wofsy, X. Zhang,
634 2007. Couplings between changes in the climate system and biogeochemistry. In: *Climate Change*
635 *2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report*
636 *of the Intergovernmental Panel on Climate Change*, 499-588.

637 Di, H.J., Cameron, K.C., Podolyan, A., Robinson, A., 2014. Effect of soil moisture and a nitrification
638 inhibitor, dicyandiamide, on ammonia oxidizer and denitrifier growth and nitrous oxide emissions in
639 a grassland soil. *Soil Biology and Biochemistry* 73: 59–68.

640 Dijkstra, J., Oenema, O., van Groenigen, J.W., Spek, J.W., van Vuuren, A.M., Bannink, A., 2013. Diet
641 effects on urine composition of cattle and N₂O emissions. *Animal* 7: 292–302.

642 Doak, B.W., 1952. Some chemical changes in the nitrogenous constituents of urine when voided on
643 pasture. *Journal of Agricultural Science* 42: 162–171.

644 Dobbie, K. E. and K. A. Smith, 2001. The effects of temperature, water-filled pore space and land use
645 on N₂O emissions from an imperfectly drained gleysol. *European Journal of Soil Science* 52 (4): 667-
646 673.

647 Duffy, P., Hyde, B., Hanley, E., Core, C., O'Brien, P., Cotter, E., Black, K., 2014. Ireland National
648 Inventory Report 2014, Greenhouse Gas Emissions 1990–2012 Reported to the United Nations
649 Framework Convention on Climate Change. Environmental Protection Agency, Wexford.

650 EPA, 2014. Ireland's provisional greenhouse gas emissions in 2013. Environmental Protection
651 Agency, Wexford.

652 Haynes, R.J., Williams, P.H., 1993. NUTRIENT CYCLING AND SOIL FERTILITY IN THE GRAZED PASTURE
653 ECOSYSTEM. In: Sparks, D.L. (Ed.), *Advances in Agronomy*, Elsevier Academic Press Inc, San Diego,
654 49: 119-199.

655 Howard, H., O'Kiely, P., Moloney, A.P., O'Mara, F.P., 2007. Intake, digestibility, N metabolism and
656 growth in growing steers offered zero-grazed grass supplemented with sucrose. *Proceedings of*
657 *the Agricultural Research Forum, Tullamore, 12 & 13 March*, page 12.

658 IPCC, 2006. IPCC Guidelines for National Greenhouse Gas Inventories. Prepared by the National
659 Greenhouse Gas Inventories Programme. B. L. Eggleston H.S., Miwa K., Ngara T. and Tanabe K.
660 Hayama, IGES, Japan.

661 IPCC, 2013. Climate Change 2013. The Physical Science Basis Working Group I Contribution to the
662 Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: Stocker, T.F., Qin, D.,
663 Plattner, G.K., et al. (Eds.), *Cambridge University Press, Cambridge, United Kingdom and New York,*
664 *NY, USA.*

665 Jarvis, S.C.W., R.J. and Pain, B.F, 1996. Opportunities for reducing the environmental impact of dairy
666 farming management: a systems approach. *Grass and Forage Science* 51: 21-31.

667 Kool, D.M., Hoffland, E., Hummelink, E.W.J., van Groenigen, J.W., 2006a. Increased hippuric acid
668 content of urine can reduce soil N₂O fluxes. *Soil Biology and Biochemistry* 38 (5): 1021–1027.

669 Kool, D.M., Hoffland, E., Abrahamse, S.P.A., van Groenigen, J.W., 2006b. What artificial urine
670 composition is adequate for simulating soil N₂O fluxes and mineral N dynamics? *Soil Biology and*
671 *Biochemistry* 38 (7): 1757–1763.

672 Krol, D.J., Forrester, P.J., Lanigan, G.J., Richards, K.G., 2015. In situ N₂O emissions are not mitigated
673 by hippuric and benzoic acids under denitrifying conditions. *Science of the Total Environment* 511:
674 362-368.

675 Lambie, S. M., Schipper, L. A., Balks, M. R., Baisden, W. T., 2012. Solubilisation of soil carbon
676 following treatment with cow urine under laboratory conditions. *Soil Research* 50(1): 50-57.

677 Linn, D.M. and Doran, J.W., 1984. Effect of water-filled pore space on carbon dioxide and nitrous
678 oxide production in tilled and nontilled soils. *Soil Science Society of America Journal* 48 (6): 1267-
679 1272.

680 Lessa, A. C. R., B. E. Madari, D. S. Paredes, R. M. Boddey, S. Urquiaga, C. P. Jantalia, B. J. R. Alves,
681 2014. Bovine urine and dung deposited on Brazilian savannah pastures contribute differently to
682 direct and indirect soil nitrous oxide emissions. *Agriculture Ecosystems and Environment* 190: 104-
683 111.

684 Luo, J., de Klein, C.A.M., Ledgard, S.F., Saggar, S., 2010. Management options to reduce nitrous oxide
685 emissions from intensively grazed pastures: a review. *Agriculture Ecosystems and Environment* 136
686 (3–4): 282–291.

687 Luo, J. F., Hoogendoorn, C., van der Weerden, T., Saggar, S., de Klein, C., Giltrap, D., Rollo, M., Rys G.,
688 2013a. Nitrous oxide emissions from grazed hill land in New Zealand. *Agriculture Ecosystems and*
689 *Environment* 181: 58-68.

690 Luo, G. J., R. Kiese, B. Wolf and K. Butterbach-Bahl, 2013b. Effects of soil temperature and moisture
691 on methane uptake and nitrous oxide emissions across three different ecosystem types.
692 *Biogeosciences* 10 (5): 3205-3219.

693 Luo, J., Lindsey, S. B., Ledgard, S. F., 2008. Nitrous oxide emissions from animal urine application on a
694 New Zealand pasture. *Biology and Fertility of Soils* 44 (3): 463-470.

695 Maljanen, M., Martikkala, M., Koponen, H.T., Virkajarvi, P., Martikainen, P.J., 2007. Fluxes of nitrous
696 oxide and nitric oxide from experimental excreta patches in boreal agricultural soil. *Soil Biology and*
697 *Biochemistry* 39: 914-920.

698 McCarthy, B., Pierce, K.M., Delaby, L., Brennan, A., Fleming, C., Horan, B., 2012. The effect of
699 stocking rate and calving date on grass production, utilization and nutritive value of the sward during
700 the grazing season. *Grass and Forage Science* 68: 364–377.

701 ME, 2015. New Zealand's Greenhouse Gas Inventory 1990-2013. Ministry for the Environment.

702 Meade, G., K. Pierce, J. V. O'Doherty, C. Mueller, G. Lanigan, T. McCabe, 2011. Ammonia and nitrous
703 oxide emissions following land application of high and low nitrogen pig manures to winter wheat at
704 three growth stages. *Agriculture Ecosystems and Environment* 140 (1-2): 208-217.

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

707 Mosier, A.R., 1989. Chamber and isotope techniques. In: Andreae, M.O., Schimel, D.S.
708 (Eds.), *Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere*.
709 John Wiley, Chichester, 175–187.

710 Mosier, A., C. Kroeze, C. Nevison, O. Oenema, S. Seitzinger, O. van Cleemput, 1998. Closing the
711 global N₂O budget: nitrous oxide emissions through the agricultural nitrogen cycle - OECD/IPCC/IEA

712 phase II development of IPCC guidelines for national greenhouse gas inventory methodology.

713 Nutrient Cycling in Agroecosystems 52 (2-3): 225-248

714 O'Donovan, M., Lewis, E., O'Kiely, P., 2011. Requirements of future grass-based ruminant production

715 systems in Ireland. Irish Journal of Agricultural and Food Research 50: 1–21.

716 Oenema, O., G. L. Velthof, S. Yamulki and S. C. Jarvis, 1997. Nitrous oxide emissions from grazed

717 grassland. Soil Use and Management 13 (4): 288-295.

718 Oenema, O., Wrage, N., Velthof, G.L., van Groenigen, J.W., Dolfing, J., Kuikman, P.J., 2005. Trends in

719 global nitrous oxide emissions from animal production systems. Nutrient Cycling in Agroecosystems

720 72 (1): 51–65.

721 Ravishankara, A.R., Daniel, J.S., Portmann, R.W., 2009. Nitrous oxide (N₂O): the dominant ozone-

722 depleting substance emitted in the 21st century. Science 326 (5949): 123–125.

723 Saggar, S., Giltrap, D. L., Davison, R., Gibson, R., de Klein, C. A. M., Rollo, M., Ettema, P., Rys, G.,

724 2015. Estimating direct N₂O emissions from sheep, beef, and deer grazed pastures in New Zealand

725 hill country: accounting for the effect of land slope on the N₂O emission factors from urine and dung.

726 Agriculture Ecosystems and Environment 205: 70-78.

727 Saggar, S., Jha, N., Deslippe, J., Bolan, N.S., Luo, J., Giltrap, D.L., Kim, D.-G., Zaman, M., Tillman, R.W.,

728 2013. Denitrification and N₂O:N₂ production in temperate grasslands: processes, measurements,

729 modelling and mitigating negative impacts. Science of the Total Environment 465: 173–195.

730 Saggar S., Luo J., Giltrap D.L., Maddena M., 2009. Nitrous oxide emission from temperate grasslands:

731 process, measurements, modelling and mitigation. In: Sheldon AI, Barnhart EP, editors. Nitrous oxide

732 emissions research progress. New York: Nova Science Publishers, 1-66.

733 Saggar S., Luo J., Kim D.-G., Jha N., 2011. Intensification in pastoral farming: impacts on soil
734 attributes and gaseous emissions. In: Singh B.P., Cowie A., Chan Y., editors. Soil health and climate
735 change (soil biology series). Berlin, Heidelberg, Germany: Springer-Verlag, 207–36.

736 Schaufler, G., B. Kitzler, A. Schindlbacher, U. Skiba, M. A. Sutton, S. Zechmeister-Boltenstern, 2010.
737 Greenhouse gas emissions from European soils under different land use: effects of soil moisture and
738 temperature. *European Journal of Soil Science* 61 (5): 683-696.

739 Schulte, R.P.O., Diamond, J., Finkle, K., Holden, N.M., Brereton, A.J., 2005. Predicting the Soil
740 Moisture Conditions of Irish Grasslands. *Irish Journal of Agricultural Research* 44: 95-110.

741 Selbie, D. R., K. C. Cameron, H. J. Di, J. L. Moir, G. J. Lanigan, K. G. Richards, 2014. The effect of
742 urinary nitrogen loading rate and a nitrification inhibitor on nitrous oxide emissions from a
743 temperate grassland soil. *Journal of Agricultural Science* 152: S159-S171.

744 Selbie, D. R., G. J. Lanigan, R. J. Laughlin, H. J. Di, J. L. Moir, K. C. Cameron, T. J. Clough, C. J. Watson,
745 J. Grant, C. Somers, K. G. Richards, 2015. Confirmation of co-denitrification in grazed grassland.
746 *Scientific Reports* 5: 17361.

747 Šimůnek, J. and Nimmo, J.R., 2005. Estimating soil hydraulic parameters from transient flow
748 experiments in a centrifuge using parameter optimization technique. *Water Resources Research*. 41:
749 1-9.

750 Touhy P., Humphreys, J., Holden, N.M., Fenton, O. 2016. Runoff and subsurface drain response from
751 mole and gravel mole drainage across episodic rainfall events. *Agricultural Water Management* 169:
752 129-139.

753 USDA, 1999. USDA Soil Quality Test Kit Guide. Edited by U.S.D. o. Agriculture. Washington DC: USA

754 Valk, H., 1994. Effects of partial replacement of herbage by maize silage on N-utilization and milk
755 production of dairy cows. *Livestock Production Science* 40: 241–250.

756 van der Weerden, T.J., Luo, J.F., de Klein, C.A.M., Hoogendoorn, C.J., Littlejohn, R.P., Rys, G.J., 2011.
757 Disaggregating nitrous oxide emission factors for ruminant urine and dung deposited onto pastoral
758 soils. *Agriculture Ecosystems and Environment* 141 (3–4): 426–436.

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

762 Wachendorf C., Lampe, C., Taube, F., Dittert, K., 2008. Nitrous oxide emissions and dynamics of soil
763 nitrogen under N-15-labeled cow urine and dung patches on a sandy grassland soil. *Journal of Plant*
764 *Nutrition and Soil Science* 171: 171-180.

765 Welten, B.G., Ledgard, S.F., Schipper, L.A., Waller, J.E., Kear, M.J., Dexter, M.M., 2013. Effects of
766 prolonged oral administration of dicyandiamide to dairy heifers on excretion in urine and efficacy in
767 soil. *Agriculture Ecosystems and Environment* 173, 28–36.

768

769 Table 1 Physical and chemical soil characteristics at 0-10 cm depth (n=5).

Site	MP	JC	HB
Soil Texture	Sandy Loam	Sandy Loam	Clay Loam
Drainage	good	moderate	imperfect
Sand (%)	57.8	52.9	47.1
Silt (%)	28.2	33.2	31.9
Clay (%)	14.1	13.9	21.0
LOI (%)	7.9	7.3	12.4
pH	5.4	5.5	5.8
Total C (%)	3.02	3.16	4.95
Total N (%)	0.32	0.30	0.47
CEC (cmol kg ⁻¹)	18.4	15.6	23.6
Field capacity (%)	30.3	35.8	46.1

770

771

Table 2 N application rate and chemical characteristics of applied excreta.

Site	Season	Treatment	Application rate (kg N ha ⁻¹)	Total N (g N L ⁻¹ or kg)	Urea N (mg N L ⁻¹)	Nitrate N	Ammonium N
MP ¹	spring	Urine	660	5.28	1335.0	0.0	2517.0
		S urine*	1166	9.33	7851.0	0.0	157.0
		Dung	490	3.92	-	-	-
	summer	Urine	774	6.19	4308.4	0.0	1564.6
		S urine	1218	9.74	9636.4	0.0	57.9
		Dung	469	3.75	-	-	-
	autumn	Urine	739	5.91	4902.6	4.1	894.6
		S urine	1194	9.55	9708.0	4.4	34.9
		Dung	420	3.36	-	-	-
JC ²	spring	Urine	638	5.10	3688.0	0.0	290.0
		S urine	1139	9.11	7559.0	0.0	72.0
		Dung	490	3.92	-	-	-
	summer	Urine	725	5.80	3495.0	34.1	2280.5
		S urine	1181	9.45	8992.0	10.4	34.0
		Dung	469	3.75	-	-	-
	autumn	Urine	718	5.74	4704.4	0.0	870.4
		S urine	1210	9.68	9701.3	0.0	35.8
		Dung	420	3.36	-	-	-
HB ³	spring	Urine	507	4.06	366.0	13.7	3155.0
		S urine	1229	9.83	8100.0	122.9	44.5
		Dung	280	2.24	-	0.6	325.8
	summer	Urine	840	6.72	1650.0	20.6	5193.3
		S urine	1240	9.92	9172.5	119.3	109.1
		Dung	651	5.21	-	1.1	676.3
	autumn	Urine	897	7.17	2872.5	40.9	2650.0
		S urine	1214	9.71	7257.4	140.8	116.8
		Dung	441	3.52	-	0.7	469.2

¹Moorepark site²Johnstown Castle site³Hillsborough site

*S urine stands for 'Synthetic urine' treatment

780 Table 3 Minor constituents of urine applied at the Hillsborough site.

Site	Season	Treatment	Allantoin (mg N L ⁻¹)	Creatinine	Uric acid	781 Hippuric acid782 783
HB ³	spring	urine	<100	90.9	62	33.8784
		S urine	1457.7	317.8	76.2	515
	summer	urine	<100	<30	20	<30785
		S urine	1263.4	321.6	79.6	522.3786
	autumn	urine	<100	83	56.1	<30
		S urine	1366	330.4	80.5	540787

788 ³Hillsborough site
789

790

791

792 Table 4 Mean cumulative N₂O emissions (kg N ha⁻¹yr⁻¹) for 365 days of measurements by site x season x treatment (n=3). All averages reported are
793 calculated from back-transformations of total emissions.

		Control			Dung			Urine			Synthetic urine		
MP ¹	Spring	0.76	B	ab	1.05	B	b	2.77	A	d	4.56	A	d
	Summer	1.04	B	a	0.94	B	b	3.05	A	d	4.49	A	d
	Autumn	0.62	C	abc	1.25	B	b	2.79	A	d	2.26	AB	e
JC ²	Spring	0.66	C	ab	1.17	C	b	4.61	B	cd	9.09	A	c
	Summer	0.43	C	bc	1.09	B	b	2.86	A	d	5.03	A	cd
	Autumn	0.32	C	c	1.36	B	b	8.63	A	bc	7.54	A	cd
HB ³	Spring	0.76	C	ab	1.40	C	b	6.83	B	c	22.3	A	b
	Summer	0.76	C	ab	3.89	B	a	14.3	A	b	8.68	A	c
	Autumn	0.59	C	abc	7.01	B	a	43.1	A	a	44.4	A	a

794 Means followed by same letter are not significantly different at P<0.05

795 Upper-case lettering refers to comparisons within rows

796 Lower-case lettering refers to comparisons within columns

797 ¹Moorepark site

798 ²Johnstown Castle site

799 ³Hillsborough site

800

801

Table 5 Emission factors (%) by site x season x treatment (n=3).

		Dung		Urine		Synthetic urine	
MP ¹	Spring	0.03	(0.04)	0.32	(0.09)	0.34	(0.10)
	Summer	-0.02	(0.03)	0.31	(0.14)	0.34	(0.13)
	Autumn	0.13	(0.04)	0.30	(0.09)	0.13	(0.02)
JC ²	Spring	0.06	(0.06)	0.65	(0.26)	0.84	(0.35)
	Summer	0.16	(0.06)	0.34	(0.06)	0.43	(0.11)
	Autumn	0.24	(0.06)	1.16	(0.10)	0.59	(0.05)
HB ³	Spring	0.15	(0.18)	1.12	(0.26)	1.79	(0.31)
	Summer	0.54	(0.22)	1.63	(0.39)	0.82	(0.33)
	Autumn	1.48	(0.21)	4.81	(0.97)	3.82	(0.98)
Average		0.31	(0.16)	1.18	(0.48)	1.01	(0.39)

Values in brackets are the SEM

¹Moorepark site

²Johnstown Castle site

³Hillsborough site

Table 6 Mean N₂O emission factors (%) by site x season, site x treatment and season x treatment.

Site x Season		EF (%)	Site x Treatment		EF (%)	Season x Treatment		EF (%)
MP ¹	Spring	0.22 <i>ab</i>	MP ¹	Dung	0.04 <i>a</i>	Spring	Dung	0.09 <i>a</i>
	Summer	0.21 <i>ab</i>		Urine	0.32 <i>bc</i>		Urine	0.67 <i>bc</i>
	Autumn	0.18 <i>a</i>		Synthetic Urine	0.27 <i>b</i>		Synthetic Urine	0.91 <i>cd</i>
JC ²	Spring	0.49 <i>bc</i>	JC ²	Dung	0.16 <i>ab</i>	Summer	Dung	0.21 <i>a</i>
	Summer	0.32 <i>ab</i>		Urine	0.70 <i>d</i>		Urine	0.71 <i>bc</i>
	Autumn	0.62 <i>cd</i>		Synthetic Urine	0.61 <i>cd</i>		Synthetic Urine	0.51 <i>b</i>
HB ³	Spring	0.91 <i>d</i>	HB ³	Dung	0.64 <i>cd</i>	Autumn	Dung	0.52 <i>b</i>
	Summer	0.95 <i>d</i>		Urine	2.21 <i>e</i>		Urine	1.56 <i>e</i>
	Autumn	3.12 <i>e</i>		Synthetic Urine	1.91 <i>e</i>		Synthetic Urine	1.09 <i>d</i>
SEM		1.07			1.07			1.05

Means followed by same letter not significantly different at P<0.05

¹Moorepark site

²Johnstown Castle site

³Hillsborough site

Table 7 Full fixed model of multiple regression analysis for cumulative N₂O flux (g N₂O-N ha⁻¹ yr⁻¹) from urine and synthetic urine treatments using soil and weather data (a), and weather data only (b).

a) Parameter	DF	Estimate	Std. Error	Adjusted R-Square	t	F	Pr > F
1	1	-383.9	181	0.49	-2.12	87.79	<0.0001
1^2	1	15.99	3.09	0.69	5.18	9.17	0.003
2	1	1443	313	0.71	4.62	5.22	0.025
3	1	7.780	3.62	0.72	2.15	4.63	0.034

Parameters:

- 1 Cumulative precipitation five days prior application
- 2 1^2 + soil clay content
- 3 2 + N application rate

b) Parameter	DF	Estimate	Std. Error	Adjusted R-Square	t	F	Pr > F
1	1	-0.12	0.02	0.50	-5.24	89.12	<.0001
2	1	-0.11	0.03	0.68	-3.74	5.68	0.0193
1 ^2	1	0.00	0.00	0.70	7.83	5.12	0.0262
3	1	0.07	0.02	0.72	4.09	8.57	0.0044

Parameters:

- 1 Cumulative precipitation five days prior application
- 2 1^2 + soil temperature seven days prior application
- 3 2 + soil moisture deficit five days post-application

Figure 1. The variation in daily N_2O emissions, soil mineral N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ shown as mirror image), and daily rainfall, mean daily air temperature and water-filled pore space (WFPS) during the first 60 days of measurements following dung and urine application at Moorepark site in spring (a, d, g), summer (b, e, h), and autumn (c, f, i). Inset graphs show daily N_2O emissions over 365 days. Pooled standard error of the mean for $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, respectively, was 10.1 and 7.2 (d), 15.6 and 20.0 (e), and 16.2 and 7.7 (f), while pooled standard error of the mean for N_2O was 11.9 (g), 18.6 (h), and 8.8 (i).

Figure 2. The variation in daily N₂O emissions, soil mineral N (NH₄⁺-N and NO₃⁻-N shown as mirror image), and daily rainfall, mean daily air temperature and water-filled pore space (WFPS) during the first 60 days of measurements following dung and urine application at Johnstown Castle site in spring (a, d, g), summer (b, e, h), and autumn (c, f, i). Inset graphs show daily N₂O emissions over 365 days. Pooled standard error of the mean for NH₄⁺-N and NO₃⁻-N, respectively, was 5.9 and 8.2 (d), 26.4 and 6.3 (e), and 41.5 and 6.2 (f), while pooled standard error of the mean for N₂O was 41.0 (g), 24.9 (h), and 25.9 (i).

Figure 3. The variation in daily N₂O emissions, soil mineral N (NH₄⁺-N and NO₃⁻-N shown as mirror image), and daily rainfall, mean daily air temperature and water-filled pore space (WFPS) during the first 60 days of measurements following dung and urine application at Hillsborough site in spring (a, d, g), summer (b, e, h), and autumn (c, f, i). Inset graphs show daily N₂O emissions over 365 days. Pooled standard error of the mean for NH₄⁺-N and NO₃⁻-N, respectively, was 18.6 and 10.7 (d), 13.8 and 10.2 (e), and 45.2 and 8.7 (f), while pooled standard error of the mean for N₂O was 87.2 (g), 40.9 (h), and 180.0 (i).

Supplementary information

Table 8 Weather parameters included in the multiple regression analysis and corresponding with the 60 days temporal N₂O figures 1-3 g-i.

Site	Season	5 days prior to application	7 days prior to application	0 - 5 days post- application	0 - 60 days post-application		
		Precipitation*	Soil temperature ⁺	SMD ⁺⁺	Precipitation*	Soil temperature ⁺	SMD ⁺⁺
MP ¹	Spring	15.4	11.0	4.3	139.1	13.2	7.5
	Summer	14.0	20.9	15.7	74.1	16.7	25.1
	Autumn	5.4	15.5	1.0	318.8	10.3	0.4
JC ²	Spring	29.6	10.6	6.0	95.2	13.3	11.9
	Summer	0.0	23.4	5.6	142.8	17.1	20.9
	Autumn	1.0	15.4	0.4	349.2	10.6	0.4
HB ³	Spring	4.2	9.2	7.5	122.0	11.8	2.0
	Summer	13.8	12.5	20.4	214.2	15.1	8.5
	Autumn	58.6	12.8	0.0	248.2	9.5	0.4

* Cumulative precipitation

⁺ Average soil temperature at 0-5cm

⁺⁺ Average SMD

¹Moorepark site

²Johnstown Castle site

³Hillsborough site

Figure 4. Departure of temperature and rainfall from a long-term average for Moorepark (MP), Johnstown Castle (JC) and Hillsborough (HB) sites at all three seasons during the experimental period.