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Soil–plant retention dominated nitrogen fate from cattle excreta under low N input in a tropical pasture system

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This study quantified ammonia (NH₃) volatilization, nitrous oxide (N₂O) emissions, and urinary nitrogen (N) mass balance across different pasture types in southeastern Brazil. Beef cattle excreta from Mixed pastures (*Urochloa brizantha* cv. Marandu + *Macrotyloma axillare* cv. Java) or Grass monoculture pasture (Marandu palisade grass) were applied in a randomized block design with six replications, during the wet and dry seasons. Although Grass pasture resulted in a tendency for excreta with higher N concentration, reflecting greater levels of mineral N, forage production, and N recovery in forage mass and litter, no significant differences were observed between pasture types in gaseous losses (NH₃, N₂O). Seasonality influenced N fate. During the wet season, a higher concentration of urinary N was observed in the 10–20 cm soil layer, and greater recovery by Marandu palisade grass at the chamber border, together with lower total ¹⁵N recovery, suggested greater hydrological N transport (~23% of non-recovered N). During the dry season, there was a difference in losses due to NH₃ volatilization, mainly from urine (~3.7%). Emission factors were lower than the IPCC's averages. The soil represented the main destination of urinary N (~54%), and the soil–plant system recovered 96.2% of the N during the dry season and 75.5% during the wet season. Under the conditions of this study, NH₃ and N₂O losses were low, and the ¹⁵N mass balance indicated limited amounts of non-recovered N under most conditions, with stronger evidence of hydrological N transport during the wetter period.

KEYWORDS

ammonia volatilization, emissions factor, livestock excreta, nitrogen fertilizer application, nitrous oxide, urine labeled, warm-season legume

1 Introduction

Nitrogen (N) not used by cattle is excreted and becomes susceptible to nutrient losses, such as nitrate (NO_3^-) leaching, ammonia (NH_3) volatilization, and nitrous oxide (N_2O) emissions. In addition to representing losses, these processes contribute to the global balance of greenhouse gas (GHG) emissions, resulting in significant environmental impacts and accelerating environmental degradation processes (Robertson and Vitousek, 2009). Although beef cattle are efficient at using N under conditions of low nutritional value of ingested forage, the utilization of this nutrient averages only 5–15% of the N ingested in pastures (Boddey et al., 2004), with most of the N consumed being excreted. Depending on the diet, cattle feces and urine can have high N concentrations, especially when protein is overformulated, compromising N use efficiency. Urine deposition in the soil can contain the equivalent of 500–1,000 kg N ha^{-1} in the area where excreta are deposited, a quantity well above the absorption capacity of plants in the areas receiving excreta (Haynes and Williams, 1993).

The high N load in the soil provides a substrate for nitrification and denitrification processes, intensifying the production and emission of N_2O . N_2O contributes substantially to the destruction of stratospheric ozone (Ravishankara et al., 2009). Furthermore, N_2O plays a critical role in atmospheric warming. The warming potential from N_2O is 273 times greater than that of CO_2 and 12 times greater than that of methane (CH_4) over 100 years (IPCC, 2023). It is estimated that the pre-industrial atmospheric concentration of N_2O was 270 nL L^{-1} , reaching 336 nL L^{-1} in Anualpec, 2022 and increasing at an annual rate of 1.3 nL L^{-1} in 2020 and 2021 (Tian et al., 2024).

The main form of N in urine is urea, which, upon contact with the soil, is rapidly converted to NH_3 by the enzyme urease. Feces also have a significant N content, but most of it is in the form of complex organic compounds that take a long time to decompose and produce mineral forms of N (Weerden et al., 2021). The NH_3 present in any form of excreta can be converted into NO_3^- by the nitrification process, in which N_2O is emitted. However, when NO_3^- formed in excreta is transported by rainwater to microsites with higher water saturation and low oxygen availability, either within the fecal plaque itself or in the adjacent soil, denitrification can become significant. This process reduces NO_3^- to N_2O and then to N_2 , which is generally considered the primary source of N_2O in soils (Butterbach-Bahl et al., 2013). Results from several studies show that when the water-occupied pore space (WFPS) exceeds 60%, if NO_3^- is present and there is a carbon (C) source to supply soil respiration, N_2O emissions are favored (Butterbach-Bahl et al., 2013; Dobbie and Smith, 2003; Oertel et al., 2016).

The Intergovernmental Panel on Climate Change (IPCC) considers that N_2O emissions from excreta occur directly or indirectly. Indirect forms derive from the emission of NH_3 , leached N, or surface runoff (Buendia et al., 2019). Indirect estimates of N_2O are highly uncertain, especially in tropical regions, and can lead to significant errors in national inventories (Lessa et al., 2014). Furthermore, the data used to populate the databases come from a few countries with temperate climates (Zhu et al., 2021), whose diets cattle differ from those commonly used in tropical countries, such as Brazil, which influences GHG emissions from bovine excreta. Brazil has one of the largest cattle herds in the world, with approximately 93% of the animals raised on pasture (Anualpec, 2022). According to Brazil's national GHG inventory, it is presumed that the deposition of cattle excrement on pastures was the primary source of N_2O emissions from

agriculture in 2020, accounting for about 50% of total emissions from N_2O (MCTI, 2022). According to the inventory, agriculture is estimated to have emitted approximately 409 Gg of N_2O . Of this amount, 173 Gg of N_2O are direct emissions from bovine excreta, and 23.1 and 24.8 Gg of N_2O are indirect emissions, through atmospheric deposition and leaching, respectively.

In Brazil, most beef cattle production occurs on unfertilized pastures of the genus *Urochloa*, *Megathyrsus*, and *Andropogon*, many of which are undergoing degradation (LAPIG, 2023). Sustainable intensification with legumes is a feasible way to recover degraded pastures and reduce GHG emissions in low-technology systems (Cardoso et al., 2016), especially in Latin America (Dubeux Jr et al., 2017). Diets that reduce total N excretion, better match supply to animal requirements, and shift N excretion from urine to feces are desirable. Such effects have been observed with forage legumes containing moderate levels of condensed tannins, which increase fecal N, reduce urinary N (Homem et al., 2021b), and decrease GHG intensity per unit of product (Homem et al., 2024). Furthermore, grass/legume mixed pastures can achieve greater animal liveweight gains than fertilized grass monocultures (Jaramillo et al., 2021; Pereira et al., 2020). Therefore, the use of legumes can reduce dependence on N fertilizers (Eugène et al., 2021), increasing the sustainability and circularity of the systems. Despite this, data on how tropical legumes affect N balance in excreta and associated emissions remain scarce. For *Macrotyloma axillare* (perennial horse gram), there are no reports of GHG emissions from the excreta of cattle fed with this forage legume. Finally, the season of year modulates forage nutritive value and the concentration of N excreted by animals (Homem et al., 2021b), reinforcing the need for seasonal studies.

Some studies have analyzed the impact of pasture system with fertilizers application, forage legumes, supplements, and concentrates on GHG emissions in tropical and subtropical regions (Bretas et al., 2020; Guimarães et al., 2022; Oliveira et al., 2022; Rigobello et al., 2023). However, more knowledge is needed regarding losses, leaching, immobilization rates, and mineralization of N present in urine and feces. The importance of each of these processes is influenced by urine characteristics and environmental factors after its deposition in the soil (Selbie et al., 2015). Therefore, we hypothesize that the fate and losses of N from livestock excreta in tropical pastures are primarily influenced by seasonal conditions rather than pasture type, with the soil–plant system evaluated here expected to retain a large proportion of excreted N, resulting in lower losses than IPCC reference values under the evaluated conditions. Furthermore, the inclusion of perennial horse gram in Marandu palisade grass pastures does not increase N losses from livestock excreta as greenhouse gas emissions. Thus, we assessed the influence of seasonal conditions and excreta origin (grass monoculture vs. mixed pasture) on the fate of N from beef cattle excreta when applied to a common Marandu palisade grass pasture, focusing on N retention in soil–plant systems and losses via gaseous emissions and leaching.

2 Materials and methods

2.1 Site description

The experiment was carried out in the experimental area of the Department of Animal Nutrition and Pastures of the Institute of

Animal Science of the Federal Rural University of Rio de Janeiro, in the Seropédica - Rio de Janeiro (22° 47'04"S, 43° 41'10"W; 33 m a.s.l.). According to the Köppen classification, the region's climate is Aw, with the wet season from November to March, an average annual rainfall of 1,213 mm, and an average annual temperature of 24.5 °C. During the experimental period, climate data were obtained from the INMET Ecology automatic weather station (Figure 1). The soil in the experimental area is a Red Yellow Acrisol with transition to Planosols (FAO IUSS, 2015). The soil texture class is sandy clay loam. More details on the experimental area can be found in Borré et al. (2025).

2.2 Pasture systems

The experimental area was divided into eight paddocks, to which two pasture systems were randomly distributed in four blocks, considering the topography of the area. The pasture systems were: (1) pure Marandu palisade grass [*Urochloa brizantha* (Hochst. ex A. Rich.) R. D. Webster cv. Marandu] without N fertilizer application (Grass); and (2) Marandu palisadegrass mixed with perennial horse gram [*Macrotyloma axillare* (E. Mey.) Verdc. cv. Java] without N fertilizer application (Mixed).

In 2015, the area was ploughed and harrowed, and dolomitic limestone was incorporated at a rate of 1.5 Mg ha⁻¹ to correct acidity and neutralize toxic aluminum. Subsequently, 33 kg ha⁻¹ of P in the form of mono ammonium phosphate was applied together with 5 kg of Marandu palisade grass seeds with the aid of a PHS3 type planter (Tatu Marchesan, Matão, SP) at a sowing depth of 2 to 4 cm. In the respective pastures of the Mixed pasture treatment, perennial horse gram was broadcast together with Marandu grass seed at 5 kg of pure viable seeds ha⁻¹. Due to the low persistence of this legume in the pasture system, it was necessary to carry out reseeding during the 2021 wet season, in addition to supplying perennial horse gram seeds mixed with salt supplied to the animals ad libitum (salt lick) for ingestion and propagation in their feces (endozoochoric dispersal). In September 2020, another 1 Mg ha⁻¹ of dolomitic limestone was applied to all pastures. In November of the same year, 15 kg of P ha⁻¹ was applied in the form of single superphosphate along with 50 kg of K ha⁻¹ in the form of potassium chloride.

In May 2020, the beef cattle (Nellore steers) were introduced into the experimental area to adapt to management and subsequently the pastures were maintained under continuous stocking with a variable stocking rate during the whole experimental period. In each

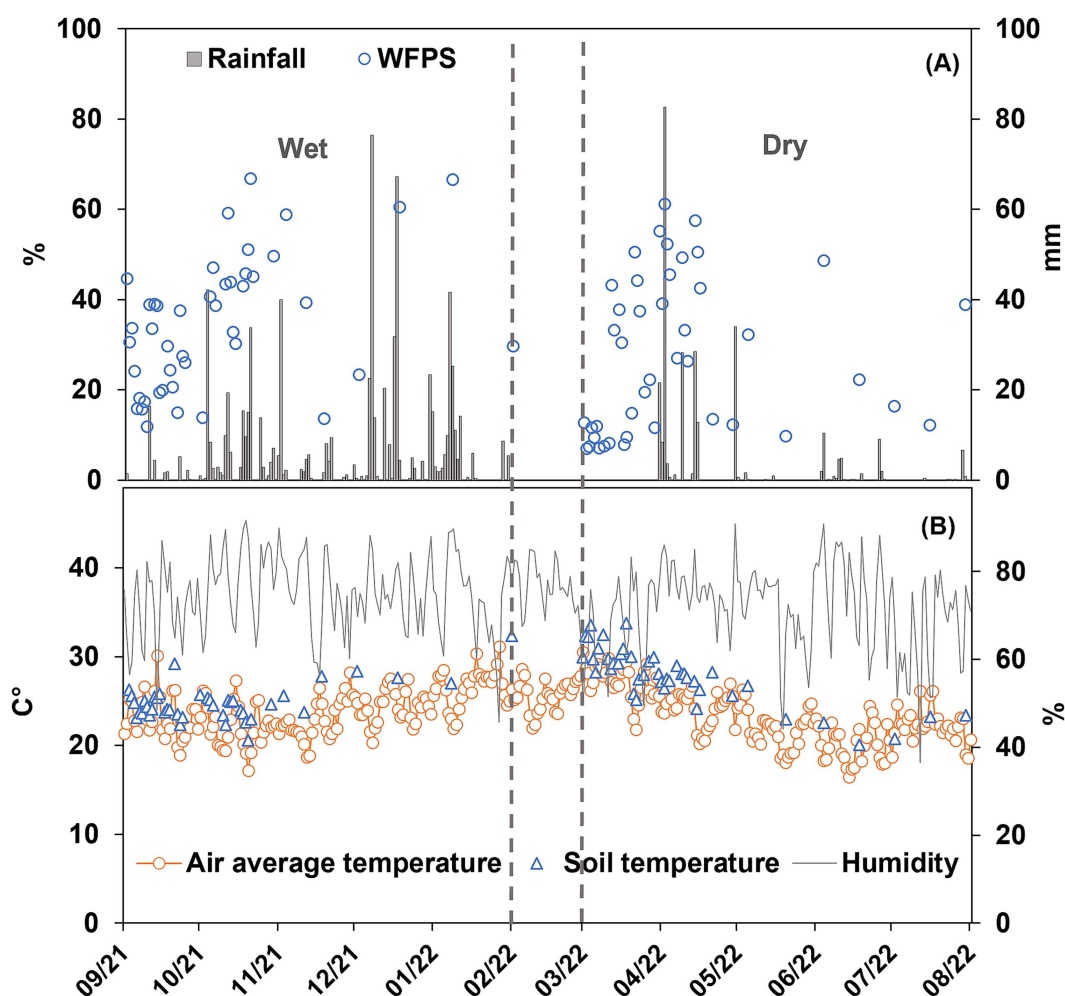


FIGURE 1

Weather data during the wet season (01/09/2021–01/02/2022) and dry season (01/03/2022–31/07/2020) experiments. The dashed line separates the experiments; no evaluation was performed across it. (A) Rainfall in mm and water-filled pore space (WFPS, in %). (B) Relative air humidity in %, average air and soil temperature in °C. Rainfall, air temperature, and relative air humidity data were obtained from the INMET Ecology automatic weather station located 3 km from the experiment.

experimental year, two Nellore steers (355 ± 7.9 kg of initial BW and 12 ± 1.3 months of age) were used as tester animals in each pasture. Herbage allowance was adopted as a grazing management criterion. The animal stocking rate was adjusted monthly to the intended target of 3 kg of green forage kg body weight⁻¹ of herbage allowance for each treatment. Water and mineral supplementation were supplied *ad libitum*.

2.3 The greenhouse gas emission from the feces and urine, and mass balance of urinary N

In the Brazilian agricultural calendar, especially in tropical regions with a well-defined dry season, the agricultural year is conventionally divided into two distinct periods: a wet season and a dry season. In the Rio de Janeiro state, the wet season extends from October/November to March/April, and the dry season from May to September. The main objective of this study was to evaluate the effect of pasture type on the fate of N from bovine excreta under contrasting rainfall patterns. To this end, excreta were collected at times that characteristically reflected the diets consumed during the dry and wet seasons, which were directly influenced by the forage nutritive value available to the animals in each period.

The samples were collected during transition periods of the seasons. Therefore, the climatic conditions during the 5 months following installation did not fully correspond to those prevailing at the time of excreta collection. Although the sampling dates occurred near the seasonal transition, forage conditions still reflected the diets typically associated with each period. However, because N fate was evaluated over the subsequent 5 months, the environmental conditions during each trial more closely reflected the following prevailing season than the exact climatic conditions on the sampling date. Thus, for interpretation purposes, the experiments were classified into two periods: Wet season (installed on September 1, 2021) and Dry season (installed on March 1, 2022). During the wet season (September 1, 2021–February 1, 2022), the accumulated rainfall was 751 mm, while during the Dry season (March 1, 2022–August 1, 2022), it totaled 271 mm, highlighting the contrast in water between the seasons.

In this experiment, N₂O emissions, NH₃ volatilization, and urinary N balance were quantified using the ¹⁵N isotopic labeling technique. Excreta were collected from steers grazing either Marandu palisade grass pastures in monoculture or mixed pasture with perennial horse gram. The experiment was took place in a Marandu palisade grass pasture without animals. All excreta were applied to the same receiving pasture, which was Marandu palisade grass without animals. Therefore, 'pasture type' refers to the origin of the excreta—the pasture grazed by the animals during the excreta collection—not the direct

effect of the receiving pasture system. Thus, experimental design isolates differences in excreta composition from pasture origin only; it does not test excreta × pasture-system interactions. The chemical and physical descriptions of the soil are presented in Table 1. Before setting up the experiment, the forage was harvested to maintain the pasture height at 30 cm. A randomized block design was used in a 2 × 2 factorial scheme + 1 control treatment and six replications. The factors were: pasture type (Grass and Mixed pastures), excreta type (feces and urine), and the control (no excreta addition). In the dry season experiment, treatment without the addition of excreta and with the addition of urea at a dose of 5 g N m⁻², equivalent to a fertilization application at a dose of 50 kg of N ha⁻¹, was added to assist as a parameter for losses (this treatment was not included in the statistics).

2.4 Collection and storage of livestock excreta

After the steers had adapted to the paddocks for each diet, livestock excreta were collected fresh from animals grazing in each pasture type and stored on the same day. Urine samples were obtained over three consecutive days and then composited by pasture type for subsequent storage and analysis. The animals were trapped in the morning for the collection of fresh feces and urine. The feces were collected directly from the rectum, and urine was collected in a plastic bucket during spontaneous urination when the animals entered the corral; when necessary, gentle manual stimulation of the prepuce was used only to facilitate urination. For storage, feces were kept in the freezer (−20 °C) and urine in the refrigerator (5 °C). One day before implementing the experiment, the excreta were thawed, visually homogenized in a container, and sampled.

Feces samples were oven dried at 55 °C for 72 h to determine dry matter (DM) concentration and ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass through a 1-mm screen. After grinding, the DM of each sample was obtained by oven drying at 105 °C for 16 h (method 934.01; AOAC International, 2000b). Further grinding of the samples was achieved using a roller mill similar to that described by Boddey et al. (2004). The N concentration was determined of these finely ground samples (<100 mesh) using an automated C–H–N elemental analyzer (Elementar Vario Macro Cube model; method 972.43; AOAC International, 2000d). Urine samples were diluted with 10% sulfuric acid solution in a ratio of 5 mL of acid per 45 mL of urine, and frozen until N analysis. Total N concentration in urine was obtained using the Kjeldahl procedure (method 920.87; AOAC International, 2000a). Feces and forage samples were finely ground in a roller mill (Boddey et al., 2004) prior to C and δ¹³C analysis. Carbon isotope analysis from feces and forage was performed to estimate the legume proportion in the animals' diet. Aliquots containing 200–400 μg C

TABLE 1 Description of the chemical and physical attributes of the soil from the experiment area.

Soil layer cm	Al	Ca	H + Al	Mg	K	P	pH	C	N	Density g cm ⁻³	Clay	Sand	Silt
	cmol _c dm ⁻³			mg dm ⁻³				%			%		
0–10	0.09	1.56	3.46	0.96	43.13	5.75	5.31	1.68	0.11	1.51	15.5	70.5	14.0
10–20	0.30	1.52	3.56	0.74	25.02	3.96	5.16	1.15	0.09	1.65	19.0	67.0	14.0
20–30	0.40	1.28	3.96	0.40	19.24	2.80	5.00	0.89	0.07	1.71	–	–	–
30–40	0.67	1.04	4.78	0.30	14.68	2.53	4.84	0.84	0.07	1.70	–	–	–

Analyses were conducted following standard Embrapa techniques (Claessen et al., 1997).

were analyzed using an automated continuous-flow isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) coupled to a Costech elemental analyzer (model ECS4010) for C and N.

2.5 Quantification of N₂O emissions

For the evaluation of N₂O emissions, the excreta were deposited underground, within the delimited area of the chamber's fitting. Feces patches were artificially prepared by pouring 1.0 kg of feces (fresh weight) in the center of the static chamber base and manually molded to a 0.24 m diameter, 0.049 m² area, and 0.04 m height to resemble a mean feces patch of a steer (Braz et al., 2003). One liter of urine was poured onto the soil surface delimited by the walls of the static chamber base (~0.24 m²) taking care to wet the entire area inside the chamber limits (Whitehead, 1995). In the treatment with urea, on the day of excreta application, 3.35 g of fertilizer was added per chamber, equivalent to 50 kg of N ha⁻¹. Each experimental plot consisted of a closed chamber. Each chamber had its base pre-installed. The base is a hollow box with the same dimensions as the chamber and a trough-shaped fitting that seals when water is added. The base was inserted into the ground to a depth of 8 cm, remaining in place until the end of the evaluation.

The N₂O fluxes were measured daily for 15 days, every 3 days for the following 2 weeks, weekly until the 12th week, and then monthly until the end of the experiment. When rainfall events occurred, additional samplings were performed for the next three consecutive days. The static closed chamber technique was used for N₂O monitoring, as described by Alves et al. (2012). The chambers were closed at the time of N₂O sampling by coupling the top of the chamber to the base. The top of the base was equipped with a trough which was filled with water to ensure the seal after coupling the top portion of the chamber. Chamber tops had the same dimensions as the base (0.40 m width × 0.60 m length × 0.09 m height) and were made of polyethylene and covered with a thermal insulation mantle to minimize temperature increase after deployment.

A four-way valve ("spider") was fitted inside the chamber for gas sampling. Gas samples were taken between 08:30 and 10:30 a.m., which is considered to represent the daily mean flux (Alves et al., 2012). During the 60-min incubation period, samples were collected every 20 min (times 0, 20, 40, and 60 min after the chamber was closed) to estimate the N₂O flux. The gas accumulated in the headspace of each chamber was sampled using 60 mL polyethylene syringes and transferred to 20 mL chromatographic vials within an hour after gas sampling. The chromatography vials were evacuated to -93.3 kPa just before use with an electric vacuum pump. Temperature inside the chamber was measured using digital thermometers at the time of gas collection for further corrections of gas concentrations.

Analyses of N₂O concentration were performed using a Shimadzu GC 2014 gas chromatograph equipped with an electron capture detector and a back-flush system with a packed Porapak Q column. Soil N₂O fluxes were calculated considering a linear increase in gas concentration in the chamber during the deployment period, according to the following Equation 1 described in Mazzetto et al. (2014):

$$f = \frac{dC}{dt} \times \frac{V}{A} \times \frac{M}{V_m} \times \frac{273}{273 + T} \quad (1)$$

Where *f* is the gas flux in μg N₂O-N m⁻² h⁻¹; *dC* is the change in gas concentration in the chamber during the incubation period

in μL L⁻¹; *dt* is the incubation time in hours; *V* is the chamber volume in L; *A* is the area of soil covered by the chamber in m²; *M* is the molecular weight of N in N₂O gas (g mol⁻¹); *V_m* is the molecular volume (standard temperature and pressure [STP]) in L mol⁻¹; and *T* is the internal temperature of the chamber at sampling time in Celsius degree. This equation assumes a linear relationship between N₂O concentration and time during the incubation period (Hutchinson and Mosier, 1981; Parkin and Venterea, 2010).

To estimate gas fluxes in each sampling, the zero time for each plot was used as the initial reference point for calculating the detection limit, as proposed by Parkin et al. (2012), who consider sampling and analytical variability as determinants of the minimum detection capacity of chamber-based methods. This approach minimizes the occurrence of type I errors, in which non-existent flows could be erroneously classified as significant due to analytical noise or random variation. Following chamber-based methodological recommendations, the minimum detectable flux approach was adopted to avoid assigning biological meaning to fluxes close to zero, including small negative values, which may arise from analytical noise rather than true detectable N₂O uptake or emission (Cowan et al., 2014). Thus, fluxes within the detection limit and with *R*² < 0.7 were set to zero, while fluxes above the detection limit but with *R*² < 0.7 were discarded as unreliable.

To determine the cumulative emissions of N-N₂O over the experimental period, the sum of emissions from each interval between successive collections was calculated. The emission in each interval was estimated using the trapezoidal area method, obtained by multiplying the average flux of N-N₂O from the two adjacent collections by the time interval between them. The percentage of N applied as excreta that was lost as N₂O was calculated using Equation (2):

$$EF(\%) = \frac{E_{emitted} - E_{control}}{N_{input}} \times 100 \quad (2)$$

Where EF is the emission factor in percentage from N-N₂O; *E_{emitted}* is the cumulative N-N₂O emissions from urine- or feces-treated plots during the study period (mg N m⁻²); *E_{control}* is the cumulative N-N₂O emissions from the control plot (no excreta) during the study period (mg N m⁻²); and *N_{input}* is the urine-N or feces-N application rate (mg N m⁻²).

2.6 Quantification of NH₃ volatilization

Ammonia volatilization was monitored for 41 days, starting on the same day as the N₂O measurements. The semi-open static chamber method was used for measurement of NH₃ volatilization from urine and feces, according to Martins et al. (2021). For that technique, a chamber was made from a transparent 2 L plastic (PET) bottle without the bottom, with a diameter of 0.10 m, covering an area of 0.008 m². Inside the chamber, NH₃ was captured by a polyethylene foam strip (0.025 m wide × 0.25 m long × 3 mm thick) that was hung vertically by a stainless-steel wire and moistened with 10 mL of a 1.0 mol dm⁻³ H₂SO₄ + 2% glycerin (v/v) solution (Jantalia et al., 2012). The lower end of the foam strip was inserted into a 60 mL plastic flask containing the volume of the acid solution that was not absorbed by the foam strip.

To apply the urine treatment, a wet ring was made with water outside the base of the chamber. The outer mold for pouring the water had a diameter of 0.26 m, and the inner mold of the chamber that received the urine had a diameter of 0.1 m. A volume of 0.033 L of urine was poured into the area at the base of the chamber. To reduce the chamber's effect on the soil, the same urine volume was applied at three different locations within the experimental unit, and the chamber was repositioned after each collection. In the fecal treatment, 1 kg of feces was deposited, and the chamber was repositioned within the fecal plaque after each collection. Changes in the chamber's position occurred due to rainfall. The control treatment with urea was applied to an area of 0.25×0.30 m, with 0.815 g of urea applied.

The chambers for determining NH_3 volatilization were inserted in the area adjacent to the N_2O collection chamber. Foam strips containing volatilized NH_3 were collected concurrently with the N_2O samplings daily until day 10, followed by three additional collections at intervals of approximately 3 days, and thereafter at weekly intervals until approximately day 41. The H_2SO_4 solution and the foam were transferred to 125 mL Erlenmeyer flasks and weighed to determine mass. Subsequently, to remove the solution impregnated in the foam, 40 mL of distilled water was added to each Erlenmeyer flask, which was then transferred to a horizontal shaker at 220 RPM for 30 min. Then, a 10 mL aliquot was analyzed by steam distillation and subsequent titration to quantify the ammoniacal N. The emission was corrected by a factor of 1.74 to account for NH_3 not captured by the chamber (Martins et al., 2021). The cumulative emissions of N-NH_3 over the experimental period was determined as described to the $\text{N-N}_2\text{O}$.

2.7 Urine labeling with ^{15}N -enriched urea

Urine samples used to quantify N_2O were labeled with enriched urea. The urea labeling was 10% of the ^{15}N atoms. Based on the urine N concentration, labeled urea was added at a level corresponding to 20% of the total N. For this, 0.6661 g urea L^{-1} was added for the Grass pasture and 0.3908 g urea L^{-1} for the Mixed pasture in the wet season, and 0.8954 g urea L^{-1} for the Grass pasture and 0.7486 g urea L^{-1} for the Mixed pasture in the dry season, respectively. Labeled urea was diluted in 1 L of urine in each N_2O application plot. In total, i.e., $\text{N-urea} + \text{N-urine}$, the equivalent of 71.9 and 42.2 kg N ha^{-1} was applied for Grass and Mixed pastures in the wet season; and 77.8 and 65.5 kg N ha^{-1} for Grass and Mixed pastures for the dry season, respectively.

Three collections of Marandu palisade grass were carried out at ground level within each base to estimate the N recovery derived from urine (Lessa et al., 2014). At the end of each experiment, the Marandu palisade grass mass was also collected up to 20 cm from the edge of the chamber. Harvests in the wet season were on 10/08/2021, 11/16/2021, and 02/01/2022; and in the dry season, harvests were on 04/04/2022, 05/10/2022, and 07/31/2022. Forage samples were oven-dried at 55°C for 72 h to a constant weigh. These samples were ground in a Cyclotec mill (Tecator, Herndon, VA) to pass a 1-mm screen.

At the end of each experiment, litter was sampled from the base of each chamber. The soil inside each base was removed entirely from the 0–10 cm and 10–20 cm layers in the dry and wet seasons. The

total soil sample was weighed and sieved to separate soil from roots. The separation of soil from roots was performed using a 2 mm sieve and manual picking. The DM and organic matter (OM) content of the roots and litter was determined. The DM was obtained after drying at 100°C for 18 h (method 934.01; AOAC International, 2000b). Ash concentration was determined by incineration in a muffle furnace at 600°C for 4 h (method 942.05; AOAC International, 2000c).

In the experiment conducted during the dry season, soil and root subsamples were collected at 10 cm intervals from 20 to 40 cm depth. Soil density was determined in each layer using samples obtained with a Kopeck ring measuring 79.5 cm^3 . Roots samples were oven-dried at 55°C for 72 h to a constant weigh. Soil and plant samples were finely ground in a roller mill (Boddey et al., 2004) and analyzed for N and $\delta^{15}\text{N}$ using the same isotope ratio mass spectrometry procedures described in section 2.4 for fecal samples. The N from the urine recovered by the plants and the remaining in the soil was determined by Equation 3:

$$\%Rur = \frac{(\% \text{ } ^{15}\text{N}_{xst} \times M \times N)}{\%^{15}\text{N}_{xsU} \times U_N} \quad (3)$$

Where $^{15}\text{N}_{xst}$ is the % of excess ^{15}N atoms, M is the dry mass of soil or plant, N is the fraction of N (%) of the plant or soil; $\%^{15}\text{N}_{xsU}$ is the % of excess ^{15}N atoms in the labeled urine, and U_N is the total N applied via labeled urine.

The C and N content of the forage samples of the other treatments was analyzed using a CN elemental analyzer (Vario MACRO Cube). For the N mass balance, emission factor from N_2O and roots below 20 cm were excluded because their N content was less than 0.01%. The only gas added to the total N fate was NH_3 , which is the main gas responsible for N loss (Cai and Akiyama, 2016). Thus, the unrecovered fraction was defined as the difference between the total N applied and the combined recovery of ^{15}N in the measured soil–plant compartments together with NH_3 -N losses quantified independently. This fraction may reflect hydrological losses, including leaching and/or surface runoff, but these pathways were not directly measured.

2.8 Supporting variables

Soil samples from the 0–0.10 m layer was taken weekly in the first month and then monthly until the end of the experiment. One soil sample from each plot was taken in the area adjacent to the chamber base that received the same amount of excreta. In the feces area, samples were carefully taken from below the feces pats. After collecting, approximately 30 g of soil was immediately cooled, transported to the laboratory, and frozen at -20°C . Subsequently, these samples were used to analyze mineral N (NH_4^+ and NO_3^-) as described by de Campos et al. (2017).

Soil temperature and moisture were measured at each gas collection event. Soil temperature was measured using a digital thermometer inserted approximately 10 cm into the soil. Three surface soil samples (0–5 cm) were collected and oven-dried at 105°C to determine moisture content. Using the surface soil density, the percentage of water filled pore space (WFPS)

was estimated according to Equations 4–6, respectively, as follows:

$$\theta v(\%) = \theta g \times Bd \quad (4)$$

$$TP(\%) = \left(1 - \frac{Bd}{Pd}\right) \times 100 \quad (5)$$

$$WFPS(\%) = \left(\frac{\theta v}{TP}\right) \times 100 \quad (6)$$

Where θv is the volumetric water content in the soil (%); θg is the gravimetric water content in the soil (%); Bd is the soil density in g cm^{-3} ; Pd is the particle density, in this case considered 2.65 g cm^{-3} ; TP is the total porosity of the soil in %; $WFPS$ is the water filled pore space in %.

2.9 Statistical analysis

The experimental design adopted was a randomized block design with five treatments (urine from the Grass and Mixed pastures; feces from the Grass and Mixed pastures, and control), with six replicates. For the urine recovery analysis, only two treatments were considered (Grass and Mixed pastures). The data were analyzed using mixed models, with the MIXED procedure in SAS (SAS Institute, Cary, NC). Treatment, season, and their interaction were considered fixed effects, while blocks were considered random effects. Covariance structures were compared using Akaike's information criterion, and the final model was fitted using a variance components covariance structure. All variance components were estimated using the restricted maximum likelihood method. Means were estimated using LSMEANS, and comparisons were made using Fisher's LSD test at a 5% probability level. The N-Fert treatment was included only in the fertilized season and was therefore presented only as a descriptive reference, not included in the inferential analyses across seasons.

3 Results and discussion

3.1 Climatic and soil conditions

The mean air temperature in the wet and dry seasons was 23.9°C and 23.1°C , with maximums of 39.0°C and 37.4°C and minimums of 12.8°C and 11.0°C , respectively (Figure 1B). The mean soil temperature was 24.8°C and 27.9°C for the wet and dry seasons. The accumulated rainfall throughout the study period was 767 mm and 271 mm in the wet and dry seasons, respectively. Over the 30 days after treatment application, rainfall of 34 mm and 0 mm was recorded during the wet and dry seasons, respectively (Figure 1A). The mean daily relative humidity was 74.7 and 71.0% during the wet and dry seasons. The mean WFPS was 34.4 and 27.7% for the wet and dry seasons, with maximums value of 66.8 and 61.2%, respectively. The automatic weather station was located approximately 3 km from the experimental area. During the study period, precipitation events occurred at the experimental site that the station did not record.

TABLE 2 Nitrogen concentration in the urine of steers grazing Marandu palisade grass monoculture pasture or mixed pasture with perennial horse gram during the wet and dry seasons.

Pasture types	Seasons of the year					
	Wet	Dry	Wet	Dry	Wet	Dry
	g N L ⁻¹		g N m ⁻²		% ¹⁵ N in excess	
Grass	1.576	2.412	5.06	7.74	1.877	1.645
Mixed	1.150	2.017	3.69	6.47	1.504	1.645

Values for the Urine treatment, with urine labeled with 20% of the N concentration, using urea labeled with 10% ¹⁵N atoms.

However, an increase in WFPS was observed at the beginning of the dry-season experiment, indicating the occurrence of these localized rainfalls.

3.2 Livestock excreta characterization

The N concentration in the urine of this study ranged from 1.15 to 2.4 g L^{-1} with application rates of 3.69 to $7.74 \text{ g of N m}^{-2}$ for the wet and dry seasons, respectively (Table 2). In general, the N concentration was greater in the Grass pasture than in the Mixed pasture. It is important to note that no statistical analysis was performed on the N concentration of livestock excreta; however, it was possible to detect a difference in soil mineral N, forage production, and ¹⁵N recovery in relation to pasture types, as will be discussed (topics 3.4 and 3.5). In the literature, the N concentration in urine ranges from 1 to 20 g N L^{-1} (Selbie et al., 2015). Therefore, low N concentrations and application rates were observed in this study (Table 2). Zhu et al. (2021) reported that low N levels are common in pasture-based livestock in tropical regions, particularly during dry seasons when diets provide minimal or suboptimal metabolic protein. In the present study, crude protein levels were within a borderline range, with 8.8% in the Mixed pasture and around 7.0% in the Grass pasture, the latter being close to the critical threshold below which protein may limit animal performance in tropical grazing systems (Reis et al., 2009). Additional information on forage nutritive value is provided in Supplementary Table S1. However, N levels were approximately 50 and 75% higher during the dry season than during the wet season for Grass and Mixed pastures, respectively. This pattern is consistent with the intake data, which showed higher CP and N intakes during the dry season than during the wet season (Supplementary Table S1). Thus, although this period was classified as a drought based on accumulated rainfall, the forage available at the time of excreta collection had greater nutritive value, which likely increased N intake and helped explain the higher N levels observed.

The N concentration from feces ranged across seasons and pasture types. In the Grass pasture, N concentration in feces was 2.15 and $2.43 \text{ g of N kg}^{-1}$ in the wet and dry seasons, with application rates of 6.90 and $7.82 \text{ g of N m}^{-2}$, respectively (Table 3). In the Mixed pasture, N concentration in feces was 1.80 and $2.32 \text{ g of N kg}^{-1}$, with application rates of 5.79 and $7.44 \text{ g of N m}^{-2}$ between the wet and dry seasons, respectively. As with urine, the values were lower when compared to data from the literature, where values typically range from 1.86 to 26.6 g kg^{-1} (Almeida et al., 2020; Tully et al., 2017). The $\delta^{13}\text{C}$ of feces from steers grazing the Mixed pasture averaged -14.46‰ . The estimated legume proportion in forage intake averaged 12.0%.

TABLE 3 Nitrogen concentration and ¹³C abundance (carbon isotope composition) in the feces of steers grazing Marandu palisade grass monoculture pasture or mixed pasture with perennial horse gram during the wet and dry seasons.

Pasture types	Seasons of the year									
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
	g N kg ⁻¹		g N m ⁻²		DM (%)		δ ¹³ C (‰)		Legume (%)	
Grass	2.150	2.435	6.90	7.82	18.43	16.60	–	–	–	–
Mixed	1.803	2.319	5.79	7.44	15.01	15.84	–14.85	–14.06	14.58	9.42

N concentration based on dry matter; applied to 1.0 kg of fresh feces; Legume proportion in feces calculated with δ¹³C of –12.63‰ and –27.82‰ for Marandu palisade grass and perennial horse gram, respectively; Abbreviations: Legume (%) legume proportion in the forage intake, DM dry matter.

Despite seasonal variation, the inclusion of perennial horse gram did not increase N excretion compared to the Grass pasture, indicating a more efficient use of N in the grass/legume mixed pasture, consistent with the findings of [Borré et al. \(2025\)](#).

3.3 N₂O and NH₃ emissions

The NH₃ volatilization was influenced by the N source and the season of year, with greater losses observed in urine applications during the dry season. Daily fluxes peaked in the first week after N source application, whereas urea treatment maintained high emissions for a more extended period. In contrast, N₂O emissions were low and close to baseline levels under all conditions, resulting in EFs well below the reference values ([Buendia et al., 2019](#)). These results indicate that lower soil moisture, lower temperature, and acidic pH, as well as differences in the concentration and form of applied N, were determinants of the observed variations in NH₃ volatilization and reduced N₂O fluxes.

Daily NH₃ volatilization was more intense in the first week after the application of N sources in both experiments, except for the treatment fertilized with N-urea at a dose of 50 kg N ha⁻¹ in the dry season, where the greatest emission lasted until 30 days after application, when it reached basal emission ([Figure 2](#)). The urea treatment averaged 37.57 ± 5 mg NH₃-N m⁻² day⁻¹ during the dry season. The average daily volatilization of NH₃-N per m² for the excreta during the dry season was 33.77 ± 2.24, 26.59 ± 1.24, and 16.68 ± 2.90 mg NH₃-N m⁻² day⁻¹ for urine, feces, and control, respectively ([Figure 2](#)). During the wet season, the average daily volatilization of NH₃-N per m² for the livestock excreta was 17.81 ± 0.10, 29.15 ± 2.06, and 16.40 ± 2.62 mg NH₃-N m⁻² day⁻¹ for urine, feces, and control, respectively. It was observed that, in treatments with excreta, volatilization was greater for the urine than with feces. This is due to the volatilization process occurring more rapidly in urine, because of its physical and chemical characteristics, in which N is predominantly in the form of urea ([Longhini et al., 2020](#); [Rigobello et al., 2023](#)). In feces, N is mainly present in stable organic forms derived from indigestible forage fractions and microbial residues ([Haynes and Williams, 1993](#); [Sordi et al., 2014](#)). After drying, fecal pats form a hydrophobic crust that slows decomposition and limits NH₃ diffusion, thereby reducing volatilization ([Mulvaney et al., 2008](#)).

Regarding the N concentration of the applied source, depending on the season of year or the N source, the NH₃ volatilization tended to increase proportionally with the increase in the doses of N applied. The greater N application rate during the dry season compared to the wet season, especially for urea (7.1 and 4.4 g m⁻², respectively), and the higher temperatures associated with this period may have favored

greater volatilization losses during this trial. These factors likely contributed to the greater NH₃ volatilization observed during the dry season, as increased substrate availability and temperature are known to enhance urease activity and N losses ([Dharmakeerthi and Thenabadu, 1996](#); [da Cruz Corrêa et al., 2021](#)).

The NH₃ emission factor (FE_{NH3}) showed a statistical difference for N source (*p* < 0.001) and seasons (*p* < 0.001; [Table 4](#) and [Figure 3](#)). Urine showed an average FE_{NH3} of 1.9%, and feces showed an average of 0.068%. The NH₃ volatilization and the FE_{NH3} were lower during the wet season compared to the dry season, consistent with previous studies highlighting the influence of climatic conditions on NH₃ losses ([Bretas et al., 2020](#); [Cardoso et al., 2019](#); [Guimarães et al., 2022](#)). Urinary N loss was nearly twice as high in the dry season compared to the wet season (33.77 vs. 17.81 mg NH₃-N m⁻² day⁻¹). In the wet season, 10 mm of rainfall occurred over the 3 days preceding N application, and 22 mm accumulated over the following 12 days. In contrast, no rainfall was recorded during the first 12 days of the dry season trial. Because peak emissions typically occur between 1 and 8 days after application ([Cassim et al., 2022](#)), the absence of rainfall likely limited N incorporation into the soil and increased volatilization. Seasonal effects were significant for urine treatments ([Supplementary Figure S1](#)), while fecal losses remained comparatively stable ([Guimarães et al., 2022](#)).

According to the IPCC ([Buendia et al., 2019](#)), to estimate NH₃ volatilization, indirect N₂O emissions, excreta deposited in the field, manure, or corral slurry are not disaggregated, assuming an average volatilization of 19.7%. The urine FE_{NH3} from published data in Brazil varies considerably from 23.6 to 1.7%. Regarding feces, there is less uncertainty, ranging from 7.2 to 0.99% ([Bretas et al., 2020](#); [Cardoso et al., 2019](#); [Guimarães et al., 2022](#); [Lessa et al., 2014](#); [Longhini et al., 2020](#)). Compiled data show averages of 8.76% for feces and urine, without distinction by excreta ([Cai and Akiyama, 2016](#)). The FE_{NH3} of urea found in this study (0.9%) was 17 times lower than the standard emission proposed by the IPCC ([Buendia et al., 2019](#)). This value, when compared with literature, can be justified by the low N concentration in the applied sources and the low soil pH (pH = 5.3 in the first 10 cm, dropping below 5 at depth; [Table 1](#)). It is well known that soil attributes such as pH and cation exchange capacity (CEC) can influence NH₃ volatilization losses ([Zhenghu and Honglang, 2000](#)). Low pH keeps NH₄⁺ more stable and less susceptible to conversion to NH₃, reducing losses due to NH₃ volatilization ([Fenn and Hossner, 1985](#)).

The N₂O flux was near baseline in both seasons and the N source ([Figure 3](#)). No prominent emission peak was observed, except for a slight peak for the dry season, 21 days after application, coinciding with more intense rainfall, which raised the WFPS from 15 to 50.5%. In fact, 95% of the fluxes were below the minimum detectable flux,

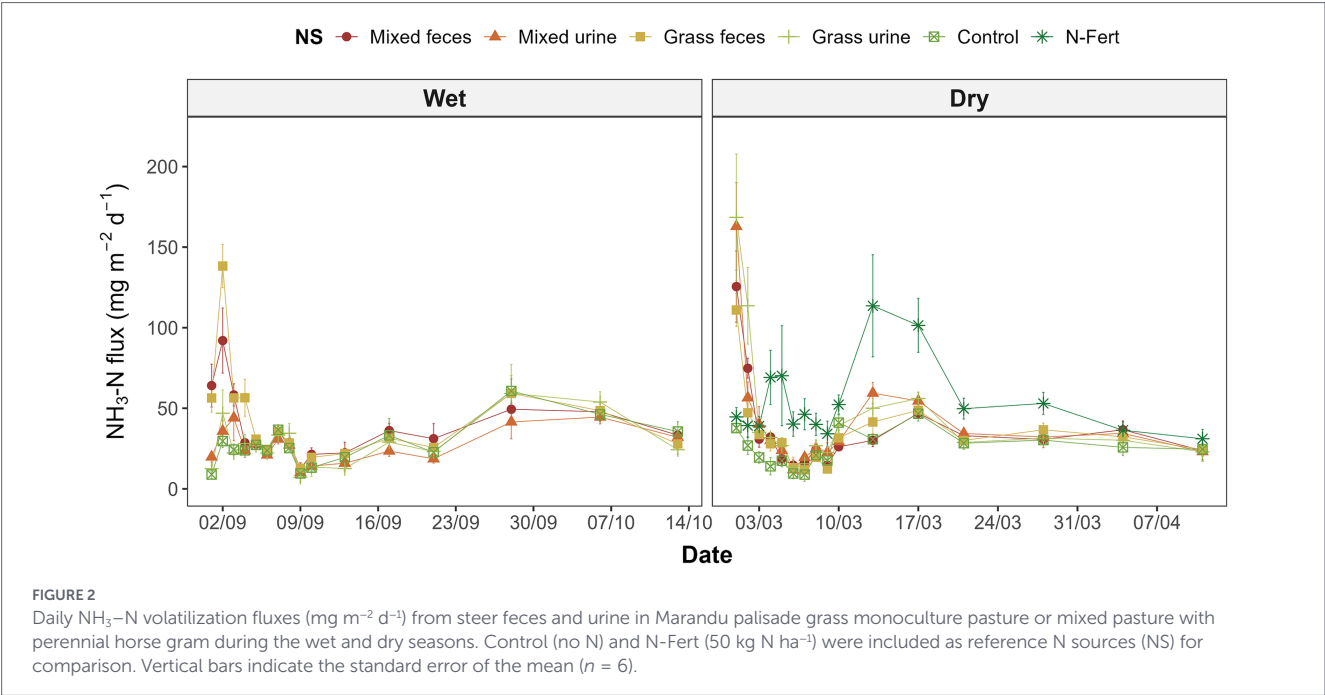


TABLE 4 Emission factors from $\text{NH}_3\text{-N}$ ($\text{FE}_{\text{NH}_3}\%$) and $\text{N}_2\text{O-N}$ ($\text{FE}_{\text{N}_2\text{O}}\%$) for the excreta of steers grazing Marandu palisade grass monoculture pasture or mixed pasture with perennial horse gram during the wet and dry seasons.

Variable	Nitrogen source (NS)				Season of the year (SY)		SEM	NS	SY	NS * SY	
	Urine		Feces		Urea	Wet					Dry
	Grass	Mixed	Grass	Mixed							
EF _{N₂O} %	−0.065	0.083	−0.054	−0.080	−0.009	−0.019	−0.039	0.041	0.3097	0.7684	0.9488
EF _{NH₃} %	1.975a	1.838a	0.068b	0.068b	0.905	0.107b	1.862a	0.158	<0.0001	<0.0001	<0.0001

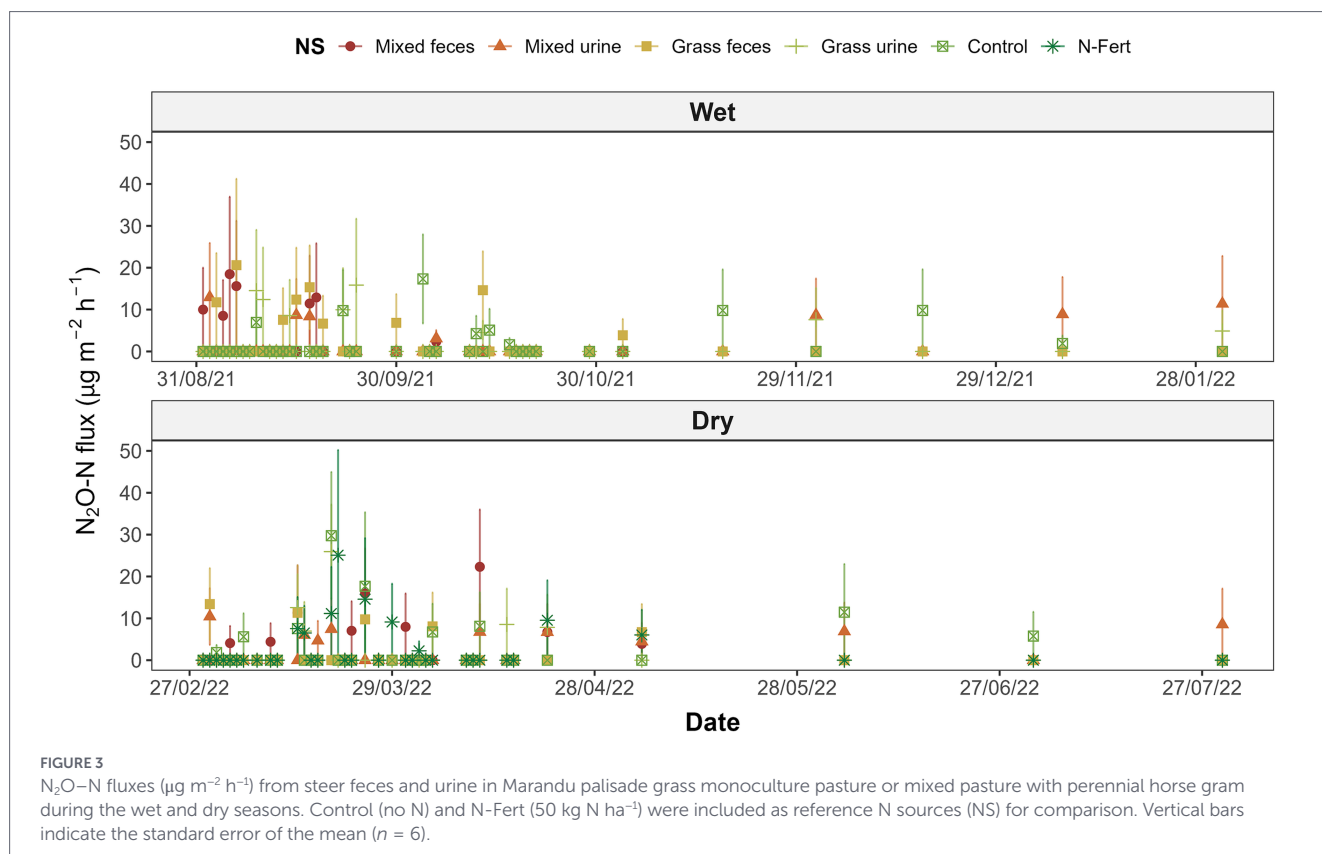
The Urea fertilization treatment was carried out only in the dry season and was not included in the statistics; in the rows, mean values followed by different letters indicate statistically significant differences ($p < 0.05$) by the LSD test; Abbreviations: Standard error of the mean (SEM; $n = 6$); Nitrogen fertilization with 50 kg N-urea (Urea); emission factor (EF).

indicating near-baseline emissions and greater uncertainty in individual flux estimates (Lammirato et al., 2018). The average $\text{N}_2\text{O-N}$ flux was 2.12 ± 0.35 , 1.71 ± 0.10 , 2.01 ± 0.12 , $2.55 \pm 0.43 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ for control, urine, feces and urea, respectively. The $\text{FE}_{\text{N}_2\text{O}}$ did not differ among livestock excreta from Grass or Mixed pastures, or seasons, nor was there an interaction between the factors (Table 4). Emission factors remained substantially lower than IPCC default values. While the predominance of low fluxes reduces the precision of individual estimates and the calculated emission factors, it is important to note that low-flux datasets may still support the overall emission pattern and treatment comparisons when interpreted cautiously (Brümmer et al., 2017; Christiansen et al., 2015). Furthermore, several studies conducted in tropical regions have reported $\text{FE}_{\text{N}_2\text{O}}$ values below 0.01% for the livestock excreta (Byrnes et al., 2017; Durango Morales et al., 2021; Zhu et al., 2021). According to these authors, factors such as urine properties, plant community composition, soil characteristics, and climatic conditions at the time of application, as well as low rainfall after application and the short duration of the experiments, may explain these low emissions.

In the present study, the low rainfall in the first 30 days after the application of the N sources (34.2 and 0 mm, in the wet and dry seasons, respectively), the consequent low WFPS (27% on average in both

experiments), and the low N concentration in livestock excreta likely contributed to the reduced N_2O emissions. This limitation should be explicitly recognized, because urinary N concentration in the present study was lower than values commonly reported for cattle urine, which likely reduced the immediate N substrate available for nitrification and denitrification. This interpretation is reinforced by Lessa et al. (2014), who evaluated cattle excreta deposited on a *Urochloa brizantha* pasture in the Brazilian Cerrado under a low-input pasture context. In that study, urine in the wet season experiment contained approximately 9.8–10.2 g of N L^{-1} and was associated with markedly greater gaseous N losses than those observed here, with urine-derived N_2O emission factors of 0.013–0.026 g N g^{-1} urine N and NH_3 losses of 16.8–30.4% of the applied urine N. The authors also reported that cattle were supplied with soybean and corn meal, which supports the interpretation that N-rich excreta were likely associated with greater dietary N supply and can substantially increase gaseous N losses even under tropical pasture conditions.

In addition to plant uptake, microbial immobilization may also have played a central role in constraining N losses. Under N-limited conditions, soil microorganisms can rapidly immobilize a substantially greater share of added N than plants. Liu et al. (2016) reported that microorganisms took up at least seven times more N than plants



shortly after ¹⁵N addition in a temperate grassland. Therefore, the relationship between N input and N₂O emissions should not be assumed to be universally linear. A growing body of evidence suggests that N₂O responses to N addition may be nonlinear, particularly across contrasting ranges of N supply (Kim et al., 2013). Based on the conceptual model proposed by Kim et al. (2013), low N inputs may remain within an initial phase in which plants and soil microorganisms compete effectively for available N, thereby limiting N losses, whereas greater emissions are expected only after this biological demand is exceeded. This interpretation is consistent with Chirinda et al. (2019), who showed that adequate vegetative cover reduced urine-induced N₂O emissions in grazed pastures, likely by strengthening the soil–plant sink for deposited N. Furthermore, as discussed below, the labeled urine balance experiment demonstrated that soil and Marandu palisade grass strongly retained excreta-derived N, substantially reducing the amount of N available for loss as N₂O or NH₃. Therefore, the very low N₂O fluxes observed here likely reflect the combined effects of low urinary N concentration, limited soil moisture after excreta deposition, and efficient microbial immobilization and plant uptake in the soil–plant system.

3.4 N-mineral

The N source significantly affected soil mineral N, with greater NH₄⁺ and NO₃[−] concentrations observed after urine application, particularly at the beginning of the dry season (Table 5; Supplementary Figure S2). Across evaluation periods, the control consistently showed the lowest values. During the first 10 days, NH₄⁺ concentrations in urine treatments were approximately twice as high in the dry season as in the wet season, coinciding with greater NH₃ volatilization (Bretas et al., 2020). A similar seasonal pattern was observed for NO₃[−] during

the first 20 days, although concentrations remained low and N₂O emission factors were negligible, suggesting limited denitrification and probable microbial immobilization. By the end of the 70 days, mineral N increased in the wet season but declined in the dry season, reflecting seasonal differences in mineralization dynamics (Thangarajan et al., 2015).

3.5 Forage production and shoot N concentration

Throughout the three harvests, forage production and shoot N concentration were influenced by N sources and seasonality ($p < 0.001$). Urine application resulted in the greatest productivity and N concentrations in the first two harvests, while feces reduced production due to a physical covering effect (Haynes and Williams, 1993). Urea (50 kg N ha^{−1}) showed intermediate performance and forage production similar to the control in the first harvest and approaching urine treatments in the second harvest (Supplementary Figure S3A).

Although there were more N in feces per area than in urine (Table 2 and Table 3), urine treatments promoted greater forage growth and greater shoot N concentration (Table 6), reflecting the rapid conversion of urinary N to plant-available mineral forms. This pattern is consistent with the higher NH₄⁺ concentrations observed shortly after urine application (Supplementary Figure S2B) and with the greater NH₃ volatilization detected during the same period, particularly in the dry season. In contrast, fecal N is predominantly organic and becomes available more gradually through mineralization processes (Weerden et al., 2021), resulting in lower short-term plant response.

Seasonal modulation was also evident. During the dry season, there was greater forage production compared to the wet season

TABLE 5 Concentration of mineral N in the form of nitrate (NO₃⁻) and ammonium (NH₄⁺) in the soil (mg N kg⁻¹ of dry soil) for the excreta of steers grazing Marandu palisade grass monoculture pasture or mixed pasture with perennial horse gram during the wet and dry seasons.

Variable	Days	Nitrogen source (NS)				Season of the year (SY)							
		Urine		Feces		Control	Urea	Dry	Wet	SEM	NS	SY	NS * SY
		Grass	Mixed	Grass	Mixed								
NO ₃ ⁻ , mg N kg soil ⁻¹	01–10	1.017 a	1.016 a	0.947 a	0.886 a	0.606 b	0.825	0.885 a	0.825 a	1.233	0.0028	0.4506	0.0122
NO ₃ ⁻ , mg N kg soil ⁻¹	11–20	1.041 a	0.935 ab	0.884 b	0.868 b	0.749 c	0.825	0.885 a	0.894 a	1.077	0.0036	0.8299	<0.0001
NO ₃ ⁻ , mg N kg soil ⁻¹	21–32	0.885 b	0.810 b	0.786 bc	1.139 a	0.674 c	0.799	0.764 b	0.943 a	1.082	<0.0001	0.0004	<0.0001
NO ₃ ⁻ , mg N kg soil ⁻¹	33–70	0.802 ab	0.796 ab	0.951 a	0.969 a	0.680 b	0.675	0.760 b	0.897 a	1.242	0.0389	0.0409	0.9468
NH ₄ ⁺ , mg N kg soil ⁻¹	01–10	27.740 a	21.665 b	16.357 c	17.291 c	10.419 d	6.863	26.525 a	11.392 b	1.470	<0.0001	<0.0001	<0.0001
NH ₄ ⁺ , mg N kg soil ⁻¹	11–20	33.727 a	29.349 ab	26.108 abc	23.183 bc	19.625 c	36.305	26.118 a	25.441 a	1.043	0.0142	0.7776	0.1009
NH ₄ ⁺ , mg N kg soil ⁻¹	21–32	17.175 a	17.138 a	13.343 a	20.127 a	13.335 a	19.521	25.686 a	9.321 b	1.687	0.2637	<0.0001	0.1442
NH ₄ ⁺ , mg N kg soil ⁻¹	33–70	30.140 ab	26.974 bc	36.046 a	28.839 bc	24.259 c	9.151	7.873 b	74.148 a	1.279	0.0048	<0.0001	0.5341

The urea-fertilized treatment was carried out only in the dry season and was not included in the statistics; in the rows, mean values followed by different letters indicate statistically significant differences ($p < 0.05$) by the LSD test; Standard error of the mean (SEM), $n = 6$.

TABLE 6 Forage mass (g m⁻²) and shoot N concentration (%) of forage inside the gas collection chambers affected by the excreta of steers grazing Marandu palisade grass monoculture pasture or mixed pasture with perennial horse gram during the wet and dry seasons.

Variable	Harvest	Nitrogen source (NS)										Season of the year (SY)				SEM	NS	SY	NS * SY	
		Urine				Feces				Control		Urea	Dry		Wet					
		Grass		Mixed		Grass		Mixed		p-value										
Forage mass, g m ⁻²	1st	174.56	a	145.75	b	82.86	d	81.74	d	114.65	c	215.34	209.51	a	58.90	b	1.248	<0.0001	<0.0001	0.0262
	2nd	130.21	a	107.44	b	85.71	c	88.40	c	88.93	c	144.49	120.34	a	80.46	b	2.378	<0.0001	<0.0001	0.0421
	3rd	90.54	a	80.91	a	87.77	a	87.77	a	93.99	a	69.61	65.66	b	116.33	a	1.287	0.7052	<0.0001	0.764
N Shoot, (%)	1st	1.679	a	1.592	a	0.945	b	0.951	b	0.882	c	1.194	1.17	a	1.12	b	1.025	<0.0001	0.0437	0.0019
	2nd	1.331	a	1.390	a	0.968	b	0.937	b	0.986	b	1.036	1.25	a	0.98	b	1.035	<0.0001	<0.0001	0.3306
	3rd	0.957	a	1.022	a	0.678	c	0.760	b	0.749	b	0.775	0.96	a	0.71	b	1.060	<0.0001	<0.0001	0.0045

Forage mass based on dry matter; Urea fertilization treatment was carried out only in the dry season and was not included in the statistics analysis; in the rows, mean values followed by different letters indicate statistical differences ($p < 0.05$) by the LSD test; abbreviations: standard error of the mean (SEM), $n = 6$.

($p < 0.0001$; Table 6), due to the higher N concentration found in the excreta in this period. During the wet season, differences among N sources were smaller, and shoot N concentration declined across harvests (Supplementary Figure S3B), suggesting lower N availability and a dilution effect under more favorable growth conditions. By the third harvest, seasonal effects predominated, and differences among N sources diminished, indicating climatic limitations on forage accumulation. Overall, the strong plant response to urinary N, especially in the first two harvests, supports the ^{15}N mass balance results, which show substantial recovery of applied N in the plant compartment (Table 7), reinforcing the role of rapid plant uptake as a key pathway limiting N losses in this tropical pasture system.

3.6 Mass balance of urine labeled with ^{15}N

In the wet and dry seasons, 76.6 and 96.2% of the applied N were in the soil–plant system. In both seasons, almost 50% of this N was found in the soil at 0–10 cm depth (Table 7). Of the fraction recovered in the soil, about 88% was present at 0–10 cm depth. Lessa et al. (2014) in tropical pasture applied with cattle urine during the wet season found that about 65% of the applied N was in the soil–plant system, where 17.4% was presented in the shoot and root *Urochloa*, 23% in the soil at 0–10 cm depth, and a substantial loss of 23.6% due to NH_3 volatilization. When urine labeled with ^{15}N was applied to a lysimeter, Fraser et al. (1994) found that 79% of the N recovered from the urine was within the first 20 cm of soil layers.

The C and N concentrations in the soil showed a progressive decrease with depth, being C: 1.68; 1.15; 0.89 and 0.84% and N: 0.11; 0.09; 0.07 and 0.07% in the 0–10, 10–20, 20–30 and 30–40 cm layers, respectively. It is important to highlight that these values were obtained in control areas, without the application of urine or feces, and therefore reflect the natural distribution of C and N in the soil profile. The surface layer (0–10 cm), besides being the first to receive urine and therefore the

central zone of initial interception and retention of applied N, naturally presents greater levels of C and N due to the accumulation of organic matter. Greater C concentration provides a greater substrate supply to microorganisms, favoring microbial immobilization of N and restricting its mineral form and vertical mobility. Thus, the greater recovery of N from urine in the surface layers results not only from direct deposition but also from the chemical and biological conditions inherent to those layers, which are more favorable to N retention and immobilization, as described by Templer et al. (2012).

Regarding the fine roots, despite the effort to recover as many as possible, some remained in the soil. It is expected that finer roots have a higher N concentration than coarser roots, which may represent a potential source of overestimating the N considered as immobilized in the soil (Roth et al., 2022). It is estimated that approximately 54% of the N was immobilized in the soil and 31% in the plant during the 150 days of the experiment, which was very close to that found in a study with barley in microplots, where 54% was immobilized in the soil and 35% in the plant (Jensen et al., 1999). Despite this, the literature reports significant variation in N immobilization in soil, ranging from 10 to 63% with an average of 26% (Selbie et al., 2015). Unfortunately, most of the data available in the literature were recorded in temperate regions and with the use of high N doses.

In low-N tropical pasture, the simultaneous high ^{15}N recovery in soil, substantial plant uptake, low NO_3^- concentrations, and negligible N_2O emissions likely reflect a conservative N cycle rather than a contradiction between soil immobilization and plant uptake. Urine deposition generates a short-lived pulse of mineral N that can be rapidly partitioned between microbial immobilization and plant uptake, thereby limiting NO_3^- accumulation and helping explain the low N_2O emissions observed here. In tropical pastures, low soil NO_3^- may result not only from reduced nitrification, but also from high inorganic N immobilization under low-N conditions (Vázquez et al., 2020). In addition, short-term tracer studies indicate that microorganisms may temporarily

TABLE 7 Recovery of N from urine labeled with ^{15}N by the excreta of steers grazing Marandu palisade grass monoculture pasture or mixed pasture with perennial horse gram during the wet and dry seasons.

Variable		Pasture type (PT)		Season of the year (SY)		SEM	p -value		
		Mixed	Grass	Wet	Dry		PT	SY	PT * SY
Soil, %	0–10 cm	53.005	48.287	49.227	52.064	2.679	0.232	0.466	0.063
	10–20 cm	2.997	4.005	5.207a	1.794 b	0.572	0.224	<0.001	0.215
	20–30 cm	0.565	0.610	–	0.588	0.143	0.843	–	–
	30–40 cm	0.242	0.262	–	0.252	0.096	0.900	–	–
Root, %	0–10 cm	0.321	0.339	0.302 b	0.358 a	0.011	0.266	0.040	0.059
	10–20 cm	0.115	0.127	0.093 b	0.149 a	0.009	0.370	<0.001	0.336
Shoot, %	1st Harvest	19.968 b	23.958 a	11.812 b	32.113 a	0.978	0.006	<0.001	0.658
	2nd Harvest	6.978	7.867	6.678 b	8.167 a	0.463	0.111	0.012	0.220
	3rd Harvest	1.745	2.035	2.222 a	1.558 b	0.138	0.138	<0.001	<0.001
	Border	0.849	0.826	1.474 a	0.201 b	0.128	0.899	<0.001	0.729
Litter	–	0.164b	0.229a	0.246 a	0.148 b	0.020	0.034	0.012	0.019
FE_{NH_3} , %	–	1.827	1.975	0.132 b	3.669 a	0.223	0.605	<0.001	0.640
Total, %		88.418	89.970	77.167 b	101.220 a	3.367	0.722	<0.001	0.171

In the rows, mean values followed by different letters indicate statistically significant differences ($p < 0.05$) by the LSD test; $\text{FE}_{\text{N}_2\text{O}}$ of urine and roots below 20 cm were not added as they are less than <0.1%; abbreviations: Emission factor for ammonia (FE_{NH_3}); Standard error of the mean (SEM).

outcompete plants for available N, whereas subsequent remobilization may contribute to plant recovery over time (Liu et al., 2016).

The N recovery from urine showed a significant difference depending on the pasture type in the first harvest of the shoot ($p = 0.006$) and in the litter ($p = 0.034$). For season, there was no difference in the soil at 0–10 cm layer, but differences were observed for all other variables ($p < 0.05$). An interaction between pasture type and season was observed for litter and the third harvest of the shoot (Table 7). The season proved to be the most significant mediating factor in the fate of urine-derived products. The soil at a depth of 10–20 cm during the wet season showed a greater N proportion derived from urine than during the dry season ($p < 0.001$), with values of 5.2 and 1.8%, respectively. This pattern is consistent with greater downward movement of urine-derived N during the wet season, although leaching was not directly measured. After the urine application during the wet season, the accumulated rainfall was 751 mm, while during the dry season it was only 271 mm. Surface layers may be unable to retain mineral forms of N, including NH_4^+ , which can be leached under conditions of high rainfall, sandy soils, low CEC, and low base saturation (Selbie et al., 2015).

There was a statistically significant difference ($p < 0.001$) in N recovery by the forage mass at the chamber border between the seasons. In this case, recovery was greater at the border during the wet season compared to the dry season. The greater recovery of ^{15}N at the chamber border during the wet season is consistent with hydrological redistribution of urine-derived N, potentially involving lateral transport and/or downward movement within the soil profile. Several mechanisms contribute to solute transport in soil, including convective, diffusive, and dispersive processes. The principles that may have displaced N from the base area into deeper layers could have been diffusive, in which the substance moves from higher to lower concentration, or dispersive, in which the substance moves passively following the mass flow (Ruiz et al., 2010).

Total N recovery also corroborates the greater loss during the wet season compared to the dry season ($p < 0.001$). During the wet season, N total recovery was 77.2%, while during the dry season it was approximately 100%. Notably, the first harvest of the shoot accounted for approximately a proportion of this difference. There was a difference of about 20% in recovery between the seasons (approximately 11.8% for the wet season and 32.1% for the dry season). Thus, under the conditions of this study, rainfall during the field may have been a leading cause of N loss from urine in pasture via leaching or surface runoff as early as the first month of evaluation.

Approximately all the N was recovered in the dry-season trial, indicating little or no N was lost under those conditions. In contrast, about 22.8% of the applied ^{15}N was not recovered in the wet-season trial. Although this non-recovered fraction cannot be attributed exclusively to leaching or surface runoff, the available evidence suggests that hydrological transport was more important during the wetter period. In a mass balance study using ^{15}N -labeled urine, a 5% unaccounted-for recovery was considered possible due to leaching losses (Lessa et al., 2014). In a review of emission factors and combined N losses from feces and urine, the average percentage of N lost by leaching is 24.5% for NH_4^+ and NO_3^- from the soil. Furthermore, N loss by leaching is notably reduced by plant absorption (Cai and Akiyama, 2016). The IPCC (2019) adopts a value of 24% (1–73%) as the fraction of N lost via leaching. Considering that Lessa et al. (2014) reported a non-recovered fraction of approximately 5%, and that in the present study this fraction ranged from 0 to 22.8%, both under tropical conditions, the reference value adopted by the IPCC for leaching-related losses (Buendia et al., 2019) may overestimate the magnitude of these losses in tropical pastures by nearly two and a half times. However,

because leaching was not directly measured in either case, this comparison should be interpreted with caution.

Another route of N loss that is frequently not accounted for is the N_2 emission. Clough et al. (1998) estimated that about 25% of the applied N was lost as N_2 , representing the main fraction of unrecovered N. Greater N_2 emissions compared to N_2O occur when there is a large availability of electron acceptors (such as NO_3^-) together with a supply of electron donors (organic C), conditions that intensify denitrification (Gillam et al., 2008). Soil aeration regulates the microbial choice of the final electron acceptor, following the preference sequence $\text{O}_2 > \text{NO}_3^- > \text{N}_2\text{O} > \text{N}_2$. Thus, environments with prolonged soil saturation by water and high C availability favor complete denitrification, in which N_2O is reduced to N_2 , the final product of the process. In the present study, during the wet season, although rainfall was constant, the soil's good drainage capacity in the surface layers prevented WFPS from exceeding 70%. Furthermore, the Marandu palisade grass litter presented a high C:N ratio, limiting the immediate supply of labile C to the microbial community. Therefore, it is presumed that environmental conditions did not favor complete denitrification, so N_2 probably did not represent a significant pathway for N loss. This inference is reinforced by the low N_2O emission observed experimentally, indicating a general limitation on denitrifying activity. Thus, under conditions of low denitrification intensity, N_2 emissions tend to be proportionally reduced relative to N_2O , reflecting a process that is not very active in either gaseous form (Ciarlo et al., 2008).

Nitrogen recovery in litter showed a significant effect of pasture type and seasons of the year, as well as an interaction between these factors ($p = 0.019$; Table 7). The urine from the Grass pasture showed the greatest recovery during the wet season ($0.3233\% \pm 0.044$), surpassing that of the Mixed pasture in both the wet season ($0.168\% \pm 0.044$) and the dry season ($0.160\% \pm 0.007$). Following this, the urine from the Grass pasture in the dry season ($0.135 \pm 0.007\%$) did not differ statistically from that of the Mixed pasture in the wet season. Litter mass also varied between seasons, with greater mass for the dry season than in the wet season ($p = 0.0074$; Supplementary Table S2). This greater litter accumulation during the dry season may have proportionally reduced the fraction of N derived from urine, diluting the recovery signal compared to the wet season, characterized by less litter deposition. It is also possible that the litter collected at the end of the experiment was associated with the newly deposited fraction, considering that, in the third harvest, the proportion of N derived from urine was greater during the wet season. This result suggests that during the wet season, urine treatments under N-deficient conditions and increased plant growth favored the recycling of leached N at depth via incorporation into the litter. It is noteworthy that forage mass was greater during the wet season than during the dry season in the third harvest (Table 6). Litter production is dynamic, directly related to growth rate and forage accumulation, and can range throughout the 28-day grazing cycle (Ramalho et al., 2019). Furthermore, the effects of urine deposition can persist for up to 150 days, and environmental conditions at the time of application influence the magnitude and duration of N uptake during plant growth (Welten et al., 2019).

The amount of N recovered from the Marandu palisade grass of urine treatment from animals in the Mixed pasture was lower than that from the Grass pasture animals during the first harvest (Table 6). The higher N concentration in the urine of the Grass pasture animals compared to the Mixed pasture in both seasons likely contributed to a greater N availability. As a result, the Grass pasture showed greater N recovery. Mixed pastures increased the efficiency of N use by steers, enabling greater animal productivity without increasing N excretion, mainly in urine (Borré et al., 2025). Although the greater amount of N in the livestock excreta from

the Grass pasture and greater values of mineral N, forage production, and N recovery in the forage and litter associated with this pasture type, no significant differences were observed in gaseous losses, i.e., NH_3 volatilization, N_2O emission, or possibly leaching. This behavior is probably related to the N immobilization in the soil–plant system, which limits the effective loss of N to the environment. In many unfertilized tropical grass pastures, background N availability is low, particularly as NO_3^- , and when mineral N is released into the soil, NO_3^- accumulation may remain limited due to rapid microbial immobilization and plant uptake. In this situation, there will be no accumulation of NO_3^- in high concentrations in the soil, reducing denitrification reactions and, consequently, N_2O production (Jensen et al., 2012). Mixed pastures, in contrast to fertilized pastures, exhibit greater N retention in the system. Nitrogen input through biological nitrogen fixation (BNF) occurs indirectly; after N assimilation in the legume mass, the plant residues deposited in the soil are mineralized (Borré et al., 2025; Homem et al., 2021b). In this scenario, litter mineralization makes N available more slowly and gradually, indicating significant reductions in N losses. However, it is recommended to conduct additional trials with higher N inputs and more intensive management in tropical environments to better understand the system's capacity to immobilize N. Such studies are fundamental to identifying strategies that reconcile productive intensification with the environmental sustainability of pasture systems.

According to urinary excretion data from the same experimental station presented by Borré et al. (2025), with a fixed stocking rate of two head ha^{-1} and urinary excretion of 30.5 and 29.1 g of N $\text{head}^{-1} \text{day}^{-1}$, for the dry season (120 days) and wet season (245 days), an excretion via urine of 21.6 kg of N $\text{ha}^{-1} \text{year}^{-1}$ would be expected. Considering the values found in urine recovery, the leading destination of N would be immobilization in the soil, where $\sim 11.8 \text{ kg of N ha}^{-1} \text{year}^{-1}$ would remain in the soil and litter, and $\sim 7.72 \text{ kg of N ha}^{-1} \text{year}^{-1}$ would be absorbed by Marandu palisade grass. Losses would correspond to approximately 10% of the excreted urine, of which about 1.52 kg of N $\text{ha}^{-1} \text{year}^{-1}$ would represent a non-recovered fraction potentially associated with hydrological losses such as leaching and/or surface runoff, in addition to $\sim 0.53 \text{ kg of N ha}^{-1} \text{year}^{-1}$ lost as NH_3 volatilization. Under the conditions of this study, N_2O losses were very low, and N_2O -N losses from urine were expected to be close to zero. Studies of N cycling in tropical pastures have assumed urinary N losses of around 50% and fecal N losses of 5% (Boddey et al., 2004; Homem et al., 2021a). These results suggest that commonly assumed urinary N losses may be high for this system. The study conducted by Lessa et al. (2014) in the Cerrado region during the rainy season, using urine labeled with ^{15}N , recorded 35% of urinary N not recovered in the soil/plant system.

4 Conclusion

In this study, the season of the year proved to be a determining factor in the fate of N from livestock excreta. The seasons exerted a more pronounced influence on NH_3 losses from urine than on those from feces, whose values remained relatively stable over time. Immobilization of N in the soil and its absorption by Marandu palisade grass accounted for the main fate of the applied N, reaching up to 96.2% in the experiment conducted during the dry season. Regardless of the season, the soil was the largest retention

compartment for N from urine, accounting for approximately 50% of the total applied. This high retention capacity resulted in low losses due to NH_3 volatilization and N_2O emissions in both evaluated periods, with N_2O emission factors lower than the IPCC averages. While the ^{15}N mass balance indicated limited non-recovered N overall and stronger evidence of hydrological N transport during the wetter period. This indicates that livestock excreta in tropical pastures may represent lower losses than those commonly used as references for GHG emission calculations and N balances.

Although urine from Grass pastures tended to have higher N concentration than urine from the Mixed pasture, no significant differences in gaseous losses were observed between excreta sources under the common Marandu palisade grass pasture used in this study. Similar gaseous losses suggest that either the receiving pasture or soil conditions played a central role in moderating emissions, masking differences in excreta N concentration. Thus, it is concluded that: (i) the introduction of perennial horse gram legume in Marandu palisade grass pastures does not promote an increase in GHG emissions from livestock excreta. These findings should not be interpreted as a full pasture-system effect, since all excreta were applied to a single receiving pasture.; (ii) under the low urinary-N and low-input conditions evaluated here, the soil–plant system retained a large proportion of excreta-derived N, indicating efficient uptake or immobilization processes that limited non-recovered N under most conditions. However, the results also indicate a potential vulnerability to N losses as N inputs and excretion rates increase, possibly because higher N loads could surpass the soil–plant system's retention capacity. Thus, these findings are most directly applicable to the experimental conditions evaluated in this study and should be extrapolated with caution to more intensive tropical grazing systems.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal studies were approved by the Comissão de Ética da Universidade Federal Rural do Rio de Janeiro. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

IR: Data curation, Conceptualization, Writing – review & editing, Investigation, Formal analysis, Writing – original draft, Visualization. JB: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. RM: Writing – review & editing, Conceptualization, Supervision, Writing – original draft, Investigation. BH: Supervision, Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Formal analysis. SS:

Writing – original draft, Investigation, Writing – review & editing. WS: Writing – original draft, Writing – review & editing, Investigation. CS: Writing – review & editing, Writing – original draft, Investigation. RM: Writing – original draft, Supervision, Writing – review & editing. BA: Conceptualization, Funding acquisition, Writing – original draft, Project administration, Writing – review & editing, Supervision. SU: Conceptualization, Writing – original draft, Supervision, Funding acquisition, Project administration, Writing – review & editing. RB: Project administration, Conceptualization, Supervision, Writing – review & editing, Writing – original draft, Funding acquisition.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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