

## The contribution of cattle urine and dung to nitrous oxide emissions

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1 **The contribution of cattle urine and dung to nitrous oxide emissions: quantification of**  
2 **country specific emission factors and implications for national inventories.**

3  
4 *Chadwick, D.R.<sup>1\*</sup>, Cardenas, L.M.<sup>2</sup>, Dhanoa, M.S.<sup>2</sup>, Donovan, N.<sup>2</sup>, Misselbrook, T.<sup>2</sup>,*  
5 *Williams, J. R.<sup>3</sup>, Thorman, R.E.<sup>3</sup>, McGeough, K.L.<sup>4</sup>, Watson, C.J.<sup>4</sup>, Bell, M.<sup>5</sup>, Anthony, S.G.<sup>6</sup>,*  
6 *Rees, R.M.<sup>5</sup>.*

7 <sup>1</sup>School of Environment, Natural Resources and Geography, Bangor University, Bangor LL57  
8 2UW, UK

9 <sup>2</sup>Rothamsted Research, North Wyke, Devon, EX20 2SB, UK

10 <sup>3</sup>ADAS Boxworth, Battlegate Rd., Cambridge, CB23 4NN, UK

11 <sup>4</sup>Agri-Food and Biosciences Institute, 18a, Newforge Lane, BT9 5PX, Belfast, UK

12 <sup>5</sup>Scotland's Rural College (SRUC), West Mains Road, Edinburgh, EH9 3JG, UK

13 <sup>6</sup>ADAS Wolverhampton, Titan 1 offices, Coxwell Avenue, Wolverhampton Science Park,  
14 Wolverhampton, WV10 9RT, UK

15  
16 **\*Corresponding author**

17  
18 **Abstract**

19 Urine patches and dung pats from grazing livestock create hotspots for production and  
20 emission of the greenhouse gas, nitrous oxide (N<sub>2</sub>O), and represent a large proportion of total  
21 N<sub>2</sub>O emissions in many national agricultural greenhouse gas inventories. As such, there is  
22 much interest in developing country specific emission factors (EFs) for excretal nitrogen  
23 (EF<sub>3</sub>, pasture, range and paddock) deposited during grazing. The aims of this study were to  
24 generate separate N<sub>2</sub>O emissions data for cattle derived urine and dung, to provide an  
25 evidence base for the generation of a country specific EF for the UK from this nitrogen  
26 source. The experiments were also designed to determine the effects of site and timing of  
27 application on emissions, and the efficacy of the nitrification inhibitor, dicyandiamide (DCD)  
28 on N<sub>2</sub>O losses. This co-ordinated set of 15 plot-scale, year-long field experiments using static  
29 chambers was conducted at five grassland sites, typical of the soil and climatic zones of  
30 grazed grassland in the UK. We show that the average urine and dung N<sub>2</sub>O EFs were 0.69%  
31 and 0.19%, respectively, resulting in a combined excretal N<sub>2</sub>O EF (EF<sub>3</sub>), of 0.49%, which is  
32 <25% of the IPCC default EF<sub>3</sub> for excretal returns from grazing cattle. Regression analysis  
33 suggests that urine N<sub>2</sub>O EFs were controlled more by composition than was the case for  
34 dung, whilst dung N<sub>2</sub>O EFs were more related to soil and environmental factors. The urine  
35 N<sub>2</sub>O EF was significantly greater from the site in SW England, and significantly greater from  
36 the early-season urine application than later applications. DCD reduced the N<sub>2</sub>O EF from  
37 urine patches by an average of 46%. The significantly lower excretal EF<sub>3</sub> than the IPCC  
38 default has implications for the UK's national inventory and for subsequent carbon  
39 footprinting of UK ruminant livestock products.

40  
41 **Keywords**

42 Grassland, greenhouse gas, nitrous oxide, cattle, urine patch, dung pat, nitrification inhibitor,  
43 dicyandiamide, inventory

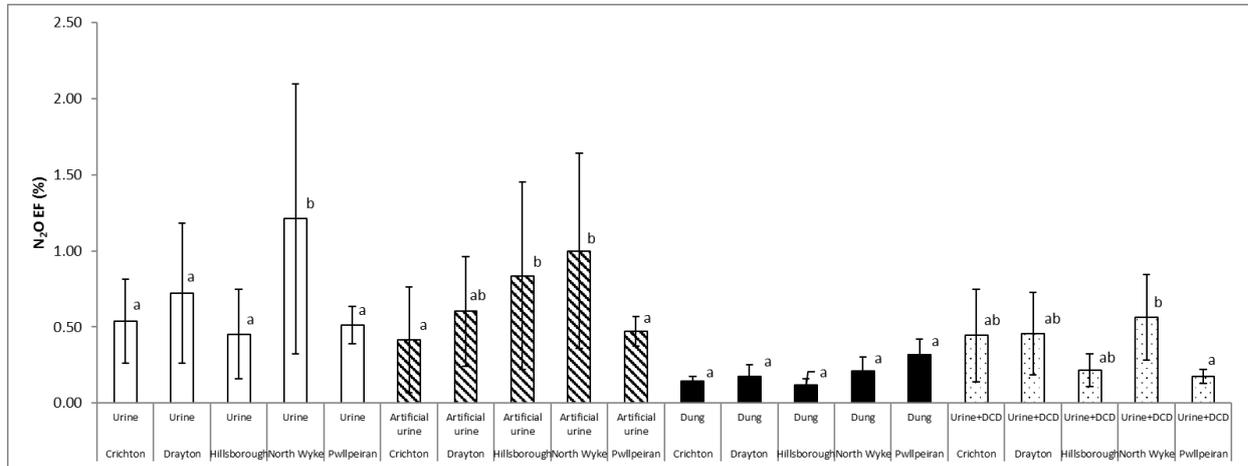
44  
45 **Highlights**

- 46 • First co-ordinated experiments in UK to generate data for country specific grazing  
47 excretal N<sub>2</sub>O EF
- 48 • Urine had a significantly greater average N<sub>2</sub>O EF (0.69%) than dung (0.19%)
- 49 • The combined excretal N<sub>2</sub>O EF was 0.49%, <25% of the IPCC default value for cattle
- 50 • DCD reduced the N<sub>2</sub>O EF from urine patches by an average of 46%

- N<sub>2</sub>O from urine was controlled by its composition, whilst N<sub>2</sub>O from dung was related to soil and environmental factors
- The urine N<sub>2</sub>O EF was significantly greater from the site in SW England and from early season application

56 **Graphical abstract**

57



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60

## 61 1. Introduction

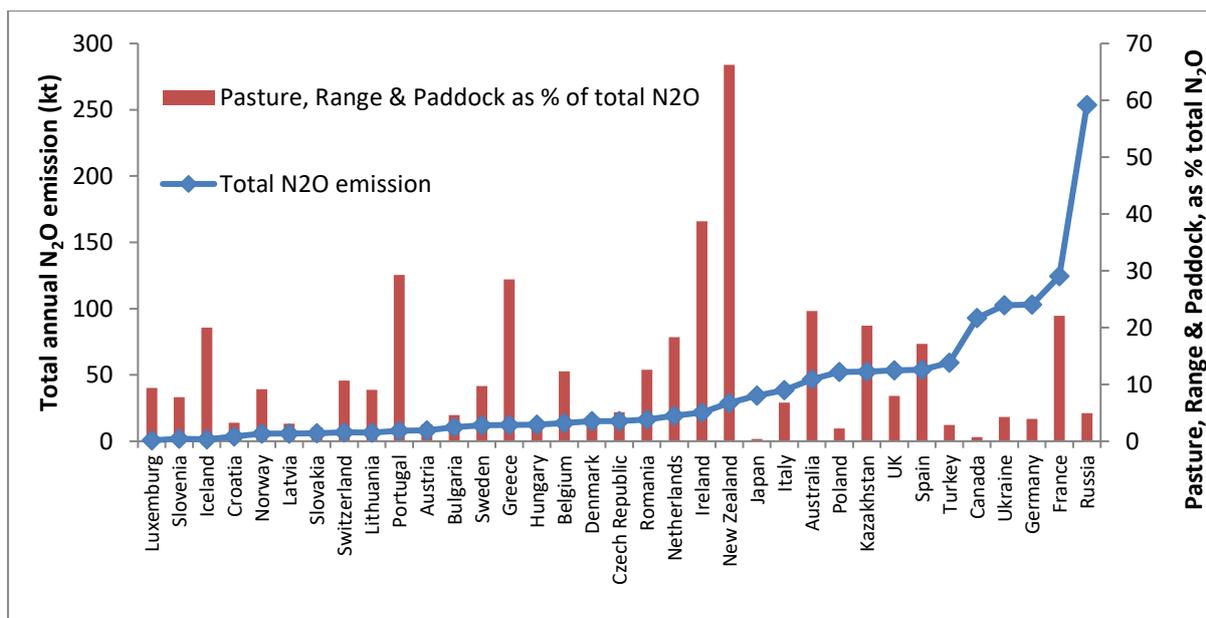
62 Grazed grasslands support a significant proportion of sheep and cattle production throughout  
63 Europe and other parts of the World, converting human-inedible plant biomass into human  
64 edible animal products but with generally low nitrogen (N) use efficiencies. The ruminant  
65 animal converts much of the organic N in plant biomass into highly reactive and bioavailable  
66 N (Nr), particularly as excreted in the urine. It is thought that 3.08 Mt of N is deposited by  
67 grazing livestock in Europe, and this value is thought to be as much as ca. 0.61 Mt N in the  
68 UK (UNFCCC, 2016). It is well documented that urine additions to grassland soils result in  
69 significant quantities of N<sub>2</sub>O production and emission, mainly due to the soil microbial  
70 processes of nitrification and denitrification (Selbie et al., 2015), following the addition of  
71 readily available N and carbon (C), and the effects of significantly increased percentage of  
72 water filled pore space (WFPS) within the urine patch (van der Weerden et al., 2012).

73  
74 Deposition of N in urine patches can represent an equivalent application rate of 200-2000 kg  
75 N ha<sup>-1</sup> (Selbie et al., 2015), depending on the protein content of the sward, livestock type, age  
76 and stage of lactation. A meta-analysis by Selbie et al. (2015) indicates average urine patch N  
77 loading rates for dairy cows and beef cattle of 613 kg N ha<sup>-1</sup> and 345 kg N ha<sup>-1</sup>, respectively.  
78 Clearly, N loading rates in urine patches are in excess of optimal plant use efficiency,  
79 increasing the risk of excess N being lost to the environment via nitrate (NO<sub>3</sub><sup>-</sup>) leaching (de  
80 Klein and Ledgard, 2001; Di and Cameron, 2007), ammonia (NH<sub>3</sub>) volatilisation (Lockyer  
81 and Whitehead, 1990; Laubach et al., 2013; Burchill et al., 2017), N<sub>2</sub>O (Di and Cameron,  
82 2008; Krol et al., 2016; Van der Weerden et al., 2017; Minet et al., 2018) and N<sub>2</sub> (Clough et  
83 al., 1998) emissions. All of these N loss pathways (except N<sub>2</sub>O losses) typically represent a  
84 significant agronomic loss, and all but N<sub>2</sub> loss have detrimental effects on the environment.

85  
86 At these high rates of N loading, the N<sub>2</sub>O emission is likely to be disproportionately greater  
87 than emissions from N sources applied at lower N loading rates, e.g. typical fertiliser N  
88 applications at agronomic rates. A curvilinear response of N<sub>2</sub>O emissions to N loading has  
89 been shown previously, e.g. Cardenas et al (2010) for fertiliser N (NH<sub>4</sub>NO<sub>3</sub>) applications  
90 between 0-375 kg N ha<sup>-1</sup> to grazed swards. Bell et al. (2015) also showed a non-linear  
91 response of N<sub>2</sub>O fluxes to NH<sub>4</sub>NO<sub>3</sub> applications (0-400 kg N ha<sup>-1</sup>) to cut grass. More  
92 specifically for urine applications, de Klein et al (2014) demonstrated greater N<sub>2</sub>O emission  
93 factors (EFs) (0.34%) from urine patches receiving an N loading of 1200 kg ha<sup>-1</sup> compared to  
94 urine patches with a lower N loading (0.10% from a loading of 200 kg ha<sup>-1</sup>) on a freely  
95 draining soil, although a linear relationship between N<sub>2</sub>O EFs and urine N loading was  
96 observed on a poorly drained soil. van Groenigen et al. (2005) found no effect of N loading in  
97 urine patches on the N<sub>2</sub>O EF.

98  
99 For excretion during cattle grazing, the default IPCC N<sub>2</sub>O EF (pasture, range and paddock) is  
100 2% for (combined excretal urine + dung EF) (cf to 1% for fertiliser N), whilst the N<sub>2</sub>O EF for  
101 sheep excretal N during grazing is only 1% (IPCC, 2006). UNFCCC submissions for 2015  
102 from different countries (using IPCC Tier 1 / 2, 2006 Guidelines) show that direct N<sub>2</sub>O  
103 emissions following N deposited to soil by grazing livestock represents from <5% (e.g. in  
104 Japan) to >65% (in New Zealand) of total national direct soil N<sub>2</sub>O emissions (Figure 1), with  
105 greater contributions coming from countries where livestock graze for significant periods of  
106 the year (UNFCCC, 2016). As this source of direct N<sub>2</sub>O emissions is significant to many  
107 national agricultural greenhouse gas inventories, there is increasing interest in developing  
108 country specific EFs that better reflect national soils and climatic conditions (e.g. Krol et al.,  
109 2016 for Ireland).

110



**Figure 1.** Annual (year 2014) agricultural N<sub>2</sub>O emissions and direct N<sub>2</sub>O emissions from excreta deposited by grazing livestock (pasture, range and paddock), expressed as a percentage of the total agricultural N<sub>2</sub>O emission, from different nations (source: UNFCCC, 2016).

Most N<sub>r</sub> excreted during grazing is in the urine, which is mostly comprised of urea that requires hydrolysis to free NH<sub>4</sub><sup>+</sup> (Selbie et al., 2015). In dung, most N is in the organic form, and requires mineralisation over a longer time period to provide a pool of NH<sub>4</sub><sup>+</sup> for nitrification and NO<sub>3</sub><sup>-</sup> for denitrification. The split between urine and dung for total excretal N will depend on dietary protein intake compared with requirement by the animal (as protein intake increases above requirement proportionally more N will be excreted as urine (Broderick et al., 2003; Reed et al., 2016), and partially on the digestibility of the protein in the diet (with a higher proportion of less digestible protein being excreted as faecal N). The UK GHG and ammonia emission inventories to date have assumed 60% of total N excretion by cattle to be as urine and 40% as dung (Webb and Misselbrook, 2004), in common with other Western European countries (Reidy et al., 2008). Disaggregating emissions to urine and dung offers an improved understanding of the sources of N<sub>2</sub>O from grazed pastures, and hence how they could be mitigated.

Since direct N<sub>2</sub>O emissions from grazing livestock represent such a large term in national agricultural greenhouse gas inventories, there has been significant interest in understanding factors that contribute to N<sub>2</sub>O production and emission from this source, e.g. soil type (Clough et al., 1998), urine composition (Kool et al., 2006; Gardiner et al., 2016), weather conditions (Krol et al., 2016), and in exploring strategies to reduce emissions. For example, Monaghan and de Klein (2014) have suggested restricting the duration of autumn and winter grazing to reduce higher N<sub>2</sub>O fluxes associated with urine deposition to wet soils (Qui et al., 2010; Krol et al., 2016). Other studies have explored how manipulating the natural urine composition, e.g. hippuric acid content, can reduce N<sub>2</sub>O production from the urine patch (Clough et al., 2009), and there has been much interest in the use of synthetic nitrification inhibitors to reduce both NO<sub>3</sub><sup>-</sup> leaching and N<sub>2</sub>O emissions from urine patches (Hatch et al., 2005; Di and Cameron, 2012; Barneze et al., 2015). New Zealand and Irish research groups have taken this a step further, in exploring how the nitrification inhibitor DCD can be delivered to urine patches to reduce N<sub>2</sub>O emissions, e.g. through boluses (Ledgard et al.,

145 2008), in drinking water (Welten et al., 2014), and in feed (Luo et al., 2015; Minet et al.,  
146 2016, 2018). However, recent publicity and research has demonstrated that there are potential  
147 unintended consequences of using nitrification inhibitors, such as contamination of milk  
148 products, e.g. via root or foliar uptake (Marsden et al., 2015; Pal et al., 2016) and increased  
149 ammonia emissions (Lam et al., 2016), so researchers are exploring new inhibitor products,  
150 including biological nitrification inhibitory compounds targeted at ruminant production  
151 (Gardiner et al., 2016) that may be deemed more acceptable to the public in the future.

152

153 The UK greenhouse gas R&D community undertook a large number of field trials to quantify  
154 N<sub>2</sub>O EFs from a range of different N sources (viz, different fertiliser N forms, different  
155 manure types, and urine and dung deposited by grazing livestock (Chadwick et al., 2011), as  
156 part of a larger programme to improve the reporting tool for the national inventory of  
157 agricultural greenhouse gas emissions that better represents the soils, climate and N  
158 management in the UK. In this paper we summarise the results of the first co-ordinated set of  
159 plot-based experiments aimed at generating new N<sub>2</sub>O emissions data for disaggregated urine  
160 and dung deposition to soil, from which country specific N<sub>2</sub>O EFs can be derived that are  
161 relevant to UK soils and climate. Some of the individual site experimental results can be  
162 found in Bell et al. (2015) and Cardenas et al. (2016). In the experiments, we tested whether  
163 season of urine and dung deposition (early grazing, mid grazing, later grazing period)  
164 influenced the N<sub>2</sub>O EF. We also tested the efficacy of a nitrification inhibitor, dicyandiamide  
165 (DCD), to reduce N<sub>2</sub>O emissions. An additional reference treatment was included in each  
166 experiment, a standardised artificial urine treatment, with the aim of using the information  
167 from this treatment to help disentangle the effects of urine composition from soil and climate  
168 effects on N<sub>2</sub>O EFs.

169

170 The specific aims of this study were to: i) determine separate direct N<sub>2</sub>O EFs for cattle urine  
171 and dung, ii) determine if season of urine and dung deposition affected the direct N<sub>2</sub>O  
172 emission, iii) assess the effects of site on direct N<sub>2</sub>O emissions from urine, iv) evaluate the  
173 efficacy of the nitrification inhibitor, DCD, to reduce direct N<sub>2</sub>O emissions from urine, and v)  
174 assess the influence of using the combined experimentally derived urine and dung N<sub>2</sub>O EF on  
175 national N<sub>2</sub>O emissions.

176

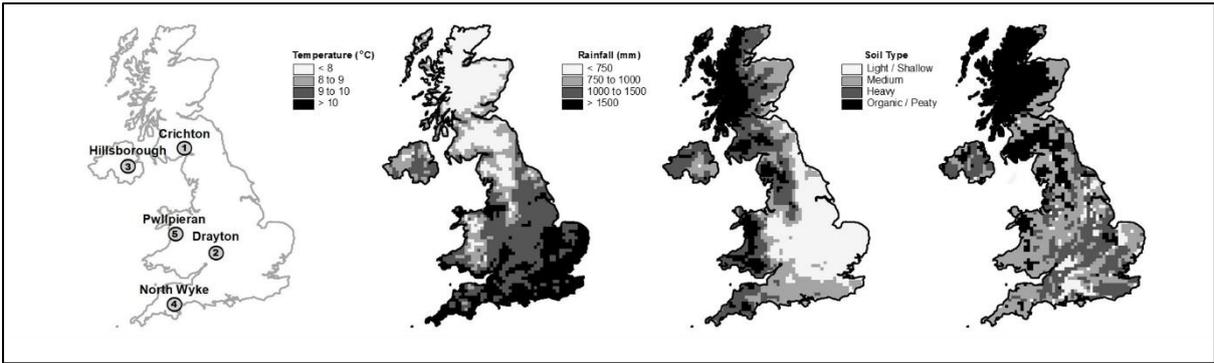
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## 178 **2. Materials and Methods**

### 179 **2.1 Site selection**

180 Five experimental sites were selected to cover the range of typical grassland soils and climate  
181 throughout the UK, with two sites in England, one in Scotland, one in Wales and one in  
182 Northern Ireland (see locations in Figure 2). Descriptions of the sites are shown in Table 1.  
183 There have been so few previous studies in the UK where N<sub>2</sub>O EFs have been quantified  
184 from urine and dung deposition that are IPCC compliant (IPCC, 2000; 2006) (i.e. where  
185 emission measurements were also made from control plots, and where measurements lasted  
186 for up to 365 days), that these sites needed to provide an appropriate range of soil texture and  
187 climate. However, some practicality was also considered in site selection; location could not  
188 be excessively far from a research base to ensure timely measurements, since >30  
189 measurement occasions were needed during each 12 month experimental period. Four  
190 measurement teams, from different UK organisations, ADAS, AFBI, Rothamsted Research -  
191 North Wyke and SRUC, conducted the 15 experiments, following an agreed joint  
192 experimental protocol to ensure aspects of the urine and dung management, chamber  
193 deployment, and ancillary measurements were made in a similar way.

194 Experiments were conducted on established grasslands where the dominant pasture plant was  
195 *Lolium perenne*, which is typical of UK livestock systems (Figure 2). Each experiment  
196 comprised three replicate blocks with five treatments, so a total of 15 plots were sampled on  
197 every occasion. There were 5 urine patches or 5 dung pats per plot (to account for variability  
198 in soil conditions) with one chamber per patch/pat, hence 45 chambers per experiment. There  
199 were also control plots that received no treatment application. Applications were made in the  
200 spring, summer and autumn (to separate plots), to simulate excretal deposition in early-, mid-  
201 and late- grazing season. Livestock were excluded from grazing the experimental areas at  
202 least 6 months prior to the start of any experiment. This minimised any direct effect of  
203 previous urine patches on N<sub>2</sub>O emissions.  
204



205

206 **Figure 2.** Site location, climate average (1981 to 2010) rainfall and temperature, and  
207 distribution of dominant soil types.

208

209

210

Site	Country	Altitude (m)	30 yr average annual rainfall (mm)	30 yr average annual air temperature (°C)	Clay content (%)	Soil pH	Organic matter content (%)	Bulk density (g cm <sup>3</sup> )
Crichton	Scotland	50	1140	9.1	13	5.6		1.07
Drayton	England	47	628	10.3	59	7.6	4.84	0.90
Hillsborough	Northern Ireland	128	908	9.0	23	6.0	9.82	0.90
North Wyke	England	185	1042	10.0	37	5.7	5.40	0.62
Pwllpeiran	Wales	213	1570	10.0	29	5.5	5.40	0.92

211 **Table 1.** Site and soil characteristics. Soil parameters for the 0-10cm layer.

212 2.2 Urine and dung provision

213 The experimental design resulted in the need for ca. 200 litres of fresh cattle urine and ca.  
 214 300 kg dung for each experiment. Urine and dung were collected from the institutions  
 215 summarised in Table 2 within 7 days of an experiment starting, and stored in sealed  
 216 containers (un-acidified) at <4°C. Table 2 summarises the origin of the urine and dung used  
 217 in each experiment.

218

	Cattle type	Age and approx. live weight	Diet
Crichton	Lactating dairy cows	3-7 years old (ca 600 kg)	Grass silage + concentrates (6.5 kg DM head <sup>-1</sup> day <sup>-1</sup> )
Drayton	Lactating dairy cows	6 years old (ca. 600 kg)	Concentrate blend, hay, straw, grass silage, maize silage
Hillsborough	Lactating dairy cows	3-5 years old (ca. 600 kg)	Grass silage + concentrates (4 kg DM head <sup>-1</sup> day <sup>-1</sup> )
North Wyke	Lactating dairy cows	6 years old (ca. 600 kg)	Concentrate blend, hay, straw, grass silage, maize silage
Pwllpeiran	Lactating dairy cows	6 years old (ca. 600 kg)	Concentrate blend, hay, straw, grass silage, maize silage

219 **Table 2.** Sources of urine and dung for the experiments.

220

221 2.3 Treatments

222 Urine and dung were removed from cold storage at least 12 hours before application to the  
 223 soil, to allow them to attain ambient temperature prior to application to the soil. Urine and  
 224 dung were applied at typical N loading rates and volumes. The volumetric loading rate was  
 225 based on a typical 1.8 litres per urination event, (Misselbrook et al., 2016). Since the N  
 226 content of the collected urine varied between feeding trials, the N loading rate varied between  
 227 an equivalent rate of 340 and 570 kg ha<sup>-1</sup>, with an average loading rate of 455 kg N ha<sup>-1</sup> (see  
 228 Table 4a). Dung was applied at an average loading rate of 835 kg N ha<sup>-1</sup> (range 625 – 1020  
 229 kg N ha<sup>-1</sup>; Table 4b). Since urine composition could not be controlled between experiments, a  
 230 standard artificial urine treatment was included at each site as a reference treatment. This was  
 231 to allow the effects of soil and climate to be determined. The artificial urine recipe of Kool et  
 232 al. (2006) was used in all experiments.

233

234 A urine treatment containing DCD was added, with DCD applied at a rate of 10 kg ha<sup>-1</sup>  
 235 equivalent (supplying 6.5 kg N ha<sup>-1</sup> equivalent), and was mixed with urine (only) just before  
 236 application, to maximise initial co-location of DCD and NH<sub>4</sub><sup>+</sup> in the soil profile. This  
 237 approach also simulated the effect of delivering DCD via boluses (Ledgard, 2008), feed (Luo  
 238 et al., 2015; Minet et al., 2016, 2018) and via water troughs (Welten et al., 2014). The  
 239 following treatments were established:

240

- 241 • Urine (target 500 kg N ha<sup>-1</sup>)
- 242 • Urine + DCD (target 500 kg N ha<sup>-1</sup> + 6.5 kg N ha<sup>-1</sup> in DCD)
- 243 • Artificial urine (500 kg N ha<sup>-1</sup>; Kool et al., 2006 recipe)
- 244 • Dung (target 800 kg N ha<sup>-1</sup>)
- 245 • Control (no additions)

246

247 Tables 4a and 4b shows application rates for urine and dung at each site.

248

249

## 250 2.4 Treatment applications

251 Urine treatments were applied to an area of 0.6 m x 0.6 m within a frame to facilitate  
252 infiltration (rather than runoff) using a watering can. After application, static chambers were  
253 inserted centrally into this area. Dung pats were spread to cover the entire area within the  
254 chamber. Five chambers were set up for each treatment plot, and three replicate plots per  
255 treatment were arranged in three blocks. We recognise that urine and dung patches are not  
256 normally this large, and have 'edges', but this method of application was deemed the most  
257 appropriate to simulate the urine patch and dung pat. It is possible that by applying the N  
258 source across the whole area of the chamber that N<sub>2</sub>O production and emission may have  
259 been affected, but there is no evidence to suggest that this would result in either an under- or  
260 over-estimate of the true emission (Marsden et al., 2016). In addition to the urine and dung  
261 patches that were established for the N<sub>2</sub>O chamber measurements, larger areas of grassland (2  
262 m x 2 m) on each plot were treated with either urine or dung at the same rate, allowing  
263 multiple soil sampling occasions for soil NO<sub>3</sub><sup>-</sup>, soil NH<sub>4</sub><sup>+</sup> and soil moisture.

264

## 265 2.5 Nitrous oxide measurements

266 We used the non-steady state static chamber approach to measure N<sub>2</sub>O fluxes (Cardenas et  
267 al., 2016). The shape and size of the chambers were 0.4 m x 0.4 m x 0.25 m (high) for the  
268 ADAS, North Wyke and AFBI experiments, and 0.4 m diameter x 0.3 m (high) for the SRUC  
269 experiments, with individual chamber areas of 0.16 and 0.13 m<sup>2</sup>, respectively. Chambers  
270 were opaque. Chamber headspace sampling followed the protocol detailed in Chadwick et al.  
271 (2014), whereby chambers were closed for a period of 40 minutes and a headspace sample  
272 taken at this time (T<sub>40</sub>). Ten ambient air samples (5 at the start and 5 at the end of the  
273 chamber closure period) were used to provide the T<sub>0</sub> concentration. Gas samples were placed  
274 in pre-evacuated 20 ml vials and transported back to individual laboratories for analysis by  
275 gas chromatography. Five chambers were assigned randomly per plot; these generated one  
276 mean flux per plot. The headspace sampling assumed a linear increase in headspace N<sub>2</sub>O  
277 concentration (as evidenced by previous research; Chadwick et al., 2014). This linear  
278 response was checked on each sampling occasion by measuring the headspace concentration  
279 at 10 minute intervals up to 60 minutes after closure, from one chamber per block.

280

281 Sampling frequency was 4-5 times in the first week after treatment application, 4-5 times in  
282 the second week, 2 times per week for the next two weeks, then once per week for 1 month.  
283 Sampling frequency was then reduced further, eventually to once per month until the end of  
284 the experiment (12 months), resulting in ca. 30 samples over the 12 month period following  
285 application in order to comply with IPCC recommendations (IPPC, 1996).

286

## 287 2.6 Other measurements

### 288 2.6.1 Dung and Urine Composition

289 Dung and urine sub-samples were taken on the day of application and characterised by  
290 measuring pH, dry matter (DM), total N (by Kjeldahl) and total organic carbon content, either  
291 using a modified Walkley-Black approach, or analysis by a TOC analyser (uv persulphate  
292 oxidation). The readily available N content was also determined, i.e. ammonium N (NH<sub>4</sub><sup>+</sup>-N)  
293 and nitrate N (NO<sub>3</sub><sup>-</sup>-N). In addition, two 30 ml sub-samples of urine were taken from each  
294 block and preserved by diluting 1:3 with HPLC grade deionised water. The first sample was  
295 acidified by adding 1M H<sub>2</sub>SO<sub>4</sub> to reduce the pH to 3 (using a pH meter). To the second  
296 sample, 100 µl chloroform was added. Both sub-samples were stored at -20°C before analysis  
297 for urea, hippuric acid, allantoin, uric acid and creatinine, by HPLC (using methods described  
298 in Kool et al., 2006).

299

300 2.6.2 Soil Mineral N and Moisture Determination

301 Soil  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N: Soil samples (0-10 cm) were taken from the dedicated sampling  
302 areas of each plot on 10-12 occasions during the 12 month experiment. Soil was passed  
303 through a 5 mm sieve before extracting with 2M KCl and filtering. Filtrates were frozen prior  
304 to analysis for  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N concentrations using segmented flow analysers.

305

306 2.6.3 Soil moisture content: Soil samples (0-10 cm) were taken from the dedicated areas of  
307 each plot on every occasion an  $\text{N}_2\text{O}$  measurement was made. Soils were weighed (fresh  
308 weight) before oven drying at  $105^\circ\text{C}$  overnight, and then reweighed. Soil moisture content  
309 was converted to %WFPS using the bulk density of the site (see below) and a particle size  
310 density of  $2.65 \text{ g cm}^3$ .

311

312 2.6.4 Bulk density

313 Three representative bulk density measurements were made per site, one per block (walking  
314 and sampling a 'W' route across each block), at the start of the experiment, using  $100 \text{ cm}^3$   
315 bulk density rings, and drying at  $105^\circ\text{C}$  overnight.

316

317 2.6.5 Weather data

318 Daily rainfall and hourly air and soil (0-5 cm) temperature were recorded on site, or daily  
319 data used from a nearby weather station (within 1 km) (Table 3).

320

321

322

Site	Grazing season period	Measurement period	Whole measurement period		Initial 30d after application			Day of application		WFPS (%)
			Total rainfall (mm)	Average temperature (°C)	Total rainfall (mm)	Average temperature (°C)	Average WFPS (%)	Daily rainfall (mm)	Average daily temperature (°C)	
Crichton	Early	03/04/12 - 18/03/13	1325	8.6	60	7.5	51.9	0.1	5.3	41.4
	Mid	27/06/12- 10/06/13	1262	8.7	125	14.7	58.3	2.3	12.9	54.8
	Late	08/10/12 - 25/09/13	1142	9.1	137	7.6	69.8	0.0	8.4	64.6
Drayton	Early	02/05/13- 17/04/14	726	10.7	78	10.9	40.1	0.0	10.0	43.6
	Mid	15/08/13- 29/07/14	769	9.4	50	15.8	31.0	1.4	20.5	27.5
	Late	17/10/13- 14/10/14	787	8.0	123	9.0	45.2	0.0	12.5	42.5
Hillsborough	Early	2/04/12 – 1/04/13	1191	8.0	77	6.3	84.1	1.4	8.3	88.5
	Mid	25/06/12 – 24/06/13	1110	8.2	102	14.0	89.3	0.0	12.1	83.1
	Late	17/09/12 – 16/09/13	1080	8.2	135	8.8	89.9	0.8	10.9	89.7
North Wyke	Early	15/05/12 – 09/05/13	1405	9.6	143	13.9	57.5	0.6	7.4	58.3
	Mid	03/07/12 – 11/06/13	1246	9.4	103	13.6	62.0	7.6	15.4	62.4
	Late	26/09/12 – 10/09/13	1288	9.5	160	9.1	63.1	11.1	12.4	71.6
Pwllpeiran	Early	11/04/13- 27/03/14	1878	10.4	96	8.4	44.8	5.7	7.8	44.1
	Mid	04/07/13- 16/06/14	1844	10.8	61	17.2	36.7	0.0	14.4	44.1
	Late	12/09/13- 27/08/14	1841	10.6	98	13.6	44.8	13.6	14.4	42.2

324 **Table 3.** Weather and soil data for the different urine and dung applications.  
325 Archived data sources: Bell et al (2017); Cardenas et al (2017); McGeough et al (2017); Thorman et al (2017a, 2017b)  
326

## 327 2.7 Data processing and Statistics

328 The N<sub>2</sub>O flux for each chamber was calculated by entering data for the sample vials N<sub>2</sub>O  
329 concentration, air temperature, closure period and chamber heights into a standard  
330 spreadsheet used by all project partners. The mean of the 5 chambers per plot was calculated  
331 and used for subsequent calculations of cumulative emissions, using the trapezoidal rule  
332 (Cardenas et al. 2010). EFs were calculated by subtracting cumulative N<sub>2</sub>O emissions from  
333 control plots from treatment plots in the same block. For the urine treatment with DCD the N  
334 content in the DCD was taken into account for the calculation of the EF. EFs uniformity of  
335 distribution were checked and, if necessary, Box Cox transformation was used on all N<sub>2</sub>O  
336 data to normalise distribution. Statistical analyses were designed to test:

- 337 i) the effect of geographical site on N<sub>2</sub>O EFs for the different treatments
- 338 ii) the effect of season of application on N<sub>2</sub>O EFs for different treatments
- 339 iii) the difference between urine and dung N<sub>2</sub>O EFs
- 340 iv) the effect of DCD in reducing N<sub>2</sub>O EFs from urine application

341  
342 Treatment effects and their interactions were evaluated using the F-test in analysis of  
343 variance (ANOVA) of each site according to the randomised block design. Multiple  
344 comparison of treatment means, if significant, were tested using the Tukey method (Hsu,  
345 1996). When ‘treatment x season’ interaction was significant then treatments were compared  
346 within each season, and seasons were compared with each treatment. In addition, all five sites  
347 were combined using REML Meta-analysis in Genstat (VSN International, 2015) to test site  
348 effects.  
349

350  
351 Multiple regression analysis (forward selection procedure in Genstat) was used to explore the  
352 key soil (% clay, pH, initial % WFPS, average WFPS for first 30 days), environment  
353 (average temperature for the first 30 days, average temperature for 365 days after application,  
354 total rainfall for the first 30 days, total rainfall for 365 days after application) and urine/dung  
355 composition (total urine/dung N content, total urine urea content, total urine/dung ammonium  
356 content, uric acid content, hippuric acid content, allantoin content, creatinine content, N  
357 application rate) factors that controlled the cumulative N<sub>2</sub>O fluxes and N<sub>2</sub>O EFs. The main  
358 effects of up to (maximum) 10 terms was estimated. No interaction terms were included for  
359 selection. In developing a multiple regression model, correlation among the predictor factors  
360 (known as multicollinearity) can affect model equation stability. For this modelling exercise  
361 we used the statistical package Genstat (Genstat 18th Ed.; VSN International, 2015), which  
362 has the built in facility to check for any multicollinearity issues (any such problem can be  
363 dealt with by using Genstat Procedure ‘Ridge’ regression which incorporates Principal  
364 Component (PCA) regression.  
365

## 366 3. Results

### 367 3.1 Urine and Dung composition

368  
369 The N content of the urine used in the 15 experiments (Table 4a) were typical for cattle urine  
370 (Dijkstra et al., 2013; Selbie et al., 2015; Gardiner et al., 2016), ranging from 6.8 to 11.4 g l<sup>-1</sup>  
371 (average 9.11 ± 0.35). In most cases urea-N represented between 60-100% of the total N  
372 content. However, for the three experiments at Hillsborough, the low urea-N content of the  
373 urine was linked to a high urine ammonium-N content (Table 4a), indicating hydrolysis of  
374 urea prior to application to the soil. Since urea hydrolysis is such a rapid process once urine  
375

376 has been deposited on the soil, we do not consider the N<sub>2</sub>O emissions from the three  
377 Hillsborough experiments to have been directly affected by this.

378

Site	Grazing season period	Urine							Artificial urine						
		Total N loading (kg ha <sup>-1</sup> )	pH	DM (%)	Total N (g l <sup>-1</sup> )	Urea-N (mg l <sup>-1</sup> )	NH <sub>4</sub> -N (mg l <sup>-1</sup> )	NO <sub>3</sub> -N (mg l <sup>-1</sup> )	Total N loading (kg ha <sup>-1</sup> )	pH	DM (%)	Total N (g l <sup>-1</sup> )	Urea-N (mg l <sup>-1</sup> )	NH <sub>4</sub> -N (mg l <sup>-1</sup> )	NO <sub>3</sub> -N (mg l <sup>-1</sup> )
Crichton	Early	480	-	4.9	9.60	6332	120	-	180	-	1.4	3.60	1318	-	-
	Mid	420	-	4.6	8.40	8127	240	-	425	-	3.5	8.50	9264	-	-
	Late	435	-	4.9	8.70	6231	100	-	425	-	3.5	8.50	9264	-	-
Drayton	Early	540	7.5	5.5	10.80	10780	825	0.5	501	7.1	4.6	10.01	9820	25	0.5
	Mid	454	9.0	4.5	9.07	8540	4870	1.5	495	7.3	3.9	9.91	8340	39	0.0
	Late	471	8.1	5.2	9.43	8480	315	0.0	495	7.1	4.7	9.90	10040	25	0.0
Hillsborough	Early	432	9.0	5.5	8.64	2900	6917	0.0	510	7.7	4.6	10.20	7335	100	115.0
	Mid	338	8.9	5.3	6.75	375	5862	27.0	502	7.6	5.0	10.04	8035	55	126.0
	Late	354	9.0	4.2	7.07	767	6216	41.0	504	8.2	4.5	10.08	8048	88	163.0
North Wyke	Early	405	8.3	5.3	8.10	6521	554	0.0	440	8.2	4.3	8.80	7079	18	0.1
	Mid	429	7.3	4.8	8.57	6284	1230	1.0	481	7.5	4.2	9.61	6833	<50	0.4
	Late	435	9.2	4.5	8.70	7382	2020	2.5	423	7.4	3.4	8.45	7774	<50	0.8
Pwllpeiran	Early	565	9.3	5.6	11.30	10100	2743	0.3	495	9.3	4.4	9.91	9620	315	0.3
	Mid	568	7.8	5.5	11.37	6840	822	0.3	498	7.4	3.9	9.96	7840	25	0.2
	Late	505	7.8	4.7	10.10	8820	115	0.3	508	7.5	4.1	10.15	10040	25	0.0

379

**Table 4a.** Average urine composition, and N and C loading rates for each experiment.

380 For urine + DCD treatments, an additional 6.5 kg N ha<sup>-1</sup> was supplied in the inhibitor. DM = dry matter.

381 Archived data sources: Bell et al (2017); Cardenas et al (2017); McGeough et al (2017); Thorman et al (2017a, 2017b)

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387

	Grazing season period	Total N loading (kg ha <sup>-1</sup> )	pH	DM (%)	Total N (g kg <sup>-1</sup> DM)	NH <sub>4</sub> -N (mg kg <sup>-1</sup> DM)	NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> DM)
Crichton	Early	1020	-	12.9	5.10	410	0.0
	Mid	680	-	11.5	3.40	260	0.0
	Late	720	-	10.6	3.60	230	0.0
Drayton	Early	840	7.6	18.9	22.2	5020	24.5
	Mid	736	7.6	36.2	10.2	3680	6.9
	Late	802	7.8	27.0	14.8	4443	0.0
Hillsborough	Early	980	6.9	14.5	4.90	500	0.0
	Mid	976	7.3	14.3	4.90	669	0.0
	Late	1008	7.7	14.3	5.00	683	0.0
North Wyke	Early	911	7.0	14.5	31.4	3035	0.1
	Mid	625	7.4	21.1	48.0	4310	21.6
	Late	771	7.5	20.5	18.8	2940	20.8
Pwllpeiran	Early	769	7.5	24.5	15.7	5095	25.2
	Mid	823	7.3	23.3	17.7	6497	7.9
	Late	866	7.5	24.1	18.0	5833	3.5

389 **Table 4b.** Average dung composition, and N and C loading rates for each experiment.

390 DM = dry matter.

391 Archived data sources: Bell et al (2017); Cardenas et al (2017); McGeough et al (2017);

392 Thorman et al (2017a, 2017b)

393

394 Concentrations of the purine derivatives in the urine varied markedly between the different

395 seasons of collection for the different experiments at each site, and between sites (Table 5).

Site	Grazing season period	Hippuric acid	Allantoin	Uric acid	Creatinine
Crichton	Early	9.17	2.42	0.30	0.68
	Mid	1.57	1.70	0.45	1.29
	Late	7.69	3.89	0.41	0.77
Drayton	Early	<0.50*	2.83	0.48	0.58
	Mid	<0.50*	<0.40*	0.55	0.62
	Late	8.02	3.51	0.54	0.68
Hillsborough	Early	<0.50*	0.74	0.15	0.40
	Mid	<0.50*	<0.40*	0.06	<0.10*
	Late	<0.50*	<0.40*	0.12	<0.10*
North Wyke	Early	3.92	1.91	0.37	0.76
	Mid	<0.50*	<0.40*	0.40	0.52
	Late	4.86	<0.40*	0.35	0.52
Pwllpeiran	Early	5.13	0.84	0.36	0.81
	Mid	<0.50*	<0.40*	0.31	0.25
	Late	8.92	3.67	0.03	0.73

396 **Table 5.** Concentrations (g l<sup>-1</sup>) of purine derivatives in cattle urine used in the experiments.

397 \*detection limit of the analytical approach.

398 To convert from mg molecule l<sup>-1</sup> to mg N l<sup>-1</sup>, multiply hippuric acid by 0.078138, allantoin by  
399 0.354161, uric acid by 0.333115, and creatinine by 0.371287.  
400 Archived data sources: Bell et al (2017); Cardenas et al (2017); McGeough et al (2017);  
401 Thorman et al (2017a, 2017b).  
402

403 This reflects differences in the diets that cattle were fed prior to collection of the urine on  
404 each occasion (see Table 2 for a summary of the diets), and differences between cattle groups  
405 at each collection site. However, concentrations are typical of those reported in the literature  
406 (Dijkstra et al., 2013; Selbie et al. 2015; Gardiner et al., 2016). The measured N contained in  
407 the purine derivatives represented from 3-28% of the total N content of the urine (average  
408 12.5% ± 0.02).  
409

410 The total N content of the dung ranged from 3.4 to 48.0 g kg<sup>-1</sup> (DM), whilst the DM content  
411 ranged from 10.6-36.2% (Table 4b). The total N loadings in the urine and dung treatments  
412 were typical for cattle, 338-568 kg ha<sup>-1</sup> (average 455 ± 17.6) and 625-1020 kg ha<sup>-1</sup> (average  
413 835 ± 31.9), respectively. These values are within reported ranges (Selbie et al., 2015).  
414

### 415 3.2 Weather

416 Annual rainfall was greater than the 30-year mean in two (of the three) Crichton experiments,  
417 and all three experiments at Drayton, Hillsborough, North Wyke and Pwllpeiran. Average  
418 annual air temperature was similar to the 30-year mean at Crichton and Pwllpeiran, cooler at  
419 Hillsborough and North Wyke, and warmer at Drayton. However, it is more likely that the  
420 weather conditions immediately before urine and dung application, and within the first three  
421 months after application would have the most influence on N<sub>2</sub>O production and emission (see  
422 Table 3).  
423

### 424 3.3 Nitrous oxide emissions

#### 425 3.3.1 Controls

426 Background (control) cumulative N<sub>2</sub>O emissions ranged from -0.03 – 1.26 kg N<sub>2</sub>O-N ha<sup>-1</sup> for  
427 all sites and all experiments, with an average from the data in Table 6 of 0.49 kg N<sub>2</sub>O-N ha<sup>-1</sup>  
428 (± 0.10). From the meta-analysis, we find that across all seasons, the N<sub>2</sub>O emissions from the  
429 controls were significantly greater from the Crichton, North Wyke and Pwllpeiran sites  
430 compared to the Drayton site (p<0.05). Within an individual site, emissions from controls  
431 also varied between seasons of application, particularly at the North Wyke site. There was no  
432 statistically significant relationship between the urine N<sub>2</sub>O EF and the cumulative annual N<sub>2</sub>O  
433 emission from the control plots (p>0.05). Across all sites, N<sub>2</sub>O emissions from the control  
434 plots at the early grazing application timing were significantly greater than from the late-  
435 grazing application (p<0.05). Regression modelling indicated that the key factors controlling  
436 the magnitude of the annual N<sub>2</sub>O fluxes from control plots were soil organic carbon content,  
437 clay content, bulk density, WFPS during the first 30d after application, and average annual  
438 temperature, with these factors accounting for ca. 56% of the variance in emissions. The  
439 resulting full regression equation was: Cumulative N<sub>2</sub>O flux (kg N ha<sup>-1</sup>) = 3.981 - 0.0846  
440 SOC - 0.02220 initial WFPS + 0.01052 x 30d WFPS - 1.683 Bulk density - 0.01807 Clay  
441 content - 0.0408 x 365d average temperature.  
442

443

Site	Grazing season period	Cumulative emissions of N <sub>2</sub> O (kg N <sub>2</sub> O ha <sup>-1</sup> )					N <sub>2</sub> O EF (% of applied N)			
		Control	Urine	Urine +DCD	Artificial Urine	Dung	Urine	Urine +DCD	Artificial Urine	Dung
Crichton	Early	0.96a <i>0.23</i>	1.92a <i>0.20</i>	1.25a <i>0.09</i>	0.93a <i>0.09</i>	2.15a <i>0.51</i>	0.20a <i>0.06</i>	0.06a <i>0.03</i>	-0.02a <i>0.13</i>	0.12a <i>0.03</i>
	Mid	0.61a <i>0.17</i>	5.18b <i>0.82</i>	5.07b <i>1.03</i>	5.29b <i>0.30</i>	2.00a <i>0.09</i>	1.09b <i>0.18</i>	1.05b <i>0.28</i>	1.10b <i>0.06</i>	0.20a <i>0.03</i>
	Late	0.79a <i>0.32</i>	2.21a <i>0.66</i>	1.79a <i>0.24</i>	1.49a <i>0.60</i>	1.55a <i>0.12</i>	0.33a <i>0.15</i>	0.23a <i>0.12</i>	0.16a <i>0.18</i>	0.11a <i>0.03</i>
Drayton	Early	0.18a <i>0.06</i>	2.02a <i>0.11</i>	1.35a <i>0.14</i>	1.86a <i>0.05</i>	0.85a <i>0.17</i>	0.34a <i>0.03</i>	0.21a <i>0.02</i>	0.34a <i>0.01</i>	0.08a <i>0.02</i>
	Mid	0.03a <i>0.09</i>	0.86a <i>0.08</i>	0.74a <i>0.07</i>	0.82a <i>0.08</i>	0.95a <i>0.05</i>	0.18a <i>0.00</i>	0.15a <i>0.00</i>	0.16a <i>0.01</i>	0.12a <i>0.01</i>
	Late	-0.03a <i>0.05</i>	7.68d <i>1.79</i>	4.73bc <i>1.35</i>	6.47cd <i>0.42</i>	2.56b <i>0.35</i>	1.64c <i>0.37</i>	1.00b <i>0.28</i>	1.31bc <i>0.08</i>	0.32a <i>0.04</i>
Hillsborough	Early	0.36a <i>0.10</i>	4.78a <i>1.11</i>	1.46a <i>0.42</i>	10.87b <i>4.84</i>	1.98a <i>0.20</i>	1.02a <i>0.26</i>	0.25a <i>0.12</i>	2.06b <i>0.96</i>	0.17a <i>0.03</i>
	Mid	0.23a <i>0.04</i>	1.20a <i>0.15</i>	1.52a <i>0.44</i>	1.96a <i>0.61</i>	1.73a <i>0.45</i>	0.29a <i>0.05</i>	0.38a <i>0.14</i>	0.34a <i>0.13</i>	0.15a <i>0.04</i>
	Late	0.15a <i>0.05</i>	0.31a <i>0.07</i>	0.20a <i>0.04</i>	0.67a <i>0.11</i>	0.51a <i>0.15</i>	0.05a <i>0.03</i>	0.01a <i>0.00</i>	0.10a <i>0.03</i>	0.04a <i>0.01</i>
North Wyke	Early	1.26a <i>0.13</i>	13.26d <i>0.50</i>	5.54b <i>0.69</i>	11.06c <i>0.43</i>	2.50a <i>0.43</i>	2.96c <i>0.14</i>	1.09b <i>0.20</i>	2.23c <i>0.12</i>	0.14a <i>0.06</i>
	Mid	0.80a <i>0.07</i>	3.19b <i>0.51</i>	2.93b <i>0.50</i>	4.16b <i>0.89</i>	3.24b <i>0.52</i>	0.56b <i>0.11</i>	0.49ab <i>0.10</i>	0.70b <i>0.18</i>	0.39a <i>0.08</i>
	Late	0.03a <i>0.13</i>	0.52a <i>0.29</i>	0.59a <i>0.11</i>	0.34a <i>0.23</i>	0.82a <i>0.17</i>	0.11a <i>0.04</i>	0.12a <i>0.02</i>	0.07a <i>0.02</i>	0.10a <i>0.01</i>
Pwlpeiran	Early	0.49a <i>0.18</i>	3.45c <i>0.34</i>	1.59ab <i>0.31</i>	3.15c <i>0.23</i>	2.17bc <i>0.31</i>	0.52b <i>0.07</i>	0.19a <i>0.07</i>	0.54b <i>0.03</i>	0.22a <i>0.05</i>
	Mid	0.42a <i>0.04</i>	2.11b <i>0.13</i>	0.94ab <i>0.03</i>	1.81b <i>0.15</i>	2.13b <i>0.33</i>	0.30b <i>0.03</i>	0.09a <i>0.01</i>	0.28b <i>0.03</i>	0.21ab <i>0.04</i>
	Late	0.52a <i>0.07</i>	4.14b <i>0.39</i>	1.78a <i>0.13</i>	3.52b <i>0.36</i>	5.08c <i>0.81</i>	0.72c <i>0.09</i>	0.25a <i>0.02</i>	0.59bc <i>0.08</i>	0.53b <i>0.09</i>

445 **Table 6.** Average cumulative N<sub>2</sub>O emissions and N<sub>2</sub>O EFs from the urine and dung  
446 treatments at each experimental site for each application. (Values in italics are standard errors  
447 of the mean).

448 Archived data sources: Bell et al (2017); Cardenas et al (2017); McGeough et al (2017);  
449 Thorman et al (2017a, 2017b)

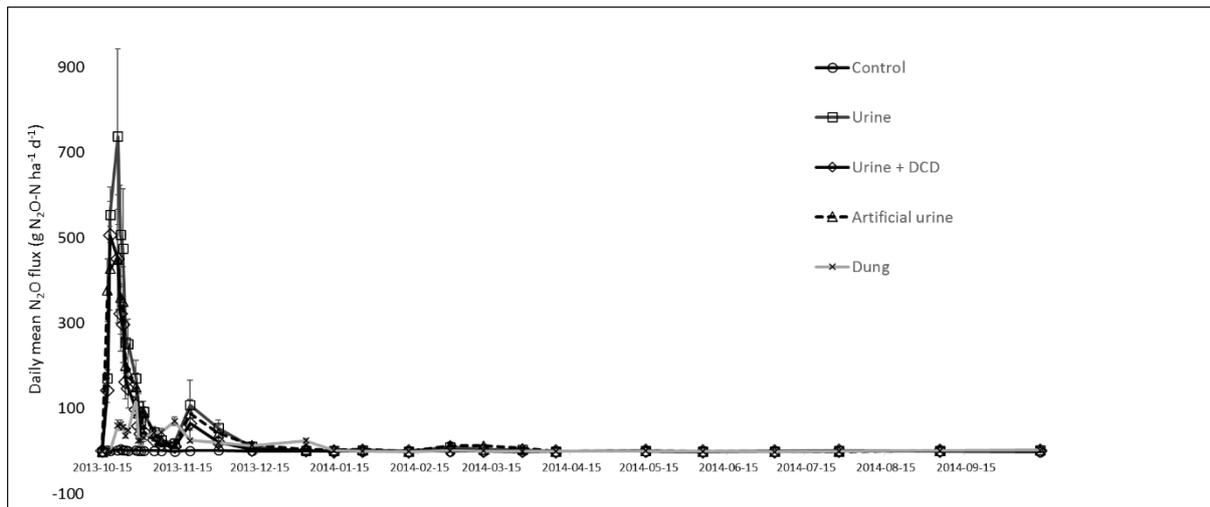
450 Within each site/timing experiment (rows), average total N<sub>2</sub>O emissions or N<sub>2</sub>O EFs between  
451 excretal N sources with different letters are significantly different (p<0.05, N=3).

452

### 453 3.3.2 Urine

454 Examples of daily N<sub>2</sub>O fluxes are shown in Figure 3 for the late-season urine, dung and  
455 control treatments at the Drayton site. These data show two distinct peaks in N<sub>2</sub>O fluxes,  
456 something observed in several of the experiments (e.g. Cardenas et al., 2016), suggesting the  
457 peaks in emission are associated either with different processes (e.g. denitrification of soil  
458 NO<sub>3</sub> during the first peak as a result of the carbon addition in the urine, and nitrification of  
459 the urine NH<sub>4</sub> source during the second peak), or different pools of N being the substrate for  
460 denitrification (e.g. the first peak associated with the urine-derived NH<sub>4</sub>, and the second peak  
461 associated with other more recalcitrant pools, e.g. N contained in purine derivatives). Further  
462 research using labelled urine N compounds would help reveal the underpinning processes  
463 and/or N sources responsible for the two peaks in emission.

464



465 **Figure 3.** Daily mean N<sub>2</sub>O fluxes following urine and dung treatments at Drayton after a late-  
 466 season application.  
 467

468  
 469 The mean urine N<sub>2</sub>O EF was 0.69% ( $\pm 0.20$ ), ranging from 0.05 – 2.96 (Table 6). Across all  
 470 seasons of application, the meta-analysis showed that the N<sub>2</sub>O EF was significantly greater  
 471 from the North Wyke site than other sites ( $p < 0.05$ ) (Figure 4). Whilst across all sites, the N<sub>2</sub>O  
 472 EF was significantly greater following an early-grazing application ( $p < 0.05$ ) (Figure 5). DCD  
 473 reduced the N<sub>2</sub>O EF from urine in 13 of the 15 experiments, although this reduction was only  
 474 significant in 5 of these experiments (Table 6). The average N<sub>2</sub>O EF for the urine + DCD  
 475 treatment was 0.37% ( $\pm 0.09$ ) (Table 6). So, the use of DCD resulted in an average reduction  
 476 in the N<sub>2</sub>O EF of 46%, although the range in efficacy was wide, i.e. from an increase in the  
 477 N<sub>2</sub>O EF of 32% (mid-season application at Hillsborough) to a reduction of 75% (at the same  
 478 site from the early-season application).

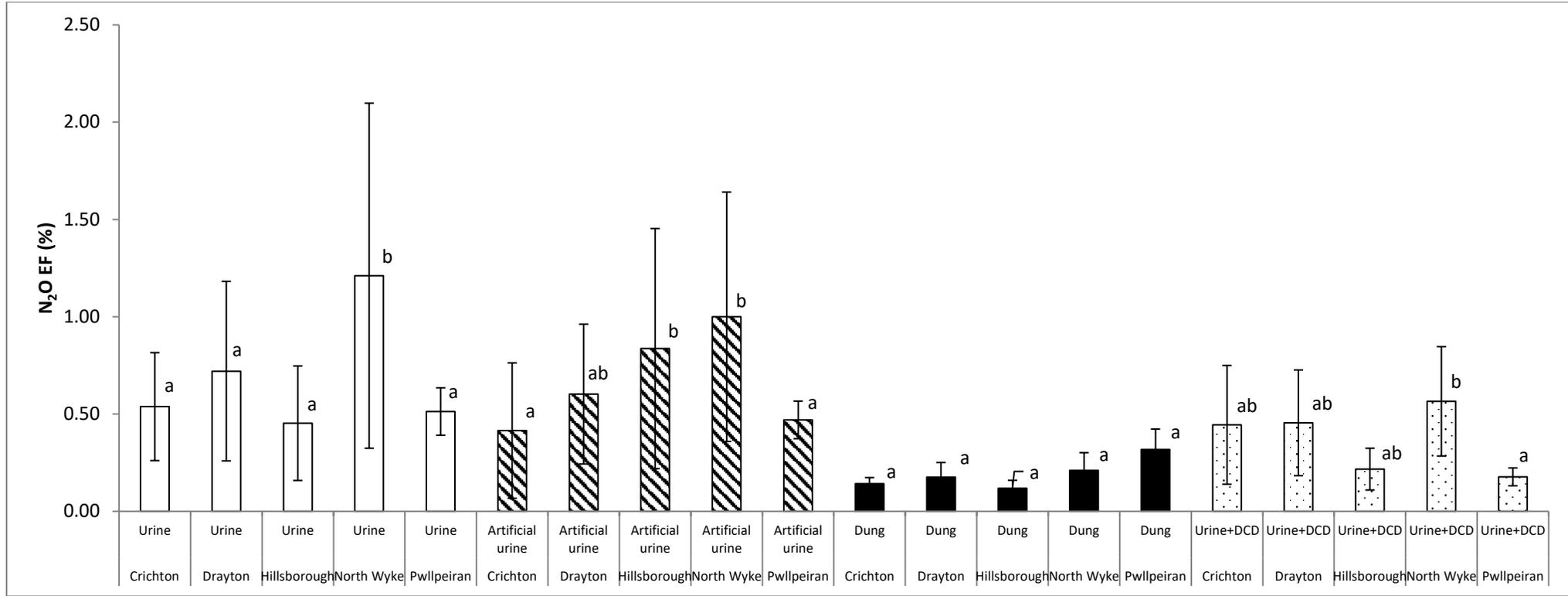
479  
 480 *3.3.3 Artificial urine*

481 The mean artificial urine N<sub>2</sub>O EF was similar to that of the real urine, 0.66% ( $\pm 0.18$ ) (Table  
 482 6), and there was a good relationship between the N<sub>2</sub>O EFs for real and artificial urine  
 483 ( $r^2 = 0.77$ ). Across all seasons, the meta-analysis showed that the N<sub>2</sub>O EF from the artificial  
 484 urine was significantly greater at North Wyke and Hillsborough ( $p < 0.05$ ) than the other sites  
 485 (Figure 4). Across all sites, the greatest N<sub>2</sub>O EF occurred following the early-grazing  
 486 application ( $p < 0.05$ ) (Figure 5).

487  
 488 *3.3.4 Dung*

489 The mean N<sub>2</sub>O EF for dung (from the meta-analysis) was 0.19% ( $\pm 0.03$ ), with a range of 0.04  
 490 – 0.53 (Table 6), which was significantly lower than for urine ( $p < 0.05$ ). The meta-analysis  
 491 showed there was no effect of site or season of application on the N<sub>2</sub>O EF from dung  
 492 ( $p > 0.05$ ) (Figures 4 and 5).

493  
 494  
 495



497

498 **Figure 4.** Average N<sub>2</sub>O EF (across three seasons of application) for each site, for urine and dung treatments.

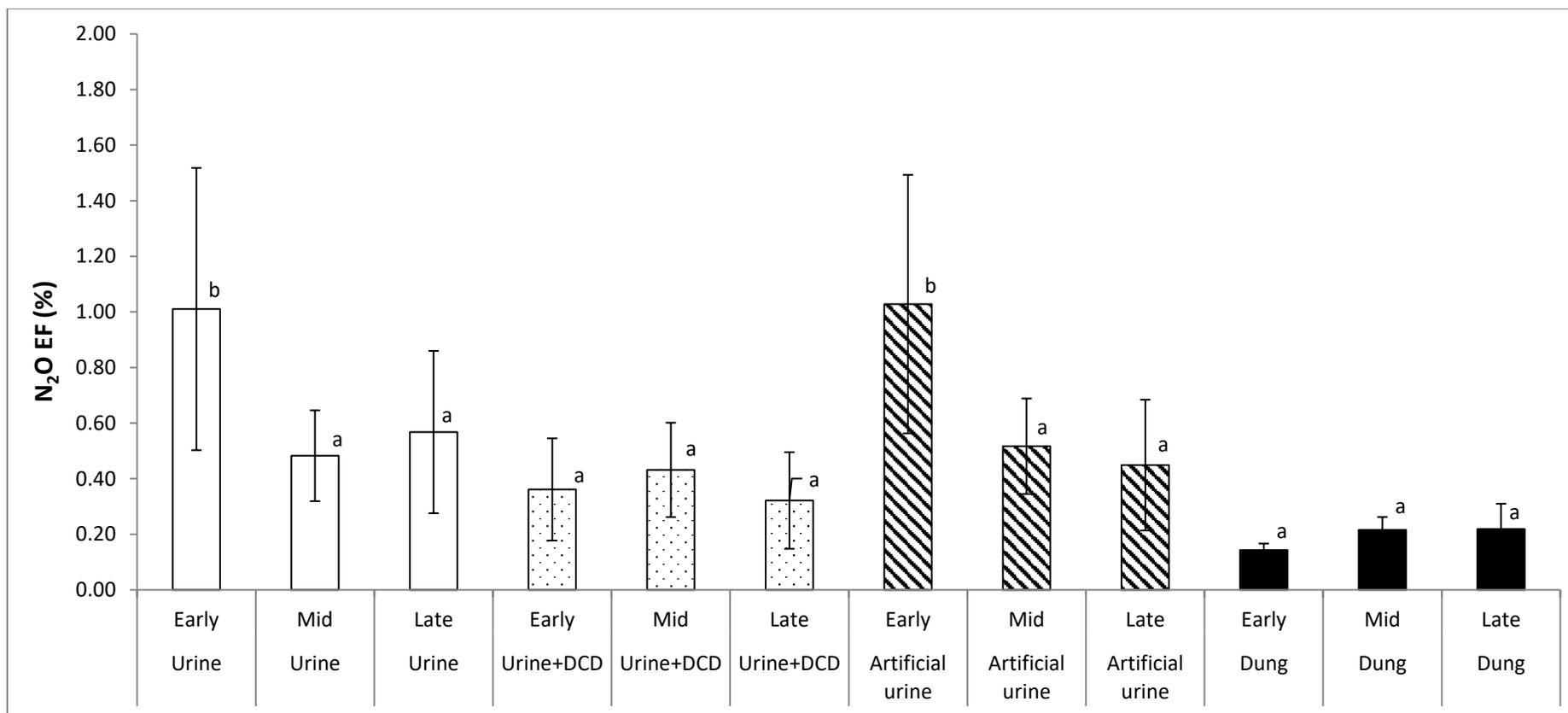
499

500 Within each urine/dung treatment, average N<sub>2</sub>O EFs (from meta-analysis) between sites with different letters are significantly different (N=3).

501

502

503



504

505 **Figure 5.** Effect of urine/dung treatment application timing (across all sites) on average N<sub>2</sub>O EF.

506

507 Within each treatment, average N<sub>2</sub>O EFs (from meta-analysis) between timings of application with different letters are significantly different

508 (N=3).

509

510

511 3.4 Factors affecting N<sub>2</sub>O fluxes from urine and dung

512

513 It is clear that there were significant (p<0.05) effects of excretal N source and season of  
514 application at each site, as well as ‘treatment’ x ‘season’ interactions (Table 7).

515

		Treatments	Application Time	Interaction
Crichton	Total N <sub>2</sub> O	<0.001	<0.001	<0.001
	EF	0.012	<0.001	0.019
Drayton	Total N <sub>2</sub> O	<0.001	<0.001	<0.001
	EF	<0.001	<0.001	0.002
Hillsborough	Total N <sub>2</sub> O	0.006	<0.001	0.014
	EF	0.035	0.002	0.042
North Wyke	Total N <sub>2</sub> O	<0.001	<0.001	<0.001
	EF	<0.001	<0.001	<0.001
Pwllpieran	Total N <sub>2</sub> O	<0.001	<0.001	<0.001
	EF	<0.001	<0.001	0.093

516 **Table 7.** Significance F-test probabilities for cumulative N<sub>2</sub>O emission and N<sub>2</sub>O EF, by  
517 timing of application, site, and timing of application x site interactions, from randomised  
518 block design ANOVA for each experiment.

519 3.4.1 Urine

520 Multiple regression analysis showed that the factors that best explained cumulative N<sub>2</sub>O  
521 emissions from urine application mainly included urine composition and soil clay content.  
522 The factors explaining 91.1% of the variance in cumulative N<sub>2</sub>O emissions from urine  
523 patches are shown via this equation: Cumulative N<sub>2</sub>O flux (kg N ha<sup>-1</sup>) = -61.94 + 38.50 urine  
524 creatinine content - 0.0042 urine urea N content + 0.003310 urine ammonium N content +  
525 0.002801 urine total nitrogen content + 4.115 soil pH - 1.036 urine hippuric acid content +  
526 4.340 urine pH - 8.06 urine uric acid content. >75% of the variance in total N<sub>2</sub>O flux was  
527 explained by the urine total N, urea-N, ammonium-N, uric acid and creatinine content.

528

529 The full equation of factors explaining 91.1% of the urine N<sub>2</sub>O EF was; EF% = -15.9 + 8.776  
530 urine creatinine content - 0.0009595 urine urea N content - 0.0007965 urine ammonium N  
531 content + 1.014 soil pH + 0.0005941 urine total nitrogen content - 0.2563 urine hippuric acid  
532 content + 1.116 urine pH -2.059 urine uric acid content. >75% of the variance in N<sub>2</sub>O EF was  
533 explained by the urine total N, urea-N, ammonium-N, uric acid and creatinine content.

534

535 3.4.2 Dung

536 In contrast to urine, multiple regression showed that the factors that best explained  
537 cumulative N<sub>2</sub>O emissions from dung application included environmental and soil factors (as  
538 well as dung factors). The full equation, explaining 68.3% of the variance in cumulative N<sub>2</sub>O  
539 emissions from dung in this study was; Cumulative N<sub>2</sub>O flux (kg N ha<sup>-1</sup>) = 4.15 - 0.0579  
540 initial % WFPS - 0.308 365d average temperature - 0.805 soil pH - 0.0408 dung nitrate N  
541 content - 0.00082 total nitrogen applied + 1.053 soil organic carbon - 10.50 soil dry bulk  
542 density + 1.927 dung pH.

543

544 The full equation of factors explaining 66.5% of the dung N<sub>2</sub>O EF was; EF% = -0.295 +  
545 0.0001187 dung ammonium N content + 0.01784 30d % WFPS - 0.01473 dung nitrate N  
546 content - 0.002143 total nitrogen applied - 0.02343 30d average temperature + 0.1159 soil  
547 organic carbon + 0.1747 dung total nitrogen content + 0.0452 365d average temperature.

548

549

#### 550 **4. Discussion**

551 Urine N<sub>2</sub>O EFs were significantly greater (average 0.69%) than the dung N<sub>2</sub>O EFs (average  
552 0.19%), signifying the importance of the Nr content as a substrate for the soil processes,  
553 nitrification and denitrification, responsible for N<sub>2</sub>O production. Our urine and dung N<sub>2</sub>O EFs  
554 are similar to some of those measured by New Zealand researchers, summarised by Kelliher  
555 et al. (2014). In New Zealand, urine N<sub>2</sub>O EFs are categorised by livestock species and  
556 farming system (lowland, hill country low and high slope), and our results are more similar to  
557 the N<sub>2</sub>O EFs for the hill-country low slope dairy cattle urine (average of 0.84%) and dung  
558 (average of 0.20%). By contrast, Krol et al. (2016) reported larger average urine and dung  
559 N<sub>2</sub>O EFs for nine experiments conducted in Ireland of 1.18% (urine) and 0.39% (dung); EFs  
560 approximately double the values we have measured. In this series of experiments, Krol et al  
561 (2016) applied urine at a higher N loading rate (average of 720 kg N ha<sup>-1</sup>) than in our study  
562 (average of 455 kg N ha<sup>-1</sup>). However, the greater N<sub>2</sub>O EF from the dung in the Irish study  
563 (0.39%) was despite using a lower N loading rate (average of 459 kg N ha<sup>-1</sup>) than in our study  
564 (835 kg N ha<sup>-1</sup>), suggesting that N loading was not the only factor resulting in the greater  
565 urine N<sub>2</sub>O EFs in these Irish experiments. Soil and environmental factors appeared to have  
566 been more conducive to N<sub>2</sub>O production and emission in this Irish study.

567

568 In our study, DCD reduced the urine N<sub>2</sub>O EFs by an average of 46%, although there was  
569 considerable variability in its efficacy to reduce N<sub>2</sub>O emissions (between sites and between  
570 seasons). In a related study, McGeough et al. (2016) took soil from these five UK grassland  
571 sites, and an additional four arable sites, and demonstrated that the efficacy of DCD to inhibit  
572 nitrification was controlled by the interaction between temperature, soil clay content and soil  
573 organic matter. Moreover, this study concluded that DCD was more effective in arable soils  
574 than in these grassland soils (McGeough et al., 2016). The average DCD N<sub>2</sub>O mitigation  
575 efficacy we measured (46%), and the range of efficacy that we measured are similar to other  
576 studies. For example, Selbie et al. (2014) showed that DCD increased the urine N<sub>2</sub>O EF by an  
577 average of +4% (a small increase) for urine applied at a loading rate of 500 kg N ha<sup>-1</sup>, but  
578 resulted in a 30% reduction for urine applied at 1000 kg N ha<sup>-1</sup> (in New Zealand).  
579 Misselbrook et al. (2014) reported a greater efficacy of DCD to reduce the urine N<sub>2</sub>O EF, by  
580 70% on a sandy clay loam in SW England. Recently, Minet et al. (2018) showed DCD,  
581 applied at 10 kg ha<sup>-1</sup>, could reduce the urine N<sub>2</sub>O EF by 34% (from 0.80% to 0.52%), but that  
582 DCD applied at 30 kg ha<sup>-1</sup> reduced the urine N<sub>2</sub>O EF further, by 64%. Note: efficacy of DCD  
583 is often reported for cumulative emissions, with reported values being much higher than the  
584 efficacy of reducing the EF itself (e.g. Selbie et al., 2014). However, the efficacy of DCD to  
585 reduce N<sub>2</sub>O EFs is needed if national inventories are to be modified accordingly.

586

587 There were insufficient data to be able to explore the relationships between N<sub>2</sub>O emissions  
588 and climate/soil with certainty, although the limited regression analysis showed that N<sub>2</sub>O  
589 emissions associated with urine were more related to urine composition than environmental  
590 and soil factors, whilst for dung which has a relatively low inorganic N content, N<sub>2</sub>O  
591 emissions were also controlled by soil and environmental factors. We recognise the  
592 limitations of conducting regression analysis on such a small data set. However, there is  
593 potential to generate a much larger data set by combining these data with those from other  
594 studies where soils and climate are similar and where similar protocols were followed, e.g.  
595 Krol et al. (2016), Minet et al. (2018) and data from some New Zealand experiments, to  
596 explore the controls of N<sub>2</sub>O emissions from urine and dung deposition, and generate  
597 improved EFs. Importantly, our unique dataset of daily N<sub>2</sub>O fluxes, cumulative emissions and  
598 emission factors, as well as soil mineral N and moisture data with weather, soil and site  
599 information have all been archived for future use by researchers (Bell et al., 2017; Cardenas  
600 et al., 2017; McGeough et al., 2017; Thorman et al., 2017a; Thorman et al., 2017b), and to  
601 allow integration with future datasets that become available.

602

603 To calculate a provisional excretal N<sub>2</sub>O EF, based on the data presented in this study, we  
604 assume a 60:40 split between the total N excreted in urine and dung (Webb and Misselbrook,  
605 2004). We estimate a combined excretal N<sub>2</sub>O EF, based on our mean urine and dung N<sub>2</sub>O  
606 EFs data of 0.49%. These UK data have now been combined with the very few additional  
607 IPCC compliant UK experimental datasets (see Misselbrook et al., 2014) to generate a new  
608 country specific N<sub>2</sub>O EF of 0.44%. This is <25% of the IPCC (2006) default EF for cattle  
609 grazing excreta (EF<sub>3</sub>), and ca. 50% of the default EF for sheep grazing excreta. If we  
610 substitute this new pasture, range and paddock EF for both cattle and sheep into the IPCC  
611 2006 methodology for calculating the UK inventory, we estimate a reduction of 11.6 kt N<sub>2</sub>O  
612 (18% less N<sub>2</sub>O for UK agriculture for 2015) and for total UK agricultural GHG emissions, a  
613 reduction of 3.4 Mt CO<sub>2</sub>e, or 7% for UK agriculture for 2015. This new EF is used in back-  
614 casting to 1990, and so has no bearing on meeting the UKs ambitious greenhouse gas  
615 mitigation target. However, a reduced GHG emission from agriculture means that a greater  
616 proportion of the emission can be 'offset' by carbon sequestration, and suggests that e.g. land  
617 sparing strategies may be more realistic (Lamb et al., 2016). The lower country specific  
618 pasture, range and paddock EF<sub>3</sub> also has implications for calculating carbon footprints of  
619 ruminant livestock products in the UK.

620

621 Clearly, this study focussed on cattle urine and dung where applications were made to  
622 lowland mineral soils, and where urine and dung were collected from cattle fed 'lowland'  
623 diets. So, questions arise about a) extrapolating the N<sub>2</sub>O EF data to sheep; indeed the IPCC  
624 default sheep urine N<sub>2</sub>O EF (1%) is greater than the new combined cattle excreta N<sub>2</sub>O EF  
625 from our study, and b) extrapolating the new N<sub>2</sub>O EF data to beef and sheep grazing in the  
626 uplands, on much more organic and potentially acidic soils, and where weather and soil  
627 conditions as well as urine/dung composition may be very different.

628

629

## 630 **5. Conclusions**

631 This was the first co-ordinated study in the UK to generate data to develop a country specific  
632 grazing excreta N<sub>2</sub>O EF for cattle. Results confirmed that urine is the greatest source of N<sub>2</sub>O  
633 compared to dung, and that the nitrification inhibitor, DCD, offers the potential to reduce  
634 N<sub>2</sub>O emissions from urine patches, although its efficacy across the sites and seasons was  
635 variable. Understanding what controls this variability, and the development of cost effective

636 delivery mechanisms need to be addressed if this technology is to be adopted. Importantly,  
637 the results of this study provide evidence that for the UK soil and climatic conditions, the  
638 N<sub>2</sub>O EF for grazing excreta for cattle is significantly lower (0.49%) than the IPCC default  
639 (2%) with implications for both government and the ruminant livestock industries as they  
640 seek to meet challenging greenhouse gas mitigation targets and greenhouse gas emission  
641 roadmaps, respectively. Further questions arise in terms of the validity of extrapolating these  
642 data from cattle to sheep grazing, and from mineral to organic soils.

643

644

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652

653

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