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Ammonia Volatilization, Forage Accumulation, and Nutritive Value of Marandu Palisade Grass Pastures in Different N Sources and Doses

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Abstract: The reduction in ammonia (NH_3) losses from volatilization has significant implications in forage production. The objective of this study was to evaluate the impact of N fertilizers (urea, ammonium nitrate, and ammonium sulfate) and four doses (0, 90, 180 and 270 kg N ha^{-1}) on N losses by NH_3 volatilization, accumulation, and forage chemical composition of *Urochloa brizantha* cv Marandu. Two field experiments were conducted to measure NH_3 losses using semi-open chambers. The forage accumulation and chemical composition were evaluated in the third experiment; the response variables included forage accumulation, crude protein (CP), and neutral detergent fiber (NDF). Compared to urea, ammonium nitrate and ammonium sulfate reduced NH_3 losses by 84% and 87% and increased total forage accumulation by 14% and 23%, respectively. Forage accumulation rate and CP increased linearly with the N levels, while NDF contents decreased linearly with the N levels. In both experiments, NH_3 losses and forage characteristics were different according to the rainfall pattern and temperature variations. Our results indicate that the use of nitric and ammoniacal fertilizers and the application of fertilizer in the rainy season constitute an efficient fertilizer management strategy to increase forage yield and decrease losses from volatilization of NH_3.

Keywords: ammonia volatilization; N pollution; ammonium sulfate; pastures; crude protein

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1. Introduction

Nitrogen (N) fertilization is one of the main strategies used to intensify the use of pastures to address the low natural availability of N in soils. However, the type, quantity, form, and timing of application of N fertilizers can have significant implications for N losses, mainly due to ammonia (NH_3) volatilization, affecting pasture productivity [1,2].

The ammonia volatilization is the main cause of low efficiency in using N fertilizers. Urea is the most used inorganic nitrogen fertilizer in the world [3], but it has the potential for NH_3 volatilization from up to 80% of the applied N, depending on the management practices adopted, soil type, and local environmental conditions mainly occurrence of rains [4,5].

Nitrogen fertilization is known to increase forage dry matter yield and nutritional value, improving the chemical composition of the plant by increasing the crude protein content and decreasing the neutral detergent fiber (NDF) content [6–8]. However, NH_3 losses after fertilization decreasing N use efficiency in plants present a threat to the environment; it can acidify the soil from ammonia oxidation [9–11]. In addition, NH_3 acts as a secondary source of N_2O, an important greenhouse gas [12–14].

Among the practices used to minimize NH_3 volatilization is fertilization splitting [9], incorporating fertilizer into the soil [14], and the use of N fertilizers in nitric and ammoniacal form, such as ammonium nitrate and ammonium sulfate [15,16]. However, the results of research on NH_3 emission factors (EFs) obtained in different regions are often inconsistent and conflicting, generating the need to evaluate new strategies to mitigate NH_3 losses for regions and specific crop systems [5,13,14].

The use of NH_3 EF from other countries, mainly in temperate climates, leads to uncertainties in Brazilian national inventories, requiring more studies to develop country-specific EF [2,11,17]. Furthermore, the potential for NH_3 losses from different nitrogen fertilizers in tropical pastures has not yet been clearly determined.

Urochloa brizantha cv Marandu is one of the most used species in regions with different soil and climate conditions in tropical Latin America, particularly in Brazil [18–20], which indicates more regional variation in response to fertilizer management practices. However, in Marandu palisade grass, the potential N loss from volatilization and the characteristics of the forage in response to three main N fertilizers used in the country are still unknown.

Furthermore, to evaluate different fertilization strategies for crop yields, it is important to study the environmental effects of this fertilization to contribute to the development of more sustainable systems. Therefore, the objective of this study was to investigate the effect of three N fertilizers (urea, ammonium nitrate, and ammonium sulfate) and four doses of N (0, 90, 180, and 270 kg N ha^{-1}) on forage accumulation and chemical composition, and NH_3 emissions in tropical Marandu grass pastures in the Brazil's Cerrado region.

2. Materials and Methods

2.1. Experimental Site

The field study was carried out in a Marandu grass pasture, *Urochloa brizantha* cv. Marandu, established in 2001, and located in the Forage and Grasslands sector of São Paulo State University—UNESP, Jaboticabal, São Paulo, Brazil (21°15′22″ S and 48°18′58″ W; 595 m altitude). According to the Köppen climatic classification, the climate in the region is of the Aw type (tropical, characterized by dry winters). The annual rainfall is 1424 mm, and the average air temperature is 22.3 °C. The soil is a Rhodic Ferralsol [21]. Soil properties (0–20 cm depth) were bulk density of 1.20 g cm^{-3}, contains 420 g kg^{-1} sand, 140 g kg^{-1} silt and 440 g kg^{-1} clay [22], pH ($CaCl_2$) 5.4, organic matter 31 g kg^{-1}, cation exchange capacity 85 mmolc dm^{-3}, P (ion-exchange resin extraction method) 16.5 mg dm^{-3}, Mehlich-1 extractable Ca 36.5 mmolc dm^{-3}; Mehlich-1 extractable Mg 14 mmolc dm^{-3}, Mehlich-1 extractable K 4.2 mmolc dm^{-3}, base saturation 55%, respectively.

Meteorological data were measured throughout the experimental period, including temperature, rainfall and water balance data being obtained from the Agrometeorological Station, Department of Exact Sciences, FCAV/UNESP, located 1.5 km from the experiment site.

2.2. Experimental Design and Treatments

Three experiments were performed on Marandu grass pastures: the first and second for the evaluation of volatilized NH_3, and the third for the evaluation of forage accumulation and chemical composition. The experimental area during the evaluation period was not allowed to graze to avoid disturbance or influence of animal excretions. In each experiment, 40 experimental plots were used.

The experiment 1 was conducted from 13 December 2017 to 3 January 2018, and experiment 2 was from 27 November 2018 to 9 March 2019. The experiment was arranged in a randomized block design, with 4 repetitions, in a 3 × 3 + 1 factorial scheme. The treatments were three N fertilizers: urea, ammonium nitrate, and ammonium sulfate; three doses of nitrogen: 90, 180, and 270 kg ha^{-1} $year^{-1}$, and one treatment without nitrogenous fertilization (control).

In experiment 1, the N doses were applied in a single fertilization (13 December 2017). In experiment 2, conducted in another area, the doses were split into three applications (27 November 2018, 12 January 2019, and 16 February 2019), representing 30, 60, and 90 kg N ha^{-1} $year^{-1}$, distributed manually after the standardization cuts, carried out to a residue of 15 cm from the soil. In the experiment 1 and 2 a treatment without N application was included to measure NH_3 background volatilization.

The experiment 3 was held from 27 November 2018 to 28 November 2019. The doses of N were divided into three applications, and applied on the same dates, similar to experiment 2. The experimental design was randomized blocks, with 4 repetitions, in a 3 × 4 × 4 factor scheme. Three N fertilizers were used: urea, ammonium nitrate, and ammonium sulfate, in four N doses: 0, 90, 180, and 270 kg N ha^{-1} $year^{-1}$, and four experimental cuts.

In the experimental 3, the pasture was managed in a cutting regime. Therefore, the experimental cuts refer to the cutting of forage, whenever the experimental plot reaches 30 cm in canopy height, with the cut being made to a residue of 15 cm from the soil. The management of pasture under a cutting regime at a height of 30 cm canopy (when marandu grass intercepts 95% of the light) and 15 cm of residue, refers to the most favorable growth pattern (crop growth rates, relative growth and net assimilation) for this cultivar.

2.3. Ammonia Volatilization Measurement

After the application of fertilizers in 21 days, evaluations of N losses by volatilization of NH_3 were carried out on the samples 1, 3, 5, 9, 14, and 21 days after application (DAA) of treatment, with the plots that did not receive fertilization as reference. Volatilized N quantification was performed according to the methodology described by Araújo et al. [23] and Longhini et al. [11]. NH_3 was captured by a semi-open chamber that consists of a transparent polyethylene terephthalate bottle (PET, soda bottle 2 L) with no bottom.

To absorb the volatilized NH_3, a foam strip (3 mm thick, 2.5 cm wide, and 25 cm long) was moistened in an acidic solution of 1 M H_2SO_4 ± 2% glycerin, and hung inside the flask, with its lower end inserted in a plastic jar (100 mL) containing the same solution of H_2SO_4. Samples were evaluated using ammonia analysis by steam distillation [24]. The amount of N recovered in the foam and plastic jar was multiplied by a factor of 1.74 calculated from the recovery of NH_3 (1/0.63), according to the calibration of the technique [23].

Ammonia emission for treatment in each sampling interval was calculated following Equation (1)

$$\text{Ammonia emission (\%)} = (NH_3\ \text{(treatment)} - NH_3\ \text{(soil background)})/(\text{N (applied)}) \quad (1)$$

where NH_3 (Treatment) is total cumulative NH_3 emission (% total N applied lost as NH_3) for the fertilizers treatments, NH_3 (Soil background) is total cumulative NH_3 emission (% total N applied lost as NH_3) for the air + soil + palisade grass without N addition, and N(applied) is N application in the area covered by the chamber (kg N ha^{-1}).

2.4. Forage Accumulation

The experimental area for forage evaluation was 40 plots of 12 m^2 (3 m × 4 m); using 6.0 m^2 of usable area, with 2 m plot spacing between plots. To begin the experiment, a uniform cut of the plots was made to 15 cm residue from the soil, on 27 November 2018. After standardization, the treatments were applied, and the experiment started. When the mean height of the plot reached 30 cm, a forage sample was manually removed at a residue of 15 cm using a 0.25 m^2 metal frame, and the rest of the plot was leveled at the same height.

The forage accumulation rate was calculated by dividing the accumulated dry mass (DM) by the number of days between evaluations (FAR, kg DM^{-1} day^{-1}), and the total forage accumulation was calculated through the sum of productions in all cuts (TFA, kg ha^{-1} $year^{-1}$).

2.5. Forage Chemical Composition

To determine the chemical composition of forage, the samples of the portion kept intact were oven-dried for 72 h at 55 °C weighed and ground in a 1 mm sieve in a mill (Thomas-Wiley Laboratory Mill Model 4, H. Thomas Co., Swedesboro, NJ, USA), and taken to the laboratory for analysis. The samples were submitted to dry matter (DM) analysis (AOAC 934.01), crude protein (CP) content was estimated using a LECO® FP 528 device (Leco Corporation, MI, USA) [25]. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the procedures described by ANKOM Technology [26].

2.6. Statistical Analysis

The data of NH_3 volatilization, forage accumulation, and chemical composition were submitted to analysis of variance (ANOVA). In experiments 1 and 2, the statistical model included effects of N fertilizers, doses, and their interaction according to the statistical model:

$$\text{Model 1: } \mu + ßi + \mathrm{F}j + \mathrm{D}k + (\mathrm{FD})jk + \varepsilon jki$$

In experiment 3, the statistical model included the effects of N fertilizers, doses, cuts, and interactions:

$$\text{Model 2: } \mu + ßi + \mathrm{F}j + \mathrm{D}l + \mathrm{C}j + (\mathrm{FD})kl + (\mathrm{FC})kl + (\mathrm{DC})kl + (\mathrm{FDC})kl + \varepsilon ijkl$$

where, μ was the mean; *ßi* were the effects of the blocks; F*j*, D*k* + (FD)*jk* were the effects of N fertilizers, dose, and dose and N fertilizers interaction, respectively; F*j*, D*l* and C*j* were the effects of N fertilizers, dose, and cut, respectively; and (FD)*kl* + (FC)*kl* + (DC)*kl* + (FDC)*kl* were the effects of interactions, while *εijkl* was the random error.

When significant, the N fertilizers and doses were compared by Tukey-HSD test at 5% probability and orthogonal polynomial contrasts, respectively. The analyses were conducted using the R program (Version 3.4.5).

3. Results

3.1. Environmental Factors

Meteorological data were measured throughout the experimental period; the distribution of rainfall after fertilization differed between experiments (Figure 1a–c). In experiment 1, the accumulated amount of rainfall was 138 mm, and the average air temperature varied from 21.1 to 26.1 °C (Figure 1a). There was no accumulated rainfall greater than 5 mm until the 8th DAA, an event that during the rainy season is considered veranico (i.e., lack of rainfall), according to Sansigolo [27].

In experiment 2, within 21 days of evaluation after each fertilization, the accumulated rainfalls were 39.6, 102.5, and 202.7 mm in the first, second, and third fertilization, respectively, and the average temperatures were 25.2. 25.8, and 23.6 °C, respectively (Figure 1b). In experiment 2 and 3, one veranico occurred after the first fertilization (from 2 December to 12 December 2018), while two veranico occurred between the second and third fertilizations (from 15 January to 24 January 2019, and from 27 January to 2 February 2019).

In experiment 3, the total annual rainfall was 994.8 mm, of which 39.6, 572.3, 157.2, 48.1, and 177.6 mm fell during the late spring of 2018, and summer, fall, winter, and spring of 2019, respectively. The average temperatures of those periods were 25.3, 24.7, 22.3, 21.6, and 25.2 °C (Figure 1c), respectively. The average annual rainfall during the experimental period was lower than the historical average in the period from 1956 to 2015, which was 1398 mm [28]. In cut 1, the period of growth until the cutting of forage included the months of December and January; the cuts 2 were made in February and March; March, April, and May in cuts 3, and May to November in cuts 4.

In the water balance of experiment 3, the water deficit and excess water values were 0 and 163 mm in November, −10 and 0 mm in December 2018, 0 and 0 mm in January, 0 and 171 mm in February, 0 and 0 mm in March, 0 and 0 in April, −16 and 0 mm in May, −29 and 0 mm in June, −32 and 0 mm in July, −53 and 0 mm in August, −27 and 0 mm in September, −58 and 0 mm in October and −20 and 0 mm in November 2019 (UNESP/FCAV Agroclimatologic Station, 2019).

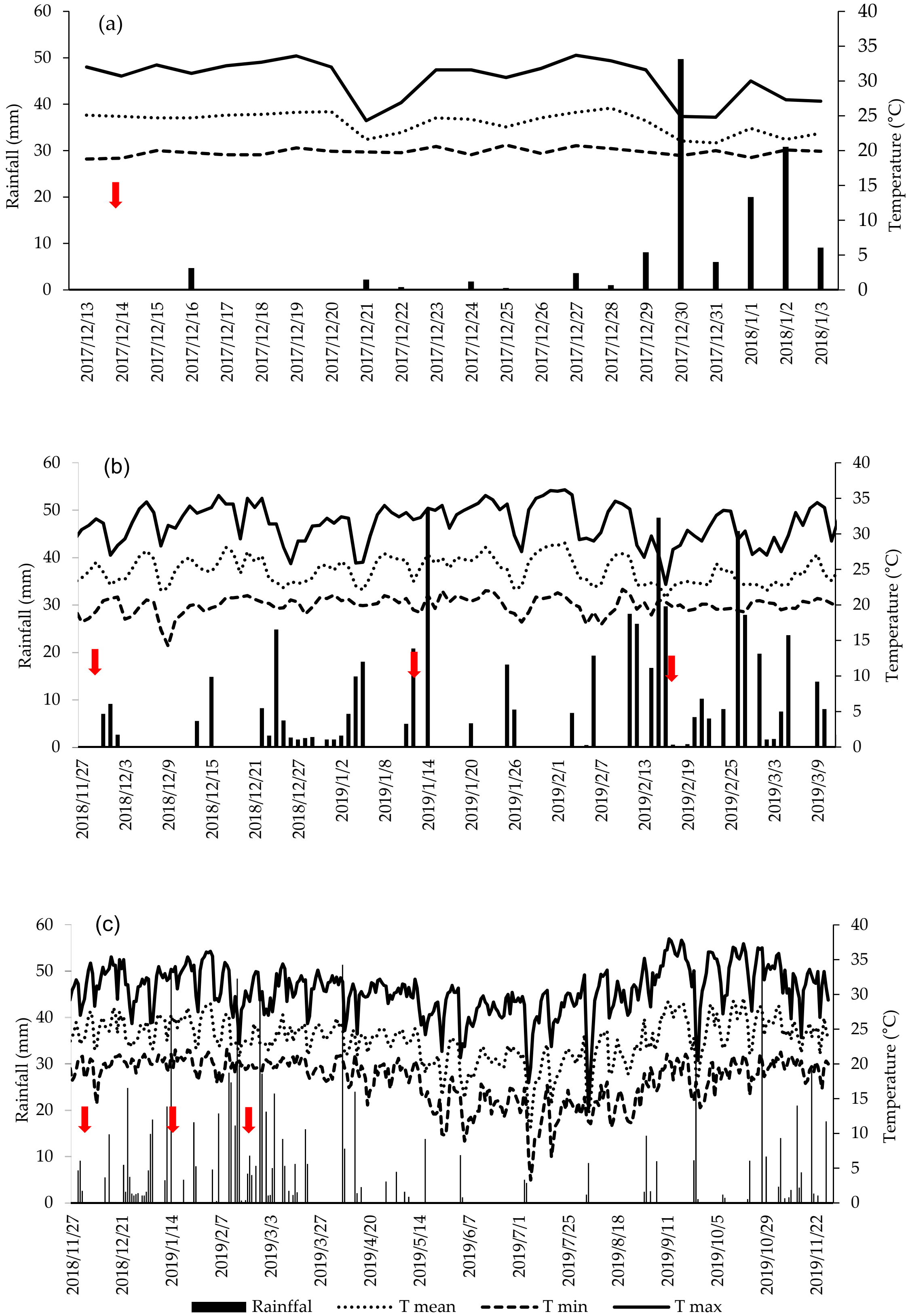

Figure 1. Daily air temperature (minimum, mean, and maximum T; °C) and daily rainfall (R; mm). Data from the Agrometeorological Station, Department of Exact Sciences, FCAV/UNESP. (**a**) Experiment 1, (**b**) experiment 2, and (**c**) experiment 3. Arrows indicate fertilization events.

3.2. Ammonia Volatilization

In both experiments, the NH_3 volatilization varied according N fertilizers ($p < 0.0001$) and nitrogen doses ($p < 0.0001$), and the interaction between fertilizers and doses had significant effect in experiment 1 ($p = 0.0003$) and experiment 2 ($p < 0.0001$) (Table 1). The N doses had linear effect on NH_3 volatilization in all fertilizers evaluated in experiment 1 ($p < 0.0265$) and experiment 2 ($p < 0.0025$).

Table 1. Emission factors (% N applied lost as NH_3) in marandu palisade grass pastures in fertilization with different N sources and doses in two experiments.

		Experiment 1	**Experiment 2**
N Fertilizers (F)	Doses (kg ha^{-1} year^{-1}) (D)	% NH_3	
Urea	90	13.92	6.30
	180	24.37	10.87
	270	44.58	24.10
	Mean	27.62	13.76
Ammonium nitrate	90	2.02	1.05
	180	3.17	2.58
	270	7.71	5.67
	Mean	4.30	3.10
Ammonium sulfate	90	2.22	0.78
	180	3.10	1.88
	270	5.27	3.65
	Mean	3.53	2.10
	SEM	0.04	0.03
	p-value		
	F	<0.0001	<0.0001
	D	<0.0001	<0.0001
	F × *D*	0.0003	<0.0001

Abbreviations: SEM, standard error of the mean. Experiment 1: N applied in a single dose. Experiment 2: N applied in three doses.

The decreasing sequence of the accumulated loss of N was: urea > ammonium nitrate > ammonium sulfate, in both experiments. When N fertilizers were applied in a single dose (experiment 1), the highest percentages of losses was observed in urea, which reached almost 45% of total N loss as NH_3 at a rate of 270 kg N ha^{-1} year $^{-1}$ (Table 1).

In experiments 1 and 2, the greatest NH_3 losses occurred until the 9th DAA, referring to the 4th collecting (Figure 2a–d), which gradually decreased until the 15th DAA; the losses were negligible after that period. In experiment 1, there was a similar pattern of daily NH_3 losses in all treatments, varying only in quantity of volatilized NH_3. The maximum peak of volatilization occurred on the 4th DAA. In experiment 2, peak emissions occurred in the 4th DAA in the first fertilization (Figure 2b); the peak was not observed in the second and third fertilization (Figure 2c,d).

The EF of NH_3 obtained in this study were differentiated according to N doses and sources, which ranged from 6.30% to 44.58% in urea, 1.05% to 7.71% in ammonium nitrate, and 0.78% to 5.27% in ammonium sulfate (Table 1).

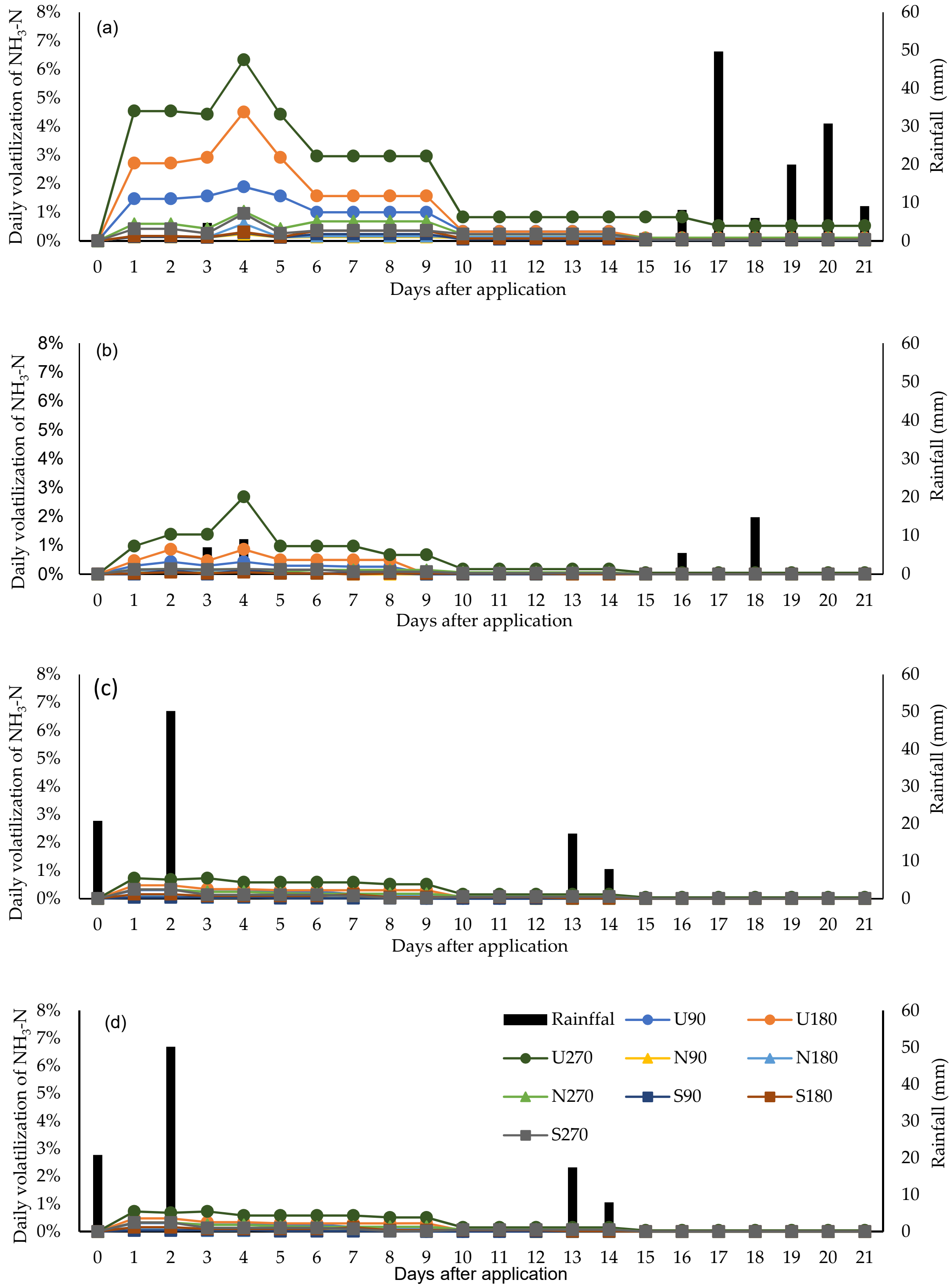

Figure 2. Daily ammonia volatilization in marandu palisade grass pastures under fertilization with fertilizers and doses of N. (**a**) experiment 1, single application (**b**) experiment 2, 1st fertilization, (**c**) experiment 2, 2nd fertilization, and (**d**) experiment 2, 3rd fertilization. U: urea, N: ammonium nitrate, S: ammonium sulfate.

3.3. Forage Accumulation

Cuts ($p < 0.001$), N fertilizers ($p < 0.001$), and N doses ($p < 0.001$) had a significant effect on FAR (Figure 3a–c). The FAR in ammonium sulfate was significantly higher by 36% than in urea (Figure 3a). The FAR had a linear increase in response to N application, so the treatments with 90, 180, and 270 kg N ha^{-1} increased the FAR by 46%, 102%, and 106%, respectively, in relation to the control treatment (Figure 3b), with a quadratic effect of the experimental cuts, with lower accumulation rates observed in cuts 1 and 4 (Figure 3c).

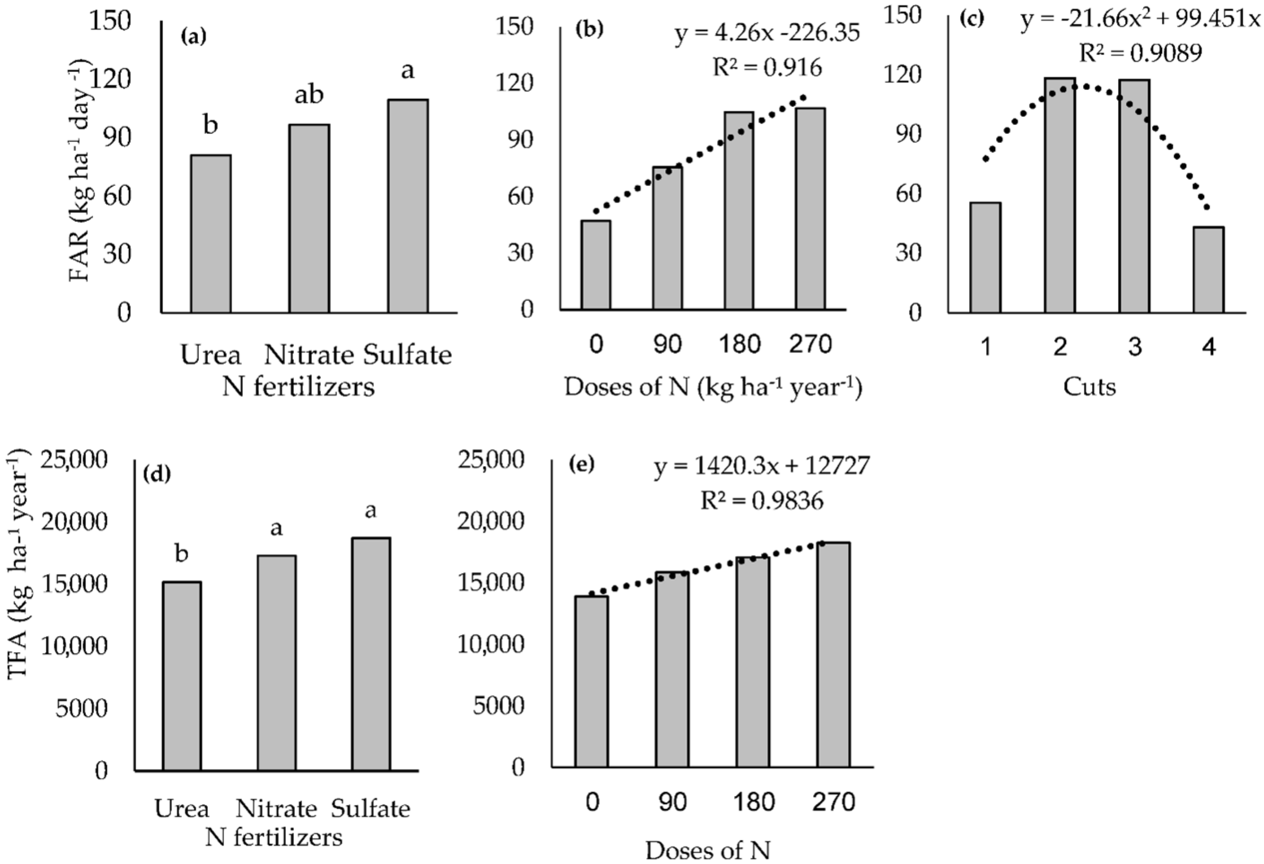

Figure 3. Forage accumulation rate (FAR, kgDM ha^{-1} day^{-1}) and total forage accumulation (TFA, kg ha^{-1} $year^{-1}$) of marandu palisade grass pasture in fertilization with N fertilizers and doses. (**a**) effect of N fertilizers on the FAR, (**b**) effect of doses of N on the FAR, (**c**) effect of cuts on the FAR, (**d**) effect of N fertilizers on the total accumulation, (**e**) effect of doses of N on the total accumulation.

TFA was affected by N fertilizers and doses ($p < 0.05$); the ammonium nitrate and ammonium sulfate provided significantly higher mean TFA of 17,299 and 18,713 kg DM ha^{-1} year, respectively, than urea, with a mean of 15,178 kg DM ha^{-1} $year^{-1}$ (Figure 3d). TFA had a positive linear increase in response to the N doses, with percentage increases of 14%, 23%, and 32% at doses 90, 180, and, 270 kg N ha^{-1}, respectively, compared to the control treatment (Figure 3e).

3.4. Chemical Composition of Forage

The effect of N fertilizers ($p = 0.001$) and doses ($p = 0.0027$) on CP content was dependent on the cuts; the means of cut 4 were the lowest, differing significantly from cut 2, which had the highest CP content (Table 2). There was a positive linear increase in CP content with N levels in cuts 1, 2, and 3. In cut 4, a cubic increase was observed (Table 2).

Table 2. Crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) of marandu palisade grass pastures in fertilization with different N fertilizers and doses.

Factors				
	Crude Protein (g kg^{-1} DM)			
Cuts	1	2	3	4
N fertilizers				
Urea	132.0 abcd	147.1 ab	121.9 cde	105.9 e
Ammonium Nitrate	142.1 abc	142.5 abc	130.5 bcd	111.1d e
Ammonium Sulfate	145.3 ab	153.2 a	140.4 abc	108.4 e
Doses of N (kg ha^{-1} $year^{-1}$)				
0	126.0	100.3	99.4	119.4
90	133.3	133.2	111.1	103.2
180	141.0	146.7	137.0	112.8
270	145.1	163.0	144.8	109.3
Mean	136.3	135.8	123.1	111.2
Effect	Linear	Linear	Linear	Cubic
	Neutral Detergent Fiber (g kg^{-1} DM)			
N fertilizers				
Urea	634.4 ab	634.4 ab	638.6 ab	657.8 a
Ammonium Nitrate	630.6 ab	625.5 ab	629.1 ab	644.9 ab
Ammonium Sulfate	634.3 ab	624.5 b	624.2 b	650.4 ab
Doses of N (kg ha^{-1} $year^{-1}$)				
0	643.3	658.0	657.3	601.1
90	638.4	641.8	656.2	643.8
180	633.5	621.0	619.0	653.3
270	627.4	621.6	616.7	655.9
Mean	635.6	635.6	637.3	638.5
Effect	Linear	Linear	Linear	Linear
	Acid Detergent Fiber (g kg^{-1} DM)			
N fertilizers				
Urea	305.4 a	288.0 abc	299.4 abc	295.4 abc
Ammonium Nitrate	302.7 ab	280.2 c	296.9 abc	285.6 abc
Ammonium Sulfate	297.0 abc	283.0 bc	290.6 abc	301.2 abc
Doses of N (kg ha^{-1} $year^{-1}$)				
0	301.7	298.3	299.5	263.5
90	302.8	286.2	299.4	280.8
180	300.5	278.4	292.2	304.9
270	301.7	286.5	295.3	296.6
Mean	301.7	287.4	296.6	286.4
Effect	NS	Quadratic	NS	Quadratic

Abbreviation: SEM, standard error of the mean. NS, not significant. CP: SEM = 7.9 (g kg^{-1} DM). NDF: SEM = 9.1 (g kg^{-1} DM). ADF: SEM = 3.9 (g kg^{-1} DM). Means not followed by the same letter differ based on Tukey-HSD test at 5% probability (comparisons between the 12 means).

The effect of N fertilizers and doses on NDF and ADF concentration were dependent on the cuts ($p < 0.01$). The N dose decreased linearly the NDF concentration in all cuts except in cut 4, which increased linearly. The N doses had a quadratic effect on ADF in cuts 2 and 4, with no significant effect in cuts 1 and 3. Within each experimental cuts, no significant difference was observed between the N fertilizers for the variables CP, NDF, and ADF.

4. Discussion

4.1. Ammonia Volatilization

In the interaction between N fertilizers and doses, there was an increasing linear effect of N doses on NH_3 losses by volatilization in both experiments, showing that the increase in amount of fertilizer applied to the soil favors losses by volatilization. According to Vlek and Stumpe [29], NH_3 loss was a first order reaction, which was directly related to N concentration in the soil solution. This reaction also explains the lower losses by volatilization observed in experiment 2, because the amount of NH_4^+ in the soil available for hydrolysis was less when the fertilization was done [11].

Reduction of volatilization losses observed in ammonium nitrate and ammonium sulfate occur essentially by the difference between the physical-chemical reactions of fertilizer volatilization. Higher losses were observed in urea, because, according to Fenn and Hosnner [30], fertilizers that produce ammonium carbonate ($(NH_4)_2CO_3$) were more susceptible to NH_3 loss. Hydrolysis of urea by the enzyme urease results in $(NH_4)_2CO_3$, which decomposes rapidly into bicarbonate (HCO_3^-), hydroxyl (OH^-), and ammonium (NH_4^+), and pH elevation due to increased HCO_3^- concentration [31].

The losses from volatilization of different fertilizers depend mainly on NH_4^+ and HCO_3^- concentrations, inducing an increase in pH; thus, due to the acidity of ammonium nitrate and ammonium sulphate, NH_4^+ ions remained stable and were not converted to NH_3, and the losses in these fertilizers were lower than in urea, which depends on soil alkalinity [30]. The significant reduction in NH_3 emissions from nitric and ammonia fertilizers was similar to that reported for sugarcane in tropical regions [32], ryegrass [33], and subtropical maize in Brazil [34].

In both experiments, the greatest losses occurred until the 9th DAA, referring to the 4th collection (Figure 2a–d), because of the higher concentration of ammoniacal nitrogen in soil solution [35]. In contrast, the difference in rainfall between fertilization events (Figure 1a,b) caused varied results in volatilization peak and accumulated NH_3 emissions. This was the main variable that regulated the volatilization potential in both experiments, demonstrating the large influence of climatic conditions on NH_3 emissions [35].

In experiment 1 and first fertilization in experiment 2, there was no occurrence of rain on the day of fertilization, which occasionally led to a peak volatilization on the 4th DAA, one day after the first rainfall, which were 4.7 and 7 mm respectively (Figure 2a,b). This was because under dry soil conditions, volatilization increases when a small amount of water of around 3 mm, was added to the soil [11,36]. According to Kissel et al. [37], low rainfall may stimulate urea volatilization, because it was not sufficient to incorporate the fertilizer. According to Sanz-Cobena et al. [36], adding 14 mm of water to the soil after fertilization can reduce volatilization losses by up to 89%, which justifies the absence of volatilization peaks in the second and third fertilizations of experiment 2, where there were rainfalls greater than 20 mm until the 2nd DAA (Figure 2c,d).

The mean EFs values in experiment 1 and 2 were 27.62% and 13.76% in urea, 4.30% and 3.10% in ammonium nitrate, and 3.53% and 2.10% in ammonium sulfate, respectively. According to the International Panel on Climate Change (IPCC) [38], the standard EFs for the respective fertilizers are 14.2%, 3.0%, and 9.5%, indicating that there was divergence between the EFs obtained under experimental conditions and the standard established by the IPCC, which was calculated in temperate climate.

Although there was a large database with estimates of NH_3 volatilization based on the types of N fertilizers and their uses, most of these studies were conducted under temperate and subtropical conditions [9,39,40]. Because NH_3 emissions after fertilizer application vary with soil type, pH, and temperature, inventories should assign emission factors for different fertilizers in different countries according to the prevailing soil and climate category in order to obtain reliable estimates of nitrogen fertilizer impact analysis for different crops.

Studies conducted in the state of São Paulo pointed out the differences between the EFs obtained in pastures and the IPCC default EFs. Cardoso et al. [13] obtained EF of 16.9% in Marandu grass pastures fertilized with 80 kg of N from urea and at similar conditions Longhini et al. [11] found EFs varying from 11.2% to 20.5% (mean 14.8%), While Morais et al. [17] reported a mean EF of 49% in elephant grass (*Pennisetum purpureum* Schum.) fertilized with 80 and 100 kg of N ha^{-1} from urea. Thus, the results of this study are important for the development of specific EFs for gas emissions according to the climatic conditions and characteristics of ruminant production systems, especially in tropical regions.

4.2. Forage Accumulation

Compared to urea, higher FAR (Figure 3a) and TFA (Figure 3d) observed in ammonium sulfate was due to the intrinsic factors of this source, since plants treated with fertilizers containing sulfur (S) use N more efficiently, and the balance between the amounts of N and S has a direct impact on forage growth [41,42]. De Bona et al. [43] highly recommended the use of fertilizers containing NH_4^+ and NO_3^- for cultivation of *U. brizantha* or similar species due to increased absorption of N and S, improving growth, nutritional value, and dry matter accumulation. We observed higher FAR (Figure 3b) and TFA (Figure 3e) with the increase in the amount of fertilizer used which was expected when N demands of grasses was met, since N fertilization is known to increase plant productivity and growth [2,6,7].

The effect of cuts in the FAR (Figure 3c) is due to the rainfall pattern throughout the experiment, which can be exemplified by the water balance, for this variable, we observed that cuts 2 and 3, which had the highest FAR, were performed between February and May, which corresponded to the period with better water conditions, with greater water excess and low water deficit; while the opposite was observed for cuts 1 and 4, which, consequently, had the lowest FAR. According to Lima et al. [44], the availability of water in the soil has a significant effect on the rate of development of *Urochloa* forage, with less plant development being observed in the period of lower precipitation and lower temperatures, corroborating the results of the lowest FAR in the cut 4, which was performed in the coldest months and with greater water deficit.

4.3. Chemical Composition of Forage

The CP, NDF and FDA contents were affected by the interaction between N fertilizers, N doses and cuts (Table 2). It was observed that the effect of fertilization was dependent on climatic conditions, mainly rainfall and temperature, resulting in high availability of forage of good nutritional value during rainy season, and decrease in quantity and quality during dry season, as noted by other authors [44,45].

It should be noted that, in addition to the amount of nutrient in the soil, intrinsic aspects of forage species, management factors and environmental factors also regulate the chemical composition of the plant [46,47]. In the tropics, temperature and water deficit are the main limiting factors for forage production [2,21,33], thus environmental fluctuations modify the morphology, development rate and alter the chemical composition of the plant.

The effect of N doses on the linear increase in CP contents and on the linear decrease in NDF contents in cuts from 1 to 3 are due to the more favorable conditions for plant development in the cutting period (December to May), where it is observed higher temperatures, higher rainfall occurrence (Figure 1) and low water deficit. On the contrary, in cut 4 (May to November), all the evaluation months had water deficit and days with minimum temperatures below 15 °C. Similar results were reported by Campos et al. [48], where N fertilization caused linear increase in CP content and linear decrease in NDF content, stimulating cell wall formation with better quality fibers, resulting to greater digestibility of the forage, depending on the climatic conditions.

The temperature regulates the meristematic activity and, in effect, the plant growth, the ideal temperature for the growth of tropical grasses varies from 30 to 35 °C while from 10 to 15 °C photosynthesis decreases and the growth practically ceases [49]. Likewise, rainfall is a key factor for grass development, considering that N is a nutrient absorbed by mass flow [21], rainier months, which consequently increase the amount of water in the soil, favor the absorption of this nutrient by the plant.

Nitrogen fertilization in periods with favorable climatic conditions promotes significant increases in dry matter yield [44,45] and affects the chemical composition and nutritional value of pastures [2,48] as observed in this work. Climatic factors also impact the morphological and chemical composition of the plant. During the period of better plant growth, N fertilization promotes acceleration in tissue renewal and an increase in the proportion of leaves, due to N increasing the elongation rate and leaf area of grasses [50].

Environmental stresses such as water deficit and low temperatures cause growth arrest and death of the aerial part of forages, as a result of the lack of organ and tissue renewal, there is an increase in structural compounds characterized by the fiber and, in parallel, a decrease in cell content levels, as protein. These factors explain the higher NDF and lower CP contents in cut 4 (Table 2). The environmental conditions did not intensely impact the ADF levels, which had smaller variations in the mean, while the effect of fertilization with N on this variable is diverse, being able to increase [46], decrease [51], cause a quadratic effect [50] or have no effect [6,8].

5. Conclusions

This study is new in reporting the agronomic and chemical variables of forage and the losses due to volatilization of NH_3 as a function of the main commercial sources of N in tropical pastures. Our results reveal that plant responses and N losses in the system vary not only due to the amount and type of fertilizer applied, but mainly due to climatic fluctuations related to precipitation and temperature. The NH_3 emission factors of the fertilizers differed from the IPCC standards, being on average 68% lower in ammonium sulphate, and 97% higher in urea with single fertilization, so that the IPCC standard EFs show little adequate depending on of fertilization management in tropical pastures. As a fertilization strategy for lower volatilization losses and higher forage productivity, we suggest fertilization in the rainy season and the use of ammonium sulfate fertilizer.

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