Received: 24 October 2024 | Revised: 25 February 2025 | Accepted: 19 May 2025

DOI: 10.1111/grs.70007

ORIGINAL ARTICLE

GRASSLAND SCIENCE | WILEY

Understanding the impacts of intensity and harvest frequency on *Tithonia diversifolia* for use in tropical silvopastoral systems

Vagner Ovani[1] | Simón Pérez-Márquez[2] | Alyce Monteiro[1] | Ana Maria Kruger[1] | Paulo de Mello Tavares Lima[3] | Beatriz Elisa Bizzuti[1] | Lumena Souza Takahashi[1] | Rogério Martins Maurício[4] | Helder Louvandini[1] | Adibe Luiz Abdalla[1]

[1]Laboratory of Animal Nutrition, Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, Sao Paulo, Brazil

[2]Net-zero and Resilient Farming, Rothamsted Research, Okehampton, UK

[3]Animal Science Department, University of Wyoming, Laramie, WY, USA

[4]Federal University of São Jõao del Rei, São João del Rei, Minas Gerais, Brazil

Correspondence
Vagner Ovani, Laboratory of Animal Nutrition, Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, Sao Paulo, CEP: 13400-970, Brazil.
Email: vagnerovani@usp.br

Funding information
National Council for Scientific and Technological Development, Grant/Award Numbers: 140200/2020-7, 200207/2023-7, 304023/2022-1, 306116/2023-5; São Paulo Research Foundation, Grant/Award Number: 2024/07657-0

Abstract

Tithonia diversifolia (Hemsl.) A. Gray has significant potential as a forage source in silvopastoral systems, particularly in tropical conditions. However, its intensity and frequency management differ from those commonly applied to grasses. This study aims to evaluate *T. diversifolia* under two harvest intensities (30 and 40 cm stubble height) and four harvest frequencies (21, 28, 35 and 42 days), aligning with Brazil's traditional tropical grass management practices. Biomass production and nutritive value of forage, as well as in vitro rumen fermentation parameters, were assessed. We observed that the tested harvest intensities have a limited impact on the biomass production and nutritional value of *T. diversifolia*. Despite the linear increase in biomass production, lower harvest frequencies (i.e., longer regrowth period) significantly affect nutritional value, impacting the products of ruminal fermentation, as indicated by the in vitro assay. The methane production per gram of degraded organic matter (OM) and the OM allocated for microbial biomass production are affected by the harvest frequencies. Additionally, we observed that rainfall and canopy height have a stronger correlation with biomass production than harvest frequencies controlled by days. We conclude that *T. diversifolia* is minimally affected by harvest intensities and, for Brazilian edaphoclimatic conditions, it can be harvested with higher frequencies than those suggested for other regions.

KEYWORDS
agroforestry, methane, nutritive value, pasture, shrub

1 | INTRODUCTION

Pastures formed by African grasses (e.g., *Urochloa* and *Megathyrsus* genera) have been the main source of feed for ruminant production in Brazil (Domiciano et al., 2021; Jank et al., 2014). However, due to inadequate soil, plant or herd management, more than half of Brazilian pastures present some level of degradation (Feltran-Barbieri & Féres, 2021; LAPIG, 2022), causing a decrease in regrowth vigor, consequently reducing the stocking rate and animal productive performance, resulting in great economic and environmental damages (Euclides et al., 2022). Thus, reversing the economic and environmental losses of pasture degradation still remains as an important

challenge for the current livestock industry in Brazil (Oliveira Silva et al., 2017).

The challenges of this scenario are intensified by climate variation, which affects biomass production and the nutritional value of pastures (Mbow et al., 2019), due to well-defined rainy and dry seasons in tropical regions of South America throughout the year (Tambara et al., 2021). Among the strategies with the potential to overcome these challenges is the adoption of silvopastoral systems (SPS), which involves utilizing land for both forest products and animal production through the browsing on shrubs and trees and/or grazing of co-existing forage crops (Allen et al., 2011).

Several advantages have been documented concerning the adoption of SPS in comparison to extensive grazing systems based on forage monocultures, emerging as a promising strategy to mitigate the environmental impact of livestock farming and enhance the resilience of tropical pasture systems toward climate fluctuations. These benefits include improvements in soil physical, chemical and biological properties (Santos et al., 2022), soil organic carbon storage (Filho et al., 2024; Resende et al., 2020), as well as similar total forage accumulation and greater nutritive value (Brunetti et al., 2022; Gomes et al., 2022), protein supply during the dry season (Ovani et al., 2023), weed reduction (Munaro et al., 2023), shaded pasture for animals (Reis et al., 2021), promotion of animal welfare (Cândido et al., 2023), improved animal performance (Oliveira et al., 2022), greenhouse gas emission mitigation (Monteiro et al., 2024) and, consequently, provision of ecosystem services (Schmitt Filho et al., 2023). These benefits depend on the characteristics or application of each chosen tree or shrub resource.

Among the shrub resources for SPS is the utilization of *Tithonia diversifolia* (Hemsl.) A. Gray, a perennial shrub belonging to the *Asteraceae* family, native to Mexico and Central America and widely distributed in the tropics and subtropics (Rai et al., 2023; Sampaio et al., 2016). *T. diversifolia* has been used as a forage source in silvopastoral arrangements or as cut-and-carry forage (Mauricio et al., 2014; Palmer, 2014). It exhibits greater biomass production and nutritional value compared to tropical grasses and demonstrates broad adaptability to different edaphoclimatic conditions, with the potential to enhance animal productivity by improving fermentative efficiency and reducing enteric methane (CH_4) emissions (Mahecha et al., 2022; Rivera et al., 2021; Rivera et al., 2022).

A wide range of harvest intensities has been investigated in previous studies on *T. diversifolia*, spanning from 10 to 100 cm of stubble height (García, 2017; Londoño et al., 2019; Partey, 2011). The suggested harvest or grazing frequencies typically occur after the plants reach a height of 100 cm, with regrowth intervals of 60 days during the rainy season and 90 days during the dry season (Alonso et al., 2013; Alonso et al., 2015; García, 2017). This recommended frequency is notably higher compared to the grasses commonly used in Brazil, which usually have harvest frequencies of less than 28 days in the rainy season in a rotational stocking method (Carnevalli et al., 2006; Giacomini et al., 2009; Pedreira et al., 2007). These distinctive intensities and harvest frequencies pose challenges in establishing management protocols for these plants within an integrated system, such as a silvopastoral arrangement.

Based on the aforementioned information regarding *T. diversifolia*, we hypothesize that the plant is minimally affected by harvest intensity, enabling management with intensities similar to those applied to traditional tropical grasses. Additionally, we propose that the harvest frequencies may potentially be higher (i.e., shorter regrowth time) than suggested in the literature, without significant detrimental effects on biomass production and with improved nutritive value. Therefore, this study aimed to assess the intensity and harvest frequency for *T. diversifolia*, focusing on forage mass production and nutritional value, for its utilization in tropical SPS.

2 | MATERIAL AND METHODS

2.1 | Study location and experimental design

The experiment was performed at the facilities of the Animal Nutrition Laboratory (LANA), of the Center for Nuclear Energy in Agriculture (CENA), University of São Paulo (USP), Piracicaba, Sao Paulo, Brazil (22°42′22.53“ South; 47°38′41.49” West; 512 m elevation). Weather data during the first and second biomass harvest cycles (Figure 1a and Figure 1b, respectively) were collected at the Piracicaba meteorological station, at the Luiz de Queiroz College of Agriculture (ESALQ - USP), 2.2 km away from the study site. All procedures involving animal use were approved by the Ethics Committee on Animal Use (CEUA) of CENA/USP (Protocol n° 006–2018).

A total of 96 plants were distributed in randomized blocks in a 2 × 4 factorial design with 4 replications (each consisting of 3 plants): 2 harvest intensities (30 and 40 cm of stubble height) and 4 harvest frequencies (21, 28, 35 and 42 days after the standardization cut), totaling 32 experimental units.

2.2 | Field management and plant implantation

Before starting the experiment, the area was utilized as pasture (*Cynodon* spp.) for sheep. Soil samples collected within the 0 to 20 cm profile were analyzed for chemical and physical attributes at the Department of Soil Science (ESALQ/USP) (Table 1), with texture characterized by 398 g/kg of clay, 202 g/kg of silt and 400 g/kg of sand.

The pasture was desiccated using the herbicide Glyphosate (1 L per 100 L of water, N-[phosphonomethyl] glycine), and the soil was subsequently tilled after a 14-day period. Given the natural fertility of the soil (Table 1), no chemical corrections or establishment fertilization were applied. To obtain *T. diversifolia* seedlings, approximately 1.5 kg of seeds were sown in a bed measuring approximately 300 × 90 cm, containing a mixture of commercial substrate and local soil. The bed was covered with a shading screen during the initial 15 days after emergence. Thirty-nine days after sowing, the seedlings were transplanted into the experimental area.

The *T. diversifolia* seedlings were transplanted manually, with a spacing of 3 m between rows and 1 m between plants. The first

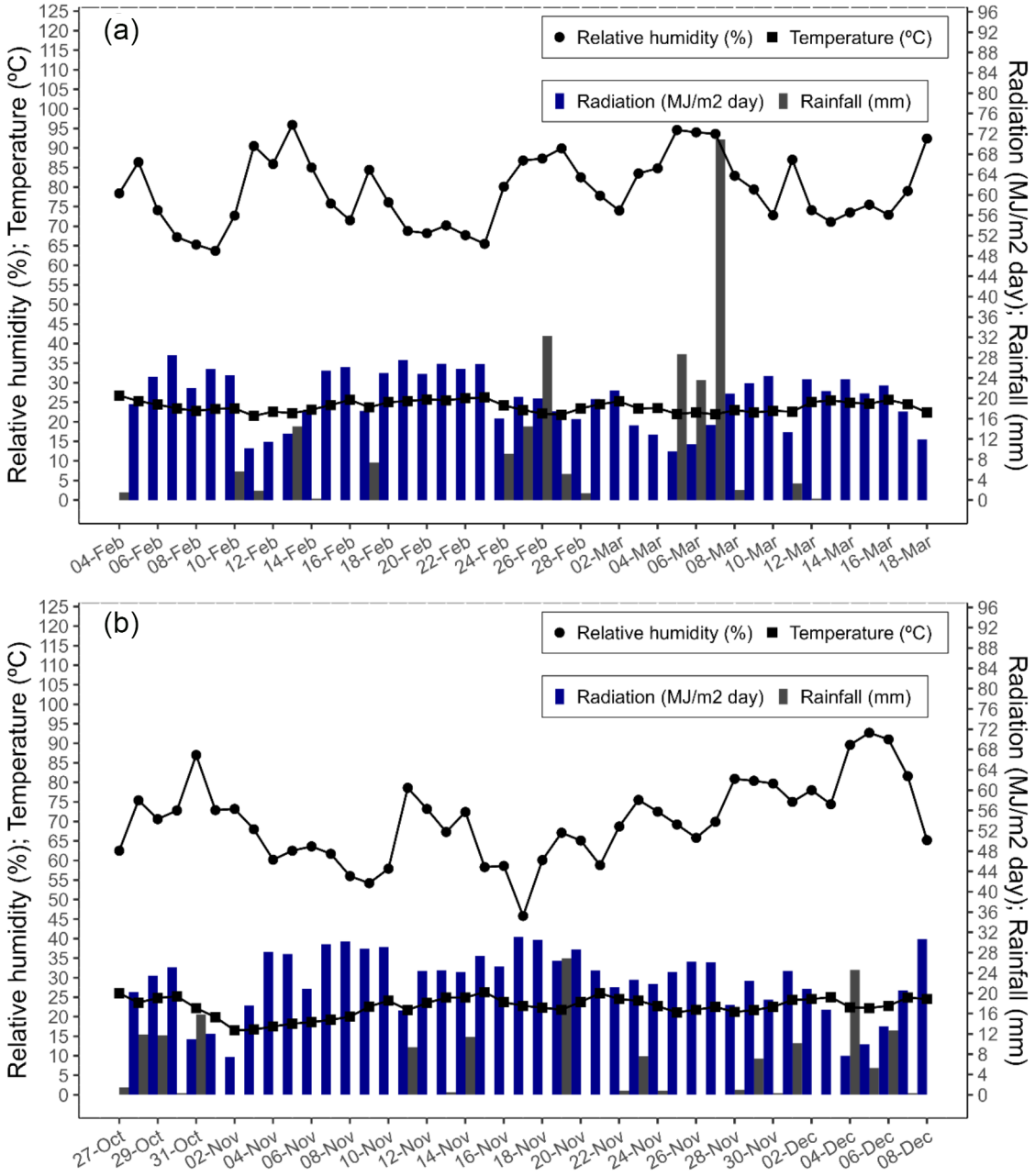

FIGURE 1 Weather conditions during the first (a) and second (b) years of biomass harvest.

biomass harvest was performed 71 days after seedling transplantation. In the first two years post-implantation, biomass was cut at a stubble height of 45 cm, with an approximate interval of 60 days between cuts. All harvested biomass was removed from the site and utilized to feed sheep.

Two biomass harvest cycles were conducted, with the first cycle occurring from February 4th to March 18th, 2021, and the second cycle from October 27th to December 8th, 2022. Prior to each biomass harvest cycle, a standardization cut was performed on all plants, following the intensities specified in the experimental design (30 and 40 cm of stubble height).

2.3 | Biomass and data collection

After reaching the defined harvest frequencies as outlined in the experimental design (21, 28, 35 and 42 days after standardization cut), measurements were taken for canopy height, number of branches per plant and biomass production. These measurements were conducted in accordance with the specified cutting intensities for each plant (30 and 40 cm of stubble height).

The harvested biomass from each plant was weighed and divided into two equal subsamples. The first subsample was separated into stem, leaves and dead material, dried in an air-forced circulation oven

TABLE 1 Soil chemical properties in the experimental area.

Variables	Before the implementation	Before the second harvest
CEC ($mmol_c/dm^3$)	121.0	166.8
BCS (%)	77	88
AS (%)	0	0
SB ($mmol_c/dm^3$)	93.0	146.4
pH ($CaCl_2$)	6.00	6.28
OM (g/dm^3)	37.0	47.7
Resin P (mg/dm^3)	174.0	478.4
K^+ ($mmol_c/dm^3$)	9.30	6.25
Ca^{2+} ($mmol_c/dm^3$)	65.0	114.4
Mg^{2+} ($mmol_c/dm^3$)	19.0	25.7
$H^+ + Al^{3+}$ ($mmol_c/dm^3$)	28.0	20.4
Al^{3+} ($mmol_c/dm^3$)	<0.1	<0.1

Abbreviations: Al^{3+}, trivalent aluminum; AS, aluminum saturation; BCS, base-cation saturation, Ca^{2+}, calcium; CEC, cation exchange capacity; H^+, hydrogen ions; K^+, potassium; Mg^{2+}, magnesium; OM, organic matter; P, phosphorus; SB, sum of bases.

at 40 °C (Model MA 037 – Marconi, Piracicaba – SP, Brazil) for 72 h, and weighed to determine the morphological composition. The second subsample was also dried, as described before, then ground to 1 mm using a knife mill (Marconi, Piracicaba – SP, Brazil) and stored under refrigeration at −20 /°C until subsequent analysis.

2.4 | Chemical analysis

The biomass samples were thawed and analyzed in duplicates to determine the content of dry matter (DM; N°: 934.01), crude protein (CP; N°: 2001.11), ether extract (EE; N°: 2003.5) and ash (N°: 942.05) (AOAC, 2011). The neutral detergent fiber fraction (assayed with a heat stable amylase and expressed exclusive of residual ash - aNDFom), acid detergent fiber (expressed exclusive of residual ash - ADFom) and lignin (determined by solubilization of cellulose with sulfuric acid - Lignin [as]) were determined according to the methodology described by Van Soest et al. (1991) and adapted by Mertens et al. (2002). The fractions of aNDFom, ADFom and Lignin (as) were determined in a sequential assay in Ankom F-57 bags (Ankom®, Macedon, NY, USA) in a fiber analyzer (TE-149 Tecnal, Piracicaba – SP, Brazil).

2.5 | In vitro gas production technique

Biomass samples were analyzed using the in vitro gas production technique, following the methodology developed by Theodorou et al. (1994). Two gas production assays were conducted, one for each biomass harvest cycle. Three male rumen-cannulated Santa Inês sheep served as rumen content donors. The animals were ad libitum fed on a diet consisting of 60% hay (Tifton 85 - *Cynodon* spp.), 40% concentrate (soybean meal and ground corn), water and mineral salt. The solid fraction was collected using a crucible tong, while the liquid fraction was collected using a silicone tube adapted to a 60 ml syringe. Both fractions were preserved in thermal containers and transported immediately for the preparation of inoculum (Lima et al., 2018).

The three inocula were prepared by blending equal volumes of solid and liquid fractions of the rumen content of each animal for approximately 10 seconds. The content was then strained through nylon gauze and maintained in a water bath at 39 °C with CO_2 saturation until inoculation (Bueno et al., 2005). An incubation medium was prepared following the solutions described by Menke et al. (1979). The substrates (biomass samples from each plant) were weighed (1 g) in Ankom F-57 bags.

Incubation was conducted in 160 ml glass bottles, each containing the substrate in Ankom F-57 bags, 50 ml of incubation medium and 25 ml of inoculum. The bottles were sealed with rubber stoppers and placed in an oven with forced air circulation (MA 035 – Marconi, Piracicaba-SP, Brazil) at 39 °C for 24 hours. In addition to the tested substrates, a blank bottle (with an empty Ankom F-57 bag) and a bottle with an internal standard sample (Tifton 85 - *Cynodon* spp.) were included in each inoculum. The volume of gas produced by the blank bottles served as a correction factor to determine the net gas production of each bottle.

At 0, 2, 4, 8, 12, 16 and 24 hours after the bottles were sealed, the pressure (psi) was measured using a semi-automatic system following the approach proposed by Mauricio et al. (1999). Pressure measurements were conducted with a pressure transducer and a datalogger (PD800–2005; Transducer: 6 pins; Sensor: SLANA001, CENA/USP, Piracicaba – SP, Brazil). The corresponding gas volume was determined by recording the volume of gas displaced into a 20 ml syringe barrel upon withdrawal of the plunger until the head-space gas pressure returned to ambient pressure (Theodorou et al., 1994). This process was performed for the first 54 bottles until the 24-hour measurements, out of a total of 216 bottles from the first gas production assay. A linear regression ($R^2 = 0.99$, n = 321) was obtained by plotting the gas pressure readings against their corresponding gas volumes (Figure 2) (Theodorou et al., 1994).

The volume of gas produced in the remaining bottles was estimated using equation (1) derived from the linear regression:

$$y = 6.1432x + 0.045143 \quad (1)$$

where y represents the volume of gas produced (mL) and x corresponds to the pressure measured with the transducer (psi).

Following each pressure measurement, 2 ml of gas was extracted from each bottle and preserved in 10 ml vacuum tubes for the quantification of CH_4 concentration using a gas chromatograph (GC – 2010, Shimadzu, Tokyo, Japan), as described by Lima et al. (2018).

At the end of 24 h, the incubation was interrupted to quantify the in vitro degraded organic matter (IVDOM). The bottles were immersed in ice water, and the Ankom F-57 bags were removed using tongs and washed with a neutral detergent solution in a fiber analyzer (TE-149 Tecnal, Piracicaba – SP, Brazil). The liquid content samples of

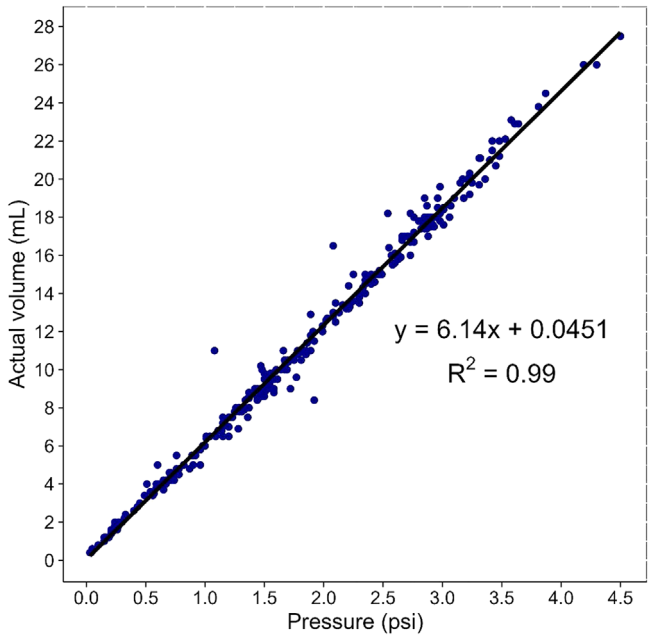

FIGURE 2 Relationship between gas pressure (psi) and gas volume (mL) from 54 bottles read during a 24 h incubation period.

each bottle were collected to measure the concentration of ammoniacal N (NH_3-N), short-chain fatty acids (SCFA) and pH, as described by Lima et al. (2018). The OM of each incubated substrate was partitioned into undegraded OM, OM for gas production (CO_2, CH_4 and H_2O), OM for SCFA and OM for microbial biomass (MB), based on stoichiometric principles as proposed by Blümmel et al. (1997) and adapted by Ovani et al. (2023).

2.6 | Statistical analysis

All data were analyzed using R software version 4.3.2 (R Core Team, 2023) and the following packages: lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017), emmeans (Lenth, 2021), ggplot2 (Wickham, 2016), cowplot (Wilke, 2020), tidyr (Wickham et al., 2023), dplyr (Wickham et al., 2021) and corrplot (Wei & Simko, 2021). The variables were analyzed using the following model:

$$Yijk = \mu + Ii + Fj + (I\,x\,F)ij + Bk + Hk + eijk \quad (2)$$

where *Yijk* represents the dependent variables for each plant "*k*" at two intensities "*i*" and different harvest frequencies "*j*"; *μ* is the general average; *Ii* is the fixed effect of intensity; *Fj* is the fixed effect of harvest frequencies; (*I x F*)*ij* is the interaction effect between intensity and harvest frequencies; *Bk* is the random effect of blocks; *Hk* is the random effect of harvest cycle (or of gas production assays for in vitro gas production parameters); and *eijk* is the experimental error.

Before the statistical analysis, normality and homoscedasticity assumptions were assessed using the Shapiro–Wilk test and Bartlett test, respectively. Data were subjected to analysis of variance (ANOVA), and for significant variables at a 5% confidence interval, means were compared by linear regression for quantitative factors (variables with a 10% confidence interval were considered a tendency). Correlation analysis was also performed with the variables related to intensity, harvest frequencies, biomass production, morphological and weather parameters. The accumulation of bromatological, morphological fractions and in vitro fermentation parameters was calculated by multiplying the fraction concentration (g/kg DM) by the biomass produced (kg DM).

3 | RESULTS

3.1 | Biomass production

No interaction effect was observed between intensity and harvest frequencies ($P > 0.05$) on biomass production and morphological composition variables (Table 2). There were no significant differences ($P > 0.05$) between the two tested intensities, except for canopy height, which was significantly greater at the intensity of 40 cm ($P < 0.01$).

A linear increase ($P < 0.01$) was observed for canopy height, biomass production and the proportion of stems and dead material as the harvest frequencies decreased (i.e., longer regrowth time). Conversely, the proportion of leaves exhibited a linear decrease ($P < 0.01$) with the extended harvest frequencies (Table 2). Despite the increase in the proportion of stems and dead material and the reduction in the proportion of leaves, a linear increase in leaf and stem accumulation (g/plant DM) was observed as the harvest frequencies decreased. Proportionally, the difference in biomass accumulation (g/plant DM) between intensities was +62.2% from 21 to 28 days, +43.7% from 28 to 35 days and +33.6% from 35 to 42 days. Meanwhile, for leaf accumulation, it was +57.9% from 21 to 28 days, +34.7% from 28 to 35 days and +20.3% from 35 to 42 days.

A positive correlation of 0.68 was observed between harvest frequencies and biomass production (Figure 3). However, biomass production showed stronger positive correlations with canopy height and rainfall (0.92 and 0.82, respectively). There were negative correlations between stem proportion, canopy height and biomass production with leaf proportion, with coefficients of −0.99, −0.92 and −0.92, respectively. However, when considering leaf production, a positive correlation was observed between canopy height and biomass production, with coefficients of 0.92 and 0.97, respectively.

3.2 | Nutritive values and in vitro gas production parameters

No interaction effect was observed for nutritive values between intensity and harvest frequencies ($P > 0.05$), except for aNDFom (Table 3). In terms of intensity, a significant difference ($P < 0.05$) was observed in aNDFom (567 and 551 g/kg, respectively) and lignin content (175 and 164 g/kg, respectively) between the 30 and 40 cm intensities. For the harvest frequencies effects, there was a linear

TABLE 2 Biomass production and morphological composition of *Tithonia diversifolia* under two intensities and four harvest frequencies.

									P-value				
	Intensity (cm)			Frequency (days)						Frequency			
Variables	30	40	SE	21	28	35	42	SE	Int.	L	Q	R^2	IxF
Canopy height (cm)	74	85	16.1	51	69	90	106	16.3	0.004	<0.001	0.898	0.48	0.977
Biomass production (g/plant FM)	1,089	1,237	538	302	779	1,479	2092	549	0.404	<0.001	0.741	0.42	0.874
Biomass production (g/plant DM)	122	136	50.4	33	88	157	236	51.9	0.423	<0.001	0.559	0.47	0.910
Branches (n)	62	67	14.6	57	68	73	60	14.8	0.176	0.562	0.036	0.08	0.458
Leaf (g/kg DM)	742	737	61.7	854	780	696	628	62.9	0.775	<0.001	0.890	0.48	0.939
Leaf (g/plant DM)	79	86	22.1	29	68	104	130	22.8	0.401	<0.001	0.505	0.51	0.921
Stems (g/kg DM)	247	252	66	146	212	287	353	67.1	0.764	<0.001	0.990	0.43	0.954
Stems (g/plant DM)	41	48	27.8	4.9	20	51	101	28.6	0.492	<0.001	0.143	0.40	0.903
Dead material (g/kg DM)	11	11	4.64	0.0	8.4	17	19	5.23	0.989	<0.001	0.329	0.24	0.932
Dead material (g/plant DM)	2.0	1.7	0.67	0.0	0.5	1.6	5.3	0.88	0.881	<0.001	0.047	0.31	0.951

Abbreviations: DM, dry mass; FM, Fresh mass; Int.: Intensity; IxF: Intensity vs Frequency interaction; L: Linear regression; Q: Quadratic regression; SE: Standard error. Variables deemed significant within a 5% confidence interval.

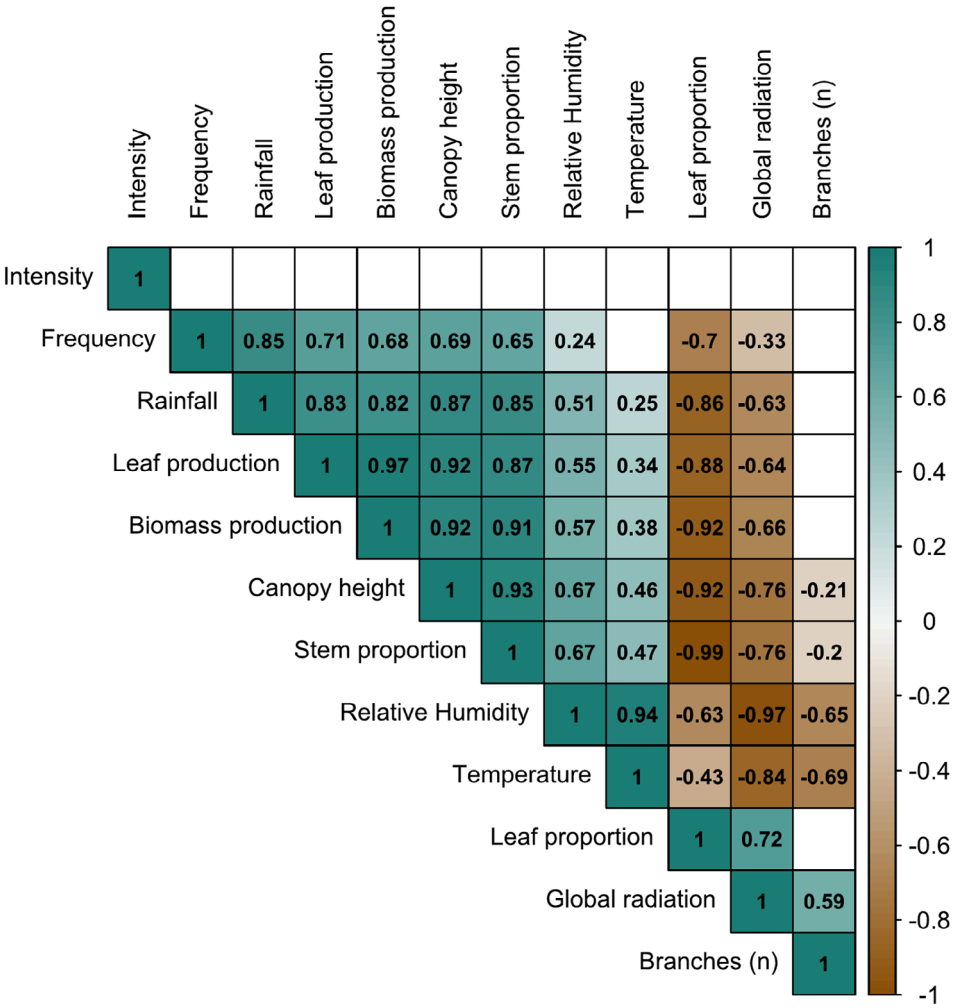

FIGURE 3 Correlation coefficient of harvest intensity, harvest frequency, biomass production, morphological and weather parameters.

increase ($P < 0.01$) in aNDFom and ADFom fractions with the decrease in harvest frequencies, while CP and ash decreased linearly ($P < 0.01$).

For the in vitro gas production parameters (Table 4), no interaction effect was observed between intensity and harvest frequencies ($P > 0.05$). However, significant differences were found for the two

TABLE 3 Biomass nutritive values (in DM basis) of *Tithonia diversifolia* under two intensities and four harvest frequencies.

									P-value				
	Intensity (cm)			Frequency (days)						Frequency			
Variables	30	40	SE	21	28	35	42	SE	Int.	L	Q	R^2	IxF
DM (g/kg)	910	912	11.8	906	906	916	915	11.9	0.461	0.020	0.864	0.08	0.439
Ash (g/kg)	101	100	1.24	101	106	99	98	1.6	0.491	0.026	0.054	0.13	0.549
CP (g/kg)	231	223	17	291	239	198	180	17.7	0.310	<0.001	0.047	0.64	0.949
EE (g/kg)	40.9	41.3	10.9	41.6	41.7	43.3	37.7	11.0	0.838	0.476	0.374	-	0.372
aNDFom (g/kg)	567	551	9.45	532	542	563	599	10.9	0.045	<0.001	0.152	0.35	0.009
ADFom (g/kg)	398	389	15.8	350	384	403	437	17.0	0.351	<0.001	0.993	0.40	0.171
Lignin (sa) (g/kg)	175	164	14.2	167	168	175	167	14.6	0.030	0.786	0.488	-	0.277

Abbreviations: ADFom, acid detergent fiber expressed exclusive of residual ash; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; CP, crude protein; DM, dry matter; EE, ether extract; Int., Intensity; IxF, Intensity vs Frequency interaction; L, Linear regression; Q, Quadratic regression; SE, Standard error. Variables deemed significant within a 5% confidence interval.

TABLE 4 Parameters of in vitro gas production technique during the 24-h incubation period of *Tithonia diversifolia* collected under two intensities and four harvest frequencies.

									P-value				
	Intensity (cm)			Frequency (days)						Frequency			
Variables	30	40	SE	21	28	35	42	SE	Int.	L	Q	R^2	IxF
GP (mL/g OM)	83.7	87.1	8.43	87.6	84.7	86.1	83.2	8.48	0.012	0.179	0.845	-	0.927
GP (mL/g DOM)	149	155	26.8	135	142	159	173	26.9	0.055	<0.01	0.559	0.08	0.963
CH_4 (mL/g OM)	4.30	4.86	0.708	4.61	4.27	4.69	4.76	0.720	<0.01	0.368	0.340	-	0.907
CH_4 (mL/g DOM)	7.71	8.65	1.750	7.08	7.35	8.54	9.74	2.150	<0.01	<0.01	0.303	0.07	0.977
IVDOM (g/kg)	543	553	41.9	631	587	518	456	42.4	0.245	<0.01	0.574	0.40	0.298
NH_3-N (mg/dl)	37.3	37.1	1.36	43.8	39.9	32.9	32.2	1.50	0.852	<0.01	0.108	0.38	0.109
pH	6.86	6.86	0.189	6.89	6.87	6.84	6.85	0.189	0.877	0.505	0.292	-	0.825

Abbreviations: CH_4, methane production; DOM, degraded organic matter; GP, Gas production; Int., Intensity; IVDOM, in vitro degradability of organic matter; IxF, Intensity vs Frequency interaction; L, Linear regression; NH_3-N, Ammoniacal N; OM, organic matter; Q, Quadratic regression; SE, Standard error. Variables deemed significant within a 5% confidence interval.

tested intensities, with the 40 cm harvest intensity leading to greater total gas production (GP) and CH_4 per incubated and per degraded OM. Regarding harvest frequencies effects, a linear increase in GP and CH_4 production per degraded OM was observed with the decrease in harvest frequencies, along with a decrease in IVDOM and NH_3-N.

No significant interaction effect ($P > 0.05$) was observed for the SCFA proportion between intensity and harvest frequencies (Table 5). However, there was a greater production of propionate by the biomass of *T. diversifolia* harvested at 40 cm intensity ($P < 0.05$), followed by a tendency ($P < 0.10$) for a reduction in acetate. For the harvest frequencies effects, a linear reduction was observed in the proportions of valerate, isobutyrate and isovalerate ($P < 0.05$) according to decreasing harvest frequencies.

The partitioning of OM indicated a linear reduction in degraded OM with the decrease of harvest frequencies ($P < 0.05$), so that after 35 days, the concentration of undegraded OM exceeded that of degraded OM (Figure 5a). Concerning the degraded fraction, a uniform portion is designated for gas and SCFA production, with no observed effect from the harvest frequencies, while the fraction of OM allocated to MB decreases linearly.

No interaction effect ($P > 0.05$) was observed between intensity and harvest frequencies when evaluating the accumulation (g/plant DM) of nutritive values and in vitro fermentation parameters (Table 6). Additionally, no significant difference ($P > 0.05$) was observed for all variables regarding the harvest intensity. Concerning the effect of harvest frequencies, a linear increase in the accumulation of all nutritive values and in vitro fermentation parameters was observed ($P < 0.05$) according to decreasing harvest frequencies.

4 | DISCUSSION

4.1 | Biomass production

In the management of traditional tropical grasses, careful consideration of stubble height post-grazing or cutting is essential since many pasture species lose vitality when frequently cut to a short stubble

TABLE 5 Short-chain fatty acid (SCFA) concentrations after in vitro incubation period of *Tithonia diversifolia* cultivated at two intensities and four harvest frequencies.

									P-value				
	Intensity (cm)			Frequency (days)						Frequency			
Variables	30	40	SE	21	28	35	42	SE	Int.	L	Q	R^2	IxF
Total (mmol/L)	109	111	19.7	113	111	110	107	19.7	0.161	0.354	0.881	-	0.912
Acetate (%)	71.8	71.2	2.90	71.0	71.8	71.3	71.9	2.91	0.061	0.501	0.944	-	0.897
Propionate (%)	16.9	17.4	1.52	17.4	16.9	17.5	16.7	1.53	0.039	0.408	0.732	-	0.906
Butyrate (%)	8.16	8.20	1.140	7.98	7.90	8.34	8.49	1.140	0.773	0.172	0.760	-	0.789
Valerate (%)	0.81	0.81	0.036	0.94	0.85	0.74	0.72	0.038	0.974	<0.01	0.067	0.40	0.320
Isobutyrate (%)	0.80	0.87	0.342	0.96	0.91	0.68	0.78	0.344	0.128	0.047	0.412	0.02	0.844
Isovalerate (%)	1.57	1.56	0.173	1.71	1.61	1.50	1.44	0.175	0.657	<0.01	0.837	0.08	0.835

Abbreviations: Int., Intensity; IxF, Intensity vs Frequency interaction; L, Linear regression; Q, Quadratic regression; SE, Standard error. Variables deemed significant within a 5% confidence interval.

TABLE 6 Accumulation of nutritive values, and in vitro fermentation parameters of *Tithonia diversifolia* biomass cultivated under two intensities and four harvest frequencies.

									P-value				
	Intensity (cm)			Frequency (days)						Frequency			
Variables	30	40	SE	21	28	35	42	SE	Int.	L	Q	R^2	IxF
DM (g/plant)	122	136	50.4	33.4	88.3	156.9	236.3	51.9	0.423	<0.001	0.559	0.47	0.910
CP (g/plant)	25.1	27.3	2.09	9.78	21.0	31.2	42.9	2.96	0.480	<0.01	0.923	0.73	0.871
aNDFom (g/plant)	72.3	76.1	5.95	17.9	47.8	88.4	142.6	8.41	0.658	<0.01	0.130	0.83	0.866
ADFom (g/plant)	51.7	54.7	4.17	11.8	34.0	63.1	103.8	4.88	0.613	<0.01	0.099	0.84	0.848
Lignin (sa) (g/plant)	21.6	22.1	1.72	5.63	14.7	27.2	39.8	2.40	0.838	<0.01	0.441	0.82	0.862
EE (g/plant)	4.80	5.58	0.349	1.39	3.69	6.76	8.93	0.493	0.127	<0.01	0.895	0.82	0.413
IVDOM (g/plant)	43.5	50.4	3.06	15.0	36.5	58.3	78.1	4.33	0.123	<0.01	0.847	0.80	0.532
SCFA (g/plant)	13.0	14.6	0.98	3.68	9.61	17.0	24.9	1.39	0.255	<0.01	0.484	0.83	0.703
Gas (g/plant)	8.57	9.58	0.635	2.40	6.31	11.1	16.5	0.90	0.272	<0.01	0.414	0.84	0.700
MB (g/plant)	21.9	26.3	1.64	8.94	20.6	30.0	36.8	2.32	0.071	<0.01	0.313	0.72	0.435

Abbreviations: ADFom, acid detergent fiber expressed exclusive of residual ash; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; CP, crude protein; DM, dry matter; EE, ether extract; Gas, dry matter potentially partitioned for CO_2, CH_4 and H_2O production; Int.: Intensity; IVDOM, in vitro degraded organic matter; IxF: Intensity vs Frequency interaction; L: Linear regression; MB, dry matter potentially partitioned for Microbial Biomass production; Q: Quadratic regression; SCFA, dry matter potentially partitioned for Short-Chain Fatty Acid production; SE: Standard error. Variables deemed significant within a 5% confidence interval.

height. The key factor influencing the vitality of pasture is the amount of remaining leaf and buds post-cutting (Shaw et al., 1976). Pasture regrowth following defoliation relies on reserves stored in the stubble, leading to a decrease in pasture biomass during the initial days of regrowth until sufficient leaf area sustains positive carbon balance in the canopy (Thomas, 1980). Consequently, in these tropical forages, harvest or defoliation intensity is deemed a more crucial factor than harvest frequency since maintaining control over stubble height preserves the number of leaves, buds and stored reserves essential for pasture resilience, defoliation intervals.

Studies have employed *T. diversifolia* harvest intensities ranging from 10 to 100 cm of stubble height (García, 2017; Londoño et al., 2019; Partey, 2011), indicating tolerance to varying harvest intensities, which justifies the lack of influence on biomass and morphological production (Table 2) in our study.

There is no clear pattern in the literature regarding the influence of harvest intensity on *T. diversifolia* biomass production. Ziegler et al. (2022) observed an interaction between intensity and harvest frequencies concerning biomass production. These authors stated that lower intensity results in greater residual leaf area and intact meristems, which stimulate regrowth. Partey (2011) tested three stubble heights and observed an increase in dry matter production from 25 cm to 50 cm; however, there was a reduction from 50 cm to 100 cm. Uu-Espens et al. (2023) did not observe any difference in

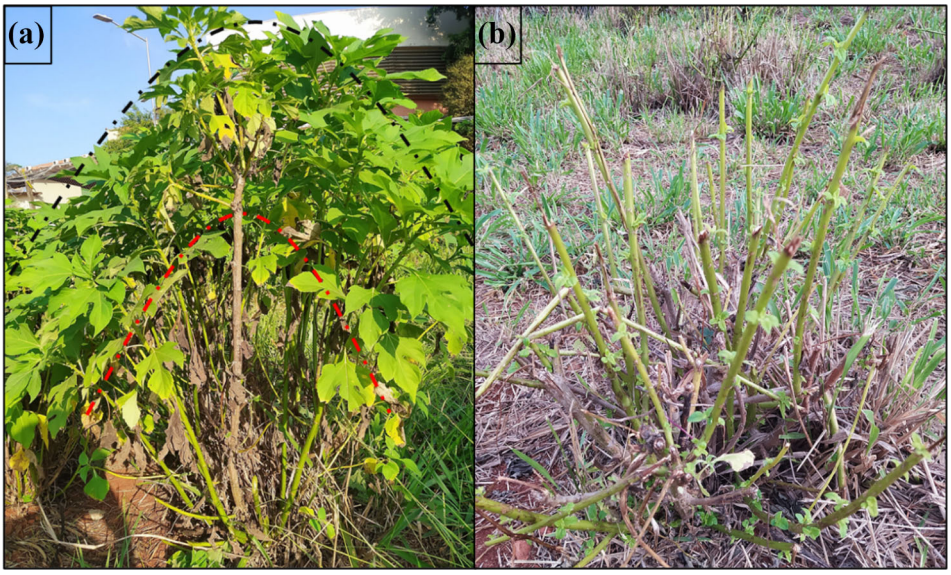

FIGURE 4 Canopy of the *Tithonia diversifolia* plant in a field condition, before (a) and after (b) grazing by ruminants. Red and black lines: lower and upper limits of branches with leaves, respectively.

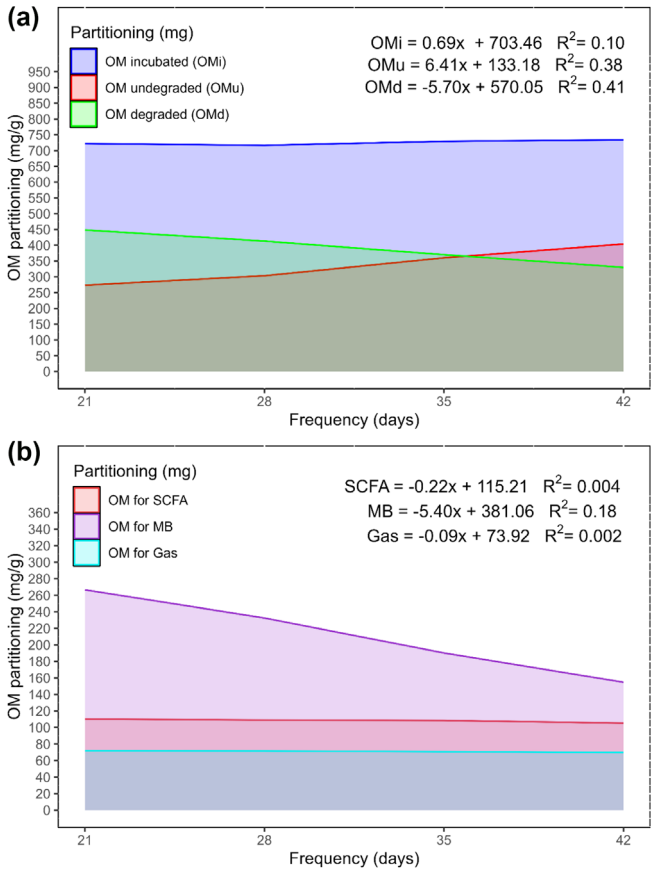

FIGURE 5 Partitioning of organic matter (OM) (a) and degraded OM (b) of the *Tithonia diversifolia* biomass after a 24-hour in vitro gas production incubation period.

the total biomass production of *T. diversifolia* at 40 cm, 60 cm and 80 cm of stubble height, but leaf production was greater at 80 cm. These authors suggested that this might be due to the remaining stems maintaining a greater number of buds, which favors shoot growth.

We posit that the intensity of harvest or grazing for tropical grasses and *T. diversifolia* may not be similar due to morphophysiological differences between these forages. *T. diversifolia* typically has a predominance of leaves at branch extremities (Figure 4a) and buds along the branches. Consequently, after defoliation by animals, there is a probable predominance of stems and senescent material in the stubble height. Thus, stubble height as a method of intensity regulation seems to be more applicable for *T. diversifolia* in cut-and-carry systems, since in an intercropped system under animal grazing, just tender stems and leaves are used to be grazed, as observed under field conditions (Figure 4b). Given the absence of differences in biomass production between the two intensities tested and in accordance with the previously described characteristics, determining stubble height for *T. diversifolia* in a grazing system is unnecessary, as its regrowth primarily relies on the buds in the mature stems that are not grazed (Figure 4b). Therefore, stubble height control should be applied only to the utilized intercropped forage.

Regarding the influence of harvest frequencies on biomass production of *T. diversifolia*, our results indicate that, despite the reduction in leaf proportion (g/kg DM), the linear increase in biomass production remains more substantial, maintaining a continuous linear increase in leaf accumulation until 42 days (g/plant DM). Verdecia et al. (2018) also observed an increase in total biomass during dry and rainy seasons up to 180 days of age. Additionally, Partey (2011) reported greater dry matter production, more than double, when comparing a 4-week harvest interval with an 8-week interval.

We also noted a stronger positive correlation between biomass production and rainfall as compared to intensity, illustrating how edaphoclimatic conditions affect plant growth during the season and how determining harvest frequencies solely based on days may not be effective. It has been reported that a fixed harvest frequency can

be inflexible for tropical forages, leading to grazing at inappropriate times, reduced harvesting efficiency and lower forage nutritional quality (Moura et al., 2021; Pedreira et al., 2007, 2009).

In this context, Mahecha et al. (2022) observed *T. diversifolia* biomass production of 188 and 229 g/plant DM for 40 and 60 days of harvest frequencies, respectively. Ziegler et al. (2022) reported *T. diversifolia* biomass production below 100 g/plant DM with a 42-day harvest frequency, whereas they observed production exceeding 200 g/plant DM with a 72-day harvest frequency. In our study, a biomass production of 236.3 g/plant DM was observed at 42 days, highlighting the ineffectiveness of fixed harvest frequencies. Nonetheless, Alonso et al. (2013), when evaluating *T. diversifolia* productivity at different growth stages, suggested that canopy height may serve as a simple management tool for this plant in SPS. Ziegler et al. (2022) also noted a significant relationship ($R^2 = 0.81$) between biomass production (g/plant DM) and plant height. In line with these findings, our study revealed a high correlation of 0.92 between plant height and biomass production values, indicating that plant height may be a more effective variable for determining harvest frequency compared to fixed harvest intervals based on days.

Our results indicate that *T. diversifolia* reached an average canopy height of 100 cm in just 42 days (Table 2), which is earlier than the previously recommended timeframe for the rainy season (i.e., with 60 days of regrowth). Moreover, heights exceeding the aforementioned range may compromise biomass utilization efficiency, as a portion of the available material could be out of reach for animals (Alonso et al., 2013; Alonso et al., 2015; García, 2017). This aspect should be closely monitored when utilizing *T. diversifolia* as a feed source for small ruminants.

Nevertheless, the 42-day period required for *T. diversifolia* to reach the cutting height (i.e., above 100 cm) is still longer than the period seen in Brazilian grasses, which would probably result in a reduction in the nutritive value of these plants, as these forages are grazed with an interval of harvest equal to or less than 28 days during the rainy season in a rotational stocking method (Lara & Pedreira, 2011; Pedreira et al., 2007, 2009; Pequeno et al., 2015). For *T. diversifolia*, these harvest frequencies (i.e., between 28 and 35 days) result in a reduction of 20 to 30% in leaf accumulation compared to 42-day harvest frequencies. Despite the reduction in biomass production, the nutritive value must also be assessed in order to elucidate the feasibility of utilizing *T. diversifolia* at harvest frequencies similar to those of forages.

4.2 | Nutritive values and in vitro gas production parameters

Despite the absence of effect on biomass production at two harvest intensities, in terms of nutritive value, an increase in the aNDFom and lignin fractions was observed in plants harvested at 30 cm intensity (Table 3), which was probably the factor underlying the slight reduction in the propionate proportion (Table 5), GP, and CH_4 produced per gram of incubated DM, and per gram of degraded OM during 24 hours of incubation (Table 4) observed for this intensity.

It is essential to note that an increase in fibrous fractions (structural carbohydrates) results in a decrease in non-structural carbohydrates (NSC) (Van Soest, 1994). The NSC degradation occurs almost entirely in the first hour of fermentation, as it is readily available for rumen microorganisms, predominantly contributing to gas production during this period (Azevêdo et al., 2003; Sniffen et al., 1992). In contrast, structural carbohydrates undergo slower degradation, requiring particle size reduction, microorganisms attachment to fiber particles, overcoming barriers posed by lignin and undergoing slow multiplication of fibrolytic bacteria, which are generally impacted by the pH reduction due to rapid NSC degradation in the first hour of fermentation (Nussio et al., 2006).

The reduction in propionate is also an effect of the increase in the fibrous fraction, as slow-fermenting carbohydrates, such as structural ones, may reduce propionate production (Amanzougarene & Fondevila, 2020; Getachew et al., 1998). However, these differences observed due to harvest intensity seem to be applicable only in cases of cut-and-carry. In a grazing system, animals are likely to be selective, not following a stubble height and consuming only tender stems and leaves, as evidenced by Figure 4b.

It was observed that nutritive value is affected by harvest frequencies (Table 3), consequently influencing rumen fermentation products, as evidenced by the in vitro gas production parameters (Tables 4 and 5). According to previous studies, the CP content of *T. diversifolia* ranges from 140 to 280 g/kg DM (Londoño et al., 2019; Medina et al., 2009; Osuga et al., 2012). In this study, the CP content of *T. diversifolia* ranged from 180 to 291 g/kg DM (Table 3), indicating a reduction of 37.9% from 21 to 42 days of harvest frequencies, with an average daily reduction rate of approximately 0.5%. There is also an observed linear increase in aNDFom and ADFom content with the increase in harvest frequencies (Table 3), likely due to the increase in stems and dead material, coupled with decreased leaves (Table 2). Consistent with these findings, Londoño et al. (2019) observed a reduction in the leaf: stem ratio in relation to the increase in biomass. Gualberto et al. (2011) stated that the CP content is influenced by the vegetative stage and by the leaf: stem ratio. Ortiz and Vega (2020) also mentioned that the reduction in forage mass quality is influenced by the increase in maturity and the relative proportion of structural components.

As a consequence of CP reduction followed by an increase in aNDFom and ADFom, there is also an observed reduction in IVDOM and NH_3-N (Table 4) with the decrease in harvest frequencies. The reduction in IVDOM is probably associated with the increase in ADFom content, caused by the reduction in leaf: stem ratio and plant maturity. In the early stages of growth, tropical forages tend to have a thin cell wall with less fiber, allowing easy rupture and short digestion times. Over time, cell walls thicken, and both leaves and stems lignify, reducing easy rupture (Ortiz & Vega, 2020), and consequently, MB degradability. Regarding the reduction in NH_3-N, it is known that its concentration is a balance between feed protein degradation and the ammonia uptake (in an in vitro scenario) for microbial protein

synthesis (Hariadi & Santoso, 2010). Therefore, with the reduction of CP in the plant biomass, a reduction in NH_3-N content is also expected.

The main detriment of this reduction in CP and NH_3-N caused by harvest frequencies is observed in the partitioning of OM obtained by the in vitro fermentation assay (Figure 5b), where a linear reduction in OM allocated to MB was observed. It is important to note that the by-products of an in vitro feed incubation with buffered rumen fluid are SCFA, gases (mainly CO_2 and CH_4) and MB (Getachew et al., 1998).

To produce new microbial amino acids (AA) and, consequently, MB synthesis to occur, some dietary constituents and fermentation by-products are necessary. In general, bacteria require the availability of energy, nitrogen (N) and carbon chains. Energy is obtained mainly from rumen-available carbohydrates or AA deamination, providing ATP for cell material biosynthesis (Aldrich et al., 1993; Bach et al., 2005). The NH_3-N is the main supplier of N for AA synthesis, and it is the only source of N for bacteria fermenting structural carbohydrates. However, bacteria fermenting NSC can also use peptides and AA as sources of N (Bach et al., 2005). Carbon chains are provided by end products of carbohydrate fermentation or also by AA deamination (Demeyer & Fievez, 2004) such as branched-chain fatty acids (e.g., isovalerate, isobutyrate, 2-methylbutyrate) (Kozloski, 2009). Thus, the reduction in MB according to decreasing harvest frequencies is related to the decrease in NH_3-N (Table 4), given that it is the main N source for cellulolytic bacteria. Additionally, this reduction is associated with the reduction of branched-chain fatty acids (Table 5) since these are utilized as a carbon skeleton for the new AA formation and the production of new bacterial cells.

The energy derived from carbohydrate degradation that was not used in the MB synthesis will be released as SCFA and gas (mainly CH_4) (Kozloski, 2009). The GP is primarily the result of carbohydrate fermentation, whereas GP from protein fermentation is relatively small (Getachew et al., 1998). Thus, the increase in CH_4 per gram of degraded OM according to decreasing harvest frequencies is presumably correlated with the increase in structural carbohydrates, as observed in the values of aNDFom and ADFom (Table 3).

The increase or reduction in GP generally follows two hypotheses: (1) the effect of the change in the acetate:propionate:butyrate ratio, which consequently alters the availability of CO_2 and H_2 in the rumen (both byproducts of CH_4 production), or (2) the allocation of degraded OM into MB (Pérez-Márquez et al., 2023). It is already known that there is a strong relationship between carbohydrate and protein metabolism in the rumen (Nocek & Russell, 1988). When the rate of protein degradation exceeds the rate of carbohydrate fermentation, large amounts of N can be lost as NH_3-N. Conversely, when the rate of carbohydrate fermentation exceeds the rate of protein degradation, microbial protein synthesis may decrease (Bach et al., 2005; Nocek & Russell, 1988). We did not observe an effect of harvest frequencies on the concentrations of SCFA. So, this indicates that the increase in GP and CH_4 per gram of degraded OM was caused by the reduction of OM allocated to MB, which is associated with an increase in structural carbohydrates and a decrease in CP.

Despite the reduction in CP and the increase in fiber fraction caused by the decrease in harvest frequencies, which consequently affected in vitro fermentation parameters (e.g., IVDOM, NH_3-N and MB), in cumulative terms, this reduction was diluted by the significant increase in biomass (Table 6). Thus, these variables continued to increase linearly (in gplant DM) until 42 days when *T. diversifolia* reached the recommended harvest height of 100 cm, as previously described. However, when reaching around 90 cm in height at 35 days (10 cm less than the recommended harvest height), the biomass still had approximately 50% of degraded OM.

After 35-day harvest frequencies, the concentration of undegraded OM surpassed the degraded OM (Figure 5a). Furthermore, at 35 days, when the plant height was 90 cm, 29.2% of the accumulated IVDOM was allocated to SCFA, 19.0% to gas and 51% to MB. At 42 days, when the plant reached 100 cm, these allocations shifted to 31.9% for SCFA, 21.1% for gas and 47.1% for MB. Additionally, the OM allocated for gas increased by 32.7% from 35 to 42 days, while for MB, the increase was only 18.5% during the same period. This suggests that the increase in OM allocated for MB is accompanied by a notable rise in OM destined for gas production, particularly for harvest frequencies exceeding 35 days. Furthermore, harvest frequencies exceeding 35 days may not only affect the nutritional value of biomass but also likely impact animal preference, as reported by Velázquez et al. (2022), who observed that sheep preferred *T. diversifolia* at 42 days over older plants, indicating that consumption decreases with plant age. These authors attributed this to nutritional value and a higher leaf: stem ratio.

Based on these findings, we propose that *T. diversifolia* can be managed with a harvest frequency determined by the pre-grazing height of 90 cm, which, in this case, was achieved with a 35-day regrowth period during the rainy season. At this height, *T. diversifolia* already exhibits high biomass production. Moreover, from the produced biomass, there is still a greater proportion of degraded OM compared to the undegraded fraction. This allocation could provide acceptable values of OM for MB with the amount of OM designated for gas production lower than the values observed for biomass collected at 42-day harvest frequencies.

It is important to note that, despite reports of *T. diversifolia*'s rapid recovery capacity following cutting (Londoño et al., 2019), and the demonstration in this study that *T. diversifolia* can be managed at harvest frequencies higher than those recommended in the literature, it remains crucial to assess plant persistence with consecutive cuts. As noted by Partey (2011), in many agroforestry species, reserve carbohydrates play a pivotal role in biomass production. Increasing the harvest frequency may lead to a reduction in these reserves due to their mobilization for the reconstruction of photosynthetic tissue after cutting or grazing. This, in turn, can decrease the regrowth rate, biomass production and long-term persistence of *T. diversifolia*. Therefore, it is essential to emphasize the significance of using canopy height as a determining criterion for selecting the appropriate harvest frequency. This parameter provides flexibility in adjusting the regrowth period in response to edaphoclimatic fluctuations throughout the year. Additionally, it is

important to highlight that these results were obtained under ideal soil cultivation conditions, without any chemical or physical limitations. Therefore, it is advisable for the producer to assess soil fertility and make necessary corrections, if needed, before establishing the pasture area.

5 | CONCLUSION

Harvest intensity had a limited impact on the biomass production and nutritive value of *T. diversifolia*. Therefore, assuming animals predominantly consume leaves and tender branches in grazing systems, often leaving mature branches behind, harvest intensity may also have a lesser impact since regrowth is ensured by the presence of buds and stored energy in mature branches. Furthermore, *T. diversifolia* can be managed with higher harvest frequencies under Brazilian edaphoclimatic conditions than those typically recommended for tropical regions. We recommend a management practice that incorporates defoliation intensities between 30 and 40 cm stubble height and a pre-grazing canopy height of 90 cm, which can be achieved within a 35-day period during the rainy season in these experimental conditions.

ACKNOWLEDGMENTS

This study was supported by the Brazilian National Council for Scientific and Technological Development (CNPq), under grant numbers 140200/2020-7, 200207/2023-7, 304023/2022-1 and 306116/2023-5 and by the São Paulo Research Foundation (FAPESP), grant number 2024/07657-0. The Article Processing Charge for the publication of this research was funded by the CoordenaÃ§Ã£o de AperfeiÃ§oamento de Pessoal de NÃ-vel Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Vagner Ovani https://orcid.org/0000-0003-2179-5861
Simón Pérez-Márquez https://orcid.org/0000-0003-3108-8159
Alyce Monteiro https://orcid.org/0000-0002-1049-7597
Ana Maria Kruger https://orcid.org/0000-0002-7789-1345
Paulo de Mello Tavares Lima https://orcid.org/0000-0002-6354-3139
Beatriz Elisa Bizzuti https://orcid.org/0000-0001-7535-408X
Lumena Souza Takahashi https://orcid.org/0000-0003-3823-8244
Rogério Martins Maurício https://orcid.org/0000-0002-5688-2255
Helder Louvandini https://orcid.org/0000-0001-7129-8463
Adibe Luiz Abdalla https://orcid.org/0000-0002-5440-9974

REFERENCES

Aldrich, J. M., Muller, L. D., Varga, G. A., & Griel, L. C. (1993). Nonstructural carbohydrate and protein effects on rumen fermentation, nutrient flow, and performance of dairy cows. *Journal of Dairy Science*, *76*(4), 1091–1105. https://doi.org/10.3168/jds.S0022-0302(93)77438-X

Allen, V. G., Batello, C., Berretta, E. J., Hodgson, J., Kothmann, M., Li, X., McIvor, J., Milne, J., Morris, C., Peeters, A., & Sanderson, M. (2011). An international terminology for grazing lands and grazing animals. *Grass and Forage Science*, *66*(1), 2–28. https://doi.org/10.1111/j.1365-2494.2010.00780.x

Alonso, J., Achang, G., Santos, L. D. T., & Sampaio, R. A. (2013). Productividad de tithonia diversifolia y conducta animal a diferentes momentos de comenzar el pastoreo. *Livestock Research for Rural Development*, *25*(11), 1–7.

Alonso, J., Fraga, G. A., Santos, L. D. T., & Sampaio, R. A. (2015). Comportamiento productivo de Tithonia diversifolia en pastoreo con reposos diferentes en ambas épocas del año. *Livestock Research for Rural Development*, *27*(6), 1–7.

Amanzougarene, Z., & Fondevila, M. (2020). Fitting of the in vitro gas production technique to the study of high concentrate diets. *Animals*, *10*(10), 1935. https://doi.org/10.3390/ani10101935

AOAC. (2011). In W. Horwitz & G. W. Latimer (Eds.), *Official methods of analysis of AOAC international* (18th ed.). AOAC International.

Azevêdo, J. A. G., Pereira, J. C., de Queiroz, A. C., Carneiro, P. C. S., Lana, R. D. P., Barbosa, M. H. P., Fernandes, A. M., & Rennó, F. P. (2003). Chemical-bromatological composition, fractionation of carbohydrates and in vitro fiber degradation kinetics of three sugarcane (*Saccharum* spp.) varieties. *Revista Brasileira de Zootecnia*, *32*(6), 1443–1453. https://doi.org/10.1590/S1516-35982003000600019

Bach, A., Calsamiglia, S., & Stern, M. D. (2005). Nitrogen metabolism in the rumen. *Journal of Dairy Science*, *88*(July 2004), E9–E21. https://doi.org/10.3168/jds.S0022-0302(05)73133-7

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Blümmel, M., Makkar, H. P. S., & Becker, K. (1997). In vitro gas production: A technique revisited. *Journal of Animal Physiology and Animal Nutrition*, *77*(1–5), 24–34. https://doi.org/10.1111/J.1439-0396.1997.TB00734.X

Brunetti, H. B., Pezzopane, J. R. M., Bonani, W. L., Bosi, C., Pasquini Neto, R., de Bernardi, A. C. C., & de Oliveira, P. P. A. (2022). Productive and nutritive traits of Piatã palisadegrass after thinning the forest component of a silvopastoral system in southeastern Brazil. *The Journal of Agricultural Science*, *160*(5), 338–348. https://doi.org/10.1017/S002185962200051X

Bueno, I. C. S., Cabral Filho, S. L. S., Gobbo, S. P., Louvandini, H., Vitti, D. M. S. S., & Abdalla, A. L. (2005). Influence of inoculum source in a gas production method. *Animal Feed Science and Technology*, *123*, 95–105. https://doi.org/10.1016/J.ANIFEEDSCI.2005.05.003

Cândido, A. C. T. F., Guerreiro Martorano, L., Cândido, B. U. F., Nascimento, W., Dias, C. T. d. S., Lisboa, L. S. S., Fernandes, P. C. C., Silva, A. R., Dias-Filho, M. B., & Beldini, T. P. (2023). Infrared thermal profiles in silvopastoral and full-sun pastures in the eastern Amazon, Brazil. *Forests*, *14*(7), 1463. https://doi.org/10.3390/f14071463

Carnevalli, R. A., Da Silva, S. C., Bueno, A. A. O., Uebele, M. C., Bueno, F. O., Hodgson, J., Silva, G. N., & Morais, J. P. G. (2006). Herbage production and grazing losses in *Panicum maximum* cv. Mombaça under four grazing managements. *Tropical Grasslands*, *40*(3), 165–176.

Demeyer, D., & Fievez, V. (2004). Is the synthesis of rumen bacterial protein limited by the availability of pre-formed amino acids and/or peptides? *British Journal of Nutrition*, *91*(2), 175–176. https://doi.org/10.1079/BJN20031073

Domiciano, L. F., dos Santos, M. L., Boote, K. J., dos Santos, P. M., Pereira, D. H., & Pedreira, B. C. (2021). Physiological responses and forage accumulation of Marandu palisadegrass and Mombaça guineagrass to nitrogen fertilizer in the Brazilian forage-based systems. *Grassland Science*, *67*(2), 93–101. https://doi.org/10.1111/GRS.12291

Euclides, V. P. B., Montagner, D. B., de Araújo, A. R., de Aragão Pereira, M., dos Santos Difante, G., de Araújo, I. M. M., Barbosa, L. F., Barbosa, R. A., & Gurgel, A. L. C. (2022). Biological and economic responses to increasing nitrogen rates in Mombaça Guinea grass pastures. *Scientific Reports*, *12*(1), 1937. https://doi.org/10.1038/s41598-022-05796-6

Feltran-Barbieri, R., & Féres, J. G. (2021). Degraded pastures in Brazil: Improving livestock production and forest restoration. *Royal Society Open Science*, *8*(7), 201854. https://doi.org/10.1098/RSOS.201854

Filho, J. F. L., de Oliveira, H. M. R., de Souza Barros, V. M., dos Santos, A. C., & de Oliveira, T. S. (2024). From forest to pastures and silvopastoral systems: Soil carbon and nitrogen stocks changes in northeast Amazônia. *Science of the Total Environment*, *908*, 168251. https://doi.org/10.1016/j.scitotenv.2023.168251

García, I. R. (2017). Potential of *Tithonia diversifolia* (Hemsl.) gray as animal feed. *Livestock Research for Rural Development*, *29*(4), 1–11.

Getachew, G., Blümmel, M., Makkar, H. P. S., & Becker, K. (1998). In vitro gas measuring techniques for assessment of nutritional quality of feeds: A review. *Animal Feed Science and Technology*, *72*(3–4), 261–281. https://doi.org/10.1016/S0377-8401(97)00189-2

Giacomini, A. A., da Silva, S. C., Sarmento, D. O. d. L., Zeferino, C. V., Souza Júnior, S. J., da Trinde, J. K., Guarda, V. d. A., & do Nascimento Júnior, D. (2009). Growth of marandu palisadegrass subjected to strategies of intermittent stocking. *Scientia Agricola*, *66*(6), 733–741. https://doi.org/10.1590/s0103-90162009000600003

Gomes, F. J., Cavalli, J., Pedreira, B. C., Pedreira, C. G. S., Holschuch, S. G., & Pereira, D. H. (2022). Forage nutritive value of marandu palisade grass under clipping in a silvopastoral system. *Agroforestry Systems*, *96*(1), 79–88. https://doi.org/10.1007/S10457-021-00696-6

Gualberto, R., Júnior, O. F. S., Costa, N. R., Braccialli, C. D., & Gaion, L. A. (2011). Influence of spacing and plant development stadium in biomass yield and nutritional value of *Tithonia diversifolia* (Hemsl.) gray. *Nucleus*, *8*(1), 242–256. https://doi.org/10.3738/1982.2278.362

Hariadi, B. T., & Santoso, B. (2010). Evaluation of tropical plants containing tannin on in vitro methanogenesis and fermentation parameters using rumen fluid. *Journal of the Science of Food and Agriculture*, *90*(3), 456–461. https://doi.org/10.1002/JSFA.3839

Jank, L., Barrios, S. C., do Valle, C. B., Simeão, R. M., & Alves, G. F. (2014). The value of improved pastures to brazilian beef production. *Crop and Pasture Science*, *65*(11), 1132. https://doi.org/10.1071/CP13319

Kozloski, G. V. (2009). *Bioquímica de ruminantes* (2nd ed.). UFMS.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). LmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13

LAPIG. (2022). *Atlas Digital das Pastagens Brasileiras* https://atlasdaspastagens.ufg.br/map

Lara, M. A. S., & Pedreira, C. G. S. (2011). Estimativa da assimilação potencial de carbono em dosséis de espécies de braquiária. *Pesquisa Agropecuária Brasileira*, *46*(7), 743–750. https://doi.org/10.1590/S0100-204X2011000700010

Lenth R. V. (2021). *emmeans: Estimated Marginal Means, aka Least-Squares Means* (R package version 1.6.0) https://cran.r-project.org/package=emmeans

Lima, P. M. T., Moreira, G. D., Sakita, G. Z., Natel, A. S., Mattos, W. T., Gimenes, F. M. A., Gerdes, L., McManus, C., Abdalla, A. L., & Louvandini, H. (2018). Nutritional evaluation of the legume Macrotyloma axillare using in vitro and in vivo bioassays in sheep. *Journal of Animal Physiology and Animal Nutrition*, *102*(2), e669–e676. https://doi.org/10.1111/JPN.12810

Londoño, J. M. B., Carabalí, A. G., & Londoño, M. A. B. (2019). Yield, agronomic parameters and nutritional quality of *Tithonia diversifolia* in response to different fertilization levels. *Revista Mexicana de Ciencias Pecuarias*, *10*(3), 789–800. https://doi.org/10.22319/rmcp.v10i3.4667

Mahecha, L., Londoño, J. D., & Angulo, J. (2022). Agronomic and nutritional assessment of an intensive Silvopastoral system: Tithonia Diversifolia, Sambucus nigra, Cynodon nlemfuensis, and Urochloa plantaginea. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *92*(1), 37–47. https://doi.org/10.1007/s40011-021-01282-7

Mauricio, R. M., Mould, F. L., Dhanoa, M. S., Owen, E., Channa, K. S., & Theodorou, M. K. (1999). A semi-automated in vitro gas production technique for ruminant feedstuff evaluation. *Animal Feed Science and Technology*, *79*(4), 321–330. https://doi.org/10.1016/S0377-8401(99)00033-4

Mauricio, R. M., Ribeiro, R. S., Silveira, S. R., Silva, P. L., Calsavara, L., Pereira, L. G. R., & Paciullo, D. S. (2014). *Tithonia diversifolia* for ruminant nutrition. *Tropical Grasslands-Forrajes Tropicales*, *2*(1), 82–84. https://www.tropicalgrasslands.info/index.php/tgft/article/view/147

Mbow, C., Rosenzweig, C., Barioni, L. G., Benton, T. G., Shukla, P. R., Skea, J., Calvo Buendia, E., Masson-Delmotte, V., Pörtner, H.-O., Roberts, D. C., Zhai, P., Slade, R., Connors, S., Van Diemen, R., Ferrat, M., Haughey, E., Luz, S., Neogi, S., Pathak, M., … Malley, J. (2019). Food security. In P. R. Shukla, J. Skea, E. C. Buendia, & V. Masson-Delmot (Eds.), *Climate change and land: An IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems* (Vol. 2019). Intergovernmental Panel on Climate Change (IPCC).

Medina, M. G., García, D. E., González, M. E., Cova, L. J., Moratinos, P., Pampanito, T., & Venezuela, T. (2009). Variables morfo-estructurales y de calidad de la biomasa de Tithonia diversifolia en la etapa inicial de crecimiento. *27*(2), 121–134.

Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D., & Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. *The Journal of Agricultural Science*, *93*(1), 217–222. https://doi.org/10.1017/S0021859600086305

Mertens, D. R., Allen, M., Carmany, J., Clegg, J., Davidowicz, A., Drouches, M., Frank, K., Gambin, D., Garkie, M., Gildemeister, B., Jeffress, D., Jeon, C. S., Jones, D., Kaplan, D., Kim, G. N., Kobata, S., Main, D., Moua, X., Paul, B., … Wolf, M. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *Journal of AOAC International*, *85*(6), 1217–1240. https://doi.org/10.1093/jaoac/85.6.1217

Monteiro, A., Barreto-Mendes, L., Fanchone, A., Morgavi, D. P., Pedreira, B. C., Magalhães, C. A. S., Abdalla, A. L., & Eugène, M. (2024). Crop-livestock-forestry systems as a strategy for mitigating greenhouse gas emissions and enhancing the sustainability of forage-based livestock systems in the Amazon biome. *Science of the Total Environment*, *906*, 167396. https://doi.org/10.1016/j.scitotenv.2023.167396

Moura, A. M., Tomich, T. R., Pereira, L. G. R., Paciullo, D. S. C., Gomide, C. A. d. M., & Gonçalves, L. C. (2021). Nutritive value and in vitro methane production of *Urochloa brizantha* cv. Marandu under fixed time or variable stocking cycles. *Grass and Forage Science*, *76*(3), 427–439. https://doi.org/10.1111/gfs.12509

Munaro, M., Barroso, A. A. M., Dominschek, R., Roncatto, E., Michelon, T. B., & Schuster, M. Z. (2023). Silvopastoral system in

subtropical Brazil keeps forage cover stable and prevent weed outbreaks. *Agroforestry Systems*, *97*(6), 985–994. https://doi.org/10.1007/s10457-023-00841-3

Nocek, J. E., & Russell, J. B. (1988). Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *Journal of Dairy Science*, *71*(8), 2070–2107. https://doi.org/10.3168/jds.S0022-0302(88)79782-9

Nussio, L. G., Campos, F. P., & Lima, M. L. M. (2006). Metabolismo de carboidratos estruturais. In *Nutrição de Ruminantes* (p. 583). Funep.

Oliveira, A. F., Menezes, G. L., Gonçalves, L. C., de Araújo, V. E., Ramirez, M. A., Guimarães Júnior, R., Jayme, D. G., & Lana, Â. M. Q. (2022). Pasture traits and cattle performance in silvopastoral systems with *eucalyptus* and *Urochloa*: Systematic review and meta-analysis. *Livestock Science*, *262*, 104973. https://doi.org/10.1016/j.livsci.2022.104973

Oliveira Silva, R., Barioni, L. G., Hall, J. A. J., Moretti, A. C., Fonseca Veloso, R., Alexander, P., Crespolini, M., & Moran, D. (2017). Sustainable intensification of brazilian livestock production through optimized pasture restoration. *Agricultural Systems*, *153*, 201–211. https://doi.org/10.1016/j.agsy.2017.02.001

Ortiz, C. A. N., & Vega, M. L. R. (2020). Determination of in vitro digestibility of forage species used in ruminant feeding. *Tropical Animal Health and Production*, *52*(6), 3045–3059. https://doi.org/10.1007/S11250-020-02325-3 /FIGURES/6

Osuga, I. M., Abdulrazak, S. A., Muleke, C. I., & Fujihara, T. (2012). Potential nutritive value of various parts of wild sunflower (*Tithonia diversifolia*) as source of feed for ruminants in Kenya. *Journal of Food, Agriculture & Environment*, *10*(2), 632–635. www.world-food.net

Ovani, V. S., Pérez-Márquez, S., da Silva, B. T. N., Louvandini, H., Abdalla, A. L., & Olival, A. d. A. (2023). Potential of *Chloroleucon acacioides* trees as an alternative feed supplement for grazing ruminants in a tropical silvopastoral system. *Journal of Agriculture and Food Research*, *11*(February), 100524. https://doi.org/10.1016/j.jafr.2023.100524

Palmer, L. (2014). A new climate for grazing livestock. *Nature Climate Change*, *4*(5), 321–323. https://doi.org/10.1038/nclimate2215

Partey, S. T. (2011). Effect of pruning frequency and pruning height on the biomass production of *Tithonia diversifolia* (Hemsl) a. gray. *Agroforestry Systems*, *83*(2), 181–187. https://doi.org/10.1007/S10457-010-9367-Y

Pedreira, B. C. e., Pedreira, C. G. S., & da Silva, S. C. (2007). Estrutura do dossel e acúmulo de forragem de Brachiaria brizantha cultivar Xaraés em resposta a estratégias de pastejo. *Pesquisa Agropecuária Brasileira*, *42*(2), 281–287. https://doi.org/10.1590/S0100-204X2007000200018

Pedreira, B. C. e., Pedreira, C. G. S., & da Silva, S. C. (2009). Acúmulo de forragem durante a rebrotação de capim-xaraés submetido a três estratégias de desfolhação. *Revista Brasileira de Zootecnia*, *38*(4), 618–625. https://doi.org/10.1590/S1516-35982009000400005

Pequeno, D. N. L., Pedreira, C. G. S., Sollenberger, L. E., De Faria, A. F. G., Silva, L. S., Pequeno, D. N. L., Pedreira, C. G. S., Faria, A. F. G., Silva, L. S., & Zootecnia, D. (2015). Forage accumulation and nutritive value of brachiariagrasses and Tifton 85 bermudagrass as affected by harvest frequency and irrigation. *Agronomy Journal*, *107*(5), 1741–1749.

Pérez-Márquez, S., Ovani, V. S., de Lima, P. M. T., Lana, Â. M. Q., Louvandini, H., Abdalla, A. L., & Maurício, R. M. (2023). Tithonia diversifolia improves in vitro rumen microbial synthesis of sheep diets without changes in Total gas and methane production. *Agronomy*, *13*(11), 2768. https://doi.org/10.3390/agronomy13112768

R Core Team. (2023). *R: A Language and Environment for Statistical Computing* (4.1.1). R Foundation for Statistical Computing; R Foundation for Statistical Computing. https://www.r-project.org/

Rai, P. K., Lee, S. S., Bhardwaj, N., & Kim, K.-H. (2023). The environmental, socio-economic, and health effects of invasive alien plants: Review on Tithonia diversifolia (Hemsl.) a. gray in Asteraceae. *South African Journal of Botany*, *162*, 461–480. https://doi.org/10.1016/j.sajb.2023.09.038

Reis, N. S., Ferreira, I. C., Mazocco, L. A., Souza, A. C. B., Pinho, G. A. S., da Fonseca Neto, Á. M., Malaquias, J. V., Macena, F. A., Muller, A. G., Martins, C. F., Balbino, L. C., & McManus, C. M. (2021). Shade modifies behavioral and physiological responses of low to medium production dairy cows at pasture in an integrated crop-livestock-Forest system. *Animals*, *11*(8), 2411. https://doi.org/10.3390/ani11082411

Resende, L. d. O., Müller, M. D., Kohmann, M. M., Pinto, L. F. G., Cullen Junior, L., de Zen, S., & Rego, L. F. G. (2020). Silvopastoral management of beef cattle production for neutralizing the environmental impact of enteric methane emission. *Agroforestry Systems*, *94*(3), 893–903. https://doi.org/10.1007/S10457-019-00460-X/FIGURES/1

Rivera, J., Chará, J., Arango, J., & Barahona, R. (2021). Effect of different genotypes of *Tithonia diversifolia* on fermentation of feed mixtures with *Urochloa brizantha* cv. Marandú. *Crop and Pasture Science*, *72*(10), 850–859. https://doi.org/10.1071/CP21102

Rivera, J. E., Villegas, G., Chará, J., Durango, S. G., Romero, M. A., & Verchot, L. (2022). Effect of *Tithonia diversifolia* (Hemsl.) a. gray intake on in vivo methane (CH4) emission and milk production in dual-purpose cows in the Colombian Amazonian piedmont. *Translational Animal Science*, *6*(4), txac139. https://doi.org/10.1093/tas/txac139

Sampaio, B. L., Edrada-Ebel, R., & Da Costa, F. B. (2016). Effect of the environment on the secondary metabolic profile of Tithonia diversifolia: A model for environmental metabolomics of plants. *Scientific Reports*, *6*(1), 29265. https://doi.org/10.1038/srep29265

Santos, J. V., Raimundo Bento, L., Dias Bresolin, J., Corso Mitsuyuki, M., Perondi Anchão Oliveira, P., Macedo Pezzopane, J. R., de Carlos Campos Bernardi, A., Carvalho Mendes, I., & Martin-Neto, L. (2022). The long-term effects of intensive grazing and silvopastoral systems on soil physicochemical properties, enzymatic activity, and microbial biomass. *Catena*, *219*, 106619. https://doi.org/10.1016/j.catena.2022.106619

Schmitt Filho, A. L., Kretzer, S. G., Farley, J., Kazama, D. C., Sinisgalli, P. A., & Deniz, M. (2023). Applied nucleation under high biodiversity silvopastoral system as an adaptive strategy against microclimate extremes in pasture areas. *International Journal of Biometeorology*, *67*(7), 1199–1212. https://doi.org/10.1007/s00484-023-02488-2

Shaw, N. H., Jones, R. M., Edye, L. A., & Bryan, W. W. (1976). Developing and testing new pastures. In N. H. Shaw & W. W. Bryan (Eds.), *Tropical pasture research: Principles and methods* (51st ed.) (p. 454). Commonwealth Agricultural Bureaux.

Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., & Russell, J. B. (1992). A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *Journal of Animal Science*, *70*(11), 3562–3577. https://doi.org/10.2527/1992.70113562X

Tambara, A. A. C., Härter, C. J., Rabelo, C. H. S., & Kozloski, G. V. (2021). Effects of supplementation on production of beef cattle grazing tropical pastures in Brazil during the wet and dry seasons: A meta-analysis. *Revista Brasileira de Zootecnia*, *50*, 1–22. https://doi.org/10.37496/RBZ5020210020

Theodorou, M. K., Williams, B. A., Dhanoa, M. S., McAllan, A. B., & France, J. (1994). A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology*, *48*(3–4), 185–197. https://doi.org/10.1016/0377-8401(94)90171-6

Thomas, H. (1980). Terminology and definitions in studies of grassland plants. *Grass and Forage Science*, *35*(1), 13–23. https://doi.org/10.1111/j.1365-2494.1980.tb01488.x

Uu-Espens, C. E., Pozo-Leyva, D., Aryal, D. R., Dzib-Castillo, B. B., Villanueva-López, G., Casanova-Lugo, F., Chay-Canul, A. J., & Canúl-Solís, J. R. (2023). Biomass production and chemical composition of *Tithonia diversifolia* by the date of harvesting at different cutting heights. *Tropical and Subtropical Agroecosystems*, *26*(3), 1–11. https://doi.org/10.56369/tsaes.4888

Van Soest, P. J. (1994). *Nutritional ecology of the ruminant* (2nd ed.). Cornell University Press. https://doi.org/10.7591/j.ctv5rf668

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*(10), 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Velázquez, V. T. V., Pérez Hernández, P., López Ortiz, S., Castillo Gallegos, E., Cruz Lazo, C., & Jarillo Rodríguez, J. (2022). Producción y calidad nutritiva de Tithonia diversifolia (Hemsl.) A. Grey en tres épocas del año y su efecto en la preferencia por ovinos Pelibuey. *Revista Mexicana de Ciencias Pecuarias*, *13*(1), 240–257. https://doi.org/10.22319/rmcp.v13i1.5906

Verdecia, D. M., Herrera, R. S., Ramírez, J. L., Bodas, R., Leonard, I., Giráldez, F. J., Andrés, S., Santana, A., Méndez-Martínez, Y., & López, S. (2018). Yield components, chemical characterization and polyphenolic profile of *Tithonia diversifolia* in Valle del Cauto, Cuba. *Cuban Journal of Agricultural Sciences*, *52*(4), 457–471.

Wei, T., & Simko, V. (2021). R package "corrplot": Visualization of a correlation matrix. https://github.com/taiyun/corrplot

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag New York.

Wickham, H., François, R., Henry, L., & Müller, K. (2021). dplyr: A grammar of data manipulation. https://cran.r-project.org/package=dplyr

Wickham, H., Vaughan, D., & Girlich, M. (2023). Tidyr: Tidy messy data. https://cran.r-project.org/package=tidyr

Wilke C. O. (2020). *cowplot: Streamlined Plot Theme and Plot Annotations for "ggplot2."* https://cran.r-project.org/package=cowplot

Ziegler, A., Rossner, M. B., & Yáñez, E. A. (2022). Defoliation frequency and intensity effects on biomass production and the morphology of *Tithonia diversifolia* (Hemsl.) a. gray. *Livestock Research for Rural Development*, *34*, 62.

How to cite this article: Ovani, V., Pérez-Márquez, S., Monteiro, A., Kruger, A. M., de Mello Tavares Lima, P., Bizzuti, B. E., Takahashi, L. S., Maurício, R. M., Louvandini, H., & Abdalla, A. L. (2025). Understanding the impacts of intensity and harvest frequency on *Tithonia diversifolia* for use in tropical silvopastoral systems. *Grassland Science*, 1–15. https://doi.org/10.1111/grs.70007