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

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Abstract

Purpose Grazing livestock has strong impact on global nitrous oxide (N_2O) emissions by providing N sources through excreta. The scarcity of information on factors influencing N_2O emissions from sheep excreta in subtropical ecosystems such as those of Southern Brazil led us to conduct field trials in three different winter pasture seasons on an integrated crop–livestock system (ICL) in order to assess N_2O emission factors (EF-N_2O) in response to variable rates of urine and dung.

Materials and methods The equivalent urine-N loading rates for the three winter seasons (2009, 2010, and 2013) ranged from 96 to 478 kg ha^{-1}, and the dung-N rates applied in 2009 and 2010 were 81 and 76 kg ha^{-1}, respectively. Air was sampled from closed static chambers (0.20 m in diameter) for approximately 40 days after excreta application and analyzed for N_2O by gas chromatography.

Results and discussion Soil N_2O-N fluxes spanned the ranges 4 to 353 μg m^{-2} h^{-1} in 2009, − 47 to 976 μg m^{-2} h^{-1} in 2010, and 46 to 339 μg m^{-2} h^{-1} in 2013. Urine addition resulted in N_2O-N peaks within for up to 20–30 days after application in the 3 years, and the strength of the peaks was linearly related to the N rate used. Emission factors of N_2O (EF-N_2O, % of N applied that is emitted as N_2O) of urine ranged from 0.06 to 0.34% and were essentially independent of N rate applied. By considering a ratio of N excreted by urine and dung of 60:40, a single combined excretal EF-N_2O of 0.14% was estimated.

Conclusions Our findings showed higher mean EF-N_2O for sheep urine than that for dung (0.21% vs 0.03%), irrespective of the occurrence or not of urine patches overlap. This value is much lower than default value of 1% of IPCC's Tier 1 and reinforces the needs of its revision.

Keywords Greenhouse gases · Livestock · Nitrous oxide · Subtropical environment

1 Introduction

Historically, Brazil has had one of the largest livestock production in the world, based on approximately 175 million hectares of native and cultivated grassland (IBGE 2010). In the current scenario of global warming, this major activity accounts for about 30% of total greenhouse gas (GHG) emissions in Brazil (MCTI 2014). Specifically, livestock accounts for more than 40% of the nitrous oxide (N_2O) emissions from the national agricultural sector (MCTI 2014), ascribed to direct deposition of animal excreta on soil. Although N_2O is released in smaller amounts than carbon dioxide (CO_2), the former gas is also important because its global warming potential is 298 times higher than that of the latter; also, N_2O has a longer atmospheric residence time than CO_2 (IPCC 2007).

Integrated crop–livestock systems (ICL) have been proposed to optimize the use of natural resources in agricultural

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land, and also to reduce GHG emissions (Carvalho et al. 2010). However, in addition to N fertilizer, many grazed systems are supported by livestock manures that can be a major source of atmospheric N_2O emissions. This is a result of excreta-N being deposited on the soil in equivalent amounts of 200 to 2000 kg per hectare (Selbie et al. 2015), and of 0.1–3.8% of urine-N and 0.01–1.5% of dung-N being potentially released as N_2O to the atmosphere (Oenema et al. 1997; Krol et al. 2016; Chadwick et al. 2018).

Nitrous oxide production in soil is derived from processes such as nitrification, denitrification, and denitrifying nitrification (Firestone and Davidson 1989; Wrage et al. 2001). These processes are governed by diverse pedoclimatic variables including soil moisture, temperature, mineral N contents, and soluble C contents (Gomes et al. 2009). In grassland, however, N_2O production and emission from soil is additionally influenced by the presence of animals. During grazing, animals use N contained in plants and convert it into animal protein for their own development. Because conversion process is highly inefficient, 56–77% of ingested N by animals is excreted (Jarvis et al. 1989; Bohnert et al. 2011), which impact soil N_2O production (Barneze et al. 2014). According to Luo et al. (2008), N_2O emissions from animal excreta (urine, largely) are a result of their biochemical composition, which is rich in urea that is easily converted to ammonium and subsequently to nitrate through microbial processes. This is probably one of the reasons for the increased N_2O emissions from soil receiving urine in relation to dung (Yamulki et al. 1998; Luo et al. 2009). These differences on N_2O emission from urine and dung should be considered to improve the estimation of the default N_2O emission factor (viz., EF-N_2O, % of N applied emitted as N_2O) (Sordi et al. 2014; Chadwick et al. 2018; Simon et al. 2018), that is standardized by IPPC's Tier 1 (2% for cattle and 1% for sheep) and are not disaggregated for urine and dung (IPCC 2013).

Studies aimed at determining the EF-N_2O for tropical and subtropical ecosystems of Brazil are scant even though roughly one-third of the overall emissions of this gas from agricultural soils come from grazing animal excreta (MCTI 2016). In fact, the high variability of EF-N_2O values (0.1–3.8% of N applied) observed in a range of studies has exposed the need of efforts to determination of regional EF-N_2O. For example, evidence gathered in New Zealand has led this country to adopt EF-N_2O values adapted to their specific pedoclimatic conditions (de Klein et al. 2003).

The primary purposes of this study were as follows: (i) determining EF-N_2O for sheep urine and dung under the regional pedoclimatic conditions of Southern Brazil; (ii) confirming whether overlapped urine patches increase EF-N_2O; (iii) verifying the need of disaggregating EF-N_2O for sheep urine and dung; and (iv) identifying the pedoclimatic variables driving soil N_2O emissions in a subtropical grassland ecosystem.

2 Material and methods

2.1 Site description and experimental design

This study was conducted in an area managed under an integrated crop–livestock protocol that was established in 2003 at the Experimental Agronomic Station of the Federal University of Rio Grande 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, Southern Brazil. The regional climate is humid subtropical and falls in Köppen's class Cfa. The mean annual precipitation and temperature are 1455 mm and 18.8 °C respectively. The soil in the experimental area is a Typic Paleudult (USDA 1999) containing 150 g kg^{-1} clay.

The protocol involves a rotation including soybean (*Glycine max*. L. Merr.) and/or maize (*Zea mays* L.) under no-tillage in the summer and autumn (crop period) and grazing of ryegrass (*Lolium multiflorum* L.) by sheep from early winter to the start of a new crop cycle in the spring-summer (livestock period). This study was conducted during the ryegrass grazing period in the years 2009, 2010, and 2013 (specifically, during the intermediate grazing cycle, which spanned September and October). In order to avoid animal interferences with the treatments, an area of 30 m^2 was isolated for this study and prior to the treatment application, ryegrass was cut 0.10 m above ground in order to mimic sheep grazing.

Experimental treatments involved applying variable rates of sheep urine in 2009, 2010, and 2013, and dung in 2009 and 2010, inside of circular metal collars (0.031 m^2) fixed in soil. In 2009, the treatments involved four different rates of urine (50, 75, 100, and 125 mL, equivalent to 161, 242, 323, and 403 kg N ha^{-1}, respectively) and one of dung (14 g, equivalent to 81 kg N ha^{-1}). Those applied in 2010 involved three different rates of urine (75, 150, and 300 mL, equivalent to 119, 239, and 478 kg N ha^{-1}, respectively) and one of dung (14 g, equivalent to 76 kg N ha^{-1}). Finally, only urine was applied in 2013, at a rate of 37.5, 75, and 150 mL, equivalent to 96, 192, and 384 kg N ha^{-1}, respectively. Using different urine rates each year was intended to evaluate whether urine patches overlap would lead to increased EF-N_2O values. An additional, control treatment with no excreta application was used to measure N_2O emissions from the soil. The experimental design used in the 3 years was of the randomized block type with three replications.

Dung was collected from diapers and urine with rigid plastic bucket held manually below the perineum of the sheep, when they were kept stabled during the morning. The urine and dung rates to be applied were estimated from the soil area spanned by each excreta. Thus, sheep with an average live weight of 30 kg produce an average of 75 mL of urine (2.4 L m^{-2}) and 7 g of dung (0.45 kg m^2 at 71% moisture) in each event, the excreta covering an area of radius 0.10 and

0.05 m^2, respectively. Excreta were collected from sheep on the day before application, and their N contents determined by Kjeldahl distillation according to Bremner et al. (Bremmer 1960). The average N contents of the urine samples in 2009, 2010, and 2013 were 10, 5, and 8 g N L^{-1}, respectively, and those of the dung samples in 2009 and 2010 were 9 and 8.4 g N kg^{-1}, respectively.

2.2 Measurement of soil N_2O fluxes and emissions

In September and October of each year, N-rates as urine and dung were applied inside of metal collars and soil N_2O-N fluxes monitored using the method of closed static chambers (Mosier 1989). Monitoring was carried out for approximately 40 days after application of the excreta, the elapsed time required for no significant difference in soil N_2O fluxes between treated and control plots were observed in studies under pedo climatic conditions of Southern Brazil (Sordi et al. 2014; Simon et al. 2018).

Air samples were collected in PVC chambers 25 cm high × 20 cm diameter that were placed on the metal collars in each collection event (Gomes et al. 2009). The system was sealed by filling the channel at the top of the collar with water. Samples were obtained from 09:00 to 11:00 am each day with polypropylene syringes 0, 15, 30, and 45 min after each chamber was closed (Jantalia et al. 2008; de Klein and Harvey 2015). Air was homogenized by switching on an internal fan 30 s before each sample was taken, the air temperature in each chamber being measured by using a stem thermometer with an outer display. Once collected, air samples were transferred to pre-evacuated 12-mL glass vials (LABCO Exetainers®).

The concentration of N_2O in each air sample was determined by gas chromatography on a Shimadzu GC-2014 instrument equipped with an electron capture detector (ECD). The temperature was set at 325 °C and N_2 at a flow rate of 26 mL min^{-1} as carrier gas. N_2O fluxes were calculated from the following equation:

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

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

Fluxes were all expressed relative to N_2O-N and cumulative emissions calculated by trapezoidal integration of the daily N_2O-N fluxes, assuming that the gaseous flux by 09:00 to 11:00 a.m. represents the average daily flux (Bayer et al. 2016).

2.3 Emission factor for N_2O (EF-N_2O)

The emission factor for N_2O (EF-N_2O, % N applied emitted as N_2O) was calculated from the following equation (De Klein et al. 2003):

$$EF\ (\%N\ applied) = \frac{\left(N_2O\text{–}N_{\text{urine or dung}}\right)-\left(N_2O\text{–}N_{\text{control}}\right)}{N_{\text{applied}}} \times 100 \quad (2)$$

where EF is the emission factor (percentage of N-urine or dung applied that was released as N_2O), N_2O-$N_{\text{urine or dung}}$ the cumulative emission of N_2O-N in soil after urine or dung was applied, N_2O-N_{control} the cumulative soil N_2O emission in the control treatment (no excreta), and N_{applied} the amount of N applied to the soil through urine or dung.

2.4 Soil and climate variables

Microplots of 1 m^2 were installed adjacent to each metal collar and received same excreta treatment. These microplots were monitored for ammonium (NH_4^+-N), nitrate (NO_3^--N), gravimetric moisture (GM), and water-filled pore space (WFPS) in the 0–0.1 m soil layer by each air sampling event, in order to examine their role on N_2O emissions from the soil. Soil samples were collected with a stainless-steel auger (3-cm diameter). N contents were determined with the Kjeldahl method, using 2 M KCl as extractant with horizontal stirring for 1 h according to Bremner et al. (Bremmer 1960). GM was obtained by drying the soil at 105 °C, and WFPS was calculated from GM, soil density as determined before the tests and particle density, which was assumed to be 2.65 g cm^{-3} (Gomes et al. 2009). The extraction of dissolved organic carbon (DOC) was performed with 1 g of soil and 10 mL of water with horizontal stirring for 10 h (Zanatta 2009), and C analysis by dry combustion in a Shimadzu analyser (TOC VCSH). Due to the equipment to C analysis in liquid samples to be no available in the first 2 years, the content of dissolved organic carbon (DOC) in soil was determined only in the last year (2013).

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

2.5 Statistical analyses

Because of the covariance structure present in the results, the analyses of variance of N_2O-N fluxes and soil variables (NH_4^+-N, NO_3^--N, WFPS, and DOC) for each experiment were done by using a mixed model in the software SAS v. 9.4 (SAS Institute Inc., Cary, NC, USA). The independent variables excreta (E), sampling day (day), and their

respective interaction were considered fixed effects, and blocks as random effect. The analyses of variance of cumulative emissions and EF-N_2O were performed by using a mixed model for each experimental period with the independent variable excreta (E) as fixed effect and block as random effect.

Relationships of N_2O-N with air temperature, soil temperature, WFPS, DOC, and mineral N were examined in terms of the significance of the Pearson coefficients for the experimental period each year. When Pearson's coefficient was significant, a fitted linear model was used to elucidate the response of N_2O fluxes to climate and soil variables.

The EF-N_2O for each year were subjected to analyses of variance with the independent variable urine rate as fixed effect and block as random effect. An orthogonal contrast analysis was also performed to compare the urine and dung emission factors for 2009 and 2010. Differences between treatment means were assessed with the least significant difference (LSD) test at the 5% significance level.

3 Results

3.1 Temperature and precipitation

The average daily temperature for the sampling period was 17.0 °C in 2009, 18.7 °C in 2010, and 19.4 °C in 2013 (Fig. 1). The highest and lowest daily temperatures were 10.5 °C and 24.2 °C, respectively, and observed in 2009. That was also the year with the highest cumulative precipitation during the measurement period (270 mm), followed by 2013 (262 mm) and 2010 (90 mm) (Fig. 1). In the 3 years, the first substantial precipitation, with at least 15 mm on 1 day, was observed on the 5th day after application (DAA) of the excreta (40 mm in 2009; 15 mm in 2010; 19 mm in 2013).

3.2 Soil N_2O-N fluxes and cumulative emissions

Soil N_2O-N fluxes were influenced by application of excreta, sampling day, and their interaction in 2009, 2010, and 2013 (Table 1). N_2O-N fluxes ranged from 4 to 353 μg m^{-2} h^{-1} in 2009, −47 to 976 μg m^{-2} h^{-1} in 2010, and 46.5 to 339.4 μg m^{-2} h^{-1} in 2013 (Fig. 2). Soil N_2O-N fluxes were increased especially by effect of the application of urine on pasture soil, and the highest N_2O flux each year usually resulted from the treatment with the highest N-rate.

Soil N_2O-N fluxes peaked at 976 μg m^{-2} h^{-1} 16 DAA in 2010, the peak resulting from application of the highest urine rate for the 3 years (U300, 300 mL). Fluxes returned to baseline levels 30, 15, and 25 DAA in 2009, 2010, and 2013, respectively, after which excreta application led to insubstantial differences from control treatment, without excreta. Dung application to the soil in 2009 and 2010 resulted in nonsignificant increases in comparison with the control treatment.

Cumulative soil N_2O-N emissions were influenced by application of the excreta (Table 1). In fact, using increasing urine rates led to average cumulative emissions ranging from 0.55 to 1.42 kg ha^{-1} in 2009, 0.24 to 1.55 kg ha^{-1} in 2010, and 0.14 to 0.67 kg ha^{-1} in 2013 (Fig. 3). Also, applying dung resulted in cumulative soil N_2O-N emissions of 0.16 kg ha^{-1} in 2009 and 0.11 kg ha^{-1} in 2010, but without significant effect of treatment compared with the control (Table 2).

The increase in cumulative N_2O-N emissions was linearly related to the urine rate applied each year (Fig. 3). Thus, each kg N ha^{-1} used increased N_2O-N emissions

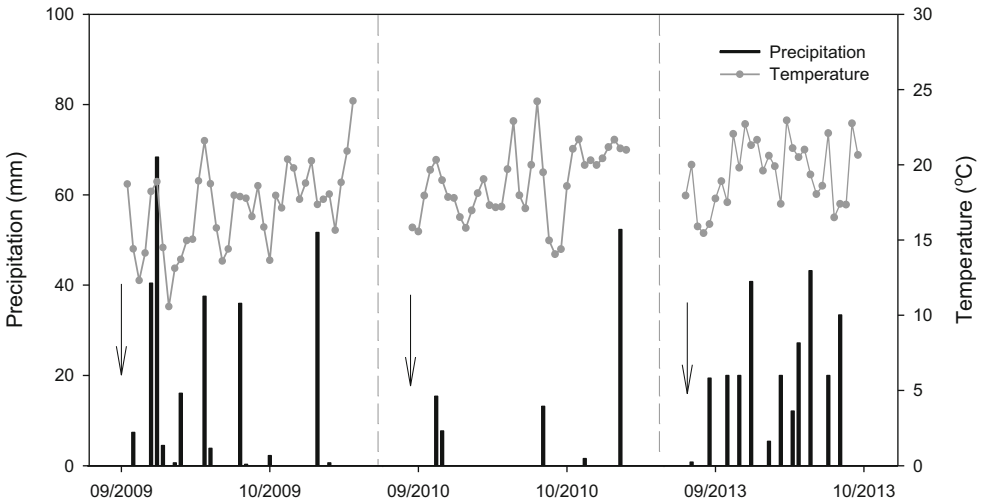

Fig. 1 Daily rainfall precipitation and the mean daily temperature for each experimental period. The arrows indicate the application of urine and dung

Table 1 Analysis of variance on the N_2O fluxes, cumulative emissions, and soil variables (WFPS, NH_4^+-N, NO_3^-N, and DOC) to each year 2009, 2010, and 2013 experiments. A p value < 0.05 is considered statistically significant

Variable		2009			2010			2013		
Dependent	Independent	df	F-value	p value	df	F-value	p value	df	F-value	p value
N_2O 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 × Day	30	2.57	0.0004	36	6.93	< 0.0001	36	3.6	< 0.0001
Cumulative N_2O	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 × Day	30	1.07	0.3881	32	1.10	0.3562	36	0.88	0.6554
NH_4^+-N	Excreta (E)	5	23.75	< 0.0001	4	4619.62	< 0.0001	3	56.09	< 0.0001
	Day	6	20.81	< 0.0001	6	2768.47	< 0.0001	12	20.42	< 0.0001
	E × Day	30	4.26	< 0.0001	24	1493.27	< 0.0001	36	3.64	< 0.0001
NO_3^--N	Excreta (E)	5	9.51	< 0.0001	4	124.36	< 0.0001	3	7.11	0.0002
	Day	6	25.04	< 0.0001	6	97.34	< 0.0001	12	32.11	< 0.0001
	E × 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 × Day							36	0.86	0.691

by 0.003, 0.005, and 0.002 kg ha^{-1} in 2009, 2010, and 2013, respectively.

3.3 Emission factor of N_2O (EF-N_2O)

Urine application rates had no significant effect on EF-N_2O, which ranged from 0.23 to 0.32% in 2009, from 0.14 to 0.34% in 2010, and from 0.06 to 0.15% in 2013 (Table 2). The N_2O-EF values for the 3 years ranged from 0.06 to 0.34% (mean value of 0.21%) with urine and on averaged of 0.03% with dung. The EF-N_2O value obtained by applying dung was 5 and 26 times lower than that for urine in 2010 and 2009, respectively (Table 2).

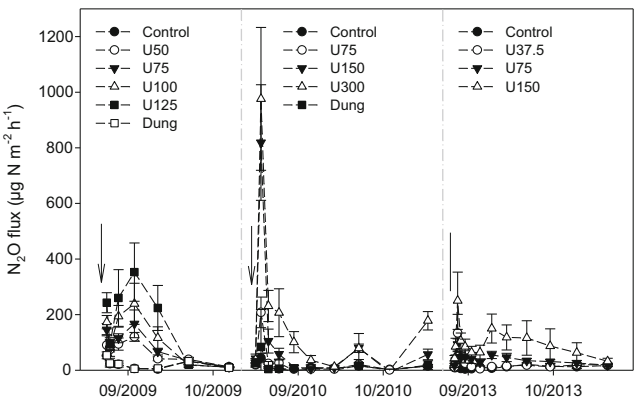

Fig. 2 Temporal nitrous oxide (N_2O) flux following urine and dung application in 2009, 2010, and 2013 experiments. The arrows indicate the application of urine and dung. Vertical bars represent standard error of the mean ($n = 3$)

3.4 Soil parameters

Water-filled porosity space (WFPS) was not altered by application of the excreta to the soil (Table 1; Figs. 1 and 4a), but a positive linear relationship ($p < 0.05$) was verified with soil N_2O-N fluxes in the 3 years (Table 3). This soil parameter ranged from 26 to 96% in 2009, 18 to 94% in 2010, and 61 to 95% in 2013 (Fig. 4a).

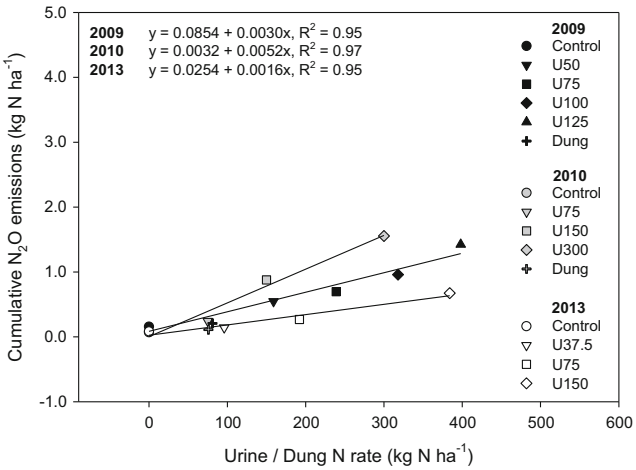

Fig. 3 Relationship between urine and dung nitrogen (N) rate and cumulative nitrous oxide (N_2O) emissions in 2009, 2010, and 2013 experiments

Table 2 Average cumulative emission of N_2O and emission factor (EF) for urine rates and dung, applied in three different year experiments ($n = 3$)

Year	Treatment	Cumulative emission N_2O-N kg ha^{-1}	EF (%)
2009	Control	0.16 ± 0.01 C	–
	U50 (161 N kg ha^{-1})	0.55 ± 0.07 BC	0.25 ± 0.04 ns
	U75 (242 N kg ha^{-1})	0.70 ± 0.09 B	0.23 ± 0.04
	U100 (323 N kg ha^{-1})	0.96 ± 0.16 AB	0.25 ± 0.05
	U125 (403 N kg ha^{-1})	1.42 ± 0.14 A	0.32 ± 0.03
	Mean Urine	**0.91 ± 0.09**	**0.26 ± 0.06 A***
	Dung (81 N kg ha^{-1})	**0.17 ± 0.02 C**	**0.01 ± 0.01 B**
2010	Control	0.07 ± 0.03 C	–
	U75 (119 N kg ha^{-1})	0.24 ± 0.05 BC	0.14 ± 0.04 ns
	U150 (239 N kg ha^{-1})	0.88 ± 0.19 AB	0.34 ± 0.08
	U300 (478 N kg ha^{-1})	1.55 ± 0.33A	0.31 ± 0.07
	Mean Urine	**0.89 ± 0.12**	**0.26 ± 0.06 A***
	Dung (76 N kg ha^{-1})	**0.11 ± 0.02 C**	**0.05 ± 0.03 B**
2013	Control	0.08 ± 0.02 B	–
	U37.5 (96 N kg ha^{-1})	0.14 ± 0.04 B	0.06 ± 0.02 ns
	U75 (192 N kg ha^{-1})	0.27 ± 0.07 B	0.10 ± 0.03
	U150 (384 N kg ha^{-1})	0.67 ± 0.21 A	0.15 ± 0.05
	Mean Urine	**0.36 ± 0.08**	**0.10 ± 0.03**

Values after the ± sign denote the standard deviation. Uppercase letters compare treatments (column) within the same year experiments, according to Tukey test ($p < 0.05$). *Contrast analyses Urine EF × Dung EF for both years was significant at $p < 0.0001$

The soil contents of NH_4^+-N and NO_3^--N were influenced by excreta application and varied throughout of the measurement period (Tables 1 and 3) with a different effect among years (Table 3). Thus, applying urine in 2009 and 2010 increased NH_4^+-N levels relative to the control treatment from the first day; by contrast, NO_3^--N content only increased after 9 days in 2009 and 6 days in 2010, when NH_4^+-N content started to fall. In 2013, the contents of NH_4^+-N and NO_3^--N evolved similarly until 20 DAA. Thus, the two peaked at 151 mg NH_4^+-N kg^{-1} soil and 44 mg NO_3^--N kg^{-1} soil within the first few days after excreta application and then decreased to near-zero levels after 30 days (Fig. 4b, c). Positive and strong relationships ($p < 0.001$) between soil contents of NH_4^+-N and NO_3^--N with N_2O-N fluxes were observed in 2009, but not in 2010 ($p > 0.05$). Positive relationship ($p < 0.001$) between N_2O-N fluxes and NH_4^+-N content was observed in 2013.

Urine increased soil contents of mineral N, especially under application of the highest urine rates (Fig. 4b, c). On the other hand, dung had no effect on such contents relative to the control treatment ($p > 0.05$). The DOC contents ranged from 4.8 to 31 mg C L^{-1} in 2013, and the highest contents were observed in the first 15 DAA (Fig. 4d). Increasing the urine application rate led to increased soil contents of DOC favoring the positive relationship ($p < 0.05$) with soil N_2O-N fluxes (Table 3).

4 Discussion

4.1 N_2O from urine and dung

The excreta increased soil N_2O-N fluxes, especially in the first few days after application but little effect was observed beyond 20 DAA (Fig. 2). Similar studies conducted under different pedoclimatic conditions stated that air samples should be collected for at least 120 DAA or until emissions fall to near-zero levels not significantly different from those of control treatment (De Klein et al. 2003). However, this required time for N added to the soil to be converted in N_2O may be highly variable depending on particular climate and soil conditions (Clough et al. 1998; Selbie et al. 2014). In this work, however, soil N_2O emissions were only observed within the first few days after application. This led us to finish sampling around 40 DAA, similarly to van Groenigen et al. (2005) in Netherlands and Lessa et al. (2014) in Brazil, who found substantial N_2O-N emissions were restricted on average of 30 days after urine application. Our results are also consistent with those of Krol et al. (2016) and Simon et al. (2018), who applied cattle excreta to pasture in different seasons of Ireland and Brazil, respectively, found N_2O-N emissions to peak on average 10 DAA in response to urine and 10–30 DAA in response to dung. The only exception was observed in 2010, when the soil N_2O fluxes in U300 and U150 became close to

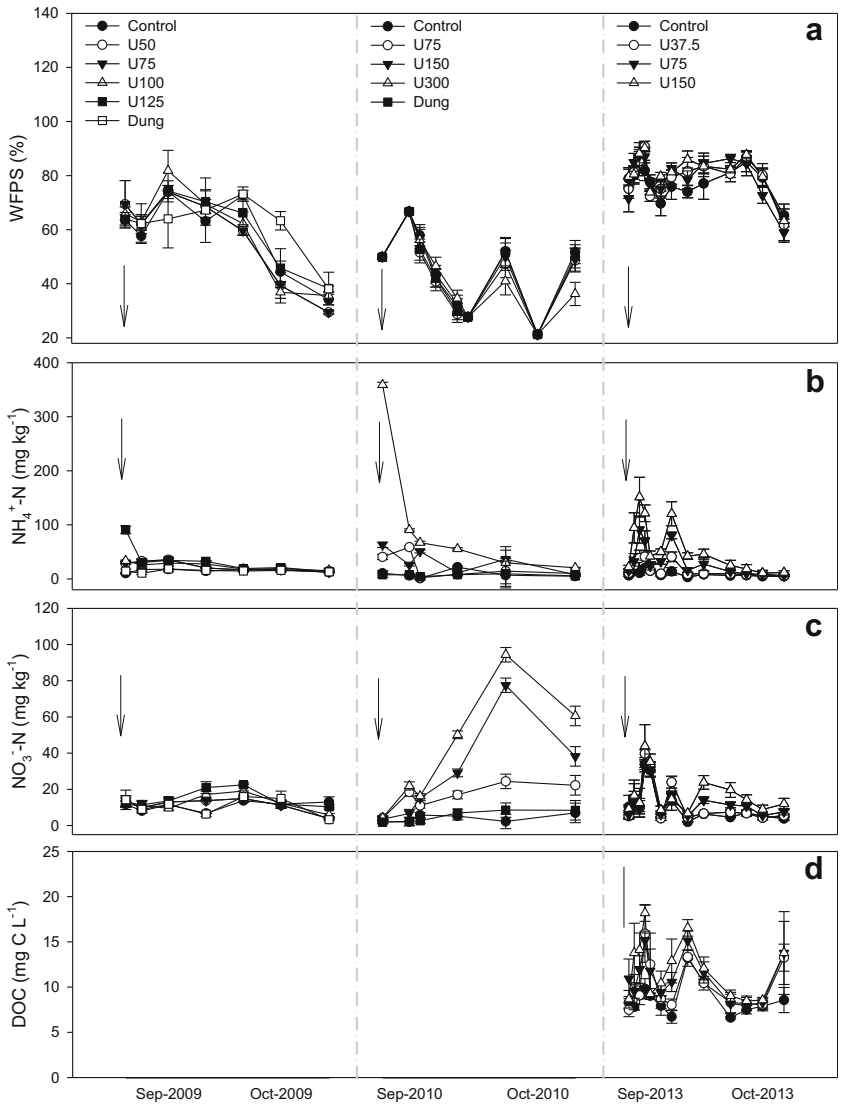

Fig. 4 Soil variable concentrations after urine and dung applications in 2009, 2010, and 2013 experiments. (A) Water filled porosity space; (B) soil ammonium (NH_4^+-N); (C) soil nitrate (NO_3^--N); (D) dissolved organic carbon. Vertical bars represent the mean standard error. The arrows indicate the application of urine and dung

Table 3 Pearson correlation coefficients of soil N_2O fluxes with soil variables (ammonium, NH_4^+-N; nitrate, NO_3^--N; water filled porosity space, WFPS; and dissolved organic carbon, DOC) for 2009, 2010, and 2013 experiments. A p value < 0.05 is considered statistically significant

Year	Variables	Regression equation	Soil N_2O fluxes	
			Coefficient	p value
2009	NH_4^+-N	$y = -34.12 + 5.72x$	0.53	< 0.0001
	NO_3^--N	$y = -79.78 + 15.29x$	0.49	< 0.0001
	WFPS	$y = -104.81 + 3.37x$	0.48	0.0003
2010	NH_4^+-N	$y = 106.65 + 0.44x$	0.02	0.5092
	NO_3^--N	$y = 117.66 + 0.26x$	0.001	0.8902
	WFPS	$y = -237.24 + 7.70x$	0.47	0.0016
2013	NH_4^+-N	$y = 11.74 + 1.13x$	0.51	< 0.0001
	NO_3^--N	$y = 33.57 + 0.86x$	0.09	0.2379
	WFPS	$y = -187.88 + 2.93x$	0.25	0.0053
	DOC	$y = -38.15 + 7.94x$	0.17	0.0025

zero around the 30th day and increase at the last sampling day (40th), with no reasonable explanation.

The rapid increase in soil N_2O-N fluxes upon urine application is consistent with results found by other authors (Hyde et al. 2005; Rochette et al. 2008; Mu et al. 2008) and can be ascribed to an increased availability of soil N and to conditions (e.g., WFPS and DOC) favoring development of microbial processes involved in the N conversion reactions occurring in soil upon excreta application (Williams et al. 1999; Smith et al. 2003; Luo et al. 2008). When urine is deployed in soil, the urea is rapidly converted into NH_4^+-N and NO_3^--N ions, which are used by nitrifying and denitrifying bacteria (Oenema et al. 1997). On the other hand, the absence of substantial N_2O-N fluxes upon application of dung (Fig. 2) was possibly associated to the low N availability and slow degradation providing a gradual supply of N at low levels (Haynes and Williams 1993). To some extent, this is confirmed by the small changes in soil NH_4^+-N and NO_3^--N contents due to

dung application, which resulted in nonsignificant difference from the control treatment.

The significant ($p < 0.05$) and positive relationship between N_2O-N fluxes and WFPS for the three evaluated years, and with DOC content in 2013 support the importance of these two factors on N_2O production and emission from soil. In addition, peaks of N_2O-N emissions were observed at WFPS > 60%, which was highlighted in 2010 when the highest N_2O emission peak was observed. Increased WFPS levels facilitate the formation of anaerobiosis sites in soil, thereby favoring denitrification (Saggar et al. 2004; Wrage et al. 2005), which is widely regarded as the most important process behind N_2O production in soil (Pimentel et al. 2015). Also, the mobility of substrates such as NO_3^- ion and soluble carbon in soil is increased by an increment in WFPS level, which boosts N_2O-N emissions (Luo et al. 1999). According to Wrage et al. (2001), denitrification requires not only adequate WFPS levels and soil nitrate contents but also a high availability of soluble C in soil. In addition, the high correlation between N_2O-N emissions and soil NH_4^+-N contents and even with WFPS (Table 3) suggests that nitrification could also be occurring here, especially in 2010 when WFPS decreased along time and favored nitrification process with consequent increase of NO_3^--N concentration, mainly under the highest urine rates (U150 and U300). N_2O production by nitrification can take place even under conditions of limited oxygen availability (WFPS > 60%), where so-called denitrifying nitrification may be favored if autotrophic bacteria oxidize ammonia (NH_3) to nitrite (NO_2^-) for subsequent reduction to N_2O (Wrage et al. 2005; Koll et al. 2011). Therefore, both denitrification and denitrifying nitrification may have occurred in parallel in our soil.

The increased amounts of mineral N supplied by the highest urine rates increased N_2O-N fluxes and consequently cumulative N_2O emissions from soil, indicating a strong and positive relationship between N_2O emissions and amount of N supplied by urine (Fig. 3). On the other hand, cumulative N_2O emissions resulting from dung application were similar to those for the control treatment ($p > 0.05$). As discussed before, this could be resulted from slow mineralization of the organic-N present in dung, which can take weeks to months, resulting in a gradual release of mineral-N which can be taken up by grass in a greater proportion than urine-N (Hoekstra et al. 2011). The decreased N_2O emissions in 2013 relative to the other years may have resulted from the more frequent precipitation events over the year, especially after urine application (Figs. 1 and 2). The fact that WFPS exceeded 60% over the year may have facilitated complete denitrification and hence favored N_2 emissions over N_2O emissions. According to Jamali et al. (2015), massive losses of mineral N resulting from leaching under heavy precipitation or in the form of N_2 can restrict N availability, thereby diminishing N_2O-N fluxes.

4.2 Emission factor of N_2O (EF-N_2O)

EF-N_2O values were not significantly influenced ($p > 0.05$) by the urine application rate (Table 2), indicating that possible occurrence of overlap of urine patches did not impact EF-N_2O. Then, our findings support the estimation of soil N_2O emission from sheep urine based on N excreted and EF-N_2O, irrespective of the occurrence or not of urine patches overlap. Despite we have not observed here, some studies evidenced that large volumes of urine application on soil may impact negatively on EF-N_2O in sites with overlapping urine application as a result of urine percolation across the soil profile and decrease of N concentration in soil surface (Luo et al. 2008).

The mean value of EF-N_2O for the 3 years and all urine application rates was 0.21%, whereas that for the 2 years under dung application was 0.03% (Table 2). A comparison of the results obtained here with the two excreta reveals that urine was the main source of N_2O-N, with emission potential 5 (2010) and 25 (2009) times higher than dung (Table 2). Even some underestimation may occur on the estimative of EF-N_2O for dung resulting from the short-term evaluation, these findings highlight a very low potential of dung as source of N_2O emission in pasture soils. This low potential is probably related to the gradual mineralization of organic N present in dung (during the evaluation period and later), resulting to mineral N forms being taken up by growing pasture, and consequently being less available to microbial processes involved in soil N_2O production.

The difference in N_2O-EF between the two excreta suggests the need to use specific EF-N_2O values for each excreta instead of default value of 1% of IPCC's Tier 1. In fact, the use of this standard value overestimates N_2O emissions from sheep urine and dung by about 80 and 94%, respectively. This overestimation statement is reinforced by other recent studies conducted with cattle in Brazil, where EF-N_2O was 0.26% for urine and 0.15% for dung under subtropical conditions (Sordi et al. 2014) and was 0.2–0.7% for urine under tropical conditions (Lessa et al. 2014; Barneze et al. 2014). Thus, these values are much lower than the default EF-N_2O of 2% for cattle urine and dung used in IPCC's Tier 1.

By considering individual EF-N_2O for urine and dung and assuming a constant ratio of N excreted by urine and dung of 60:40, respectively, a single weighted excretal EF-N_2O can be estimated and applied in national inventories (Webb and Misselbrook 2004; Chadwick et al. 2018). Based in our data, we estimated a weighted excretal EF-N_2O of 0.14%, reinforcing that default value of 1% used by IPCC's Tier 1 is not appropriate to be applied in the subtropical ecosystems in Southern Brazil.

Similar to this study conducted with sheep excreta on cultivated winter pasture in the subtropical ecosystem in

Southern Brazil, future efforts should be applied to estimative of EF-N_2O for excreta in extensive livestock on native grasslands aiming to approach these two representative regional production systems. Similarly, efforts should be applied in tropical humid and semi-arid ecosystems of Brazil, aiming to have specific excreta EF-N_2O, in order to improve the national inventories.

5 Conclusions

Urine was the main source of N_2O emissions from soil during the winter grazing season in an integrated crop–livestock system in the subtropical ecosystem in Southern Brazil. The soil N_2O emissions peaked in a few days after urine application and returned to baseline levels 20–30 days later, with no significant difference in relation to the control treatment. The positive relationship of soil N_2O emissions with inorganic N (NO_3^-N and NH_4^+-N) and with WFPS levels (> 60%) and DOC, suggests that denitrification and denitrifying nitrification processes were concurrently involved in soil N_2O production. EF-N_2O for sheep dung was much lower than for urine (0.03% vs 0.21%), and both are much lower than the default value of 1% of IPCC's Tier 1. Our findings support the estimation of soil N_2O emission from sheep urine based on N excreted and EF-N_2O, irrespective of the occurrence or not of urine patches overlap. Based in our data and assuming a ratio of N excreted by urine and dung of 60:40, respectively, we estimated a weighted excretal EF-N_2O of 0.14%, which can properly attend the regional inventories of N_2O emissions by sheep excreta in cultivated winter pastures. Future efforts should be applied to the determination of EF-N_2O in extensive livestock production system on native grasslands in Southern Brazil, as well in tropical humid and semi-arid ecosystems in Brazil.

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