

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

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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 affects 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 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.

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 (Jank et al. 2014; Domiciano et al. 2021). However, due to inadequate soil, plant, or herd management, more than half of Brazilian pastures present some level of degradation (Feltran-Barbieri and 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 (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 towards climate fluctuations. These benefits include improvements in soil physical, chemical, and biological properties (Santos et al. 2022), soil organic carbon storage (Resende et al. 2020; Filho et al. 2024), as well as similar total forage accumulation and greater nutritive value (Gomes et al. 2022; Brunetti 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 from Mexico and Central America and widely distributed in the tropics and subtropics (Sampaio et al. 2016; Rai et al. 2023). *T. diversifolia* has been used as 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 (CH₄) emissions (Rivera et al. 2021, 2022; Mahecha 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 (Partey 2011; García 2017; Londoño et al. 2019). The suggested harvest or grazing frequencies typically occurs 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, 2015; García 2017). This recommended frequency is notably higher compared to the grasses commonly used in Brazil, which usually has harvest frequencies of less than 28 days in the rainy season in a rotational stocking method (Carnevali et al. 2006; Pedreira et al. 2007; Giacomini et al. 2009). These distinctive intensities and harvest frequencies pose challenges in establishing management protocols for these plants within an integrated system such as 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 silvopastoral systems.

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 (Fig. 1a and Fig. 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 animals use were approved by the Ethics Committee on Animal Use (CEUA) of CENA/USP (Protocol nº 006-2018).

A total of 96 plants were distributed in randomized blocks in a 2 x 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.

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

CEC, cation exchange capacity; BCS, base-cation saturation, AS, aluminum saturation; SB, sum of bases; OM, organic matter; P, phosphorus; K⁺, potassium; Ca²⁺, calcium; Mg²⁺, magnesium; H⁺, hydrogen ions; Al³⁺, trivalent aluminum.

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 x 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 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, standardization cut was performed on all plants, following to 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 at 40°C (Model MA 037 – Marconi, Piracicaba – SP, Brazil) for 72h, and weighed to determine the morphological composition. The second subsample was also dried, as described before, then ground to 1mm 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 (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 inoculums 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 CO₂ 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 (Fig. 2) (Theodorou et al. 1994).

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

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

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 CH₄ concentration using a gas chromatograph (GC – 2010, Shimadzu, Tokyo, Japan), as described by Lima et al. (2018).

At the end of 24h, 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 each bottle were collected to measure the concentration of ammoniacal N (NH₃-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 (CO₂, CH₄, and H₂O), 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 and Simko 2021). The variables were analyzed using the following model:

$$Y_{ijk} = \mu + I_i + F_j + (I \times F)_{ij} + B_k + H_k + e_{ijk} \quad (02)$$

where, Y_{ijk} represents the dependent variables for each plant " k " at two intensities " i " and different harvest frequencies " j "; μ is the general average; I_i is the fixed effect of intensity; F_j is the fixed effect of harvest frequencies; $(I \times F)_{ij}$ is the interaction effect between intensity and harvest frequencies; B_k is the random effect of blocks; H_k is the random effect of harvest cycle (or of gas production assays for *in vitro* gas production parameters); and e_{ijk} 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 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⁻¹ 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 ($P < 0.01$).

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

Variables	Intensity (cm)		SE	Frequency (days)				SE	P-value				
	30	40		21	28	35	42		Int.	Regression			IxF
										L	Q	R ²	
Canopy height (cm)	74	85	16.1	51	69	90	106	16.3	0.004	<.001	0.898	0.48	0.977
Biomass production (g plant ⁻¹ FM)	1089	1237	538	302	779	1479	2092	549	0.404	<.001	0.741	0.42	0.874
Biomass production (g plant ⁻¹ DM)	122	136	50.4	33	88	157	236	51.9	0.423	<.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 ⁻¹ DM)	742	737	61.7	854	780	696	628	62.9	0.775	<.001	0.890	0.48	0.939
Leaf (g plant ⁻¹ DM)	79	86	22.1	29	68	104	130	22.8	0.401	<.001	0.505	0.51	0.921
Stems (g kg ⁻¹ DM)	247	252	66	146	212	287	353	67.1	0.764	<.001	0.990	0.43	0.954
Stems (g plant ⁻¹ DM)	41	48	27.8	4.9	20	51	101	28.6	0.492	<.001	0.143	0.40	0.903
Dead material (g kg ⁻¹ DM)	11	11	4.64	0.0	8.4	17	19	5.23	0.989	<.001	0.329	0.24	0.932
Dead material (g plant ⁻¹ DM)	2.0	1.7	0.67	0.0	0.5	1.6	5.3	0.88	0.881	<.001	0.047	0.31	0.951

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

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⁻¹ DM) was observed as the harvest frequencies decreased. Proportionally, the difference in biomass accumulation (g plant⁻¹ 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 (Fig. 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 accumulation, 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⁻¹, respectively) and lignin content (175 and 164 g kg⁻¹, respectively) between the 30 and 40 cm intensities. For the harvest frequencies effects, there was a linear increase ($P < 0.01$) in aNDFom and ADFom fractions with the decrease in harvest frequencies, while CP and ash decreased linearly ($P < 0.01$).

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

Variables	Intensity (cm)		SE	Frequency (days)				SE	P-value				
	30	40		21	28	35	42		Int.	Regression			IxF
										L	Q	R ²	
DM (g kg ⁻¹)	910	912	11.8	906	906	916	915	11.9	0.461	0.020	0.864	0.08	0.439
Ash (g kg ⁻¹)	101	100	1.24	101	106	99	98	1.6	0.491	0.026	0.054	0.13	0.549
CP (g kg ⁻¹)	231	223	17	291	239	198	180	17.7	0.310	<.001	0.047	0.64	0.949
EE (g kg ⁻¹)	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 ⁻¹)	567	551	9.45	532	542	563	599	10.9	0.045	<.001	0.152	0.35	0.009
ADFom (g kg ⁻¹)	398	389	15.8	350	384	403	437	17.0	0.351	<.001	0.993	0.40	0.171
Lignin (sa) (g kg ⁻¹)	175	164	14.2	167	168	175	167	14.6	0.030	0.786	0.488	-	0.277

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

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 tested intensities, with the 40 cm harvest intensity leading to greater total gas production (GP) and CH₄ per incubated and per degraded OM. Regarding harvest frequencies effects, a linear increase in GP and CH₄ production per degraded OM was observed with the decrease in harvest frequencies, along with a decrease in IVDOM and NH₃-N.

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

Variables	Intensity (cm)		SE	Frequency (days)				SE	P-value				
	30	40		21	28	35	42		Int.	Regression			IxF
										L	Q	R ²	
GP (mL g ⁻¹ 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 ⁻¹ 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 ⁻¹ 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 ⁻¹ 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 ⁻¹)	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 ⁻¹)	0.04	0.04	0.001	0.04	0.04	0.03	0.03	0.002	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

OM, organic matter; DOM, degraded organic matter, IVDOM, *In vitro* degradability of organic matter; NH₃-N, Ammoniacal N; GP, Gas production; CH₄, methane production; SE, Standard error; Int., Intensity; L, Linear regression; Q, Quadratic regression; IxF, Intensity vs Frequency interaction. Variables deemed significant within a 5% confidence interval.

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.

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

Variables	Intensity (cm)		SE	Frequency (days)				SE	P-value				
	30	40		21	28	35	42		Int.	Regression			IxF
										L	Q	R ²	
Total (mmol L ⁻¹)	1.65	1.68	0.282	1.70	1.68	1.66	1.62	0.282	0.206	0.360	0.853	-	0.881
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

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

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 (Fig. 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⁻¹ 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 were observed ($P < 0.05$) according to decreasing harvest frequencies.

Table 06 Accumulation (g plant⁻¹ DM) of nutritive values, and *in vitro* fermentation parameters of *Tithonia diversifolia* biomass cultivated under two intensities and four harvest frequencies

Variables	Intensity (cm)		SE	Frequency (days)				SE	P-value				
	30	40		21	28	35	42		Int.	Regression			IxF
										L	Q	R ²	
DM	122	136	50.4	33.4	88.3	156.9	236.3	51.9	0.423	<.001	0.559	0.47	0.910
CP	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	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	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)	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	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	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	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	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	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

DM, dry matter; SE: Standard error; Int.: Intensity; L: Linear regression; Q: Quadratic regression; IxF: Intensity vs Frequency interaction; CP, crude protein; EE, ether extract; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; ADFom, acid detergent fiber expressed exclusive of residual ash; IVDOM, *in vitro* degraded organic matter; SCFA, dry matter potentially partitioned for Short-Chain Fatty Acid production; Gas, dry matter potentially partitioned for CO₂, CH₄, and H₂O production. MB, dry matter potentially partitioned for Microbial Biomass production. Variables deemed significant within a 5% confidence interval.

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 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 (Partey 2011; García 2017; Londoño et al. 2019), 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 the total biomass production of *T. diversifolia* at 40 cm, 60 cm, and 80 cm of stubble height, but leaf production was greater at the 80 cm. These authors suggested that this might be due to the remaining stems maintaining a greater number of buds, which favor 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 have a predominance of leaves at branch extremities (Fig. 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 use to be grazed, as observed under field conditions (Fig. 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 matured stems that are not grazed (Fig. 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^{-1} DM), the linear increase in biomass production remains more substantial, maintaining a continuous linear increase in leaf accumulation until 42 days (g plant^{-1} 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 (Pedreira et al. 2007, 2009; Moura et al. 2021).

In this context, Mahecha et al. (2022) observed *T. diversifolia* biomass production of 188 and 229 g plant^{-1} DM for 40 and 60 days of harvest frequencies, respectively. Ziegler et al. (2022) reported *T. diversifolia* biomass production below 100 g plant^{-1} DM with a 42-day harvest frequency, whereas they observed production exceeding 200 g plant^{-1} DM with a 72-day harvest frequency. In our study, a biomass production of 236.3 g plant^{-1} 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 silvopastoral systems. Ziegler et al. (2022) also noted a significant relationship ($R^2 = 0.81$) between biomass production (g plant^{-1} 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, 2015; García 2017). This aspect should be closely monitored when utilizing *T. diversifolia* as a feed source for small ruminants.

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 time. This parameter offers flexibility in adjusting the regrowth period in response to edaphoclimatic fluctuations throughout the year.

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 (Pedreira et al. 2007, 2009; Lara and Pedreira 2011; 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 accessed 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 CH₄ 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 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 (Sniffen et al. 1992; Azevêdo et al. 2003). 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 (Getachew et al. 1998; Amanzougarene and Fondevila 2020). 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 Fig. 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* range from 140 to 280 g kg⁻¹ DM (Medina et al. 2009; Osuga et al. 2012; Londoño et al. 2019). In this study, the CP content of *T. diversifolia* ranged from 180 to 291 g kg⁻¹ 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 NH₃-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 thickens, and both leaves and stems lignify, reducing easy rupture (Ortiz and Vega 2020), and consequently, MB degradability. Regarding the reduction in $\text{NH}_3\text{-N}$, 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 and Santoso 2010). Therefore, with the reduction of CP in the plant biomass, a reduction in $\text{NH}_3\text{-N}$ content is also expected.

The main detriment of this reduction in CP and $\text{NH}_3\text{-N}$ caused by harvest frequencies is observed in the partitioning of organic matter obtained by the *in vitro* fermentation assay (Fig. 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 $\text{NH}_3\text{-N}$ 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 and Fievez 2004) such as branched-chain fatty acids (e.g., isovalerate, isobutyrate, 2-methylbutyrate) (Kozloski 2009). The reduction in MB according to decreasing harvest frequencies is related to the decrease in $\text{NH}_3\text{-N}$ (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 carbohydrates 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 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 and Russell 1988). When the rate of protein degradation exceeds the rate of carbohydrate fermentation, large amounts of N can be lost as $\text{NH}_3\text{-N}$. Conversely, when the rate of carbohydrate fermentation exceeds the rate of protein degradation, microbial protein synthesis may decrease (Nocek and Russell 1988; Bach et al. 2005). 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.

The ratios of SCFA in the rumen typically exhibit a molar proportion of 65:25:10 for acetate:propionate:butyrate in forage-based diets and 50:40:10 for diets rich in concentrates, depending on the pH (Nussio et al. 2006). Although we did not observe differences between harvest frequencies, we noticed a ratio of acetate:propionate:butyrate around 71:17:08. This is a typical effect of the high concentration of structural carbohydrates in *T. diversifolia*, as acetate is produced from the fermentation of the fibrous fraction (Amanzougarene and Fondevila 2020).

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, $\text{NH}_3\text{-N}$, 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 g plant^{-1} DM) until 42 days when *T. diversifolia* reached the recommended harvest height of 100 cm, as previously described.

However, even 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 (Fig. 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 emphasize that these results were obtained in soil presenting ideal cultivation conditions, without chemical or physical limitations. Thus, 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 using 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.

Declarations

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Author Contributions

VO: Conceptualization, Methodology, and Writing - Original Draft; **SPM, AM, AMK, PMTL, BEB, and LST:** Investigation and Writing – Review & Editing; **HL and ALA:** Resources, Supervision, and Writing – Review & Editing.

Declaration of interest

The authors report no conflict of interest.

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Figures

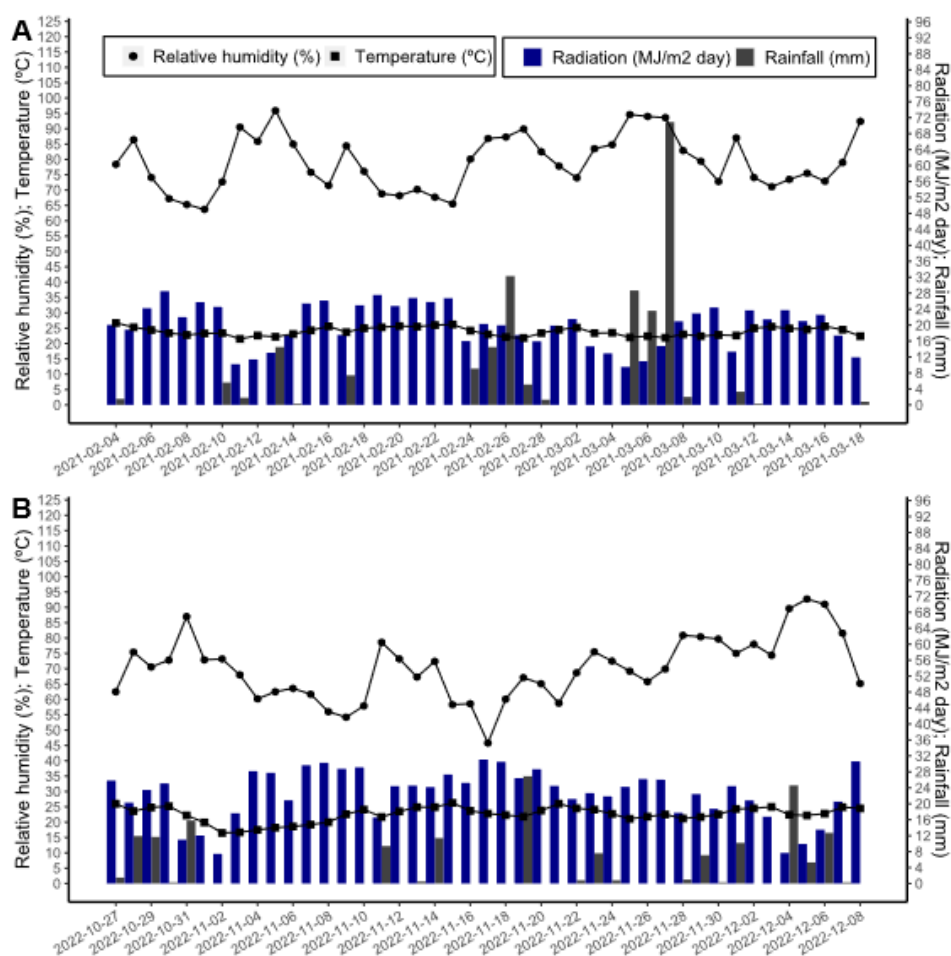


Figure 1

Weather condition during the first (a) and second (b) biomass harvest cycles

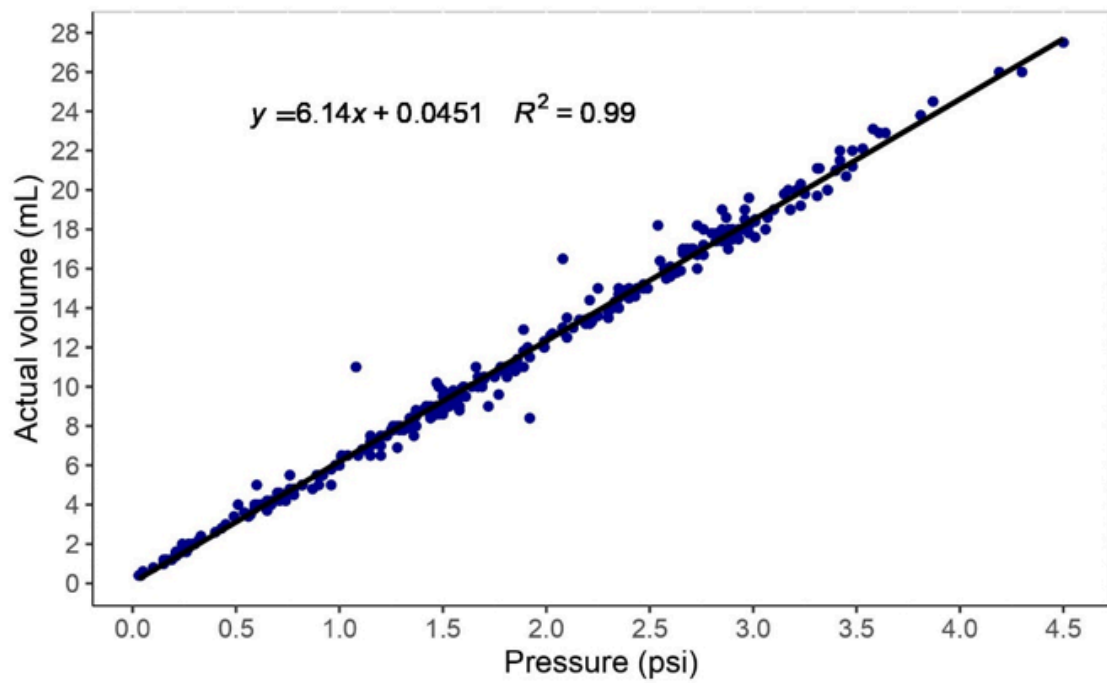


Figure 2

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

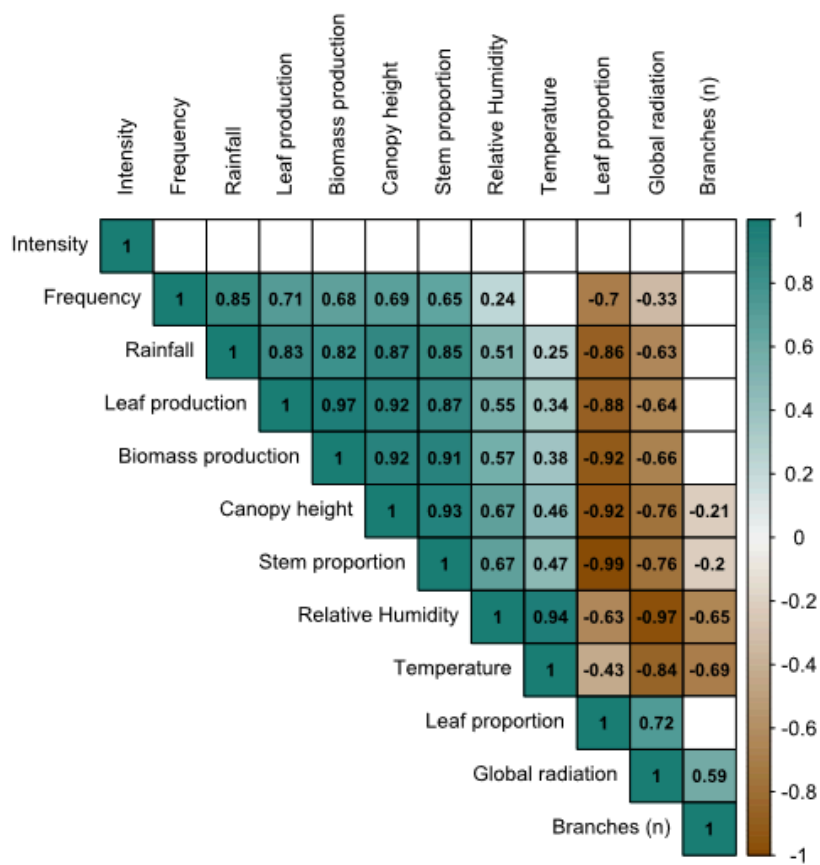


Figure 3

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

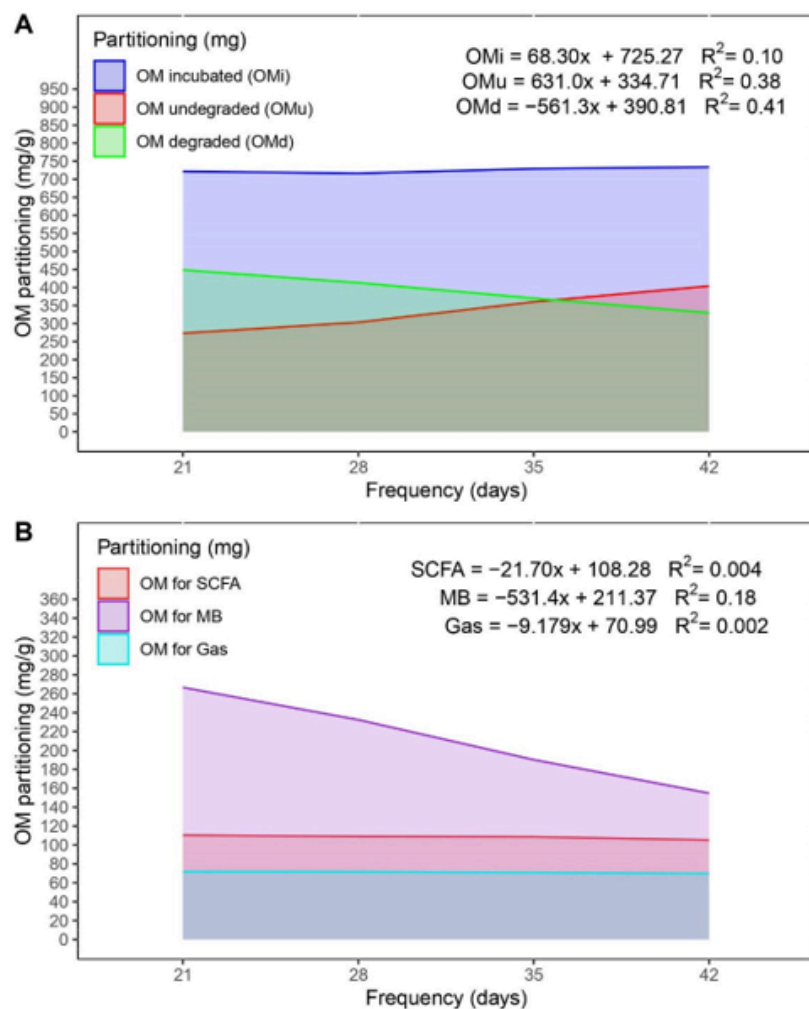


Figure 4

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

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [S1.biomassproductionandnutritionalvaluedata.xlsx](#)
- [S2.invitrogasproductiondata.xlsx](#)