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Nitrous Oxide Emissions and Ammonia Volatilization from Pasture after Cattle Dung and Urine Applications in the Dry and Rainy Seasons of the Brazilian Cerrado

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Abstract: An important source of greenhouse gases in Brazil is the nitrous oxide (N_2O) emission from pasture, and microorganisms play an important role in nitrogen transformations in the soil. This study aimed to evaluate N_2O emission and NH_3 volatilization from bovine excreta in pasture in an integrated crop–livestock system (ICL) in the Brazilian Cerrado. Three treatments (urine, dung and control) were performed in two pastures (Area 1—three-year pasture of *Urochloa ruziziensis* and Area 2—one-year pasture of *Urochloa brizantha* cv. Piatã), with two application times of the excreta (dry and rainy season), during two successive years of application. Compared to the control, the excreta deposition on ICL increased soil N_2O and NH_3 fluxes. In the dry season, N_2O fluxes were associated with higher ammonium (NH_4^+) availability. In the rainy season, these fluxes were related to NO_3^- availability and water-filled pore space (WFPS). In both areas, NH_3 volatilization was higher after urine than dung application, especially in the dry season. The highest N_2O emission factors were obtained for urine (0.32%), the rainy season (0.36%), and older pasture (Area 1: 0.24%). All these values were below the mean IPCC default values (0.77%). These results indicate that N_2O emissions in pasture should be evaluated in regional conditions.

Keywords: soil management; nitrate; ammonium

1. Introduction

Globally, livestock is responsible for 60% and 32% of total ammonia (NH_3) and N_2O emissions, respectively [1]. In Brazil, in 2020, the anthropogenic emission of greenhouse gases (GHG) in the agriculture sector was dominated by methane (CH_4) from the enteric fermentation of cattle (12,958.00 Gg CH_4), N_2O derived from nitrogen (N) fertilization (82.59 Gg N_2O), and excreta deposited in pastures (184.97 Gg N_2O) [2]. A total of 97% and 41% of the total agricultural CH_4 and N_2O emissions were attributed to livestock activity [2]. In agricultural soils, N_2O is produced through two main microbiological processes: nitrification and denitrification. In both processes, N_2O is an intermediate product. Nitrification is the process of conversion of NH_3 and ammonium (NH_4^+) mostly into nitrite (NO_2^-) and then to nitrate (NO_3^-) by ammonia-oxidizing bacteria and archaea. Denitrification is the transformation of NO_3^- and its final product is dinitrogen (N_2), and NO_2^-, nitric oxide (NO), and N_2O are intermediate products controlled by facultative anaerobic bacteria [3].

Soil moisture is the main controlling factor that determines the pathway of N_2O emissions [4,5]. In aerobic soil conditions, nitrification is the primary source of N_2O [6], while denitrification occurs in predominantly anaerobic conditions and becomes the predominant process when the water-filled pore space (WFPS) of the soil is above 60–70% [4]. Another important factor is the concentration of mineral N (NH_4^+ and NO_3^-) that determines the rate of N_2O fluxes [7].

In addition, N_2O fluxes from pasture areas are also influenced by plant biomass, bovine dung, and urine [8]. In the pastures of Brazil, dung and urine deposited on the soil are responsible for 37% of agricultural N_2O emissions [2].

Pastures are a source of protein for cattle, which excrete 70–95% of the nitrogen consumed in dung and urine [9]. Nitrogen lost from animal excreta increases NH_3 volatilization and N_2O fluxes to the atmosphere and can also be leached as nitrate [10–13]. Lessa et al. [10] measured, in a comparable environment to our study, that less than 1% of N had been lost as N_2O from cattle excreta and around 15% as NH_3. Additionally, around 10% of volatilized NH_3 is emitted as N_2O after redeposition [11], which turns this process into an indirect source of N_2O.

From the 1970s, livestock production has gradually expanded in the Cerrado, with a significant expansion of pastures. In 2023, there was 1.51–1.60 million km^2 land under pasture, two thirds of which was in some degree of degradation [14,15]. In this context, the integrated crop–livestock system (ICL) is a promising alternative to mitigate the vulnerability of livestock and agriculture through soil conservation management practices and the diversification of activities [16,17].

The Brazilian Cerrado has two seasons: a rainy and a dry one [18]. There is little information about N_2O emissions and NH_3 volatilization from cattle excreta in this region [12,19].

There are contrasting data in the literature concerning the effect of ICLs on N_2O and NH_3 emissions. According to Thomas et al. [20], no-tillage and crop rotation reduces N_2O emission from urine. However, Piva et al. [21] obtained N_2O emission three times higher in ICL under no-tillage than continuous crop, likely due to the application of N in the annual crop phase, due to N fertilizer and excreta.

Because of the rate of expansion of ICLs in Brazil, as a strategy to reduce the environmental impact of livestock, in this study we investigated N_2O emission and NH_3 volatilization from dung and urine (NH_3). We conducted this investigation in pastures within an ICL in the Brazilian Cerrado, to contribute to a better understanding of the influencing factors and magnitude of emissions.

2. Materials and Methods

2.1. Study Area and Experimental Design

The study was carried out in two adjacent areas under integrated crop–livestock system (ICL) management at the Brazilian Agricultural Research Corporation's National Rice and Bean Research Center (Embrapa Arroz e Feijão) in Santo Antônio de Goiás (latitude 6°29′59″ to 16°29′44″ W and longitude 49°17′35″ to 49°17′54″ S, altitude 804 m a.s.l).

Originally, the entire area was covered by typical native species of the Cerrado. Between 1933 and 1950 part of the original vegetation was removed, and until 1983, common bean, rice and maize were grown in the area. Thereafter, only common beans and maize were cultivated. As of 1993, alternating soybean and brachiaria species were planted (*Urochloa* sp.). In 1995, the ICL system was implemented which became consolidated in 2000 (Table 1).

Table 1. Crop rotations and soil management types of areas under integrated crop–livestock system (ICL) in the rainy and dry seasons, from 2003 to 2014.

Year/Season	Area 1		Area 2	
	Preparation	**Plant**	**Preparation**	**Plant**
2003/2004 (Rainy)	Conventional	Rice	No-tillage	Maize + *U. brizantha*
2004 (Dry)	-	Fallow	-	Pasture
2004/2005 (Rainy)	No-tillage	Maize + *U. brizantha*	-	Pasture
2005 (Dry)	-	Pasture	-	Pasture
2005/2006 (Rainy)	-	Pasture	-	Pasture
2006 (Dry)	-	Pasture	-	Pasture
2006/2007 (Rainy)	-	Pasture	-	Pasture
2007 (Rainy)	-	Pasture	-	Pasture
2007/2008 (Rainy)	-	Pasture	Conventional	Soybean–Maize
2008 (Dry)	-	Pasture	No-tillage	Bean
2008/2009 (Rainy)	-	Pasture	No-tillage	Rice
2009 (Dry)	-	Pasture	-	Fallow
2009/2010 (Rainy)	No-tillage	Maize + *Urochloa* sp.	No-tillage	Soybean
2010 (Dry)	-	Pasture	-	Pasture
2010/2011 (Rainy)	-	Pasture	-	Pasture
2011 (Dry)	-	Pasture	-	Pasture
2011/2012 (Rainy)	-	Pasture	No-tillage	Maize + *U. brizantha*
2012 (Dry)	-	Pasture	-	Pasture
2012/2013 (Rainy)	-	Pasture	-	Pasture
2013 (Dry)	-	Pasture	-	Pasture
2013/2014 (Rainy)	-	Pasture	-	Pasture

Conventional: soil preparation equals one plowing and two harrowings. No-tillage: direct planting of the seed + fertilizer mix into the planting groove without physical soil preparation.

The pasture areas were used to rear beef cattle of the zebu breed Nellore "BRGN". Animals grazed the areas at a mean stocking rate of 1.5 AU ha^{-1} in the dry season and 2.7 AU ha^{-1} in the rainy season. The mean daily weight gain in the respective seasons was 0.3 and 0.6 kg per animal.

The soil of both areas was a clay Oxisol [22]. In Area 1, maize was cultivated in consortium with *Urochloa ruziziensis* in the 2009/2010 harvest to renew the pasture within the ICL, hence at the time of the study the pasture was 3-years-old. In Area 2, maize was planted in consortium with *Urochloa brizantha*, Piatã variety, in the rainy season of 2011/12; hence, in this area the pasture was 1-year-old when our study started. Similar rotation was adopted in both areas (Table 1).

Precipitation and temperature data of the study period were measured at the Meteorological Station of EMBRAPA Rice and Beans (Figure 1).

In the study areas (Area 1 and 2), two experiments were implemented, one treated with excreta at the beginning of the dry season (March 2012), and the other in the middle of the rainy season (January 2013) (Figure 2).

In the second study year, excreta applications were repeated in the same manner. In other words, a second application was performed in May 2013 on the plots fertilized with excreta in May 2012 (dry season) and a second application in January 2014 on the plots that received excreta in January 2013 (rainy season) (Figure 2).

A total of 48 plots were established in an experimental design of four randomized blocks in 3 × 2 × 2 sub-sub plots, with three excreta applications (dung, urine and control = no excreta); two pasture areas (Area 1—third year of *Urochloa ruziziensis* pasture and Area 2—first year of *Urochloa brizantha* pasture), and two periods of excreta application (dry and rainy season) repeated in time (Year 1 and Year 2).

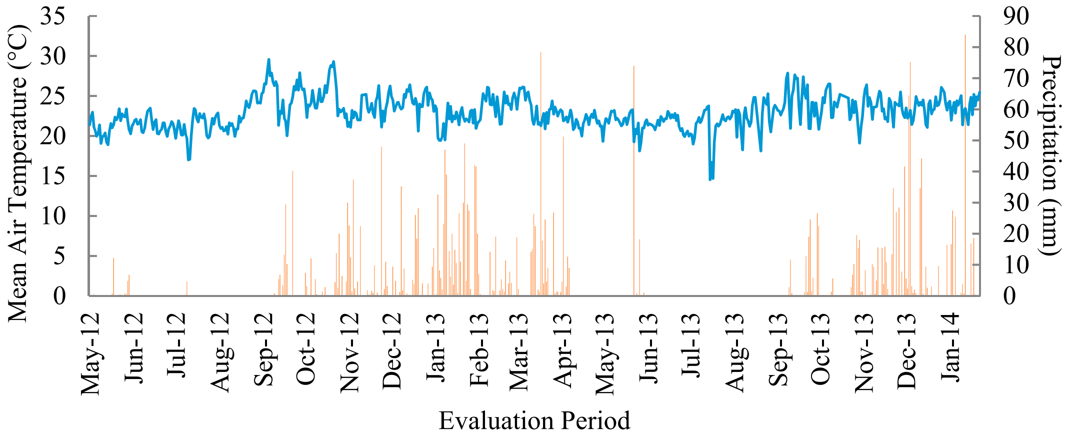

Figure 1. Rainfall index and mean daily temperature, recorded at the Meteorological Station of Fazenda Capivara of Embrapa Rice and Beans, in Santo Antônio de Goiás, from May 2012 to January 2014.

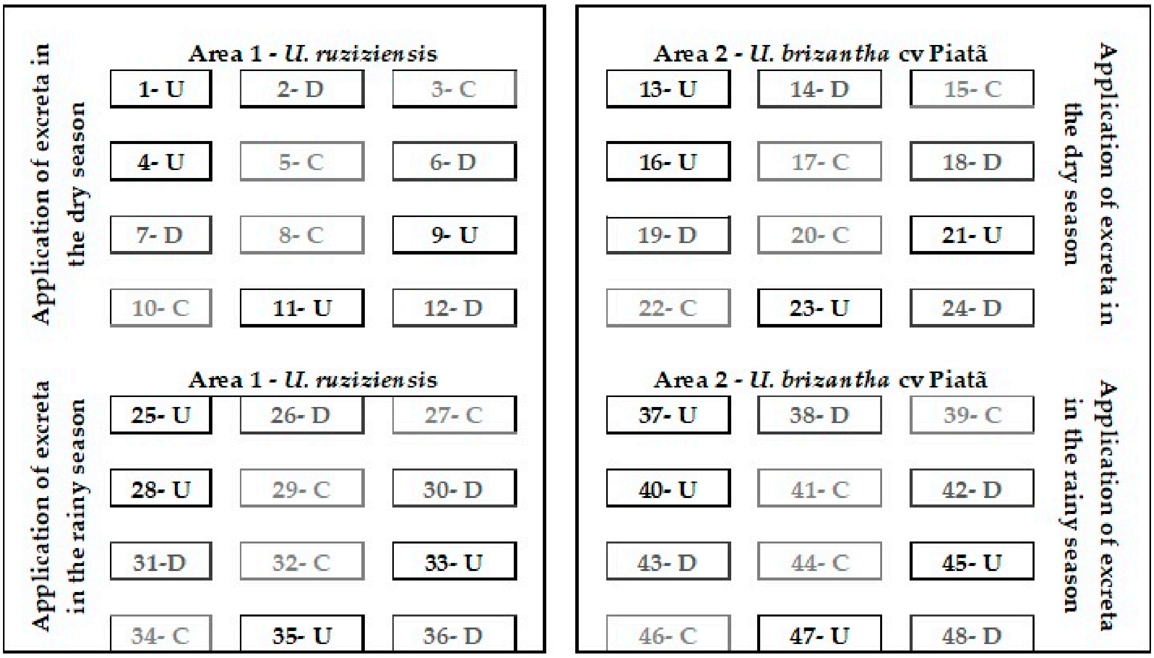

Figure 2. Distribution of the treatments in the two study areas. U: urine; D: dung; C: control (without excreta application).

Each plot consisted of a 40 × 240 cm area, where 40 × 60 cm was covered by the chamber of N_2O emission measurement, and the rest of the area (40 × 180 cm) was used for soil sampling to determine the other variables (bulk density, ammonium (NH_4^+) and nitrate (NO_3^-)).

The excreta were collected from dairy cattle. The urine volume applied in each chamber was 1/3 of the estimated urination of an adult female bovine, i.e., a volume of 0.5 L, and distributed in the chamber area (0.24 m^2). Two (2.0) kg of fresh dung were applied, corresponding to a cowpat of an adult bovine, inside the chambers (0.24 m^2), and evenly distributed on the chamber bottom. In the third treatment, no bovine excreta were applied (control).

Excreta sub-samples were collected to determine the N content and respective amount of N applied per chamber, via the Kjeldahl procedure [23] (Table 2).

Table 2. Nitrogen concentration of urine and dung and the amount per chamber in each application in the dry and rainy seasons.

Application	Season	Date of Excreta Application	N Urine		N Dung	
			$g\ L^{-1}$	$g\ m^{-2}$	$g\ kg^{-1}$	$g\ m^{-2}$
First	Dry	11 May 2012	7.80	16.25	22.50	7.42
First	Rainy	8 January 2013	6.00	12.50	31.00	10.23
Second	Dry	27 May 2013	6.10	12.71	23.50	7.75
Third	Dry	30 July 2013	5.80	12.08	21.00	6.93
Second	Rainy	26 November 2013	7.50	15.63	20.00	6.60

2.2. Gas Sampling and N_2O Analysis

One static chamber for air collection was installed per plot on the soil. Each chamber consisted of a rectangular metal base (40 × 60 cm), inserted 10 cm deep into the soil, perpendicular to the sowing line, and was left in the same place for the entire evaluation period. Around the top of the metal base ran a gutter about 1 cm wide, on which a metal cover 15 cm high and with the same dimensions of width and thickness was set at the time of sampling. To ensure airtight sealing, the gutter was filled with water. To avoid large temperature differences between the internal and external environment, the chamber was covered with a waterproof aluminized blanket. At the top of the chamber, connections were installed to transfer gas from inside the chamber to headspace vials.

Gas samples were always taken in the morning, from 09:00 to 10:00, as recommended by Alves et al. [24], to estimate the daily mean of N_2O fluxes from the soil.

After excreta application, gas was collected daily for seven days. Thereafter, sampling was performed twice a week for two weeks, then weekly until completing two months of evaluation and finally fortnightly, until urine and dung were applied again in the area. Applications were made in two periods of the year, in the dry and rainy seasons, to study how the climate interferes with the dynamics of N_2O emissions.

Air samples were collected three times (0, 10 and 20 min after chamber closure) to ensure linearity of the fluxes. A manual vacuum pump that transfers gas from the chambers to the headspace vials, through applying 70 kPa vacuum, was used to collect gas from within the chambers.

The N_2O concentration was determined using gas chromatography with a XL Auto System (PerkinElmer Inc., Waltham, MA, USA) with a packed column, containing "Porapak Q" at 65 °C and an electron capture detector 63Ni (ECD) at 375 °C. The flow of the argon–methane carrier gas mixture (argon 95%, methane 5%) in the system was 17.6 mL min^{-1}. To calibrate the chromatograph, primary N_2O standards were used, at concentrations of 350 ppbv and 1000 ppbv.

2.3. Calculation of Nitrous Oxide Fluxes

According to Parkin and Venterea [25], due to the influence of environmental conditions, particularly during sampling, fluxes may have a nonlinear pattern. Hutchinson and Mosier [26] suggested that applying linear regression to N_2O fluxes would underestimate the real flow.

The HM function is not always applicable to estimate the N_2O flow [26]. To use this function, some conditions must be fulfilled, e.g., gas sampling must have been performed at least three times and the time interval between the "zero" (C0) and second time (C1) of sampling and between the second (C1) and third sampling (C2) must be equal [26].

Based on the criteria used to indicate the variation in N_2O concentration in the chamber during the incubation interval ($\Delta C/\Delta dt$), the nitrous oxide flow per unit area (μL N_2O m^{-2} h^{-1}) was computed by multiplying the gas concentration at a given time (μL gas L^{-1} h^{-1}) by the chamber volume (L), and the resulting value divided by the chamber base area (m^2). The gas flow was then converted from the volume unit (μL gas m^{-2} h^{-1}) to mass unit (μg gas m^{-2} h^{-1}).

The total emission (TotEm) during the evaluation period at each time of year was determined by integrating daily N_2O fluxes. The emission factor was determined as the percentage of N_2O emitted of the N applied as urine or dung [24].

2.4. Sampling and Analysis of Volatilized Ammonia

Nitrogen losses via ammonia volatilization were measured in a semi-open chamber proposed by Martins et al. [27]. Each chamber consisted of one transparent 2-L PET bottle (diameter 10 cm) and was placed on the areas with excreta and control treatment. Inside the chamber, the bottle was suspended with a wire rod, where a sheet of polyethylene foam was suspended and moistened with 40 mL of H_2SO_4 capture solution (1 mol dm^{-3}) and 2% glycerin.

The ammonia collection chambers were installed near those for N_2O collection, where excreta were applied for soil sampling. The chambers were installed immediately after excreta application. The sampling pots with capture solution were exchanged every third day during the first 10 days after excreta application; later, thereafter, the sampling frequency followed that used for nitrous oxide.

To quantify N volatilized in the form of ammonia, after removal from the field, a capture solution was added in the laboratory with 30 mL of distilled water to wash the foam sheet. The capture solution that was still within the plastic bottle was shaken in a horizontal shaker at 200 rpm for 15 min; then, the foam was wrung out to remove all solution and then discarded. The entire solution was transferred to the digestion tube for distillation and subsequent titration with HCl 0.003 mol dm^{-3}. Nitrogen volatilized as ammonia was calculated based on the volume of hydrochloric acid used for titration, blank tests and samples, according to Equation (1).

$$NH_3\ (mg) = (Va - Vb) \times Nac \times PMN \quad (1)$$

where Va = volume of acid used for sample titration and Vb = volume of acid used for white titration. Nac = normality of acid and PMN = molecular weight of nitrogen.

Subsequently, the results were corrected, based on a correction factor of 1.74, to estimate the real volatilization rate of NH_3 of the soil, proposed by Martins et al. [27]. Cumulative NH_3 volatilization was estimated in mg m^{-2} by Equation (2).

$$NH_3\ (mg\ m^{-2}) = [(Naccumulated\ (mg)/0.008]/1.74 \quad (2)$$

2.5. Soil and Plant Variables

When nitrous oxide gas sampling coincided with the collection for determination of ammonia volatilization, soil sampling in the 0–0.01m layer was also performed, to determine gravimetric moisture, soil nitrate (NO_3^-), and ammonium (NH_4^+).

Nitrate and ammonium were extracted using potassium chloride solution (KCl) 1M and analyzed using an automated flow injection system (FIA, Lachat Instruments, 5600 Lindburg Drive, Loveland, CO, USA). In addition, the percentage of water-filled pore space (WFPS) of the soil was calculated by Equation (3).

$$WFPS = (U \times BD)/[1 - (BD/Dp)] \quad (3)$$

where WFPS is the water-filled pore space (%), U the soil gravimetric moisture (g g^{-1}), BD bulk density (g cm^{-3}), and Dp is particle density (g cm^{-3}).

During the experiment, when the plants hampered gas sampling in the areas of excreta treatment, plant samples were taken to determine plant dry weight per area (DW) in the same area. Parts of the samples were ground for analysis of total nitrogen (NT) using dry combustion, to later calculate N accumulation (AcN) in the forage, according to Equation (4).

$$Ac\ de\ N\ (g\ m^{-2}) = DW\ (kg\ ha^{-1}) \times TN\ (g\ m^{-2}), \quad (4)$$

where AcN is N accumulation, DW (dry weight), and TN (total nitrogen).

2.6. Statistical Analysis

Descriptive analyses were used to demonstrate the daily N_2O fluxes, cumulative ammonia volatilization and the pattern of soil variables in the same period: ammonium, nitrate, water-filled pore space, temperature, and rainfall index. The variables N_2O emission factor, total N_2O emission and cumulative ammonia volatilization were evaluated according to the sources of variation in the experiment: area (plot), excreta (subplots), times of the year (sub-sub plots) and their interactions.

The variable nitrogen accumulation was evaluated according to the sources of variation in the experiment: area (plot), excreta (subplots), collection time (sub-sub plots), and their interactions. The data were subjected to analysis of variance and means compared using the Tukey test at 5% probability. The statistical program R was used. Simple and multiple linear regression was also performed to explore the nature of the relationships between the explanatory variables of WFPS, soil nitrate, and ammonium content, and the response variable of N_2O fluxes.

3. Results

3.1. N_2O Fluxes, Soil Nitrate and Ammonium Content in the Dry Season

The N_2O fluxes from cattle excreta when applied in the annual dry season, in both study years, ranged from −22.54 to 628.53 μg N_2O m^{-2} h^{-1} in both areas. In Area 1, excreta application significantly increased N_2O fluxes in the ICL system over the control, without excreta application (−30.91 to 76.75 μg N_2O m^{-2} h^{-1}) (Figure 3A). Urine provided fluxes from −23.67 to 580.28 μg N_2O m^{-2} h^{-1}, and dung fluxes between −29.95 and 628.53 μg N_2O m^{-2} h^{-1} (Figures 3A and 4A).

In Area 2, the fluxes were close to those of the control: urine (between −22.54 and 259.37 μg N_2O m^{-2} h^{-1}), dung (−27.71 and 124.71 μg N_2O m^{-2} h^{-1}) and control (−25.02 and 204.06 μg N_2O m^{-2} h^{-1}) (Figure 4A).

After the second excreta application (27 May 2013) in the annual dry season, positive fluxes were recorded already on the third day after excreta application (DAA) (Figures 3A and 4A), exactly when another unexpected precipitation of 74 mm occurred (Figure 1). This effect lasted until 10 DAA, with emission peaks on the third DAA in all treatments, regardless of the area. In Area 1, peaks of 428.62, 411.57 and 18.94 μg N_2O m^{-2} h^{-1} were recorded for urine, dung and control, respectively (Figure 3A). In Area 2, the peaks for urine, dung and control, respectively, were 90.15, 19.15 and 2.90 μg N_2O m^{-2} h^{-1} (Figure 4A).

Owing to the 74 mm rainfall, it was decided to apply a third excreta deposition about 60 days after the second application (30 July 2013), when N_2O emissions reached baseline values. Even after N supply by means of the excreta, N_2O emissions remained null or negative until another rainfall of 11.6 mm, 48 DAA, and on this day, the peaks for urine, dung and control, respectively, were 247.25, 628.53 and 58.31 μg N_2O m^{-2} h^{-1} in Area 1 and 152.60, 49.52 and 28.13 N_2O m^{-2} h^{-1} in Area 2.

Dung application, in general, showed lower soil nitrate in ammonium than in urine and, in general, was similar to the control for Area 1 and Area 2 (Figures 5B and 6B).

After all three excreta applications, fluxes were only detected after precipitation, which increased the water-filled pore space in the annual dry period. Thus, in this study, the most intense N_2O fluxes in the dry period coincided with WFPS values close to 60% (Figures 3B and 4B) and nitrate contents higher than 21.38 mg kg^{-1} (Figures 5A and 6A). Nitrate availability in the soil increased with the increase in soil moisture (Figures 5A and 6A) and concomitantly with the decrease in ammonium (Figures 5B and 6B), which culminated in N_2O peaks in all treatments after the three applications in the dry period.

In Area 1, the intensity of N_2O fluxes from urine was higher than from dung after the first two applications. On the other hand, after the third application, fluxes from dung were 2.5 times higher than from urine (Figure 3A).

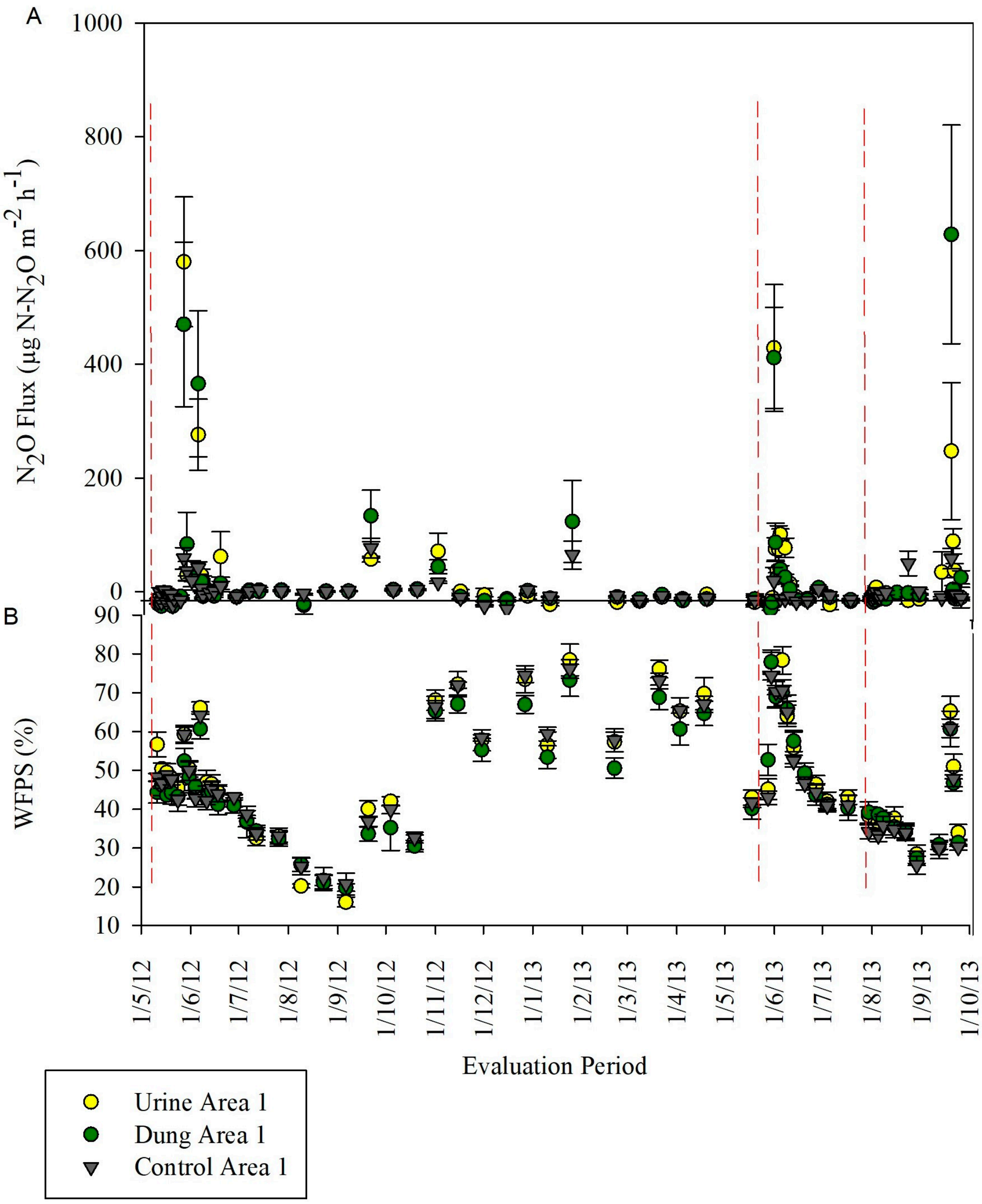

Figure 3. Fluxes of nitrous oxide (N_2O) (**A**), and water-filled pore space (WFPS) (**B**) of the soil under pasture in Area 1, with application of urine and dung in the dry season. Dashed lines indicate the applications of excreta in the pasture.

Table 3 shows the N accumulation of plants from excreta applications in the dry and rainy seasons. In both areas, N accumulation was generally higher in Area 2, regardless of the season. The period of the year with the highest N accumulation was November and April, which coincided with the beginning and end of the rainy season, respectively.

Table 3. Total nitrogen accumulation (g m^{-2}) in the forage after the application of excreta in the dry (Dry AcN) and rainy (Rainy AcN) seasons in two areas of pastures and collections at different times.

Dry Season	Area 1	Area 2	Rainy Season	Area 1	Area 2
November 2012	6.27 Ab	13.13 Aa	March 2013	5.12 Ab	6.65 Aa
March 2013	7.43 Ab	13.74 Aa	April 2013	3.38 BCb	4.83 Ba
April 2013	4.44 ABa	5.31 Ba	November 2013	4.28 ABb	6.55 Aa
July 2013	2.64 Ba	2.45 Ba	January 2014	3.02 Ca	3.33 Ca

Area 1: third year of pasture of *Urochloa ruziziensis*. Area 2: first year of pasture of *Urochloa brizantha* cv Piatã. Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ by the Tukey test at 5% probability.

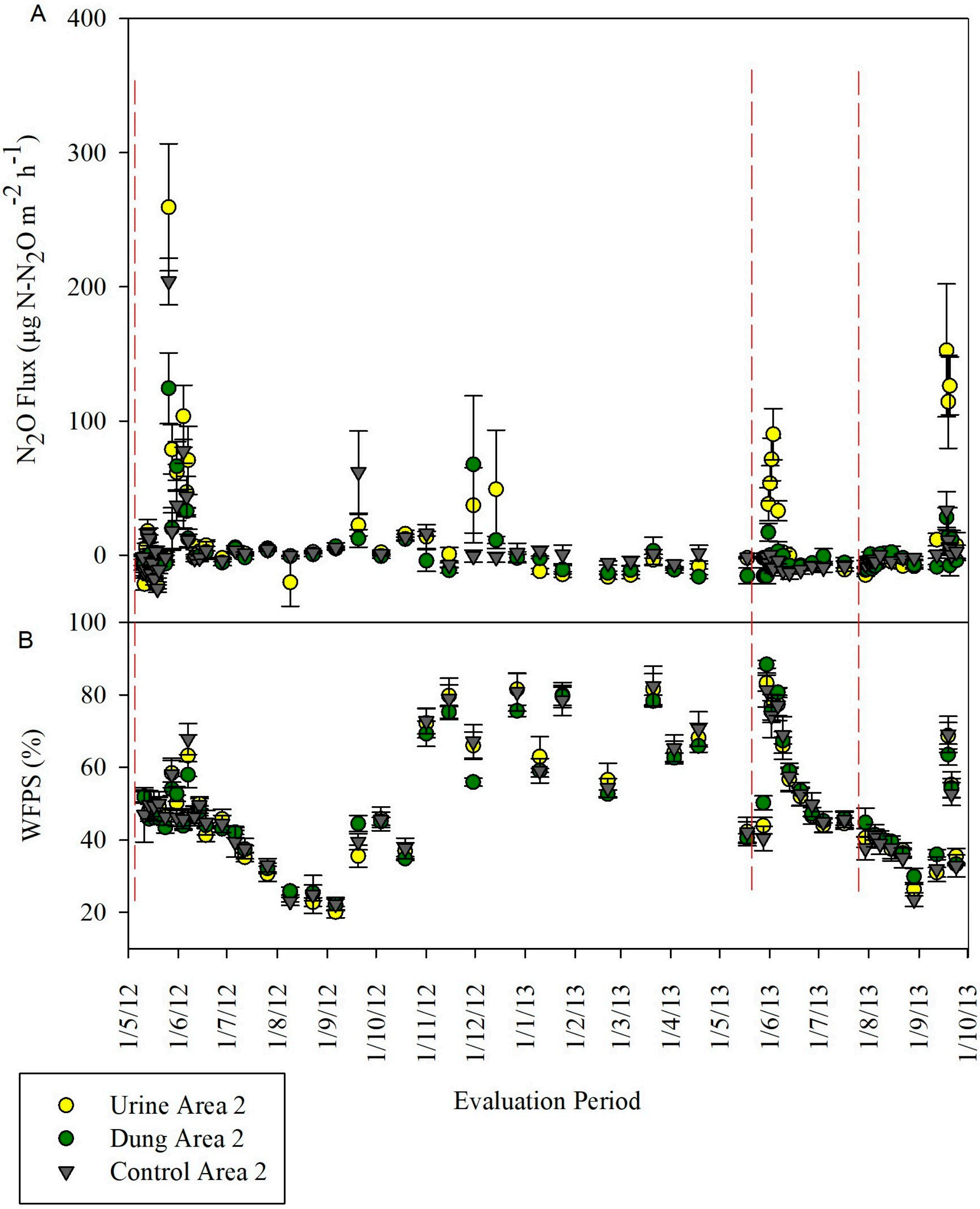

Figure 4. Fluxes of nitrous oxide (N_2O) (**A**), and water-filled pore space (WFPS) (**B**) of the soil under pasture in Area 2, with the application of urine and dung in the dry season. Dashed lines indicate the applications of excreta in the pasture.

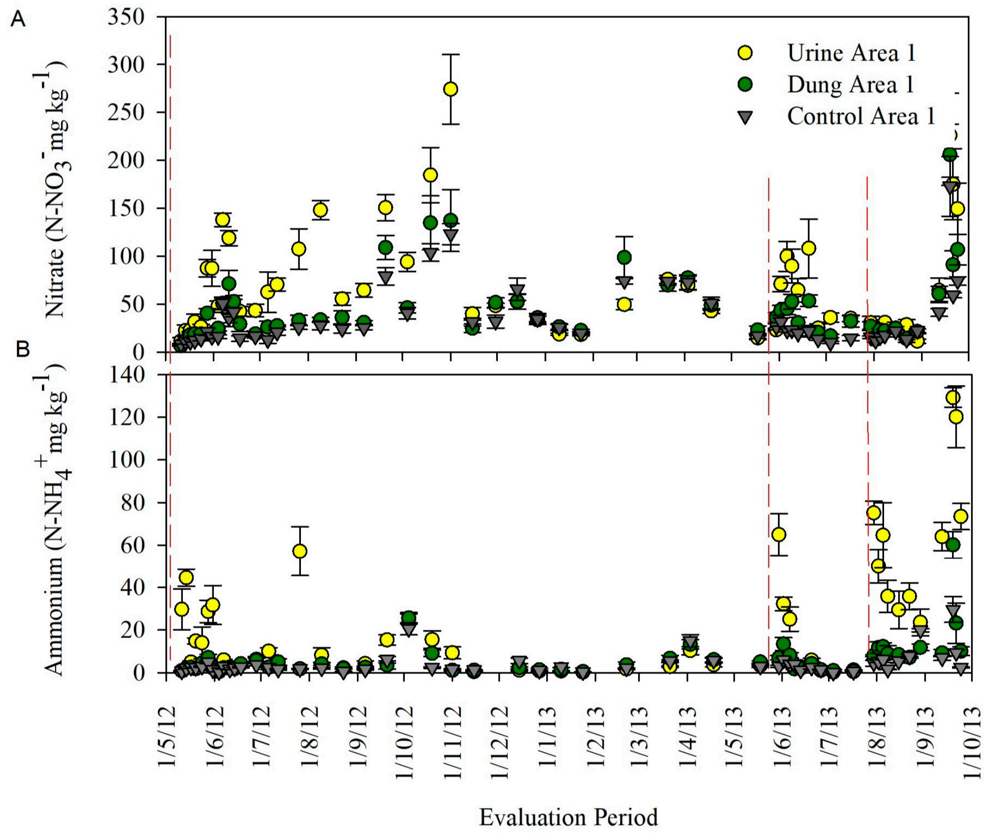

Figure 5. Nitrate (**A**) and ammonium (**B**) in the soil under pasture in Area 1, with application of urine and dung in the dry season. Dashed lines indicate the applications of excreta in the pasture.

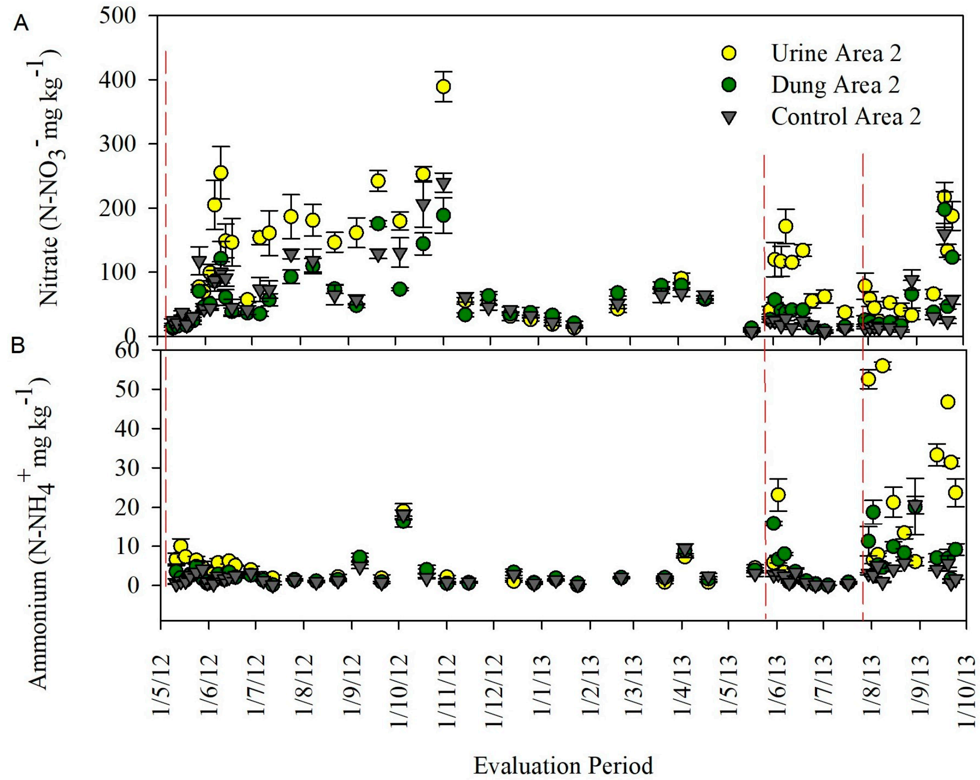

Figure 6. Nitrate (**A**) and ammonium (**B**) in the soil under pasture in Area 2, with the application of urine and dung in the dry season. Dashed lines indicate the applications of excreta in the pasture.

The entire evaluation period in the dry season for Area 1 and 2 indicated that only soil ammonium had a significant linear relationship with N_2O fluxes, with determination coefficients (R^2) of 0.14 at 5% probability (Table 4).

Table 4. Multiple and simple regression analysis for the dependent variable nitrous oxide (N-N_2O) as a function of the levels of nitrate (N-NO_3^-) and ammonium (N-NH_4^+) of the soil under two pastures and application of bovine excreta in the dry season.

Season	Linear Regression	R^2
Total	$N_2O = 2.59 + 0.06\ NO_3^- + 0.37\ NH_4^+$ *	0.15 *
	$N_2O = 4.23 + 0.09\ NO_3^-$	0.10 ^{ns}
	$N_2O = 6.01 + 0.43\ NH_4^+$ *	0.14 *
Positive fluxes	$N_2O = 33.74 - 0.11\ NO_3^- + 0.81\ NH_4^+$ *	0.21 *
	$N_2O = 37.62 - 0.07\ NO_3^-$	0.07 ^{ns}
	$N_2O = 25.07 + 0.71\ NH_4^+$ *	0.18 *

* Significant at 5% probability using the *t* test. ^{ns}: not significant.

3.2. N_2O Fluxes, Nitrate and Ammonium Content in the Rainy Season

The N_2O fluxes from cattle excreta, when applied in the annual rainy season, in both years of evaluation, varied between −40.01 and 686.68 μg N-N_2O m^{-2} h^{-1} between Area 1 and 2, respectively. Excreta application increased the soil N_2O fluxes considerably compared to the control treatment (without excreta application), with peaks of 56.20 and 32.40 μg N-N_2O m^{-2} h^{-1} in Areas 1 and 2, respectively.

In Area 1, fluxes varied between −47.72 and 353.15 μg N_2O m^{-2} h^{-1} for urine and from −52.56 to 560.83 μg N_2O m^{-2} h^{-1} for dung (Figure 7A). In Area 2, fluxes ranged from −40.01 to 686.68 μg N_2O m^{-2} h^{-1} for urine and for dung between −41.87 and 294.20 μg N_2O m^{-2} h^{-1} (Figure 8A).

On the first day after urine application in Area 1, the highest peak of the study period (353.15 μg N_2O m^{-2} h^{-1}) was obtained, while in Area 2, the emission peak (686.68 μg N_2O m^{-2} h^{-1}) in response to urine application was on the fourth DAA. The peaks related to dung deposition in both pasture areas occurred on the fourth DAA, with fluxes of 560.83 and 294.20 μg N_2O m^{-2} h^{-1} for urine and dung, respectively, in Area 1 after the second excreta application (Figure 7A).

After the second excreta application (25 November 2013), N_2O emissions were recorded from both urine and dung in the first DAA, in both areas. On the fourth day after urine application in Area 1, the highest peak of the study period (312.19 μg N_2O m^{-2} h^{-1}) was measured, and after the second application, the emission peak (364.59 μg N_2O m^{-2} h^{-1}) in response to urine application was on the first DAA.

In urine treatments, the ammonium content in the soil increased until 6 January 2013 in Areas 1 and 2. Also, nitrate increased after the second application of urine on the 26 November 2013 in both areas (Figures 9A and 10A). In dung treatment, the nitrate content, in general, was lower than urine treatment, and the highest value was obtained on the 7 February 2013, thirty days after. In the second application of dung, the higher value of nitrate was obtained at the second DAA for both areas, and was similar to the peaks of N_2O.

In urine treatments, the ammonium content in the soil was higher, especially in the first two DAA in both areas (Figures 9B and 10B).

The results of the entire evaluation period of the rainy season for Area 1 and 2 showed that only soil nitrate had a significant linear relationship with N_2O fluxes (R^2 = 0.52, at 1% probability) (Table 5), indicating that 52% of the N_2O variation can be explained via the variation in soil nitrate. Under the same conditions, WFPS was a secondary factor that explained a part of the N_2O fluxes (R^2 = 0.28).

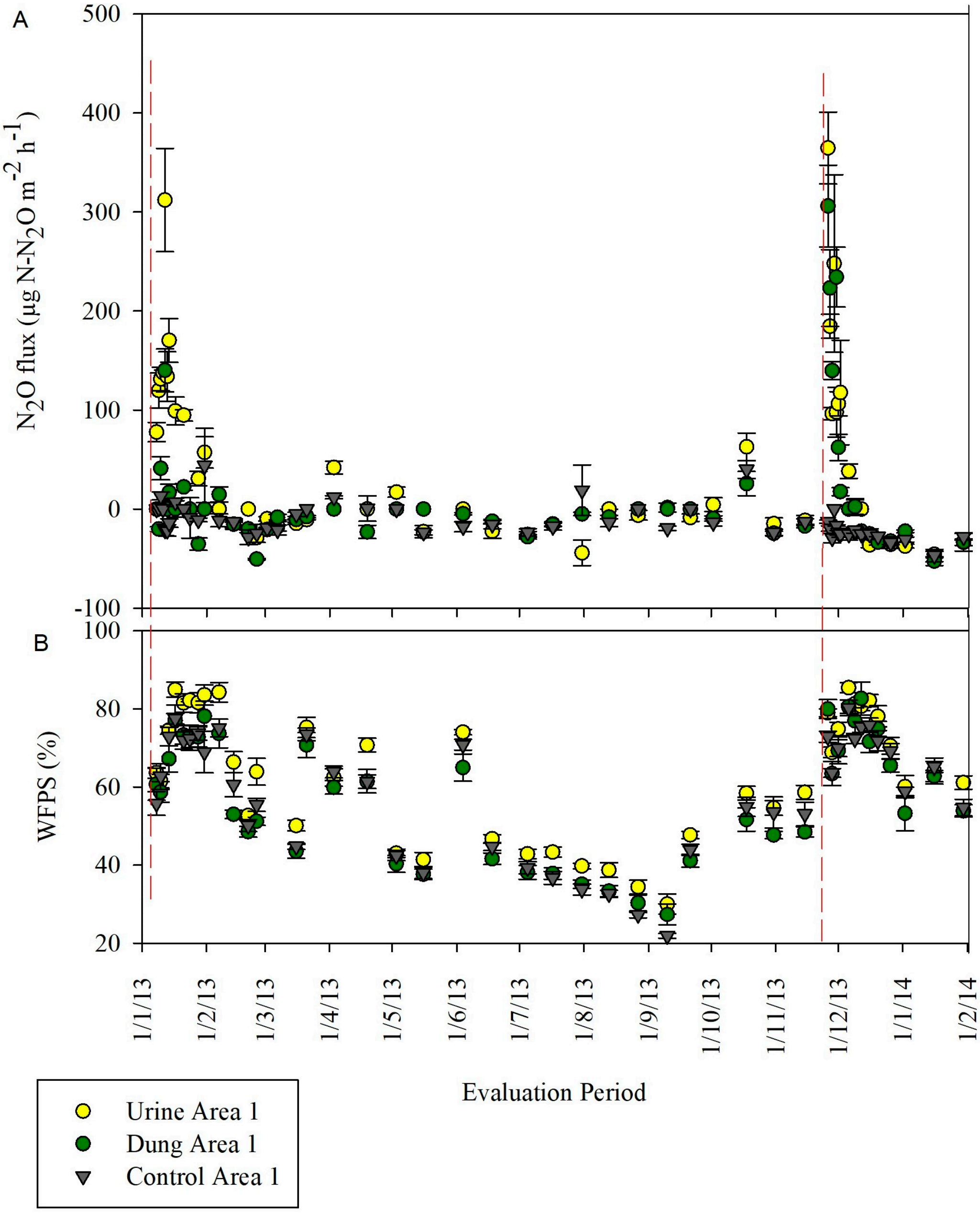

Figure 7. Fluxes of nitrous oxide (N_2O) (**A**), and water-filled pore space (WFPS) (**B**) of the soil under pasture in Area 1 (third year of *Urochloa ruziziensis* pasture), with the application of urine and dung in the rainy period. Dashed lines indicate the applications of excreta in the pasture.

The areas treated with excreta in the rainy season were evaluated for 388 consecutive days, i.e., with effective gas sampling on 53 days. In this period, 34% of the mean fluxes were positive and 51% negative. At this stage, negative fluxes were more pronounced in the treatments without excreta application, reaching 77 and 66% of the fluxes in Areas 1 and 2, respectively.

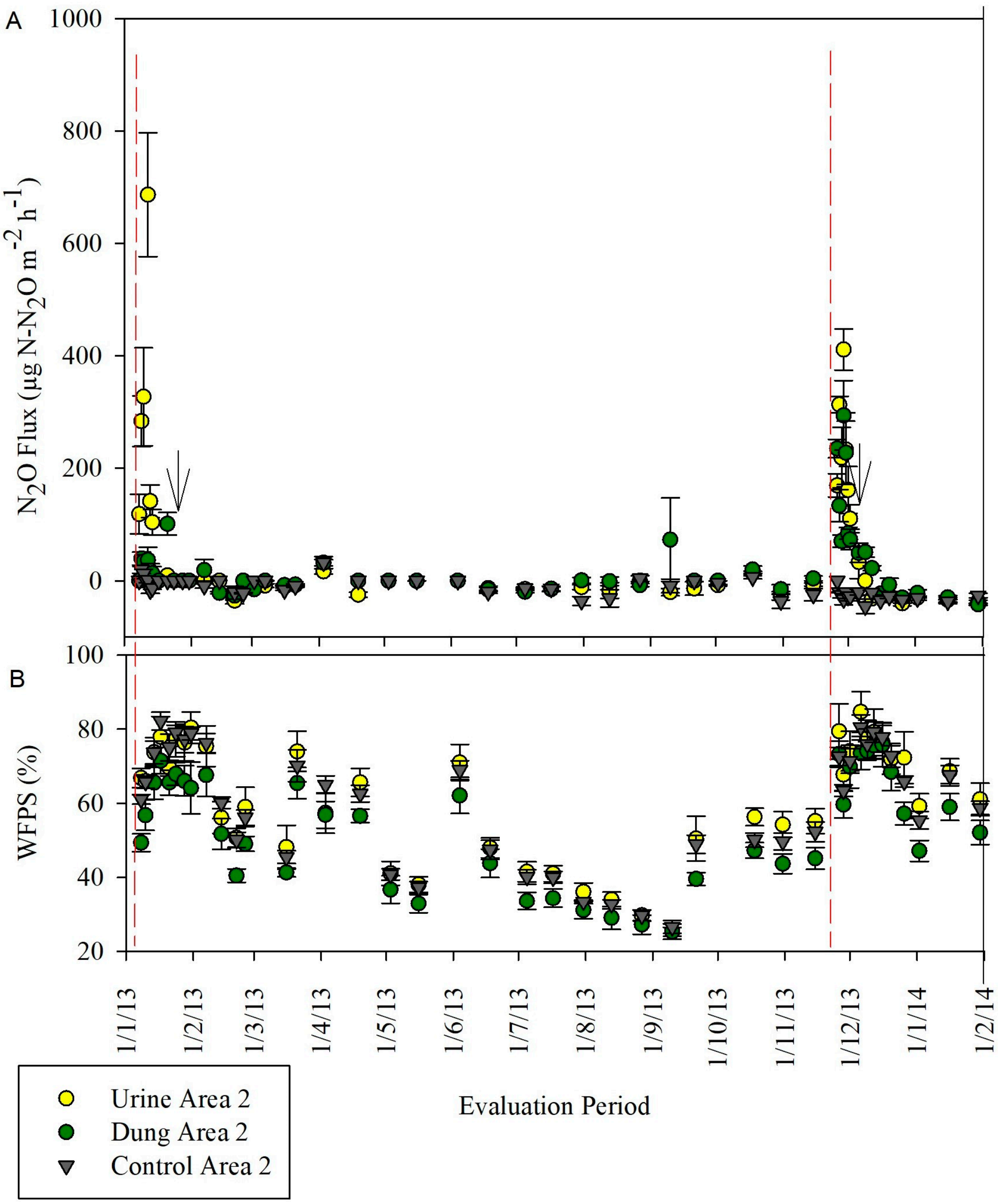

Figure 8. Fluxes of nitrous oxide (N_2O) (**A**), and water-filled pore space (WFPS) (**B**) of the soil under pasture of Area 2 (first year of pasture of *Urochloa brizantha* cv Piatã), with application of urine and dung in the rainy period. Dashed lines indicate the applications of excreta in the pasture.

Table 5. Multiple and simple regression analysis for the dependent variable nitrous oxide ($N\text{-}N_2O$) as a function of nitrate ($N\text{-}NO_3^-$) and ammonium ($N\text{-}NH_4^+$) levels of the soil and water-filled pore space (WFPS) under pastures and application of bovine excreta in the dry period.

Period	Linear Regression	R^2
Total	$N_2O = -31.42 + 0.63\ NO_3^-$ ** $-$ $0.16\ NH_4^+$	0.52 **
	$N_2O = -32.04 + 0.62\ NO_3^-$ **	0.52 **
	$N_2O = 5.33 + 0.17\ NH_4^+$	0.04 ^{ns}
	$N\text{-}N_2O = -59.47 + 1.12$ WFPS **	0.28 **
Positive fluxes	$N_2O = 24.12 + 0.53\ NO_3^{-\ **}$ $-$ $0.58\ NH_4^+$	0.46 **
	$N_2O = 22.22 + 0.51\ NO_3^-$ **	0.45 **
	$N_2O = 67.43 + 0.09\ NH_4^+$	0.02 ^{ns}
	$N_2O = -34.43 + 1.57$ WFPS *	0.28 *

*, ** Significant at 1% and 5% probability using "*t*" test, respectively. ^{ns}: not significant.

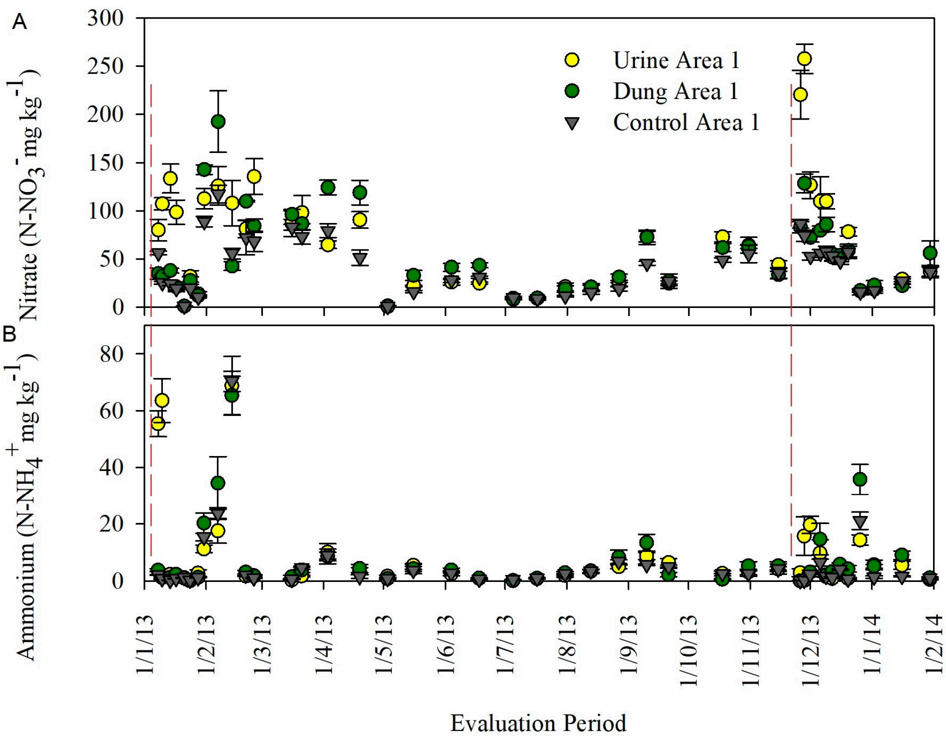

Figure 9. Nitrate (**A**) and ammonium (**B**) in the soil under pasture in Area 1 (third year of pasture of *Urochloa ruziziensis*), with the application of urine and dung in the rainy season. Dashed lines indicate the applications of excreta in the pasture.

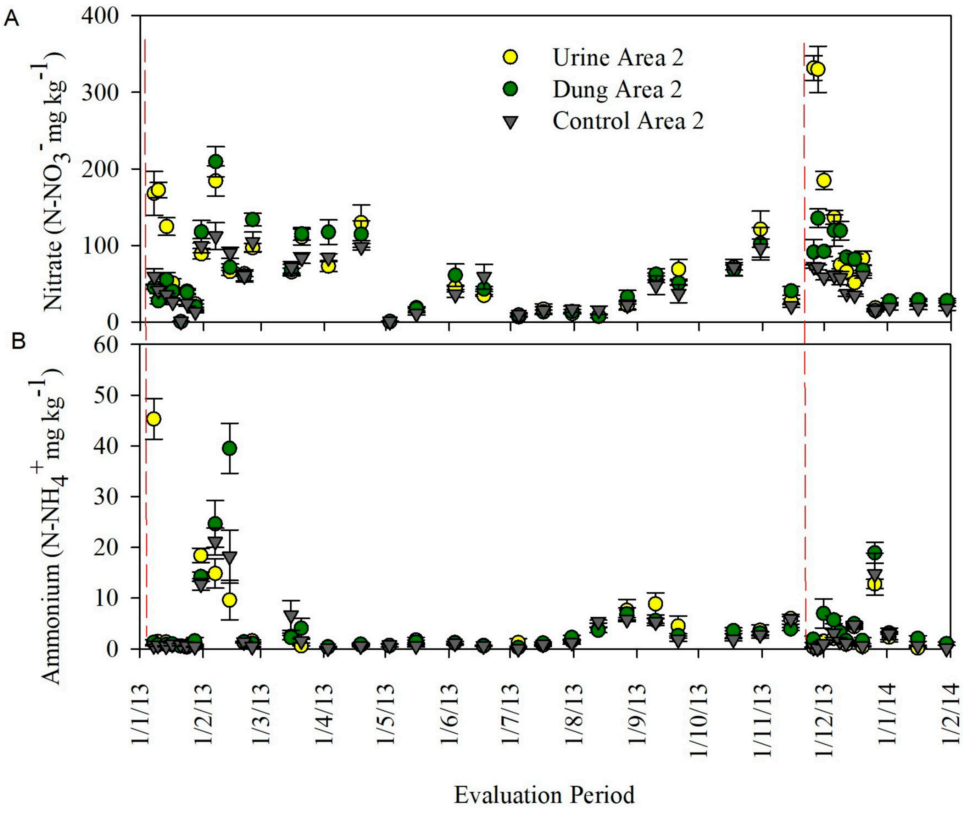

Figure 10. Nitrate (**A**) and ammonium (**B**) in the soil under pasture of Area 2 (first year of pasture of *Urochloa brizantha* cv Piatã), with the application of urine and dung in the rainy season. Dashed lines indicate the applications of excreta in the pasture.

3.3. Ammonia Volatilization in the Dry and Rainy Seasons

In the three applications performed in the dry season, losses from the urine treatment in the form of ammonia were greatest, especially in the first three days of monitoring (Figure 11). The mean volatilization from Area 1 was 122.89, 135.23 and 21.81 mg of NH_3 m^{-2} day^{-1} after the three applications, respectively.

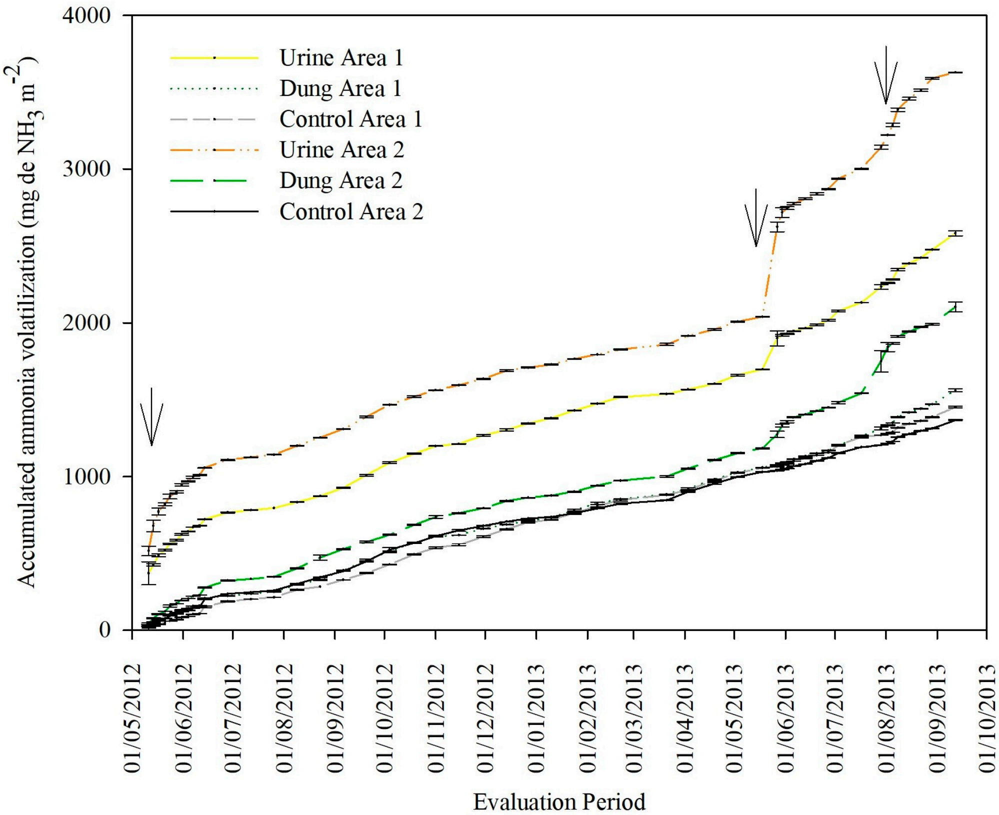

Figure 11. Accumulated ammonia volatilization refers to three applications of bovine excreta artificially deposited in the dry season of the year in pasture integrated crop–livestock system, for 502 days. The arrows indicate the application of excreta.

Volatilization rates in the dung treatment were also higher in the first days after application, but less intense and more constant than from urine, and then similar to the control throughout most of the evaluation period. The highest peaks were recorded in Area 2, with a mean volatilization of 18.27, 30.22 and 14.96 mg of NH_3 m^{-2} day^{-1} in the first three days of evaluation after the three applications, respectively.

Figure 12 shows cumulative N losses as NH_3 from cattle excreta when applied to pastures in the rainy season. The monitoring of volatilization from both excreta showed a similar pattern, with NH_3 losses soon after applications. In Area 2, the daily loss from urine in the first four days was 80.23 and 79.62 mg of NH_3 m^{-2} day^{-1} after both applications, respectively. From dung, daily losses were 57.41 and 22.16 mg of NH_3 m^{-2} day^{-1}, after both applications.

Cumulative ammonia loss from urine was lower in Area 1 than Area 2 (Table 6), and in Area 1, N_2O emissions losses tended to be higher than in Area 2, with higher emissions of NH_3 in the dry season compared to the rainy season (Table 6). Dung and Control treatments showed higher NH_3 volatilization in the rainy season.

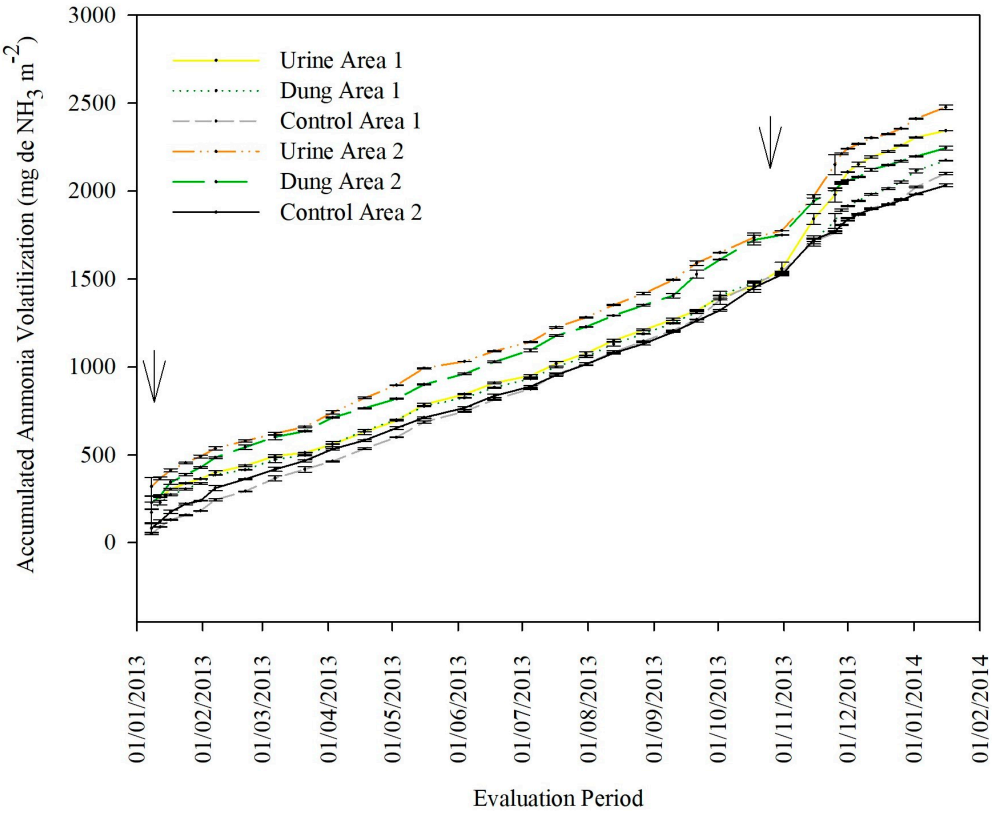

Figure 12. Accumulated ammonia volatilization refers to two applications of bovine excreta artificially deposited in the rainy season of the year in pasture in integrated crop–livestock system, for 388 days. The arrows indicate the application of excreta.

Table 6. Accumulated volatilization of ammonia (mg NH_3 m^{-2}) in two areas under pasture with the application of urine and dung in the dry and rainy seasons.

Excreta	Area 1	Area 2	Excreta	Dry Season	Rainy Season
Urine	2463.27 Ab	3053.16 Aa	Urine	3106.51 Aa	2409.92 Ab
Dung	1867.65 Ba	2174.48 Ba	Dung	1832.54 Bb	2209.59 Aba
Control	1776.14 Ba	1699.90 Ba	Controle	1409.13 Cb	2066.91 Ba

Area 1: third year of pasture of *Urochloa ruziziensis*. Area 2: first year of pasture of *Urochloa brizantha* cv Piatã. Averages followed by the same lowercase letter in the row and uppercase letter in the column do not differ by the Tukey test at 5% probability. Dry season 502 days of monitoring, and in the rainy season 388 days of monitoring.

3.4. Total N_2O Emission and Emission Factor

For total N_2O emission (TotEm), the differences among treatments were only significant for excreta and the period of the year (Table 7). The total N_2O emission from excreta was higher (1509.47 and 1285.5 g N_2O ha^{-1} for urine and dung, respectively) than in the control treatment, and total emissions were lower in the rainy than the dry season (877.59 and 1313.19 g N_2O ha^{-1}, respectively).

Emission factors were higher in the rainy season for urine application, and in Area 1, as shown in Table 8. The emission factor for urine (0.32%) was more than three times higher than for dung (0.10).

Table 7. Total emission of N_2O (g ha^{-1}) (EmTot) as function of the excreta applied and the season of the year.

Excreta	EmTot
Control	491.11 b
Urine	1509.47 a
Dung	1285.59 a
Season *	EmTot
Dry	1313.19 a
Rainy	877.59 b

* Total measurement period of 502 days in the dry season and 388 days in the rainy season. Averages followed by the same lowercase letter in the column do not differ by the Tukey test at 5% probability.

Table 8. Total emission of N_2O (g N_2O ha^{-1}) (EmTot), and emission factor (EF) from the excreta applied, pasture age and the period of the year.

Area	EmTot	EF (%)	Excreta	FE (%)	Period	EF (%)
Area 1	1115.33 a	0.24 b	Dung	0.10 b	Dry	0.11 b
Area 2	697.53 b	0.18 a	Urine	0.32 a	Rainy	0.36 a

Dry season (502 days) and rainy season (388 days) monitoring. Area 1: third year of *Urochloa ruziziensis* pasture. Area 2: first year of pasture of *Urochloa brizantha* cv Piatã. Averages followed by the same lowercase in the column do not differ in the row by the Tukey test at 5% probability.

4. Discussion

4.1. N_2O Fluxes, Nitrate and Ammonium Content in the Dry Season

This study evaluated the impact of urine and dung in N_2O emission and ammonium volatilization during the dry and rainy seasons in the Brazilian Cerrado. The application of urine and dung in the soil during the dry season in Area 1 cultivated with *U. ruziziensis* and in Area 2 cultivated with *U. brizantha.* The application of excreta was applied separately in each season of the year (dry and rainy), and for the dry season excreta were applied three times and in the rainy season it was applied twice.

Urine and dung were applied three times during the annual dry season. At 16 DAA (days after application) of the first urine and dung application (10 May 2012), positive fluxes were observed in both Area 1 and Area 2. This can be explained by a rainfall of 12.2 mm, unexpectedly high for the period (Figure 1). This effect lasted until 40 DAA, but there were emission peaks in all treatments at 16 DAA, regardless of the area (Figures 3A and 4A). In Area 2, the N_2O peaks were 259.37, 124.06 and 204.06 μg N_2O m^{-2} h^{-1} from urine, dung, and control, respectively (Figure 4A).

After two excreta applications, fluxes were only detected after precipitation (Figure 1), indicating that an increase in water-filled pore space promoted higher N_2O emissions in both areas (Figures 3B and 4B). The increase in N_2O emissions was also obtained by Yuan et al. [28]; the authors obtained higher N_2O fluxes through increasing the irrigation in grasses, indicating that variations in WFPS in the dry season affect N_2O emissions.

At the third application of excreta, N_2O emissions remained null or negative until another rainfall of 11.6 mm, and on this day, the peaks for urine, dung, and control, respectively, were 247.25, 628.53, and 58.31 μg N_2O m^{-2} h^{-1} in Area 1 and 126.20, 28.13, and 33.26 N_2O m^{-2} h^{-1} in Area 2. After all three excreta (urine and dung) applications, fluxes were only detected after precipitation, which increased the water-filled pore space in the annual dry period (Figures 3B, 4B, 5A and 6A). In Area 1 and Area 2, water-filled pore space (WFPS) was close to 60% after urine and dung application. On 1 July 2012, WFPS decreased and reached 20% at the end of dry season (1 September 2013) (Figures 3B and 4B). During the rainy season, WFPS reached 70–80% in both areas.

Thus, the most intense N_2O fluxes in the dry period coincided with WFPS values close to 80%. In addition, N_2O fluxes were higher and nitrate contents were higher than 21.38 mg kg^{-1} (Figures 5A and 6A). Nitrate availability in the soil increased with the increase in soil moisture (Figures 5A and 6A) and concomitantly with the decrease in ammonium (Figures 5B and 6B), which culminated in N_2O peaks in all treatments after the three applications in the dry period.

The effect of soil moisture on N_2O emissions has been widely discussed and recognized in the literature [7,28,29], especially when associated with higher nitrate levels [7,30] and without the limitation of readily available organic carbon content [7,31]. These conditions are required for the denitrification process of the soil. Lessa et al. [10] also applied cattle excreta to brachiaria pasture (*U. brizantha* cv. Marandú) in the Cerrado region in the annual dry season and observed that no N_2O fluxes were induced after the excreta became available. Only after an artificial irrigation of 28 mm in the area, almost 30 days after excreta application, low-intensity N_2O fluxes were recorded. In the dry season, N_2O was emitted a few days after excreta application, and more intense emissions were observed after rainfall in the dry season [32].

The higher N_2O fluxes were from urine than from dung after the first two applications. This was due to increased N from ruminants, leading to higher N in urine, especially in the form of urea which represents more than 70% of its composition [33], and up to 80% of which is hydrolyzed for up to 48 h [34,35].

After three applications of dung, N_2O fluxes were much higher than from urine, and one factor that could explain why the N_2O fluxes in areas under dung only increased after the third excreta application is the form of N in its composition. Nitrogen from dung is mainly organic [36], whose mineralization is gradual and, furthermore, its mineral N levels are lower than in urine as most of the organic compounds are insoluble in water and have a high C/N ratio [37,38]. Also, the addition of carbon content in dung may induce an increase in N_2O emissions, as Li et al. [39] obtained higher N_2O emissions in soil with the addition of several sources of organic carbon, promoting a priming effect. It is possible that there is an interaction of carbon and nitrogen content in cattle dung and urine, which would influence the priming effect of CO_2 and N_2O emissions.

After all three excreta applications, N_2O fluxes were lower in Area 2 than in Area 1. This could be explained with the history of each Area (Table 1), as Area 1, during pasture, was one-years-old and Area 1 was three-years-old. The presence of plant residues could increase N_2O fluxes, as plant-derived C supply may increase N_2O emissions [40].

At the first sampling of pasture in Area 2, N accumulation tended to be higher, which may explain the fact that the same application rates of N influenced the low N_2O fluxes in pasture Area 2 in this dry season. During the whole period of evaluation in the dry season for Area 1 and 2 and for positive fluxes of N_2O, only soil ammonium presented a linear relationship with N_2O fluxes. These results indicated that only 15% of the N_2O variation can be explained by the NH_4^+ content in the soil.

Autotrophic and heterotrophic nitrification occur under aerobic conditions and denitrification under anaerobic conditions, contributing to N_2O emissions, although these reactions are not fully understood [41]. According to Heil et al. [42], pH, soil C/N ratio, and manganese content control N_2O emissions from hydroxylamine (NH_2OH), the first intermediate compound of nitrification. Moisture pulses after dry spells may favor SOM mineralization, due to increased microbial activity in response to recent population growth or even due to the decomposition of microorganisms killed during the dry season; the authors call this process the "Birch Effect" [43].

The areas treated with excreta applications in the dry season were evaluated for 502 consecutive days, i.e., a total of 74 days of effective gas sampling. In Area 1 and 2, respectively, 39% of the mean fluxes were positive and 57% negative.

These proportions of N_2O fluxes were similar in all treatments. The factors that regulate these influxes of N_2O in the soil are not well understood, but the low availability of mineral N, low pH, and a low percentage of WFPS are known to be favorable conditions for the consumption of this gas in the soil [7,44].

4.2. N_2O Fluxes and Soil, Nitrate and Ammonium Content in the Rainy Season

In both areas and at both application times, urine deposition on the soil increased N_2O fluxes already in the first DAA. The emissions peaked on the fifth DAA, after the first application (1 July 2013) occurred, in both areas (312.16 and 686.68 µg N_2O m^{-2} h^{-1} in Area 1 and 2, respectively). For dung application, N_2O emissions were recorded after the second and third DAA, in Areas 1 and 2, respectively. However, the peaks occurred at different times. In Area 1, emissions from dung peaked on the fifth DAA (140.10 µg N_2O m^{-2} h^{-1}), and in Area 2 this peak occurred only after 13 DAA (164.17 µg N_2O m^{-2} h^{-1}). After this first excreta application, N_2O emission remained high until the 30 DAA in Area 1, when influxes were similar to the control treatment, considered the basal emission.

After the second excreta application, N_2O emissions were recorded from both urine and dung in the first DAA in both areas, and N_2O from excreta was continuously high until 18 DAA in both areas, when these became similar to the basal emission from the soil, with negative fluxes.

Several studies carried out in Brazil corroborate the results of this study. Sordi et al. [45] also observed higher N_2O fluxes soon after the application of cattle dung and urine rates in the different seasons of the year, with higher fluxes from urine in summer and from dung in spring. Lessa et al. [10] evaluated the effect of urine and dung on N_2O fluxes of a Cerrado latosol in the rainy season (rainy summer). They observed more intense fluxes in the first 30 DAA, with higher fluxes from urine than from dung.

In urine treatments, the nitrate content in the soil was higher, especially in the first two DAA in both areas in the rainy season (Figures 9B and 10B), as urine is composed mainly by urea which is converted into NH_4^+ and NO_3^-, increasing soil nitrification [46].

The data of this study reinforced that in pasture soils, N_2O emission occurs by the two microbiological processes of nitrification and denitrification [47]. However, under favorable moisture conditions, ammonium is rapidly mineralized and converted to nitrate, and high levels of nitrate, associated with increased WFPS, result in intense denitrification in the following days. This process was the cause for the highest N_2O peaks in the rainy season (Figures 7B and 9A for Area 1 and Figures 8B and 10A for Area 2).

Different from the dry season, in the rainy season, for the results of the whole period of evaluation for Area 1 and 2, only soil nitrate showed a linear relationship with N_2O emissions. Also, it was observed that 52% of the N_2O variation could be explained by the variation in soil nitrate. WFPS could explain 28% of the N_2O fluxes. These results indicate that N_2O fluxes in the rainy season are also favored when soil mineral N is NO_3^- [7,10,18].

4.3. Ammonia Volatilization in the Dry and Rainy Seasons

Ammonia (NH_3) volatilization is a pathway by which N migrates from the soil to the atmosphere in the form of gas, and this reaction accounts for the greatest N losses from the soil surface [48].

In the case of cattle excreta, urine represented a major source of ammonia volatilized to the atmosphere in the annual dry season (Figure 11). In the first three days, the daily means of 171.29, 259.98, and 52.31 mg of NH_3 m^{-2} day^{-1} were emitted from Area 2 (Figure 11). This resulted from urine application, which, due to urea hydrolysis, raised the soil pH temporarily and favored volatilization losses, as urea represents 75–90% of the N excreted and is hydrolyzed in the soil by the enzyme urease [46,49].

In the Brazilian Cerrado, Lessa et al. [10] observed in the rainy season that 80% of NH_3 volatilization occurred in the first two days of application and was almost null in the following weeks, but for dung, NH_3 volatilization increased for up to four days and ceased after 10 days. In the dry season, NH_3 volatilization was up to four times lower, and for dung, it was similar to the rainy season.

In the rainy season, different results were obtained in relation to the dry season as NH_3 volatilization from urine and cattle from both applications was similar in both study areas, indicating that there was probably an increase in precipitation, especially during excreta application (Figure 1), which could alter the pattern of NH_3 volatilization.

Regardless of the pasture area, the total NH_3 volatilization from urine was greater than in the other treatments, since 24 and 28% more ammonia was lost from Area 1 than from the dung and control treatments, respectively. In Area 2, where cumulative volatilization losses from urine were higher (3.05 kg NH_3 m^{-2}), the difference between the dung and control treatments was 29 and 44%, respectively. In the rainy season, Longhini et al. [50] found that ammonia volatilization from urine was 10 times higher than from dung.

Nitrogen content in urine may be a major source of NH_3 and N_2O [51]. Since about 56 to 93% of N in urine is in the form of urea [52], and high N losses from ammonia volatilization after urea application are common [53], urine can be an important source of NH_3 emission to the atmosphere. In addition to some sources of N volatilized as ammonia, this should also induce an increase in soil pH due to urine application to the soil [54]. Nitrogen hydrolysis that occurs in urine increases ammonium concentration in the soil, which is associated with an increase in pH, which favors potential conditions for NH_3 volatilization [55].

A lower cumulative ammonia in Area 1 than Area 2 (Table 8) suggested that losses in other forms from Area 1, e.g., through nitrate leaching or nitrous oxide emission, were higher, indicating that volatilization losses were minimized. In addition, Saggar et al. [55] suggested that plants also affect ammonia volatilization by reducing the concentration of ammonium ions in the soil solution or by changing the pH in the rhizosphere region.

Some factors that affected these ammonia losses were the pH, texture, clay fraction mineralogy, soil moisture capacity, temperature and organic matter content [55,56]. Ammonia losses increased with intensifying drought conditions, which occurs when temperatures rise [57] and relative humidity declines. This favors the diffusion of this gas into the atmosphere. In addition, infiltration is reduced in very dry soils, which facilitates ammonia emission due to the fertilizer–air contact [58]. Saggar et al. [55] reported that soil moistened by urine remains dry, and drought conditions favor NH_3 volatilization. Moreover, according to Saggar et al. [55], hot, dry or summer conditions favor volatilization, while rainy, cold or winter conditions minimize these losses. On the other hand, Lessa et al. [10] observed no differences in ammonia volatilization from dung between the dry and rainy seasons.

In both areas, the volatilization of accumulated ammonia originating from dung was similar to the control and lower than from urine, which confirmed previous studies [14,56]. According to Laubach et al. [54], the high humidity in bovine dung, associated with the elevation of pH (also demonstrated by the authors), suggests a potential loss through NH_3 volatilization. Lessa et al. [10] also indicated that this reduced gas loss from dung was because nitrogen in dung is not readily available and mineralization requires more time.

4.4. Total N_2O Emission and Emission Factor

Total N_2O emissions (ToEm) were similar for both excreta applications and were higher in the dry season than rainy season. As previously shown (Figures 7A and 8A), negative fluxes were higher and more frequent in the rainy season, especially in the control treatment, which may have influenced the total emission data.

Emission factors were 3.2 times higher in the rainy than in the dry season, and a similar trend was obtained for urine compared to dung application. Lessa et al. [10] also found a higher emission factor for urine than for dung, when applied in the rainy season. Sordi et al. [45] found low N_2O emissions and reduced emission factors for dung in both summer and winter. This low-emission factor is associated by the authors with high precipitations, as the experiment was conducted in sub-tropical conditions with events of precipitation during the whole year, and they stated that under rain, dung patches remained saturated, creating conditions to reduce N_2O to N_2.

The 2019 Refinement to the 2006 IPCC Guidelines suggests a generic factor (EF_{3PRP}) of 0.77% (0.03–3.82) for the direct emission of N_2O from urine and 0.13% (0.00–0.53) for dung in wet climate regions, to elaborate national GHG inventories. The emission factors observed in this study are all within the IPCC uncertainty range, albeit at its lower margins.

Similarly low-emission factors for excreta have been found in tropical regions, such as those obtained by other studies [10,32,44,45,59,60].

5. Conclusions

We measured and evaluated N_2O emission and NH_3 volatilization during a one year and a half period in two pasture areas, with one area being 3-years-old and the other being recently established within an ICL, with the separate application of urine and dung during the dry and rainy seasons in the Cerrado region in central Brazil. Excreta deposition in the pastures increased nitrous oxide fluxes and NH_3 volatilization, especially in the older pasture.

In the dry season of the year, positive fluxes of nitrous oxide occurred after precipitation, triggered by an increase in the water-filled pore space.

In the dry season, nitrous oxide fluxes were associated with higher ammonium availability. In the rainy season, these fluxes were related to nitrate availability and WFPS. In both seasons (dry and wet), urine promotes high losses of nitrogen as ammonium.

The area with the younger pasture component in the integrated crop–livestock system presented higher losses through ammonia volatilization and lower through nitrous oxide when the nitrogen source was urine, regardless of the season of year. Higher nitrous oxide emission factors were observed for urine (0.32%), and in the rainy season of the year (0.36%), and also in the older, 3-year-old pasture (0.24%), indicating that the contribution of cattle excreta needs to be separately considered, as does the history of pastures, and these should be evaluated in regional conditions. All emission factors were within the IPCC 95% confidence interval, albeit at its lower range.

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