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# $N_2O$ emissions from urine-treated tropical soil: Effects of soil moisture and compaction, urine composition, and dung addition

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ABSTRACT

Increasing attention is being paid to the importance of $N_2O$ emissions due to livestock activities in tropical countries. Understanding the key variables driving $N_2O$ emission could help minimize impacts of $N_2O$ release and improve the accuracy of $N_2O$ inventories. We aimed to investigate the effects of soil moisture, soil compaction, urine composition, urine volume, and dung addition on $N_2O$ emissions from a urine-treated tropical Ferralsol under controlled conditions. Manipulated soil conditions (e.g., moisture content, compaction, and dung addition) affected $N_2O$ emissions when varying quantities of urine-N ($p = 0.02$) were applied (urine volumes remained equal) and when varying urine volumes ($p = 0.04$) were applied (quantities of urine-N remained equal). When the amount of urine-N applied was varied, the estimated $N_2O$ emission factor (EF) was 3.14 ± 0.70%, 2.29 ± 1.25%, 3.90 ± 0.64%, 4.73 ± 0.88%, and 6.62 ± 1.10% for moist soil, dry soil, compacted soil, plus dung, and plus dung and compacted soil treatments, respectively. While varying the volume of urine, the estimated $N_2O$ EF was 4.96 ± 1.66%, 4.27 ± 1.42%, 3.99 ± 1.19%, 6.50 ± 0.35%, and 7.37 ± 0.76% for moist, dry soil, compacted soil, plus dung, and plus dung and compacted soils treatments, respectively. The urine-N concentration influenced $N_2O$ emissions ($p = 0.02$) [which decreased linearly ($p = 0.062$)] as well the volume of urine ($p < 0.01$) [which increased linearly ($p < 0.01$)]. The chemical form of the applied urine-N (urea, nitrate, or ammonium) did not affect $N_2O$ emissions and the emissions factor averaged 1.40 ± 0.38%. $N_2O$ production was affected by the KCl concentration in the urine ($p < 0.01$), and the effect was curvilinear. The key driving factor affecting $N_2O$ emissions was soil moisture content. The $N_2O$ response varied when the urine volume differed (in both moist and dry soil conditions), and with the addition of dung.

## 1. Introduction

Nitrous oxide ($N_2O$) is the third-largest contributor to the greenhouse gas emissions driving climate change. $N_2O$ emissions come primarily from N fertilization of soil and excretion of urea by animals (WMO, 2015). $N_2O$ emissions from livestock represent approximately 14.5% of the global anthropogenic $N_2O$ flux (Gerber et al., 2013). These fluxes may dominate the greenhouse gas budget in countries where economies depend, to a large extent, on livestock farming. In Brazil, the fraction of the anthropogenic $N_2O$ flux coming from urine and dung voided by livestock in pastures was 37% in 1995 and 57.7% in 2012 (MCTI, 2014). This fraction is expected to continue to increase in the near future.

Urea from urine is rapidly hydrolyzed to yield $NH_3$ (ammonia) or $NH_4^+$ (ammonium). Autotrophic nitrifiers oxidize these energy-rich compounds to $NO_2^-$ and subsequently to $NO_3^-$. Finally, heterotrophic denitrifiers use $NO_3^-$ and $NO_2^-$ as electron acceptors, thereby reducing these oxidized N species to NO, $N_2O$, and $N_2$ (Oenema et al., 1997). These reactions occur for both urine and dung patches, although the initial concentration of $NH_3/NH_4^+$ species is much lower in dung than in urine.

The key factors affecting $N_2O$ emission from N-fertilized soils appear to be water-filled pore space (WFPS), temperature, and mineral N concentration (Dobbie et al., 1999). The main mechanism involved in $N_2O$ emissions varies according to the soil temperature. Nitrification is the predominant process (at approximately 40% WFPS), and when

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WFPS ranges from 50 to 70%, $N_2O$ is mainly produced by denitrification (Dobbie et al., 1999). For grazed grasslands, the high N concentration from animal excretion, chemical form of N compounds, and subsequent N transformations contribute to high $N_2O$ losses (Oenema et al., 1997). Soil temperature and moisture affect $N_2O$ emission from bovine manure patches (Mazzetto et al., 2014). Uchida et al. (2011) attributed higher $N_2O$ fluxes during the rainy season to the warmer and wetter conditions. Soil compaction decreases the total pore volume, especially the number of large pores. This in turn decreases soil aeration, possibly leading to partial anaerobiosis, and to changes in N transformation and $N_2O$ production rates (Oenema et al., 1997). Soil characteristics might also affect $N_2O$ emissions in tropical grassland soils, as found in temperate soils. In this study, we tested the following hypotheses: (1) a greater proportion of the urine-N will be emitted as $N_2O$ from moist soil than from dry soil, and (2) $N_2O$ emissions will be greater from compacted soil than from moist soil.

The level of $N_2O$ emissions from cattle dung and from cattle urine differs. It has been reported that under field conditions, $N_2O$ emissions are much lower from dung patches than from urine patches (e.g., van der Weerden et al., 2011; Lessa et al., 2014; Rochette et al., 2014). In the western Amazon, Mazzetto et al. (2014) evaluated $N_2O$ emissions from dung during wet and dry tropical seasons and concluded that feces cannot be considered an $N_2O$ source under those conditions. None of these studies evaluated $N_2O$ emissions from dung plus urine and how $N_2O$ production might be affected by soil compaction. Therefore, we also investigated the following hypotheses: (3) $N_2O$ emissions will be lower from urine plus dung than from urine alone, and (4) a greater fraction of the excreta-N will be emitted as $N_2O$ from urine plus dung than from the urine-plus-dung and soil compaction treatments.

Lessa et al. (2014) found differences in the $N_2O$ emissions between seasons. They found EFs of 1.93% from urine-N in the rainy season and 0.1% in the dry season, with this variation explained by difference in the soil moisture. The urine-N concentration affected $N_2O$ losses (van Groenigen et al., 2005a, 2005b). The nitrogen concentration in urine varies greatly, depending mainly on the amount of protein in the diet, and ranging from 3.0 to 20.5 g N/L (Dijkstra et al., 2013). Urine-N may interact with other N sources applied to grassland soil as ammonium sulfate and potassium nitrate. Some chemicals present in bovine urine (like KCl) may have an inhibitory effect on $N_2O$ emissions (Agrawal et al., 1985; van Groenigen et al., 2005a). Regarding urine composition, we tested the following hypotheses: (5) $N_2O$ emissions will increase when the urine volume increases, (6) the proportion of the urine-N emitted as $N_2O$ will be greater when urine-N increases, (7) $N_2O$ emissions will differ among nitrogen sources, and (8) $N_2O$ production will be inhibited by increasing KCl concentration in the urine.

Most previous studies on the effects of soil conditions on $N_2O$ emissions from cattle excreta have been conducted on temperate grassland soils (e.g., Oenema et al., 1997; Yamulki and Jarvis, 2002; Rochette et al., 2014). Although Sordi et al. (2013), Lessa et al. (2014), and Mazzetto et al. (2014) represent the few carried out under tropical conditions, no manipulation of either soil conditions or urine characteristics was attempted in these. Different interactions between these variables and $N_2O$ emissions are expected for tropical grassland soils. In addition, there is an increasing need to understand the key variable driving $N_2O$ production from livestock in tropical regions, in order to develop $N_2O$ mitigation strategies and to improve inventories of $N_2O$ emissions.

To this end, we manipulated soil conditions of, and urine application to, a patch of tropical soil under controlled conditions, and then assessed $N_2O$ emissions for 106 d. The objective of this part of the study was to evaluate the effects, on $N_2O$ emissions, of: 1) soil characteristics, 2) the amount of urine-N applied (when the volume of urine applied was constant), 3) the volume of urine applied (when the amount of urine-N was constant), 4) the source of the N in the urine applied, and 5) the concentration of KCl added to the urine.

## 2. Material and methods

### 2.1. Location and soil characteristics

The incubation was carried out in the greenhouse facility of the Forragicultura Sector of the São Paulo State University "Júlio de Mesquita Filho" campus in Jaboticabal, São Paulo, Brazil. A 20 cm-deep layer of sandy clay Ferralsol (42% clay, 14% silt, 44% sand) was collected for the incubation study in June 2013, from a grassland in Jaboticabal, Brazil (21°15′22″S, 48°18′08″W; altitude 595 m).

The chemical characteristics of soil were pH 4.9 (in water), 0.18% total N, 2.04% total C, and, for the dry soil (11.0 mg $NH_4$-N and 4.7 mg $NO_3$-N) $kg^{-1}$. The soil was mixed and passed through a 4 mm sieve; then 500 g portions of moist soil were placed in square 1.5 L jars. The dung was from Nellore cattle and was collected immediately after defecation. The animal diet was solely grass (*Brachiaria brizantha* cv. Marandu).

### 2.2. Experimental design

To determine the effects of, and interactions between, soil characteristics and urine composition on $N_2O$ emissions, four incubations were conducted simultaneously.

a) Incubation 1
A factorial experiment was carried out in a completely randomized design. The first factor was different concentrations of urine-N (125, 250, 500, or 750 mg $kg^{-1}$ dry soil; 5 replicates) applied in equal volumes of urine (50 mL $kg^{-1}$ dry soil) under different soil conditions (moist, dry, compacted, moist plus dung, and moist plus dung plus compaction; 4 replicates). In this incubation, two treatments were included to measure background $N_2O$: moist and dry soil, without N addition.

b) Incubation 2
A second incubation studied the effect of different volumes of urine (25, 50, 100, or 200 mL $kg^{-1}$ dry soil; 5 replicates) containing equal amounts of urine-N (500 mg $kg^{-1}$ dry soil) on $N_2O$ emissions under the same soil conditions (second factor). The background and experimental design were as above.

c) Incubation 3
In the third incubation, four treatments containing different N sources (500 mg N $kg^{-1}$ dry soil of urea, ammonium sulfate, potassium nitrate, or the background with no N source applied in 100 mL urine$^{-1}$ $kg^{-1}$ dry soil; 4 replicates), were tested using a completely randomized design.

d) Incubation 4
In this incubation the treatments were different concentrations of KCl (0.0, 5.0, 10.0, or 20.0 g $L^{-1}$ urine; 4 replicates) added to the urine then applied in 100 mL urine$^{-1}$ $kg^{-1}$ dry soil, along with a background treatment without added KCl. Each treatment included four replicates in a completely randomized design.

### 2.3. Treatment preparations

The incubations were conducted under controlled conditions: temperature 25.0 ± 1.0 °C and 80% relative humidity. 500 g of soil was added to each square jar (1.5 L), in which the initial moisture was 8.0 $g^{-1}$ $H_2O$ $g^{-1}$ soil.

a) Urine treatments
Artificial urine was used in order to manipulate its characteristics. The urine was prepared according to Doak (1952) using urea, hippuric acid, creatine, allantoin, ureic acid, and $NH_4Cl$ with total N in

the proportions of 88.6%, 6.2%, 0.8%, 1.5%, 0.4% and 2.5%, respectively. 14.20 g $L^{-1}$ $KHCO_3$ and 10.50 g $L^{-1}$ KCl were added to all artificial urine, except for the treatments in which KCl concentrations were manipulated to study its effects on nitrification.
In the incubation designed to study the different sources of N, the preparation of urine-N urea was replaced by ammonium sulfate and potassium nitrate, and the same amounts of $NH_4Cl$ and KCl were added, according to Doak (1952).
In the study of the effect of the KCl concentration in the urine, instead of adding KCl at 10.50 g $L^{-1}$ the desired amount of KCl was added, and the amounts of the other urine components were the same.

b) Soil treatments
First, 500 g of soil was passed through a sieve (4 mm); then added to the jars with 8.0 g $H_2O$ $g^{-1}$ soil. The initial soil bulk density was 1.2 g $cm^{-3}$. For the soil treatment named "moist," 100 mL water $kg^{-1}$ was added, resulting in a volumetric soil moisture content of 34.1% (40.4% WFPS)·For the treatment "dry" 50 mL water $kg^{-1}$ soil was applied, to provide a volumetric moisture content of 22.1% (62.3% WFPS). The treatment "compacted" was prepared by compressing the soil in the jars (using a piece of wood) to increase the bulk to approximately 2.0 g $cm^{-3}$. After compaction, the volumetric soil moisture content increased from 34.1 to 56.8% (100% WFPS). Finally, the treatment "moist plus dung" was prepared by applying 150.0 g of fresh dung ($kg^{-1}$ dry soil) to the soil. This jar received 100 mL water $kg^{-1}$ dry soil, resulting in an initial volumetric soil moisture content of 48.5% (88.6% WFPS). The treatment labeled "moist plus dung and compaction" was prepared the same as the previous treatment, increasing the soil bulk density to 2.0 g $cm^{-3}$. This provided an initial volumetric soil moisture content of 56.8% (100% WFPS).
The jars were kept uncovered except during $N_2O$ measurement, allowing exchange with the atmosphere. Changes in the water content were corrected during the experimental period. Evaporative losses for the un-compacted and compacted treatments averaged 0.66 and 1.1 g water $kg^{-1}$ $d^{-1}$, respectively. During the first 7 d of incubation after urine and dung application, the soil moisture was corrected by weighing the jars daily and spraying deionized water onto the soil surface as required. From 7 d to 60 d after application (DAA), the soil moisture was corrected every two days; then subsequently twice a week, until the end of the experiment at 106 d.
Control treatments were prepared using deionized water instead of urine. Two background treatments were evaluated: "dry," to which 50 mL water $kg^{-1}$ dry soil was applied, and "moist," to which 100 mL water $kg^{-1}$ dry soil was applied.

### 2.4. $N_2O$ measurement and soil analysis

The static, closed-chamber technique was followed to evaluate $N_2O$ flux. The chambers were jars with 1.02 L of headspace. Sampling was carried out between 9:00 and 10:00 am, as advocated by Alves et al. (2012). $N_2O$ flux was measured 25 times during the 106 d incubation period (daily during the first week; every two days during the second week, twice a week during the rest of the first month, weekly between 30 and 60 DAA, and twice in the remaining 30 d). We stopped collecting air samples after the flux during treatments became similar to the background values.

The $N_2O$ flux was measured by closing the jar lid for 0.75 h and determining the change in the headspace concentration. Air samples were taken, with 50 mL polypropylene syringes, at the end ($T_{45}$) of the incubation period. Ambient gas samples ($T_0$) were also taken to check the initial $N_2O$ concentration in six chambers immediately after coupling the jar caps. The chamber from which the initial sample was collected varied on each new sampling day.

The air temperatures inside and outside the chamber was recorded using a digital thermometer. The air samples were transferred to 20 mL pre-evacuated vials (Shimadzu flasks) for quantitative analysis using gas chromatography (Shimadzu Green House Gas Analyzer GC-2014; Kyoto, Japan) for measurement of $N_2O$ under the following conditions: injector 250 °C, column at 80 °C, carrier gas $N_2$ (30 mL $min^{-1}$), and electron capture detector at 325 °C.

The linearity of the flux during the incubation period was previously tested successfully over a 1.5 h period, with the $N_2O$ concentrations evaluated every 10 min. Five jars were used to conduct the linearity test. Portions (500 g) of the same soil used in the incubations were placed in the jars, then the highest N concentration used in this study was applied to the soil (750 mg N $kg^{-1}$ dry soil), and the soil moisture was increased to 70% WFPS to allow the highest $N_2O$ flux expected in the incubations. Gas samples were taken over 5 d on the same schedule used during incubation.

Flux calculations (unit: ng N $g^{-1}$ dry soil $h^{-1}$) were based on the assumption that there was a linear increase in the $N_2O$ concentration with time in the closed chamber. The procedure followed was the same as in numerous recently published studies (de Klein et al., 2014; Lessa et al., 2014; Martins et al., 2015; Cardoso et al., 2016; Nichols et al., 2016; van der Weerden et al., 2016). The lowest detection limit was found using linear regression (see Parkin et al., 2012), which was compared to the quadratic methods of Hutchinson/Mosier and revised Hutchinson/Mosier. The concentrations of the samples were adjusted for dissolved gas in soil solution using the Bunsen coefficient (Moraghan and Buresh, 1977).

Cumulative emissions were calculated by plotting the daily flux through time, with linear interpolation between them, and then integrating the data. The EF representing the fraction of N in urine, or urine plus dung lost as $N_2O$, was calculated using the ratio between the $N_2O$-N emitted from the excreta (minus the corresponding background emission) and the total-N emitted from the excreta. In order to avoid biasing the results, negative fluxes were included in the calculation of cumulative emissions. The minimum detection limit was ± 0.16 ng N $g^{-1}$ of dry soil $h^{-1}$.

Soil inorganic N was measured at the beginning and at the end of the incubation period using 2 M KCl extraction and colorimetric analysis according to Kempers and Zweers (1986) for ammonium, and Miyazawa et al. (1985) for nitrate.

### 2.5. Data analysis

The effects of manipulation of urine and soil conditions on $N_2O$ EF emission were tested to determine the effect of the amount of urine-N applied in a constant volume under different soil conditions (using two-way ANOVA, with amount of urine-N and soil treatment as factors) and the effect of the volume of urine applied with amount of urine-N held constant (two-way ANOVA, with urine volume and soil treatment as factors). The effect of the form of urine-N applied, and the effect of KCl concentration in the applied urine, were also tested (both using one-way ANOVA).

When ANOVA was significant, the Tukey–HSD test was used to distinguish the means of manipulated soil and urine-N compounds; and polynomial orthogonal contrast was used to report the effect of urine volume, urine-N rates, and KCl concentration. All statistical analyses were performed using the R statistical program (version 3.1.2; R Core Team, 2014).

## 3. Results

### 3.1. Effect of soil characteristics and varying urine-N

In the experiment conducted with varying amounts of applied urine-N, the $N_2O$ flux peaked between 13 DAA in all treatments (Fig. 1a). The highest mean fluxes were ~105 ng $N_2O$-N $g^{-1}$ dry soil $h^{-1}$, which occurred in the 500 mg N $kg^{-1}$ treatments with dry and compacted soil,

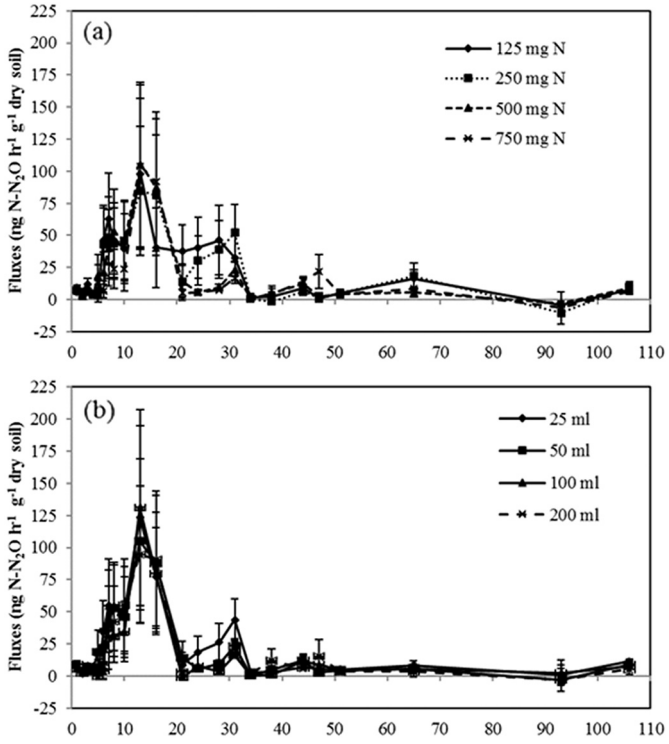


**Fig. 1.** $N_2O$ flux (ng $N_2O$-N $g^{-1}$ dry soil) by day of sampling, 106 d after application for (a) different amounts of urine-N (125, 250, 500, or 750 mg N $kg^{-1}$ dry soil), and (b) different volumes of urine (25, 50, 100, or 200 mL $kg^{-1}$ dry soil).

both with dung addition (Fig. 1a). Negative mean fluxes were found 93 DAA. The $N_2O$ emissions started 5 d after urine application, and dropped to background levels at 34 DAA (Fig. 1a).

Soil conditions (e.g., moisture content, compaction, and dung addition) significantly affected the fraction of $N_2O$ lost ($p = 0.02$) when varying amounts of urine-N were applied in equal volumes (Table 1). Applying urine-N in different amounts significantly affected $N_2O$ emissions ($p = 0.02$).

The EF decreased linearly ($p < 0.062$) from 5.10% to 1.39% as the amount of applied urine-N increased from the lowest (125 mg $kg^{-1}$ dry soil) to the highest (750 mg $kg^{-1}$ dry soil) application rate. The estimated $N_2O$ EF averaged 3.14 ± 0.70%, 2.29 ± 1.25%, 3.90 ± 0.64%, 4.73 ± 0.88%, and 6.62 ± 1.10% for moist (uncompacted), dry (uncompacted), compacted soil, plus dung (uncompacted), and plus dung (compacted) soil treatments, respectively (Table 1).

### 3.2. Effect of urine volume

In the experiment in which the volume of the applied urine was varied, $N_2O$ emissions occurred mainly in the period between 5 and 31 DAA and peaked at 13 DAA in the 100 mL urine $kg^{-1}$ dry soil. The mean flux was highest (131.1 ng $N_2O$-N $g^{-1}$ dry soil $h^{-1}$) in the treatment with 100 mL of urine $kg^{-1}$ dry soil, and lowest (− 3.3 ng $N_2O$-N $g^{-1}$ dry soil $h^{-1}$) 93 DAA in the treatment with 200 mL of urine $kg^{-1}$ dry soil. Negative fluxes were found in all treatments (Fig. 1b).

When the volume of applied urine was varied (with equal amounts of urine-N), soil conditions affected the quantity of $N_2O$ emitted ($p = 0.04$). Moreover, the urine volume affected $N_2O$ emissions ($p < 0.01$) (Table 2). The EF increased in dry soil, with compaction and dung addition, as compared to moist soil. The EF increased from 3.24 ± 1.31% to 7.45 ± 0.65% as urine volume was increased linearly ($p < 0.001$) from the lowest (25 mL $kg^{-1}$ dry soil) to the highest (200 mL $kg^{-1}$ dry soil) volume. $N_2O$ losses averaged 5.42 ± 0.60% of the total N added (Table 2). The estimated $N_2O$ EF averaged 4.96 ± 1.66%, 4.27 ± 1.42%, 3.99 ± 1.19%, 6.50 ± 0.35%, and 7.37 ± 0.76%, for moist, dry soil, compacted soil, plus dung, and plus dung and compacted soils treatments, respectively.

### 3.3. Effect of dung addition

In the treatments with dung addition, higher $N_2O$ emissions occurred in both incubations in which the urine-N or urine volumes were studied. High $N_2O$ emissions commenced at 5 DAA and persisted until 38 DAA (Fig. 2). The highest mean $N_2O$ flux when dung was added was 5–20 times greater than for the urine treatments. Negative fluxes were mainly observed in the dry and compacted soil (Fig. 2).

Nitrous oxide emissions in the two treatments with dung addition were higher than those in which only soil moisture and compaction were manipulated. The treatment “urine plus dung and compaction” differed from moist ($p = 0.05$) and dry ($p = 0.02$) soils in the experiment varying the volume of urine (with amount of urine-N constant; see Table 1). The “urine plus dung” treatment differed from compacted soil ($p = 0.03$), and “urine plus dung and compaction” also differed from the compacted soil treatment ($p = 0.04$) during the incubation in which the amount of urine-N applied was varied (with volume of applied urine constant; see Table 2).

### 3.4. Effect of urine-N source

In the incubation in which different sources of urine-N were applied, $N_2O$ emission persisted from 10 to 65 DAA and peaked first with the urea and nitrate treatment (13 DAA), and then at 28 DAA with the source ammonium. Negative fluxes were observed at the beginning and the end of the evaluations (Fig. 3a).

**Table 1**
$N_2O$ emission factor (% of applied N) for different amounts of urine-N applied in equal volumes (50 mL $kg^{-1}$ dry soil).

| Treatment* | Amount of applied urine-N (mg $kg^{-1}$) dry soil** | | | | Average |
|---|---|---|---|---|---|
| | 125 | 250 | 500 | 750 | |
| Urine | 5.10 | 2.60 | 3.45 | 1.39 | 3.14 ( ± 0.70) |
| Urine + dry soil[a] | 6.41 | 0.38 | 1.56 | 0.82 | 2.29 ( ± 1.25) |
| Urine + compaction | 5.86 | 3.77 | 2.41 | 3.55 | 3.90 ( ± 0.64) |
| Urine + dung | 5.20 | 1.94 | 6.57 | 5.20 | 4.73 ( ± 0.88) |
| Urine + dung + compaction | 9.20 | 7.80 | 5.36 | 3.92 | 6.62 ( ± 1.10) |
| Average | 6.40 ( ± 0.79) | 3.30 ( ± 1.25) | 3.87 ( ± 0.93) | 2.98 ( ± 0.82) | 4.14 ( ± 0.54) |

The data between parentheses range indicates the standard error ($n = 5$ for amount of applied urine-N, $n = 4$ for soil treatments).
[a] Only 50 mL water $kg^{-1}$ soil, as compared to 100 mL for the other treatments.
* $p = 0.02$ for the test of differences between soil condition treatments ($n = 20$).
** $p = 0.021$ for the test of differences between amount of applied urine-N treatments ($n = 20$).

**Table 2**
$N_2O$ emission factor (% of applied N) for different volumes of applied urine with equal amounts of urine-N (500 mg $kg^{-1}$ dry soil).

| Treatment* | Volume of applied urine (mL $kg^{-1}$ dry soil)** | | | | Average |
|---|---|---|---|---|---|
| | 25 | 50 | 100 | 200 | |
| Urine | 1.92 | 1.56 | 8.09 | 8.26 | 4.96 ( ± 1.66) |
| Urine + dry soil[a] | 0.77 | 2.41 | 6.75 | 7.15 | 4.27 ( ± 1.42) |
| Urine + compaction | 0.69 | 6.57 | 3.05 | 5.64 | 3.99 ( ± 1.19) |
| Urine + dung | 6.70 | 5.36 | 7.17 | 6.77 | 6.50 ( ± 0.35) |
| Urine + dung + compaction | 6.10 | 8.09 | 5.84 | 9.43 | 7.37 ( ± 0.76) |
| Average | 3.24 ( ± 1.31) | 4.80 ( ± 1.24) | 6.18 ( ± 0.86) | 7.45 ( ± 0.65) | 5.42 ( ± 0.60) |

The data range between parentheses indicates the standard error ($n$ = 5 for amount of applied urine-N, $n$ = 4 for soil treatments).
[a] Only 50 mL water $kg^{-1}$ soil, as compared to 100 mL for the other treatments.
* $p$ = 0.17 for the test of differences between soil condition treatments ($n$ = 20).
** $p$ = 0.04 for the test of differences between urine volume treatments ($n$ = 20).

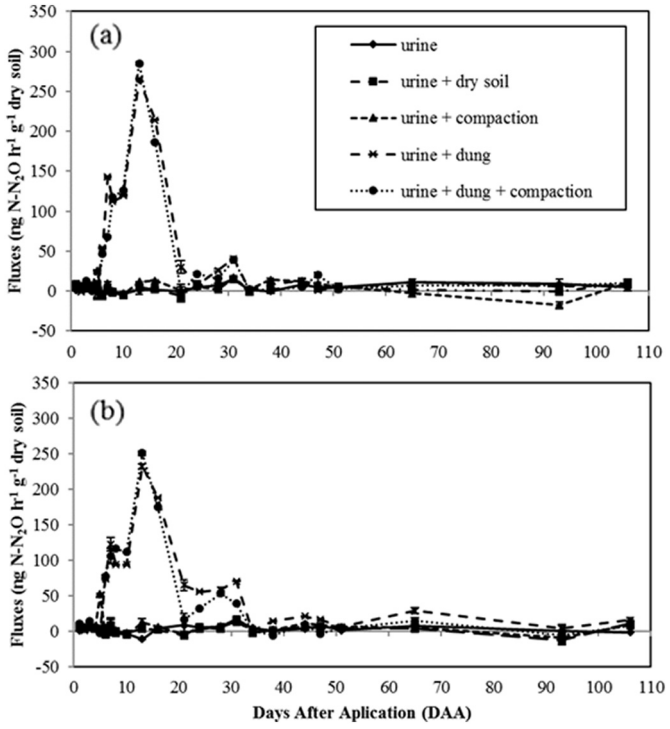


**Fig. 2.** $N_2O$ flux (ng $N_2O$-N $g^{-1}$ dry soil) by day of sampling 106 d after application from (a) manipulated soil conditions (moist soil, dry soil, compacted, with dung addition, and with dung addition plus compaction) under different urine volumes applied, with equal quantities of urine-N (500 mg N $kg^{-1}$ dry soil), (b) The same soil conditions cited above under different N-urine quantities added in equal volumes of urine (100 mL $kg^{-1}$ dry soil).

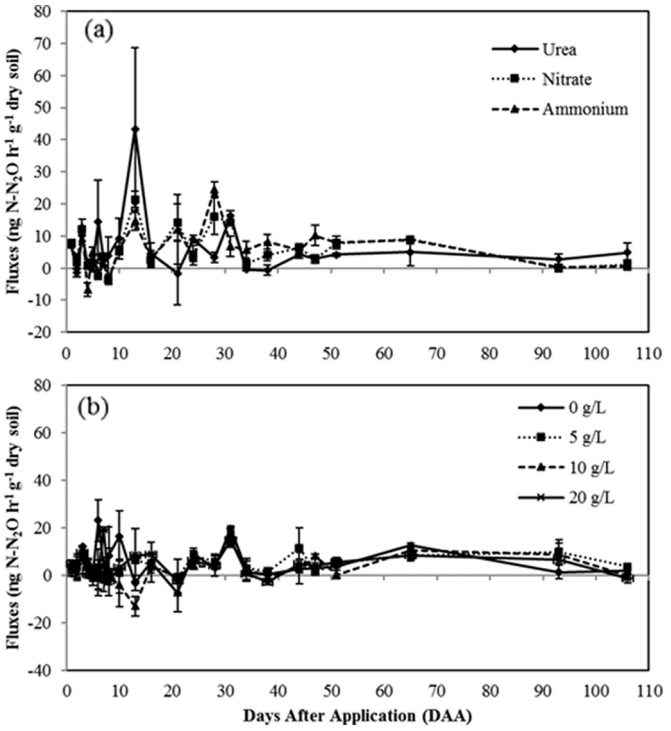


**Fig. 3.** $N_2O$ flux (ng $N_2O$-N $g^{-1}$ dry soil) by day of sampling for 106 d after application for (a) chemical N compound added in the urine (ammonium, urea, nitrate) and (b) concentration of KCl in the urine (0, 5, 10, and 20 g $L^{-1}$).

The fraction of N emitted as $N_2O$ was 0.76 ± 0.26%, 1.32 ± 0.28%, and 2.13 ± 1.08% for ammonium, urea, and nitrate, respectively (Table 3). However, the chemical form of N in the urine did not influence $N_2O$ emissions ($p$ = 0.38).

### 3.5. Effect of KCl concentration

Variation in the KCl concentration in the urine-$N_2O$ flux peaked at 5 DAA and 31 DAA. The lowest flux was measured at 21 DAA. However, $N_2O$ fluxes were lower in this incubation compared to those analyzing the effect of urine-N, urine volume, and N source; $N_2O$ production persisted until 91 DAA (Fig. 3b).

The KCl concentration in the urine was found to have a significant effect ($p$ < 0.05). The estimated $N_2O$ EF averaged 3.22 ± 1.21%, 4.44 ± 0.65%, 3.03 ± 0.42%, and 1.17 ± 0.23%, for the concentrations of 0.0, 5.0, 10.0, and 20.0 g $L^{-1}$, respectively. The effect of KCl concentration on $N_2O$ emissions was curvilinear ($p$ < 0.01).

**Table 3**
$N_2O$ emission factor (% of applied N) from different N-compounds.

| Treatment* | Amount of applied N (mg $kg^{-1}$ dry soil) | | | | Mean |
|---|---|---|---|---|---|
| | 125 | 250 | 500 | 750 | |
| Ammonium | 0.19 | 0.70 | 1.46 | 0.70 | 0.76 ( ± 0.26) |
| Urea | 1.56 | 2.01 | 0.79 | 0.94 | 1.32 ( ± 0.28) |
| Nitrate | 5.34 | 1.04 | 0.73 | 1.42 | 2.13 ( ± 1.08) |
| Mean | | | | | 1.40 ( ± 0.38) |

The data between parentheses range the standard error ($n$ = 4).
* $p$ = 0.38. Significance for the test of differences between treatments with different chemical forms of urine-N ($n$ = 12).

### 3.6. Mineral N concentration

Mean soil concentrations of mineral N measured in the urine and dung treatments after 106 days of incubation were close to or below background levels in all of the studied treatment combinations, with values of (5.7, 5.1, and 3.0) mg N-$NH_4$ $kg^{-1}$ dry soil and (1.7, 1.9, and

2.4) mg $N\text{-}NO_3\ kg^{-1}$ dry soil for the background, urine, and dung treatments, respectively.

## 4. Discussion

### 4.1. Effect of soil moisture

In this 106-d incubation study, cumulative $N_2O$ emissions from moist soil (62.3% WFPS) were 73% higher than from dry soil (40.4% WFPS) when the amount of applied urine N was varied (Table 1) and 16% when the urine volume was varied (Table 2). However, when the moist and dry treatments were compared using the Tukey test, no significant difference was found. $N_2O$ production and consumption is regulated by interactions between the $O_2$ concentration and soil moisture content (Wu et al., 2013). Differences in $N_2O$ emission between soil types are largely the result of differences in their water holding capacity, which directly influences their aeration status (Rochette et al., 2014). High $N_2O$ emissions normally occur when neither WFPS nor temperature is limiting (Dobbie et al., 1999).

Our data are compatible with those of van Groenigen et al. (2005a), who did not find an effect of urine volume on $N_2O$ emissions from either applied urine-N or from soil organic N in a field study. In contrast, Velthof and Oenema (1995) showed that $N_2O$ emissions were highest when soil WFPS was > 70% and lowest when WFPS was < 50%. Likewise, Orwin et al. (2010) found that $N_2O$ emissions were 100 times greater in a silty loam soil incubated at 70%, than at 30% WFPS.

### 4.2. Effect of soil compaction

Soil compaction increases the emission of $N_2O$. Yamulki and Jarvis (2002) reported a 3.5-fold increase in $N_2O$ emissions after compaction, compared to un-compacted soil, while a 1.4-fold increase was reported by Hansen et al. (1993). In a field study, van Groenigen et al. (2005a) reported a highly significant effect of soil compaction on $N_2O$ emissions from applied urine ($p = 0.002$) and a marginal effect when dung was added ($p = 0.054$). They observed that with soil compaction, $N_2O$ emissions increased by a factor of 2.2 (from 1.30% to 2.92% of applied N) and that with dung application, $N_2O$ production was augmented by a factor of 1.8 (from 1.60% to 2.82%).

In our study, soil compaction had no effect when compared to the treatment “moist.” $N_2O$ emissions increased 1.25 times in the experiments with varying urine-N concentration (Table 1), and when the urine volume was varied, it decreased by 25% (Table 2). However, $N_2O$ emissions from the compacted treatment differed from that with dung addition, and for “dung plus compaction” in the experiment varying the urine-N concentration. However, when the urine volume was varied, $N_2O$ emissions differed from “dung plus compacted” and moist or dry soil.

The lowest EF (0.69%) was found for the smallest urine volume tested (25 mL $kg^{-1}$ dry soil), against an average of 5.0% for the other urine volume treatments, suggesting a combined effect, with percent WFPS, on $N_2O$ emissions. We found that soil compaction with dung addition did not affect $N_2O$ loss, when compared to not compacted, given that EFs averaged 6.50% and 7.37% with and without soil compaction, respectively (Table 2). Taken together, our data suggest that the soil compaction effect on $N_2O$ emissions was masked and outweighed by the presence of dung.

### 4.3. Effect of dung addition

Nitrous oxide emissions from urine patches have been reported to be much higher than from dung patches (van der Weerden et al., 2011; Sordi et al., 2013; Lessa et al., 2014). Cardoso (2016), in a field study, applied cattle urine together with dung and observed that $N_2O$ emissions were similar to that from urine patches. Under our experimental conditions, $N_2O$ emissions were higher with the addition of dung plus urine than with urine only (Tables 1 and 2). This implies that further study should be done to quantify $N_2O$ emissions from dung and urine applied together, instead of only considering urine or dung separately.

The ratio of C:N can affect $N_2O$ loss. Klemedtsson et al. (2005) reported that the $N_2O$ emissions rapidly increased with reduction in the soil C:N below a threshold ratio of 25. The dung applied had this C:N ratio, which combined with moist conditions and N availability, probably stimulated microbial activity and created an ideal environment for higher $N_2O$ emissions. Different cattle diets can result in variation of biochemical composition of the dung and it is possible that this might affect $N_2O$ emissions. More experiments need to be conducted to investigate this possible effect.

### 4.4. Influence of urine N concentration and volume

In incubation experiments, van Groenigen et al. (2005b) did not find that either the amount of urine-N or the urine volume had an overall significant effect on $N_2O$ emissions. Here, it was observed that the EF declined from 6.40% to 2.98% as the amount of urine-N was augmented (Table 1), whereas the EF increased linearly from 3.24 to 7.35% with increasing urine volume (Table 2). Despite these data, our results disagree with the conclusions of van Groenigen et al. (2005b).

Although many factors are known to control $N_2O$ emissions (Signor and Cerri, 2013 - review), Oenema et al. (1997) suggested that the relationship between N availability in the soil and $N_2O$ emission is the most useful indicator for evaluating total emissions from a certain area, and which might explain the effect of different amounts of urine-N applied. However, Mazzetto et al. (2014) argued that the soil mineral N concentration is the key factor for regulating $N_2O$ emission from soil, because when soil mineral N reaches levels as high as those found in urine patches, it no longer limits the amount of $N_2O$ released.

The 2006 guidelines for national greenhouse gases inventories from the Intergovernmental Panel on Climate Change (IPCC) assumed that $N_2O$ emissions increase linearly with the N rate applied to the soil. In our study, we found that EF varied according to the urine-N rate and presented a linear effect ($p < 0.062$) consistent with the IPCC figure. Cardoso et al. (2016), in a study of $N_2O$ emissions from subtropical pastureland, varied the urine volume applied to the grassland soil. They found that $N_2O$ EF decreased linearly from 4.9% to 2.43% when the volume of urine was increased from 1.0 L to 2.0 L, differing from our findings. They attributed the result obtained for the higher volume to possible percolation of the urine deep into the soil, resulting in less N being available for $N_2O$ production in the topsoil.

However, in incubation assays N leaching is limited, which may explain the different pattern reported here, where EF increased linearly; and by van Groenigen et al. (2005b), who found no effect from the volume of urine applied, when compared to field studies that show an effect of urine volume on $N_2O$ emissions.

### 4.5. Effects of nitrogen source

Nitrous oxide emissions from soil are influenced by the chemical form of the N present in the soil. Indeed, nitrate can be denitrified immediately, whereas ammonia needs to be nitrified before denitrification takes place. This explains why the increase in $N_2O$ losses induced by ammoniacal fertilizers occurs more slowly than that observed for nitric fertilizers, as has been consistently observed by other researchers (e.g., Signor and Cerri, 2013).

In the present study, the N-containing compound in the urine did not influence $N_2O$ emissions; however, the losses induced by nitrate were 2.8 and 1.6 times greater, respectively, than those induced by ammonium and urea (Table 3). Dobbie and Smith (2003) measured $N_2O$ emissions after application of ammonium nitrate or urea to an intensively managed grassland in Scotland, and found that the emissions were, on average, 2.4 times higher from the former than from the latter. Delaune et al. (1998) reported that the $N\text{-}NH_4$ and $N\text{-}NO_3$

**Table 4**
$N_2O$ emission factor (% of applied N emitted as $N_2O$) for different KCl concentrations in applied urine.

| KCl concentration ($g\ L^{-1}$) | $N_2O$ emission % N applied emitted |
|---|---|
| 0.0 | 3.22 ( ± 1.21) |
| 5.0 | 4.44 ( ± 0.65) |
| 10.0 | 3.03 ( ± 0.42) |
| 20.0 | 1.17 ( ± 0.23) |
| Effect[a] | Quadratic |

[a] The data between parentheses range the standard error ($n = 4$). $p < 0.01$ for the test of differences between KCl treatments ($n = 16$). The data fit the equation $f(x) = -0.01\ K^2 - 0.13\ K + 3.46$, where f(x) = % $N_2O$ EF and K = concentration of KCl in urine in $g\ L^{-1}$; $R^2 = 0.8697$; $p < 0.01$.

fertilizers they studied increased the amount of N released as $N_2O$ by 15% and 56%, respectively. Studying tropical soils in southern Brazil, Zanatta et al. (2010) found that nitrate fertilizers induced more $N_2O$ emissions than urea or ammonium fertilizers.

### 4.6. Inhibitory effect of KCl

It has been suggested that KCl may inhibit $N_2O$ release from soil through its inhibition of nitrification (Monaghan and Barraclough, 1992). In the present study, $N_2O$ emissions showed a curvilinear effect from KCl concentration on $N_2O$ emissions. The KCl concentration expected in the bovine urine range from 5 to 10.5 $g\ L^{-1}$. In this study the highest $N_2O$ losses were observed in the treatment 10.5 g KCl $L^{-1}$ urine (Table 4). While the lowest $N_2O$ emission was found in the highest KCl concentration. A possible explanation for this reduction is that KCl can inhibit nitrification. Agrawal et al. (1985) observed that increasing the concentration of $K^+$ ions affected nitrification. van Groenigen et al. (2005a) found an effect of KCl on $N_2O$ emissions comparable to ours, but because their data did not fit any equation, they did not draw a conclusion.

### 4.7. Implications

According to the IPCC (2006), the default emission factor for cattle excreta voided in grasslands is 2%. To compare the EFs calculated from an incubation study with the IPCC default emission factor is perhaps not appropriate. However, we can indicate the potential emissions in relation to the different conditions studied. We found an overall average EF of 4.14% when urine-N content was varied (Table 1) and a mean EF of 5.42% when urine volume was varied (Table 2). Our use of artificial urine possibly also led to higher $N_2O$ emissions than if we had used real urine (de Klein et al., 2003; van Groenigen et al., 2005a).

Nevertheless, Cardoso et al. (2016) used cow urine and found an EF of 4.9%, which is similar to that found in the current study. In a subtropical soil, Sordi et al. (2013) found an EF of 0.26% for urine and 0.15% for dung, while Lessa et al. (2014), in a study of a Cerrado pastureland, estimated an average EF of 0.7% for excreta-N. These studies were conducted in exclusion-areas that were not grazed and probably did not represent the average $N_2O$ losses typical of grassland.

Animal urination and defecation occurred principally in the areas where cattle tend to congregate, such as pasture corners, shadows, and areas near feeders and water tanks. In this study we simulated the conditions cited above, and perhaps this explains why $N_2O$ emissions were much higher than reported by Sordi et al. (2013) and Lessa et al. (2014). Additional research is needed to better quantify $N_2O$ emissions under various soil conditions, and to indicate the variation in the composition of excreta found in the cattle production systems of the tropics.

In the present study, mean EF was 3.76% and 6.30% for urine and urine plus dung addition, respectively. We suggest that this effect of dung application is summed with that of the urine and cannot be compared to measurement of $N_2O$ losses performed using dung patches alone. Finally, the strategies for mitigation of $N_2O$ emissions suggested by van Groenigen et al. (2006), of avoiding a combination of soil compactions, dung and urine patches found in so-called “camping areas” in pastures, by rotating food and water supply locations or shad areas and avoiding grazing under wet conditions, are supported by our results, in which the EFs were 1.25, 1.2, and 2 times greater in moist, compacted, and dung-added soils, respectively, than in un-compacted dry soil.

## 5. Conclusions

Soil conditions affected $N_2O$ emissions when different amounts of urine-N were applied (in equal urine volumes) and when different volumes of urine were added (containing equal amounts of urine-N). Moist soil did not differ as much from dry soil as from compacted soil. However, when soil was compacted and dung was added, the $N_2O$ emissions were higher than when it was only compacted.

When different amounts of urine-N were applied, the moist and dry soils emitted less $N_2O$ than the compacted soil with dung application. Nitrous oxide emissions were affected by dung addition. The mean EF for urine was 3.76% and for urine plus dung, the average was 6.03%.

Urine-N concentrations affected $N_2O$ emissions, which decreased linearly, and the urine volumes presented a significant effect on $N_2O$ production, which increased linearly. Incubation with varying amounts of urine-N and varying urine volumes resulted in mean EFs of 4.14% and 5.42%, respectively.

The chemical form of urine-N added (i.e., urea, nitrate, or ammonium) did not affect $N_2O$ emissions and the EF was 1.40 ± 0.38%. The concentration of KCl added to the urine affected $N_2O$ losses.

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