



Grazing intensity and seasonality regulate N cycling via litter and excreta in tropical grass–legume pastures

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Abstract Integrating forage legumes into grasslands reduces the need for N fertilization, but its effectiveness depends on grazing management. This 2 years study evaluated N cycling in *Urochloa brizantha* and *Arachis pintoi* pasture under severe (10 cm stubble height), moderate (15 cm stubble height), and light (20 cm stubble height) grazing intensities. Nitrogen cycling from litter and livestock excreta were assessed. Moderate grazing decreased in 243 kg ha⁻¹ existing litter, but increased legume proportion in the litter (13.4%) compared to severe (7.8%). Pre-grazing legume mass was lower under moderate grazing than under light grazing by 785 kg ha⁻¹, and post-grazing legume mass under light grazing was 57% greater than the mean value observed under moderate and

severe grazing. Moderate grazing led to a lower litter deposition rate but reduced C/N ratios in both existing and deposited litter compared to light grazing. Nitrogen intake was 73% greater in the rainy season than in the dry season. Furthermore, severe grazing increased N intake and excretion by 47.7 kg ha⁻¹ season⁻¹ due to the higher stocking rate associated with this treatment. Nitrogen cycled from litter was greatest under light grazing during the dry season but peaked under severe grazing in the rainy season (+41 kg ha⁻¹ season⁻¹). Mixed pastures should be managed with a pre-grazing height of 24–30 cm and a stubble height of approximately 15 cm to improve N cycled via litter with lower C:N ratio and greater fecal over urinary N excretion, potentially reducing N losses and improving nutrient recycling efficiency.

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Introduction

Nitrogen (N) is a crucial component of nucleotides and proteins, forms the backbone of chlorophyll, and is one of the primary macronutrients for plants, and yet it is one of the most limiting nutrients for plant growth (Moreau et al. 2019). In a tropical pasture, the N moves through several pathways, both biological and chemical (Russelle 1992). The balance between nutrient inputs and outputs in a grazing system impacts its long-term sustainability. The inputs come from fertilization, biological N fixation, atmospheric deposition, feeds, and supplements. Nutrients can leave the grassland ecosystem through various pathways, including the export of animal products like beef, milk, and wool, or losses through processes such as ammonia volatilization, denitrification, leaching, and runoff (Dubeux and Sollenberger 2020). Besides the inputs and outputs, some transformations can make the nutrients unavailable for certain periods. Once nutrients are absorbed by grassland plants, they can return to the soil mainly through two pathways: litter deposition or livestock excreta. Forages consumed by cattle return nutrients via excreta, while ungrazed, senescent forages return nutrients via litter (Dubeux and Sollenberger 2020).

Understanding N cycling in grazed ecosystems requires a comprehensive evaluation of all major N pools and fluxes. Although grazing removes substantial aboveground biomass, total plant N pools may remain stable because grazing commonly increases herbage N concentrations, which compensates for the biomass loss (Wang et al. 2016). Such increases in tissue N concentration arise from greater soil N availability driven by manure deposition, enhanced mineralization, shifts in plant functional composition, and faster nutrient turnover (Biondini et al. 1998; Wang et al. 2016; Dubeux and Sollenberger 2020).

Inadequate management of herds, vegetation, and soil in both natural and planted grasslands can reduce productivity and degrade grazing land. This is typically linked to overgrazing, insufficient weed and pest control, and a lack of fertilization (Boddey et al. 2004; Feltran-Barbieri and Féres 2021). The

use of inorganic N fertilizers is an option to overcome reduced productivity. However, there are some environmental and economic concerns regarding their use in grazing lands, since their production and application depend on a great amount of energy and generate considerable amounts of greenhouse gas (GHG) emissions (Sabry 2015). Thus, the use of forage legumes emerges as an alternative to inorganic N fertilizers to increase the amount of forage and N in grazing lands (Homem et al. 2021b; Pereira Neto et al. 2024). Over the past decade, research has highlighted the benefits of optimal legume management in tropical and subtropical regions for improving pasture and animal performance, as well as N cycling. For instance, studies employing rotational stocking systems with moderate defoliation intensities (e.g., 30–50% biomass removal to retain sufficient leaf area for photosynthesis and N fixation) have shown increased forage yields and livestock weight gains in legume-grass mixtures (Gomes et al. 2018, 2020; dos Santos Oliveira et al. 2023).

Grass-legume mixed pastures produce a better-quality litter with a shorter half-life time (Sollenberger et al. 2019). The N cycling in a grazing system is influenced by the grazing management practices adopted (Gomes et al. 2020; Depablos et al. 2021). For instance, in a rotational stocking, the amount of litter returned to the soil depends on the stocking rate, with greater amounts returning under light grazing intensities. The nutrient return is determined by the amount of deposited litter, its composition, and decay rates (Dubeux and Sollenberger 2020). Conversely, N returned via livestock excreta tends to be lower and more spatially heterogeneous. Gomes et al. (2020) reported that in a rotational stocking system of palisade grass (*Urochloa brizantha*) and forage peanut (*Arachis pintoï*) managed between 90 and 95% of light interception, the N cycling via litter increased due to a greater amount of forage peanut in the litter and a lower forage removal rate. Similar results were also observed by Depablos et al. (2021) for pastures of palisade grass and calopo (*Calopogonium mucunoides*).

Although the effects of grazing intensity on N cycling have been widely documented in tropical environments (Boddey et al. 2004; Wang et al. 2019), and the impacts of legume inclusion and grazing frequency on tropical grass pastures have also been explored (Gomes et al. 2020; Depablos et al. 2021;

Homem et al. 2021c; Borré et al. 2025), the combined effects of grazing intensity and mixed pastures on N cycling in tropical systems have not yet been studied. Grazing can increase surface soil N pools—through manure deposition, enhanced mineralization, and shifts in plant community composition—while simultaneously reducing N stored in plant litter due to litter removal and faster decomposition. These contrasting effects highlight the tight coupling of carbon (C) and N cycles, whereby increased belowground C inputs can stimulate long-term soil N accumulation (Han et al. 2008; Wang et al. 2016).

In tropical grass–legume systems, such as palisade grass–forage peanut pastures, these dynamics may be further influenced by the contrasting growth patterns, litter quality, and N inputs from biological fixation (Gomes et al. 2020; Depablos et al. 2021; Homem et al. 2021c). Therefore, quantifying plant litter, biomass, and excreta in grazing studies is critical to capture the redistribution of N among ecosystem compartments, assess nutrient returns via dung and urine, and elucidate the mechanisms through which grazing intensity regulates nutrient cycling and soil fertility.

Accordingly, evaluating N cycling dynamics in rotationally stocked palisade grass–forage peanut pastures under varying grazing intensities is necessary to understand how forage intake, livestock excreta, and litter contributions from both species shape N cycling pathways and overall ecosystem functioning. We hypothesize that increasing grazing intensity would favor the N cycling through livestock excreta and reduce litter deposition. We aimed to determine which range of grazing intensity would combine balanced N cycling through excreta and litter in a mixed pasture composed of palisade grass and forage peanut.

Material and methods

Experimental site

All experimental procedures were approved by the Ethics and Animal Welfare Committee of the Federal University of Lavras (protocol number 026/2019). The study was carried out at the Experimental Farm of the Federal University of Lavras, Brazil (21°14'S, 45°00'W; 918 m above sea level). The area presents a

subtropical humid mesothermal climate with dry winters [Köppen-Geiger climate classification: Cwa; (de Sá Júnior et al., 2012)]. Meteorological data were obtained from a weather station located 1,000 m from the experimental area (Supplementary Fig. 1).

The pasture used was established in December 2006 by joint seeding of palisade grass [*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster cv. Marandu (syn. *Brachiaria brizantha*)] and forage peanut (*Arachis pintoi* Krapov. & W.C. Greg. cv. BRS Mandobi). The seeding rates were 7.6 and 7.2 kg ha⁻¹ of pure live seed for palisade grass and forage peanut, respectively.

The site was managed for experimental purposes until 2015. Since then, it had been mostly idle, occasionally grazed, and used to balance stocking rates in other experiments. Before establishing the present trial, herbage mass was measured and averaged 8,877 kg ha⁻¹.

A 2-yr experimental period (from September 2019 to May 2021) was adopted. The soil in the area is a Ferralsol (WRB/FAO classification). Soil texture is clayey (55.1% clay, 6.8% silt, and 38.1% sand) and had the following properties at the start of the experiment: pH_(H2O) = 5.6, OM = 45.8 g kg⁻¹, P = 2.8 mg kg⁻¹, K⁺ = 69.4 mg kg⁻¹, Ca²⁺ = 382.7 mg kg⁻¹, Mg²⁺ = 63.2 mg kg⁻¹, and cation exchange capacity = 57.1 cmolc kg⁻¹. Thirty days before the experiment started, agricultural gypsum (1.39 t ha⁻¹) and limestone (278 kg ha⁻¹) were applied. Yearly, at the beginning of the spring, fertilizers were applied as single superphosphate (17.5 kg of P ha⁻¹), and potassium chloride (44 kg of K ha⁻¹).

Treatments and experimental management

The grazing management treatments were three levels of grazing intensity: severe (10 cm of stubble height), moderate (15 cm of stubble height), and light (20 cm of stubble height). A rotational stocking system was implemented, with each experimental unit being grazed for nine days, followed by a period of regrowth that ranged according to the treatment (Supplementary Table 1). The pasture was divided into 12 experimental units, each further split into three equally sized paddocks (see Supplementary Fig. 2; Rodrigues da Cruz et al. 2024). This setup resulted in replicate sizes of 1080 m² for light grazing, 660 m² for moderate grazing, and 480 m² for intense grazing.

Grazing started when the canopy reached a height of 25 ± 1 cm, corresponding to 90–95% light interception (Gomes et al. 2018). A minimum of two purebred Tabapua heifers, initially weighing 210 ± 20 kg, were used for grazing trials. Canopy height was monitored daily, and additional heifers were introduced to meet the stubble height target within three days (using a put-and-take technique; Allen et al. 2011).

Because the total experimental area was limited (0.8 ha), paddock sizes differed among grazing intensities. For comparability with other studies, stocking rates (SR) are expressed on a per-hectare basis; however, these values do not represent the actual number of heifers physically present on one ha. Stocking rate for each treatment and season was calculated as the total animal units (AU; 1 AU=500 kg BW) present during the grazing days divided by the paddock area and the total area required to complete one full grazing cycle (grazing + regrowth). All paddocks began with a base stocking of two tester heifers, and additional animals were introduced or removed using the put-and-take technique (Allen et al. 2011) to reach the target stubble height within three days. Because regrowth intervals varied among grazing intensities, the SR values reported in Table 4 represent the mean AU ha⁻¹ averaged across all cycles within each season, rather than instantaneous animal numbers per hectare.

During the dry season, only one grazing cycle was carried out to decrease the stubble height of all paddocks to 15 cm before initiating the second grazing year. This management was performed to remove the excessive dead material, allow radiation to get to the base of the canopy, and stimulate tillering (Costa et al. 2016).

Experimental evaluations

Herbage characteristics

Canopy height was measured using a sward stick (Barthram 1985) at 80 randomly selected points within each paddock, both before and after grazing. In mixed pastures managed based on canopy characteristics rather than individual species, these measurements reflect the overall canopy structure, integrating contributions from both grass and legume components, and were averaged to represent the pre- and post-grazing canopy heights

of each paddock. Herbage mass was sampled using three square frames (0.5×0.5 m) per experimental unit at ground level, once per grazing cycle in the pre- and post-grazing condition (see Supplementary Fig. 2). Following forage harvest, the botanical composition was analyzed by manually separating grass from legumes. All samples were oven-dried at 55 °C for 72 h until they reached a constant weight. The herbage mass (kg ha⁻¹) was calculated as the total of both grass and legume mass.

Litter dynamics

Existing and deposited litter were measured every 14 days throughout the experiment. Prior to the first grazing cycle, three square frames (0.5×0.5 m) were randomly placed in each experimental unit, with the corners marked by wooden stakes for accurate sampling of deposited litter. Litter was defined as all detached dead plant material accumulated on the soil surface (Allen et al. 2011). The initial sample was considered the existing litter (Rezende et al. 1999). Every 14 days, deposited litter was collected from the same locations where the previous existing litter samples were taken. The frames were repositioned and marked again, and new existing litter samples were collected. Deposited litter was calculated as the total amount of litter collected throughout the season.

All samples were oven-dried at 55 °C for 72 h until they reached a constant weight, then ground through a 1-mm screen using a Cyclotec mill (Tecator, Herson, VA). Dry matter (DM) concentration was determined by further drying the samples at 100 °C for 16 h (method 934.01: AOAC, 2000). Ash concentration was measured by incinerating the samples in a 600 °C muffle furnace for 4 h (method 942.05: AOAC, 2000). Data were reported on an organic matter (OM) basis to account for mineral effects on nutrient concentration, with nutrient ratios expressed per kg of OM.

The decomposition constant was calculated according to the following equation, described by Rezende et al. (1999):

$$k = [\ln(Le(n-1) + Ldn) - \ln(Le_n)]/t$$

where $Le_{(n-1)}$ is the amount of existing litter in the previous cycle, Ld_n is the amount of deposited litter in the current cycle, Le_n is the existing litter in the current cycle, and t is the cycle time (d). The half-time

($t^{1/2}$) was estimated according to the following equation (Rezende et al. 1999):

$$t^{1/2} = \ln(2)/k$$

where k was the decomposition constant ($\text{g m}^{-2} \text{d}^{-1}$). Litter disappearance was estimated by the equation (Rezende et al. 1999):

$$\text{Litter disappearance} = Le(n - 1) + Ldn - Len$$

The proportion of grass and legume in the litter samples was estimated according to the ratio of natural ^{12}C and ^{13}C isotopes by the equation:

$$\% \text{legume} = 100 \times (\delta^{13}\text{C}_G - \delta^{13}\text{C}_S) \div (\delta^{13}\text{C}_G - \delta^{13}\text{C}_L)$$

where % legume is the proportion of carbon (C) from a legume in the litter sample, and $\delta^{13}\text{C}_G$, $\delta^{13}\text{C}_L$, and $\delta^{13}\text{C}_S$ are the values of $\delta^{13}\text{C}$ abundance of the dead material from the grass (-12.98‰), legume (-27.28‰), and the litter samples, respectively. For the analysis of $\delta^{13}\text{C}$, C and N concentration, samples were ground to a fine powder in a roller mill. Sub-samples containing between 300- to 500 μg C were analyzed for total C and ^{13}C abundance using an automated continuous-flow isotope-ratio mass spectrometer consisting of a Finnigan DeltaV mass spectrometer coupled to the output of a Carlo Erba EA 1108 total C and N analyzer (Finnigan MAT).

Herbage intake and nutritive value

Forage samples were hand-plucked for N analysis following the method of Vries (1995). Samples were collected separately for each species on the first and third days, both pre- and post-grazing. After collecting, the samples were oven-dried at 55°C for 72 h, then ground through 2 mm and 1-mm screens using a Cyclotec mill (Tecator, Herndon, VA). Dry matter (DM) and ash concentrations were measured as previously described for litter samples. Crude protein (CP) concentration was calculated from the N content using the formula $\text{CP} = \text{total N} \times 6.25$, with N determined by the Kjeldahl method (method 920.87: AOAC, 2000).

Titanium dioxide (TiO_2) was used as an external marker to estimate fecal production (Titgemeyer et al., 2001). Daily, 10 g of TiO_2 per animal was administered directly into the rumen using a flexible

garden hose as a probe for nine consecutive days: six days for adaptation and three days for collection. Fecal samples were collected daily at varied times (6:00 am, 12:00 pm, and 6:00 pm) over the three-day collection period, and composite samples were prepared for each animal (see Fig. Supplementary Fig. 2). During sampling days, heifers were restrained in a chute, and feces were collected directly from the rectum. Fecal samples were oven-dried at 55°C for 72 h to determine DM concentration, then air-equilibrated and ground through 2-mm and 1-mm screens. Titanium dioxide concentration in the fecal samples was analyzed following Myers et al. (2004).

The TiO_2 mass (mg) in each fecal sample was calculated based on absorbance readings using a standard calibration curve. The concentration of TiO_2 was first expressed on an air-dry basis (ASA) and subsequently corrected to a dry matter basis (DM) at 105°C using the following equations:

$$[\text{TiO}_2]_{\text{ASA}} = \frac{M_{(\text{TiO}_2)}}{\text{ASA}} \times 100$$

$$[\text{TiO}_2]_{\text{DM}} = \frac{[\text{TiO}_2]_{\text{ASA}}}{\% \text{DM}} \times 100$$

The fecal output (kg DM day^{-1}) was calculated based on the ratio between the amount of TiO_2 supplied and its concentration in feces, according to the following equation:

$$\text{Fecal production (kg DM day}^{-1}\text{)} = \frac{10}{([\text{TiO}_2]_{\text{DM}}/100)} \times \frac{1}{1000}$$

where 10 represents the daily TiO_2 dose (g day^{-1}), $[\text{TiO}_2]_{\text{DM}}$ is the TiO_2 concentration in fecal dry matter (%), and the division by 1000 converts grams to kilograms.

For the estimation of indigestible neutral detergent fiber (iNDF), fecal and hand-plucked forage samples, ground through a 2-mm screen, were incubated in the rumen for 288 h, using two cannulated cows grazing on a mixture of palisade grass and forage peanut (Huhtanen et al., 1994). The iNDF was used as an internal marker to estimate forage intake and the contribution of each component (grass and legume) in the diet. The proportion of palisade grass and forage peanut in the iNDF residual fecal samples was quantified using $\delta^{13}\text{C}$ isotopes by the equation:

$$\%legume = 100 \times (\delta^{13}CG - \delta^{13}CS) \div (\delta^{13}CG - \delta^{13}CL)$$

where %legume is the proportion of C from a legume in the iNDF residual fecal samples (Lopes de Sá, 2017) and $\delta^{13}C_G$, $\delta^{13}C_L$, and $\delta^{13}C_S$ are the values of $\delta^{13}C$ abundance of the iNDF residual in hand-plucking samples of the grass (−12.97‰), legume (−28.34‰), and the iNDF residual in fecal samples, respectively. These samples were processed as described above for litter samples.

Fecal excretion was used to estimate the total amount of iNDF excreted daily, in this way, it was possible to obtain the iNDF intake. After that, iNDF concentrations from hand-plucked samples of each component were used to estimate forage intake. Total forage intake was calculated as the sum of grass and legume intakes, according to their proportions in the diet. Nitrogen intake ($\text{g animal}^{-1} \text{d}^{-1}$) was estimated by multiplying the grass and legume intake by the N concentration from hand-plucking samples of each plant species. Total N intake ($\text{g animal}^{-1} \text{d}^{-1}$) was calculated as the sum between N intake derived from grass and legume, respectively.

N excretion

The N excretion was determined by summing the total N excreted in the feces and urine per animal. To achieve this, the diet components, as well as fecal and urine samples, were analyzed for N concentration using the method 920.87 from AOAC (2000). To determine the fecal N excretion, the fecal production was multiplied by the total N concentration in the feces.

Spot sampling was used to assess the excretion of urinary nitrogenous compounds (Valadares et al. 1999). Spot urine samples were obtained by vulval stimulation at the same time as fecal sample collection. A 16 mL aliquot was taken, and 4 mL of 0.002% sulfuric acid (H_2SO_4) was added. A 3-d composite sample was created and stored in a plastic flask at -20°C . Urinary creatinine concentration was determined using a commercial kit (Creatinine K016, Bioclin, Belo Horizonte, Brazil). Urine volume (L d^{-1}) was estimated using creatinine concentration as a marker and assuming a daily creatinine output according to equation (Silva et al. 2012):

$$UV(\text{Ld}^{-1}) = (0.0345 \times BW^{0.9491}) \div UCC$$

where UV (L d^{-1}) is daily total urinary production, BW (kg) is body weight, and UCC (g L^{-1}) is urine creatinine concentration. Urinary N excretion was calculated using urinary volume and total N concentration in these samples. Urine and feces were collected during each grazing cycle and then pooled by season.

The N intake was obtained based on the value of forage intake by the animals and the total N concentration found in the hand-plucking samples. The N cycling ($\text{kg ha}^{-1} \text{season}^{-1}$) was calculated by summing deposited litter and the excreta from feces and urine per hectare per season.

Statistical analysis

The experimental design was entirely randomized with three treatments (grazing intensities), four replications, and repeated measurements over time (seasons of the year). Data were analyzed using the mixed model method (Littell et al., 2000), performed by the MIXED procedure of SAS (SAS Institute, Cary NC). The effects of grazing intensity and season were considered fixed effects, and year and replicate as random effects. The Akaike information criterion was used to choose the best (co)variance structure (Akaike, 1974). All variance components were estimated using the restricted maximum likelihood method. The treatment averages were estimated using the LSMEANS statement and compared using Student's T-test with $P \leq 0.10$. The statistical model for data analysis was as follows:

$$Y_{ijk} = \mu + GI_i + \gamma_i + Y_j + S_k + (GI \times S)_{ik} + \epsilon_{ijk}$$

where Y_{ijk} = value observed in the i th GI of the k th season of the j th year; μ = overall average; GI_i = fixed effect associated with i th grazing intensity, $i = 1, 2, 3$; γ_i = random error of i th GI nested with replication; Y_j = random effect associated with j th year, $j = 1, 2$; S_k = fixed effect associated with k th season, $k = 1, 2$; $(GI \times S)_{ik}$ = fixed effect of interaction of i th GI with the k th season. ϵ_{ijk} = random error associated with the i th GI, the k th season, the j th year.

Table 1 Herbage characteristics of mixed pastures of palisade grass and forage peanut as affected by grazing intensities and seasons

Item	Grazing intensity (GI)			Seasons (S)		SEM ^a	P-value		
	Light	Moderate	Severe	Dry	Rainy		GI	S	GI×S
Herbage mass, kg ha ⁻¹	8,097	7,307	8,117	7,976	7,705	817	0.362	0.518	0.756
Grass mass, kg ha ⁻¹	5,698	5,693	6,205	6,369	5,362	525	0.712	0.025	0.993
Legume mass, kg ha ⁻¹	2,399 a	1,614 b	1,912 ab	1,607	2,343	464	0.020	0.007	0.351
Herbage mass, kg ha ⁻¹	6,900 a	4,977 b	4,899 b	5,064	6,121	349	<0.001	0.026	0.718
Grass mass, kg ha ⁻¹	5,149 a	3,980 b	3,889 b	4,067	4,612	287	<0.001	0.155	0.913
Legume mass, kg ha ⁻¹	1,751 a	997 b	1,010 b	997	1,509	386	<0.001	0.011	0.637

Means followed by different letters in the row differ at $P \leq 0.10$ by Tukey's test. Values are least squares means ($n = 4$ replications per treatment)

^aStandard error of the means

Results

Herbage characteristics

The pre-grazing legume mass (kg ha⁻¹) was affected only by grazing intensity and season ($P \leq 0.020$; Table 1). Moderate grazing intensity was 785 kg less pre-grazing legume mass than light intensity, but they do not differ from severe. However, the pre-grazing herbage and grass mass did not differ among grazing intensities, the average were 7,840 and 5,865 kg ha⁻¹, respectively ($P \geq 0.362$), even being managed with the same average pre-grazing canopy height

(25 ± 1 cm). Post-grazing herbage mass was greater for light intensity compared to moderate and severe ($P < 0.001$), and it was 1,057 kg ha⁻¹ greater during the rainy season compared to the dry season ($P = 0.026$). In the post-grazing condition, the grass mass under light grazing intensity was 29% greater than moderate and severe ($P < 0.001$). Legume mass post-grazing followed the same pattern and was 57% greater under light intensity than the average of moderate and severe ($P < 0.001$). The greatest legume mass in the pre- and post-grazing conditions was recorded during the rainy season ($P \leq 0.011$).

Table 2 Dynamics of the existing litter of mixed pastures of palisade grass and forage peanut as affected by grazing intensities and seasons

Item	Grazing intensity (GI)			Seasons (S)		SEM ^a	P-value		
	Light	Moderate	Severe	Dry	Rainy		GI	S	GI×S
Total existing litter, kg OM ^b ha ⁻¹	1,371 a	1,154 b	1,423 a	1,576	1,055	219	0.011	<0.001	0.939
Grass existing litter, kg OM ha ⁻¹	1,224 a	1,000 b	1,312 a	1,387	958	217	0.007	<0.001	0.809
Legume existing litter, kg OM ha ⁻¹	147 b	154 a	111 c	189	97.1	9.1	0.003	0.011	0.908
$\delta^{13}\text{C}$ existing litter, ‰	-14.5 a	-14.9 b	-14.1 a	-14.7	-14.3	0.3	0.004	0.041	0.707
% legume in existing litter ^c	10.7 ab	13.4 a	7.8 b	12.0	9.2	1.9	0.045	0.089	0.661
Existing litter N concentration, g kg ⁻¹	7.0	7.9	7.3	7.3	7.6	0.43	0.134	0.224	0.373
Existing litter C concentration, g kg ⁻¹	338 ab	334 b	347 a	344	335	5.8	0.087	0.103	0.211
Existing litter C/N ratio	48.1 a	41.9 b	47.2 a	47.1	44.1	3.3	0.018	0.036	0.357
Existing litter N stock, kg ha ⁻¹	9.6	9.2	10.4	11.5	8.0	1.8	0.530	<0.001	0.672

Means followed by different letters in the row differ at $P \leq 0.10$ by Tukey's test. Values are least squares means ($n = 4$ replications per treatment)

^aStandard error of the means

^bOrganic matter

^cCalculated from ^{13}C abundance of the grass (-12.98‰) and legume (-27.28‰) dead material

Litter dynamics

The total and grass existing litter (kg of OM ha⁻¹) under moderate grazing intensity were 243 and 268 kg less than the average of light and severe intensities ($P \leq 0.011$; Table 2). The total and grass existing litter were 49.3 and 44.7% greater during the dry season than during the rainy season ($P < 0.001$). Moderate grazing intensity resulted in a significantly lesser $\delta^{13}\text{C}$ (‰) in the existing litter ($P = 0.004$), which corresponded to greater existing litter from legume for this treatment ($P = 0.003$). The legume in the existing litter was 92 kg OM ha⁻¹ greater during the dry season than in the rainy season. Even with greater legume proportion in the existing litter under moderate grazing compared to severe (13.4 vs 7.8%), the N concentration was not different among grazing intensities ($P = 0.134$) and averaged 7.4 g kg⁻¹. However, there was a difference in C concentration in the existing litter, which was 13 g kg⁻¹ less for moderate grazing compared to severe ($P = 0.087$). The decrease in C concentration led to a 5.75 lower C/N ratio under moderate grazing intensity compared to the average of the other two intensities ($P = 0.018$). There was no difference between grazing intensities for the existing litter N stock (an average of 9.7 kg ha⁻¹; $P = 0.530$). There were no differences between seasons for the

existing litter N and C concentrations ($P \geq 0.103$); however, lowest existing litter C/N ratio and N stock in the existing litter was recorded for the rainy season ($P \leq 0.036$).

Moderate grazing intensity resulted in a lower overall deposition rate and grass deposition rate compared to the other two grazing intensities ($P = 0.001$; Table 3); however, the legume deposition rate did not differ among the grazing intensities and averaged 5.1 kg OM ha⁻¹ d⁻¹ ($P = 0.456$). The $\delta^{13}\text{C}$ (‰) value was also lower under moderate grazing intensity ($P = 0.027$), indicating a greater contribution of legume to the deposited litter compared to severe grazing intensity ($P = 0.099$; 14.1 vs 9.1%). The greatest total, grass and legume deposition rates were recorded for the dry season ($P \leq 0.016$).

Similarly to the C/N ratio of the existing litter, moderate grazing intensity also had a lower C/N ratio of deposited litter compared to light and severe intensities ($P = 0.047$). Despite the difference in C/N ratio identified, there were no statistical differences in the deposited litter N and C concentrations among grazing intensities (7.8 and 343 g kg⁻¹, respectively; $P \geq 0.526$). The half-life time remained unaffected by the evaluated factors, averaging 31.2 days ($P \geq 0.293$). The greatest N concentration and lowest C/N ratio in the deposited litter was recorded for the rainy season

Table 3 Dynamics of litter deposition, decomposition, and N cycling of mixed pastures of palisade grass and forage peanut as affected by grazing intensities and seasons

Item	Grazing intensity (GI)			Seasons (S)		SEM ^a	P-value		
	Light	Moderate	Severe	Dry	Rainy		GI	S	GI × S
Deposition rate, kg OM ^b ha ⁻¹ d ⁻¹	51.9 a	38.0 c	45.7 b	49.4	41.0	9.0	0.001	0.004	0.336
Grass deposition rate, kg OM ha ⁻¹ d ⁻¹	46.0 a	32.8 b	41.2 a	43.1	36.7	8.9	0.001	0.016	0.293
Legume deposition rate, kg OM ha ⁻¹ d ⁻¹	5.9	5.3	4.5	6.3	4.2	0.8	0.456	0.008	0.715
$\delta^{13}\text{C}$ deposited litter, ‰	-14.6 a	-15.0 b	-14.4 a	-14.8	-14.5	0.3	0.027	0.041	0.749
% legume in deposited litter ^c	11.3 ab	14.1 a	9.9 b	12.7	10.6	2.0	0.099	0.234	0.780
Half-life, d	29.7	32.5	31.6	32.0	30.4	2.7	0.365	0.293	0.959
Deposited litter N concentration, g kg ⁻¹	7.6	8.3	7.5	7.0	8.5	0.7	0.610	0.003	0.717
Deposited litter C concentration, g kg ⁻¹	349	342	337	346	340	10.7	0.526	0.620	0.347
C/N ratio of deposited litter	45.9 a	41.2 b	44.9 a	49.4	40.0	1.7	0.047	0.015	0.120
Legume deposited litter N, kg ha ⁻¹ season ⁻¹	6.0	8.2	5.1	6.2	6.6	1.3	0.119	0.653	0.201

Means followed by different letters in the row differ at $P \leq 0.10$ by Tukey's test. Values are least squares means (n = 4 replications per treatment)

^aStandard error of the means

^bOrganic matter

^cCalculated from ^{13}C abundance of the grass (-12.98‰) and legume (-27.28‰) dead material

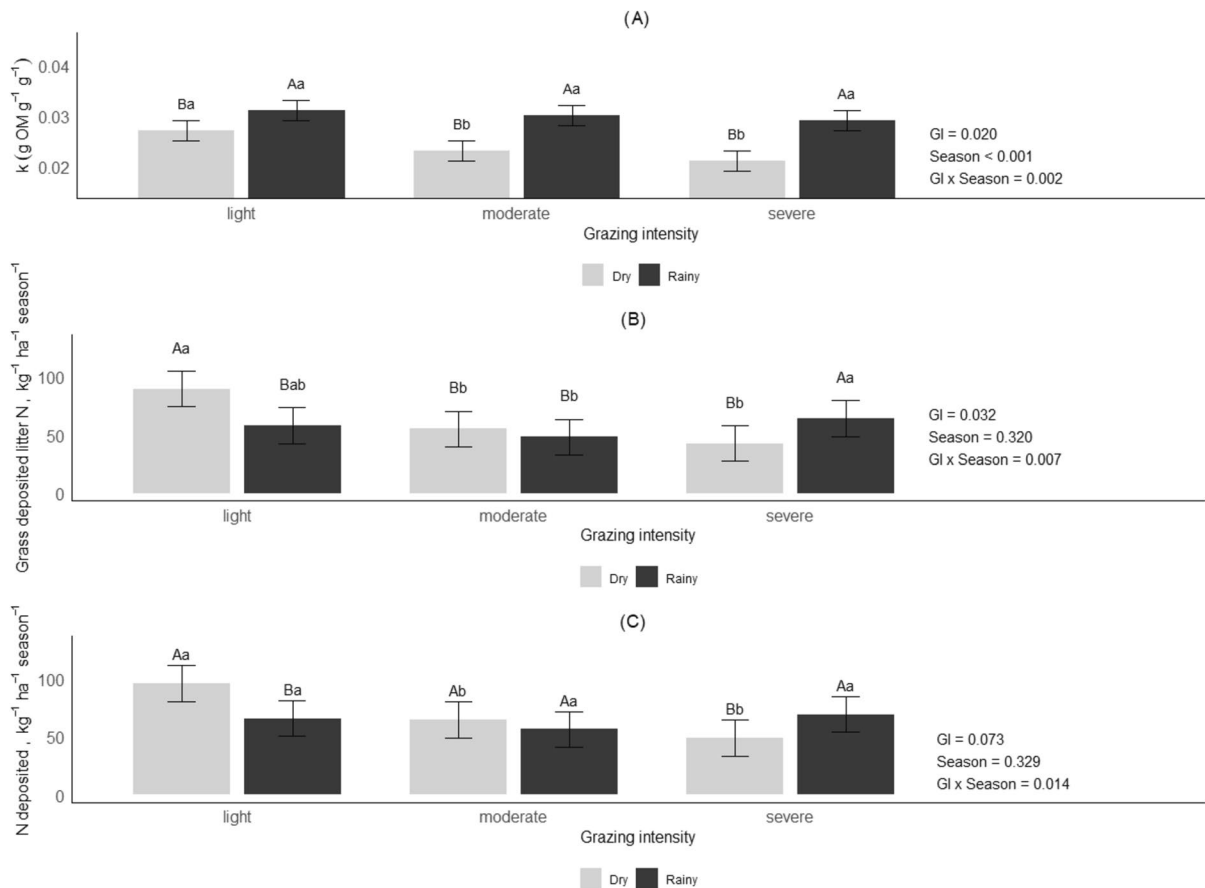


Fig. 1 Litter decomposition rate (A), N cycled per area via grass deposited litter (B), and N deposited per area and season (C) of mixed pastures of palisade grass and forage peanut as affected by grazing intensity and season. Bars with differ-

ent uppercase letters compare seasons, and lowercase letters compare grazing intensities by Tukey's test ($P < 0.10$). Values are least squares means ($n = 4$ replications per treatment). Error bars represent $\pm$ standard error of the means

($P \leq 0.015$). There was no difference between seasons for the half-life and C concentration ($P \geq 0.293$) in the deposited litter.

A faster decomposition rate (k , g OM $g^{-1} d^{-1}$) was recorded during the rainy season across all grazing intensities ($P = 0.002$; Fig. 1A). In the dry season, light grazing led to a faster decomposition rate compared to the other treatments. However, during the rainy season, no significant differences in decomposition rates were recorded. The N cycled per area from the palisade grass in the deposited litter (kg $ha^{-1} season^{-1}$) was greatest during the dry season under light grazing intensity, did not change under moderate, and was greatest during the rainy season under severe grazing intensity ($P = 0.007$; Fig. 1B). Among treatments within the dry season, the N cycled via

palisade grass litter was greater for light grazing intensity, followed by moderate and severe, which did not differ between each other. During the rainy season, the greatest N cycled from palisade grass litter was recorded for the light and severe intensities. Total N deposited (kg $ha^{-1} season^{-1}$) showed an interaction between grazing intensity and season ($P < 0.001$; Fig. 1C). During the dry season, light intensity favored the deposition of N through litter compared to the other two intensities. However, it was not observed during the rainy season, which averaged 64 kg $ha^{-1} season^{-1}$ for the three intensities. Within seasons, light intensity was greater during the dry season compared to rainy season and severe presented the opposite response. Moderate intensity averaged 60 kg $ha^{-1} season^{-1}$, not differing between seasons. The N

cycled from legume deposited litter was on average 6.4 kg N ha⁻¹ season⁻¹ and was unaffected by grazing intensities and season ($P \geq 0.119$; Table 3).

Nitrogen intake and excretion

The CP and N intake (g d⁻¹ heifer⁻¹) was 73% greater during the rainy season compared to the dry season ($P = 0.005$). However, grazing management intensities did not affect them ($P = 0.506$; Table 4). The N intake per animal from legume and from grass were not affected by grazing management, they were respectively 8.6 and 38.1 g N d⁻¹ heifer⁻¹. More N intake per animal from legume and grass was recorded for the rainy season ($P \leq 0.016$). The urinary N excretion per animal was not affected by grazing management and season ($P \geq 0.240$; an average of 26.6 g N d⁻¹ heifer⁻¹). The fecal N excretion per animal was on average 28.6 g N d⁻¹ heifer⁻¹, not differing among grazing management and season ($P \geq 0.140$). Total individual N excretion averaged 55.2 g of N d⁻¹ heifer⁻¹ for all grazing management and season ($P \geq 0.574$).

The stocking rate (AU ha⁻¹) was greatest under severe grazing intensity ($P = 0.006$; Table 4), but with no difference between seasons ($P = 0.460$). Total N intake and grass N intake (kg N ha⁻¹ season⁻¹) were greater under severe intensity compared to light grazing intensity due to greater stocking rate ($P \leq 0.062$). Nonetheless, the N intake from legume did not differ among intensities and was 8.6 kg N ha⁻¹ season⁻¹ on average. The greatest total N intake and from grass and legume (kg N ha⁻¹ season⁻¹) were recorded during the rainy season ($P \leq 0.003$). The urinary N excretion per area (kg N ha⁻¹ season⁻¹) were not affected by grazing management intensities and seasons ($P \geq 0.193$). However, fecal N excretion per area was 17.8 kg N ha⁻¹ season⁻¹ less for light intensity compared to moderate and severe ($P = 0.006$). Total N excretion had a different response, being 43.5 kg N ha⁻¹ season⁻¹ greater under severe intensity compared to light and moderate ($P = 0.066$). There were no differences between seasons for the urinary, fecal and total N excretions per area ($P \geq 0.216$).

There was a significant interaction between grazing intensities and seasons for N cycling ($P = 0.009$;

Table 4 Nitrogen intake and excretion by beef heifers grazing mixed pastures of palisade grass and forage peanut as affected by grazing management and season

Item	Grazing intensity (GI)			Season (S)		SEM ^a	<i>P</i> -value		
	Light	Moderate	Severe	Dry	Rainy		GI	S	GI×S
CP ^b intake, g d ⁻¹ heifer ⁻¹	319	306	252	214	371	64.3	0.506	0.005	0.542
N intake g d ⁻¹ heifer ⁻¹	51.0	48.9	40.4	34.2	59.4	10.3	0.506	0.005	0.542
N intake from legume g d ⁻¹ heifer ⁻¹	8.8	10.6	6.5	4.8	12.5	2.4	0.431	0.003	0.686
N intake from grass g d ⁻¹ heifer ⁻¹	42.2	38.3	33.9	29.4	46.9	8.5	0.494	0.016	0.586
Urinary N excretion, g d ⁻¹ heifer ⁻¹	33.3	19.5	27.0	22.6	30.6	8.0	0.318	0.240	0.878
Fecal N excretion, g d ⁻¹ heifer ⁻¹	27.5	33.2	25.0	30.9	26.3	4.2	0.140	0.245	0.956
Total N excretion, g d ⁻¹ heifer ⁻¹	60.8	52.7	52.0	53.5	56.9	9.0	0.744	0.574	0.828
Stocking rate, AU ha ⁻¹	1.6 c	2.6 b	4.5 a	2.7	3.1	0.78	0.006	0.460	0.803
N intake, kg ha ⁻¹ season ⁻¹	29.5 b	43.8 ab	66.0 a	24.9	67.9	12.1	0.062	0.003	0.270
N intake from legume, kg ha ⁻¹ season ⁻¹	5.2	9.7	10.9	3.2	14.0	2.8	0.280	0.002	0.412
N intake from grass, kg ha ⁻¹ season ⁻¹	24.3 b	34.1 ab	55.1 a	21.7	53.9	8.7	0.040	0.002	0.150
Urinary N excretion, kg ha ⁻¹ season ⁻¹	22.2	16.0	47.3	20.8	36.2	12.2	0.193	0.260	0.765
Fecal N excretion, kg ha ⁻¹ season ⁻¹	16.3 b	29.8 a	38.3 a	25.4	30.8	4.9	0.006	0.357	0.491
Total N excretion, kg ha ⁻¹ season ⁻¹	38.5 b	45.8 b	85.6 a	46.2	67.0	14.4	0.066	0.216	0.602

Means followed by different letters in the row differ at $P \leq 0.10$ by Tukey's test. Values are least squares means ($n = 4$ replications per treatment)

^aStandard error of the means

^bCP: crude protein; AU: Animal unit, 500 kg body weight (Allen et al. 2011)

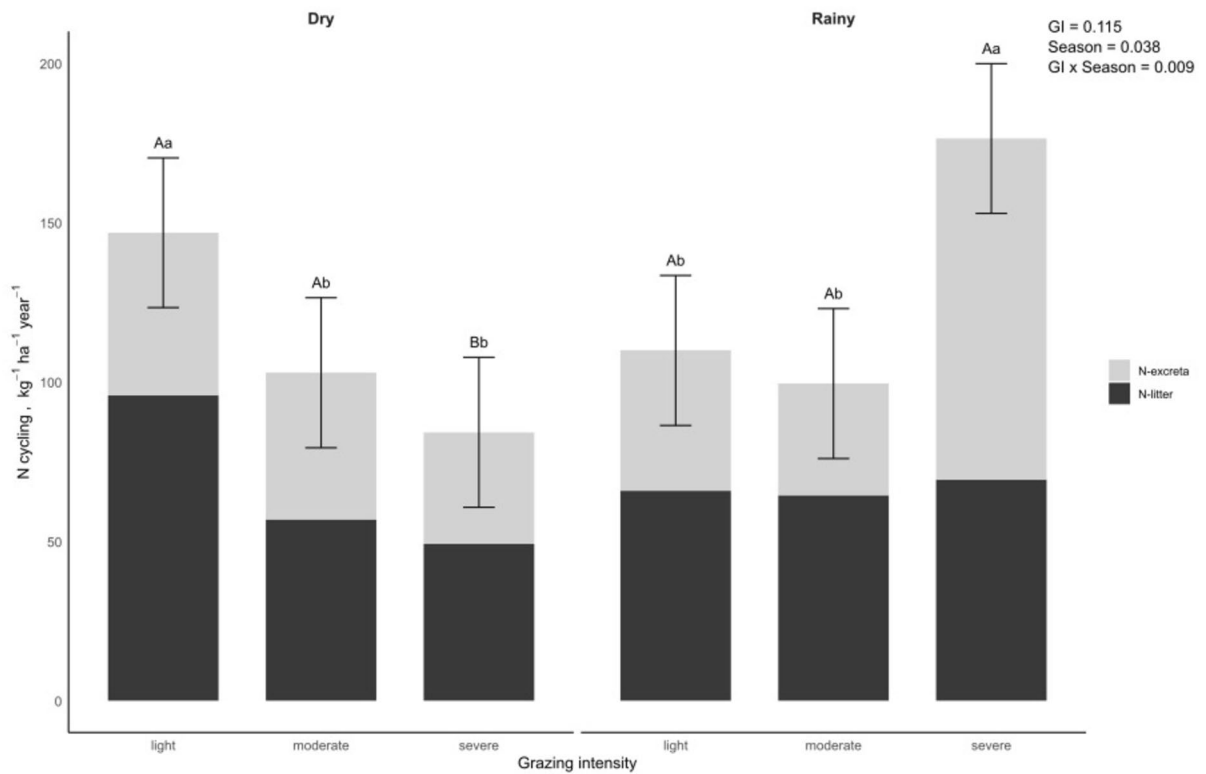


Fig. 2 N cycling via litter and livestock excretion of beef heifers grazing mixed pastures of palisade grass and forage peanut as affected by grazing intensity and season. Bars with different uppercase letters compare seasons, and lowercase letters

compare grazing intensities by Tukey's test ($P < 0.10$). Values are least squares means ($n = 4$ replications per treatment). Error bars represent $\pm$ standard error of the means

kg N ha⁻¹ season⁻¹). Nitrogen cycling was unaffected by season under light and moderate grazing; however, it was greater in the rainy season under severe intensity (Fig. 2). Among grazing intensities within dry season, light grazing had the greater N cycling per area when compared to moderate and severe intensities. During the rainy season, however, severe grazing had the greatest N cycling compared to the average of the other two ($+ 70$ kg N ha⁻¹ season⁻¹).

Discussion

Herbage characteristics and litter dynamics

Tropical grazing lands are frequently limited in N, so using legume-grass mixtures can be a viable alternative to fertilizers for enhancing N cycling through plant litter (Kohmann et al. 2018). Plant litter, which results from leaf abscission, stem lodging, and the

trampling of aerial plant parts by animals, plays a crucial role in nutrient cycling (Vendramini et al. 2014). Its decomposition is a key process that contributes to the nutrient supply for plants in grazing systems, with excreta being the other major source of nutrients. According to Thomas (1992), as stocking rates increase or stubble height decreases, a greater proportion of plant tissue is consumed by grazing, which reduces the role of plant litter in nutrient cycling and enhances the importance of animal excreta. In the current experiment, moderate grazing intensity had lower existing litter and deposition rate than light and severe intensities. Moderate grazing intensity allowed greater grass and legume intake among the other intensities (Rodrigues da Cruz et al. 2024), which explains the lesser senescent material available to become part of the litter. When the grazing intensity is severe, there will be more trampling over the pasture, causing the drop of stems and green material, which will be part of the deposited litter. In ungrazed

pastures, the deposited litter comes solely from natural leaf and shoot senescence. Thus, Apolinário et al. (2017) evaluated the effect of increasing stocking rate and N fertilizer on litter biomass in Napier grass pastures reported that increasing the stocking rate generally led to less litter deposited. The amount of legume (kg OM ha^{-1}) in the existing litter was greater under moderate grazing intensity than under the other two levels of intensities, resulting in a lower C/N ratio. This difference may have been due to the greater legume proportion in the post-grazing herbage mass under moderate grazing, particularly during the dry season, which was 264 kg ha^{-1} greater than the average of the other two intensities (data not shown).

The Liu et al. (2011) reported an interaction between season and stocking rate on litter deposition. The authors demonstrated a linear increase in litter mass as stubble height increases, during summer and fall, but not during spring. Existing and deposited litter were lowest during periods that coincided with greater rainfall and temperatures. Similar results were also observed by Apolinário et al. (2017) and Dubeux Jr. et al. (2006).

In a meta-analysis, Su et al. (2022) exemplify why severe grazing can slow litter decomposition rate (k) compared to moderate and light. Severe grazing can increase bulk density, thus reducing soil oxygen and water content, which decreases microbial decomposition (Lin et al. 2017; Lai and Kumar 2020). In addition, other studies also found that severe grazing led to a marked reduction in the catalase to polyphenol oxidase ratio compared to the control plots, subsequently affecting the decomposition of labile substances (Ho et al. 2017; Li et al. 2022). In the current experiment, litter decomposition was similar among grazing intensities during the rainy season. However, it was slower during the dry season for moderate and severe grazing intensities.

The same meta-analysis also reports the differences in litter decomposition caused by litter type and the environment, especially elevation and precipitation (Su et al. 2022). Precipitation also showed to be one of the main drivers of the decomposition rate in a study conducted in Colorado with litter from C_4 grasses (Brandt et al. 2007). The authors reported that during simulated rainy season conditions, there were no differences between ultraviolet exposure, the same occurred with low C/N ratio litter. However, under drought conditions and high C/N ratio litter, the

k was slower when ultraviolet was blocked. It is worth noting that the pastures were grazed once during the dry season to reduce the dead material and stimulate tillering at the beginning of the spring. This management practice could have contributed to greater ultraviolet exposure and caused faster k to light grazing.

Another factor that might have contributed to a faster k for the light intensity was the litter composition during the dry season. During the dry season, there was a greater amount of N in the deposited litter from the grass ($\text{kg ha}^{-1} \text{ season}^{-1}$) in paddocks managed under light grazing. Some studies have shown that N concentration in litter increases as it decomposes, once dry matter is lost faster than N (Thomas and Asakawa 1993; Wachendorf et al. 1997; Cantarutti et al. 2002). Ratios of C/N and C/lignin are considered important components of decomposition models, with lower values associated with faster decomposition (Thomas and Asakawa 1993; Dubeux et al. 2006a, b). Although moderate grazing showed a lower C/N ratio in the present study, light grazing had a faster decomposition rate during the dry season.

Although the $\delta^{13}\text{C}$ values and the corresponding calculations confirmed a contribution of legume material to the litter, the overall proportion of legumes was relatively low, which was somewhat unexpected considering the canopy composition. The litter sampling procedure performed every 14 days following Rezende et al. (1999), allowed for consistent estimates of existing and deposited litter, but it did not fully account for the disappearance of lighter or more degradable fractions between collections. This limitation may have led to an underestimation of legume litter, as legume residues generally decompose faster than grass residues. According to Thomas and Asakawa (1993), litter from tropical forage legumes can decompose two to three times faster than that from *Urochloa* grasses due to lower C:N and lignin:N ratios. Therefore, much of the legume-derived litter may have mineralized before subsequent sampling. Additionally, the low proportion of legumes in the litter could also be associated with preferential grazing of forage peanut, which is more acceptable and has higher nutritive value compared to palisade grass (Homem et al. 2021a; Rodrigues da Cruz et al. 2024). These combined factors: rapid decomposition, potential litter loss during the 14-day interval, and selective grazing, help explain the low $\delta^{13}\text{C}$ -based legume

proportion observed in the litter, despite a substantial presence of legumes in the sward and diet.

Nitrogen intake, excretion, and cycling in mixed pastures

The total N excretion per animal, along with N coming from feces and urine was not affected by grazing intensities or season. In an experiment evaluating temporal excretion patterns from two grazing intensities of MG6 pastures (*Lolium perenne*–*Cynosurus cristatus*), the authors also did not find differences between moderate and light grazing (Orr et al. 2012a, b). Furthermore, research has shown that the proportion of N excreted in urine increases with increasing N concentrations in the diet (Meer 1983). However, the N proportions excreted in urine were 57%, 36%, and 53% for light, moderate, and severe grazing, respectively, and showed no correlation with N intake. One possible explanation is related to the greater proportion of legume that animals ingested under moderate grazing (Rodrigues da Cruz et al. 2024). Although we did not measure in this study, forage peanut managed with a pre-grazing height between 90 to 95% light interception can contain 1.96 to 2.10% dry matter of condensed tannins (Gomes et al. 2018). Condensed tannins in legumes bind to proteins, protecting them from degradation in the rumen and resulting in higher fecal-to-urinary N ratios in excretion (Mueller-Harvey 2006; Ebert et al. 2017; Lagrange and Villalba 2019). A shift in N excretion from urine to feces results in more stable N fractions in manure, as N is primarily bound to organic compounds like neutral detergent and acid detergent insoluble N. This may help reduce N losses to the environment in the form of ammonia (Duval et al. 2016).

Light grazing consistently exhibited a lower stocking rate regardless of the season. The total N excretion per area was 45.8 kg ha⁻¹ season⁻¹ under moderate grazing, which is comparable to 45.2 kg ha⁻¹ season⁻¹ reported by Gomes et al. (2020) for heifers grazing mixed pastures of forage peanut and palisade grass at 90 to 95% light interception and a stubble height of 15 cm.

The N cycling (kg ha⁻¹ season⁻¹) was calculated by summing the N from the deposited litter, and the N from feces and urine per hectare per season. The N cycling had an interaction between season and grazing intensities. In comparison to data from Gomes

et al. (2020) from the same field, we observed lower N cycling. As mentioned above, litter deposition and decomposition are associated with several factors, among them, precipitation, aboveground biomass, and grazing (Semmartin et al. 2004; Ramalho et al. 2019). Ramalho et al. (2019) noted that during each 28-day grazing cycle, trampling leads to increased litter deposition on grazing days (animals are present in the paddock), followed by the first half of the rest period when grazing ceases and regrowth begins. In the final cycle, leaf production and senescence reach an equilibrium, resulting in greater litter deposition compared to the previous cycle. Thus, as a pasture undergoes more grazing cycles, trampling leads to increased litter deposition. In this study, we experienced lower precipitation and, consequently, fewer grazing cycles compared to the research by Gomes et al. (2018), which may explain the reduced N cycling observed.

Conclusion

The hypothesis of greater grazing intensity would favor N cycling through greater excreta deposition was true only during the rainy season. Mixed pastures of Marandu palisade grass and forage peanut managed with moderate grazing intensity (approximately 15 cm stubble height) exhibited lower existing and deposited litter, reduced litter C:N ratio, and a shift in N excretion from urine to feces. These responses indicate improved internal N recycling and greater potential for N retention within the pasture system compared with lighter or more severe grazing. Although total N removed through animal growth and potential gaseous or leaching losses were not measured, moderate grazing appears to promote a more balanced and environmentally efficient N cycling dynamic rather than simply maximizing total N turnover. Therefore, maintaining Marandu palisade grass/forage peanut mixed pastures with 24–30 cm pre-grazing height and 15 cm stubble height can enhance nutrient use efficiency and system sustainability in tropical pastures.

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Author contribution P.J.R.C. and D.V.S. were responsible for project execution, with part of the data included in their respective theses. Í.B.G.L. and I.O.R. collaborated in project execution, both in the laboratory and in the field. B.J.R.A. was one of the project's creators and was responsible for the EMBRAPA AGROBIOLOGIA laboratory. R.M.B. was also one of the project's creators, responsible for stable isotope analyses and part of the research funding. B.G.C.H. was responsible for statistical analysis and contributed to writing and laboratory analyses. A.J.P. was one of the project's creators, contributing to experimental management, data analysis, and writing. D.R.C. coordinated the project and supervised the theses. All authors reviewed the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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