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The influence of tropical pasture improvement on animal performance, nitrogen cycling, and greenhouse gas emissions in the Brazilian Atlantic Forest

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Abstract

This study evaluated beef cattle performance, nitrogen (N) dynamics, and potential greenhouse gas emissions (GHG) in tropical pastures receiving N fertilization or mixed with legumes. During the cool season, 54 Nellore heifers were randomly allotted to continuous grazing in three plots of each pasture type: T1 (Marandu grass with 150 kg N/ha), T2 (Marandu grass with 120 kg N/ha), T3 (Marandu grass with *Arachis pintoi*), T4 (Marandu grass with *Desmodium heterocarpo*), T5 (Marandu grass without N fertilization), and T6 (degraded pasture). The stocking rate was periodically adjusted to achieve the target canopy height of 20–25 cm. Animal performance and N livestock excretion were estimated by variation in live weight and spot samples of urine and feces, respectively. GHG emissions were calculated using the methodology of the Intergovernmental Panel on Climate Change. Animal performance was higher in fertilized and mixed pastures (e.g., T1 to T4) than in T5 and T6 pastures. Heifers grazing on T1, T2, T3, and T4 excreted lower amounts of urine with higher N concentrations than heifers grazing on T5 and T6. Heifers grazing in T2 and T4 pastures had a lower concentration of fecal N than those grazing on other pastures, reflecting lower N excretion in this form. The potential for direct nitrous oxide emissions per unit area in pastures mixed with legumes was approximately four times lower than that in pastures with mineral N fertilization. The recovery of degraded pastures and the inclusion of forage legumes contributed to maintaining the sustainability of animal production in pastures.

Keywords: Mixed pasture, fertilized pasture, urinary nitrogen, emission mitigate, beef cattle.
Abbreviations: ADG_average daily gain; AU_animal unit; CE_creatinine excretion; DMI_dry matter intake; LW_live weight; N_2O_nitrous oxide; SR_stocking rate; UV_urine volume.

Introduction

The high percentage of unproductive grasslands and the encroachment of new pastures on natural tropical forests are the main obstacles to improving sustainability in Brazilian livestock production (dos Reis et al., 2021; Oliveira et al., 2020; Soares et al., 2020). In addition to carbon (C) emitted during the conversion of natural forests to grasslands, livestock activity produces greenhouse gases (GHGs), principally in the form of methane (CH_4) from enteric fermentation; nitrous oxide (N_2O) emitted from the use of nitrogenous fertilizers; and CH_4 and N_2O released from manure management and deposition of animal excreta on pastures (Bretas et al., 2020; Chadwick, 2000; Piva et al., 2019). This contributes significantly to GHG emissions worldwide (IPCC, 2006).

These emissions are mainly influenced by environmental conditions, animal category, and the quality of the animal diet (Beauchemin et al., 2020; Nascimento et al., 2021). Under tropical pasture conditions, studies have shown that increasing the quantity and quality of forage in pastures can positively affect animal productivity and mitigate emissions (Barneze et al., 2014; Cardoso et al., 2016; Oliveira et al., 2020). Moreover, it can help maintain or increase C stocks and nutrient cycling in the soil (Cerri et al., 2016; dos Santos et al., 2019; Soussana and Lemaire, 2014). These factors highlight the benefits of recovering degraded pastures for mitigating GHG emissions (dos Reis et al., 2021).

One of the main approaches for recovering degraded pastures and intensifying production systems is to increase the availability of nitrogen (N) cycling in the system. However, the effects of N losses and cycling in pasture ecosystems are typically complex and involve diverse factors, such as climate, soil, plants, microorganisms, and ruminant animals (Scholefield et al., 1991), with N circulating among various components at widely differing rates (Jarvis, 1993). Thus, studies worldwide have sought to determine the dynamics of N in the soil, plants, animals, and atmospheric systems to characterize and quantify the main processes and propose alternative production technologies (Jarvis, 1993; Kebreab et al., 2001; Ledgard, 2001; Boddey et al., 2004; Box et al., 2017; Lagrange et al., 2020; Marshall et al., 2021; Homem et al., 2021a). Consequently, an evaluation of tropical pastures based on the study of N cycling and potential GHG emissions (N_2O and CH_4) can contribute to understanding the dynamics of C and N and formulating strategies to mitigate environmental impact.

This study evaluated the effects of different forms of N fertilization on the tropical pastures of *Brachiaria brizantha* cv. Marandu, including mixed pastures with legumes, for beef cattle performance, N content in urine and feces, and potential N_2O and CH_4 emissions. The objectives were to assess practices that could improve N dynamics and reduce the impacts of cattle breeding concerning climate change and environmental degradation.

Results and discussion

Forage quality and stocking rate (ST)

No significant differences were detected in the N content of the forage consumed by animals in the Marandu grass monoculture fertilized with 150 kg N/ha/year (T1); Marandu grass monoculture fertilized with 120 kg N/ha/year (T2); mixed pasture of Marandu grass and *Arachis pintoi* cv. Belmonte (T3); mixed pasture of Marandu grass and *Desmodium heterocarpon* (T4); and Marandu grass monoculture without N fertilizer application (T5) ($p > 0.05$; Table 1). In contrast, there were significant differences between each of the five pasture types and the degraded pasture type (T6) ($p < 0.05$). Digestibility values showed a similar pattern, in which pasture degradation reduced forage digestibility by 18% when compared to the other pasture types ($p < 0.05$). The most distinct effect of pasture improvement was reflected in the stocking rate (SR), with values ranging from 0.7 to 3.1 AU/ha for T6 and T3 pastures, respectively.

The N content of tropical grasses ranged from 0.8% to 2.1% with an average value of 1.3% (Detmann et al., 2014), whereas digestibility values were generally within the range of 55% to 60% (Barbero et al., 2015; Dupas et al., 2016; McRoberts et al., 2018). These values are directly affected by system type, forage plant type, and seasonality (Delevatti et al., 2019; Phelan et al., 2015; Dupas et al., 2016). However, different management techniques can improve the nutritional value of forage throughout the year. These include rotational grazing, N fertilization, and intercropping with legume plants (Gomes et al., 2018; Boddey et al., 2020), which can enhance plant growth, density, and renewal, thereby improving the overall forage quality (Yasuoka et al., 2018). More significant inputs of N to the pasture directly affect the quality and quantity of forage (Berça et al., 2019; Brambilla et al., 2012) and consequently facilitate a higher SR (Boddey et al., 2004; Pereira et al., 2020; Homem et al., 2021b).

Animal performance

The average daily gain (ADG) per animal ranged from 0.404 to 0.228 kg/day for the T6 and T1 pastures, respectively (Table 1). Although no significant difference in ADG was found between treatments, there was a trend towards higher ADG values in pastures fertilized with N or mixed with legumes than in pastures without N input ($p = 0.09$). In pasture systems, live weight (LW) gain per area is determined by ADG and SR (Pereira et al., 2020). Increasing the availability and nutrient intake capacity of grazing animals could maximize animal production (Chapman et al., 2007). N fertilization is the fastest way to produce more meat in smaller areas (Cardoso et al., 2016). During the experimental period, animal production in pastures with N fertilization (T1 and T2) was 189 and 168 kg/ha, respectively (Table 1), which was higher than the values observed in pastures T5 and T6 (99 and 29 kg/ha, respectively ($p < 0.05$)). The animal production was 217 and 147 kg/ha in pastures mixed with legumes, even without the addition of N fertilizer.

Currently, sustainable intensification of livestock production is a worldwide need and has attracted the attention of several research groups, mainly in pasture production systems. For example, Homem et al. (2021b) conducted a study to understand the effects of N fertilization via the introduction of a legume in Marandu grass pastures on beef cattle performance during the spring-summer season. They found significantly greater gains in LW per area on pastures fertilized with N and mixed with legumes (219 and 143 kg/ha, respectively) than on pastures without N input (106 kg/ha). Additionally, they concluded that N input affected productivity per unit area more strongly than ADG and that mixed legume and Marandu grass pastures are sustainable and have a high potential for use in the tropics. Thus, mixed legume and grass pastures must be promoted as a critical technology to improve beef production, particularly for low-technology producers, resulting in a higher economic yield.

Livestock N excretion

Compared with the other pasture types, animals grazing on T6 pasture ingested an average of 12% less N ($p < 0.05$; Table 2). With an increase in the N content of pastures and higher animal N intake, there may be greater daily N excretion through urine per animal (Van Soest, 1994; Valadares et al., 1999; De Oliveira et al., 2016). However, heifers grazing on T1, T2, T3, and T4 excreted smaller daily volumes of urine (15 vs. 21 L/AU. day; Table 2) with a higher N concentration (5.5 vs. 4.0 g/L) than heifers grazing on T5 and T6 pasture ($p < 0.05$); therefore, not altering the daily N excretion ($p > 0.05$). According to Chopa et al. (2016), N intake was significantly higher in Holstein steers grazed on pasture with N fertilization than on pasture without fertilization (136 vs. 114 g/animal. day; $p < 0.05$). However, no significant differences in urinary N excretion (53 vs. 48 g/animal. day; $p > 0.05$) were found and N input was concluded to improve the absorption efficiency of the animals; thereby, reflecting greater ADG.

The fecal N concentrations ranged from 1.3% to 1.8%, with the lowest values recorded in the T2 and T4 pastures (Table 2). Thus, cattle grazed on the T2 and T4 pastures excreted lower amounts of fecal N ($p < 0.05$) compared to animals grazed on the other pasture types. According to Cheng et al. (2016), fecal production per animal is relatively constant over time, indicating that the concentration of N in feces should be considered an essential variable for N losses from this source. Authors who have evaluated the effects of N input in pastures on daily N excretion have reported no significant differences in N excretion when compared to pastures without N addition, with values ranging from 46 to

Table 1. Effects of nitrogen (N) inputs on forage quality and animal performance during the cool season in pastures of the *Brachiaria brizantha* cultivar Marandu subjected to different forms of N fertilization.

Parameters	Pasture					
	T1	T2	T3	T4	T5	T6	SEM
Pasture							
N (%)	1.6 a	1.6 a	1.6 a	1.5 a	1.4 ab	1.3 b	0.1 **
Digestibility (%)	60.4 a	61.9 a	60.6 a	62.4 a	60.5 a	50.0 b	2.1 **
Stocking rate (AU/ha)	2.6 b	2.7 b	3.1 a	2.3 b	2.3 b	0.7 c	0.2 **
Animal performance							
DMIR (kg/AU.day)	11.2	10	10.2	11.6	11.2	10.9	0.2 ns
ADG (kg/day)	0.404	0.347	0.389	0.356	0.240	0.228	0.078 ns
LW gain (kg/ha.season)	189 ab	168 b	217 a	147 b	99 c	29 d	4 **

T1 (Marandu grass with 150 kg N/ha); T2 (Marandu grass with 120 kg N/ha); T3 (Marandu grass with *Arachis pintoi*); T4 (Marandu grass with *Desmodium heterocarpo*); T5 (Marandu grass without N fertilization); T6 (degraded pasture).
DMIR: Dry Matter intake required; ADG: Average daily gain; LW: Live weight; AU: Animal unit (450 kg live weight);
SEM: Standard error of the mean.
ns: Differences between means were not significant.
∗ Significant at p = 0.05.
∗∗ Significant at p = 0.01
Mean data followed by the same letter did not differ in the column according to the Tukey-HSD test at 5% probability.

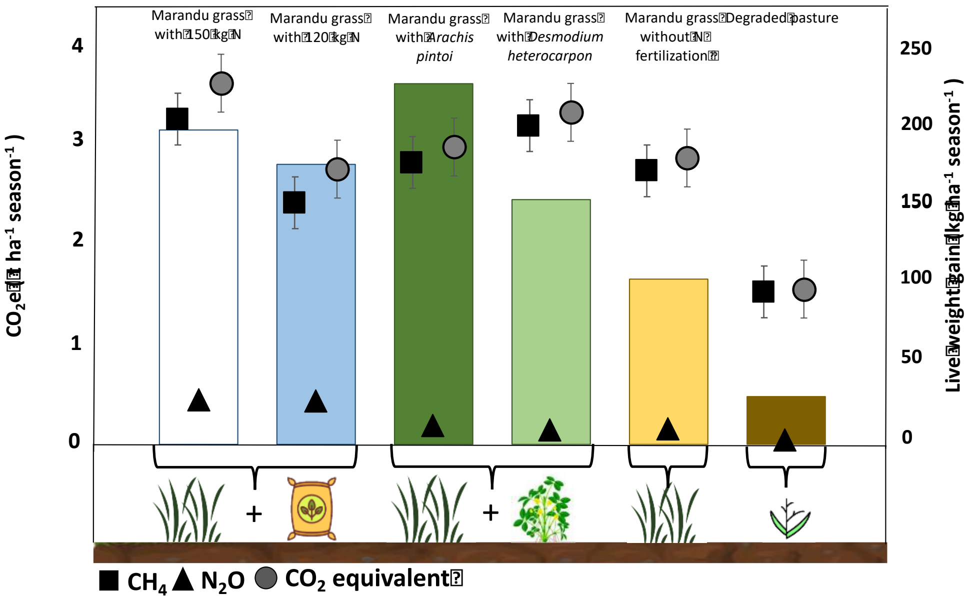


Fig 1. Animal production and greenhouse gas emissions (N_2O and CH_4) from cattle pastures during the cool period in southern Bahia, Brazil.

Table 2. Effects of nitrogen (N) inputs on N livestock excretion during the cool season in pastures of the *Brachiaria brizantha* cultivar Marandu subjected to different forms of N fertilization.

Parameters	Pasture						
	T1	T2	T3	T4	T5	T6	SEM
N intake (g/AU.day)	182 a	164 b	161 b	183 a	159 b	143 c	7 *
Urine							
Volumen (L/AU.day)	15.7 b	14.1 b	14.0 b	16.0 b	22.2 a	20.0 a	1.2 *
N concentration (g/L)	5.4 a	5.9 a	5.7 a	5.2 a	4.1 b	3.8 b	0.6 *
N urina (g/AU.day)	79	82	77	80	93	70	10 nsa
Feces							
Production (kg/AU.day)	4.5 b	3.8 b	4 b	4.4 b	4.4 b	5.5 a	0.2 **
N concentration (%)	1.6 b	1.4 c	1.6 b	1.3 c	1.8 a	1.6 b	0.1 **
N feces (g/AU.day)	69 b	52 c	64 b	57 c	78 a	91 a	5 *

T1 (Marandu grass with 150 kg N/ha); T2 (Maranu grass with 120 kg N/ha); T3 (Marandu grass with *Arachis pintoi*; T4 (Marandu grass with *Desmodium heterocarpo)* T5 (Marandu grass without N fertilization); T6 (degraded pasture).
SEM: Standard error of the mean;
AU: animal unit (AU) = 450 kg live weight.
ns Differences between means were not significant.
∗ Significant at p = 0.05.
∗∗ Significant at p = 0.01
Mean data followed by the same letter did not differ in the column according to the Tukey-HSD test at 5% probability.

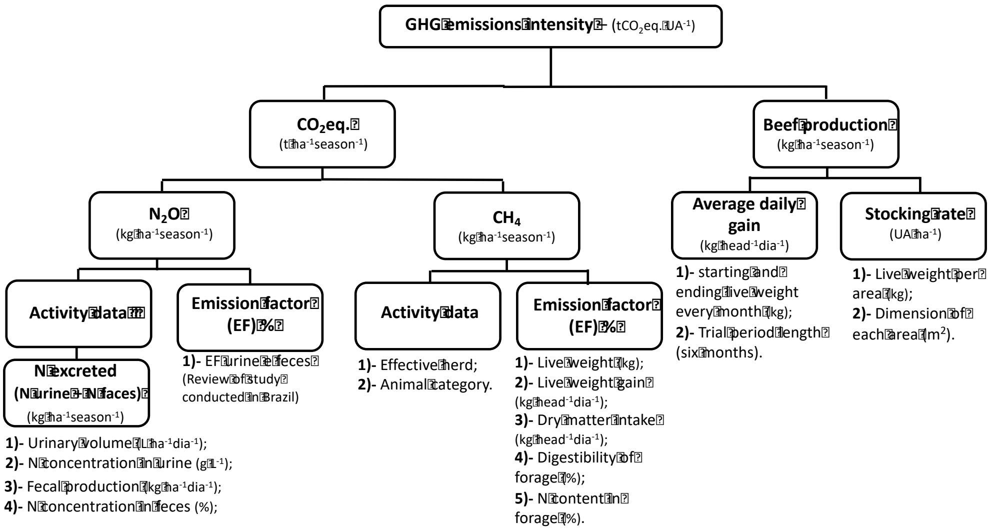
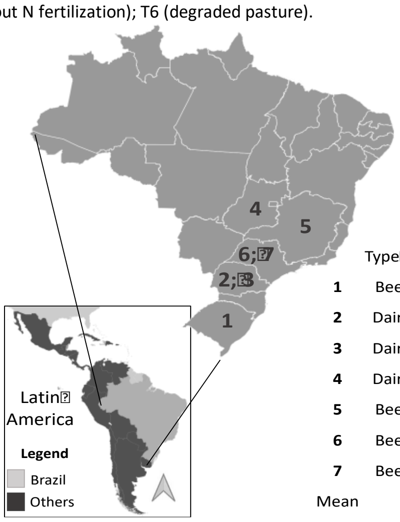


Fig 2. Conceptual model for quantifying emissions from livestock production considering N_2O emissions from animal excreta and CH_4 from enteric fermentation and feces deposited in the soil (Tier 2, IPCC).

Table 3. Emissions of N_2O and CH_4 per unit area and per unit animal (AU) measured over six consecutive months during the cool season in pastures of the *Brachiaria brizantha* cultivar Marandu subjected to different forms of nitrogen fertilization.

	GHG emissions per area and livestock unit											
Source	T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6
	kg/ha.season						kg/AU.season					
CH_4												
Enteric fermentation	104	72	87	101	84	38	40	27	28	44	37	55
Feces	5.2	3.6	4.4	5.1	4.2	1.9	2.0	1.3	1.4	2.2	1.8	2.7
CO_2 eq.	3046	2118	2564	2973	2472	1131	1171	784	827	1293	1075	1616
N_2O												
Direct												
Urine	0.19	0.19	0.21	0.15	0.16	0.04	0.07	0.07	0.07	0.07	0.07	0.06
Feces	0.05	0.03	0.04	0.04	0.03	0.02	0.02	0.01	0.01	0.02	0.02	0.02
Fertilizer	0.75	0.75	-	-	-	-	0.75	0.28	-	-	-	-
CO_2 eq.	292	288	74	56	59	17	249	107	24	24	26	25
Indirect (excreta and fertilizer)												
Volatilization	0.07	0.07	0.08	0.06	0.06	0.01	0.03	0.02	0.02	0.03	0.03	0.02
Leaching	0.16	0.15	0.17	0.14	0.14	0.03	0.06	0.05	0.05	0.06	0.06	0.04
CO_2 eq.	70	63	73	60	61	12	27	23	24	26	26	17
Total CO_2 eq. (t/season)	3.4	2.5	2.7	3.1	2.6	1.2	1.4	0.9	0.9	1.3	1.1	1.7

T1 (Marandu grass with 150 kg N/ha); T2 (Maranu grass with 120 kg N/ha); T3 (Marandu grass with *Arachis pintoi*; T4 (Marandu grass with *Desmodium heterocarpo)* T5 (Marandu grass without N fertilization); T6 (degraded pasture).



Emission factors

	Type of cattle	Urine (%)	Feces (%)	Locality	Reference
1	Beef cattle	0.23	0.08	Rio Grande do Sul (RS)	Mazzetto et al. (2015)
2	Dairy cattle	0.26	0.15	Paraná (PR)	Sordi et al. (2014)
3	Dairy cattle	0.3	0.11	Paraná (PR)	Simon et al. (2020)
4	Dairy cattle	1.2	0.01	Goiás (GO)	Lessa et al. (2014)
5	Beef cattle	0.06	0.03	Minas Gerais (MG)	Bretas et al. (2020)
6	Beef cattle	1.02	0.36	São Paulo (SP)	Cardoso et al. (2019)
7	Beef cattle	0.2	-	São Paulo (SP)	Barneze et al. (2014)
Mean	-	0.48	0.12		

Fig 3. Review of the N_2O emission factors (EF) for excreta on pasture soils in Brazil was reported in the literature (only field studies) and this localization.

59 g N/animal (Chopa et al., 2015; Berça et al., 2019). However, other studies on the effect of protein supplementation in beef steers grazing on tropical pastures have found higher amounts of excreted N in the feces of animals receiving supplementation than in animals that did not (45 vs. 35 g N/animal) (Rocha et al., 2016). Thus, 80% of the ingested N was excreted in the urine and feces (Table S1), and the non-absorbed N may enter one of several pathways, including reabsorption by plants, leaching as nitrate (NO_3^-), volatilization as ammonia (NH_3), and emission to the atmosphere as N_2O (Selbie et al., 2015).

GHG emissions

The potential emissions of N_2O during the cool season from the assessed pastures ranged from 0.2 to 0.9 kg/AU, with the highest values recorded in T1 and T2 pastures (0.9 and 0.4 kg/AU, respectively) (Table 3). Although chemical N fertilization is essential for improving pasture quality and increasing animal productivity, it also increased N_2O emissions compared to other pasture treatments. In contrast, whereas cattle grazed on mixed pastures showed an increase in productivity, no emissions from synthetic fertilizer inputs were detected (Figure 1). The direct emission of N_2O per unit area in mixed pastures containing legume plants was three times lower than that in pastures receiving N fertilization with urea (Table 3), indicating that this is one of the most promising technologies for increasing the sustainability of beef cattle production systems (Boddey et al., 2020; Homem et al., 2021b).

Assessing only N_2O and CH_4 emissions in terms of CO_2eq, on average, 85% of the emissions were associated with enteric fermentation (Figure 1), which is consistent with previously reported values (Mazzetto et al., 2015; Cardoso et al., 2016). Furthermore, the plots of intensively cultivated pastures emitted twice the amount of these gases compared to the degraded pasture per unit area (Figure 1). However, when considering the emissions of CH_4 and N_2O in terms of kilograms of LW produced, substantially lower emissions are estimated for intensive pastures than for degraded pastures (Table 3). In some studies conducted in Brazil, Brunes and Couto (2017) and Mazzetto et al. (2015) reported that the relative reduction in emissions from intensive systems can be attributed primarily to the more rational use of forage, the production area, and a reduction in the age of livestock at slaughter.

Materials and methods

Study site characterization

The study was conducted at the *Estação Experimental de Zootecnia do Extremo Sul da Bahia* (ESSUL-CEPLAC), Northeastern Brazil (Itabela, Bahia). The area lies within the Atlantic Forest biome with an average annual precipitation of 1,311 mm, no defined dry season, an average temperature of 25ºC, and the lowest temperatures in June, July, and August (Fig S1). The climate of the region is transitional and characterized by tropical rainforest (Af) and tropical monsoon (Am) climates, as defined by the Köppen classification system (Peel et al., 2007). Meteorological data covering the study period was obtained from a weather station located 1,000 m southeast of the experimental area. The soils in the region are formed from the Coastal Tablelands (*Tabuleiros Costeiros*) geological formation and comprise loose sedimentary deposits of Miocene to Pliocene age (Vilas Bôas et al., 2001), which are mainly classified as oxisols and ultisols. The soils are predominantly sandy (> 700 g sand/kg) to a depth of approximately 20 cm and are characterized by low natural fertility and acidity, with phosphorus (P) being the most limiting element.

Pasture implantation and fertilization

To obtain a representative assessment of the Brazilian production scenario, two experimental areas (areas 1 and 2) of tropical pastures (20 ha in total) within the vicinity of the experimental station were examined. The establishment of pasture in Area 1 began in 1995. In 2002, the entire area (eight hectares) transformed into a mixed pasture of Marandu grass and *Arachis pintoi* cv. Belmonte which was seeded randomly in half of the plots. Since 2002, plots containing monoculture Marandu grass pasture have been annually fertilized with 120 kg of N, 40 kg of P_2O_5, and 50 kg of K_2O per hectare, whereas the mixed pasture plots received 40 kg of P_2O_5 and 50 kg of K_2O per hectare with no N application.

Similar to area 1, area 2 was initially seeded with Marandu grass. However, the area was converted to a mixed pasture containing *Desmodium heterocarpon* (subsp. *ovalifolium*) in 1994. In this area, the annual fertilization consisted of 150 kg of N, 40 kg of P_2O_5, and 50 kg of K_2O per hectare for plots of the monoculture pasture, and 40 kg of P_2O_5 and 50 kg of K_2O per hectare for plots in the grass-legume mixed pasture area. In addition, degraded pasture plots located adjacent to the two areas were assessed. These plots were characterized by low forage production, exposed soil, and the growth of non-forage plants.

Experiment

The experiment was conducted during the cool season (May to November 2017) within the Mata Atlântica biome. The assessments included animal performance, N livestock excretion, and potential GHG emissions from 54 Nelore (*Bos taurus indicus*) heifers with an initial LW of 370 (± 15) kg and ages of 24 months. The animals belonged to the experimental station and were randomly distributed for continuous grazing in three plots of each type of tropical pasture. ST was periodically adjusted to achieve a target canopy height of 20–25 cm.

Treatments were six pasture types replicated three times in a randomized complete design: Marandu grass monoculture fertilized with 150 kg N/ha/year (T1), Marandu grass monoculture fertilized with 120 kg N/ha/year (T2), a mixed pasture of Marandu grass and *Arachis pintoi* cv. Belmonte (T3), a mixed pasture of Marandu grass and *Desmodium heterocarpon* (T4), a Marandu grass monoculture without N fertilizer application (T5), and a degraded pasture with low forage production, exposed soil, and growth of non-forage plants (T6).

Animal performance

ADG per animal and LW gain per area were estimated by weighing all the animals at the same time of the day at 28-day intervals (Pereira et al., 2020), during the cool season (May to November 2017). The SR was calculated by dividing the sum of the total weight of the animals grazing the pastures by the area of the pasture. For conversion to animal units (AU = 450 kg), SR values were divided by 450, which represents the weight of an adult animal at maturity. The LW gain per area (kg/ha.season) was calculated by multiplying the AU in each pasture by the ADG.

The dry matter intake (DMI) of individual animals in each treatment was estimated by applying the Beef Cattle Nutrient Requirements Model (NRC 2016), using the individual ADG values of each animal and the *in vitro* digestibility of each diet. Software was used to estimate the DMI required by each animal (DMIR) to achieve the observed ADG.

Forage quality

Using a simulated grazing technique (Prohmann et al., 2012), forage samples were collected monthly from May to September (2017) for assessment of *in vitro* digestibility (Tilley and Terry, 1963) and N content (Alves et al., 1999). Single samples collected from each plot were mixed to obtain three composite samples from each plot of a given pasture. Subsequently, the samples were air-dried at a temperature of 65°C for 72 h.

Livestock N excretion

N intake was calculated as the product of N content in the forage and the DMIR of the animal. The N excreted in feces and urine was quantified in spot samples from six heifers from each pasture from May to September, measured twice a day (09:00 am and 3:00 pm) between the 23^{rd} and 26^{th} days of each month.

The amount of N excreted in feces (g/day) was measured as the product of fecal production multiplied by the concentration of N (%). To quantify the concentration of N in feces, fecal grab samples were collected from the rectum of the animals. Fecal production was determined based on individual DMIR and the *in vitro* digestibility of the diets (Mayes and Dove, 2000; Lagrange et al., 2020). The N concentration in feces and urine was determined using the Kjeldahl digestion and steam distillation method (Alves et al., 1999).

The average amount of N excreted in urine by each AU was calculated from the daily urine volume (UV) per animal unit (L/day.AU) and N concentration (g/L). Urine samples (approximately 100 ml) were collected during spontaneous urination. Urine volume (L/d) was estimated based on the concentration of urinary creatinine (a waste product of muscle metabolism) as daily urinary creatinine excretion (CE, mg/d) divided by the urinary creatinine concentration (mg/L) (Valadares et al., 1999; Chizzotti et al., 2008).

GHG emissions

Estimates of potential emissions were obtained following the methods proposed by the Intergovernmental Panel on Climate Change (IPCC, 2006) (Figure 2), which are based on activity data and emission factors (Tier 2).

Potential N_2O emissions were assessed from urine and feces deposited on pastures (Figure 1) (Lessa et al., 2014; Bretas et al., 2020). The total N within the excreta is determined and the average N_2O emission factor for urine and feces obtained in studies carried out in Brazil is applied (Figure 3).

Potential emissions of enteric CH_4 were calculated based on gross energy intake. This method computes the LW, ADG, DMIR, digestibility, and protein content of consumed forage. For all pastures, the proportion of gross energy and energy consumption converted to CH_4 and the Y_m value was assumed 6.5%.

The values obtained for the potential emissions of N_2O and CH_4 were converted to C equivalents (CO_2eq) by applying global warming potential factors of 25 for CH_4 and 298 for N_2O (IPCC, 2006). Thus, a balance was drawn between emissions and animal productivity by assessing emissions per kilogram of LW produced. Although the potential of pastures to sequester C in the soil is recognized (Cerri et al., 2007; dos Santos et al., 2019), changes in C content after 20 years under the same management conditions do not imply gains or losses for the system (IPCC, 2006).

Data analyses

The data obtained in this study was analyzed using replicates of the experimental units and temporal replicates. The statistical model included pasture and period as fixed effects, whereas the animal or plot were random effects. All calculations were performed using the *Nortest* package of the R software, version 3.5.2, and the data was analyzed using the LME procedure (R core Team, 2020). Prior to analysis, the data was assessed for homoscedasticity and normality of the residues using the BoxCox and Cramer–von Mises tests, respectively. Multiple comparison tests (Student–Newman–Keuls test) were performed for all ANOVA values to determine differences among the mean values obtained for different pastures. Differences were considered statistically significant at a p-value < 0.05.

Conclusions

Mixing tropical pastures with legume plants is a critical tool for reducing the contribution of beef production to climate change. This technique may increase the N content in forage similar to that of pastures fertilized with chemical N, and enhance the LW gain per unit area. Although heifers in grazing pastures with N inputs excreted less urine with higher N concentrations, the amount of N excreted daily was equivalent to that recorded in treatments without N inputs. Tropical pastures comprising a mixture of grass and legumes may contribute to greater reductions in the emission of N_2O per unit area and per unit animal than in pastures fertilized with chemical N. However, further investigation is required to constrain N dynamics in legume-augmented tropical pastures.

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