Nitrous oxide emissions from cattle excreta applied to a Scottish grassland: Effects of soil and climatic conditions and a nitrification inhibitor

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HIGHLIGHTS

- N_2O emissions were measured from cattle excreta applied to grassland.
- Emissions varied depending on environmental conditions at the time of application.
- Emissions from urine were greater than dung following summer application.
- N_2O emission factors were lower than the 2% value used by the IPCC.
- Addition of DCD did not reduce annual N_2O emissions from urine.

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ABSTRACT

Dung and urine excreted onto grasslands are a major source of nitrous oxide (N_2O). These N_2O emissions stem from inefficient utilisation of nitrogen (N) ingested by ruminants, and the inability of pasture to utilise the deposited N. Predicted growth in dairy and meat consumption means that there is a requirement to quantify N_2O emissions, and investigate emission reduction mechanisms. Three 12 month 'seasonal' experiments were undertaken at Crichton, SW Scotland, where N_2O emissions were measured from applications of cattle urine, dung, artificial urine and urine + a nitrification inhibitor (NI), dicyandiamide (DCD). The three application timings were 'spring', 'summer' and 'autumn', representative of early-, mid- and late grazing seasons. N_2O emissions were measured from static chambers for 12 months. The aim was to quantify emissions from cattle excreta, and determine their dependence on the season of application, and the respective contribution of dung and urine to total excreta emissions. Measurement from NI amended urine was made to assess DCD's potential as an emission mitigation tool. Emissions were compared to the IPCC's default emission factor (EF) of 2% for cattle excreted N. Mean annual cumulative emissions from urine were the highest when applied in summer (5034 g N_2O-N ha^{-1}), with lower emissions from spring (1903 g N_2O-N ha^{-1}) and autumn (2014 g N_2O-N ha^{-1}) application, most likely due to higher temperatures and soil moisture conducive to both nitrification and denitrification in the summer months. Calculated EFs were significantly greater from urine (1.1%) than dung (0.2%) when excreta was applied in summer, and EFs varied with season of application, but in all experiments were lower than the IPCC default of 2%. These results support both lowering and disaggregating this EF into individual EFs for dung and urine. Addition of DCD to urine caused no significant reduction in emissions, suggesting that more research is required into its use as a mitigation option.

1. Introduction

The efficient use of nitrogen (N) in agricultural systems provides many challenges (Zaman and Blennerhassett, 2010). Spanning over 25% of the earth's surface, and 40% of European agricultural land

(Carter, 2007), grasslands support as many as 1800 million units of livestock (Saggar et al., 2013), and so the impact that grazing animals are having on greenhouse gas (GHG) emissions and climate change is of global significance. Agriculture is responsible for approximately 70% of the UK's total nitrous oxide (N_2O) emissions (Rees et al., 2013), and of all agricultural land, grazed grasslands have higher N_2O emissions than un-grazed grasslands and arable cropland (van Groenigen et al., 2005a). Excretal N deposited by grazing animals on pastures (grazing

returns) is a major source of direct and indirect N_2O emissions from soil, an extremely powerful GHG. On grasslands used for grazing livestock little of the N ingested by cattle is utilised efficiently (Zaman and Blennerhassett, 2010), with 75–95% being excreted (Saggar et al., 2013; Eckard et al., 2010), with often less than 30% converted into milk and meat protein (Zaman et al., 2013). The N deposited in one urination patch can be as high as 600–1000 kg N ha^{-1} (Welten et al., 2013; Zaman and Blennerhassett, 2010), and is 2–3 times more than the pasture is able to utilise (Zaman et al., 2009). 1.5 Tg of total global anthropogenic N_2O emissions (6.7 Tg N year^{-1}) are estimated to be emitted from grazing animal excreta (Taghizadeh-Toosi et al., 2011; Oenema et al., 2005) through both direct and indirect (from leached and volatilised excreta-N) emissions. The deposition of livestock urine and resultant cycling of soil N is said to be the largest driver of N_2O emissions in agriculture (Wakelin et al., 2013; Welten et al., 2013). As human consumption of dairy and meat products is expected to increase into the future (Dijkstra et al., 2013) environmental impacts associated with grazing returns are also expected to increase as a result of agricultural intensification.

Despite the importance of the N_2O emissions associated with these grazing returns, and the requirement to reduce them, there are high levels of uncertainty associated with their measurement and quantification. Better measurements of N_2O emissions in the field using controlled experiments can help to identify environmental drivers and processes responsible for soil N_2O emissions from grazing returns, and enable more effective mitigation. The current approach to quantifying and reporting N_2O emissions in national inventories uses emission factors (EFs) for dung and urine that do not take account of influences from contrasting soils, climates, and season of deposition on the magnitude of emissions. Neither do they disaggregate emissions from dung with those from urine. When quantifying the impact that grazing animals have on direct soil N_2O emissions the IPCC guidelines apply a 2% EF to cattle excreta, assuming that 2% of the N deposited is emitted to the atmosphere (IPCC, 2006). This value is however based on measurements and evidence from only a few countries (Lessa et al., 2014), and the publication of much variation around this value (urine EF range of 0.1–4%; dung EF range of 0–0.7% (Rochette et al., 2014)), perhaps due to factors such as soil type, climate and season of deposition, calls for more research. There is a specific need for measurements from field experiments rather than laboratory trials, and from across a range of climates and seasons. Williams et al. (1999) and Lovell and Jarvis (1996) reported direct N_2O EFs as high as 7% and 14%, but these were based on laboratory experiments undertaken at 13 °C or 16 °C. With air and soil temperature, precipitation, and soil moisture all known to have an influence on the size of N_2O emissions from soil, the timing of grazing and dung and urine deposition could play a large role in the extent of emissions and N loss from the system. Although GHG production in soils is largely a biological process, environmental and soil chemical and physical conditions have an influence on these biological processes, and can thus be an important influence on the extent of emissions (Ball, 2013). The 3 major controls identified by Conen et al. (2000) on direct N_2O emissions from agricultural soils were water filled pore space (%WFPS), soil mineral N content, and temperature. Kelly et al. (2008) reported higher N_2O emissions at temperatures >15 °C, and Allen et al. (1996) observed high emissions from poorly drained soils. In countries with temperate climates, like the UK, high soil moisture and heavy rainfall often observed in late autumn and winter could be responsible for greater denitrification rates and N_2O emissions following deposition of animal excreta at this time of year. Along with soil and climate, the timing of deposition needs to be included to improve EF calculation. The amount of N excreted is also dependent on the type of cattle or sheep and diet quality, with beef cattle found to excrete less N than dairy cattle, and a lower quality diet leading to greater N excreted in dung (Lessa et al., 2014). Van Groenigen et al. (2005b) attribute greatest emissions to dairy cattle bred in intensive systems in developed countries- but this is not taken into account in IPCC tier 1 estimates. In

addition to concerns regarding the application of the same default EF value to all areas, climates and soil types, is concern over the chosen value of 2% itself for cattle excreta. Some of the studies used when determining this default value used artificial urine, or were incubation studies rather than field trials. Van Groenigen et al. (2005a) raise concerns that use of these artificial urines may lead to overestimation of EF values, and de Klein et al. (2003) found that emissions from artificial urine were not always as high as from real urine. New Zealand no longer uses this IPCC default value of 2% for cattle, but instead uses a country specific value of 1% (de Klein et al., 2011).

In addition to improving the quantification of emissions, and identifying the processes responsible, the requirement to reduce emissions has led to research into the use of nitrification inhibitors (NIs) as a potential N_2O mitigation measure. The performance of NIs applied to animal excreta and grassland soils in reducing N_2O emissions and nitrate leaching has been reported in several studies; however the rate of success has been variable (Barneze et al., 2014; Wakelin et al., 2014; Zaman et al., 2013; Dennis et al., 2012; Kelliher et al., 2007). Other grassland studies by Merino et al. (2001) and McTaggart et al. (1997) have shown DCD to have variable success in reducing N_2O emissions depending on the type of fertiliser to which DCD is applied; emphasising that the success of NIs at reducing emissions from urine patches on grasslands could be very different to that following application of nitrate based inorganic fertilisers. It is clear that there is much variability in the emission reduction success rate of DCD, and that results relating to mineral fertiliser studies or those undertaken in different soil and environmental conditions cannot be assumed to apply to cattle urine on grasslands in Scotland. The timing of NI application could be of major importance in their success at reducing emissions, with rainfall a potential cause of NI leaching from the soil, and soil temperature a potential factor in the degradation of DCD. The importance of undertaking longer term experiments to clarify the longevity of the effect is also recognised (Clark et al., 2005). Further uncertainty stems from the impact of DCD on crop production and pasture growth, with Moir et al. (2003) reporting increases in pasture production of 25% following application of DCD to an artificial urine treatment, but others, including Li et al. (2014) and Cookson and Cornforth (2002) finding no significant effect.

The aim of this work was to reduce uncertainty in the national agricultural N_2O inventory through an increased understanding of the factors and processes responsible for direct N_2O emissions from cattle grazing returns to Scottish grasslands. The aim was to assess the extent of the emissions associated with excreta deposited in different seasons, and to investigate drivers such as environmental and soil conditions and grassland management activities. These emission measurements allowed EFs to be calculated, and compared to the IPCC default value of 2%, to add to support in either maintaining or altering this current value. In addition to measuring the impact of grazing season on N_2O emissions, the success in reducing N_2O emissions from urine through use of the NI DCD was investigated. Measurements from artificial urine produced in the laboratory were made to assess its suitability in similar research experiments, when 'real' urine is not available. The impact of the seasonal deposition of dung and urine, and the use of NIs on grassland yield was also investigated. The study presented here is one of 5 experiments in the UK following a consistent protocol when measuring N_2O emissions from urine and dung. This work forms part of the UK GHG Platform Project, and comparison of results from these different sites will allow the extent of emissions from urine and dung to be assessed, along with the impact of variations in soil, climate and location on these emissions.

2. Materials and methods

2.1. Field site and experimental design

A field experiment was established in South-West Scotland in April 2012, at Crichton Royal Farm in Dumfries (55°02.5′N, 3°35.3′W). The

specific field site is part of a network of sites in the UK at which comparative studies are being undertaken, each in a different soil and climatic zone. The Crichton site was chosen as it is representative of a wet climate zone, with a free-draining sandy to sandy-loam light soil texture and no history of long-term manure application. The soil has an organic matter (OM) content of 4.8–7.3%, and the site has a 30 year (1971–2000) long-term average rainfall of 1140 mm, and mean annual temperature of 9.1 °C.

To enable assessment of the effects of seasonal dung and urine application the study consisted of three individual 12-month measurement periods, all located within the same field, but treated as three separate simulated experiments, with each one blocked and randomised and including a control. The first experimental period ran from April 2012 to April 2013, with urine and dung application representative of 'spring' N returns. The 'summer' N returns period began in June 2012 and was completed in June 2013, and the 'autumn' N returns period was established in October 2012, and completed in October 2013. Each of the seasonal experiments consisted of three replicate blocks and five treatments (plots) per block (dung, urine, urine + DCD, artificial urine, and a control) positioned in a completely randomised plot layout. Emissions of N_2O were measured from five replicate static closed chambers on each plot, totalling 15 N_2O measurements per treatment on each sampling occasion.

2.2. Dung and urine application

Samples of fresh dung and urine applied to plots in spring, summer and autumn were analysed for total N and C, NH_4^+-N and NO_3^--N and dry matter contents. These data and calculated rates of N application for all treatments are displayed in Table 1. Artificial urine was made in the laboratory using chemicals following the method outlined in D.A. Kool et al. (2006) for recipe 2 (R2), and the dung and real urine were collected from Holstein dairy cattle (aged 2–7 years) <7 days prior to the experiment start date. The cattle had been fed on a mixed diet of homegrown and byproducts, and the real urine was stored in a refrigerator at <4 °C prior to application to the experimental plots. Chemical constituents of the urine were also measured to enable assessment of the role of hippuric acid and other chemical characteristics in N_2O emission generation. This was undertaken following inconsistent findings in the literature regarding the ability of hippuric acid to inhibit nitrification (Bertram et al., 2009; Clough et al., 2009; D.M. Kool et al., 2006; Van Groenigen et al., 2005a). Note: artificial urine was inadvertently applied at lower than the intended N concentration in the spring experiment (Table 1). In compliance with the IPCC guideline advice all experiments were monitored for a 12-month period to determine annual EFs.

Five patches on each plot were prepared for gas sampling measurements. The amounts of dung and urine applications were based upon typical N loadings reported by Yamulki et al. (1998). The individual urine patches measured 60 cm × 60 cm, and were located randomly across the plot. All of the urine treatments (real urine, artificial urine, urine + DCD) were applied at a rate equivalent to 5 L/m^2 (1.8 L of urine/patch), using a watering can fitted with a rose attachment. Prior to application a wooden frame was placed around the patches to avoid any seepage or runoff of the urine from the treated area. This frame was removed once all of the urine had soaked into the soil. Dung was applied to patches with a circular area of 0.126 m^2 at a rate equivalent to 20 kg/m^2, and spread to an even thickness across the patch. In addition to the patch areas within the plot, an additional area measuring 2 m × 2 m was set aside for soil mineral N, grass yield and grass N uptake sampling. Dung and urine were applied to these areas respectively, at the same rate as applied to the gas sampling patches. For the urine + DCD treatment a 1% solution of DCD was mixed with the urine to ensure an even distribution of the DCD over the patch, with the DCD applied at an equivalent rate of 10 kg ha^{-1}.

2.3. N_2O emission sampling

The same protocol was followed for each seasonal experiment, to allow a direct comparison of results. Here a closed static chamber technique was used (e.g. Chadwick et al., 2014; Louro et al., 2013), where five circular chambers made of opaque polypropylene (400 mm diameter, 300 mm height and soil surface area coverage of approximately 0.126 m^2) were placed on each plot (15 replicate chambers/treatment) and inserted 5 cm into the soil at the beginning of the experimental period. Five ambient air samples were collected prior to chamber measurements, and lids were then placed onto chambers, and sealed. Chamber lids were left in place for 40 min and a 50 mL sample of gas was then extracted from the chamber using a syringe and a valve with a three-way tap, and transferred to a pre-evacuated 20 mL glass vial so that it was under pressure. A further five ambient samples were taken at the end of the 40 minute closure period. All gas sampling events were undertaken between 10 am and 12 pm, with the order in which the blocks were sampled randomised each day, to avoid bias resulting from diurnal variation.

Measurements were made for 12 months following treatment application, along with one set of background N_2O measurements in the week prior to application. Eight sets of measurements were made over the first two weeks following treatment application, with sampling frequency reduced to two times a week, and then once every two weeks one month post-application. Sampling frequency was reduced to once per month after 25 weeks of measurements. In the laboratory a needle was used to release excess pressure within the vials, and gas samples were analysed for N_2O concentration using an Agilent 7890A Gas Chromatograph (GC) fitted with an electron capture detector (Agilent Technologies, Berkshire, UK), with an N_2O detection limit of 0.025 ppmv. GC response was calibrated using certified standard N_2O gas mixtures of 0.35, 1.1, 5.1, and 10.7 ppmv. N_2O flux from each chamber was calculated by measuring the increase in chamber headspace concentration at the end of the 40 minute closure period, above that of the average concentration in ambient air samples. This procedure assumes that gas

Table 1
Nitrogen application rates and chemical characteristics (analysed on a fresh weight basis) of excreta used in each seasonal experiment.
A urine = artificial urine.

		Application rate (kg N ha^{-1})	DM %	Total N %	Nitrate N %	Ammonium N %	Allantoin (g N/L)	Creatinine (g N/L)	Uric acid (g N/L)	Hippuric acid (g N/L)
Spring	Urine	480	4.9	0.96	<0.010	0.012	0.85	0.25	0.10	0.72
Summer	Urine	420	4.6	0.84	<0.010	0.024	0.60	0.48	0.15	0.12
Autumn	Urine	435	4.9	0.87	<0.010	0.010	1.38	0.29	0.14	0.60
Spring[a]	A urine	180	1.4	0.36	<0.010	<0.010	0.35	0.06	0.02	0.09
Summer	A urine	425	3.5	0.85	<0.010	<0.010	1.26	0.33	0.06	0.59
Autumn	A urine	425	3.5	0.85	<0.010	<0.010	1.26	0.33	0.06	0.59
Spring	Dung	1020	11.5	0.34	<0.010	0.026				
Summer	Dung	680	12.9	0.51	<0.010	0.041				
Autumn	Dung	720	10.6	0.36	<0.010	0.023				

[a] Artificial urine was inadvertently applied at the incorrect N concentration in spring.

accumulation in the chamber is linear over the 40 min, and this was checked for three random chambers on each sampling occasion by extracting a sample every 10 min, for a total of 60 min after closure. This assumption of linearity is further supported by evidence from previous research (Chadwick et al., 2014; Lessa et al., 2014).

2.3.1. N_2O flux calculations

The N_2O flux was calculated using N_2O concentration, chamber height, the ideal gas law, and the air temperature and chamber closure time. These details were entered into a standard spreadsheet used by all sites in the UK GHG Platform Project. The mean flux for each plot was calculated and then used to derive the mean flux and standard error (SE) for each treatment on any sampling occasion. Plot values rather than individual chamber values were used in all statistical analysis to avoid pseudoreplication. Cumulative fluxes were calculated by interpolating the area under the curve between sampling points, and a mean cumulative flux and SE was calculated for each treatment using plot means. If emission measurements were a few days short of the complete 365 day annual period then the flux was extrapolated to the full 365 day period to ensure that a direct comparison could be made between seasons. Emission Factors were calculated by subtracting the cumulative emission from the control treatment in each block from the cumulative emission from individual treatments in the same block, as in the IPCC methodology, displayed in Eq. (1).

$$EF = \left(\frac{\text{Cumulative } N_2O \text{ flux (kg } N_2O\text{-N)} - \text{cumulative } N_2O \text{ flux from control (kg } N_2O\text{-N)}}{N \text{ applied (kg N)}} \right) \times 100 \qquad (1)$$

2.4. Grass yield and N uptake measurements

Grass yields from all of the treatments and the control plots were measured at several points throughout the year for each experiment. A cut was taken every time the grass reached the top of the gas sampling chambers, with yield measurements taken from a 1 m × 1 m area within the 2 m × 2 m patch set aside for soil and grass sampling. The fresh yield for each patch was measured, and a representative sample of the grass was dried and weighed at 65 °C to allow conversion of fresh weight to dry matter yields. A subsample of the grass from each plot at each grass cut was milled and analysed for C/N to determine N uptake.

2.5. Soil mineral N

Five randomly selected soil samples were taken from the 0–10 cm layer of each plot using a soil auger, and bulked to give one representative sample per plot. The soils were analysed for NH_4^+-N and NO_3^--N by colorimetric analysis (Singh et al., 2011), using a Skalar SAN^{++} segmented flow analyser, after 2 M KCl extraction of a sieved (<4 mm) sample, with a soil: extractant ratio of 1:2. Soils were collected 14 times throughout the year, with four sets of samples taken in the first four weeks of the experiment. Sampling frequency then declined to once per month for the next six months, and then once every seven weeks for the remainder of the experiment and coincided with N_2O emission measurements.

2.6. Meteorological and additional soil data

Soil samples for the determination of gravimetric soil moisture were collected on every N_2O emission sampling day. Five samples from the 0–10 cm soil layer were taken randomly from each block, and then bulked to give a total of three soil moisture samples per day. Bulk density samples were also collected to convert these results to volumetric soil moisture, and an assumed particle density value of 2.65 g cm^{-3} was used to convert to %WFPS. Further soil samples were taken at the beginning of the experiment to measure field capacity and permanent

wilting point, pH (in water), extractable P, K, S and Mg, total N, TOC and particle size distribution, and meteorological stations were set to record daily air temperature and precipitation. Many of these parameters are required for site characterisation for future mechanistic modelling of N_2O fluxes, e.g. DNDC and DayCent (Abdalla et al., 2010).

2.7. Data analysis

All statistical analysis was undertaken using GENSTAT (GenStat 16th Edition. Release 16.1., VSN International Ltd., Oxford). Initial analysis of annual cumulative emissions, EFs and grass yield was analysed as a mixed model using the REML (restricted maximum likelihood) algorithm. The random effect model was block nested within season with a separate residual term (block by treatment interaction) for each season. Effects of treatment, season of application and their interaction were tested using the Wald statistic. The cumulative emissions were transformed using the natural logarithms before analysis to more closely satisfy the assumption that residuals and random effects are normally distributed. In addition, the EFs were transformed using the square root function. In this case, because of the negative EFs, 0.21 was added to the measurements before being transformed. Results were considered statistically significant at p < 0.05.

3. Results and discussion

3.1. Weather and soil moisture

The highest rainfall in the first 30 days post application was observed following autumn application (137 mm), with 125 mm recorded following summer application, and only 60 mm following spring application (Fig. 1a). The generation of an N_2O emission peak, followed by a return to background N_2O flux within the first month of the experiments (Fig. 2 and Section 3.4) meant that a decision was made to analyse not only the annual soil and environmental conditions for each experimental period, but also the conditions in the first 30 days, when greatest emissions were observed. Although the total amount of rainfall recorded in these 30 day periods was highest in the autumn experiment, this corresponds with the long-term average (1971–2000) trend, with rainfall in October generally higher than in the summer or spring months. The rainfall in this particular year was however higher than the long-term average for this time (Fig. 1a). Although the rainfall recorded in the 30 day post application period during the summer experiment was lower than in the autumn, this rainfall total was much greater than the 30-year average for this time of year (Fig. 1a). The low amount of rainfall in the 30 days post-application in the spring experiment was typical of long-term rainfall for this month (Fig. 1a). These results suggest that the summer and autumn experiments took place in conditions atypical of those usually experienced at these times of year.

When rainfall totals from each of the experimental periods is assessed (Fig. 1b) it can be seen that the spring and summer experiments received greater rainfall than the long-term average, with the autumn experiment experiencing the driest conditions, more typical of this location. Reference to Fig. 2 shows that air temperatures experienced during the period of treatment application were similar in the spring and autumn experiments, but much greater in the summer experiment, with a mean temperature over the first 30 days of 14.8 °C in summer compared to 7.5 °C in spring and autumn. Comparison of these temperatures to the long-term average for April of 7.7 °C, July of 15.5 °C and October of 9.6 °C indicates that temperatures for the spring and summer experiments were typical of those expected in this location, but that temperatures during the autumn experiment were lower than average. Calculated %WFPS values over each experimental period are also displayed in Fig. 2, where higher soil moisture conditions of >60%WFPS are evident for the first five months of the autumn experiment, in comparison to %WFPS values of 40–62% in the first few weeks after treatment application in spring and summer, when the largest
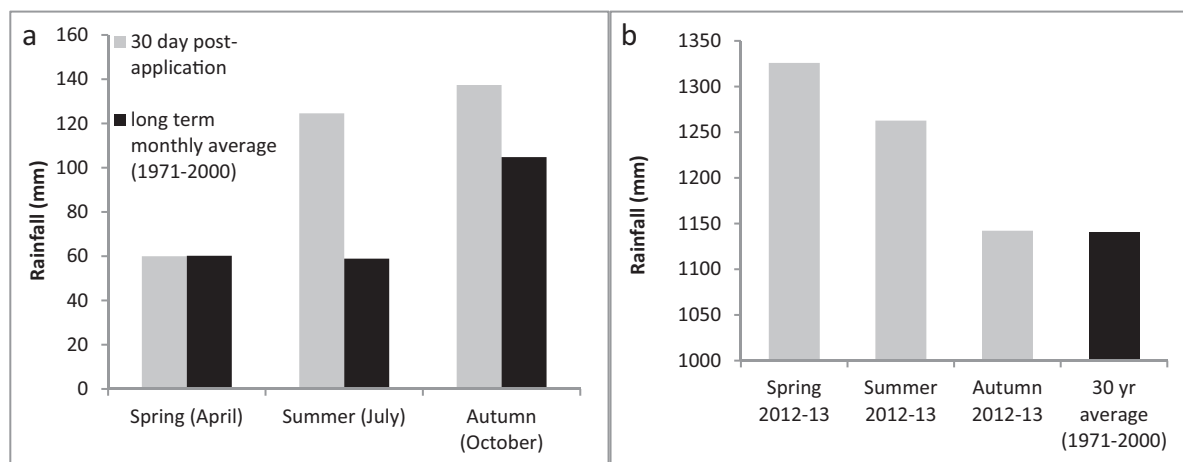
Fig. 1. a. A comparison of rainfall totals in the 30 days after dung and urine treatment applications for each seasonal experiment, and a comparison with the 30 year long-term average (1971–2000) for the month of application; b. A comparison of annual rainfall totals over each experimental period with the 30 year annual average (1971–2000) at this site.

daily fluxes of N_2O occurred. The mean %WFPS recorded over the 1st 30 days of the experiment was highest following autumn deposition (69%), 57% following summer deposition, and lowest (51%) after spring deposition.

3.2. Cumulative N_2O emissions

Statistical analysis revealed a significant difference in cumulative emissions caused by the season of excreta application (p = 0.005, SED = 0.1689), the type of excreta (p < 0.001, SED = 0.1734), and a significant interaction between season and excreta type (p < 0.001, SED = 0.3065). This significant interaction indicates that treatment had a different effect on cumulative N_2O emissions depending on the season of application. The variation in emissions with treatment and season is displayed in Fig. 3. With relation to treatment differences, in the spring experiment emissions from artificial urine (916 g N_2O-N ha^{-1}) were significantly lower than from both urine (1902 g N_2O-N ha^{-1}) and from dung (2035 g N_2O-N ha^{-1}). This would be expected due to the lower N concentration of artificial urine used in the spring application and the high % N content of the dung applied in this season compared to summer and autumn (Table 1). This is confirmed by a lack of difference in EFs between dung and artificial urine in spring (Section 3.3), where N application rate is taken into account in the EF calculation. There was no significant difference due to addition of DCD to urine (1246 g N_2O-N ha^{-1}), and no significant difference between emissions from dung and those from urine. Summer application of treatments resulted in significantly lower emissions from dung (1996 g N_2O-N ha^{-1}) than from all of the urine treatments (urine: 5034 g N_2O-N ha^{-1}; artificial urine: 5276 g N_2O-N ha^{-1}; urine + DCD: 4827 g N_2O-N ha^{-1}), but there was no difference between urine and artificial urine, or between urine and urine with added DCD. Application of treatments in the autumn resulted in no significant difference in cumulative emissions between urine (2014 g N_2O-N ha^{-1}) and dung (1538 g N_2O-N ha^{-1}), or between any of the urine treatments (Table 2). The interaction also reveals that the difference in emissions resulting from season of application varied depending on the type of excreta. With relation to seasonal differences, emissions from all of the urine treatments (urine, artificial urine, urine + DCD) were significantly higher following summer application than they were following spring or autumn application. In relation to artificial urine it is possible that this could be the result of its low %N in the spring. A higher %N concentration of urine in the spring than summer experiment (Table 1) indicates however that the higher summer flux from urine is not the result of a higher N application rate, and that other factors are responsible. This is confirmed in Section 3.3 where urine EFs which account for N application rate are also

significantly higher in summer. Season of application did not cause any difference in emissions from dung. There was no difference in emissions between spring and autumn application for any of the treatments (Table 2). Log-transformed and back-transformed cumulative N_2O emissions for all seasonal experiments and excreta types are displayed in Table 2.

3.3. Emission factors

Statistical analysis revealed a significant difference in EF caused by the season of excreta application (p = 0.005, SED = 0.09339), the type of excreta (p = 0.010, SED = 0.06339), and a significant interaction between season and excreta type (p = 0.011, SED = 0.1269). This significant interaction indicates that treatment had a different effect depending on the season of application. The variation in EFs with season and treatment is displayed in Fig. 4. In the spring application experiment the mean EF calculated for urine (0.2) was significantly greater than that calculated for artificial urine (−0.08), but there was no difference between EF for any other types of excreta (the addition of DCD did not reduce the annual EF (urine + DCD = 0.06), and EFs for dung (0.11) and urine (0.2) were not significantly different). Following summer application the dung EF (0.20) was significantly lower than the EFs calculated for all types of urine application (urine 1.07; artificial urine 1.10; urine + DCD 1.03). There was no difference in EFs calculated for any types of urine application, again indicating no reduction in emissions resulting from the use of DCD, and a similar response in emissions from artificial and real urine. Following autumn application there was no difference in EFs calculated for any type of excreta (dung 0.10; urine 0.31; artificial urine 0.11; urine + DCD 0.21). The interaction also reveals that the difference in emissions resulting from season of application varied depending on the type of excreta. Season of application caused no significant difference in the EF calculated for dung (spring 0.11; summer 0.20; autumn 0.10). There was no significant difference in the EF calculated for any type of excreta when application in spring was compared to application in autumn (Table 3). EFs calculated for all types of summer urine applications were all significantly higher than those calculated for spring and autumn applications (Table 3). The horizontal dashed line in Fig. 4 indicates the current IPCC default EF value of 2% for cattle grazing returns, emphasising that the EFs calculated in this study are much lower than those used to prepare inventories according to the IPCC methodology. Square root-transformed and back-transformed EFs for each seasonal experiment and type of excreta application are displayed in Table 3.
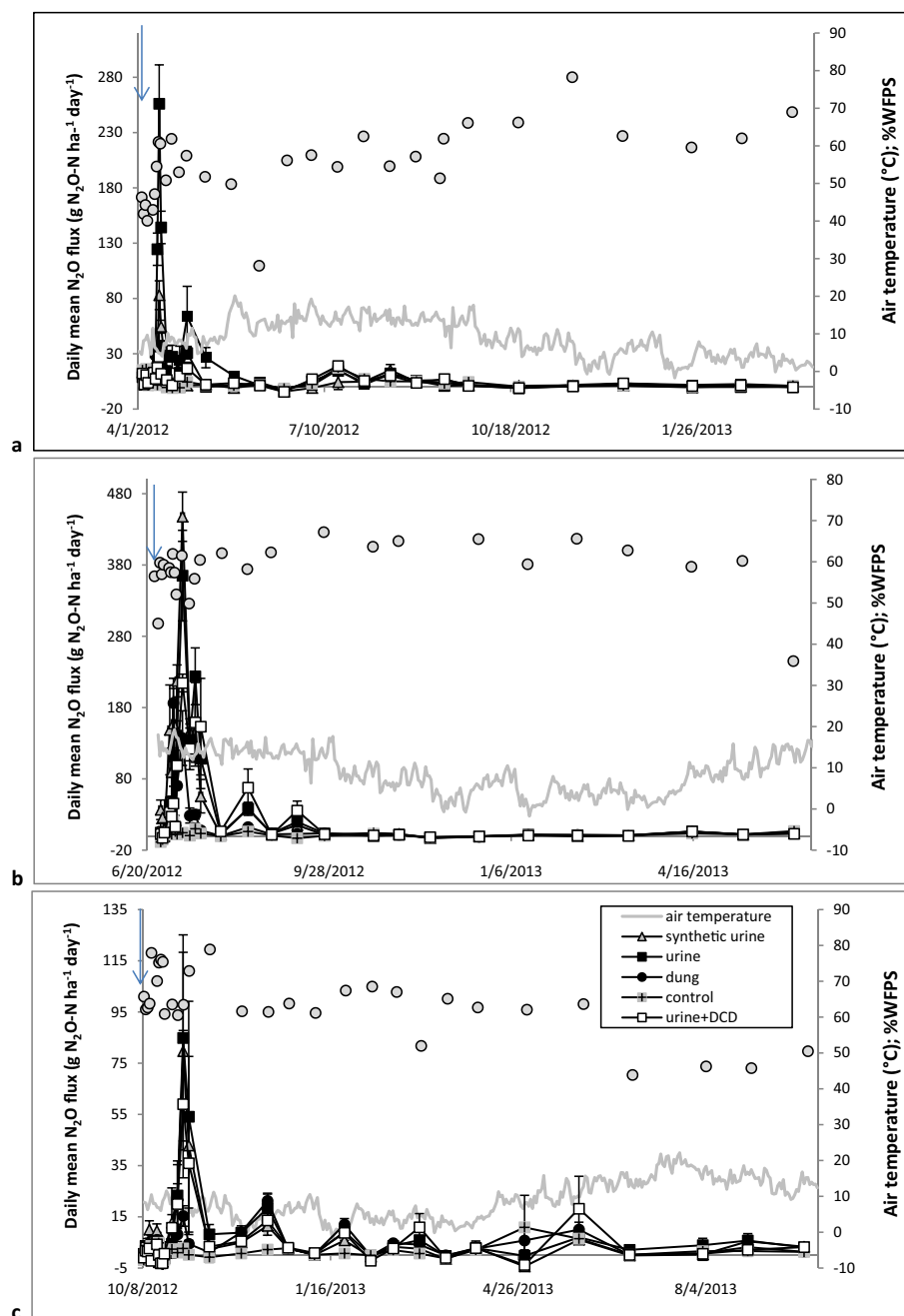
Fig. 2. The variation in air temperature, %water filled pore space (WFPS), and daily N$_2$O emissions during the one year measurement periods following dung and urine application in: a. spring; b. summer; c. autumn. Vertical arrows indicate timing of application. Note — the use of different scales on the Y axis to provide clarity of N$_2$O fluxes for the three experiments. Error bars on the N$_2$O emission data indicate the standard error of the mean (n = 3).

3.4. Daily N$_2$O and temporal trends: relationship with soil and environmental variables

A large peak in emissions is observed at all sites within the first three weeks following application of the grazing returns, however the magnitude of this peak varies with season (Fig. 2). The largest single peak in emissions was observed following summer application, when a flux of 448 g N$_2$O-N ha^{-1} was observed from the artificial urine treatment on 9th July, 13 days after deposition. This is in comparison to the highest peak in the spring experiment of 256 g N$_2$O-N ha^{-1} from the urine treatment on 12th April (10 days post application), and 84 g N$_2$O-N ha^{-1} from the urine treatment in the autumn experiment on 29th October (21 days post application). The peak in emissions after application

in the spring experiment occurred after an increase in %WFPS, however this was not true for summer or autumn application (Fig. 2).

Peak emissions of N$_2$O in all experiments occurred soon after dung and urine application, and the increase in soil mineral N concentration (Fig. 5). A peak in soil NO$_3^-$-N content on 22nd May followed the peak in soil NH$_4^+$-N content (6th April) in the spring experiment, suggesting that nitrification was the dominant soil process, responsible for N$_2$O production, as the N$_2$O emission peak (12th April) was observed before the rise in soil NO$_3^-$-N (Fig. 5a). A smaller second peak in N$_2$O emissions on 27th April again preceded the rise in soil NO$_3^-$-N, indicating a further episode of nitrification. No peaks in N$_2$O emissions were observed following the rise and decline in soil NO$_3^-$-N, suggesting that denitrification did not contribute to N$_2$O emissions on this occasion, supported
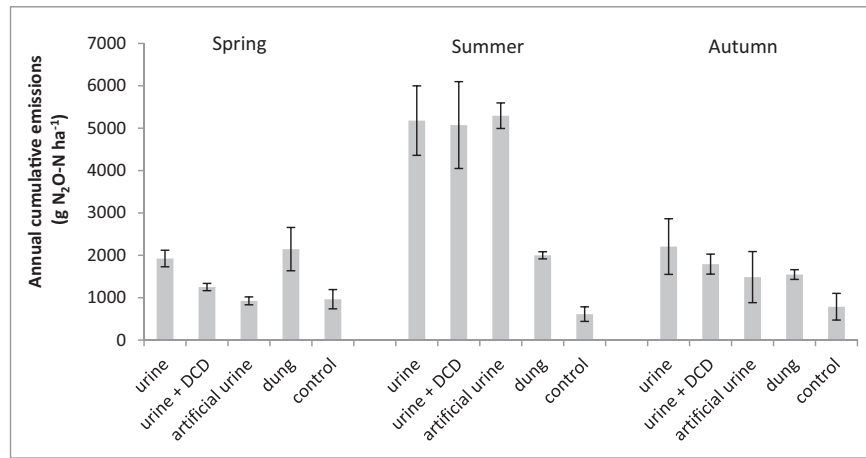
Fig. 3. The variation in annual measured mean cumulative N_2O emissions from each treatment for each application season. Error bars illustrate the standard error of the mean (n = 3).

by the %WFPS values at this time of approximately 50% (Fig. 2). Measured soil mineral N contents in the summer experiment were lower than in the spring and autumn experiments (Fig. 5b), but the peak in N_2O emissions on 9th July similarly followed the rise in soil NH_4^+-N content (9th July). Unlike in the spring experiment, the N_2O emissions peak also followed a peak in soil NO_3^--N content (3rd July), and was indicative of a period of denitrification which may have contributed to N_2O emissions. This is supported by %WFPS values rising above 60% at this time (Fig. 2), which would have been conducive to the processes of both nitrification and denitrification. A second peak in soil NO_3^--N was measured on 16th July, along with a second peak in N_2O emissions on this date, suggesting a further episode of denitrification. Low soil mineral N levels in the summer combined with high N_2O emissions suggest that the processes of both nitrification and denitrification occurred in combination, and at a very quick rate. In the autumn experiment soil NH_4^+-N and NO_3^--N levels peaked immediately following grazing deposition, and the peak in N_2O emissions was similar to the 2nd peak in the summer experiment, in that it followed rather than preceded a rise in soil NO_3^--N, indicating a process of denitrification, which is again supported by %WFPS values conducive to this process (Fig. 2). High soil NH_4^+-N contents were maintained until 4th April 2013, with no further observed peaks in soil NO_3^--N over the experimental period, indicative of much reduced nitrification in this seasonal experiment. This lack of nitrification is thought to be the result of high soil %WFPS, which will have prevented this process from occurring. Although the observed soil %WFPS values of 60–80% would have been conducive to denitrification in the autumn experiment, the lack of nitrification will have limited the soil NO_3^--N available for this process, and can help explain the low emissions of N_2O observed in this experiment.

There was a variation in temporal emissions between types of excreta, with peak emissions occurring from the urine/artificial urine in all experiments, and lowest emissions from the control (Fig. 2). At

the time of peak emissions, N_2O fluxes from DCD amended urine patches were lower than those from the un-amended urine in all experiments. In the spring experiment the urine + DCD flux peaked at 27.3 g N_2O-N ha^{-1} day^{-1} compared to a much greater flux of 256 g N_2O-N ha^{-1} day^{-1} from urine. In the summer experiment 215 g N_2O-N ha^{-1} day^{-1} was emitted from the DCD amended urine, compared to 365 g N_2O-N ha^{-1} day^{-1} from the urine without DCD, and in the autumn 55.0 g N_2O-N ha^{-1} day^{-1} from the urine + DCD treatment compared to 84.9 g N_2O-N ha^{-1} day^{-1} from urine alone. These results suggest that DCD was successful in inhibiting nitrification, however, analysis of the temporal N_2O emissions over the entire experimental periods (Fig. 2) showed that the reduction in emissions with DCD was not consistent throughout the year, with greater emissions from the DCD treatment at certain times of the year resulting in non-significant differences in annual cumulative fluxes (Section 3.2).

3.5. Grass yield

There was a significant difference in grass yield caused by the season of excreta application (p = 0.004, SED = 0.8222), the type of excreta (p = < 0.001, SED = 0.6521), and a significant interaction between season and excreta type (p = 0.019, SED = 1.243). This significant interaction indicates that treatment had a different effect on grass yield depending on the season of application. The variation in yield with season and treatment is displayed in Fig. 6. In the spring experiment grass yield was significantly lower from the control treatment than from any of the other applications, but there was no significant difference in the grass yield produced between any of the other treatments, indicating that DCD had no impact on yield. Following summer applications there was no significant difference in any of the yields produced under the different treatments, and following autumn applications the yield produced from the dung was significantly lower than from the artificial urine, but there was no difference between any other treatments. Analysis from all three seasonal experiments illustrates no variation in grass yield resulting from the addition of DCD to urine. Although there was a difference in yield resulting from season of application, this varied depending on the type of excreta applied. Season of application caused no significant difference in grass yield from the control treatment. A significantly greater yield was produced following spring application than summer applications from all of the urine treatments and from the dung. The yields produced following spring applications from the urine, urine + DCD and dung treatments were also significantly greater than from the autumn applications, but there was no difference between spring and autumn applications of artificial urine. There was no difference in yield produced between summer and autumn applications from any of the treatments.

Table 2
A comparison of cumulative N_2O emissions between seasons and treatments: Log transformed and back transformed mean cumulative N_2O emissions. Means that do not share a letter are significantly different.

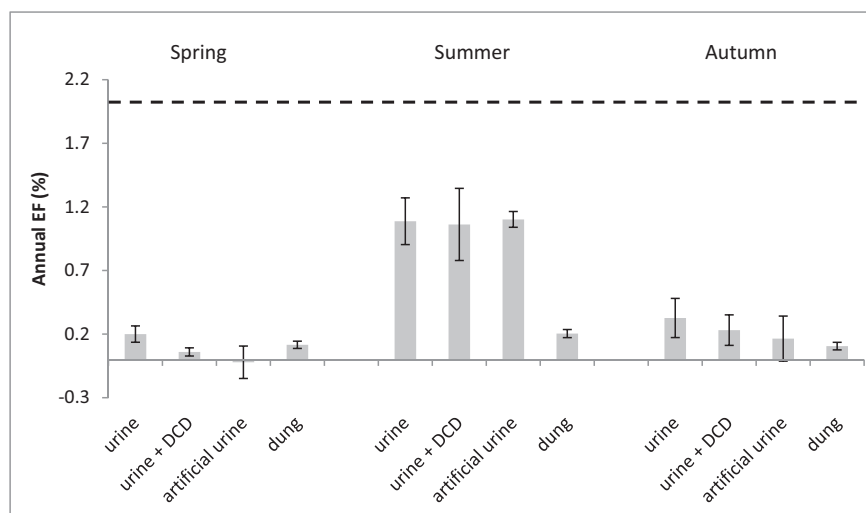|	Log transformed cumulative N_2O-N emission (g N_2O-N ha^{-1})			Back transformed cumulative N_2O-N emission (g N_2O-N ha^{-1})		
	Spring	Summer	Autumn	Spring	Summer	Autumn
1. Control	6.82[ab]	6.35[a]	6.48[a]	912	570	653
2. Urine	7.55[c]	8.52[d]	7.61[c]	1903	5034	2014
3. Urine + DCD	7.13[bc]	8.48[d]	7.47[c]	1246	4827	1760
4. A urine	6.82[ab]	8.57[d]	7.15[bc]	916	5276	1273
5. Dung	7.62[c]	7.60[c]	7.34[bc]	2035	1996	1538

Fig. 4. The variation in measured annual EFs depending on the season of application and type of excreta. Error bars illustrate the standard error of the mean (n = 3); the dashed line represents the default IPCC EF (2%) for excretal returns for grazing cattle.

4. General discussion

The highest N_2O fluxes in this study were recorded following summer urine applications; however the greatest amount of rainfall over the first 30 days was highest for the autumn application experiment (Fig. 1a). Soil %WFPS in the summer was also lower than in the autumn (Fig. 2). This conflicts with other research that suggests that avoiding anaerobic soil conditions provides the potential to reduce emissions (Kelly et al., 2008) and that greatest fluxes will be observed in winter when soils are at their wettest (Di et al., 2014). The 60% threshold usually associated with denitrification and the generation of high fluxes of N_2O was exceeded for the greatest number of days in the autumn experiment, however this seasonal experiment generated the smallest peak fluxes. The high emissions recorded in summer did occur following a period of heavy rainfall, but the soil at this time was drier than that in autumn when very low fluxes were recorded. These results suggest that nitrification rather than denitrification was the most dominant source of N_2O emissions, indicating that decisions made to reduce emissions should not be based purely on the avoidance of conditions conducive to denitrification. The lower N application rate of urine applied in the summer than in the spring or autumn experiment (Table 1), and the significant difference between EFs calculated for these seasonal applications indicates that the rate of N application was not the cause of this variation in emissions.

The low level of soil NH_4^+-N recorded in the summer experiment after application (Fig. 5b) suggests that rapid nitrification took place in the soil before any soil mineral N measurements were made in this experiment. This would correspond with the high rates of N_2O emissions recorded at this time, indicating a rapid loss of N from the soil system. Low levels of soil NO_3^--N were also recorded in the summer experiment (Fig. 5b) suggesting that high rates of denitrification could also have contributed to the high N_2O emissions in this experiment. Carter

(2007) observed equal amounts of nitrification and denitrification at %WFPS of 45%, confirming that both processes are likely to have produced N_2O emissions following summer deposition when %WFPS was 57% in this study. This rapid loss of soil mineral N following summer deposition was in contrast to the much higher concentrations measured in the soil in the autumn experiment (Fig. 5c), where soil NH_4^+-N remained at a high level for more than four months following deposition. This observation suggests that the process of nitrification did not occur immediately after autumn deposition, or at a great rate, confirmed by the low levels of NO_3^--N also recorded. Although soil NO_3^--N levels were low (<75 kg ha^{-1}), there was a peak recorded on 10th October, which then fell to background levels and preceded the peak in N_2O emissions, indicating that in this experiment denitrification was likely to be the dominant source of the observed low N_2O emissions. Reduced nitrification following autumn deposition indicates that there were environmental constraints, and the observation of nitrification at a similar soil temperature in the spring experiment (Figs. 2 and 5a.) implies that soil temperature did not limit this process in the autumn. It is most likely therefore that soil conditions in the autumn were too wet for nitrification. Although soil moisture conditions were conducive to denitrification, the lack of nitrification meant that soil NO_3^--N levels were too low for this process and the resultant production of N_2O to occur. Soil mineral N results from the spring deposition experiment (Fig. 5a), and the peak in N_2O emissions preceding the peak in soil NO_3^--N imply that soil and environmental conditions were conducive to the process of nitrification, and that this was the main contributor to emissions in spring. Although denitrification did occur in the spring it does not appear to have been the dominant source of N_2O emissions. With similar soil moisture conditions observed at the time of peak emissions following spring and summer application (spring: 60%; summer: 62%) soil temperature appears to be the most influential factor in the production of greater emissions in the summer. This is supported by Dijkstra et al. (2013), who found that temperature increases from 5 °C to 15 °C can result in increased emissions greater than an order of magnitude. Although it is possible that the low hippuric acid content of summer applied urine (Table 1) could have caused the generation of high N_2O emissions in the summer, this is unlikely, as using this explanation would suggest that the lowest peak emissions should be observed from spring applied urine, rather than the observed autumn applied urine. The role of hippuric acid in nitrification inhibition from urine patches does though require more investigation.

The results of this study thus suggest that the generation of N_2O following the application of dung and urine is strongly influenced by weather conditions in the few weeks post deposition, and that

Table 3
A comparison of EFs between seasons and treatments: Square root transformed and back transformed mean EFs. Means that do not share a letter are significantly different.

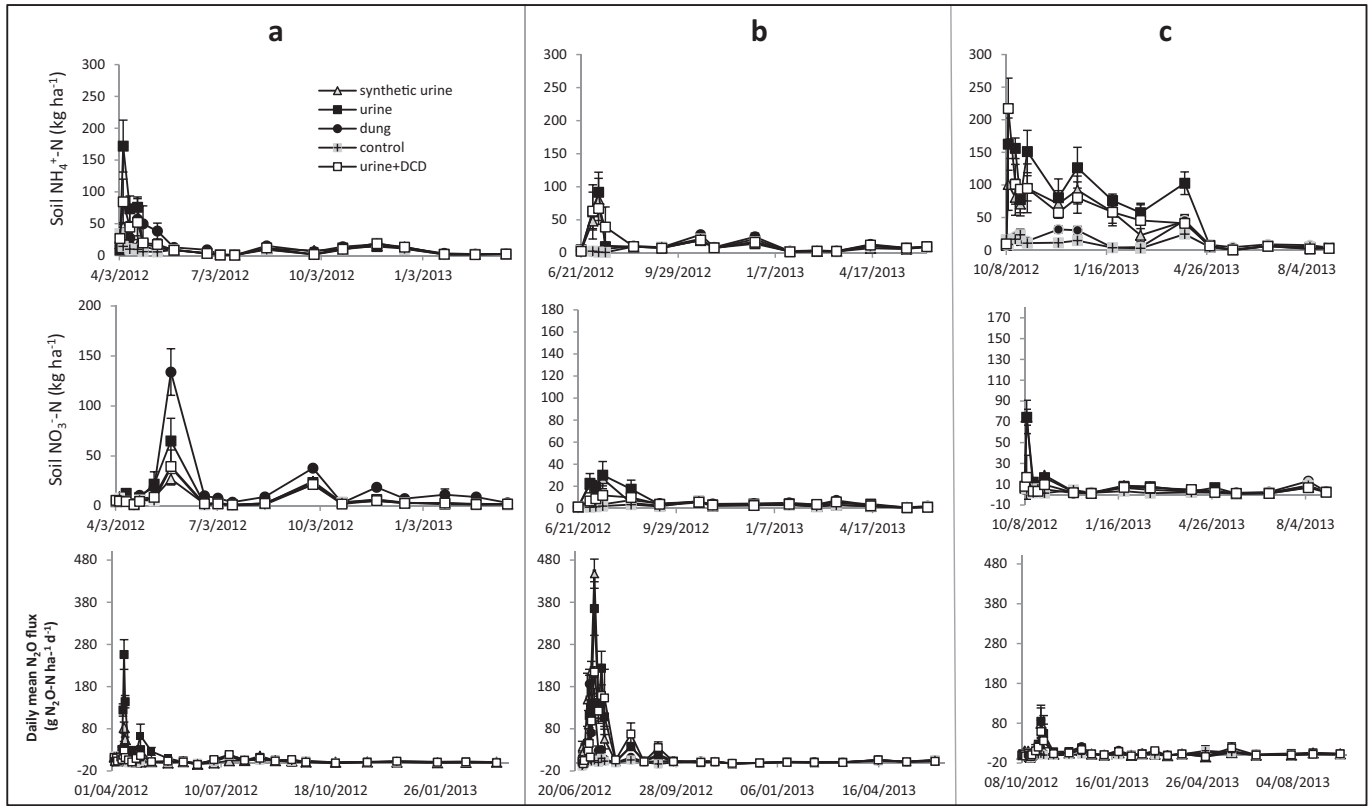|	Square root transformed EF (N_2O-N as a % of N applied)			Back transformed EF (N_2O-N as a % of N applied)		
	Spring	Summer	Autumn	Spring	Summer	Autumn
1. Urine	0.64[b]	1.13[c]	0.72[b]	0.20	1.07	0.31
2. Urine + DCD	0.52[ab]	1.11[c]	0.65[b]	0.06	1.03	0.21
3. A urine	0.37[a]	1.14[c]	0.56[ab]	−0.08	1.10	0.11
4. Dung	0.57[ab]	0.64[b]	0.56[ab]	0.11	0.20	0.10

Fig. 5. Annual variation in soil NH_4^+-N, soil NO_3^--N contents and daily measured N_2O emissions following application of excreta in: a. spring; b. summer; c. autumn. Error bars indicate the standard error of the mean (n = 3).

minimum emissions will occur with lower temperatures and when soil water conditions are too wet for nitrification to take place. Although the highest fluxes were recorded following summer application, it must be recognised that the seasonal cumulative emission and EF estimates calculated in this study were derived from the prevailing weather conditions that occurred during the experiments. It would however be possible to use the data generated by this study and linked to an appropriate spatially explicit model such as DNDC (Giltrap et al., 2008) to estimate regional differences in N_2O emissions linked to grazing returns.

The significantly lower mean annual cumulative flux observed from summer dung than from summer urine applications, despite a higher N application rate (Tables 1 and 2) supports the results found in other studies (e.g. Lessa et al., 2014; van der Weerden et al., 2011). Lessa et al. (2014) found emissions from dung to be 1–2 orders of magnitude less than those from urine, and Dijkstra et al. (2013) found much greater

losses of N_2O from urine than from dung. This has implications when calculating EFs from grazed grasslands, and suggests that estimates should be weighted according to the proportions of dung and urine deposited in excreta. These results support the argument for disaggregation of N_2O EFs for urine and for dung proposed by van der Weerden et al. (2011). The mean dung EF of 0.14 measured in this study corresponds well with those of 0.04 and 0.15 for dung quoted by van der Weerden et al. (2011) and Rochette et al. (2014) respectively, and the mean EF of 0.53 measured for urine corresponds with quoted values of 0.29 (van der Weerden et al., 2011) and 0.31 (Rochette et al., 2014) for urine. This also has implications for any future mitigation strategies to reduce emissions that focus on altering animal diets to alter the proportion of urine and dung N in the total N excreted (e.g. Luo et al., 2013). In addition to disaggregating EFs for dung and urine, comparison of the mean EFs measured in this study with the IPCC default value of 2% for
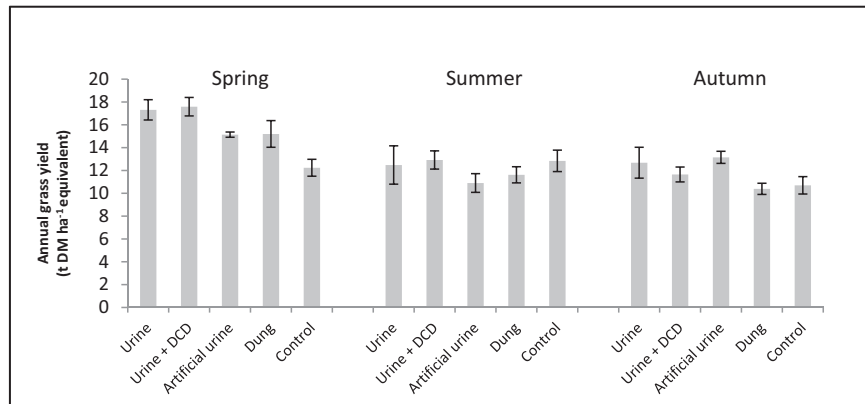

Fig. 6. The variation in mean annual grass yield depending on the season of application and type of excreta applied. Error bars indicate the standard error of the mean (n = 3).

cattle excreta suggests that the IPCC value should be reduced for countries like the UK with temperate climates.

The lack of significantly lower annual cumulative fluxes resulting from the addition of DCD to urine in these three experiments suggests that using DCD as a mitigation method is not consistently effective. Although it was not significant, there was a greater reduction in annual cumulative N_2O emissions following spring application than from summer or autumn applications of urine and DCD (Fig. 3c). Kim et al. (2012) found the DCD half life to vary with season, and observed a linear decrease in half-life with increasing temperature. This was supported by the findings of de Klein et al. (2011), and other reports that DCD has a half life of only 20 days at temperatures of 25 °C, suggesting that the poor performance of DCD as a mitigation option in our summer deposition experiment was to be expected, as temperatures were approaching 20 °C at the time of treatment application (Fig. 2). The short half-life at these high temperatures could be an argument for later application of DCD to grasslands after grazing starts, to correspond with the time of greatest flux, which in all seasonal experiments studied here was between 10 and 21 days post application. This however, would be in contrast to the advice of Kelliher et al. (2007), who call for immediate application post deposition. Another argument in support of DCD application immediately following deposition comes from Kim et al. (2012), who observed interception of DCD by the plant canopy for a period of 16 days, suggesting that if application is delayed then the DCD may not have time to enter the soil before the processes of nitrification and denitrification take place. Zaman and Nguyen (2012) attribute the less effective performance of DCD in their spring urine patch experiments to lower soil temperatures in autumn. This is supported by Kelliher et al. (2007), who argue that DCD will be most effective and last the longest at low soil temperatures.

Soil conditions such as pH, temperature and other environmental variables are known to be important controls on the success and effectiveness of DCD at mitigating N_2O emissions (Li et al., 2014). The organic matter (OM) and clay content of soils is also thought to play a role in its effectiveness, as DCD adsorption to OM (Li et al., 2014) and clay minerals increases the rate of its decomposition, suggesting that the results of this study should not be assumed to be representative of UK grazed grasslands. The location of this study in a cool, wet area in southern Scotland means that soil OM contents are generally higher than at other grazed grassland sites in the UK, and results from other sites in this experimental platform will allow assessment of the effectiveness of DCD in soils lower in OM and higher clay contents. The nonsignificant difference identified in this study resulting from DCD addition to urine was however only relevant when assessing annual cumulative flux. Results in Fig. 2. show that N_2O emissions are reduced when only the period of peak emissions is assessed, and the success of DCD in inhibiting nitrification is displayed further in Fig. 5, supporting the results of Welten et al. (2013), who found DCD to be effective at inhibiting NH_4^+-N conversion to NO_3^--N. This has implications with respect to the length of the experimental period over which we assess such results, and could be the reason for a conflict with results produced from other short-term experiments. A lack of any significant difference in the grass yield produced with the addition of DCD to urine suggests that use of this NI would have neither a positive or negative impact on grass production, and that this cannot be used as an argument to promote its use until further research is undertaken.

No difference in cumulative emissions between real and artificial urine was observed in this study (other than in the spring experiment when artificial urine was applied at a lower N concentration), in contrast to the findings of de Klein et al. (2003) where EFs calculated for artificial urine were lower than for real urine at two of their sites. This conflict in findings suggests that more research is required before we can make firm conclusions on the suitability of artificial urine as a substitute for real urine in future experimentation; however it does suggest that the artificial urine produced and used in this study could be useful for exploring differences between different geographical areas. As the

NH_4^+-N in urine is a source of NH_3 it must be recognised that NH_3 volatilisation from urine patches could be large in magnitude (Laubach et al., 2013), and thus any future experiments would benefit from measurement of these emissions to quantify the true impact of grazing animal excreta on atmospheric quality and climate change.

5. Conclusion

Research presented in this study highlights the variation in N_2O emissions from grazing animal excreta depending on the weather and soil water conditions at the time of deposition. Greatest emissions from summer simulated urine patches in this experiment may be attributable to warmer conditions and a soil moisture status conducive to both nitrification and denitrification. This variation with season implies that use of a universal and constant EF to calculate emissions is inappropriate, and that the extent of emissions can vary greatly depending on the weather conditions at the time of deposition. Unpredictable and changeable weather in the UK suggests that rather than creating seasonal EFs, focus should now be on gaining a better understanding of relationships between climatic and environmental variables and N_2O emissions, and to build these into an appropriate spatially explicit model at a national scale, in order to predict and model N_2O. EFs were significantly higher from urine than dung after application in the summer, suggesting that EFs for cattle excreta should be disaggregated into those from dung and those from urine, with emission calculations then weighted accordingly to the amount of dung and urine deposited. In all seasonal application experiments calculated EFs were well below the IPCC default of 2% for cattle excreta, with the greatest EF from urine of 1.07%, and from dung of 0.2%. Use of the NI DCD had no significant impact on N_2O emissions over an annual period in any experiment, however high temperatures and rainfall following deposition could have caused dislocation of DCD and NH_4^+, and degradation, and more research is required into the use of this NI before firm conclusions can be drawn.

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