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Nitrous oxide emission factor from cattle urine and dung in native grassland of the Pampa biome, South Brazil

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Nitrous oxide emission factor from cattle urine and dung in native grassland of the Pampa biome, Southern Brazil

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1 **TITLE PAGE**

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4

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20 SUMMARY TEXT FOR THE TABLE OF CONTENTS

21 Cattle excreta are the main source of nitrous oxide in world grasslands. The emissions were
22 assessed in subtropical native grasslands and we verified that emission factor for dung was
23 approximately one-tenth lower than that for urine (0.08% vs 0.74%). Our findings highlight
24 that both were much lower than the default 2% of IPCC's Tier 1, which needs to be revised
25 aiming to avoid overestimation in national inventories of greenhouse gases.

26

For Review Only

27 **Nitrous oxide emission factor from cattle urine and dung in native grassland of the**
28 **Pampa biome, Southern Brazil**

29

30 **Abstract**

31 Native grassland supports extensive livestock production in the Pampas, South America, but
32 the impact of cattle excreta on nitrous oxide (N₂O) emissions remains unknown in this biome.
33 Aiming to determine the N₂O emission factor (EF-N₂O, % of N applied that is emitted as
34 N₂O) for urine and dung from beef cattle grazing on native grassland, we conducted a field
35 study under low and moderate forage allowances (FA4 and FA12; 4 and 12 kg DM/100 kg
36 live weight, respectively) during the 30th year of a long-term grassland experiment on a Typic
37 Paleudult soil in Southern Brazil. Urine and dung were applied onto separate patches, at rates
38 equivalent to one average urination or defecation; and N₂O fluxes were monitored with closed
39 static chambers over 338 days. In adjacent microplots, water-filled pore space (WFPS),
40 nitrate, ammonium and extractable dissolved organic carbon (DOC) were monitored in the top
41 0.1 m soil. Averaged across the forage allowances, daily N₂O fluxes were low in soil without
42 excreta (1.3 g N ha⁻¹), but increased upon application of dung (3.8 g N ha⁻¹) and, especially,
43 of urine (66 g N ha⁻¹). The annual N₂O emission and the EF-N₂O for urine were greater under
44 FA12 than FA4; but for dung such difference was not observed. The positive relationship
45 between N₂O emission and both soil ammonium and nitrate intensities did not allow the
46 identification of the main microbial processes involved in N₂O production, but the higher
47 slope coefficient of the linear regression with nitrate (0.37) than with ammonium (0.19)
48 suggests that denitrification was more prominent to N₂O production than nitrification and
49 nitrifier denitrification. On average, the EF-N₂O was almost 10-times higher for urine than for
50 dung (0.74% vs 0.08%), being both much lower than the default value of 2% of IPCC's Tier
51 1. The results reinforce the needs of disaggregating the EF-N₂O for urine and dung, and of
52 revising the IPCC's Tier 1 EF-N₂O considered in national inventories of livestock greenhouse
53 gas emissions.

54

55 **Additional Keywords:** cattle excreta, N₂O, subtropical, livestock, forage allowance.

56

57

58

59 Introduction

60 The Pampa biome covers 750.000 km² including Uruguay, Northern Argentina and Southern
61 Brazil. In Brazil, the native grasslands of Pampa biome support a grazing herd of 13 million
62 cattle (Carvalho and Batello 2009), with impacts on ecosystem nitrogen cycling. Cattle have a
63 very low nitrogen (N) use efficiency, releasing 90–95% of their N intake through N-enriched
64 excreta (Whitehead 2000), whose deposition onto soil can supply rates equivalent to 2000 kg
65 N ha⁻¹. That exceeds the N uptake capacity of plants and rapidly increases the concentration
66 of soil inorganic N, leading to N₂O production by nitrification, nitrifier denitrification or
67 denitrification (Oenema *et al.* 1997). Because N-fertilizer is not applied in most of Pampa's
68 grasslands, cattle excreta constitute the main source of N for N₂O production in soils of this
69 biome.

70 Although animal excreta contribute with approximately 30% of the soil N₂O emissions
71 in Brazil (Brazil 2016), the country has no specific emission factor of nitrous oxide (EF-N₂O,
72 % of N applied that is emitted as N₂O) for urine and dung. The Brazilian national greenhouse
73 gas inventory is still based on the default EF-N₂O of 2% recommended in IPCC's Tier 1
74 (IPCC 2006). However, this default 2% is derived from studies carried out mainly in
75 temperate environments (De Klein 2004; IPCC 2006), and so its suitability for tropical or
76 subtropical conditions of Brazil is uncertain and should be investigated. Also questionable is
77 its indiscriminate use for both urine and dung. Recent studies conducted in subtropical Brazil
78 suggested that the EF-N₂O for cattle urine and dung is less than 1% (Sordi *et al.* 2014; Simon
79 *et al.* 2018). The same studies also showed that the EF-N₂O was lower for dung relative to
80 urine, suggesting that those two excreta types should have disentangled EF-N₂O in national
81 and regional greenhouse gas (GHG) inventories.

82 Grazing on native grassland of the Pampa biome is usually intense, with high stocking
83 rate and low forage allowances (Modernel *et al.* 2016) that can impair soil properties

84 (Taboada *et al.* 2011), alter the botanical composition of forage (Cruz *et al.* 2010), interfere
85 with the C and N cycles (Piñeiro *et al.* 2010), the microbial population (Bardgett *et al.* 1998)
86 and activity of nitrifying and denitrifying bacteria (Patra *et al.* 2005), all of which impacting
87 soil N₂O production.

88 The scarcity of information about N₂O emissions from cattle excreta deposited on
89 native grassland in the Pampa biome led us to determine the EF-N₂O for urine and dung from
90 beef cattle on a subtropical Typic Paleudult in southern Brazil. Our hypotheses were: (a)
91 native grassland under a high stocking rate (i.e., a low forage allowance) leads to increased
92 N₂O emissions from excreta; (b) the default EF-N₂O of 2% in IPCC's Tier 1 overestimates
93 the N₂O-N emissions from cattle excreta in the target production system; (c) EF-N₂O is
94 greater for urine than it is for dung.

95

96 **Material and Methods**

97 *Experimental site*

98 The study was conducted during the 30th year of a long-term experiment on the native
99 grassland of the Pampa biome, at the experimental station of the Federal University of Rio
100 Grande do Sul (30°05'27" S and 51°40'18" W), Eldorado do Sul-RS, Southern Brazil. The
101 local climate is humid subtropical (Cfa in Köppen's classification), with annual precipitation
102 of 1455 mm well distributed throughout the months and with mean annual temperature of
103 18.8 °C (Bergamaschi *et al.* 2013). The soil is classified as Typic Paleudult according to Soil
104 Taxonomy, and as sandy clay loam Acrisol according to WRB/FAO.

105 The experiment represents an extensive system of livestock production, so that soil
106 was not amended with lime nor fertilizer, and vegetation was not mowed since the experiment
107 implantation in 1986. The native grassland was grazed by heifers on continuous grazing for
108 30 years (1986–2016), under forage allowances of 4, 8, 12 or 16 kg dry matter/100 kg animal

109 live weight; being the stocking accordingly adjusted. Only forage allowances of 4 and 12 kg
110 dry matter/100 kg live weight (FA4 and FA12) were used in this study, to represent intensive
111 and moderate grazing levels, respectively. In FA4, the homogeneous creeping vegetation of
112 prostate or rhizomatous growth habit comprised essentially *Paspalum*, *Piptochaetium* and
113 *Andropogon* grasses. In FA12, a lower vegetation stratum of *Paspalum*, *Axonopus* and
114 *Piptochaetium* interacted with an upper stratum of *Andropogon* and *Aristida* tussocks (Cruz et
115 al., 2010). The main soil characteristics of top 0-0.2 m layer under FA4 and FA12 are
116 presented in Table 1.

117

118 *Treatments and experimental design*

119 Each of the two forage allowances was combined with three cattle excreta treatments: (a)
120 control, without excreta; (b) deposition of urine; and (c) deposition of dung. Treatments were
121 arranged in a randomized block design, with 3 replicates, and were applied in a 5 × 5 m area
122 fenced in each plot of FA4 and FA12. Grazing was simulated with manual cutting and plant
123 material removal. Urine and dung were collected from heifers (aged 1.6 years) grazing
124 exclusively on native grassland, under the same respective FA, and were stored at 4 °C.
125 Excreta were applied onto the soil surface inside circular metal collars of 0.30 m diameter, in
126 October 10, 2013. Urine was applied at a rate of 1 L per patch (average of 17 urinations) and
127 dung at a rate of 1.8 kg of fresh weight per patch (331 g dry matter, average of 6 defecations).
128 The N concentration in urine and dung was determined by Kjeldahl method (Keeney and
129 Nelson 1982), while the C concentration in dung and the extractable dissolved organic carbon
130 (DOC) concentration in urine were determined by dry combustion in a Shimadzu TOC-VCSH
131 analyzer (Nelson and Sommers 1982). Urine N concentration was 6.5 g L⁻¹ (Table 2), lower
132 than the range of 6.7-15 g L⁻¹ reported by Whitehead (1970) and Chadwick *et al.* (2018), but
133 within the 1-20 g L⁻¹ range (average 7.2 to beef cattle) reported by Selbie *et al.* (2015). Dung

134 N concentration was 12.8 g kg^{-1} (Table 2), which is comparable to the range of $12\text{-}40 \text{ g kg}^{-1}$
135 reported by Whitehead (1970) and $3.4\text{-}48 \text{ g kg}^{-1}$ reported by Chadwick *et al.* (2018). The
136 characteristics of urine and dung and total N loading in the treatments are present in Table 2.

137

138 *Air sampling and chromatographic determination of N_2O*

139 Nitrous oxide fluxes were measured by using closed static chambers (Mosier *et al.* 2006) of
140 $0.3 \text{ m high} \times 0.3 \text{ m diameter}$, made of polyvinyl chloride, deployed on the circular metal
141 collars that were anchored 0.05 m in the soil during the whole measurement period. Each
142 chamber was water-sealed in a gutter at the top of the collars. In treatment FA12, the collars
143 were placed between tussocks, on the native grassland lower stratum.

144 Immediately after application of the excreta, we started the monitoring of $\text{N}_2\text{O-N}$
145 fluxes, which lasted 338 days. The interval between air-sampling sessions was 2 days until the
146 16th day after application, then 7 days until the 92th day after application, and 15 days
147 afterwards. In each sampling session, air samples were collected from 09:00 to 11:00 am by
148 using 20 mL polypropylene syringes, at 0, 15, 30 and 45 min after the chamber closure.
149 Chambers were equipped with an internal fan, to homogenize the headspace air in the 30 s
150 before sample removal, and with a digital thermometer. Air samples were stored in pre-
151 evacuated 12 mL vials (LABCO Extainers[®]) and analyzed for N_2O by gas chromatography on
152 a Shimadzu GC-2014 instrument equipped with an electron capture detector (ECD). The
153 temperature was set at $325 \text{ }^\circ\text{C}$ and N_2 used as carrier gas.

154 The $\text{N}_2\text{O-N}$ fluxes ($\text{g ha}^{-1} \text{ day}^{-1}$) (Eq. 1) were calculated according to changes in gas
155 concentration in the chamber headspace during the deployment period ($\Delta C/\Delta t$), to atmospheric
156 pressure within the chamber (P , 1 atm), to chamber volume (V , 0.0212 m^3), to gas constant
157 (R , $0.08205 \text{ atm mol}^{-1} \text{ k}^{-1}$), to temperature within the chamber (T , kelvin), to gas molar mass

158 (M, g mol⁻¹), to soil area covered by the chamber (A, 0.049 m²), and for the N/N₂O (14/44)
 159 molecular ratio:

$$160 \quad N_2O - N = \frac{\Delta C}{\Delta t} \times \times \frac{M}{A} \times \frac{N}{N_2O} \quad \text{Eq. 1}$$

161 Annual cumulative N₂O-N emissions were calculated by trapezoidal integration of the
 162 daily N₂O-N fluxes, assuming that the gaseous flux by 09:00 to 11:00 a.m. represents the
 163 average daily flux (Bayer *et al.* 2016).

164 The N₂O emission factor (EF-N₂O) for urine or dung, i.e. the percentage of the applied
 165 N emitted as N₂O, was calculated according to Eq. 2 (De Klein *et al.* 2003):

$$166 \quad EF - N_2O = \frac{N_2O - N_{urine\ or\ dung} - N_2O - N_{control}}{N_{applied}} \times 100 \quad \text{Eq. 2}$$

167 where EF-N₂O is the emission factor (% of N applied as urine or dung released as N₂O), **N₂**
 168 **O - N_{urine or dung}** cumulative N₂O-N emission (kg N ha⁻¹) from urine or dung, **N₂**
 169 **O - N_{control}** that from the control plots, and **N_{applied}** the N rate applied as urine or dung (kg
 170 N ha⁻¹).

171

172 *Soil properties*

173 Ammonium (NH₄⁺-N), nitrate (NO₃⁻-N), extractable dissolved organic carbon (DOC), and
 174 water-filled pore space (WFPS) in the 0–0.1 m soil layer were monitored by each air sampling
 175 session, during 92 days after application of the excreta. Soil samples were collected with a
 176 stainless steel auger (3 cm diameter) from a rectangular microplot of 0.5 × 1.0 m size that was
 177 installed adjacent to each metal collar and subjected to the same treatment.

178 Gravimetric soil moisture content was determined by oven drying (105 °C for 48 h).

179 Soil NH₄⁺-N and NO₃⁻-N were extracted with 1 M KCl [ratio 1:10 (m/v)] shaken for 30 min.

180 After decantation for 30 minutes, the supernatant was collected and kept frozen until analysis.

181 Inorganic N in the supernatant was quantified by distillation, with sequential addition of MgO

182 and Devarda's alloy, and titration with H₂SO₄ (Keeney and Nelson 1982). Soil DOC was

183 extracted by shaking 10 g of field-moist soil in 20 mL of CaCl₂ 5mM, for 1 minute. After
184 decantation for 30 minutes, the supernatant was filtered through regenerated cellulose
185 membrane filter (0.4 µm) in a vacuum pump (Chantigny *et al.* 2008). The DOC concentration
186 was determined by dry combustion using Shimadzu TOC-VCSH Analyser. NO₃⁻, NH₄⁺ and
187 DOC intensity (kg ha⁻¹ day⁻¹) was determined by trapezoidal interpolation of the NH₄⁺-N,
188 NO₃⁻-N and extractable DOC concentration in the sampling period (92 days) (Zebarth *et al.*
189 2012). WFPS was calculated as the ratio between the volumetric water and total soil porosity,
190 that was estimated from the soil density assuming soil particle density of 2.65 g cm⁻³ (Paul
191 2007).

192

193 *Statistical analyses*

194 After confirmed the normality of data by means of the Kolmogorov-Smirnov test and the
195 homogeneous experimental errors by Levene test, we performed a joint analysis of
196 experiments, considering the effects of forage allowance (*FA*) and excreta (*E*) on the
197 response variables using the MIXED procedure. This procedure considers the main factors
198 and their interactions as fixed factors and the block variable and the experimental errors as
199 random variables. The data of annual N₂O-N emission and EF-N₂O-N were subjected to
200 analysis of variance (ANOVA) and, significant results ($p < 0.05$) were compared using the
201 Tukey test ($p < 0.05$). The statistical model used in the analysis of variance to evaluate annual
202 N₂O-N emission and EF-N₂O-N was as follows:

$$203 \quad Y_{ijk} = \mu + FA_i + E_j + FA_iE_j + B_k + \text{Error}(ij)k$$

204 where μ denotes the overall mean of the experiment; B denotes block ($k = 1, 2, 3$); $FA =$
205 forage allowance ($j = 1, 2$); E denotes excreta type ($k = 1, 2, 3$) and Error denotes
206 experimental error.

207 The relationship between soil variables and N₂O-N emissions was evaluated by the
208 significance of determination coefficient (r^2) of linear equations. All analyses were performed
209 with the software SAS v. 9.4 (SAS Institute for Advanced Analytics, Cary, NC, USA).

210

211 **Results and Discussion**

212 *Soil N₂O fluxes and cumulative emissions*

213 Soil N₂O fluxes from dung pats (averaged 3.8 g N ha⁻¹ day⁻¹) were similar to those from
214 control soil, but urine increased fluxes considerably, to an average of 66 g N ha⁻¹ day⁻¹. This
215 result is consistent with previous reports and can be attributed to differences in the N form
216 between the two excreta (Oenema *et al.* 1997; Yamulki *et al.* 1998), to the greater percolation
217 of urine N in soil (Van Der Weerden *et al.* 2011) and to the lower equivalent rate of N loading
218 in dung (Rochette *et al.* 2014; Sordi *et al.* 2014).

219 Nitrogen in urine is present mainly as urea, which is highly soluble in soil and quickly
220 undergoes hydrolysis and ammonification to increase soil NH₄⁺ contents (Fig. 2). On the other
221 hand, the insoluble organic N forms in dung (Oenema *et al.* 1997; Van Der Weerden *et al.*
222 2011) are slowly mineralized by soil microbiota, hardly altering NH₄⁺ contents (Fig. 2). The
223 slow mineralization of dung N may be associated to its relatively high C/N ratio (31.8). The
224 mineralization of organic materials with high C/N ratio is usually slower because it depends
225 on the availability of mineral N in soil, which is used by microbes to decompose low-quality
226 plant debris (Paul 2007). The high C/N ratio of dung might be related to animals feeding
227 exclusively on native grassland, which contains low N content (6.9-8.7% crude protein or 1.1-
228 1.4% N, Moojen and Maraschin 2002). Previous study also shows that dung from animals fed
229 on extensive pasture in Brazil, where fertilization is rarely practiced, had low N
230 concentrations and high chemical recalcitrance (Lessa *et al.* 2014).

231 The greater contact of urine with soil owing to percolation also accelerates N
232 transformations and favors N₂O production. On the other hand, dung has limited, surface-only
233 contact with soil, which results in slow mineralization and ammonification of N, and then in
234 lower N₂O fluxes (Oenema *et al.* 1997; Simon *et al.* 2018). Rainfall after dung deposition
235 might promote percolation of manure-compounds into the soil (Van Der Weerden *et al.* 2011)
236 and then N mineralization; but, in our study, the earliest rainfall occurred almost two weeks
237 after manure deposition (Fig. 1), when the dung patches were partially dry and covered by a
238 surface crust.

239 Our hypothesis that N₂O emissions increases with high stocking rate (low forage
240 allowance) was not confirmed. The average daily flux of N₂O from urine was about 20%
241 higher under FA12 (73.1 g N ha⁻¹) than under FA4 (58.1 g N ha⁻¹). The N₂O fluxes in urine
242 patches increased gradually after a short period of 4 days under FA4, peaked at 14 and 24
243 days and amounted 292 and 623 g N ha⁻¹ day⁻¹, respectively (Figure 1). While under FA12,
244 the N₂O fluxes took 14 days to rise, peaked at 24 days after urine application and amounted
245 900 g N ha⁻¹ day⁻¹. The earlier N₂O flux in FA4 may be related with the impact of high
246 grazing intensity on soil, which can have larger populations of nitrifying and denitrifying
247 bacteria (Le Roux *et al.* 2003; Patra *et al.* 2005) resulting from the increased amounts of labile
248 substrate produced by more frequent plant defoliation and root exudation (Bardget *et al.*
249 1998). Moreover, the soil bulk density as affected by FA may have also influenced the
250 dynamics and quantity of N₂O emitted. Studies had observed a delay in N₂O production
251 followed by a strong peak when soil is compacted (Van Groening *et al.* 2005a; Bhandral *et al.*
252 2007), as observed in FA12. The soil compaction in FA12 might have hindered the
253 nitrification process (Figure 2), thus retarding N₂O emission. Some studies showed that
254 nitrification of NH₄⁺ was slowed down due to compaction (De Neve and Hoffmann 2002;
255 Bhandral *et al.* 2007). Furthermore, an increased bulk soil density reduces soil porosity and

256 gas diffusivity, increasing the activity of anaerobic microorganisms and facilitating N₂O
257 production (Van Groenigen *et al.* 2005a). Usually, a higher stocking rate increases soil bulk
258 density (Taboada *et al.* 2011), but in our study the moderate stocking rate in FA12 led to the
259 highest density (1.52 g cm⁻³) in 0–0.05 m soil layer relative to FA4 (1.43 g cm⁻³). This
260 apparent contradiction in FA12 may have resulted from the presence of tussocks covering
261 29% of the grazing area (Da Trindade *et al.* 2012) and forcing animals to trample between
262 them. Additionally, the lower bulk density under FA4 could be related to greater root growth
263 in the top layer relative to FA12 (López-Mársico *et al.* 2015).

264 There was no correlations between daily soil N₂O-N fluxes and NH₄⁺-N ($r^2=0.04$;
265 $p<0.05$), NO₃⁻-N ($r^2=0.02$; $p=0.07$), DOC ($r^2=0.02$; $p=0.10$) or WFPS ($r^2=0.07$; $p<0.01$) after
266 excreta application. The lack of relationship between N₂O fluxes and soil attributes is a
267 generally common result (Anger *et al.* 2003) and may have two reasons. First, the soil
268 sampling methods are poor at discriminating the microsites/hotspots where N₂O are in fact
269 produced (Velthof *et al.* 1996); and, second, in some occasions N₂O production may be
270 favored by one factor but hindered by another, resulting in poor relationship between N₂O
271 fluxes and soil variables (Dobbie and Smith 2003).

272 Both reasons were softened by estimating weighted averages for fluxes and soil
273 properties. Integrated temporal measurements of N₂O fluxes and soil variables are known as
274 “intensity” or “exposure” of the variables (Zebarth *et al.* 2012). In this approach, cumulative
275 N₂O-N emission during the 92 days period were positively correlated to NH₄⁺-N ($r^2 = 0.99$),
276 NO₃⁻-N ($r^2 = 0.95$) or DOC intensities ($r^2 = 0.77$) (Fig. 3). The relationship of N₂O-N
277 emissions with NH₄⁺-N and with NO₃⁻-N intensities suggests that N₂O was produced
278 concurrently by nitrification, nitrifier/denitrification and denitrification. However, the higher
279 angular coefficient of the linear regression of cumulative N₂O-N emissions with nitrate
280 ($a=0.37$) than with ammonium ($a=0.19$) suggests that denitrification was more prominent to

281 N₂O production than nitrification and nitrifier denitrification (Fig. 3) in this oxidative and
282 well drained environment.

283 The extractable DOC intensity after urine application was similar in both forage
284 allowances (Fig. 3), but cumulative N₂O emissions from soil were greater under FA12 than
285 FA4 (Table 4). This suggests the potential influence of alternative factors on N₂O emissions.
286 Weier *et al.* (1993) found that, in soil where C availability and WFPS are not limiting factors,
287 NO₃⁻-N was the factor driving N₂O production and the N₂/N₂O ratio. Thus, the greater the
288 soil NO₃⁻-N content, the greater was N₂O emission relative to N₂ and thus, the lower was the
289 N₂/N₂O ratio. In our study, the increased intensity of the NO₃⁻ content under FA12 may thus
290 have boosted N₂O emission through a decreased N₂/N₂O ratio.

291 The annual N₂O emissions for urine were greater under FA12 than FA4, but
292 essentially identical under both forage allowances for dung (Table 3). Under FA12 the soil
293 released 12.38 kg N ha⁻¹ yr⁻¹, which is 4 kg ha⁻¹ yr⁻¹ more than under FA4 (Table 4). Our
294 results contradicted Cardoso *et al.* (2017), which observed grazing intensity had a negative
295 linear effect on annual cumulative N₂O emissions in tropical grassland. However, our results
296 are in line with Yan *et al.* (2016) that demonstrated increasing grazing rate decreased soil N₂O
297 fluxes. Some possible reasons could explain our results: (1) The higher soil compaction in
298 FA12 than FA4, as previously explained; (2) The lower availability of mineral-N in the soil of
299 FA4 compared to FA12 (Figure 3). Xu *et al.* (2008) also observed long-term of intensive
300 grazing reduced soil nitrate concentrations. Possibly, there is a higher plant and microbial N
301 competition in FA4, due to the frequent plant defoliation by grazing and the lower N organic
302 content in the soil than FA12 (Table 1).

303 Soil N₂O emissions for dung was statically similar to the control treatment (1.13 vs
304 0.41 kg N ha⁻¹ yr⁻¹ on average) (Table 4). The low emissions with the control treatment is
305 consistent with the results of other studies on unfertilized pasture and related to the low

306 availability of soil inorganic N (Rochette *et al.* 2014), and the prevalence of grasses with thick
307 roots and containing biomass with a high C/N ratio (Glatzel and Stahr 2001). The low N₂O
308 emissions for dung can also be ascribed to a low availability of N as a result of the
309 composition of the excreta and, as noted earlier, the limited contact of the solid excreta with
310 the soil.

311

312 *N₂O emission factors for bovine urine and dung*

313 The EF-N₂O for urine was greater under FA12 than under FA4 (0.90 vs 0.58%), but did not
314 differ under both forage allowances for dung (Table 4). As stated earlier, the higher soil bulk
315 density under FA12 may have resulted in the increased EF for urine relative to FA4. Van
316 Groenigen *et al.* (2005 a, b) observed that soil compaction significantly affected EF-N₂O from
317 urine, raising it by a factor of 2.2-5.4 times, but did not consistently effected the EF-N₂O from
318 dung. Although EF-N₂O was higher in FA12 than FA4, that does not imply that FA12 should
319 not be used in native grassland in Pampa Biome. Other aspects of FA12, like better indices of
320 animal productivity (Carvalho *et al.* 2015), higher soil organic C stock (Modernel *et al.* 2016)
321 and floristic diversity (Cruz *et al.* 2010), should be pondered.

322 The average N₂O emission factors for the two forage allowances with urine was
323 0.74%, which is roughly 9 times greater than the average value for dung (0.08%, Table 4). As
324 previously suggested by other authors (Lessa *et al.* 2014; Rochette *et al.* 2014; Sordi *et al.*
325 2014; Van Der Weerden *et al.* 2011), these results reinforce the needs of disaggregation of
326 EF-N₂O for urine and dung. Our EF-N₂O values fall within the ranges of previously reported
327 values for urine (0.2-3.6%) and dung (0.10-0.18%) in Brazilian subtropical and tropical
328 pastures (Sordi *et al.* 2014; Lessa *et al.* 2014; Barneze *et al.* 2014; Mazzeto *et al.* 2015;
329 Cardoso *et al.* 2016) and are much lower than the default value of 2% of IPCC's Tier 1 for
330 cattle excreta (IPCC, 2006). In addition to disaggregation of EF-N₂O for urine and dung, the

331 lower EF-N₂O for animal excreta than IPCC's Tier 1 seems to be a global trend and not a
332 exclusivity for tropical and subtropical pastures of Brazil, reinforcing the needs of revision of
333 values to be adopted in developing national inventories of greenhouse gas emissions from
334 agricultural soils.

335 The IPCC does not state the N₂O sampling time to be used in order to estimate
336 emission factors. According to Bouwman (1996), soil N₂O fluxes should be monitored for at
337 least 1 year after application of the N source for reliable estimates to be obtained. Klein *et al.*
338 (2003) highlights the influence of soil type, soil drainage and region on N₂O emissions and
339 recommend measurements continue until N₂O emissions return to background levels. In
340 general, N₂O emissions on well-drained soils returning to background levels within few
341 months after urine application (Krol *et al.* 2016). In our study, we simulate the influence of
342 the sampling time on EF-N₂O by examining the N₂O fluxes from urine as measured over a
343 period of 338 days and found that 3 months and 20 days is adequate to obtain an EF-N₂O that
344 was equivalent to 95% of the value achieved considering the whole sampling period (Figure
345 4). Dung required a monitoring of 7 months and 20 days in order to obtain a similarly close
346 EF-N₂O value. The longer time needed for dung was a result of slower decomposition and
347 mineralization of N in the soil compared to urine.

348

349 **Conclusions**

350 Urine deposition on soil is the main source of N₂O-N emissions in unfertilized native
351 grassland in the Pampa biome. The N₂O emission factor for urine (0.74%) is much higher
352 than for dung (0.08%), and both are much lower than the default value of 2% of IPCC's Tier
353 1. These results reinforce the needs of disaggregation of EF-N₂O for the two excreta types
354 and to consolidate regional emission factor values for use in national inventories of
355 greenhouse gas from agricultural soils. Contrary to our hypothesis, cumulative N₂O

356 emissions and EF-N₂O values were greater under moderate than low forage allowance, what
357 was possibly related to soil bulk density and soil N content.

358

359 **Conflicts of interest**

360 The authors declare no conflicts of interest.

361

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366 01.10.06.001.08.00).

367

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1 Figure Captions**2**

3 Fig. 1. Soil nitrous oxide (N₂O) fluxes, daily rainfall and mean daily air temperature
4 during 338 days after cattle urine and dung application to a Typic Paleudult soil under
5 native grassland with two forage allowances (FA4 and FA12, respectively, 4 and 12 kg
6 dry matter/100 kg live weight) in the Pampa Biome, Southern Brazil. The vertical bars
7 represent least significant differences as per Tukey's test ($P = 0.05$). Control treatment:
8 no excreta.

9

10 Fig. 2. Water-filled porosity space (WFPS) and soil contents in extractable dissolved
11 organic carbon (DOC), ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N) during 92 days after
12 cattle urine and dung application to the 0–0.10 m layer of a Typic Paleudult soil under
13 native grassland with two different forage allowances (FA4 and FA12, respectively, 4
14 and 12 kg dry matter/100 kg live weight) in the Pampa biome, Southern Brazil. The
15 vertical bars represent mean standard error. Control treatment: no excreta.

16

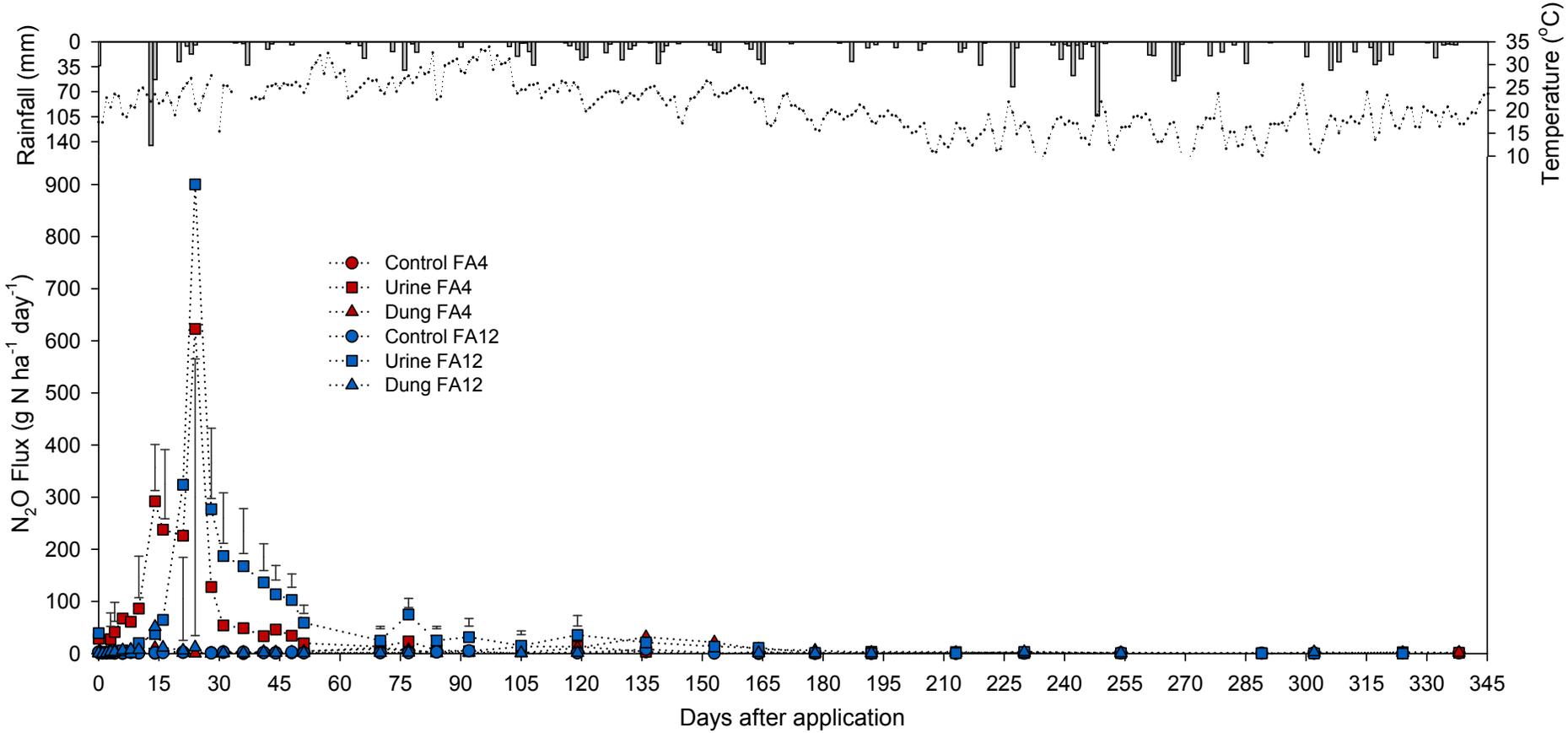
17 Fig. 3. Relationship between soil nitrous oxide (N₂O) emissions and the intensity of the
18 soil contents in ammonium- NH₄⁺ (a), nitrate – NO₃⁻(b) and extractable dissolved
19 organic C - DOC (c) over a period of 92 days following application of cattle urine or
20 dung.

21

22 Fig. 4. Simulation of the nitrous oxide (N₂O) emission factor for cattle urine and dung
23 over different assessment periods of up to 338 days after the excreta were applied to a
24 Typic Paleudult soil under native grassland in the Pampa biome, Southern Brazil.
25 Values are the means for two different forage allowances (FA4 and FA12, respectively,
26 4 and 12 kg dry matter/100 kg live weight).

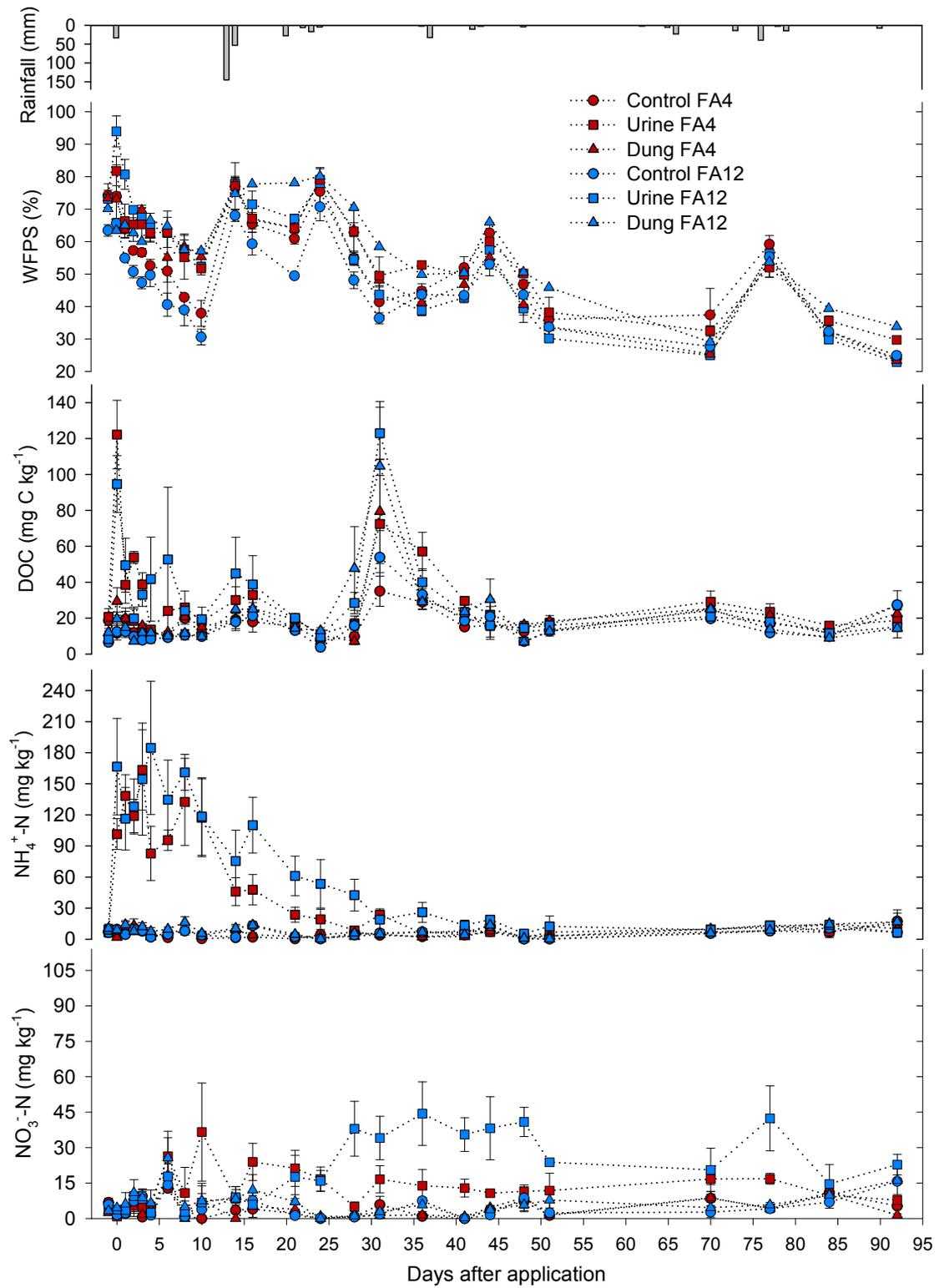
27**28**

29



30

31 Fig. 1.

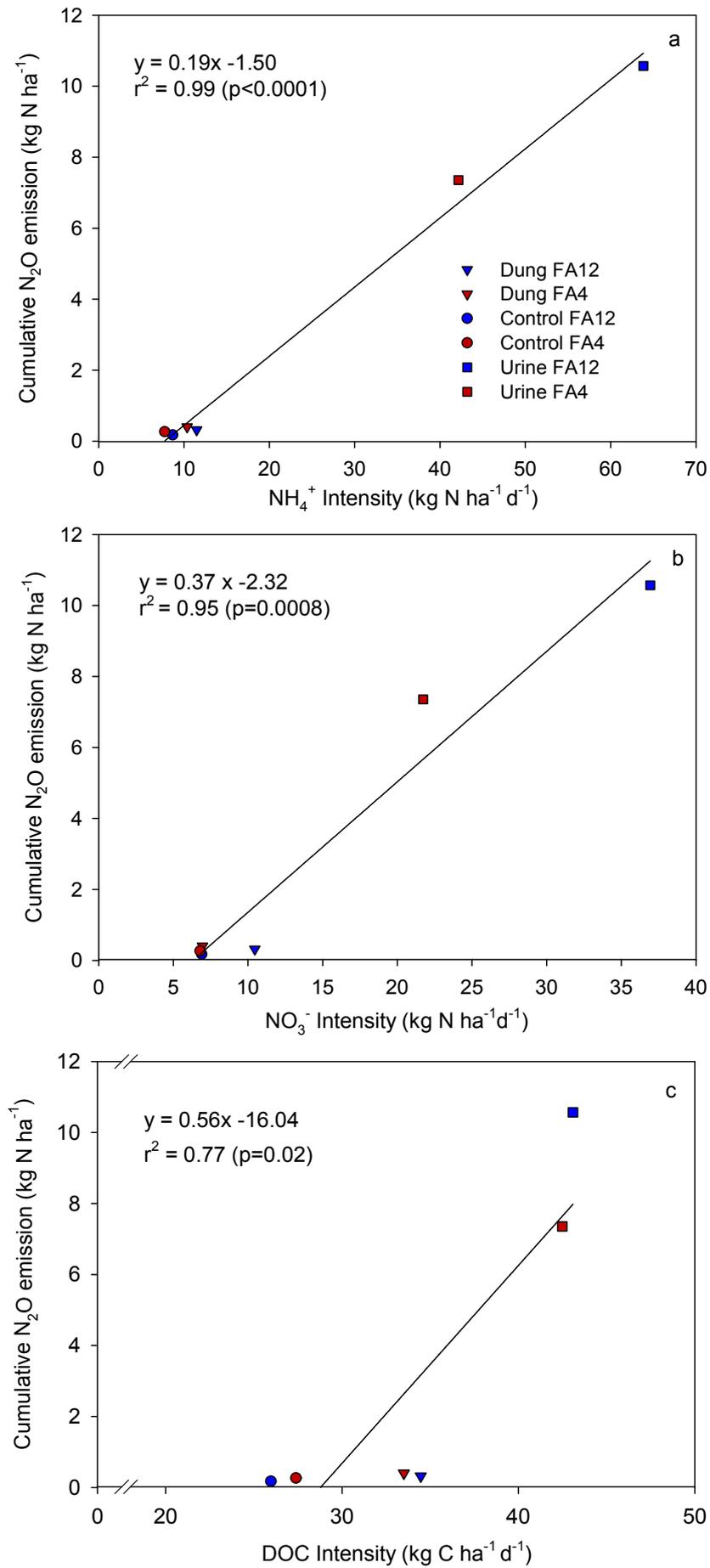


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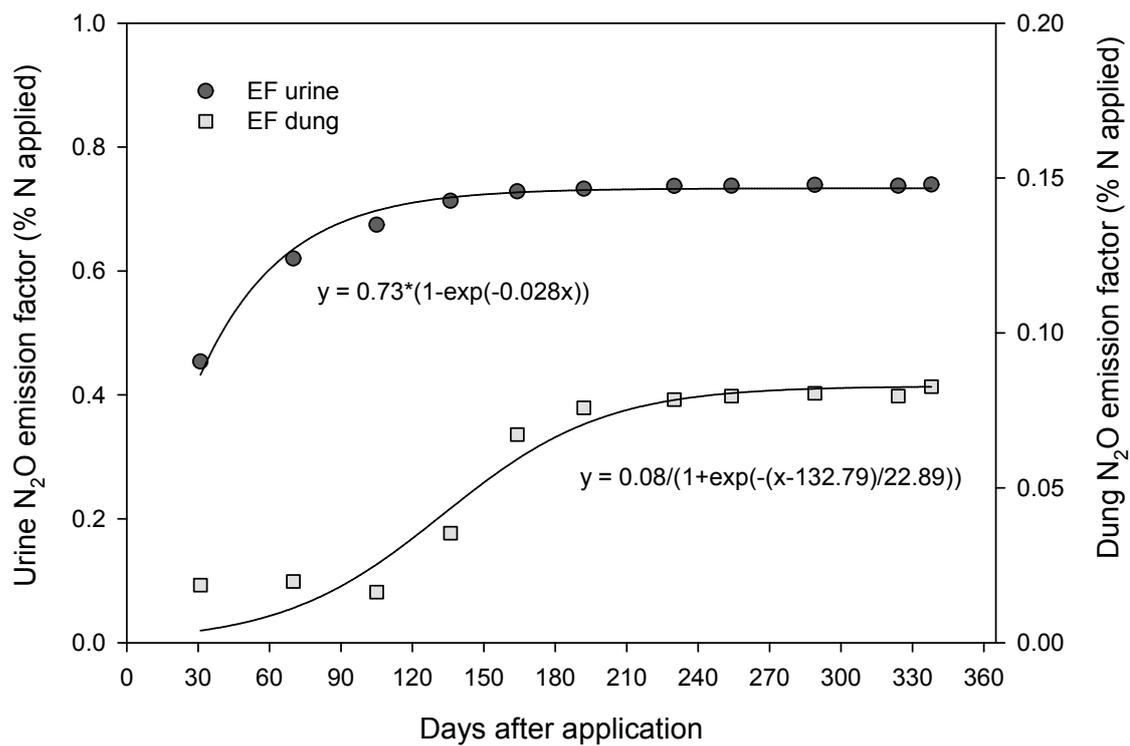
33 Fig. 2.

34

35



36 Fig. 3.



37 Fig. 4.
38

Review Only

1 **Table 1.**

2 **Main characteristics of the top 0-0.2 m layer of a Typic Paleudult of a native grassland**
 3 **under 4 and 12% forage allowances, Southern Brazil.**

4	5 Forage allowance	6 Bulk soil density		7 SOC		8 TN	
		4%	12%	4%	12%	4%	12%
6	Soil layer, cm	--- kg dm ⁻³ ---		----- g kg ⁻¹ -----			
7	0-5	1.43	1.52	15.4	16.2	1.33	1.77
8	5-10	1.65	1.61	10.4	12.1	0.84	1.21
9	10-20	1.68	1.61	9.1	10.1	0.71	1.10

9 SOC: soil organic carbon, TN: total nitrogen.

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10 **Table 2**

11 **Characteristics of the cattle urine and dung, and equivalent N rates applied through the**
 12 **excreta.**

13

Excreta	DM	TOC	Total Nitrogen	DOC	C:N ratio	pH	N rate	
	----- g kg ⁻¹ -----			----- g L ⁻¹ -----			kg ha ⁻¹	
Urine	-	-	-	6.5	23.9	3.7	8.8	1325
Dung	183.9	407.6	12.8	-	-	31.8	-	861

14 DM: Dry Matter; TOC: Total Organic Carbon; C: Dissolved Organic Carbon;

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15 **Table 3**

16 **Results of analysis of variance for annual N₂O-N emission and of N₂O emission factors**
 17 **(EF-N₂O) as a function of forage allowance and type of excreta, and interaction of the**
 18 **two factors, for a native grassland in the Pampa biome, Southern Brazil.**

19

Variable		df	<i>F</i> -value	<i>P</i> -value
Dependent	Independent			
Annual N ₂ O-N emission	Forage allowance	1	2.4	0.15
	Excretas	2	89.4	<0.0001
	FA x Excretas	2	6.4	0.02
EF-N ₂ O	Forage allowance	1	0.8	0.41
	Excretas	1	47.0	0.0005
	FA x Excretas	1	6.9	0.04

Table 4

Annual N₂O-N emissions and N₂O emission factor (EF-N₂O) for cattle urine and dung applied to a Typic Paleudult soil under native grassland with two forage allowances (4 and 12 kg dry matter/100 kg live weight) in the Pampa biome, Southern Brazil.

Forage allowance	Urine	Dung	Control
Annual N ₂ O emission (kg N ha ⁻¹)			
FA4	8.05 Ba ^a	1.78 Ab	0.38 Ab
FA12	12.38 Aa	0.48 Ab	0.45 Ab
Mean	10.21	1.13	0.41
EF-N ₂ O, % N applied			
FA4	0.58 Ba	0.16 Ab	
FA12	0.90 Aa	0.00 Ab	
Mean	0.74 a	0.08 b	

FA4 and FA12: 4% and 12% forage allowances, respectively;

$$EF - N_2O = \frac{N_2O - N_{urine\ or\ dung} - N_2O - N_{control}}{N\ applied} \times 100$$

Uppercase letters compare the forage allowances (column) for each excreta, and the lowercase letters compare excreta (line) for each forage allowance, according to Tukey test ($p \leq 0.05$).