



**HAL**  
open science

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

Diego Fernandes de Bastos, Emanuelle Cavazini Magiero, Michely Tomazi, Janquieli Schirmann, Murilo Veloso, Paulo César de Faccio Carvalho, Cimélio Bayer

### **► To cite this version:**

Diego Fernandes de Bastos, Emanuelle Cavazini Magiero, Michely Tomazi, Janquieli Schirmann, Murilo Veloso, et al.. A 3-year assessment of nitrous oxide emission factors for urine and dung of grazing sheep in a subtropical ecosystem. *Journal of Soils and Sediments*, 2020, 20 (2), pp.982-991. 10.1007/s11368-019-02456-7 . hal-04260660

**HAL Id: hal-04260660**

**<https://normandie-univ.hal.science/hal-04260660>**

Submitted on 9 Nov 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Journal of Soils and Sediments

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

<b>Manuscript Number:</b>	JSSS-D-19-00603
<b>Full Title:</b>	Three-year assessment of nitrous oxide emission factor from urine and dung of grazing sheep in a subtropical region
<b>Article Type:</b>	Research Article
<b>Section/Category:</b>	Soils
<b>Corresponding Author:</b>	Cimelio Bayer Universidade Federal do Rio Grande do Sul AGRONOMIA, Porto Alegre, RS BRAZIL
<b>Corresponding Author Secondary Information:</b>	
<b>Corresponding Author's Institution:</b>	Universidade Federal do Rio Grande do Sul
<b>Corresponding Author's Secondary Institution:</b>	
<b>First Author:</b>	Diego Fernandes Bastos
<b>First Author Secondary Information:</b>	
<b>Order of Authors:</b>	Diego Fernandes Bastos Emanuelle Cavazini Magiero Michely Tomazy Janquieli Schirmann Murilo Gomes Veloso Paulo Cesar de Faccio Carvalho Cimelio Bayer
<b>Order of Authors Secondary Information:</b>	
<b>Funding Information:</b>	
<b>Abstract:</b>	<p><b>Purpose</b> Grazing livestock has strong impact on global nitrous oxide (N<sub>2</sub>O) emissions by providing N sources through excreta. The scarcity of information on factors influencing N<sub>2</sub>O 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 N<sub>2</sub>O emission factors (N<sub>2</sub>O-EF) in response to variable rates of urine and dung.</p> <p><b>Materials and methods</b> The equivalent urine-N rates for the three winter seasons (2009, 2010 and 2013) ranged from 96 to 478 kg ha<sup>-1</sup>, and the dung-N rates applied in 2009 and 2010 were 81 and 76 N kg ha<sup>-1</sup>, respectively. Air was sampled in closed static chambers (0.20 m in diameter) and analyzed for N<sub>2</sub>O by gas chromatography.</p> <p><b>Results and discussion</b> Soil N<sub>2</sub>O-N fluxes spanned the ranges 4 to 353 µg m<sup>-2</sup> h<sup>-1</sup> in 2009, -47 to 976 µg m<sup>-2</sup> h<sup>-1</sup> in 2010 and 46 to 339 µg m<sup>-2</sup> h<sup>-1</sup> in 2013. Urine addition resulted in N<sub>2</sub>O-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 N<sub>2</sub>O (EF-N<sub>2</sub>O, % of N applied that is emitted as N<sub>2</sub>O) 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-N<sub>2</sub>O 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-N<sub>2</sub>O for sheep urine and dung, efforts should be applied to establish regional EF-N<sub>2</sub>O to be used in national GHG inventories.</p>
<p><b>Suggested Reviewers:</b></p>	<p>Diana Selbie AgResearch Ltd diana.selbie@agresearch.co.nz Expert on soil N<sub>2</sub>O emission, especially factor emission of N<sub>2</sub>O in grassland</p> <p>Bruno Alves Embrapa Solos bruno.alves@embrapa.br Expert on soil N dynamics GHG emissions in agricultural soils</p> <p>Dave Chadwick Bangor University d.chadwick@bangor.ac.uk Expert on soil N<sub>2</sub>O emission, especially factor emission of N<sub>2</sub>O in grassland</p> <p>Jiafa Luo AgResearch Ltd jiafa.luo@agresearch.co.nz Expert on N<sub>2</sub>O emission in livestock systems</p> <p>Coby Hoogendoorn AgResearch Ltd cobyjh@gmail.com Expert on soil N<sub>2</sub>O emission, especially factor emission of N<sub>2</sub>O in grassland</p> <p>Claudia Jantalia Embrapa Solos claudia.jantalia@embrapa.br Expert on soil N dynamics GHG emissions in agricultural soils</p> <p>Cecile de Klein AgResearch Ltd cecile.deklein@agresearch.co.nz Expert on N<sub>2</sub>O emission in livestock systems</p>
<p><b>Opposed Reviewers:</b></p>	

1        1    **TITLE PAGE**

2  
3        2

4  
5        3    **Three-year assessment of nitrous oxide emission factor from urine and dung of grazing sheep in a**  
6  
7        4    **subtropical region**

8  
9        5

10       6    Diego Fernandes de Bastos<sup>(1)</sup>, Emanuelle Cavazini Magiero<sup>(1,2)</sup>, Michely Tomazi<sup>(1,3)</sup>, Janquieli

11       7    Schirmann<sup>(1)</sup>, Murilo G. Veloso<sup>(1)</sup>, Paulo César de Faccio Carvalho<sup>(3)</sup>, Cimélio Bayer<sup>(1\*)</sup>

12  
13  
14  
15       8

16  
17       9    <sup>1</sup> Department of Soil Science and Graduate Program on Soil Science, Faculty of Agricultural and Life  
18       10    Sciences, Federal University of Rio Grande do Sul, 91540-000, 7712 Bento Goncalves Ave, Porto  
19       11    Alegre, RS, Brazil.

20       12  
21       13    <sup>2</sup> Secretaria for Rural Development and Cooperativism of Rio Grande do Sul State, 90110-000, Praia de  
22       14    Belas Ave., 1768, Porto Alegre, RS, Brazil.

23       15  
24       16    <sup>3</sup> Brazilian Agricultural Research Corporation (Embrapa Agropecuary West), 79804-970, Rod. BR 163,  
25       17    Km 253,6, Dourados, MS, Brazil

26       18  
27       19    <sup>3</sup>Grazing Ecology Research Group, Faculty of Agricultural and Life Sciences, Federal University of Rio  
28       20    Grande do Sul, 91540-000, 7712 Bento Goncalves Ave, Porto Alegre, RS, Brazil.

29       21  
30       22    \* **Corresponding Author:** [cimelio.bayer@ufrgs.br](mailto:cimelio.bayer@ufrgs.br), tel.: +55 51 3308 6017, (C. Bayer)

31       23    **Orcid**

32       24    Diego Fernandes de Bastos

33       25    Emanuelle Cavazini Magiero

34       26    Michely Tomazi - 0000-0002-3618-2403

35       27    Janquieli Schirmann - 0000-0002-8560-7949

36       28    Murilo G. Veloso - 0000-0001-5358-5768

37       29    Paulo César de Faccio Carvalho - 0000-0002-7020-3001

38       30    Cimélio Bayer - 0000-0001-8553-7330

39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## 31 **Abstract**

### 32 **Purpose**

33 Grazing livestock has strong impact on global nitrous oxide (N<sub>2</sub>O) emissions by providing N sources  
34 through excreta. The scarcity of information on factors influencing N<sub>2</sub>O 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 N<sub>2</sub>O emission  
37 factors (N<sub>2</sub>O-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<sup>-1</sup>, and the dung-N rates applied in 2009 and 2010 were 81 and 76 N kg ha<sup>-1</sup>, respectively. Air was  
42 sampled in closed static chambers (0.20 m in diameter) and analyzed for N<sub>2</sub>O by gas chromatography.

### 44 **Results and discussion**

45 Soil N<sub>2</sub>O-N fluxes spanned the ranges 4 to 353 µg m<sup>-2</sup> h<sup>-1</sup> in 2009, -47 to 976 µg m<sup>-2</sup> h<sup>-1</sup> in 2010 and 46  
46 to 339 µg m<sup>-2</sup> h<sup>-1</sup> in 2013. Urine addition resulted in N<sub>2</sub>O-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 N<sub>2</sub>O (EF-N<sub>2</sub>O, % of N applied that is emitted as N<sub>2</sub>O) 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-N<sub>2</sub>O 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-N<sub>2</sub>O for sheep urine and dung, efforts should be applied to establish regional EF-  
55 N<sub>2</sub>O 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 (N<sub>2</sub>O) emissions from the national agricultural sector (MCTI 2014), ascribed to direct  
65 deposition of animal excreta on soil. Although N<sub>2</sub>O is released in smaller amounts than carbon dioxide  
66 (CO<sub>2</sub>) the former gas is also important because its global warming potential is 298 higher than that of the  
67 latter; also, N<sub>2</sub>O has a longer atmospheric residence time than CO<sub>2</sub> (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 N<sub>2</sub>O 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 N<sub>2</sub>O 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, N<sub>2</sub>O 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 N<sub>2</sub>O production  
81 (Barneze 2013). According to Luo et al. (2008), N<sub>2</sub>O 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 N<sub>2</sub>O emissions from soil receiving urine in relation to dung (Yamulki et al.  
85 1998; Luo et al. 2009). Despite this difference, the default N<sub>2</sub>O emission factor (viz., EF-N<sub>2</sub>O, % of N  
86 applied emitted as N<sub>2</sub>O) 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-N<sub>2</sub>O 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-N<sub>2</sub>O 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-N<sub>2</sub>O. For  
92 example, evidence gathered in New Zealand has led this country to adopt EF-N<sub>2</sub>O 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-N<sub>2</sub>O for sheep urine and  
95 dung under the regional pedoclimatic conditions of Southern Brazil; (ii) confirming whether overlapped  
96 urine events increase EF-N<sub>2</sub>O; (iii) establish whether the use of a single EF-N<sub>2</sub>O for urine and dung is  
97 adequate for use national inventories of agricultural N<sub>2</sub>O emissions; and (iv) identifying the pedoclimatic  
98 variables driving soil N<sub>2</sub>O 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<sup>-1</sup> 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 m<sup>2</sup> 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  
4  
5 119 applied to 1 m<sup>2</sup> 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<sup>-2</sup>) and 7  
10  
11 122 g of dung (0.45 kg m<sup>2</sup> at 71% moisture) in each event, the excreta covering an area of radius 0.10 and  
12  
13 123 0.05 m, respectively.

14  
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<sup>-1</sup>, respectively) and one of dung (14 g, equivalent to 81 kg  
18  
19 126 N ha<sup>-1</sup>). 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<sup>-1</sup>, respectively) and one of dung (14 g, equivalent to 76 kg N ha<sup>-1</sup>). 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<sup>-1</sup>,  
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-N<sub>2</sub>O values. An additional, control treatment involving application  
28  
29 131 of no excreta was used to measure N<sub>2</sub>O emissions from the soil. The experimental design used in the  
30  
31 132 three years was of the randomized block type with three replications.

32  
33  
34 133 Excreta were collected from sheep on the ICL protocol on the day before application and their  
35  
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<sup>-1</sup>, respectively, and those  
39  
40 136 of the dung samples in 2009 and 2010 were 9 and 8.4 g N kg<sup>-1</sup>, respectively.

41  
42  
43 137 The sample volumes for each treatment were applied to the base area, the remainder being saved  
44  
45 138 for the microplot area, which was used to sample the soil in order to determine moisture, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>  
46  
47 139 -N and DOC in parallel with air sampling.

48  
49 140

## 50 51 141 *2.2 Measurement of soil N<sub>2</sub>O fluxes and emissions*

52  
53 142 In September and October of each year, soil N<sub>2</sub>O-N fluxes were monitored for approximately  
54  
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 m<sup>2</sup> was inserted down to 5 cm in the soil at the center of each plot



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 N<sub>2</sub>O 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 N<sub>2</sub> at a flow rate of 26 mL min<sup>-1</sup> as sweeping gas. N<sub>2</sub>O 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<sup>-1</sup> K<sup>-1</sup>),  $T$   
 161 (K) the temperature inside the chamber,  $M$  (g mol<sup>-1</sup>) the molar mass of the gas and  $A$  (m<sup>2</sup>) the area of the  
 162 chamber base.

163 Fluxes were all expressed relative to N<sub>2</sub>O-N and cumulative emissions calculated by integrating  
 164 the area by the curve for soil N<sub>2</sub>O-N by linear interpolation (Gomes et al. 2009) using the software  
 165 SigmaPlot v. 11.0.

166

### 167 2.3 Emission factor for N<sub>2</sub>O (EF-N<sub>2</sub>O)

168 The emission factor for N<sub>2</sub>O (EF-N<sub>2</sub>O, % N applied emitted as N<sub>2</sub>O) 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 N<sub>2</sub>O), N<sub>2</sub>O-  
172 N<sub>urine or dung</sub> the cumulative emission of N<sub>2</sub>O-N in soil after urine or dung was applied, N<sub>2</sub>O-N<sub>control</sub> the  
173 cumulative emission with the control treatment (no excreta), and N<sub>applied</sub> the amount of N applied to the  
174 soil through urine or dung.

175

#### 176 *2.4 Soil and climate variables*

177 Microplots 1 m<sup>2</sup> 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 (NO<sub>3</sub><sup>-</sup>  
179 -N) and ammonium (NH<sub>4</sub><sup>+</sup>-N), in order to examine their role on N<sub>2</sub>O 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<sup>-3</sup> (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 N<sub>2</sub>O-N  
192 fluxes and soil variables (NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-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-N<sub>2</sub>O 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 N<sub>2</sub>O-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.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 200 When Pearson's coefficient was significant, a fitted linear model was used to elucidate the response of  
2  
3 201 N<sub>2</sub>O fluxes to climate and soil variables.

4  
5 202 The EF-N<sub>2</sub>O 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  
8  
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.

14  
15 207

### 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  
20  
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  
26  
27 214 in the three years, with at least 15 mm on one day, occurred on the 5th day after application (DAA) of  
28  
29 215 the excreta.

30  
31 216

32  
33 217 *<insert Figure 1 here>*

34  
35 218

#### 36 219 *3.2 Soil N<sub>2</sub>O-N fluxes and cumulative emissions*

37  
38 220 Soil N<sub>2</sub>O-N fluxes were influenced by application of excreta, sampling day and their interaction  
39  
40 221 in both 2009, 2010 and 2013 (Table 1). N<sub>2</sub>O-N fluxes ranged from 4 to 353 µg m<sup>-2</sup> h<sup>-1</sup> in 2009, -47 to  
41  
42 222 976 µg m<sup>-2</sup> h<sup>-1</sup> in 2010 and 46.5 to 339.4 µg m<sup>-2</sup> h<sup>-1</sup> 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 N<sub>2</sub>O-N fluxes peaked at 976 µg m<sup>-2</sup> h<sup>-1</sup> 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

53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

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 N<sub>2</sub>O-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<sup>-1</sup> in 2009,  
235 0.24 to 1.55 kg ha<sup>-1</sup> in 2010 and 0.14 to 0.67 kg ha<sup>-1</sup> in 2013 (Fig. 3). Also, applying dung resulted in  
236 cumulative soil N<sub>2</sub>O-N emissions of 0.16 kg ha<sup>-1</sup> in 2009 and 0.11 kg ha<sup>-1</sup> in 2010, but without significant  
237 effect of treatment compared to the control (Table 2).

238 The increase in cumulative N<sub>2</sub>O-N emissions was linearly related to the urine rate applied each  
239 year (Fig. 3). Thus, each kg N ha<sup>-1</sup> used increased N<sub>2</sub>O-N emissions by 0.003, 0.005 and 0.002 kg ha<sup>-1</sup>  
240 in 2009, 2010 and 2013, respectively.

241

242 *<insert Figure 3 here>*

243

### 244 3.3 Emission factor of N<sub>2</sub>O (EF-N<sub>2</sub>O)

245 Urine application had no effect on EF-N<sub>2</sub>O, 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 N<sub>2</sub>O-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-N<sub>2</sub>O 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 N<sub>2</sub>O-N fluxes and

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

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  $\text{mg C L}^{-1}$  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).

276

277 *<insert Figure 4 here>*

278

## 279 **4 Discussion**

### 280 *4.1 N<sub>2</sub>O 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

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 285 al. 2003). However, this required time for N added to the soil to be converted in N<sub>2</sub>O may highly variable  
2  
3 286 depending on particular climate and soil conditions (Clough et al. 1998; Selbie et al. 2014). In this work,  
4  
5 287 however, emissions only were observed within the first few days after application. This led us to finish  
6  
7 288 sampling around 40 DAA, similarly to van Groenigen et al. (2005) in Netherlands and Lessa et al. (2014)  
8  
9 289 in Brazil, who found substantial N<sub>2</sub>O-N emissions were restricted on average of 30 days after urine  
10  
11 290 application. Our results are consistent with those of Sordi et al. (2014), who applied cattle excreta to  
12  
13 291 pasture in different seasons of Southern Brazil and found N<sub>2</sub>O-N emissions to peak on average 45 DAA  
14  
15 292 in response to urine. This result suggests that N added through urine is more rapidly converted in N<sub>2</sub>O  
16  
17 293 under subtropical conditions such as those of Southern Brazil.

19 294         The rapid increase in soil N<sub>2</sub>O-N fluxes upon excreta application is consistent with results found  
20  
21 295 by other authors (Hyde et al. 2005; Rochette et al. 2008; Mu et al. 2008) and can be ascribed to an  
22  
23 296 increased availability of soil N and to conditions (e.g. WFPS and DOC) favoring development of  
24  
25 297 microbial processes involved in the N conversion reactions occurring in soil upon excreta application  
26  
27 298 (Williams et al. 1999; Smith et al. 2003; Luo et al. 2008). Thus, nitrogen present as urea in urine is  
28  
29 299 rapidly converted into ammonium and nitrate ions, which are used by nitrifying and denitrifying bacteria  
30  
31 300 (Oenema et al. 1997). The significant ( $p < 0.05$ ) and positive relationship between N<sub>2</sub>O-N fluxes and  
32  
33 301 WFPS for the three evaluated years, and with DOC content in 2013 support the importance of these two  
34  
35 302 factors on N<sub>2</sub>O production and emission from soil. In addition, peaks of N<sub>2</sub>O-N emissions were observed  
36  
37 303 at WFPS > 60%. Increased WFPS levels facilitate the formation of anaerobiosis sites in soil, thereby  
38  
39 304 favoring denitrification (Wrage et al. 2005; Saggar et al. 2004), which is widely regarded as the most  
40  
41 305 important process behind N<sub>2</sub>O production in soil (Pimentel et al. 2015). Also, the mobility of substrates  
42  
43 306 such as nitrate ion and soluble carbon in soil is increased by an increase in WFPS level, which boosts  
44  
45 307 N<sub>2</sub>O-N emissions (Luo et al. 1999). According to Wrage et al. (2001), denitrification requires not only  
46  
47 308 adequate WFPS levels and soil nitrate contents, but also a high availability of soluble C in soil. Our  
48  
49 309 results, however, are inconclusive as to which process prevailed in N<sub>2</sub>O production. The high correlation  
50  
51 310 between N<sub>2</sub>O-N emissions and soil NH<sub>4</sub><sup>+</sup>-N contents and even with WFPS (Table 3) suggests that  
52  
53 311 nitrification could also be occurring here. N<sub>2</sub>O production by nitrification can take place even under  
54  
55 312 conditions of limited oxygen availability (WFPS > 60%), where so-called “denitrifying nitrification”  
56  
57 313 may be favored if autotrophic bacteria oxidize ammonia (NH<sub>3</sub>) to nitrite (NO<sub>2</sub><sup>-</sup>) for subsequent reduction  
58  
59  
60  
61  
62  
63  
64  
65

1 314 to N<sub>2</sub>O (Wrage et al. 2005; Koll et al. 2011). Therefore, both denitrification and denitrifying nitrification  
2  
3 315 may have occurred in parallel in our soil.

4  
5 316 The absence of substantial N<sub>2</sub>O-N emissions upon application of dung (Fig. 2) was possibly  
6  
7 317 associated to the low N availability and slow degradation providing a supply of N and other nutrients at  
8  
9 318 low levels (Haynes and Williams 1993). To some extent, this is confirmed by the small changes in soil  
10  
11 319 NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N contents due to dung application, which resulted in low difference from the control  
12  
13 320 treatment.

14  
15 321 The presence of readily available N in soil is determining to N<sub>2</sub>O-N emission (Luo et al. 2008).  
16  
17 322 N<sub>2</sub>O-N fluxes vary depending on the particular N form prevailing in soil (particularly, as noted earlier,  
18  
19 323 on NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N contents). In 2009, N<sub>2</sub>O-N fluxes were positively correlated with NH<sub>4</sub><sup>+</sup>-N and  
20  
21 324 NO<sub>3</sub><sup>-</sup>-N contents of soil (Table 3). However, the greatest emissions that year coincided with the highest  
22  
23 325 soil NH<sub>4</sub><sup>+</sup>-N contents relative to NO<sub>3</sub><sup>-</sup>-N, as well as the highest WFPS levels, which suggests that N<sub>2</sub>O  
24  
25 326 was formed preferentially by denitrifying nitrification. The positive correlation between N<sub>2</sub>O-N fluxes  
26  
27 327 and NH<sub>4</sub><sup>+</sup>-N contents but not NO<sub>3</sub><sup>-</sup>-N contents in 2013 further confirms the importance of nitrification in  
28  
29 328 the formation of N<sub>2</sub>O in soil (Table 3). Although the lack of relationship between N<sub>2</sub>O-N fluxes and  
30  
31 329 content of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N in 2010, N<sub>2</sub>O-N emissions increased due to the increase in soil content  
32  
33 330 of NH<sub>4</sub><sup>+</sup>-N (Table 3, Fig. 4b).

34  
35 331 The high NH<sub>4</sub><sup>+</sup>-N contents found may have resulted from hydrolysis of urea, main nitrogen-  
36  
37 332 containing compound in urine (Bolan et al. 2004); on the other hand, the low NO<sub>3</sub><sup>-</sup>-N contents observed  
38  
39 333 may have be associated to leaching process of this ion (Sordi et al. 2014). These assumptions are  
40  
41 334 consistent with the fact that NH<sub>4</sub><sup>+</sup>-N contents invariably exhibited strong and positive relationship with  
42  
43 335 N<sub>2</sub>O-N emissions, even at WFPS levels above 60% (Figs 2 and 4b).

44  
45 336 The increased amounts of mineral N supplied by the highest urine rates increased N<sub>2</sub>O-N fluxes  
46  
47 337 and consequently cumulative N<sub>2</sub>O emissions from soil, indicating a strong and positive relationship  
48  
49 338 between N<sub>2</sub>O emissions and amount of N supplied by urine (Fig. 3). On the other hand, cumulative N<sub>2</sub>O  
50  
51 339 emissions resulting from dung application were similar to those for the control treatment ( $p > 0.05$ ). As  
52  
53 340 discussed before, this could be resulted of slow mineralization of the organic-N present in dung, which  
54  
55 341 can take weeks to months, resulting in a gradual release of mineral-N which are uptake by grazing in a  
56  
57 342 greater proportion than urine-N (Hoekstra et al. 2011). The decreased N<sub>2</sub>O emissions in 2013 relative to  
58  
59  
60  
61  
62  
63  
64  
65

1 343 the other years may have resulted from the more frequent precipitation events over the year, especially  
2  
3 344 after urine application (Figs 1 and 2). The fact that WFPS exceeded 60% over the year may have  
4  
5 345 facilitated complete denitrification and hence favored  $N_2$  emissions over  $N_2O$  emissions. According to  
6  
7 346 Jamali et al. (2015), massive losses of mineral N resulting from leaching under heavy precipitation or in  
8  
9 347 the form of  $N_2$  can restrict N availability —and hence  $N_2O$  production— in soil, thereby diminishing  
10  
11 348  $N_2O$ -N fluxes.

12  
13 349

#### 14 350 *4.2 Emission factor of $N_2O$ (EF- $N_2O$ )*

15  
16  
17 351 EF- $N_2O$  values were not significantly influenced ( $p > 0.05$ ) by the urine application rate (Table  
18  
19 352 2), indicating that overlapping or not the urine application on the same site results in similar EF- $N_2O$ .  
20  
21 353 Our results suggest then that the estimation of soil  $N_2O$  emission from sheep urine just accounting  
22  
23 354 animals number and N excreted as a viable choice in national inventories for  $N_2O$  emission. However,  
24  
25 355 larger volumes of urine application on soil possibly impact negatively on EF- $N_2O$  in sites with  
26  
27 356 overlapping urine application as a result of urine percolation across the soil profile and N concentration  
28  
29 357 decrease in soil surface (Luo et al. 2008).

30  
31  
32 358 The mean value of EF- $N_2O$  for the three years and all urine application rates was 0.21%,  
33  
34 359 whereas that for the two years under dung application was 0.03% (Table 2). As explained above, the  
35  
36 360 decreased EF- $N_2O$  value for urine in 2013 relative to the other years was a result of small cumulative  
37  
38 361 emissions.

39  
40  
41 362 The EF- $N_2O$  values for urine and dung are similar to those found in other places in Brazil. Sordi  
42  
43 363 et al. (2014) reported a value of 0.26% for urine and 0.15% for dung under subtropical conditions. Also,  
44  
45 364 Lessa et al. (2014) found 0.7% for urine under tropical conditions (Brazilian cerrado), and Barneze et al.  
46  
47 365 (2014) 0.2% for urine in the Atlantic forest region (tropical conditions). These results suggest that the  
48  
49 366 default EF- $N_2O$  value used in IPCC's Tier 1 (viz., 1% of all N applied in animal excreta; IPCC 2013)  
50  
51 367 overestimates the actual values for some local conditions irrespective of the particular excreta applied.

52  
53 368 A comparison of the results obtained here with the two excreta reveals that urine was the main  
54  
55 369 source of  $N_2O$ -N, with emission potential more than 15 times higher than that of dung (Table 2). The  
56  
57 370 difference in  $N_2O$ -EF between two excreta suggests the need to use specific EF- $N_2O$  values for each  
58  
59 371 excreta instead of IPCC's default value (1%). In fact, using the standard value in IPCC's Tier 1  
60  
61  
62  
63  
64  
65



372 overestimated N<sub>2</sub>O 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 N<sub>2</sub>O 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 N<sub>2</sub>O-N emissions have a strong relationship with inorganic N  
 379 (particularly as NH<sub>4</sub><sup>+</sup>-N) and were facilitated by high WFPS levels (> 60%). Urine was the main source  
 380 of N in N<sub>2</sub>O emissions from soil during the grazing period. Our results indicate that the EF-N<sub>2</sub>O value in  
 381 IPCC's Tier 1 (1% of N applied) overestimates N<sub>2</sub>O emissions in grassland areas under the typical  
 382 subtropical conditions of Brazil and reinforce the needs to disaggregate the EF-N<sub>2</sub>O values for urine and  
 383 dung.

384

## 385 **Acknowledgments**

386 This work was funded by the Brazilian National Council for Scientific and Technological Development  
 387 (CNPq) and the Research Support Foundation of the Rio Grande do Sul state (FAPERGS).

388

## 389 **References**

- 390 Barneze AS, Mazzetto AM, Zani CF, Misselbrook T, Cerri CC (2014) Nitrous oxide emissions from soil  
 391 due to urine deposition by grazing cattle in Brazil. *Atmos. Environ.* **92**: 394-397. doi:  
 392 10.1016/j.atmosenv.2014.04.046
- 393 Bateman EJ, Baggs EM (2005) Contributions of nitrification and denitrification to N<sub>2</sub>O emissions from  
 394 soils at different water-filled pore space. *Biol. Fertil. Soils* **41**: 379-388. doi: 10.1007/s00374-005-0858-  
 395 3
- 396 Bolan NS, Sagggar S, Luo J, Bandrahl R, Singh J (2004) Gaseous emissions of nitrogen from grazed  
 397 pastures: Processes, measurements and modelling, environmental implications, and mitigation. *Adv*  
 398 *Agron.* **84**: 37- 120. doi: 10.1016/S0065-2113(04)84002-1.
- 399 Bremner JM (1960) Determination of nitrogen in soil by the Kjeldahl method. *Journal of Agricultural*  
 400 *Science*, **55**: 11-33.
- 401 Carter MS (2007) Contribution of nitrification and denitrification to N<sub>2</sub>O emissions from urine patches.  
 402 *Soil Biol. Biochem.* **39**: 2091-2102. doi: 10.1016/j.soilbio.2007.03.013.
- 403 Carvalho JLN, Avanzi JC, Silva MLN, Mello CR, Cerri CEP (2010) Potencial de sequestro de carbono  
 404 em diferentes biomas do Brasil. *Rev. Bras. Cienc. Solo* **34**: 277-289. doi: 10.1590/S0100-  
 405 06832010000200001
- 406 Clough TJ, Ledgard SF, Sprosen MS, Kear MJ (1998) Fate of <sup>15</sup>N labelled urine on four soil types.  
 407 *Plant Soil*, **199**: 195–203. doi: 10.1023/A:1004361009708.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 408 De Klein CAM, Sherlock RR, Cameron KC, Van Der Weerdent TJ (2001) Nitrous oxide emissions from  
409 agricultural soils in New Zealand – a review of current knowledge and directions for future research. *J.*  
410 *R. Soc. N. Z.* **31**: 543-544. doi: 10.1080/03014223.2001.9517667
- 411 De Klein CAM, Barton L, Sherlock RR, Li Z, Littlejohn RP (2003) Estimating a nitrous oxide emission  
412 factor for animal urine from some New Zealand pastoral soil. *Aust. J. Soil Res.* **41**: 381-399. doi:  
413 10.1071/SR02128.
- 414 Firestone MK, Davidson EA (1989) Microbiological basis of NO and N<sub>2</sub>O production and consumption  
415 in soil. In: Andreae M O, Schimel D S. eds. Exchange of Trace Gases between Terrestrial Ecosystems  
416 and the Atmosphere, *John Wiley & Sons* **1**: 7-21.
- 417 Gomes J, Bayer C, Costa FS, Piccolo MC, Zanatta JA, Vieira FCB, Six J (2009) Soil nitrous oxide  
418 emissions in long-term cover crops-based rotations under subtropical climate. *Soil Till. Res.* **106**: 36-44.  
419 doi: 10.1016/j.still.2009.10.001
- 420 Haynes RJ, Williams PH (1993) Nutrient cycling and soil fertility in the grazed pasture ecosystem. *Adv*  
421 *Agron* **49**: 119-199. doi: 10.1016/S0065-2113(08)60794-4
- 422 Hoekstra NJ, Lalor S, Richards KG, O’Hea N, Dungait J, Schulte, RPO, Schmidt O (2011) The fate of  
423 slurry N fractions in herbage and soil during two growing seasons following application. *Plant Soil.* **342**:  
424 83-96. doi: 10.1007/s11104-010-0669-7.
- 425 Jamali H, Quayle WC, Baldock J (2015) Reducing nitrous oxide emissions and nitrogen leaching losses  
426 from irrigated arable cropping in Australia through optimized irrigation scheduling. *Agric. For.*  
427 *Meteorol.* **208**: 32-39. doi: 10.1016/j.agrformet.2015.04.010.
- 428 Hyde B, Ryan M, Hawkins M, Connolly J, Carton OT (2005) Nitrous oxide emissions. In: End of project  
429 report RMIS 4974. Johnstown Castle Research Centre, *Wexford*. **1**: 0-26.
- 430 IBGE (2010) Instituto Brasileiro de Geografia e Estatística. Censo agropecuário. Rio de Janeiro.
- 431 IPCC (2013) Intergovernmental Panel on Climate Change - IPCC. Climate Change 2013: the physical  
432 science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental  
433 Panel on Climate Change Cambridge: Cambridge University Press.
- 434 IPCC (2007). In: S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor, H.L.  
435 Miller (Eds.), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to*  
436 *the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University  
437 Press, Cambridge, United Kingdom and New York, NY, USA [Technical Summary].
- 438 Jantalia CP, Dos Santos HP, Urquiaga S, Boddey RM, Alves BJR (2008) Fluxes of nitrous oxide from  
439 soil under different crop rotations and tillage systems in the South of Brazil. *Nutr. Cycl. Agroecosyst.* **82**:  
440 161-173. doi: 10.1007/s10705-008-9178-y.
- 441 Jarvis SC, Scholefield D, Pain B (1995) Nitrogen cycling in grazing systems. In *Nitrogen Fertilization*  
442 *in the Environment* (Ed. P. E. Bacon), 381–420.
- 443 Khalil MI, Baggs EM (2005) CH<sub>4</sub> oxidation and N<sub>2</sub>O emissions at varied soil water-filled pore spaces  
444 and headspace CH<sub>4</sub> concentrations. *Soil Biol. Biochem.* **37**: 1785-1794. doi:  
445 10.1016/j.soilbio.2005.02.012
- 446 Koll DM, Dolfing J, Wrage N, Van Groenigen JW (2011) Nitrifier denitrification as a distinct and  
447 significant source of nitrous oxide from soil. *Soil Biol. Biochem.* **43**: 174-178. doi:  
448 10.1016/j.soilbio.2010.09.030.
- 449 Lessa ACR, Madari BE, Paredes DS, Boddey RM, Urquiaga S, Jantalia CP, Alves BJR (2014) Bovine  
450 urine and dung deposited on Brazilian savannah pastures contribute differently to direct and indirect soil  
451 nitrous oxide emissions. *Agric. Ecosyst. Environ.* **190**: 104-111. doi: 10.1016/j.agee.2014.01.010.
- 452 Letica SA, De Klein CAM, Hoogendoorn CJ, Tillman RW, Littlejohn RP, Rutherford AJ (2010) Short-  
453 term measurement of N<sub>2</sub>O emissions from sheep-grazed pasture receiving increasing rates of fertilizer  
454 nitrogen in Otago, New Zealand. *Anim. Prod. Sci.* **50**: 17-24. doi: 10.1071/EA07281
- 455 Luo J, Tillman RW, Ball PR (1999) Factors regulating denitrification in a soil under pasture. *Soil Biol.*  
456 *Biochem.* **31**: 913–927. doi: 10.1016/S0038-0717(99)00013-9.

- 1 457 Luo J, Lindsey SB, Ledgard SF (2008) Nitrous oxide emissions from animal urine application on a New  
2 458 Zealand pasture. *Biol. Fertil. Soils*. **44**: 463–470. doi: 10.1007/s00374-007-0228-4
- 3 459 Luo J, Van Der Weerden T, Hoogendoorn C, De Klein C (2009) Determination of the N<sub>2</sub>O emission  
4 460 factor for animal dung applied in spring in three regions of New Zealand. Report for the Ministry of  
5 461 Agriculture and Forestry. Wellington: Ministry of Agriculture and Forestry.
- 6 462 MCTI, Ministério da Ciência Tecnologia e Inovação (2014) Estimativas anuais de emissões de gases de  
7 463 efeito estufa no Brasil - 2ª edição. MCTI, Brasília, 168. Disponível em <http://www.mct.gov.br>.
- 8 464 Mosier AR (1989) Chamber and isotope techniques. In: Andreae MO & Schimel DS ed. Exchange of  
9 465 traces gases between terrestrial ecosystems and the atmosphere: report of the Dahlem Workshop. Berlin,  
10 466 Wiley, 175-187.
- 11 467 Mu Z, Kimura SD, Toma Y, Hatano R (2008) Nitrous oxide fluxes from upland soils in central Hokkaido.  
12 468 *J. Envir. Sci.* **20**: 1312-1322. doi: 10.1016/S1001-0742(08)62227-5
- 13 469 National Research Council (NRC) (1985) Nutrient requirements of sheep. 6.ed. Washington, D.C.:  
14 470 National Academy Press, 99.
- 15 471 Oenema O, Velthof GL, Yamulki S, Jarvis SC (1997) Nitrous oxide emissions from grazed grassland.  
16 472 *Soil Use Manage.* **13**: 288–295.
- 17 473 Pimentel LG, Weiler DA, Pedroso GM, Bayer C (2015) Soil N<sub>2</sub>O emissions following cover-crop  
18 474 residues application under two soil moisture conditions. *J. Plant Nut. Soil Sci.* **178**: 631-640. doi:  
19 475 10.1002/jpln.201400392
- 20 476 Rafique R, Hennessy D, Kiely G (2011) Nitrous oxide emission from grassland under different  
21 477 management systems. *Ecosystems*. **14**: 563–582. doi: 10.1007/s10021-011-9434-x
- 22 478 Rochette P, Angers DA, Chantigny MH, Gagnon B, Bertrand N (2008) N<sub>2</sub>O fluxes in soils of contrasting  
23 479 textures fertilized with liquid and solid dairy cattle manures. *Canadian J. Soil Sci.* **88**: 175-187. doi:  
24 480 10.4141/CJSS06016
- 25 481 Saggari S, Bolan NS, Bhandral R, Hedley CB, Luo JA (2004) Review of emissions of methane, ammonia,  
26 482 and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. *New  
27 483 Zeal. J. Agr Res.* **47**: 513–544. doi: 10.1080/00288233.2004.9513618
- 28 484 Selbie DR, Cameron KC, Di HJ, Moir JL, Lanigan GJ, Richards KG (2014) The effect of urinary nitrogen  
29 485 loading rate and a nitrification inhibitor on nitrous oxide emissions from a temperate grassland soil. *J.  
30 486 Agric. Sci.* **152**: S159-S171. doi: 10.1017/S0021859614000136
- 31 487 Smith KA, Ball T, Conen F, Dobbie KE, Massheder J, Rey A (2003) Exchange of greenhouse gases  
32 488 between soil and atmosphere: interactions of soil physical factors and biological processes. *European J.  
33 489 Soils Sci.* **54**: 779–791. doi: 10.1046/j.1351-0754.2003.0567.x
- 34 490 Sordi A, Dieckow J, Bayer C, Albuquerque MA, Piva JT, Zanatta JA, Tomazi M, da Rosa CM, de Moraes  
35 491 A (2014) Nitrous oxide emission factors for urine and dung patches in subtropical Brazilian pastureland.  
36 492 *Agric. Ecosyst. Environ.* **190**: 94-103 doi: 10.1016/j.agee.2013.09.004
- 37 493 Tedesco MJ, Gianello C, Bissani CA, Bohnen H, Volkweiss SJ (1995) Análise de solo, plantas e outros  
38 494 materiais. 2 ed. Porto Alegre: Universidade Federal do Rio Grande do Sul - Departamento de Solos, 174.
- 39 495 Williams DL, Ineson P, Cowards PA (1999) Temporal variations in nitrous oxide fluxes from urine-  
40 496 affected grassland. *Soil Biol. Biochem.* **31**: 779-788. doi: 10.1016/S0038-0717(98)00186-2
- 41 497 Van der Weerden TJ, Luo J, de Klein CAM, Hoogendoorn CJ, Littlejohn RP, Rys GJ (2011)  
42 498 Disaggregating nitrous oxide emission factors for ruminant urine and dung deposited onto pastoral soil.  
43 499 *Agric. Ecosyst. Environ.* **141**: 426-436. doi: 10.1016/j.agee.2011.04.007
- 44 500 Van Groenigen JW, Velthof GL, van der Bolt FJE, Vos A, Kuikman PJ (2005) Seasonal variation in N<sub>2</sub>O  
45 501 emissions from urine patches: effects of urine concentration, soil compaction and dung. *Plant Soil.* **273**:  
46 502 15–27. doi: 10.1007/s11104-004-6261-2
- 47 503 Van Groenigen JW, Kool DM, Velthof GL, Oenema O, Kuikman PJ (2006) Mitigating N<sub>2</sub>O emissions  
48 504 from urine patches in pastures. *International Congress Series.* **1293**: 347-350. doi:  
49 505 10.1016/j.ics.2006.01.018
- 50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
- 506 Zanatta JA (2009) Emissão de óxido nitroso afetada por sistemas de manejo do solo e fontes de  
507 nitrogênio. Theses, Universidade Federal do Rio Grande do Sul.
- 508 Wrage N, Velthof GL, Van Beusichem ML (2001) Role of nitrifier denitrification in the production of  
509 nitrous oxide. *Soil Biol. Biochem.* **33**: 1723-1732. doi: 10.1016/S0038-0717(01)00096-7
- 510 Wrage N, Van Groenigen JW, Oenema O, Baggs EM (2005) A novel dua-isotope labelling method for  
511 distinguishing between soil sources of N<sub>2</sub>O. *Rapid Commun. Mass Sp.* **19**: 3298-3306. doi:  
512 10.1002/rcm.2191
- 513 Yamulki S, Jarvis SC, Owen P (1998) Nitrous oxide emissions from excreta applied in a simulated  
514 grazing pattern. *Soil Biol. Biochem.* **30**: 491-500. doi: 10.1016/S0038-0717(97)00145-4

15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

515

1 **Figures Caption**

2

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

5

6 Fig. 2. Temporal nitrous oxide (N<sub>2</sub>O) 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 (N<sub>2</sub>O) emissions in 2009, 2010 and 2013 trials.

11

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 (NH<sub>4</sub><sup>+</sup>-N); (C) Soil nitrate  
14 (NO<sub>3</sub><sup>-</sup>-N); (D) Dissolved organic carbon. Vertical bars represent the mean standard error.

15

16

17

18

19

20

21

22

23

24

25

26

27

28

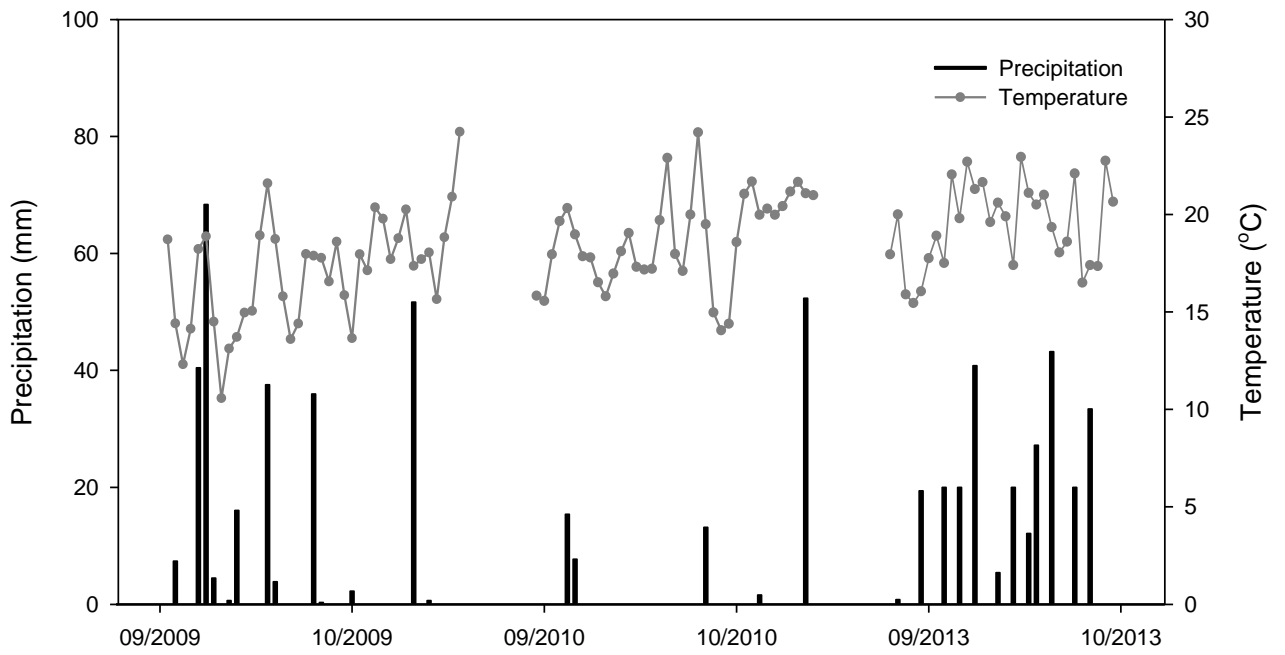
29

30

31

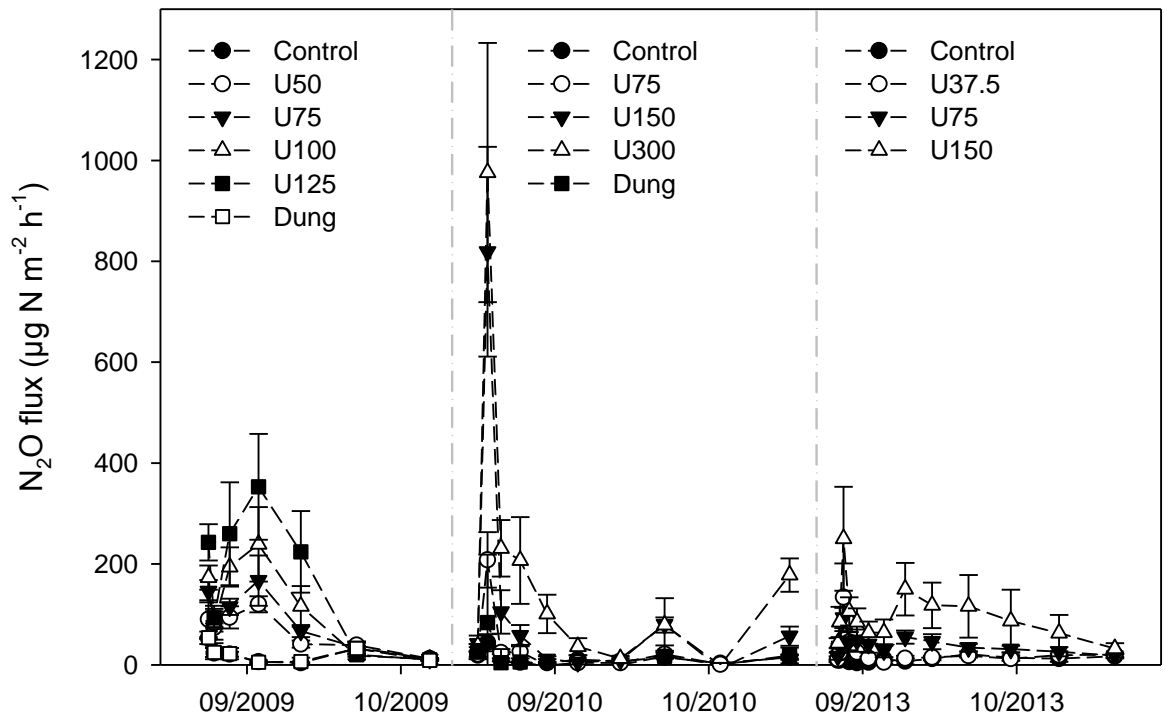
32

33



**Fig. 1.**

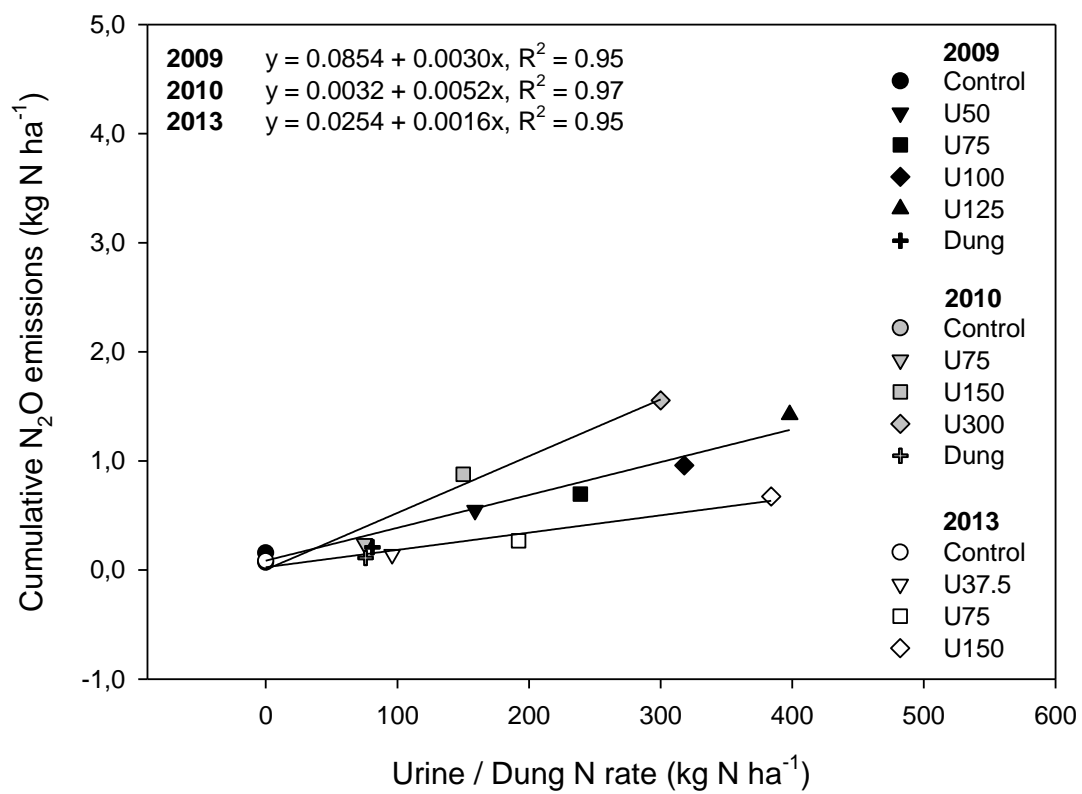
34  
 35  
 36  
 37  
 38  
 39  
 40  
 41  
 42  
 43  
 44  
 45  
 46  
 47  
 48



49  
 50  
 51  
 52  
 53  
 54  
 55  
 56  
 57  
 58  
 59  
 60  
 61

**Fig. 2.**





62

63 **Fig. 3.**

64

65

66

67

68

69

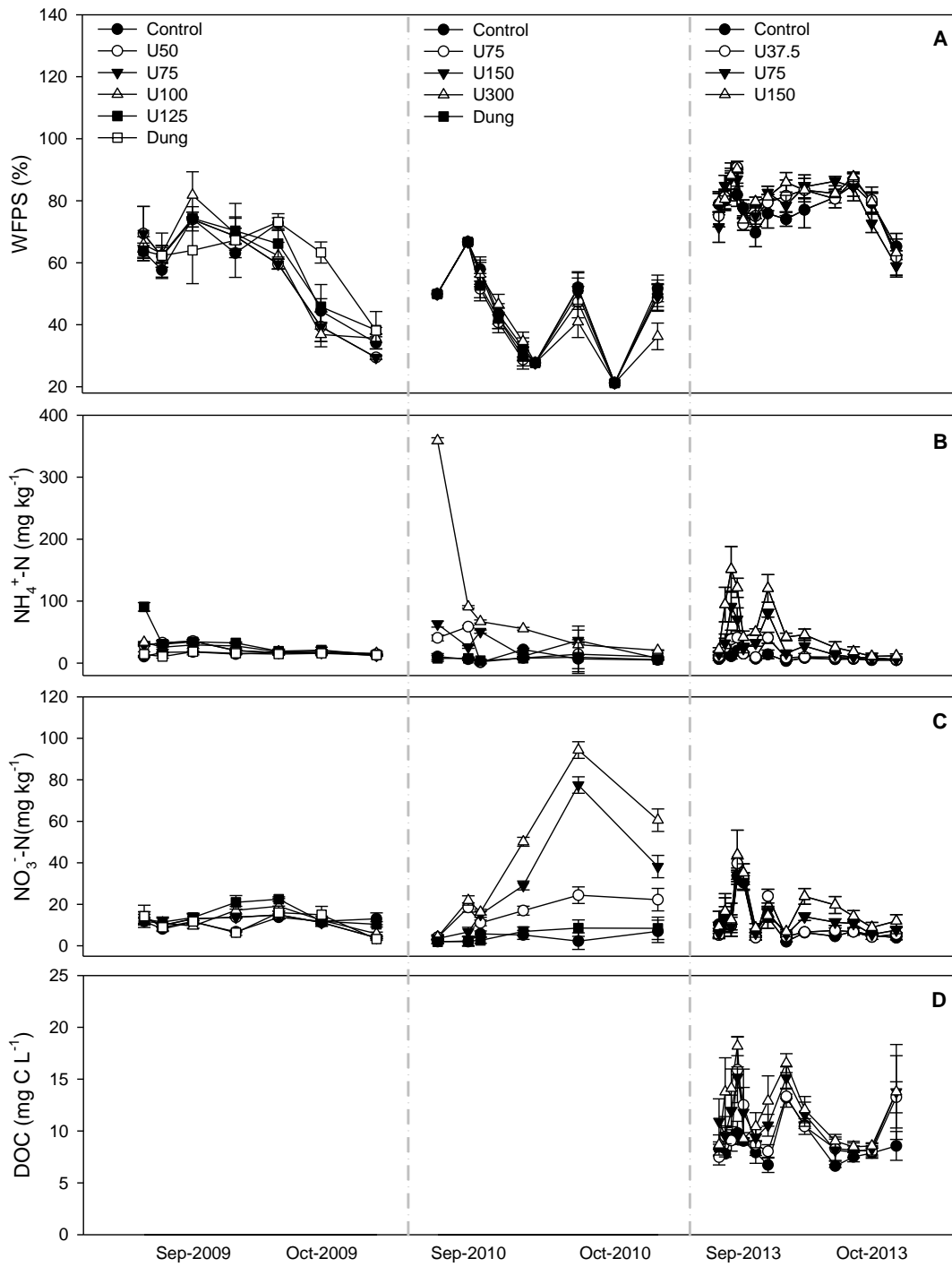
70

71

72

73

74



**Fig. 4.**

75  
76  
77

Table 1. Analysis of variance on the N<sub>2</sub>O fluxes, cumulative emissions and soil variables (WFPS, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-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 <sub>2</sub> 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 <sub>2</sub> 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 <sub>4</sub> <sup>+</sup> -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 <sub>3</sub> <sup>-</sup> -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 N<sub>2</sub>O and emission factor (EF) for urine doses and dung, applied in three different year trials.

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

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 N<sub>2</sub>O fluxes with soil variables (ammonium – NH<sub>4</sub><sup>+</sup>-N, nitrate – NO<sub>3</sub><sup>-</sup>-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 <sub>2</sub> O fluxes	
			Coefficient	<i>p</i> -value
2009	NH <sub>4</sub> <sup>+</sup> -N	$y = -34.12 + 5.72x$	0.53	<0.0001
	NO <sub>3</sub> <sup>-</sup> -N	$y = -79.78 + 15.29x$	0.49	<0.0001
	WFPS	$y = -104.81 + 3.37x$	0.48	0.0003
2010	NH <sub>4</sub> <sup>+</sup> -N	$y = 106.65 + 0.44x$	0.02	0.5092
	NO <sub>3</sub> <sup>-</sup> -N	$y = 117.66 + 0.26x$	0.001	0.8902
	WFPS	$y = -237.24 + 7.70x$	0.47	0.0016
2013	NH <sub>4</sub> <sup>+</sup> -N	$y = 11.74 + 1.13x$	0.51	<0.0001
	NO <sub>3</sub> <sup>-</sup> -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