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A 3-year assessment of nitrous oxide emission factors for urine and dung of grazing sheep in a subtropical ecosystem

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Three-year assessment of nitrous oxide emission factor from urine and dung of grazing sheep in a subtropical region --Manuscript Draft--

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Abstract:	<p>Purpose Grazing livestock has strong impact on global nitrous oxide (N₂O) emissions by providing N sources through excreta. The scarcity of information on factors influencing N₂O emissions from sheep excreta in subtropical environments such as those of Southern Brazil led us to conduct field trials in three different winter pasture seasons on an integrated crop–livestock system (ICLS) in order to assess N₂O emission factors (N₂O-EF) in response to variable rates of urine and dung.</p> <p>Materials and methods The equivalent urine-N rates for the three winter seasons (2009, 2010 and 2013) ranged from 96 to 478 kg ha⁻¹, and the dung-N rates applied in 2009 and 2010 were 81 and 76 N kg ha⁻¹, respectively. Air was sampled in closed static chambers (0.20 m in diameter) and analyzed for N₂O by gas chromatography.</p> <p>Results and discussion Soil N₂O-N fluxes spanned the ranges 4 to 353 µg m⁻² h⁻¹ in 2009, -47 to 976 µg m⁻² h⁻¹ in 2010 and 46 to 339 µg m⁻² h⁻¹ in 2013. Urine addition resulted in N₂O-N peaks within for up to 20 days after application in the three years, and the strength of the peaks was linearly related to the N rate used. Emission factors of N₂O (EF-N₂O, % of N applied that is emitted as N₂O) of urine ranged from 0.06 to 0.34% and were essentially independent of N rate applied.</p>

	<p>Conclusions</p> <p>Our findings showed higher mean EF-N₂O for sheep urine than that for dung (0.21% vs 0.03%), which are much lower than the default value of 1% of IPCC's Tier 1. Therefore, in addition to the needs of disaggregation the EF-N₂O for sheep urine and dung, efforts should be applied to establish regional EF-N₂O to be used in national GHG inventories.</p>
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1 1 **TITLE PAGE**

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5 3 **Three-year assessment of nitrous oxide emission factor from urine and dung of grazing sheep in a**
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7 4 **subtropical region**

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31 **Abstract**

32 **Purpose**

33 Grazing livestock has strong impact on global nitrous oxide (N₂O) emissions by providing N sources
34 through excreta. The scarcity of information on factors influencing N₂O emissions from sheep excreta in
35 subtropical environments such as those of Southern Brazil led us to conduct field trials in three different
36 winter pasture seasons on an integrated crop–livestock system (ICLS) in order to assess N₂O emission
37 factors (N₂O-EF) in response to variable rates of urine and dung.

39 **Materials and methods**

40 The equivalent urine-N rates for the three winter seasons (2009, 2010 and 2013) ranged from 96 to 478
41 kg ha⁻¹, and the dung-N rates applied in 2009 and 2010 were 81 and 76 N kg ha⁻¹, respectively. Air was
42 sampled in closed static chambers (0.20 m in diameter) and analyzed for N₂O by gas chromatography.

44 **Results and discussion**

45 Soil N₂O-N fluxes spanned the ranges 4 to 353 μg m⁻² h⁻¹ in 2009, -47 to 976 μg m⁻² h⁻¹ in 2010 and 46
46 to 339 μg m⁻² h⁻¹ in 2013. Urine addition resulted in N₂O-N peaks within for up to 20 days after
47 application in the three years, and the strength of the peaks was linearly related to the N rate used.
48 Emission factors of N₂O (EF-N₂O, % of N applied that is emitted as N₂O) of urine ranged from 0.06 to
49 0.34% and were essentially independent of N rate applied.

51 **Conclusions**

52 Our findings showed higher mean EF-N₂O for sheep urine than that for dung (0.21% vs 0.03%), which
53 are much lower than the default value of 1% of IPCC's Tier 1. Therefore, in addition to the needs of
54 disaggregation the EF-N₂O for sheep urine and dung, efforts should be applied to establish regional EF-
55 N₂O to be used in national GHG inventories.

57 **Keywords:** greenhouse gases, livestock, subtropical environment, nitrous oxide.

59 1 Introduction

60 Historically, Brazil has had one of the largest livestock production in the world, alicerced on
61 approximately 175 million hectares of native and cultivated grassland (IBGE 2010). In the current
62 scenario of global warming, this major activity accounts for about 30% of greenhouse gas (GHG)
63 emissions in Brazil (MCTI 2014). Specifically, Brazilian livestock accounts for more than 40% of the
64 nitrous oxide (N₂O) emissions from the national agricultural sector (MCTI 2014), ascribed to direct
65 deposition of animal excreta on soil. Although N₂O is released in smaller amounts than carbon dioxide
66 (CO₂) the former gas is also important because its global warming potential is 298 higher than that of the
67 latter; also, N₂O has a longer atmospheric residence time than CO₂ (IPCC 2007).

68 Integrated crop-livestock systems (ICLS) have been proposed to optimize the use of natural
69 resources in rural land, and also to reduce GHG emissions (Carvalho et al. 2010). However, the typical
70 livestock component of these systems can be a major source of atmospheric N₂O emissions. This is a
71 result of excreta-N being deposited on the soil in equivalent amounts of 200 to 2000 kg per hectare, and
72 of 0.1–3.8% of urine-N and 0.1–0.7% of dung-N being potentially released as N₂O to the atmosphere
73 (Oenema et al. 1997).

74 Nitrous oxide production in soil is derived from processes such as nitrification, denitrification
75 or denitrifying nitrification of N (Firestone & Davidson 1989; Wrage et al. 2001). These processes are
76 governed by diverse pedoclimatic variables including soil moisture, temperature, mineral N contents and
77 soluble C contents (Gomes et al. 2009). In grassland, however, N₂O production and emission from soil
78 is additionally influenced by the presence of animals. During grazing, animals use N contained in plants
79 and convert it into animal protein for their own development. Because of conversion process is highly
80 inefficient, more than 80% of ingested N by animals is excreted, which impact soil N₂O production
81 (Barneze 2013). According to Luo et al. (2008), N₂O emissions from animal excreta (urine, largely) are
82 a result of their biochemical composition, which is rich in N compounds that are easily converted into
83 others by nitrification, denitrification or denitrifying nitrification in soil. This is probably one of the
84 reasons for the increased N₂O emissions from soil receiving urine in relation to dung (Yamulki et al.
85 1998; Luo et al. 2009). Despite this difference, the default N₂O emission factor (viz., EF-N₂O, % of N
86 applied emitted as N₂O) standardized by IPPC's Tier 1 (2% for cattle and 1% for sheep) are identical for
87 urine and dung (IPCC 2013).

88 Studies aimed at determining the EF-N₂O for specific regions of Brazil are scant even though
89 roughly one-third of the overall emissions of this gas from agricultural soils come from grazing animal
90 excreta (MCT 2016). In fact, the high variability of EF-N₂O values (0.1–4% of N applied) observed in a
91 range of studies has exposed the need for regional studies to determination of regional EF-N₂O. For
92 example, evidence gathered in New Zealand has led this country to adopt EF-N₂O values adapted to their
93 specific pedoclimatic conditions (de Klein et al. 2003).

94 The primary purposes of this study were as follows: (i) determining EF-N₂O for sheep urine and
95 dung under the regional pedoclimatic conditions of Southern Brazil; (ii) confirming whether overlapped
96 urine events increase EF-N₂O; (iii) establish whether the use of a single EF-N₂O for urine and dung is
97 adequate for use national inventories of agricultural N₂O emissions; and (iv) identifying the pedoclimatic
98 variables driving soil N₂O emissions.

100 **2 Material and Methods**

101 *2.1 Site description and experimental design*

102 This study was conducted in an area managed under an integrated crop–livestock protocol that
103 was established in 2003 at the Experimental Agronomic Station of the Federal University of Rio Grande
104 do Sul. The station is located 46 m a.s.l. at 30°05' S, 51°39' W in the municipality of Eldorado do Sul
105 (Southern Brazil). The regional climate is humid subtropical and falls in Köppen's class Cfa. The mean
106 annual precipitation and temperature are 1455 mm and 18.8 °C respectively. The soil in the experimental
107 area is a Typic Paleudult (USDA 1999) containing 150 g kg⁻¹ clay.

108 The ICL protocol involves a rotation including soybean (*Glycine max.* L. Merr.) and maize (*Zea*
109 *mays* L.) under no-tillage in the summer and autumn (crop period) and grazing of ryegrass (*Lolium*
110 *multiflorum* L.) by sheep from early winter to the start of a new crop cycle in the spring-summer
111 (livestock period). Two different grazing intensities (moderate and low) and two stocking methods
112 (continuous and rotational) are used in the livestock period (Savian et al. 2014).

113 This study was conducted during the ryegrass grazing period in the years 2009, 2010 and 2013
114 (specifically, during the intermediate grazing cycle, which spanned September and October). In order to
115 avoid animal interferences with the treatments, the study was performed in an isolated 30 m² plot of
116 ungrazed land that was used as reference for the ICL protocol. Prior to the treatments, ryegrass in the

1 117 plot was cut 0.10 m above ground in order to mimic sheep grazing. The treatments involved applying
2
3 118 variable rates of sheep urine in 2009, 2010 and 2013, and dung the former two years. Both excreta were
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5 119 applied to 1 m² microplots.

6
7 120 The urine and dung rates to be used were estimated from the soil area spanned by each excreta.
8
9 121 Thus, sheep with an average live weight of 30 kg produce an average 75 mL of urine (2.4 L m⁻²) and 7
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11 122 g of dung (0.45 kg m² at 71% moisture) in each event, the excreta covering an area of radius 0.10 and
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13 123 0.05 m, respectively.

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15 124 The treatments used in 2009 involved four different rates of urine (50, 75, 100 and 125 mL,
16
17 125 equivalent to 161, 242, 323 and 403 kg N ha⁻¹, respectively) and one of dung (14 g, equivalent to 81 kg
18
19 126 N ha⁻¹). Those applied in 2010 involved three different rates of urine (75, 150 and 300 mL, equivalent
20
21 127 to 119, 239 and 478 kg N ha⁻¹, respectively) and one of dung (14 g, equivalent to 76 kg N ha⁻¹). Finally,
22
23 128 only urine was applied in 2013, at a rate of 37.5, 75 and 150 mL, equivalent to 96, 192 and 384 kg N ha⁻¹,
24
25 129 respectively. Using different urine rates each year was intended to help confirm whether urine overlap
26
27 130 on the soil would lead to increased EF-N₂O values. An additional, control treatment involving application
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29 131 of no excreta was used to measure N₂O emissions from the soil. The experimental design used in the
30
31 132 three years was of the randomized block type with three replications.

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33
34 133 Excreta were collected from sheep on the ICL protocol on the day before application and their
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36 134 N contents determined by Kjeldahl distillation according to Bremner et al. (1960). The average N
37
38 135 contents of the urine samples in 2009, 2010 and 2013 were 10, 5 and 8 g N L⁻¹, respectively, and those
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40 136 of the dung samples in 2009 and 2010 were 9 and 8.4 g N kg⁻¹, respectively.

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43 137 The sample volumes for each treatment were applied to the base area, the remainder being saved
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45 138 for the microplot area, which was used to sample the soil in order to determine moisture, NH₄⁺-N, NO₃⁻
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47 139 -N and DOC in parallel with air sampling.

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50 51 141 *2.2 Measurement of soil N₂O fluxes and emissions*

52
53 142 In September and October of each year, soil N₂O-N fluxes were monitored for approximately
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55 143 40 days after application of the excreta, using the method of closed static chambers (Mosier 1989). For
56
57 144 this purpose, a metal base of 0.031 m² was inserted down to 5 cm in the soil at the center of each plot
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145 before the experiment and then supplied with the volumes of urine and dung corresponding to their
 146 respective deposition areas.

147 Air samples were collected in PVC chambers 25 cm high \times 20 cm diameter that were placed on
 148 the metal bases in each collection event (Gomes et al. 2009). The system was sealed by filling the channel
 149 at the top of the base with water. Samples were obtained from 09:00 to 11:00 am each day with the aid
 150 of polypropylene syringes 0, 15, 30 and 45 min after each chamber was closed (Jantalia et al. 2008). Air
 151 was homogenized by switching on an internal fan 30 s before each sample was taken, the air temperature
 152 in each chamber being measured by using a stem thermometer with an outer display. Once collected, air
 153 samples were transferred to pre-evacuated 12 mL glass vials (LABCO Exetainers[®]).

154 The concentration of N₂O in each air sample was determined by gas chromatography on a
 155 Shimadzu GC2014 instrument (a “Greenhouse” model) equipped with an electron capture detector
 156 (ECD) and using N₂ at a flow rate of 26 mL min⁻¹ as sweeping gas. N₂O fluxes were calculated from the
 157 following equation:

$$f = \frac{\Delta Q}{\Delta t} \frac{PV}{RT} \frac{M}{A} \quad (1)$$

159 where f ($\mu\text{g m}^{-2} \text{h}^{-1}$) is the gas flux, $\Delta Q/\Delta t$ the change in gas concentration, P the pressure inside the
 160 chamber (1 atm), V (L) the chamber volume, R the universal gas constant (0.08205 atm L mol⁻¹ K⁻¹), T
 161 (K) the temperature inside the chamber, M (g mol⁻¹) the molar mass of the gas and A (m²) the area of the
 162 chamber base.

163 Fluxes were all expressed relative to N₂O-N and cumulative emissions calculated by integrating
 164 the area by the curve for soil N₂O-N by linear interpolation (Gomes et al. 2009) using the software
 165 SigmaPlot v. 11.0.

166

167 2.3 Emission factor for N₂O (EF-N₂O)

168 The emission factor for N₂O (EF-N₂O, % N applied emitted as N₂O) was calculated from the
 169 following equation (De Klein et al. 2003):

$$\text{EF (\% N applied)} = \frac{(N_{2O-N_{urine\ or\ dung}}) - (N_{2O-N_{control}})}{N_{applied}} \times 100 \quad (2)$$

170

171 where EF is the emission factor (percentage of N-urine or dung applied that was released as N₂O), N₂O-
172 N_{urine or dung} the cumulative emission of N₂O-N in soil after urine or dung was applied, N₂O-N_{control} the
173 cumulative emission with the control treatment (no excreta), and N_{applied} the amount of N applied to the
174 soil through urine or dung.

175

176 *2.4 Soil and climate variables*

177 Microplots 1 m² in size were applied urine and dung with a view to monitoring gravimetric
178 moisture (GM) in the soil, the water-filled porosity space (WFPS), and the soil contents of nitrate (NO₃⁻
179 -N) and ammonium (NH₄⁺-N), in order to examine their role on N₂O emissions from the soil. N contents
180 were determined with the Kjeldahl method, using 2 M KCl as extractant with horizontal stirring for 1 h
181 according to Bremner et al. (1960). GM was obtained by drying the soil at 105 °C, and WFPS was
182 calculated from GM, soil density as determined before the tests and particle density, which was assumed
183 to be 2.65 g cm⁻³ (Gomes et al. 2009). The content of dissolved organic carbon (DOC) in soil was
184 determined only in the last year (2013) by extracting 1 g of soil with 10 mL of water with horizontal
185 stirring for 10 h (Zanatta 2006) and using a Shimadzu TOC VCSH instrument for dry combustion
186 measurements.

187 The rainfall and average air temperature data used were obtained from an Automatic Weather
188 Station approximately 0.5 km from the experimental area.

189

190 *2.5 Statistical analyses*

191 Because of the covariance structure present in the results, the analyses of variance of N₂O-N
192 fluxes and soil variables (NH₄⁺-N, NO₃⁻-N, WFPS and DOC) for each experiment were done by using a
193 mixed model in the software SAS v. 9.4 (SAS Institute Inc., Cary, NC, USA). The independent variables
194 excreta (E), sampling day (day) and their respective interaction were considered fixed effects, and blocks
195 as random effect. The analysis of variance of cumulative emissions and EF-N₂O were performed by
196 using a mixed model for each experimental period with the independent variable excreta (E) as fixed
197 effect and block as random effect.

198 Relationship of N₂O-N with air temperature, soil temperature, WFPS, DOC and mineral N were
199 examined in terms of the significance of the Pearson coefficients for the experimental period each year.

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1 200 When Pearson's coefficient was significant, a fitted linear model was used to elucidate the response of
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3 201 N₂O fluxes to climate and soil variables.

4
5 202 The EF-N₂O for each pasture season were subjected to analyses of variance with the
6
7 203 independent variable urine rate as fixed effect and block as random effect. An orthogonal contrast
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9 204 analysis was also performed to compare the urine and dung emission factors for 2009 and 2010.
10
11 205 Differences between treatment means were assessed with the least significant difference (LSD) test at
12
13 206 the 5% significance level.

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16 208 **3 Results**

17 209 *3.1 Temperature and precipitation*

18
19 210 The average daily temperature for the sampling period was 17.0 °C in 2009, 18.7 °C in 2010
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21 211 and 19.4 °C in 2013 (Fig. 1). The highest and lowest daily temperatures were 10.5 °C and 24.2 °C,
22
23 212 respectively, and observed in 2009. That was also the year with the highest cumulative precipitation (270
24
25 213 mm), followed by 2013 (262 mm) and 2010 (90 mm) (Fig. 1). The first event of substantial precipitation
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27 214 in the three years, with at least 15 mm on one day, occurred on the 5th day after application (DAA) of
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29 215 the excreta.

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33 217 *<insert Figure 1 here>*

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35 218

36 219 *3.2 Soil N₂O-N fluxes and cumulative emissions*

37
38 220 Soil N₂O-N fluxes were influenced by application of excreta, sampling day and their interaction
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40 221 in both 2009, 2010 and 2013 (Table 1). N₂O-N fluxes ranged from 4 to 353 µg m⁻² h⁻¹ in 2009, -47 to
41
42 222 976 µg m⁻² h⁻¹ in 2010 and 46.5 to 339.4 µg m⁻² h⁻¹ in 2013 (Fig. 2). Soil fluxes were increased by effect
43
44 223 of the application of urine or dung on pasture soil. The highest flux each year was that resulting from the
45
46 224 treatment involving the highest urine rate.

47
48 225 Soil N₂O-N fluxes peaked at 976 µg m⁻² h⁻¹ 16 DAA in 2010, the peak resulting from
49
50 226 application of the highest urine rate for the three years (U300, 300 mL). Fluxes returned to baseline levels
51
52 227 30, 15 and 25 DAA in 2009, 2010 and 2013, respectively, after which excreta application led to

228 insubstantial differences from control treatment, without excreta. Dung application to the soil in 2009
229 and 2010 resulted in nonsignificant increases in the fluxes relative to urine application.

230

231 *<insert Figure 2 here>*

232

233 Cumulative soil N₂O-N emissions were influenced by application of the excreta (Table 1). In fact, using
234 increasing urine rates led to average cumulative emissions ranging from 0.55 to 1.42 kg ha⁻¹ in 2009,
235 0.24 to 1.55 kg ha⁻¹ in 2010 and 0.14 to 0.67 kg ha⁻¹ in 2013 (Fig. 3). Also, applying dung resulted in
236 cumulative soil N₂O-N emissions of 0.16 kg ha⁻¹ in 2009 and 0.11 kg ha⁻¹ in 2010, but without significant
237 effect of treatment compared to the control (Table 2).

238 The increase in cumulative N₂O-N emissions was linearly related to the urine rate applied each
239 year (Fig. 3). Thus, each kg N ha⁻¹ used increased N₂O-N emissions by 0.003, 0.005 and 0.002 kg ha⁻¹
240 in 2009, 2010 and 2013, respectively.

241

242 *<insert Figure 3 here>*

243

244 3.3 Emission factor of N₂O (EF-N₂O)

245 Urine application had no effect on EF-N₂O, which ranged from 0.23 to 0.32% in 2009, from
246 0.14 to 0.34% in 2010 and from 0.06 to 0.15% in 2013 (Table 2). The N₂O-EF values for the three years
247 ranged from 0.06 to 0.34% (mean value of 0.21%) with urine and on averaged of 0.03% with dung. The
248 EF-N₂O value obtained by applying dung was about 15 times lower than that for urine in 2009 and 2010
249 (Table 2).

250

251 *<insert Table 2 here>*

252

253 3.4 Soil parameters

254 Water-filled porosity space (WFPS) was not altered by application of the excreta to the soil
255 (Table 1; Figs 1 and 4a). However, positive linear relationship ($p < 0.05$) between soil N₂O-N fluxes and

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256 WFPS in the three years (Table 3). This parameter ranged from 26 to 96% in 2009, 18 to 94% in 2010
 257 and 61 to 95% in 2013 —where it remained above 60% throughout (Fig. 4a).

258

259 *<insert Table 3 here>*

260

261 The soil contents of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ were influenced by excreta application and GHG
 262 sampling day (Table 1) with different effect among years (Table 3). Thus, applying urine in 2009 and
 263 2010 increased $\text{NH}_4^+\text{-N}$ levels relative to the control treatment from the first day; by contrast, $\text{NO}_3^-\text{-N}$
 264 content only increased after 9 days in 2009 and 6 days in 2010, when $\text{NH}_4^+\text{-N}$ content started to fall. In
 265 2013, the contents of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ evolved similarly until 20 DAA. Thus, the two peaked at 151
 266 $\text{mg NH}_4^+\text{-N kg}^{-1}$ soil and 44 $\text{mg NO}_3^-\text{-N kg}^{-1}$ soil within the first few days after excreta application and
 267 then decreased to near-zero levels after 30 days (Figs 4b and 4c). Positive and strong relationships ($p <$
 268 0.001) between soil contents of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ with $\text{N}_2\text{O-N}$ fluxes were observed in 2009, but not
 269 in 2010 ($p > 0.05$). Positive relationship ($p < 0.001$) between $\text{N}_2\text{O-N}$ fluxes and $\text{NH}_4^+\text{-N}$ content was
 270 observed in 2013.

271 Urine increased soil contents of mineral N, especially under application of the highest urine
 272 rates (Figs 4b and 4c). On the other hand, dung had no effect on such contents relative to the control
 273 treatment ($p > 0.05$). The DOC contents ranged from 4.8 to 31 mg C L^{-1} in 2013, with overweighting
 274 contents in the first 15 DAA (Fig 4d). Increasing the urine application rate led to increased soil contents
 275 of DOC favoring the positive relationship ($p < 0.05$) with soil $\text{N}_2\text{O-N}$ fluxes (Table 3).

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277 *<insert Figure 4 here>*

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279 **4 Discussion**

280 *4.1 N₂O from urine and dung*

281 The excreta increased soil $\text{N}_2\text{O-N}$ fluxes, especially in the first few days after application but
 282 little effect was observed beyond 20 DAA (Fig. 2). Similar studies conducted under different
 283 pedoclimatic conditions unveiled that air samples should be collected for at least 120 DAA or until
 284 emissions fall to near-zero levels not significantly different from those of control treatment (De Klein et

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1 285 al. 2003). However, this required time for N added to the soil to be converted in N₂O may highly variable
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3 286 depending on particular climate and soil conditions (Clough et al. 1998; Selbie et al. 2014). In this work,
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5 287 however, emissions only were observed within the first few days after application. This led us to finish
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7 288 sampling around 40 DAA, similarly to van Groenigen et al. (2005) in Netherlands and Lessa et al. (2014)
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9 289 in Brazil, who found substantial N₂O-N emissions were restricted on average of 30 days after urine
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11 290 application. Our results are consistent with those of Sordi et al. (2014), who applied cattle excreta to
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13 291 pasture in different seasons of Southern Brazil and found N₂O-N emissions to peak on average 45 DAA
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15 292 in response to urine. This result suggests that N added through urine is more rapidly converted in N₂O
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17 293 under subtropical conditions such as those of Southern Brazil.

19 294 The rapid increase in soil N₂O-N fluxes upon excreta application is consistent with results found
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21 295 by other authors (Hyde et al. 2005; Rochette et al. 2008; Mu et al. 2008) and can be ascribed to an
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23 296 increased availability of soil N and to conditions (e.g. WFPS and DOC) favoring development of
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25 297 microbial processes involved in the N conversion reactions occurring in soil upon excreta application
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27 298 (Williams et al. 1999; Smith et al. 2003; Luo et al. 2008). Thus, nitrogen present as urea in urine is
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29 299 rapidly converted into ammonium and nitrate ions, which are used by nitrifying and denitrifying bacteria
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31 300 (Oenema et al. 1997). The significant ($p < 0.05$) and positive relationship between N₂O-N fluxes and
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33 301 WFPS for the three evaluated years, and with DOC content in 2013 support the importance of these two
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35 302 factors on N₂O production and emission from soil. In addition, peaks of N₂O-N emissions were observed
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37 303 at WFPS > 60%. Increased WFPS levels facilitate the formation of anaerobiosis sites in soil, thereby
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39 304 favoring denitrification (Wrage et al. 2005; Saggar et al. 2004), which is widely regarded as the most
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41 305 important process behind N₂O production in soil (Pimentel et al. 2015). Also, the mobility of substrates
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43 306 such as nitrate ion and soluble carbon in soil is increased by an increase in WFPS level, which boosts
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45 307 N₂O-N emissions (Luo et al. 1999). According to Wrage et al. (2001), denitrification requires not only
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47 308 adequate WFPS levels and soil nitrate contents, but also a high availability of soluble C in soil. Our
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49 309 results, however, are inconclusive as to which process prevailed in N₂O production. The high correlation
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51 310 between N₂O-N emissions and soil NH₄⁺-N contents and even with WFPS (Table 3) suggests that
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53 311 nitrification could also be occurring here. N₂O production by nitrification can take place even under
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55 312 conditions of limited oxygen availability (WFPS > 60%), where so-called “denitrifying nitrification”
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57 313 may be favored if autotrophic bacteria oxidize ammonia (NH₃) to nitrite (NO₂⁻) for subsequent reduction
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1 314 to N₂O (Wrage et al. 2005; Koll et al. 2011). Therefore, both denitrification and denitrifying nitrification
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3 315 may have occurred in parallel in our soil.

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5 316 The absence of substantial N₂O-N emissions upon application of dung (Fig. 2) was possibly
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7 317 associated to the low N availability and slow degradation providing a supply of N and other nutrients at
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9 318 low levels (Haynes and Williams 1993). To some extent, this is confirmed by the small changes in soil
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11 319 NH₄⁺-N and NO₃⁻-N contents due to dung application, which resulted in low difference from the control
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13 320 treatment.

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15 321 The presence of readily available N in soil is determining to N₂O-N emission (Luo et al. 2008).
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17 322 N₂O-N fluxes vary depending on the particular N form prevailing in soil (particularly, as noted earlier,
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19 323 on NH₄⁺-N and NO₃⁻-N contents). In 2009, N₂O-N fluxes were positively correlated with NH₄⁺-N and
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21 324 NO₃⁻-N contents of soil (Table 3). However, the greatest emissions that year coincided with the highest
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23 325 soil NH₄⁺-N contents relative to NO₃⁻-N, as well as the highest WFPS levels, which suggests that N₂O
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25 326 was formed preferentially by denitrifying nitrification. The positive correlation between N₂O-N fluxes
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27 327 and NH₄⁺-N contents but not NO₃⁻-N contents in 2013 further confirms the importance of nitrification in
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29 328 the formation of N₂O in soil (Table 3). Although the lack of relationship between N₂O-N fluxes and
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31 329 content of NH₄⁺-N and NO₃⁻-N in 2010, N₂O-N emissions increased due to the increase in soil content
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33 330 of NH₄⁺-N (Table 3, Fig. 4b).

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35 331 The high NH₄⁺-N contents found may have resulted from hydrolysis of urea, main nitrogen-
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37 332 containing compound in urine (Bolan et al. 2004); on the other hand, the low NO₃⁻-N contents observed
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39 333 may have be associated to leaching process of this ion (Sordi et al. 2014). These assumptions are
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41 334 consistent with the fact that NH₄⁺-N contents invariably exhibited strong and positive relationship with
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43 335 N₂O-N emissions, even at WFPS levels above 60% (Figs 2 and 4b).

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45 336 The increased amounts of mineral N supplied by the highest urine rates increased N₂O-N fluxes
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47 337 and consequently cumulative N₂O emissions from soil, indicating a strong and positive relationship
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49 338 between N₂O emissions and amount of N supplied by urine (Fig. 3). On the other hand, cumulative N₂O
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51 339 emissions resulting from dung application were similar to those for the control treatment ($p > 0.05$). As
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53 340 discussed before, this could be resulted of slow mineralization of the organic-N present in dung, which
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55 341 can take weeks to months, resulting in a gradual release of mineral-N which are uptake by grazing in a
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57 342 greater proportion than urine-N (Hoekstra et al. 2011). The decreased N₂O emissions in 2013 relative to
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1 343 the other years may have resulted from the more frequent precipitation events over the year, especially
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3 344 after urine application (Figs 1 and 2). The fact that WFPS exceeded 60% over the year may have
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5 345 facilitated complete denitrification and hence favored N₂ emissions over N₂O emissions. According to
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7 346 Jamali et al. (2015), massive losses of mineral N resulting from leaching under heavy precipitation or in
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9 347 the form of N₂ can restrict N availability —and hence N₂O production— in soil, thereby diminishing
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11 348 N₂O-N fluxes.

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14 350 *4.2 Emission factor of N₂O (EF-N₂O)*

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17 351 EF-N₂O values were not significantly influenced ($p > 0.05$) by the urine application rate (Table
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19 352 2), indicating that overlapping or not the urine application on the same site results in similar EF-N₂O.
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21 353 Our results suggest then that the estimation of soil N₂O emission from sheep urine just accounting
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23 354 animals number and N excreted as a viable choice in national inventories for N₂O emission. However,
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25 355 larger volumes of urine application on soil possibly impact negatively on EF-N₂O in sites with
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27 356 overlapping urine application as a result of urine percolation across the soil profile and N concentration
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29 357 decrease in soil surface (Luo et al. 2008).

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32 358 The mean value of EF-N₂O for the three years and all urine application rates was 0.21%,
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34 359 whereas that for the two years under dung application was 0.03% (Table 2). As explained above, the
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36 360 decreased EF-N₂O value for urine in 2013 relative to the other years was a result of small cumulative
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38 361 emissions.

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41 362 The EF-N₂O values for urine and dung are similar to those found in other places in Brazil. Sordi
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43 363 et al. (2014) reported a value of 0.26% for urine and 0.15% for dung under subtropical conditions. Also,
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45 364 Lessa et al. (2014) found 0.7% for urine under tropical conditions (Brazilian cerrado), and Barneze et al.
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47 365 (2014) 0.2% for urine in the Atlantic forest region (tropical conditions). These results suggest that the
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49 366 default EF-N₂O value used in IPCC's Tier 1 (viz., 1% of all N applied in animal excreta; IPCC 2013)
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51 367 overestimates the actual values for some local conditions irrespective of the particular excreta applied.

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53 368 A comparison of the results obtained here with the two excreta reveals that urine was the main
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55 369 source of N₂O-N, with emission potential more than 15 times higher than that of dung (Table 2). The
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57 370 difference in N₂O-EF between two excreta suggests the need to use specific EF-N₂O values for each
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59 371 excreta instead of IPCC's default value (1%). In fact, using the standard value in IPCC's Tier 1
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372 overestimated N₂O emissions from urine and dung by about 80 and 94%, respectively, which confirms
 373 the importance of considering the particular pedoclimatic conditions in these studies.

374

375 **5 Conclusions**

376 N₂O emissions from soil receiving sheep urine and dung during the grazing period in an
 377 integrated crop–livestock (ICL) system under subtropical conditions of Southern Brazil started an
 378 average 20 days after deposition. Soil N₂O-N emissions have a strong relationship with inorganic N
 379 (particularly as NH₄⁺-N) and were facilitated by high WFPS levels (> 60%). Urine was the main source
 380 of N in N₂O emissions from soil during the grazing period. Our results indicate that the EF-N₂O value in
 381 IPCC's Tier 1 (1% of N applied) overestimates N₂O emissions in grassland areas under the typical
 382 subtropical conditions of Brazil and reinforce the needs to disaggregate the EF-N₂O values for urine and
 383 dung.

384

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388

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1 **Figures Caption**

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3 Fig. 1. Daily rainfall precipitation and the mean daily temperature for each year trial
4 period.

5

6 Fig. 2. Temporal nitrous oxide (N₂O) flux following urine and dung application in 2009,
7 2010 and 2013 trials. Vertical bars represent standard error of the mean.

8

9 Fig. 3. Relationship between urine and dung nitrogen (N) rate and cumulative nitrous
10 oxide (N₂O) emissions in 2009, 2010 and 2013 trials.

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12 Fig. 4. Soil variables concentrations after urine and dung applications in 2009, 2010 and
13 2013 trials. (A) Water filled porosity space; (B) Soil ammonium (NH₄⁺-N); (C) Soil nitrate
14 (NO₃⁻-N); (D) Dissolved organic carbon. Vertical bars represent the mean standard error.

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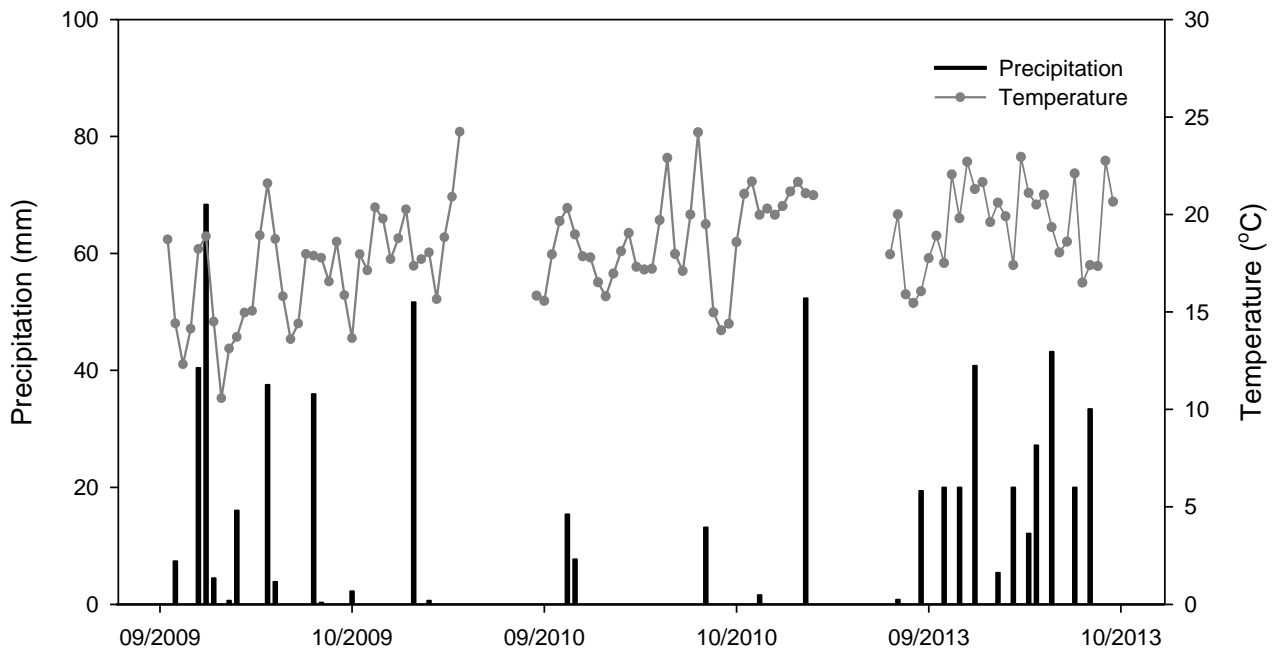
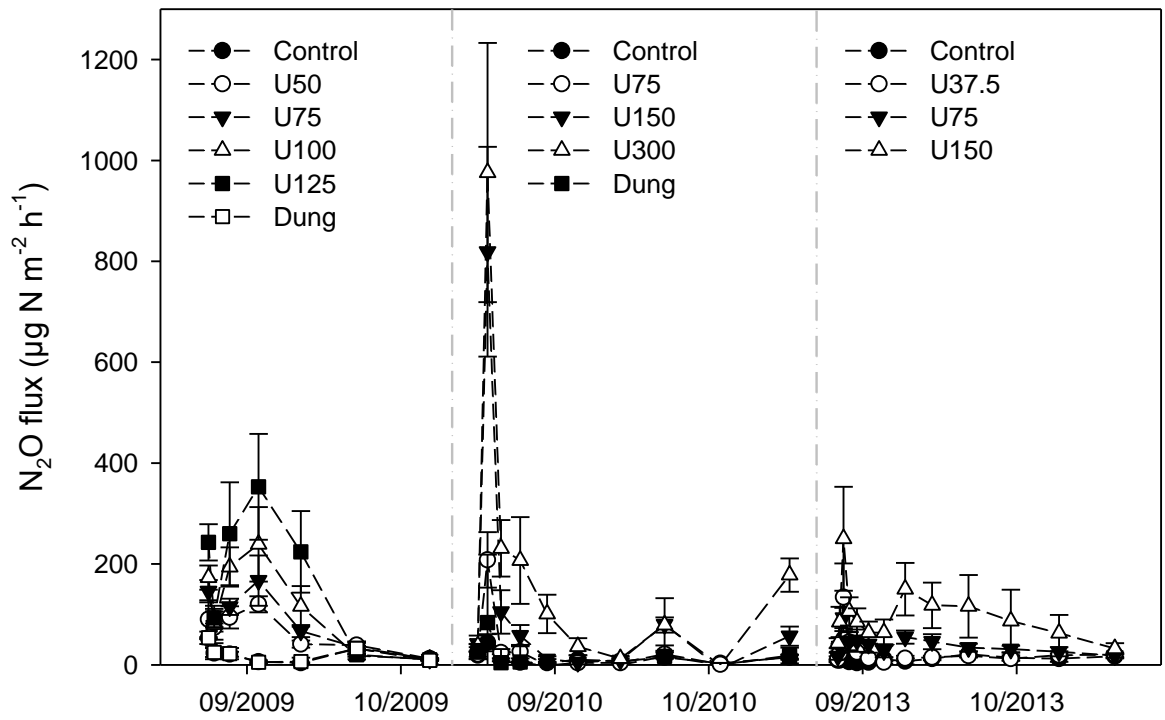


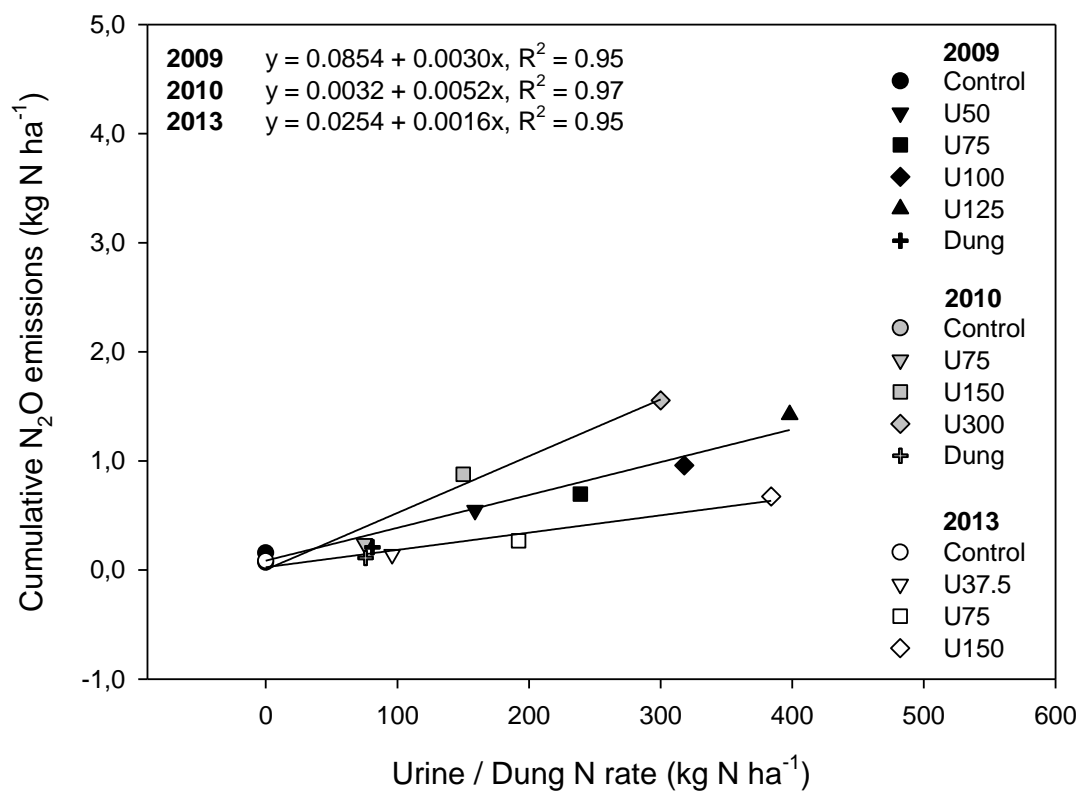
Fig. 1.

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Fig. 2.



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63 **Fig. 3.**

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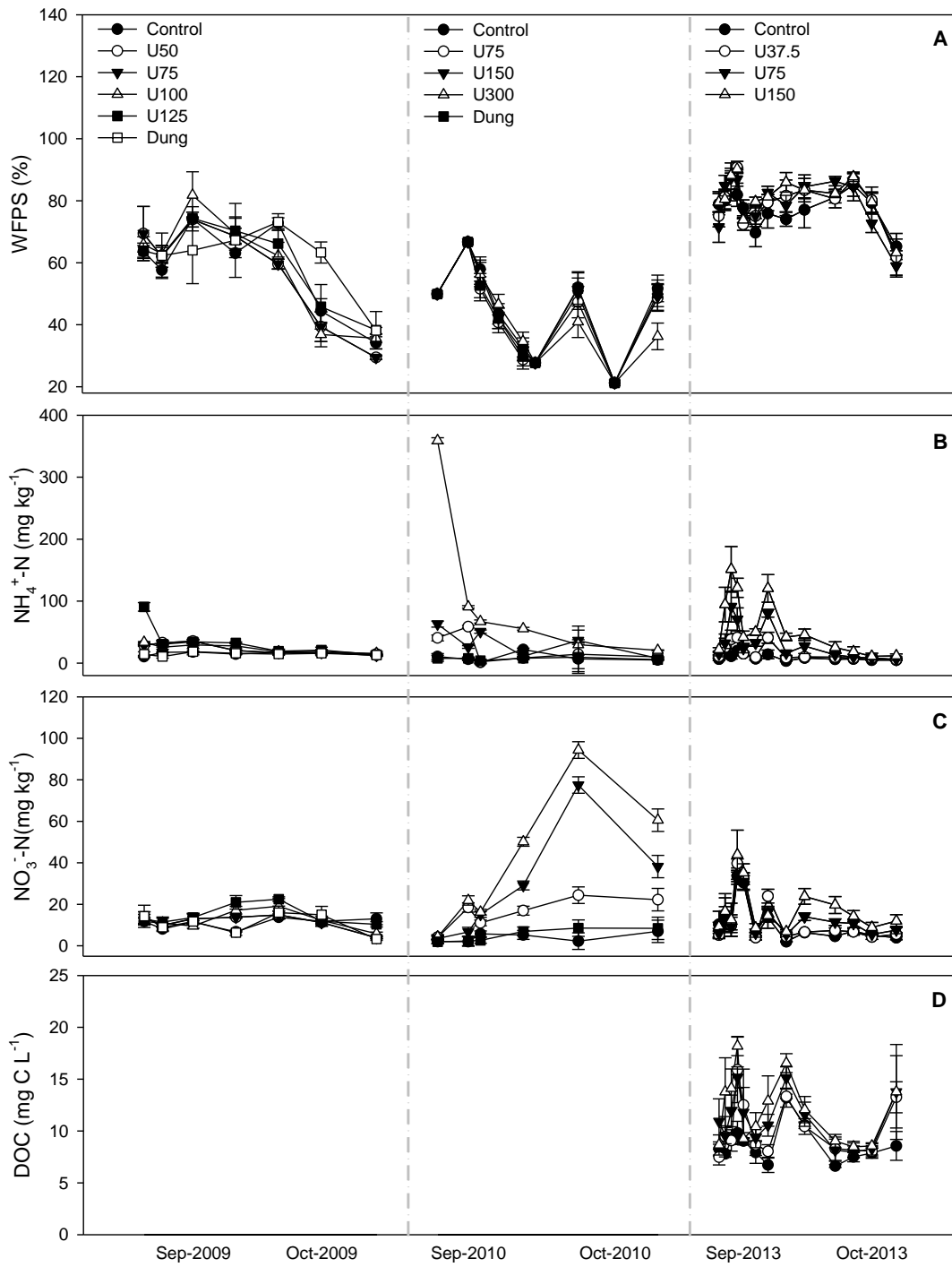


Fig. 4.

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Table 1. Analysis of variance on the N₂O fluxes, cumulative emissions and soil variables (WFPS, NH₄⁺-N, NO₃⁻-N and DOC) to each year 2009, 2010 and 2013 trials. A *p*-value < 0.05 is considered statistically significant.

Variable		2009			2010			2013		
Dependent	Independent	df	<i>F</i> -value	<i>p</i> -value	df	<i>F</i> -value	<i>p</i> -value	df	<i>F</i> -value	<i>p</i> -value
N ₂ O flux	Excreta (E)	5	21.30	<0.0001	4	22.50	<0.0001	3	70.06	<0.0001
	Day	6	14.31	<0.0001	9	31.06	<0.0001	12	12.63	<0.0001
	E x Day	30	2.57	0.0004	36	6.93	<0.0001	36	3.6	<0.0001
Cumulative N ₂ O	Excreta	5	38.39	<0.0001	4	14.08	<0.0001	3	9.33	0.0112
WFPS	Excreta (E)	5	0.41	0.8410	4	1.30	0.2759	3	2.36	0.0763
	Day	6	90.79	<0.0001	8	161.74	<0.0001	12	17.1	<0.0001
	E x Day	30	1.07	0.3881	32	1.10	0.3562	36	0.88	0.6554
NH ₄ ⁺ -N	Excreta (E)	5	23.75	<0.0001	4	4619.62	<0.0001	3	56.09	<0.0001
	Day	6	20.81	<0.0001	6	2768.47	<0.0001	12	20.42	<0.0001
	E x Day	30	4.26	<0.0001	24	1493.27	<0.0001	36	3.64	<0.0001
NO ₃ ⁻ -N	Excreta (E)	5	9.51	<0.0001	4	124.36	<0.0001	3	7.11	0.0002
	Day	6	25.04	<0.0001	6	97.34	<0.0001	12	32.11	<0.0001
	E x Day	30	2.62	0.0003	24	21.44	<0.0001	36	0.78	0.7988
DOC	Excreta (E)							3	10.87	<0.0001
	Day		-----			-----		12	8.82	<0.0001
	E x Day							36	0.86	0.691

Table 2. Cumulative emission of N₂O and emission factor (EF) for urine doses and dung, applied in three different year trials.

Year trial	Treatment	Cumulative emission N ₂ O-N	
		kg ha ⁻¹	EF (%)
2009	Control	0.16 C	-
	U50 (161 N kg ha ⁻¹)	0.55 BC	0.25 ns
	U75 (242 N kg ha ⁻¹)	0.70 B	0.23
	U100 (323 N kg ha ⁻¹)	0.96 AB	0.25
	U125 (403 N kg ha ⁻¹)	1.42 A	0.32
	Mean Urine	0.91	0.26 A*
	Dung (81 N kg ha⁻¹)	0.17 C	0.01 B
2010	Control	0.07 C	-
	U75 (119 N kg ha ⁻¹)	0.24 BC	0.14 ns
	U150 (239 N kg ha ⁻¹)	0.88 AB	0.34
	U300 (478 N kg ha ⁻¹)	1.55 A	0.31
	Mean Urine	0.89	0.26 A*
	Dung (76 N kg ha⁻¹)	0.11 C	0.05 B
	2013	Control	0.08 B
U37.5 (96 N kg ha ⁻¹)		0.14 B	0.06 ns
U75 (192 N kg ha ⁻¹)		0.27 B	0.10
U150 (384 N kg ha ⁻¹)		0.67 A	0.15
Mean Urine		0.36	0.10

Uppercase letters compare treatments (column) within the same year trial, according to Tukey test ($P < 0.05$).

*Contrast analyses Urine EF x Dung EF for both years was significant at $P < 0.0001$.

Table 3. Pearson correlation coefficients of soil N₂O fluxes with soil variables (ammonium – NH₄⁺-N, nitrate – NO₃⁻-N , water filled porosity space – WFPS and dissolved organic carbon - DOC) for 2009, 2010 and 2013 trials. A *p*-value < 0.05 is considered statistically significant.

Year trial	Variables	Regression equation	Soil N ₂ O fluxes	
			Coefficient	<i>p</i> -value
2009	NH ₄ ⁺ -N	$y = -34.12 + 5.72x$	0.53	<0.0001
	NO ₃ ⁻ -N	$y = -79.78 + 15.29x$	0.49	<0.0001
	WFPS	$y = -104.81 + 3.37x$	0.48	0.0003
2010	NH ₄ ⁺ -N	$y = 106.65 + 0.44x$	0.02	0.5092
	NO ₃ ⁻ -N	$y = 117.66 + 0.26x$	0.001	0.8902
	WFPS	$y = -237.24 + 7.70x$	0.47	0.0016
2013	NH ₄ ⁺ -N	$y = 11.74 + 1.13x$	0.51	<0.0001
	NO ₃ ⁻ -N	$y = 33.57 + 0.86x$	0.09	0.2379
	WFPS	$y = -187.88 + 2.93x$	0.25	0.0053
	DOC	$y = -38.15 + 7.94x$	0.17	0.0025