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Effect of the Interaction between Excreta Type and Nitrogen Fertilizer on Greenhouse Gas and Ammonia Emissions in Pastures

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Abstract: This study aimed to evaluate the emission factor of N_2O, CH_4, and the volatilization of NH_3 for the combination of feces or urine with increasing doses of ammonium nitrate in tropical palisade grass pastures. The emission of greenhouse gases was assessed in eight treatments combining feces and urine with doses (75 and 150 kg of N ha^{-1}) of ammonium nitrate, (32% N). The emission factor of N_2O was 0.11, 0.19, and 0.17% for feces, urine, and 75 kg N ha^{-1} $year^{-1}$ (as ammonium nitrate) and showed an additive linear effect when feces or urine were combined with increasing doses of N fertilizer. The emission factor of CH_4 of feces (0.18 kg CH_4 $animal^{-1}$ $year^{-1}$) was similar irrespective of combination with ammonium nitrate. The N loss by volatilized NH_3 has a decreasing linear effect ($p < 0.05$) for the combination of feces or urine with ammonium nitrate. We concluded that N_2O and CH_4 emission factors of feces and urine in tropical climate conditions are lower than those reported by the IPCC. However, their N_2O emission factors are sharply enhanced when combined with ammonium nitrate. These results may contribute to improvements in national and regional greenhouse gas inventories of livestock production.

Keywords: ammonium nitrate; carbon dioxide; emission factor; marandu palisade grass; methane; nitrous oxide; tropical soils

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1. Introduction

Pastoral ecosystems are vital for food production in the world due to the ecosystem services they provide, such as provisioning of feed and fresh water; supporting soil stability, nutrient cycling, and natural habitat; regulating (climate, pollination, and water purification); and cultural (recreation, ecotourism, and pleasing landscape; [1]). Otherwise, they also emit nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2), which are known as the leading greenhouse gases (GHG) in the production of beef cattle. Nevertheless, previous studies have demonstrated functional and suitable alternatives for GHG mitigation from soil and animal [2–5] that offset the negative aspects of pastoral ecosystems for ruminant production.

Signatory countries of the Kyoto Protocol should report their GHG emissions on a mandatory or optional basis. The methodology of the Intragovernmental Panel on Climate Change—IPCC [6] advisory board on climate change was developed to carry out these inventories. The IPCC default emission factor (EF) of N_2O and CH_4 for bovine excreta (feces + urine) and nitrogen fertilizers is commonly used to calculate the GHG inventories of each country. Then, the updated default EFs currently adopted by IPCC are 0.4% of total excreted N (amount of nitrogen in feces + urine, EF_{3PRP} [7], emitted directly in the form of N_2O) and 1% of N from fertilizers lost as N_2O (EF_1 [7], while the EF of CH_4 of feces is

0.6 kg CH_4 per kg volatile solid excretion [7]. However, those default EF data might not represent the actual emissions from excreta and fertilizers for several countries because of the regionalized soil and climate variations. For instance, a previous study carried out in a subtropical climate demonstrated that the emission factor of N_2O could represent less than $\frac{1}{4}$ of the value suggested by the IPCC [8]. Moreover, the default EF does not account for feces and urine separately, which may overestimate GHG emissions, considering that the EF of feces and urine may differ considerably [8,9].

Regarding the EF of fertilizers lost as N_2O (EF_1), a previous study reported EF_1 of 1.7 and 1.1% for doses of nitrogen (urea) of 75 and 150 kg ha^{-1}, respectively, in a long-term experiment with ryegrass canopy (*Lolium multiflorum*), oats (*Avena sativa*), and common beans (*Phaseolus vulgaris*) in summer [10], which were within the range recommended by IPCC. Likely, the study of Cardoso et al. [9] confirmed the IPCC emission factor of 1% for nitrogen fertilizers (urea). Although urea is the main N fertilizer used in agriculture, a previous study reported that the EF_1 of ammonium nitrate was 17% lower than that of urea in tropical conditions [11], implying that N fertilizers in the ammoniacal and/or nitric forms could be alternative fertilizers to urea to minimize N loss [11].

Another important source of N loss to the atmosphere is ammonia volatilization (NH_3). A previous study conducted in tropical conditions with three sources of N fertilizer (urea, ammonium nitrate, and ammonium sulfate) at three doses of N (90, 180, and 270 kg of N ha^{-1}) observed that the losses by volatilization of NH_3 were greater for urea followed by ammonium nitrate and ammonium sulfate, whose N losses in the form of NH_3 increased linearly with the applied N dose [12], suggesting that nitrate use may be beneficial, aiming at the sustainability of the pastoral ecosystem.

Previous studies have already reported the lowest emission of N_2O and the additive emission behavior of the excreta and N fertilizer interaction in temperate climates [13–16]. Furthermore, lower emission of CH_4 [9] from feces and lower volatilization of NH_3 [12] of different sources and doses of N in tropical soils, compared with IPCC's default, have been demonstrated. However, the emission factors of N_2O, CH_4, and NH_3 volatilized the interaction between feces or urine, and their combinations with nitrogen fertilizer in tropical climate conditions are still lacking. We hypothesize that the emission factors of N_2O, CH_4, and NH_3 volatilized of the interaction between feces or urine and their combinations with N fertilizer (as ammonium nitrate) in tropical climate conditions would be additive but lower than the IPCC default recommendation.

Therefore, the objective of this research was to quantify the emission factor of N_2O, CH_4, and the volatilization of NH_3 and the fluxes of N_2O, CH_4, and CO_2 of feces and urine of cattle, combined with doses of N fertilizer as ammonium nitrate, under tropical climate conditions and to identify the key variables that explain the production of each GHG in marandu palisade grass pastures.

2. Materials and Methods

2.1. Experimental Area

The experiment was conducted during two rainy and dry seasons, 2018/2019 and 2019/2020, to calculate the emissions of N_2O, CH_4, and CO_2 and the volatilization of NH_3 of different excretes (feces or feces urine), combined with doses of N fertilizer as ammonium nitrate. The experiment was carried out in the Beef Cattle Center of the Department of Animal Science of Sao Paulo State University (UNESP), campus Jaboticabal, located in the physiographic region of the Western Plateau of São Paulo, coordinates 21°15′22″ S, 48°18′08″ O, with an altitude of 595 m above sea level. The climate is humid subtropical with dry winters and warm summers (Aw), according to Köppen's classification, and the soil is classified as typical Hapludox with a clay texture [17]. The experimental area was established in 2005 with *Urochloa brizantha* cv. Marandu (marandu palisade grass).

The experimental area consisted of 2195 m^2, which had been restricted from animal access since September 2018. The canopy of this area was maintained at the height of 25 cm by regular cutting. The treatments were applied in two distinct sites within the

experimental area: (i) one site with the standard static chambers to evaluate N_2O, CH_4, and CO_2 emissions [9], and an adjacent area of 1 m^2 to measure porous space, moisture, ammonium (NH_4^+), and nitrate (NO_3^-) of soil (Figures S1 and S2); and (ii) another site with an open semi-static chamber to evaluate NH_3 volatilization (Figures S3 and S4) [18].

At the beginning of the experimental period, soil samples of the experimental area were collected at 20 cm depth. The mean values of soil chemical characteristics were P-resin: 11.5 mg dm^{-3}; organic matter: 28.5 g dm^{-3}; pH $CaCl_2$: 5.3; K^+: 4.6 mmol dm^{-3}; Ca^{2+}: 24 mmol dm^{-3}; Mg^{2+}: 13.5 mmol dm^{-3}; H + Al: 26 mmol dm^{-3}; the sum of bases: 42.5 mmol dm^{-3}; cation exchange capacity: 68.5 mmol dm^{-3}; base saturation: 62%. The mean values of soil physical characteristics were 27.5% of clay, 6.5% of silt, and 66% of sand.

2.2. Treatments

Treatments consisted of the combination of feces or urine with doses of N fertilizer: Feces (F), Feces + 75 kg of N ha^{-1} (F + 75), Feces + 150 kg of N ha^{-1} (F + 150), Urine (U), Urine +75 kg of N ha^{-1} (U + 75), Urine +150 kg of N ha^{-1} (U + 150), only fertilizer—75 kg of N ha^{-1} (75N) and control without excreta or fertilizer (Basal). Nitrogen fertilization doses were 75 or 150 kg of N ha^{-1} $year^{-1}$. The source used was ammonium nitrate, which has 32% nitrogen. The experiment was evaluated for two consecutive years in a randomized block design with eight treatments and five replicates (double blocking: blocks = slope of the terrain and year).

2.3. Collection of Bovine Feces and Urine

Feces and urine were collected fresh from Nellore bulls immediately after excretion from a simultaneous experiment where the animals were kept in marandu palisade grass pastures managed in a continuous and put-and-take stocking to maintain the target canopy height of 25 cm and fertilized with nitrogen (N) doses at 0, 75, and 150 kg N ha^{-1} as ammonium nitrate (32% N). The animals have been cared for according to São Paulo State University Animal Ethics and Use Committee, and all procedures were approved under protocol approval number 7979/18.

The collection of feces and urine was performed in January of each year. Feces and urine from animals managed in pastures without N fertilization (0 kg N ha^{-1}) were used in the F and U treatments; feces and urine from animals managed in pastures with N fertilization of 75 kg N ha^{-1} were used in the F + 75N and U + 75N; and feces and urine from animals managed in pastures with N fertilization of 150 kg N ha^{-1} were used in the in F + 150N and U + 150N treatments. Briefly, in the morning for three consecutive days, a group of four animals were taken to the corral and held in the squeeze chute. Feces were collected after spontaneous defecation and urine, during spontaneous urination. Feces and urine were pooled among bulls within each managed pasture as explained earlier, and pooled samples were split into replicates of each treatment.

After collection, feces and urine were stored in a refrigerator at −15 °C. Subsequently, they were thawed, a sample was taken for chemical analysis, and the remainder was used in the experiment. Feces and urine were analyzed for nitrogen content (method 978.02; [19]). Urine pH was measured using a digital pH meter (pH meter TEC 7, Tecnal, Brazil). Feces were analyzed for dry matter (method 934.01; [19]) and total carbon content [20]. The carbon–nitrogen ratio (C/N) was estimated by dividing the total carbon content of feces by the sum of the nitrogen content of feces and urine. The characteristics of the excretes are depicted in Table 1.

Table 1. Chemical characteristics of feces and urine from Nellore bulls managed in marandu palisade grass pastures fertilized with different doses of N.

Variables [a]	Doses of N Fertilization in Pastures (kg N ha^{-1})		
	0	**75**	**150**
DM (%)	15.5 ± 0.24	15.5 ± 0.19	14.8 ± 0.26
Carbon in feces (% of DM)	43.8 ± 0.81	45.1 ± 2.22	43.2 ± 0.67
N in feces (% of DM)	1.09 ± 0.04	1.18 ± 0.06	1.26 ± 0.07
N in urine (g L^{-1})	1.6 ± 0.83	1.8 ± 0.37	0.7 ± 0.38
pH—urine	7.7 ± 0.07	8.1 ± 0.01	8.2 ± 0.02
C/N	34.9	33.1	32.1

[a] DM = dry matter; C/N = carbon nitrogen ratio. Value ± standard deviation.

2.4. N_2O, CH_4, and CO_2 Flux Measurements and Emission Factors

Direct emissions of N_2O, CH_4, and CO_2 were measured using the static closed chamber technique [9]. Gas fluxes were measured from 18 February to 12 December 2019 in the first year and from 20 January to 13 October 2020 in the second year. Each chamber was composed of a base and a chamber itself. The base of the chamber consisted of a rectangular metal frame (0.6 × 0.4 m) that was inserted about 7 cm deep into the soil and left for the whole experimental period. The headspace of the chambers consisted of a rectangular polyurethane box of 0.6 × 0.4 m and 24 cm high (volume of 0.0576 m^3) coated with a thermal insulation mantle (thickness of 8 mm), equipped with an output valve for sample removal.

The experimental site consisted of 40 (8 treatments × 5 replicates) 2 × 2.5 m plots, within which the base of the chamber was placed, and an adjacent area of 1 m^2 was used for soil collection. Each plot received the same treatment in each experimental year.

At the beginning of each year (on 18 February 2019 in the first year and 20 January 2020 in the second year), the amount of 1.6 kg of fresh feces, 1 L of urine, and the equivalent of kg N ha^{-1} of ammonium nitrate fertilizer were applied in the center of the base of the chambers and the adjacent area, on the soil surface, according to the respective treatments [21]. The amount of feces and urine were based on previous study of Lessa et al. [21]. Ammonium nitrate fertilizer (as prills) was spread manually into the chambers to simulate fertilization. The total amount of N applied in each plot per treatment is depicted in Supplementary Material (Table S1).

After applying the treatments, the gas and soil samples were collected three times in the first week, twice in the second, third, and fourth weeks, and once a week in the fifth, sixth, seventh, and eighth weeks. From the nineteenth week onwards, samples were collected every fifteen days. The samples were taken between 9 and 10 a.m. [22]. For gas sampling, the top was coupled to the base of the chamber, whose headspace remained closed for 40 min, and headspace air was sampled at 0, 20, and 40 min after closure. The samples were collected with a 30 mL polypropylene syringe. Soil (at 5 cm depth) and air temperatures inside and outside the chamber were measured using digital thermometers. The air samples were transferred to pre-evacuated chromatography vials (30 mL), and then the gas concentration was determined by gas chromatography.

The samples were analyzed in a gas chromatograph (Shimadzu Green House Gas Analyzer GC-2014; Kyoto, Japan) with the following conditions to measure N_2O: injector at 250 °C, column at 80 °C, using N_2 as carrier gas (30 mL min^{-1}), and the electrical capture detector at 325 °C; for CH_4: using H_2 as carrier gas (30 mL min^{-1}), flame intake detector at 280 °C.

Fluxes of N_2O (μg N-N_2O m^{-2} h^{-1}), CH_4 (μg C-CH_4 m^{-2} h^{-1}), and CO_2 (mg C-CO_2 m^{-2} h^{-1}) were estimated assuming the linear increase of gas concentration during the sampling period, air temperature and pressure, chamber volume, and area of the metal bases [23].

The hourly fluxes of the three gases were multiplied by 24, obtaining the daily emissions, and then were integrated through linear interpolation, bringing the cumulative emission. Negative fluxes were included in the calculations to avoid bias in the results [24]. The

direct emission factor (EF) N_2O-N emitted as a function of the percentage of N applied was calculated by: EF = ((N-N_2O Treatment − N-N_2O control treatment)/N applied) × 100%, respectively, in g N m^{-2}. Cumulative emissions of CH_4 (g CH_4 kg^{-1} dry feces) were multiplied by the annual fecal production of an adult animal to obtain the emission factor of CH_4 originating from feces (kg CH_4 $head^{-1}$ $year^{-1}$). Cumulative CO_2 emissions were calculated in kg per m^{-2} year (kg m^{-2} $year^{-1}$) [23].

2.5. Evaluation of N Losses by Ammonia Volatilization

The N losses in the form of NH_3 volatilized were measured using a semi-open static chamber technique [18]. The NH_3 volatilization was measured from 18 March to 4 April 2019 in the first year and from 2 February to 3 March 2020 in the second year.

Semi-open static chambers were built from PET bottles (transparent polyethylene ethylene plastic), with a capacity of 2 L and an area of 0.008 m^2. After applying the treatments, the samples were collected at 5 p.m. on the 1st, 3rd, 5th, 9th, 14th, and 21st days by replacing acid-embedded foam strips with fresh ones.

Inside the PET bottle, the foam system to capture NH_3 consisted of a polyurethane foam strip (0.017 g cm^{-3}) with 3 mm thickness, 2.5 cm wide, and 25 cm long, moistened with an acid solution, and suspended vertically with a rigid wire of 1.5 mm in diameter. In a plastic bottle with a capacity of 50 mL, suspended by the lower end of the rigid wire, 10 mL of H_2SO_4 solution, 1 mol dm^{-3} + glycerin (2% v/v) were added. At installation, the foam strip was kept with the lower end inside the 50 mL vial to avoid splashing the acid solution on the substrate, and the other end of the foam was attached to the top of the rigid wire to keep it upright. The chamber was suspended approximately 1.5 cm from the ground surface. The determination of N-NH_3 retained in the foam was performed by distillation and titration (Kjeldahl method; method 978.02; [19]).

2.6. Soil and Meteorological Parameters

Soil bulk density was measured at the beginning of the experiment in November 2018; samples (10 cm diameter × 5 cm height) were dried at 105 °C for 72 h and then dried, obtaining soil density (Mg m^{-3}).

On the day of gas sampling, soil samples were collected at a depth of 0–10 cm (from an adjacent area of 1 m^2) to determine soil moisture, extractable ammonium, and extractable nitrate concentration. Approximately 10 g of sampled soil were mixed in 50 mL, 2 M of KCl, stirred for 30 min at 240 RPM, and filtered (filter paper circles, MN 640 d, 150 mm, Macherey–Nagel). The filtered solution was frozen until N–nitrate (plus N–nitrite) and N–ammonium were determined. The Berthelot reaction and reduction by vanadium chloride-III [25] were used to determine NH_4^+ and NO_3^-, respectively. The remaining sampled soil was dried at 105 °C for 72 h to determine gravimetric moisture. The water-filled pore soil space (% WFPS) was calculated using soil and particle density (2.65 g cm^{-3}). The daily precipitation, minimum air temperature, and maximum and average precipitation were obtained from the agrometeorological station of the Department of Engineering and Exact Sciences of Sao Paulo State University (UNESP), campus Jaboticabal, located 500 m away.

Rainfall and temperature conditions characterized the distinct seasons of the tropical climate of Jaboticabal, São Paulo, Brazil, with well-defined rainy and dry seasons. It rained 323.7 mm (2019) and 403 mm (2020) during the rainy season, while the rainfall was practically null throughout the dry season. The mean temperature was 23.7 °C (standard error of the mean (SEM) = 0.17) and 23.2 °C (SEM = 0.19) in 2019 and 2020, respectively. The water-filled soil pore space (%WFPS) was greater at the implementation of the experiment and decreased over time, averaging 36% (ranging from 8 to 90%) in 2019 and 2020 (Figure 1).

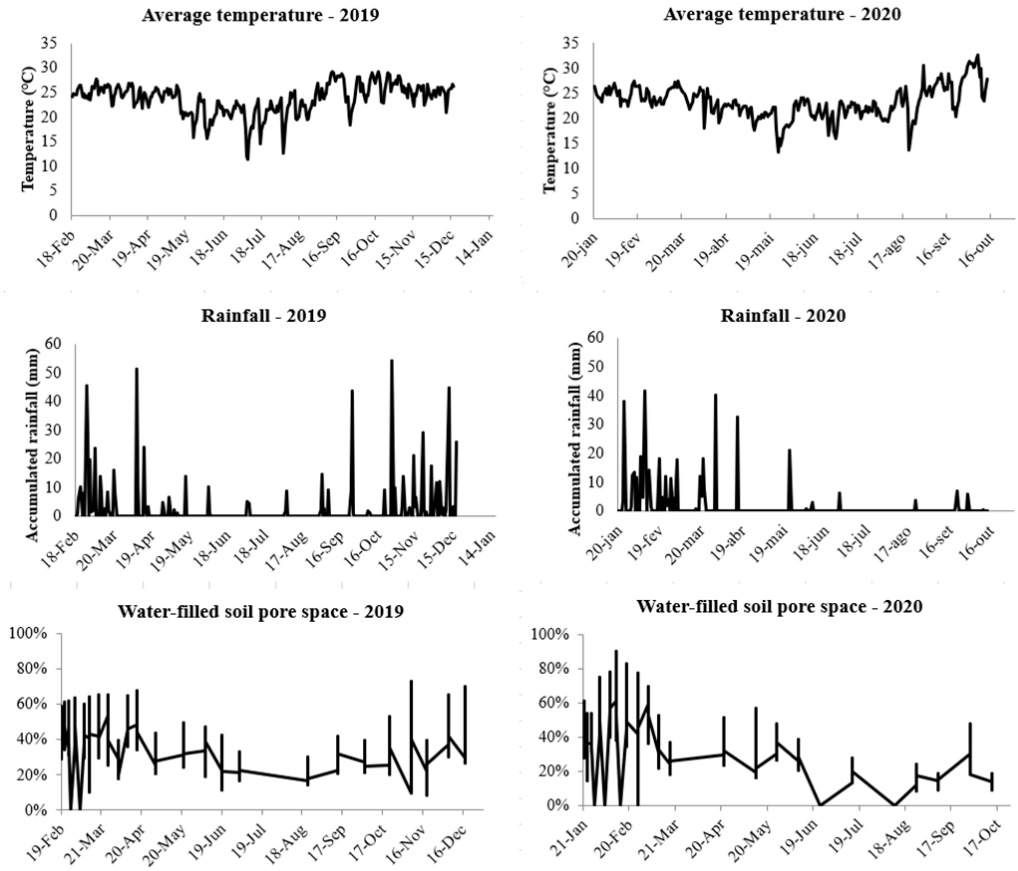


Figure 1. Average temperature and rainfall of the municipality of Jaboticabal, São Paulo, Brazil, and water-filled soil pore space of Rhodic Ferralsol in the Atlantic Forest to Cerrado transition region during the rainy and dry seasons of 2019 and 2020.

2.7. Statistical Analysis

The experiment was conducted for two consecutive years, in a randomized complete block design (double blocking: the slope of the terrain and year), with eight treatments and five replicates. Data were assessed for normality and homoscedasticity using the Shapiro–Wilk normality test and Bartlett test, respectively.

Statistical analyses were performed to compare the source of N (feces, urine, and N fertilizer) and the combination of the type of excreta (feces or urine) with the dose of N fertilizer applied. Comparisons between treatments were performed using Tukey's test. For each excreta, the effect of their combination with increasing doses of N fertilizer was performed using orthogonal contrasts to identify linear or quadratic effects. Significance was set at $p \leq 0.05$. Within each source of N, the gas fluxes (response variable) and the explanatory variables (humidity, temperature, ammonium, and soil nitrate and precipitation) were submitted to linear regression analysis (assuming p-value < 0.15). All data were analyzed using the MIXED procedure of SAS (version 9.3; SAS Institute, Cary, NC, USA).

3. Results

3.1. Seasonal Variation of N_2O, CH_4 and CO_2 Fluxes

The highest emission of N_2O occurred approximately 15 days after the application of excreta and N fertilizer on the patches, mainly in the treatment U + 150N (Figure 2). Likewise, the highest emissions of CH_4 and CO_2 occurred after the application of excreta and N fertilizer during the rainy season (from January to April; Figures 3 and 4, respectively). Conversely, during the dry season, N_2O, CH_4, and CO_2 emissions were negligible (Figures 2–4, respectively).

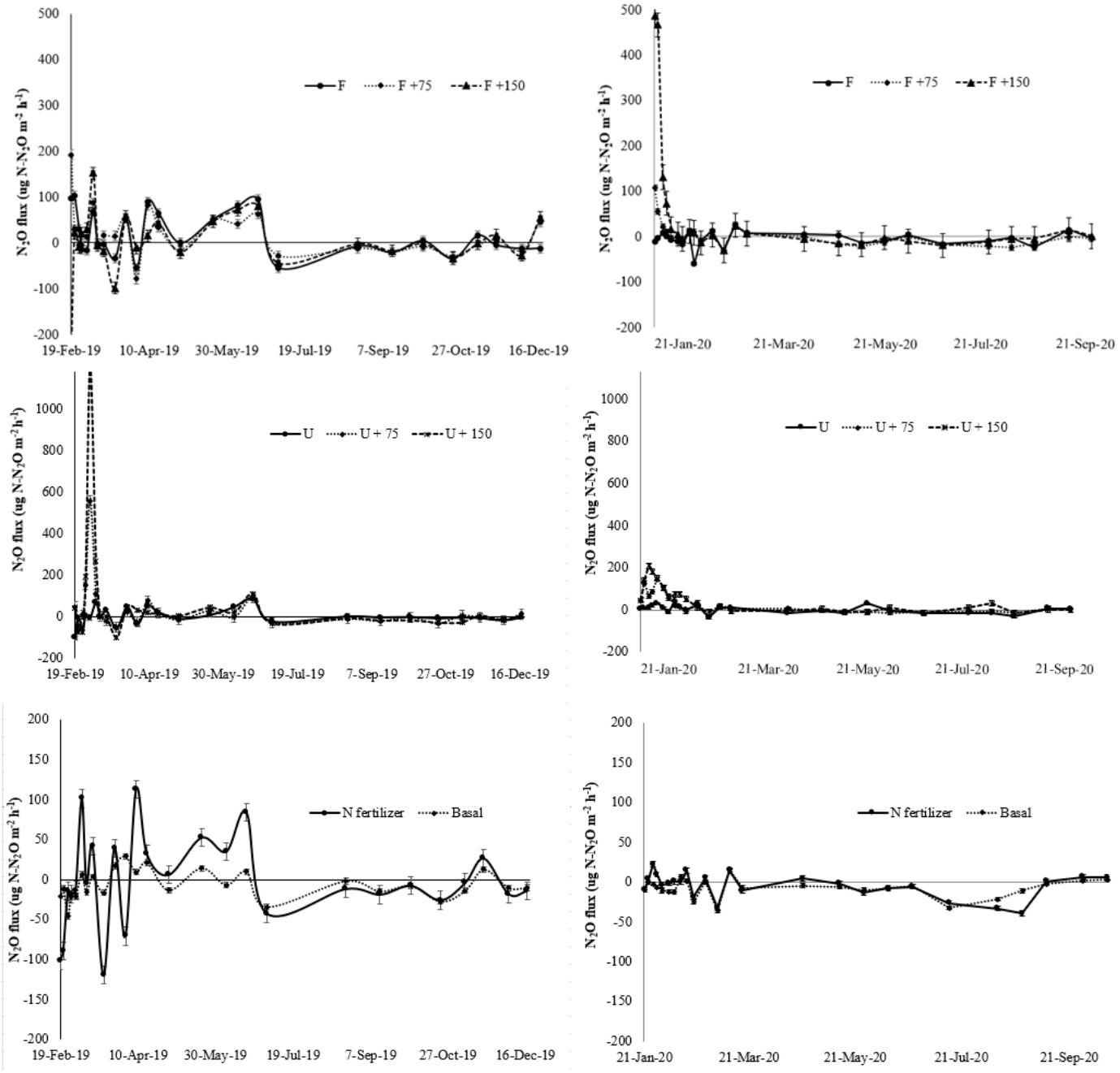


Figure 2. N_2O flux of urine, feces, N fertilizer (ammonium nitrate), basal Rhodic Ferralsol soil (control), and the combination of feces or urine with doses of N fertilizer during two years of evaluation (19 February 2019, to 13 October 2020). F: Feces; F + 75: Feces + 75 kg of N fertilizer ha^{-1}; F + 150: Feces + 150 kg of N fertilizer ha^{-1}; U: Urine; U + 75: Urine +75 kg of N fertilizer ha^{-1}; U + 150: Urine +150 kg of N fertilizer ha^{-1}; N fertilizer: only fertilizer at the dose of 75 kg of N ha^{-1} and Basal: control without excreta or fertilizer. The N fertilizer source was ammonium nitrate (32% N). (n = 5). The y axis scales may differ for the different N sources.

Among the eight treatments evaluated, the individual N_2O fluxes were correlated with individual rainfall event for six of them: F + 75 (R = 0.32 p = 0.05), F + 150 (R = 0.38, p = 0.01), U + 75 (R = 0.41, p = 0.04), U + 150 (R = 0.38, p = 0.04), 75N (R = 0.37, p = 0.11), Basal (R = 0.35, p = 0.05). The fluxes of CH_4 were correlated with the ammonium content in the soil for six treatments: F (R = 0.35, p = 0.04), F + 75 (R = 0.37, p = 0.02), F + 150 (R = 0.38, p = 0.03), U + 75 (R = 0.43, p = 0.006), 75N (R = 0.45, p = 0.004), Basal (R = 0.25, p = 0.10).

The CO_2 flux was correlated with ammonium content in the soil for treatments: F (R = 0.65, p = 0.03), U (R = 0.41, p = 0.09), U + 75 (R = 0.40, p = 0.09), U + 150 (R = 0.55, p = 0.01); and with rainfall for treatments: F (R = 0.65, p = 0.0 01), U (R = 0.41, p = 0.12), U + 150 (R = 0.55, p = 0.002), 75N (R = 0.41, p = 0.04), Basal (R = 0.46, p = 0.001).

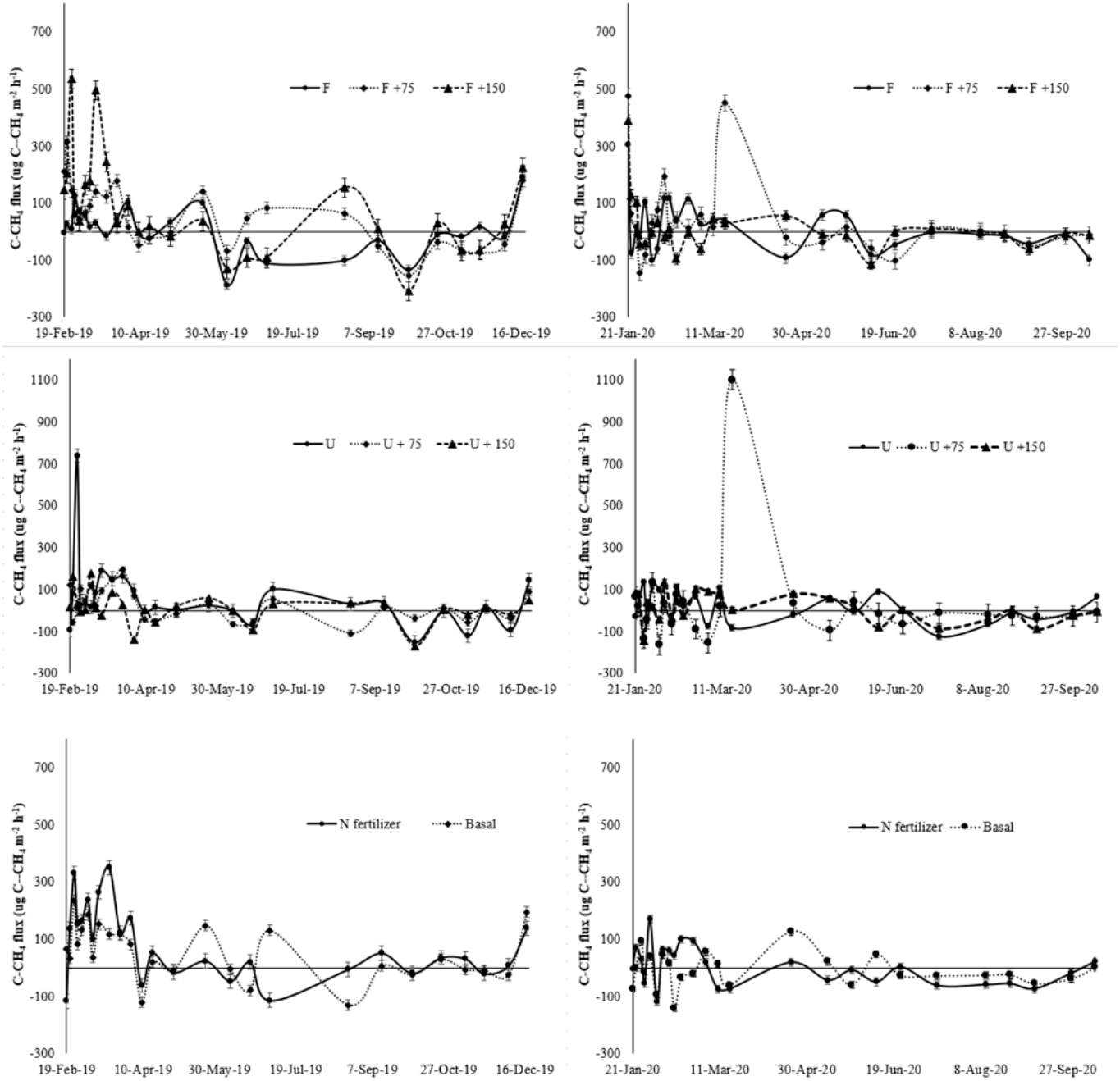


Figure 3. CH_4 flux of urine, feces, N fertilizer (ammonium nitrate), basal Rhodic Ferralsol soil (control), and the combination of feces or urine with doses of N fertilizer during two years of evaluation (19 February 2019, to 13 October 2020). F: Feces; F + 75: Feces + 75 kg of N fertilizer ha^{-1}; F + 150: Feces + 150 kg of N fertilizer ha^{-1}; U: Urine; U + 75: Urine +75 kg of N fertilizer ha^{-1}; U + 150: Urine +150 kg of N fertilizer ha^{-1}; N fertilizer: only fertilizer at the dose of 75 kg of N ha^{-1} and Basal: control without excreta or fertilizer. The N fertilizer source was ammonium nitrate (32% N). (n = 5). The y axis scales may differ for the different N sources.

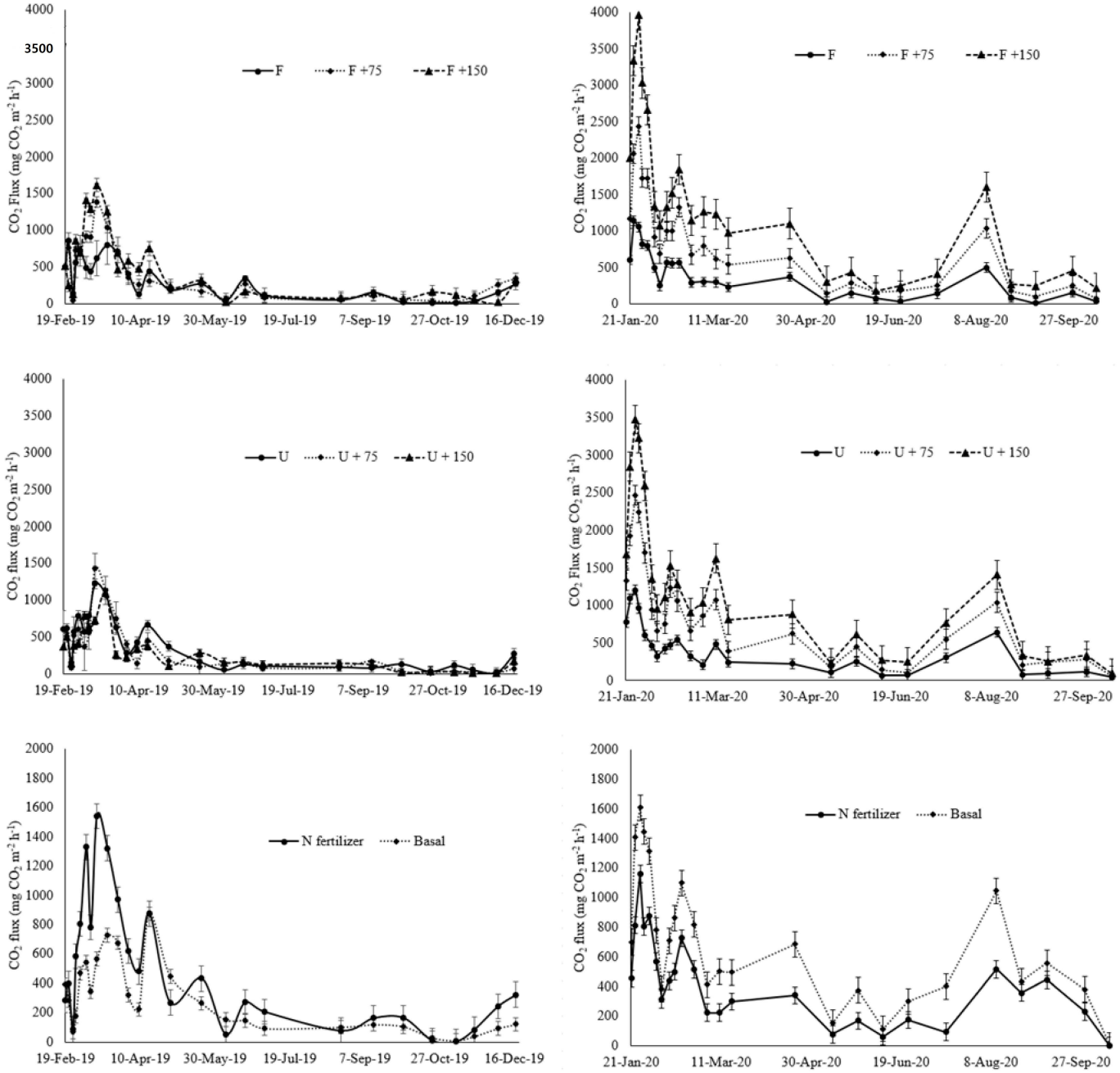


Figure 4. CO_2 flux of urine, feces, N fertilizer (ammonium nitrate), basal Rhodic Ferralsol soil (control), and the combination of feces or urine with doses of N fertilizer during two years of evaluation (19 February 2019, to 13 October 2020). F: Feces; F + 75: Feces + 75 kg of N fertilizer ha^{-1}; F + 150: Feces + 150 kg of N fertilizer ha^{-1}; U: Urine; U + 75: Urine +75 kg of N fertilizer ha^{-1}; U + 150: Urine +150 kg of N fertilizer ha^{-1}; N fertilizer: only fertilizer at the dose of 75 kg of N ha^{-1} and Basal: control without excreta or fertilizer. The N fertilizer source was ammonium nitrate (32% N). (n = 5). The y axis scales may differ for the different N sources.

3.2. Effect of N Source on Accumulated Emissions of N_2O, CH_4, and CO_2

The annual accumulated emission of N_2O (average of 1.18 mg N_2O m^2) and CH_4 (average of 47.4 mg N_2O m^2) were similar ($p > 0.05$) among N sources, e.g., feces, urine, and ammonium nitrate, and compared to Basal (without excreta or fertilizer, Table 2). On the contrary, the annual accumulated CO_2 emission was the highest for 75N (ammonium nitrate), the lowest for Basal, and intermediate for F and U (Table 2).

Table 2. Effect of type of excreta (feces and urine) and their combination with doses of N fertilizer on annual accumulated emissions of N_2O, CH_4, and CO_2.

	Treatment [1]	N_2O (mg N_2O m^{-2})	CH_4 (mg CH_4 m^{-2})	CO_2 (kg CO_2 m^{-2})
		Effect of N source		
	F	7.5	19.2	8.47 [ab]
	U	4.9	60.7	10.42 [ab]
	N fertilizer	0.40	71.4	11.51 [a]
	Basal	−8.05	38.4	7.15 [b]
	SEM	5.47	0.19	0.47
	p	0.15	0.16	0.008
		Effect of feces and doses of N fertilizer		
	F	7.5	19.2	8.47
Feces	F + 75	17.0	65.9	10.65
	F + 150	74.2	65.7	13.38
	SEM	0.08	0.29	0.252
	Effect	linear	NS	linear
	p	0.016	0.08	0.005
		Effect of urine and doses of N fertilizer		
	U	4.9	60.7	10.42
Urine	U + 75	47.2	42.4	10.18
	U + 150	75.2	28.45	9.38
	SEM	0.08	0.29	0.252
	Effect	linear	NS	NS
	p	0.003	0.37	0.74

[1] F: Feces; F + 75: Feces + 75 kg of N fertilizer ha^{-1}; F + 150: Feces + 150 kg of N fertilizer ha^{-1}; U: Urine; U + 75: Urine +75 kg of N fertilizer ha^{-1}; U + 150: Urine +150 kg of N fertilizer ha^{-1}; N fertilizer: only fertilizer at the dose of 75 kg of N ha^{-1} and Basal: control without excreta or fertilizer (n = 5). The N fertilizer source was ammonium nitrate (32% N). SEM: standard error of mean. NS: not significant, $p > 0.05$. [a,b] Means followed by the same letter in the column are not significantly different (Tukey's test, alpha = 5%).

3.3. Effect of Excreta Type Combined with Doses of N Fertilizer on Accumulated Emission of N_2O, CH_4 and CO_2

The annual accumulated emission of N_2O from feces and urine increased linearly when combined with increasing doses of N fertilizer as ammonium nitrate (Table 2). In contrast, the accumulated emission of CH_4 from feces or urine, combined with nitrogen doses, did not fit the linear or quadratic model (Table 2), with an average emission of 50.2 mg CH_4 m^{-2}, and 43.8 mg CH_4 m^{-2}, respectively. The accumulated emission of CO_2 from feces increased linearly when combined with increased ammonium nitrate doses (Table 2). However, the accumulated emission of CO_2 from urine combined with increased nitrate doses did not fit the linear or quadratic model (Table 2), with an average value of 9.99 kg m^{-2}.

3.4. Ammonia Volatilization

Nitrogen loss due to volatilization of NH_3 was similar (average of 9.5%; $p > 0.05$; standard error of mean = 0.11) for feces, urine, and N fertilizer (ammonium nitrate). However, nitrogen loss by volatilization of NH_3 of feces or urine linearly decreased when combined with N fertilizer doses (Table 3), from 6.75% (F) to 2.28% (F + 150), and from 14.1% (U) to 3.27% (U + 150), respectively.

Table 3. Effect of type of excreta (feces and urine) and their combination with doses of N fertilizer on NH_3 emission.

	Treatment [1]	NH_3 (%)
	Effect of feces and doses of N fertilizer	
	F	6.75
Feces	F + 75	5.32
	F + 150	2.28
	SEM	0.018
	Effect	linear
	p	0.0003
	Effect of urine and doses of N fertilizer	
	U	14.1
Urine	U + 75	5.00
	U + 150	3.27
	SEM	0.018
	Effect	linear
	p	<0.0001

[1] F: Feces; F + 75: Feces + 75 kg of N fertilizer ha^{-1}; F + 150: Feces + 150 kg of N fertilizer ha^{-1}; U: Urine; U + 75: Urine +75 kg of N fertilizer ha^{-1}; U + 150: Urine +150 kg of N fertilizer ha^{-1}; N fertilizer: only fertilizer at the dose of 75 kg of N ha^{-1} and Basal: control without excreta or fertilizer (n = 5). The N fertilizer source was ammonium nitrate (32% N). SEM: standard error of mean.

3.5. The Emission Factor of N_2O and CH_4

The emission factor of N_2O was 0.11, 0.19, and 0.17% for feces, urine, and 75 kg N ha^{-1} $year^{-1}$ as ammonium nitrate, respectively, not differing between sources (Table 4). Nevertheless, the emission factor showed an increasing linear effect when feces or urine were combined with an increased nitrate dose (Table 4). Conversely, the emission factor of CH_4 of the soil was similar (0.18 kg CH_4 $animal^{-1}$ $year^{-1}$; $p = 0.08$; standard error of mean = 1.24) for feces irrespective of combination with increased ammonium nitrate dose.

Table 4. Emission factor (EF) of N_2O from type of excreta (feces and urine) and their combination with doses of N fertilizer.

	Treatment [1]	EF (%)
	Effect of N source	
	F	0.11
	U	0.19
	N fertilizer	0.17
	SEM	0.0.086
	p	0.35
	Effect of feces and doses of N fertilizer	
	F	0.11
Feces	F + 75	0.13
	F + 150	0.42
	SEM	0.09
	Effect	linear
	p	0.010
	Effect of urine and doses of N fertilizer	
	U	0.19
Urine	U + 75	0.58
	U + 150	1.03
	SEM	0.09
	Effect	linear
	p	0.0006

[1] F: Feces; F + 75: Feces + 75 kg of N fertilizer ha^{-1}; F + 150: Feces + 150 kg of N fertilizer ha^{-1}; U: Urine; U + 75: Urine +75 kg of N fertilizer ha^{-1}; U + 150: Urine +150 kg of N fertilizer ha^{-1}; N fertilizer: only fertilizer at the dose of 75 kg of N ha^{-1} and Basal: control without excreta or fertilizer (n = 5). The N fertilizer source was ammonium nitrate (32% N). SEM: standard error of mean.

4. Discussion

4.1. Seasonal Variation of N_2O, CH_4, and CO_2 Fluxes

The general pattern of N_2O flux throughout the year, with higher fluxes during the rainy season and lower or null emission during the dry season, agreed with a previous study [9], which evaluated the fluxes separately by season. Indeed, rainfall was the primary key explanatory variable for N_2O emission in this study, corroborating with previous reports [26,27]. After a rainy event, there was an increase in %WFPS, which contributed to higher N_2O emissions [28].

Otherwise, the negative fluxes of N_2O can be explained by the balance between the concentration of N_2O in the soil pores and that on the pasture surface (gas diffusion). According to Cardoso et al. [29], the lack of available nitrate in the soil may be the mechanism behind the absorption of N_2O, leading denitrifying bacteria to use N_2O as an electron acceptor, which in turn contributes to the absorption network of N_2O [30]. A complete denitrification led to a higher production of N_2 instead of N_2O.

In this study, the higher emission of CH_4 after the application of excreta and N fertilizer during the rainy season (from January to April) might be the consequence of the combination of N from fertilizer and/or excreta with available soil carbon, which led to methanogenic bacteria producing more CH_4 [31]. Regarding feces, their higher CH_4 emissions in the first days after the application might also be prompted by the methanogenic bacteria contained in cattle feces [32] in combination with the high carbon content of feces, leading to higher CH_4 emissions where feces were freshly applied [31]. Generally, ammonium content in the soil was the key explanatory variable for CH_4 emission. It is well-known that nitrogen fertilizers hinder the oxidation of CH_4 by NH_3, which competes with CH_4 for methane monooxygenase in methanotrophs [33]. Li et al. [34] reported that high concentrations of ammonium (>40 mg NH_4^+ N kg^{-1} dry soil) are known to inhibit CH_4 oxidation substantially.

The highest CO_2 emission that occurred after the application of excreta and N fertilizer on the patches was also observed by Brito et al. [35]. The pattern of CO_2 flux throughout the year observed in this study, with higher fluxes in the rainy season and low or null emission in the dry season agreed with previous studies [31,35], which evaluated the fluxes separately by season. The correlation of CO_2 flux with ammonium content in the soil and rainfall might suggest that nitrogen was the main key variable for CO_2 emission because N provides substrates for soil microbiology, allowing the increase in microbiological soil activity and, therefore, higher emission. Additionally, rainfall events determined the seasonal variation of CO_2 in this study, suggesting that the increase in soil moisture, which occurs mainly in rainy summers [35], presumably led to an increase in the rate of root respiration due to plant growth and microbial processes involved in the decomposition of labile organic matter from the soil, which, in turn, led to increased production and CO_2 emission. Then, the correlation between ammonium in the soil and CO_2 flux probably was because ammonium provided N for root growth due to the high respiration rate [36].

4.2. Effect of N Source on Accumulated Emissions of N_2O, CH_4 and CO_2

The lack of difference in annual accumulated emission of N_2O and CH_4 between feces, urine, and ammonium nitrate diverged from previous studies that reported accumulated emissions of N_2O in urine sites were much higher than in feces sites [21,26,37]. Nonetheless, under similar conditions to our study (similar soil properties and climate), Cardoso et al. [9] did not observe differences in N_2O emissions between urine and feces sites, in agreement with our results. Moreover, the accumulated emission of N_2O among the three sources of N (feces, urine, and ammonium nitrate) did not differ from Basal (without excreta or fertilizer) in this experimental condition. This lack of difference might be due to the low volatilization of nitrogen fertilizer [12] and to the fact that urea from urine emits around 1% of N_2O, whose remainder is lost in the form of NH_3. Additionally, the C:N ratio of feces in this study (i.e., C:N of 33.3) was higher than the one proposed by Klemedtsson et al. [38] and Maire et al. [15] (i.e., C:N of 25). The high C: N stimulates microorganisms

to use the available N to carry out the decomposition of organic matter, which reduces the relationship with the amount of N available for the production of N_2O, consequently explaining the low N_2O.

Like N_2O emission, the annual accumulated emission of CH_4 did not differ among N sources and Basal, suggesting that the different types and sources of N might not have stimulated differences in the growth and activity of CH_4 oxidants. Indeed, Cardoso et al. [31] observed that the effect of different forms of N on CH_4 emission is probably related to the role of NH_3 in competing for methane monooxygenase enzymes [39], to increase the oxidation of NH_3 [40] or the osmotic effects [41], independent of N source. Regarding the annual accumulated CO_2 emission, we observed that various forms of N influenced the emission of CO_2, although N from feces (organic N) and N from urine (N urea) was similar. Conversely, the N fertilizer (synthetic N, as ammonium nitrate) was the highest because this form of N is the most readily available [42].

4.3. Effect of Excreta Type Combined with Doses of N Fertilizer on Accumulated Emission of N_2O, CH_4 and CO_2

The linear increase of annual accumulated N_2O emission of feces or urine when combined with increasing doses of N fertilizer as ammonium nitrate indicated a multiplicative effect of the excreta and N fertilizer, e.g., the cumulative N_2O emission from urine or feces applied with ammonium nitrate were greater than either urine or feces or ammonium nitrate alone, as observed in Ryegrass lawns *(Lolium perenne* L.) of Ireland [13]; and in marandu grass pastures in Brazil [42]. In general, the cumulative N_2O emission of feces and urine, when applied together, has been reported to be additive [12,16], e.g., their output was the sum of their individual emissions. Hyde et al. [13] summarized that the multiplicative effects observed come from the combinations of the nitrate pool from fertilizer (ammonium nitrate) with the readily available carbon of feces or urine and the moisture of applied urine. Thus, the readily available carbon compounds from feces or urine enhanced the soil C denitrification and, in turn, increased denitrification loss in the presence of nitrate [12]. Moreover, urine application increased moisture level in the presence of nitrate from fertilizer, which might boost denitrification [12].

The flux of CH_4 is the net result between the production of methanogenesis and the oxidation by methanotroph processes [43]. Usually, undisturbed soils (pastures) are considered oxidizing agents of CH_4 [44]. Thus, the lack of effect between feces or urine and N fertilizer on CH_4-accumulated emission in this study suggests that the flux of CH_4 might be independent of excreta type or nitrate dose.

Furthermore, previous studies have suggested that, after a long period of N fertilization, microbial populations can adapt to the addition of N, and no inhibitory effect on CH_4 uptake has been observed ([45,46]. On average, during the rainy period, 12 kg of N was supposed to be incorporated into the soil, as the canopy where the gas samples were collected has been established since 2005, so we hypothesized that the N contents of the soil were as elevated as to the point of not interfering in the oxidation of CH_4. Further studies with different sources of N (excreta, N fertilizers, and doses) under adverse soil conditions should be evaluated to understand the interaction between N content in soil and oxidation of CH_4.

The accumulated emission of CO_2 from feces increased linearly when combined with increased nitrate doses because the availability of carbon in the soil and the intake of synthetic nitrogen in the soil allowed more significant microbial activity, therefore, resulting in higher emission [42,47]. Conversely, the lack of difference in the accumulated emission of CO_2 of urine combined with increased nitrate might have occurred because the C:N ratio did not provide high soil microbial activity, and therefore, CO_2 emission.

In both types of excreta combined with N fertilizer, CO_2 emissions in this study were higher than the data presented by Brito et al. [35]. The soil in the work of Brito et al. [35] has a history of nutrient replacement via synthetic fertilizer of up to 20 years and resulted in CO_2 emission of 58 t ha^{-1} (in 2011) and 74 t ha^{-1} (in 2012). In the area of our study, nutrient

replacement via synthetic fertilizer has been a routine practiced only in the last four years, suggesting that soils with low fertility may be a factor that increases CO_2 emissions [48,49].

4.4. Ammonia Volatilization

The volatilized NH_3 contributes indirectly to the emission of N_2O, so proper quantification is essential to identify less volatile N sources [12]. Nitrogen loss due to volatilization of NH_3 was similar among treatments in this study. Conversely, a previous study observed greater loss due to the volatilization of NH_3 in urine compared to that of feces, which was almost insignificant [21]. Indeed, a wide range of N loss due to volatilization of NH_3 has been reported when the source of N is urine [50–52]. Notwithstanding, the losses of ammonium nitrate are in agreement with Chagas et al. [53], which reported low volatilization of NH_3 when the source is ammonium nitrate, compared to urea, urea with urea inhibitor, and urea with polymer at a dose of 100 kg of N ha^{-1}.

The combination of feces or bovine urine with different doses of nitrogen is unprecedented in studies with the volatilization of NH_3. The loss of N did not increase, possibly, due to soil acidity, which, combined with the N dose, precluded the loss due to volatilization of NH_3. A significant variation (1.5–40%) in volatilized NH_3 was found when urine was the source of N in soils of temperate pastures [9,52,54]. Otherwise, values of volatilized NH_3 ranging from 2 to 12.4% and 3.8 to 8.9% were observed when feces were combined with urine [9]. Another factor that may explain this decreasing effect is that ammonium nitrate is a less volatile source of N [12,55].

The volatilization of NH_3 is within the values observed in the literature for tropical climate and marandu palisade grass pastures [9,12]. In this study, a less volatile N source was used compared with urea [55], the most used N fertilizer source in Brazil.

4.5. The Emission Factor of N_2O and CH_4

In general, type of excreta has been reported to influence the N_2O emission factor [8,9,56]. This occurs because, immediately after the urination of the bovine, urea from urine is rapidly hydrolyzed into ammonium in the soil, increasing the pH of the soil and stimulating the release of water-soluble carbon available as a microbial food supply for denitrifying bacteria [57], increasing the emission of N_2O. In contrast, mineral N in feces is lower; consequently, the transformation of soil N by microbial activity under sites with feces is lower. In this study, the lack of difference of N_2O emission factor between feces and urine might be attributed to the high variability of accumulated N_2O emission in tandem with negative emissions from both patches (urine and feces), indicating that nitrification and the amount of available NO_3^- for N_2O emissions from urine and feces were similar.

The EF_1 of ammonium nitrate in this study was lower than that reported by the IPCC [7] and by Correa et al. [11]. Moreover, previous studies also reported higher EF_1 of other sources of N fertilizer, at a similar N dose, whose values ranged from 0.71% [9] to 1.7% [10] for urea; and EF_1 was 2.15% for calcium ammonium nitrate [13]. Indeed, a previous study reported that EF_1 of ammonium nitrate was 17% lower than that of urea and ammonium sulfate; the authors attributed the lower emission factor of ammonium nitrate to the fact that nitric and nitrate fertilizers only produce N_2O through denitrification, whereas amidic and ammoniacal sources produce N_2O through nitrification and denitrification. Thus, nitrate fertilizers, such as ammonium nitrate, are expected to reduce emissions in comparison to amidic sources, such as urea [11].

The increasing linear effect of the N_2O emission factor as a result of the combination of feces or urine with nitrate dose was also reported in a previous study under subtropical conditions [15]. As postulated by Hyde et al. [13], the available carbon of feces increases the microbial activity of the soil, together with the available N of the fertilizer, therefore increasing the emission factor. For urine and N fertilizer, other authors had separately demonstrated that sites with urine [21,37] and nitrogen doses [12] increased the emission of N_2O due to the nitrification activity that occurs in the soil, which was demonstrated in this study for the combination between urine and ammonium nitrate.

The emission factor of CH_4 observed in this study (0.18 kg CH_4 animal^{-1} year^{-1}) was lower than that observed by Cardoso et al. [9] (0.54 kg CH_4 animal^{-1} year^{-1}) under similar experimental conditions. Conversely, another study conducted under tropical climate conditions reported lower emission factors of 0.06 kg CH_4 animal^{-1} year^{-1} (winter) and 0.10 kg CH_4 animal^{-1} year^{-1} (summer; [30]).

Otherwise, in this study, the nitrogen type, dose, and C: N ratio did not influence the CH_4 emission factor of the soil. In general, the fluctuation of CH_4 emission flux is explained by the fact that the pastures can remove CH_4 from the atmosphere. Saggar et al. [58] measured the GHG emission flux for two years in New Zealand pastures and concluded that the grasslands functioned as a sink for $CH_{4,}$ with annual carbon removal in the form of CH_4 in the range of 0.64 $\pm$ to 0.14 kg ha^{-1}. Therefore, the emission and mitigation balance does not allow us to observe differences between sources and doses of N.

This research fills an important gap for the national greenhouse gas inventories calculations because it separately quantified the emission factors for feces, urine, and their interaction with nitrogen fertilizer doses in tropical soil conditions of the Southwest and Midwest, which may contribute to improvements in GHG inventories.

Further studies should quantify the emission factors of interactions between excrements of other herbivores (sheep, goats, horses) with different sources of fertilizers, as well as on the microbiology of GHG emissions from pastures. Although nitrogen fertilization increases the emission of N_2O and $CO_{2,}$ the system should be assessed systematically. Analyzing the carbon footprint, the higher meat production, and the land-saving effect due to fertilized pastures will provide greater sustainability for intensified systems [59]. These data can form the basis for further research evaluating the potential for N fertilization to achieve sustainable intensification of livestock based on pastures.

5. Conclusions

The emission factor of N_2O of feces and urine is 10% and 33% lower than that recommended by the IPCC, respectively, with a multiplicative effect when combined with ammonium nitrate hotspots.

The emission factor of CH_4 is also 20% lower than that recommended by the IPCC. The CO_2 emissions have an additive effect on the combination of feces and ammonium nitrate. The N loss due to volatilization of NH_3 decreases in hotspots when feces or urine were combined with ammonium nitrate.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/atmos14030492/s1, Table S1. Total amount of N applied in each plot per treatment; Figure S1. Layout of the standard static chambers and distribution of treatments in the field site. Treatments consisted of the combination of feces or urine with doses of N fertilizer: Feces (F), Feces + 75 kg of N ha^{-1} (F + 75), Feces + 150 kg of N ha^{-1} (F + 150), Urine (U), Urine + 75 kg of N ha^{-1} (U + 75), Urine + 150 kg of N ha^{-1} (U + 150), only fertilizer-75 kg of N ha^{-1} (75N) and control without excreta or fertilizer (Basal); Figure S2. Field site with the standard static chambers to evaluate N_2O, CH_4, and CO_2 emissions, and an adjacent area of 1 m^2 to measure porous space, moisture, ammonium (NH_4^+) and nitrate (NO_3^-) of soil; Figure S3. Layout of the open semi-static chambers and their treatments in the field site. Treatments consisted of the combination of feces or urine with doses of N fertilizer: Feces (F), Feces + 75 kg of N ha^{-1} (F + 75), Feces + 150 kg of N ha^{-1} (F + 150), Urine (U), Urine + 75 kg of N ha^{-1} (U + 75), Urine + 150 kg of N ha^{-1} (U + 150), only fertilizer-75 kg of N ha^{-1} (75N) and control without excreta or fertilizer (Basal); Figure S4. Field site with the open semi-static chambers to evaluate the N losses in the form of NH3 volatilized.

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