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Phenological and nutritional dynamics of *Tithonia diversifolia* across harvest intervals in tropical mid-altitudes

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Abstract As a protein-bank shrub for cut-and-carry use within silvopastoral agroforestry, *Tithonia diversifolia* can stabilize on-farm protein supply, yet links between its phenology, forage quality, and yield remain scarce. This study evaluated morphological (plant height, stem length and diameter, number of leaves and stems, leaf-to-stem ratio), chemical (DM, CP, aNDFn, ADFn, ADL, EE, NSCn, OM, NE_L), and yield (biomass and nutrient yields per cut and per year) responses of *T. diversifolia* across five harvest intervals (20, 30, 40, 50, and 60 days) under mid-altitude tropical conditions to identify phenological indicators of optimal harvest time. Twelve 36 m^2 plots were arranged in a randomized block design (three slope positions) and monitored using repeated measures. Growth stage significantly affected nutrient concentrations ($P < 0.05$), nutrient yields, and morphological traits ($P < 0.0001$). Crude protein declined from 268 to 134 $g \cdot kg^{-1}$ DM, while aNDFn, ADFn, and lignin increased; NE_L followed a cubic pattern, and NSCn peaked at 20 and 50 days. Biomass and nutrient yields per cut increased with age, but annual yields of DM, CP, NSCn, OM, and lignin peaked at 40 days, as longer regrowth intervals reduced harvest frequency. Plant height, stem length, and diameter correlated negatively with CP and NE_L but positively with nutrient yields ($|r| \geq 0.80$; $P < 0.01$). The optimal harvest stage was 40 days, when plant height was 122 cm, stem length 73 cm, and stem diameter 8.2 mm, balancing forage quality and productivity. These indicators offer low-cost tools to optimize cutting schedules and enhance silvopastoral sustainability.

Keywords Fodder banks · Forage quality · Nutrient yield · Optimal harvest time · Sustainable livestock feeding · Tropical forages

Abbreviations

ADFn	Acid detergent fiber corrected for nitrogen
ADL	Acid detergent lignin
aNDFn	Neutral detergent fiber treated with α-amylase and corrected for nitrogen
CP	Crude protein

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DM	Dry matter
EE	Ether extract
L:S	Leaf-to-stem ratio
NE_L	Net energy for lactation (1 × maintenance)
NSCn	Non-structural carbohydrates corrected for nitrogen
OM	Organic matter

Introduction

Feed represents the largest component of livestock production costs (Botero et al. 2019), prompting increased interest in alternative feed sources (Herrera et al. 2020). Trees and shrubs are particularly valued for their superior nutritional profile compared with tropical grasses, as well as for their high biomass yield (Uu et al. 2023). *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae), native to Mexico (Canto et al. 2023) and widely distributed across subtropical regions, has shown strong potential as a high-quality forage for ruminants due to its superior nutritive value compared with tropical grasses (Botero et al. 2019). Its high biomass production capacity under tropical silvopastoral conditions has been demonstrated (Temoche et al. 2025), supporting its integration within agroforestry systems (Escobedo et al. 2025).

As a forage resource for dairy cattle (Mejía et al. 2017), *T. diversifolia* is recognized for its high protein content (Botero et al. 2019) and relatively low fiber concentration compared with tropical grasses (Uu et al. 2023). Although it is not a legume, it accumulates significant amounts of nitrogen in its leaves (Canto et al. 2023), contributing to its elevated protein levels (Botero et al. 2019). In addition, *T. diversifolia* contains beneficial secondary metabolites (Pretti et al. 2018; Herrera et al. 2020), making it an attractive option for sustainable livestock feeding systems.

As in other forage species, the biomass yield and chemical composition of *T. diversifolia* are influenced by the timing of harvest (Uu et al. 2023). In practice, the management of *T. diversifolia* in forage banks and silvopastoral systems is still largely based on chronological age rather than explicit phenological criteria, as producers and many research trials typically schedule harvests using fixed regrowth intervals (Astúa et al. 2021; Uu et al. 2023; Arias et al. 2023). However, these fixed intervals do not account for variability in growth rate across seasons, environments, or cutting heights, leading to inconsistent forage quality and sub-optimal harvests. Because the plant's phenological stages are closely linked to its nutritional value, monitoring phenology enables producers to anticipate fluctuations in forage quality and determine the optimal harvest stage (Lemaire and Belanger 2019). Moreover, evidence from common forage crops shows that phenological indicators provide more accurate guidance for optimizing harvest timing and forage quality than calendar-based schedules (Arzani et al. 2004).

Although several studies have assessed the phenological development and biomass production of *T. diversifolia*, most have evaluated long harvest intervals typical of tropical shrub management (60–90 days) (Ruiz et al. 2021). Recent work has shown that harvest timing strongly influences both yield and nutritive value (Temoche et al. 2025). The present study focused on shorter harvest intervals (20–60 days) under tropical mid-altitude conditions to explore how more frequent cutting affects plant morphology, forage quality, and productivity. Integrating phenological and nutritional traits provides an opportunity to develop practical tools for optimizing forage management strategies (Lemaire and Belanger 2019). We hypothesize that the phenological age of *T. diversifolia* can be used to identify the optimal harvest time that maximizes biomass yield while minimizing nutrient loss. Therefore, the aim of this study was to describe how the morphological structure, chemical composition, and biomass yield of *T. diversifolia* vary with plant age, in order to identify key phenological traits that serve as indicators of the optimal harvest time based on both yield and nutritional quality.

Materials and methods

Location

The experiment was conducted from July to November 2015 at the "El Jazmín" experimental farm of UNISARC, located in Santa Rosa de Cabal, Risaralda, Colombia (4°54′49″ N, 75°37′27″ W; altitude 1,631 m). According to Holdridge's life zone classification (1982), the area is classified as Premontane Wet Forest. The region exhibits a bimodal rainfall pattern, with peak precipitation typically

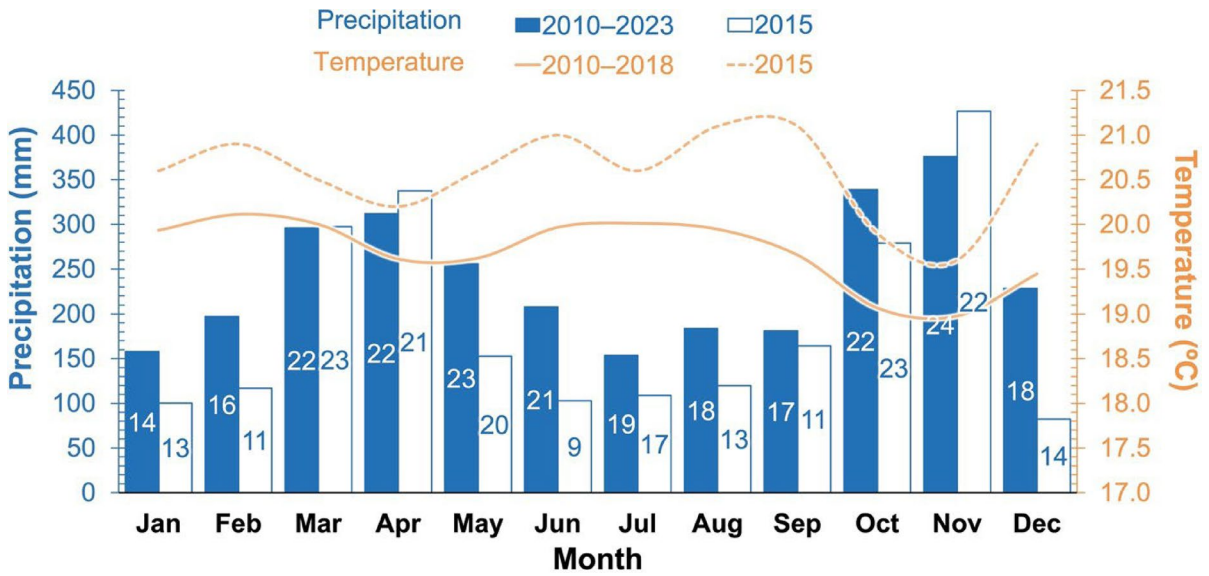


Fig. 1 Monthly precipitation and temperature at the experimental site (Santa Rosa de Cabal, Risaralda, Colombia). Bars represent average monthly precipitation from 2010 to 2023 (dark blue) and observed monthly precipitation in 2015 (white). The solid orange line indicates the average monthly temperature from 2012 to 2018, while the dashed orange line represents monthly temperature in 2015. Numbers within the bars show the average number of rainy days per month (dark blue bars: 2010–2023; white bars: 2015). Data were obtained from the on-site weather station operated by the National Coffee Research Center (CENICAFÉ)

occurring during March–April and October–November (Fig. 1).

From 2010 to 2014, the average annual temperature was 19.4 °C (range: 13.5–30.2 °C), average relative humidity was 81.6%, and average annual precipitation was 3,040 mm, with an average of 256 rainy days per year. The annual sunshine duration was 1,207 h. During the 60-day trial period, the average temperature was 20.1 °C, relative humidity was 79.9%, cumulative precipitation reached 558.3 mm (38 rainy days), and total sunshine duration was 204 h. Climatic data were obtained from the on-site weather station operated by the National Coffee Research Center (CENICAFÉ).

Establishment of plots

Twelve plots of *T. diversifolia* (36 m^2 each; 6 m×6 m) were established on experimental land with a 49° slope (approximately 115% gradient). The plots were arranged into three blocks (four plots per block) according to slope position (upper, middle, and lower), with 1 m spacing between plots.

The site received no liming, fertilization, irrigation, pesticides, or herbicides before and after seeding. Only manual weeding and superficial soil chopping were performed before planting. Soil analysis from the same field (sampled later but representative of the site conditions) indicated a sandy-loam texture, acidic pH (5.99), moderate organic matter (4.33%), and exchangeable Ca, Mg, and K of 7.00, 1.29, and 0.13 $cmol{\cdot}kg^{-1}$, respectively, with available P of 22.23 $mg{\cdot}kg.^{-1}$

Seeding was conducted in July 2015 using vegetative propagation. Cuttings were obtained from naturally established *T. diversifolia* shrubs at the mature vegetative stage on the same farm, using stem segments from the middle portion of the plant, approximately 30 cm in length and 15 mm in diameter, without leaves and containing three buds.

The planting spacing was 60 cm between rows and 30 cm between plants, resulting in a planting density of 55,555 $plants{\cdot}ha^{-1}$. This density was selected to promote rapid canopy closure and uniform biomass production under short harvest intervals. Rows were leveled, and a root growth stimulant composed of cane molasses and *Aloe vera* (1:1 w:w) was applied. Regrowth and cutting survival were monitored weekly; manual weeding was also performed weekly during the

first four weeks after establishment, and dead cuttings were replaced as needed. Two uniformity prunings were performed before the start of the experimental period.

A schematic representation of the experimental layout is provided in Fig. 2.

Procedure

A standardization pruning was performed at 20 cm above ground level before the start of the evaluation. Measurements were taken at 20, 30, 40, 50, and 60 days post-pruning, each representing a single harvest event for that regrowth interval. Only one harvest was conducted per interval; therefore, the study provides a snapshot of plant responses at each regrowth age rather than repeated harvests over time.

Flowering in *T. diversifolia* at this site typically begins after 60 days of regrowth. Because all sampling dates (20–60 days) occurred before or at the onset of flowering, all plants sampled were in the vegetative, pre-flowering stage, and no selection based on flowering status was required.

To complement calendar-based regrowth ages, thermal time was estimated using growing degree days (GDD). GDD was calculated following Jeffrey et al. (2025) and using the species-specific base temperature proposed by Paredes et al. (2025), as shown in Eq. 1.

$$GDD = \sum_{i=0}^{n} \left[\frac{T_{max,i} + T_{min,i}}{2} - T_{base} \right] \quad (1)$$

where $T_{max,\,i}$ and $T_{min,\,i}$ are maximum and minimum temperatures on day i, T_{base} is the species-specific base temperature.

The selected sampling times also reflected common farm practices, where cutting typically occurs between 40 and 50 days after pruning. On each sampling date, four shrubs per plot were randomly selected, avoiding border plants. Each shrub was considered a sampling unit, and the plot-level average was used as the experimental unit.

At 10:00 h, shrub height was measured using a graduated ruler from the base to the apex of the tallest regrowth axis. Each shrub was then pruned to 20 cm above ground level and immediately weighed. This cutting height is recommended because it preserves basal buds and supports vigorous regrowth (Guatusmal et al. 2020; Canto et al. 2023). Leaves and stems were manually separated and weighed individually for each shrub.

Five stems per shrub were randomly selected to measure stem diameter and length. Diameter was measured 10 cm above the base using a digital micrometer. The number of leaves and stems per shrub was also recorded.

After measurement, the leaves and stems from each plot were chopped using a grass chopper and mixed manually. Two 500 g subsamples (one of

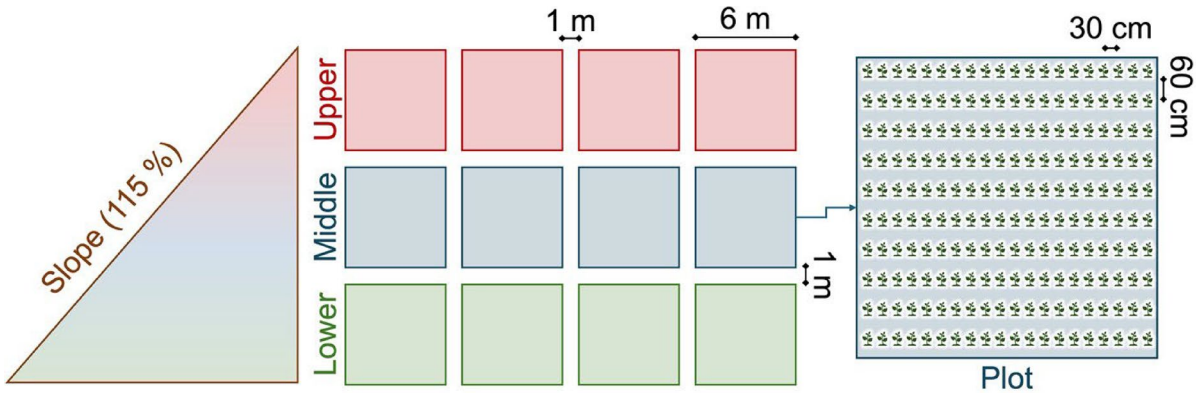


Fig. 2 Experimental layout of *Tithonia diversifolia* plots across slope positions. The experiment consisted of 12 plots (6×6 m; 36 m^2 each) established on land with a 49° slope (115% gradient). Plots were arranged into three blocks (upper, middle, and lower slope positions), with four plots per block and 1 m spacing between plots. Each plot was planted using vegetative cuttings at 60×30 cm spacing (55,555 plants·ha^{-1})

leaves and one of stems) were packed in plastic bags, stored at –20 °C, dehydrated in a forced-air oven at 60 °C for 48 h, and ground using a Udy® mill fitted with a 1 mm sieve. Dried samples were then proportionally mixed on a dry matter (DM) basis to obtain a composite sample per plot.

Calculations

Dry matter yield per cut ($DM_{y/c}$, g) was calculated using the following Eq. (2):

$$DM_{y/c} = PW \times DM_c \quad (2)$$

where PW PW is plant weight (g) and DM_c is DM concentration ($g \cdot g^{-1}$ fresh forage).

Nutrient yield per cut ($N_{y/c}$, g) and energy yield per cut ($E_{y/c}$, cal) were calculated using the following Eq. (3, 4):

$$N_{y/c} = DM_{y/c} \times N_c \quad (3)$$

$$E_{y/c} = DM_{y/c} \times E_c \quad (4)$$

where $DM_{y/c}$ is DM per cut (g), N_c is nutrient concentration ($g \cdot g^{-1}$ DM), and E_c is energy concentration ($cal \cdot g^{-1}$ DM).

Annual yields of DM ($DM_{y/yr}$, g), nutrients ($N_{y/yr}$, g), and energy ($E_{y/yr}$, cal) were obtained by multiplying the corresponding per-cut yields ($DM_{y/c}$, $N_{y/c}$, and $E_{y/c}$) by the number of harvest cycles per year. The annual number of harvest cycles was estimated as the ratio between the total number of days in a year (365) and the growth age (days) of each cycle.

The nutrient and energy yields per cut ($N_{y/c}$ and $E_{y/c}$) represent values obtained from a single harvest event at each harvest interval. The corresponding annual yields were extrapolated from these single-event results assuming uniform regrowth and survival throughout the year. Therefore, the reported annual yields should be interpreted as theoretical estimates, not as measurements from consecutive cuts, and do not account for long-term persistence or cumulative regrowth capacity.

Chemical analysis

Ground forage samples were analyzed for DM and ash content using gravimetric methods according to AOAC (2010). Crude protein (CP) was determined using the Kjeldahl method (Thiex et al. 2002), and ether extract (EE) by the Soxhlet method (AOAC 2010).

Fiber fractions were analyzed following Van Soest et al. (1991), including neutral detergent fiber with α-amylase and corrected for nitrogen (aNDFn, without sodium sulfite), acid detergent fiber corrected for nitrogen (ADFn), and acid detergent lignin (ADL). Insoluble CP in neutral detergent and acid detergent were quantified by Kjeldahl analysis of the respective aNDF and ADF residues (Licitra et al. 1996).

Non-structural carbohydrates corrected for nitrogen (NSCn) and net energy for lactation at 1 × maintenance (NE_L) were estimated according to NRC (2001). Organic matter (OM) was calculated by subtracting ash content from DM (Miyaji et al. 2014).

Statistical analysis

Data were analyzed using a repeated measures design over time, with slop location included as a blocking factor, according to the following model: $y_{ijk} = \mu + \beta_i + \delta_{ij} + \lambda_k + \varepsilon_{ijk}$, where y_{ijk} is the observed value; μ is the overall mean; β_i is the random effect of block (slope position); λ_k is the fixed effect of growth age; δ_{ij} is the random error (mean = 0 and variance σ^2_δ), representing variation among plots within blocks and the covariance between repeated measurements; and ε_{ijk} is the residual error (mean = 0 and variance σ^2), representing within-plot variation over time. Post hoc comparisons among sampling days were conducted using Tukey's test, with significance declared at $P < 0.05$. A Poisson distribution was applied for discrete variables.

When the assumption of sphericity was violated (Mauchly's Test, $P < 0.05$), Greenhouse–Geisser epsilon values were used to adjust P-values.

To model the response of variables to plant age, the following regression models were used (Eq. 5–12):

$$Linear: y_{(t)} = \beta_0 + \beta_1 \cdot t + \varepsilon \quad (5)$$

$$Quadratic: y_{(t)} = \beta_0 + \beta_1 \cdot t + \beta_2 \cdot t^2 + \varepsilon \quad (6)$$

$$Cubic: y_{(t)} = \beta_0 + \beta_1 \cdot t + \beta_2 \cdot t^2 + \beta_3 \cdot t^3 + \varepsilon \quad (7)$$

$$Gompertz: y_{(t)} = \beta_0 \cdot e(-\beta_1 \cdot e(-\beta_2 \cdot t)) + \varepsilon \quad (8)$$

$$Logistic: y_{(t)} = \beta_0 \cdot \left[1 + \beta_1 \cdot e(-\beta_2 \cdot t)\right]^{-1} + \varepsilon \quad (9)$$

$$Von\ Bertalanffy: y_{(t)} = \beta_0 \cdot \left[1 - \beta_1 \cdot e(-\beta_2 \cdot t)\right]^{3} + \varepsilon \quad (10)$$

$$Brody: y_{(t)} = \beta_0 \cdot \left[1 - \beta_1 \cdot e(-\beta_2 \cdot t)\right] + \varepsilon \quad (11)$$

$$Exponential: y_{(t)} = \beta_0 \cdot e(\beta_1 \cdot t) + \varepsilon \quad (12)$$

were evaluated, where $y_{(t)}$ is the dependent variable as a function of growth age t (days), β_0, β_1, β_2, and β_3 are model parameters, and ε is the residual error, assumed to be normally distributed with zero mean and constant variance.

The modelling objective was to describe age-related trends rather than to predict nutritional values. Models were retained only when statistically significant ($P < 0.01$), biologically plausible, and with $R^2_{adj} > 0.50$. Performance was assessed using R^2_{adj}, AIC, and BIC, with preference for simpler models to avoid overfitting.

All statistical analyses were performed in R (version 4.3.1; R-Core Team, 2023), using the packages nlme, emmeans, easynls, glmmTBM, and DHARMA.

Results

Growth age effects on nutrient concentration

Growth age influenced the concentrations of DM in the whole plant, leaves, and stems, as well as the concentrations of CP, aNDFn, ADFn, ADL, NSCn, OM, NE_L ($P < 0.0001$), and EE ($P = 0.0101$) in *T. diversifolia* (Table 1). The highest concentrations of whole-plant DM, CP, EE, and NE_L were recorded at 20 days of regrowth, coinciding with the lowest values of the fiber fractions aNDFn, ADFn, and ADL ($P < 0.05$). Stem DM peaked at 60 days, while the highest DM values in leaves were recorded at 20, 50, and 60 days ($P < 0.05$). Non-structural carbohydrates concentration reached its lowest point at 30 days and peaked at 20 and 50 days ($P < 0.05$). Organic matter concentration was lowest at 30 days and highest at 60 days ($P < 0.05$).

Table 1 Variation in the nutritional composition of *Tithonia diversifolia* (Hemsl.) A. Gray across growth ages

Ítem	Growth age (days)					SEM	P-value
	20	30	40	50	60		
DM (g·kg^{-1})							
Plant	119.3 ± 7.1^{a}	96.7 ± 7.2^{d}	93.4 ± 6.5^{d}	106.4 ± 8.6^{c}	112.1 ± 8.5^{b}	1.7	< 0.0001
Leaves	140.4 ± 7.4^{a}	125.3 ± 7.8^{b}	125.2 ± 7.6^{b}	136.5 ± 8.0^{a}	139.4 ± 7.8^{a}	2.1	< 0.0001
Stems	71.5 ± 8.2^{c}	65.1 ± 5.6^{d}	72.6 ± 6.7^{c}	90.0 ± 8.1^{b}	98.5 ± 7.2^{a}	1.8	< 0.0001
	g·kg^{-1} DM						
CP	268.4 ± 20.8^{a}	215.2 ± 22.9^{b}	170.1 ± 19.3^{c}	130.9 ± 16.6^{d}	134.3 ± 18.1^{d}	3.8	< 0.0001
aNDFn	215.7 ± 37.2^{d}	315.6 ± 36.4^{c}	348.6 ± 22.7^{b}	333.1 ± 43.0^{bc}	392.6 ± 55.8^{a}	9.4	< 0.0001
ADFn	212.7 ± 33.4^{c}	327.8 ± 48.3^{b}	365.0 ± 62.1^{b}	438.7 ± 49.8^{a}	460.6 ± 59.3^{a}	14.0	< 0.0001
ADL	81.7 ± 31.8^{e}	149.9 ± 25.9^{b}	175.9 ± 13.4^{a}	126.9 ± 18.7^{c}	106.6 ± 18.1^{d}	5.2	< 0.0001
EE	18.9 ± 5.0^{a}	12.3 ± 6.0^{b}	13.3 ± 3.5^{b}	13.6 ± 6.1^{b}	17.7 ± 6.8^{ab}	1.5	0.0101
NSCn	365.1 ± 54.2^{a}	300.5 ± 55.4^{c}	334.8 ± 16.7^{b}	391.2 ± 38.0^{a}	337.4 ± 50.3^{b}	9.5	< 0.0001
OM	868.2 ± 10.9^{b}	843.6 ± 11.9^{c}	866.7 ± 9.1^{b}	868.8 ± 6.0^{b}	882.0 ± 8.6^{a}	2.3	< 0.0001
	cal·kg^{-1} DM						
NE_L	1296 ± 185^{a}	748 ± 119^{d}	892 ± 124^{c}	1065 ± 109^{b}	1081 ± 126^{b}	29	< 0.0001

Growth ages: days after cutting; values represent mean ± standard deviation; means within rows with different superscripts (a, b, c, d) differ significantly ($P < 0.05$; Tukey test). SEM, standard error of mean. Ítem: DM, dry matter; CP, crude protein; aNDFn, neutral detergent fiber treated with α-amylase and corrected for nitrogen; ADFn, acid detergent fiber corrected for nitrogen; ADL, acid detergent lignin; EE, ether extract; NSCn, non-structural carbohydrates corrected for nitrogen; OM, organic matter; NE_L, net energy for lactation (1 × maintenance; NRC 2001).

Model fit analysis for nutrient concentration changes

Changes in the concentrations of DM (whole plant and stems), aNDFn, and ADL ($P < 0.0001$) were initially best fitted by cubic models according to statistical criteria. However, given that only five growth ages were evaluated, cubic functions produced inflection points that lacked biological justification and indicated overfitting of the limited dataset. Because the aim of the modelling was to describe general age-related tendencies rather than to generate predictive equations, simpler and biologically coherent models were preferred.

Biologically, whole-plant DM concentration declined during early regrowth (20–40 days), whereas stem DM increased progressively up to 60 days. Crude protein concentration decreased sharply with advancing age. In contrast, fiber fractions increased steadily with regrowth age: aNDFn and ADFn rose linearly across the evaluated range, while lignin (ADL) increased up to mid-regrowth and then stabilized or declined slightly at later stages. Consequently, quadratic functions were selected to summarize age-related trends in whole-plant DM, CP, and ADL, while linear models were retained for aNDFn and ADFn ($P < 0.0001$; Fig. 3).

Changes in the concentrations of leaf DM, NSCn, OM, and NE_L ($P < 0.0001$), as well as EE ($P < 0.0041$), were also best fitted by cubic models according to statistical criteria. Despite statistical significance, these variables showed relatively low R^2_{adj} values, indicating limited explanatory power; therefore, these models were not retained for interpretation. In particular, R^2_{adj} values were ≤ 0.50 for leaf DM (0.42), NSCn (0.29), EE (0.15), and OM (0.50), suggesting that the models explained less than half of the observed variability. NE_L differed from the other discarded variables in that it showed a high R^2_{adj}

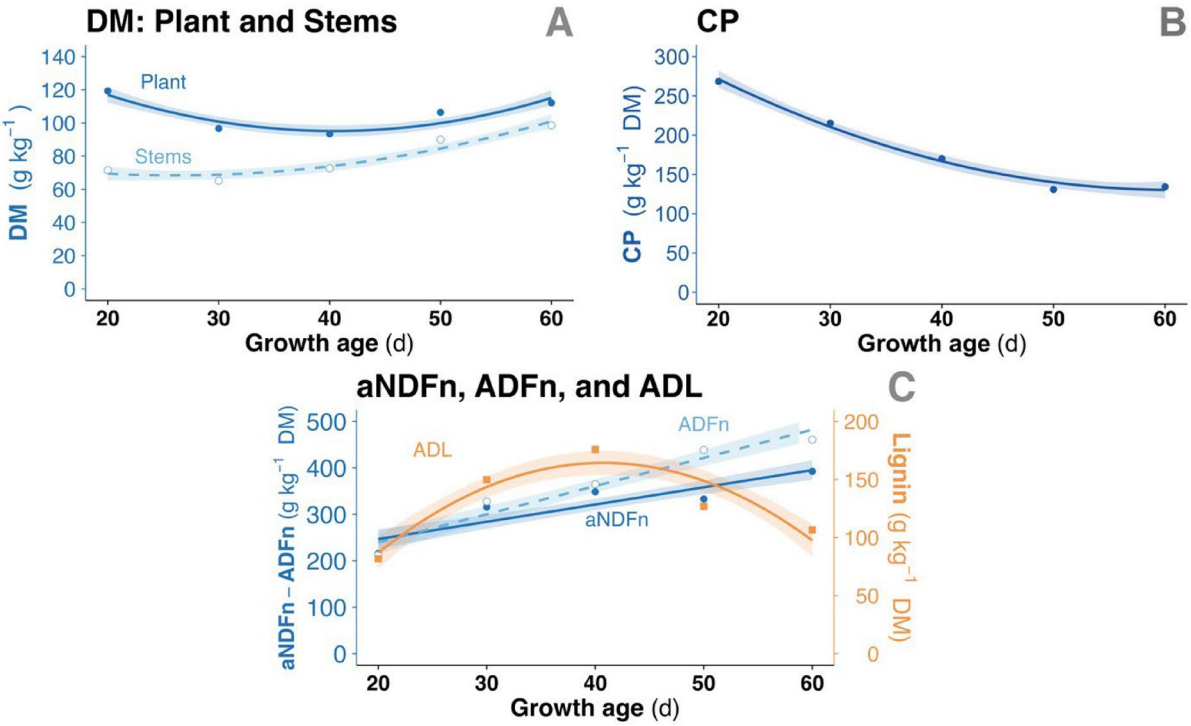


Fig. 3 Observed means and fitted curves describing changes in nutritional composition of *Tithonia diversifolia* (Hemsl.) A. Gray across growth ages. Solid and dash lines represent the fitted; ●, ○ and ■ represent observed means at each growth age, and shaded bands represent the 95% confidence interval of the fitted curves. The selected models were all statistically significant ($P < 0.001$). Model equations were: (**A**) DM whole plant: $y = 180.3 - 4.21 \cdot x + 0.052 \cdot x^2$; DM stems: $y = 88.7 - 1.48 \cdot x + 0.028 \cdot x^2$. (**B**) CP: $y = 444 - 10.34 \cdot x + 0.085 \cdot x^2$. (**C**) aNDFn: $y = 172.6 + 3.71 \cdot x$; ADFn: $y = 118.2 + 6.068 \cdot x$; ADL: $y = -134.4 + 14.66 \cdot x - 0.18 \cdot x^2$. ADFn, acid detergent fiber corrected for nitrogen; ADL, acid detergent lignin; aNDFn, neutral detergent fiber treated with α-amylase and corrected for nitrogen; CP, crude protein; DM, dry matter

under the cubic model; however, because the cubic function lacked biological support and the quadratic model showed low explanatory power ($R^2_{adj} = 0.36$), NE_L was not retained.

Both statistically and biologically supported models are presented in Supplementary Table S1 for completeness, together with estimated coefficients and model fit statistics.

Growth age effects on DM and nutrient yield

Growth age also affected the yields of DM and nutritional components, both per cut and on an annual basis ($P < 0.0001$; Table 2). The highest per-cut yields of DM and most nutritional components were observed at 60 days of regrowth, except for ADL, which peaked at 40 days ($P < 0.05$). The yield of NSCn increased up to 50 days ($P < 0.05$), with no further gains at 60 days, suggesting a biological plateau in soluble carbohydrate accumulation.

On an annual basis, the highest yields of DM, CP, aNDFn, NSCn, OM, and lignin were observed at 40 days of regrowth ($P < 0.05$). In contrast, the maximum annual yields of ADFn, EE, and NE_L were recorded at 60 days. Although DM and NSCn yields peaked at 40 days, extending the growth period to 60 days did not result in additional gains ($P > 0.05$), suggesting a biological plateau in both biomass accumulation and soluble carbohydrate yield.

Environmental conditions varied across regrowth intervals and were associated with changes in DM accumulation (Fig. 4). The reduced biomass accumulation between 41 and 50 days coincided with lower

Table 2 Variation in dry matter (DM) and nutritional component yields of *Tithonia diversifolia* (Hemsl.) A. Gray across growth ages (per cut and per year)

Ítem	Growth age (days)					SEM	P-value
	20	30	40	50	60		
Shrub per·cut							
DM (g)	12.4 ± 2.7^{e}	53.3 ± 8.8^{d}	121.9 ± 13.1^{c}	135.7 ± 22.3^{b}	181.8 ± 23.2^{a}	4.6	$< 0.0001^{*}$
CP (g)	3.3 ± 0.7^{e}	11.4 ± 1.7^{d}	20.7 ± 3.2^{b}	17.8 ± 4.0^{c}	24.4 ± 4.5^{a}	0.9	$< 0.0001^{*}$
aNDFn (g)	2.7 ± 1.0^{d}	16.9 ± 3.5^{c}	42.3 ± 3.6^{b}	45.1 ± 8.6^{b}	68.8 ± 10.5^{a}	1.8	$< 0.0001^{*}$
ADFn (g)	2.7 ± 0.9^{e}	17.4 ± 3.5^{d}	44.3 ± 8.2^{c}	58.9 ± 8.3^{b}	80.8 ± 11.6^{a}	2.2	$< 0.0001^{*}$
ADL (g)	1.02 ± 0.52^{d}	8.04 ± 2.26^{c}	21.40 ± 2.49^{a}	17.28 ± 4.22^{b}	18.73 ± 3.91^{b}	0.8	$< 0.0001^{*}$
EE (g)	0.24 ± 0.09^{c}	0.65 ± 0.31^{c}	1.62 ± 0.48^{b}	1.70 ± 0.74^{b}	3.20 ± 1.23^{a}	0.15	$< 0.0001^{*}$
NSCn (g)	4.5 ± 0.9^{d}	16.0 ± 4.1^{c}	40.9 ± 5.8^{b}	53.1 ± 10.8^{a}	56.3 ± 5.3^{a}	1.6	$< 0.0001^{*}$
OM (g)	10.7 ± 2.3^{e}	45.0 ± 7.4^{d}	105.6 ± 10.8^{c}	117.9 ± 19.8^{b}	160.3 ± 20.6^{a}	4.1	$< 0.0001^{*}$
NE_L (cal)	15.9 ± 3.2^{e}	39.6 ± 7.4^{d}	109.2 ± 21.8^{c}	145.0 ± 30.9^{b}	195.8 ± 30.0^{a}	5.8	$< 0.0001^{*}$
Shrub per year							
DM (g)	215 ± 46^{c}	628 ± 103^{b}	1084 ± 117^{a}	971 ± 160^{a}	1088 ± 139^{a}	35	$< 0.0001^{*}$
CP (g)	58 ± 12^{c}	134 ± 20^{b}	184 ± 29^{a}	128 ± 29^{b}	146 ± 27^{b}	6	$< 0.0001^{*}$
aNDFn (g)	44 ± 10^{d}	199 ± 41^{c}	377 ± 32^{a}	322 ± 62^{b}	412 ± 62^{a}	13	< 0.0001
ADFn (g)	43 ± 10^{d}	205 ± 42^{c}	395 ± 73^{b}	422 ± 59^{b}	484 ± 69^{a}	16	$< 0.0001^{*}$
ADL (g)	18 ± 9^{c}	89 ± 18^{b}	190 ± 22^{a}	124 ± 30^{b}	112 ± 23^{b}	6	< 0.0001
EE (g)	4.1 ± 1.6^{d}	7.6 ± 3.6^{c}	14.4 ± 4.3^{b}	12.2 ± 5.3^{b}	19.2 ± 7.4^{a}	1.2	< 0.0001
NSCn (g)	78 ± 15^{c}	189 ± 49^{b}	364 ± 52^{a}	380 ± 77^{a}	337 ± 32^{a}	13.3	$< 0.0001^{*}$
OM (g)	187 ± 39^{d}	530 ± 87^{c}	940 ± 96^{a}	844 ± 141^{b}	959 ± 123^{a}	32	$< 0.0001^{*}$
NE_L (cal)	276 ± 56^{d}	466 ± 87^{c}	972 ± 194^{b}	1038 ± 221^{b}	1172 ± 173^{a}	41	$< 0.0001^{*}$

Growth ages: days after cutting; values represent mean ± standard deviation; means within rows with different superscripts (a, b, c, d, e) differ significantly ($P < 0.05$; Tukey test). SEM, standard error of mean. P-value: values with superscript * indicate P-values adjusted for sphericity using Greenhouse–Geisser epsilon. Ítem: DM, dry matter; CP, crude protein; aNDFn, neutral detergent fiber treated with α-amylase and corrected for nitrogen; ADFn, acid detergent fiber corrected for nitrogen; ADL, acid detergent lignin; EE, ether extract; NSCn, non-structural carbohydrates corrected for nitrogen; OM, organic matter; NE_L, net energy for lactation (1 × maintenance; NRC 2001).

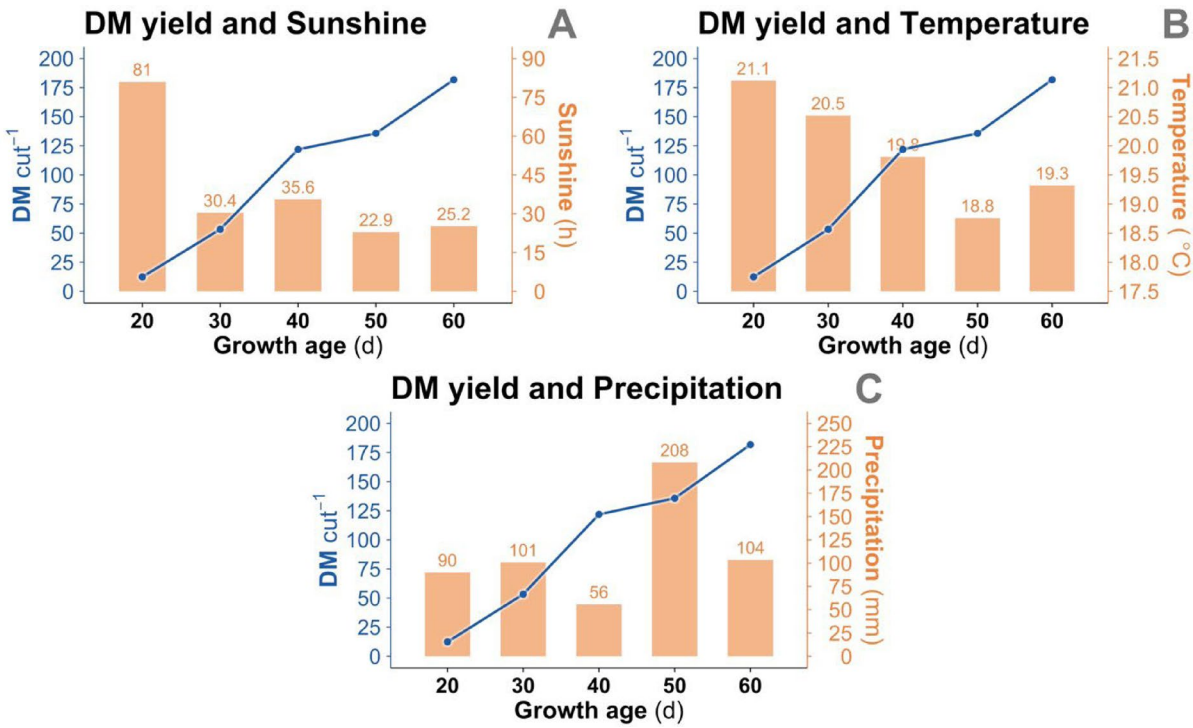


Fig. 4 Variation in dry matter yield and environmental conditions of *Tithonia diversifolia* (Hemsl.) A. Gray across growth ages. Lines represent dry matter (DM) yield (g·cut.$^{-1}$), and bars represent (**A**) sunshine (h), (**B**) temperature (°C), or (**C**) precipitation (mm) for each cutting interval (20, 30, 40, 50, and 60 days of growth)

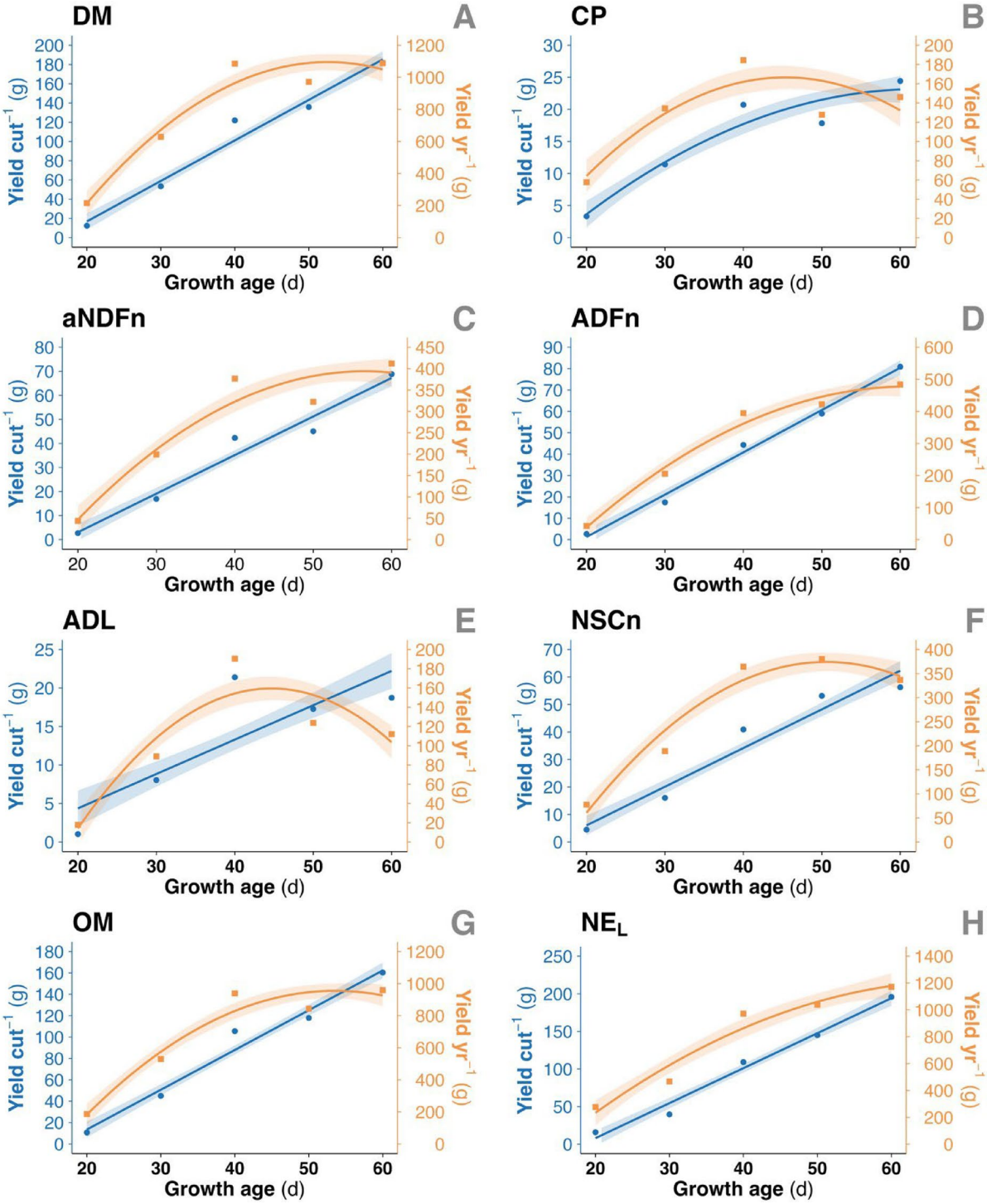
bright-sunshine duration (22.9 h) and a cooler mean temperature (18.8 °C) relative to the other intervals (25.2 – 81 h; 19.3 – 21.1 °C). GDD increased progressively with regrowth age, reaching 242, 352, 458, 555, and 655 °C·d at 20, 30, 40, 50, and 60 days, respectively. These values indicate that DM yield peaked around 458 °C·d, suggesting that this thermal threshold may represent an optimal cutting point under tropical mid-altitude conditions.

Model fit for yield changes

For yield changes per cut, statistical criteria indicated that quadratic models best described the patterns observed for DM, ADL, NSCn, and OM ($P < 0.0001$); however, from a biological perspective, these variables did not exhibit any evident inflection point. Crude protein yield was best explained by a cubic model ($P < 0.0001$); however, as noted previously, with only five growth ages evaluated, cubic functions introduced inflection points that lacked biological justification and indicated overfitting of the limited dataset.

Clear age-related patterns were observed for per-cut yields across all components. Yields of DM, ADL, NSCn, aNDFn, ADFn, OM, and NE_L increased progressively with regrowth age, without a clear biological inflection point. In contrast, CP yield increased up to mid-regrowth and then tended to level off or decline slightly at later stages, indicating a biologically meaningful curvature. Consequently, linear functions were selected to summarize age-related trends in per-cut yields for most variables, whereas a quadratic model was retained for CP ($P < 0.0001$; Fig. 5).

Regarding annual yield changes, although cubic models provided a better statistical fit for CP and aNDFn ($P < 0.0001$), these models lacked biological meaning. Annual yields of DM, CP, aNDFn, ADFn, ADL, NSCn, OM, and NE_L increased with regrowth age up to an intermediate stage and then increased more slowly or plateaued at longer intervals.


DM
A
CP
B
aNDFn
C
ADFn
D
ADL
E
NSCn
F
OM
G
NEL
H
Yield cut−1 (g)
Yield yr−1 (g)
Growth age (d)

◂**Fig. 5** Observed means and fitted curves describing changes dry matter and nutritional component yields of *Tithonia diversifolia* (Hemsl.) A. Gray across growth ages (per cut and per year). Solid and dash lines represent the fitted; ●, ○ and ■ represent observed means at each growth age, and shaded bands represent the 95% confidence interval of the fitted curves. The selected models were all statistically significant ($P<0.001$). Model equations were: (**A**) DM·cut^{-1}: $y=-67.5+4.21{\cdot}x$; DM·yr^{-1}: $y=-1201.2+87.35{\cdot}x-0.831{\cdot}x^2$. (**B**) CP·cut^{-1}: $y=-19.1+1.35{\cdot}x-0.011{\cdot}x^2$; CP·yr^{-1}: $y=-161.3+14.46{\cdot}x-0.159{\cdot}x^2$. (**C**) aNDFn·cut^{-1}: $y=-28.99+1.6{\cdot}x$; aNDFn·yr^{-1}: $y=-436.1+29.35{\cdot}x-0.259{\cdot}x^2$. (**D**) ADFn·cut^{-1}: $y=-38.3+1.98{\cdot}x$; aNDFn·yr^{-1}: $y=-493.9+31.78{\cdot}x-0.26{\cdot}x^2$. (**E**) ADL·cut^{-1}: $y=-4.57+0.44{\cdot}x$; ADL·yr^{-1}: $y=-316.7+21.32{\cdot}x-0.239{\cdot}x^2$. (**F**) NSCn·cut^{-1}: $y=-22.1+1.41{\cdot}x$; NSCn·yr^{-1}: $y=-483.5+33.91{\cdot}x-0.335{\cdot}x^2$. (**G**) OM·cut^{-1}: $y=-61.0+3.72{\cdot}x$; OM·yr^{-1}: $y=-1013+73.52{\cdot}x-0.687{\cdot}x^2$. (**H**) NE_L·cut^{-1}: $y=-85.0+4.7{\cdot}x$; NE_L·yr^{-1}: $y=-712.3+55.18{\cdot}x-0.394{\cdot}x^2$. ADFn, acid detergent fiber corrected for nitrogen; ADL, acid detergent lignin; aNDFn, neutral detergent fiber treated with α-amylase and corrected for nitrogen; CP, crude protein; DM, dry matter; NE_L, net energy for lactation (1 × maintenance) (NRC 2001); NSCn, non-structural carbohydrates corrected for nitrogen; OM, organic matter

Consequently, quadratic functions were retained to describe these annual yield trends ($P<0.0001$; Fig. 5).

The exponential model provided the best statistical fit for EE yield per cut and per year ($P<0.0001$). However, the model fit for annual EE yield was relatively weak ($R^2_{adj}=0.46$), indicating limited predictive accuracy; therefore, EE was not retained for further biological interpretation.

Both statistically and biologically supported models are presented in Supplementary Table S2 for completeness, together with estimated coefficients and model fit statistics.

Growth age effects on morphological traits

Growth age influenced plant height, fresh weight of the whole plant, leaves, and stems; dry weight of leaves and stems; the leaf-to-stem (L:S) ratio on both a fresh and dry matter basis; stem size (diameter and length); and the number of leaves and stems ($P<0.0001$; Table 3).

After pruning, *T. diversifolia* plants developed multiple basal stems per plant, and the number of stems decreased progressively with regrowth age as individual stems elongated and thickened. The greatest values for plant height, whole plant fresh weight, stem weight (both fresh and dry), and stem size (diameter and length) were observed at 60 days of regrowth. In contrast, the highest L:S ratios, on both a fresh and dry matter basis, were recorded at 20 days ($P<0.05$). Maximum fresh leaf weight occurred at 40 days, while the highest dry leaf weights were observed at 40, 50, and 60 days. The largest number of leaves was found at 40 and 50 days, whereas the greatest number of stems was recorded at 20, 30, and 40 days (Table 3).

Model fit for morphological changes

Whole-plant fresh weight increased rapidly during early regrowth and then approached a plateau at later growth stages, whereas plant height increased progressively with regrowth age. In this context, quadratic and linear models were selected, respectively ($P<0.0001$; Fig. 6).

Quadratic models best explained variation in stem weight on both a fresh and dry matter basis ($P<0.0001$); however, these trajectories did not exhibit a clear inflection point. Stem weight increased consistently across regrowth age, whereas leaf weight (on both a fresh and dry matter basis) increased rapidly during early growth and reached a plateau at approximately 40 days. The leaf-to-stem (L:S) ratio, on both fresh and dry matter bases, decreased rapidly during early regrowth and then stabilized at later growth stages. Accordingly, quadratic models were retained for leaf weight and the L:S ratio on both bases, while linear models were selected for stem weight on both bases ($P<0.0001$; Fig. 6).

Quadratic models also best explained variation in the number of leaves and stems ($P<0.0001$). However, relatively low R^2_{adj} values were observed for the number of leaves (0.48) and stems (0.47), indicating limited predictive accuracy for these variables. Stem length and stem diameter increased consistently with regrowth age, and linear models were therefore selected for these variables ($P\leq0.0001$; Fig. 6).

Both statistically and biologically supported models are presented in Supplementary Table S3 for completeness, together with estimated coefficients and model fit statistics.

Table 3 Variation in plant height, plant weight (whole, leaves, and stems), stems size, and number of leaves and stems of *Tithonia diversifolia* (Hemsl.) A. Gray across growth ages

Item	Growth age (days)					SEM	P-value
	20	30	40	50	60		
Heigth (cm)	52.6 ± 7.3^{e}	82.9 ± 5.8^{d}	122.4 ± 11.8^{c}	139.6 ± 16.7^{b}	179.0 ± 23.3^{a}	3.4	$< 0.0001^{*}$
Weight (g)							
Plant	104 ± 22^{d}	542 ± 70^{c}	1312 ± 179^{b}	1283 ± 230^{b}	1624 ± 189^{a}	41	$< 0.0001^{*}$
Leaves	76 ± 16^{d}	288 ± 38^{c}	585 ± 89^{a}	501 ± 68^{b}	551 ± 84^{ab}	18	$< 0.0001^{*}$
Stems	28 ± 7^{d}	253 ± 35^{c}	726 ± 116^{b}	782 ± 174^{b}	1073 ± 156^{a}	31	$< 0.0001^{*}$
L:Sratio	2.66 ± 0.29^{a}	1.12 ± 0.07^{b}	0.82 ± 0.12^{c}	0.66 ± 0.11^{d}	0.52 ± 0.10^{e}	0.04	< 0.0001
DM (g·plant^{-1})							
Leaves	10.6 ± 2.2^{c}	36.2 ± 5.6^{b}	73.0 ± 9.6^{a}	68.4 ± 10.4^{a}	74.7 ± 11.0^{a}	2.4	< 0.0001
Stems	2.0 ± 0.5^{e}	16.5 ± 3.0^{d}	52.4 ± 7.2^{c}	69.7 ± 14.1^{b}	102.6 ± 10.6^{a}	2.4	$< 0.0001^{*}$
L:S ratio	5.19 ± 0.58^{a}	2.21 ± 0.19^{b}	1.41 ± 0.21^{c}	1.00 ± 0.14^{d}	0.73 ± 0.09^{e}	0.07	$< 0.0001^{*}$
Stem size							
Diameter (mm)	3.81 ± 0.48^{e}	5.55 ± 0.62^{d}	8.19 ± 1.27^{c}	8.99 ± 1.11^{b}	11.09 ± 1.25^{a}	0.26	< 0.0001
Length (cm)	11.3 ± 1.4^{e}	38.0 ± 4.7^{d}	73.9 ± 9.4^{c}	90.0 ± 11.3^{b}	124.6 ± 18.5^{a}	2.5	$< 0.0001^{*}$
Number of leaves	284 ± 26^{b}	411 ± 35^{a}	373 ± 32^{a}	209 ± 21^{c}	200 ± 20^{c}	5.5	< 0.0001
Number of stems	40 ± 3^{a}	46 ± 4^{a}	40 ± 3^{a}	24 ± 2^{b}	21 ± 2^{b}	0.6	< 0.0001

Growth ages: days after cutting; values represent mean ± standard deviation; means within rows with different superscripts (a, b, c, d, e) differ significantly ($P < 0.05$; Tukey test). SEM, standard error of mean. P-value: values with superscript * indicate P-values adjusted for sphericity using Greenhouse–Geisser epsilon. Ítem: DM, dry matter; L:S, leaf-to-stem ratio, calculated as leaf weight (g) divided by stem weight (g), based on fresh or dry weight.

Optimal harvest time

Strong negative correlations were found between CP concentration and plant height, stem diameter, and stem length (–0.83, –0.82, and –0.84, respectively; $P < 0.01$; Supplementary Fig. S1). However, these correlations disappeared when regrowth age was included as a covariate, indicating that both morphological and nutritional variables were primarily driven by age. The harvest interval that maximized annual DM, CP, NSCn, and OM yields was 40 days of regrowth. At this age, plants reached approximately 122 cm in height, with stems measuring about 8.2 mm in diameter and 73 cm in length (Fig. 7).

Discussion

Dry matter concentration

Monitoring plant phenology is essential for determining the optimal harvest time, as phenological stages are directly associated with forage quality and yield (Lemaire and Belanger 2019). In this study, DM concentrations in the whole plant ranged from 93.4 to 119.3 g·kg^{-1}, aligning with values reported by some authors (Lezcano et al. 2012; Arias et al. 2023), but lower than those reported by others (Botero et al. 2019; Rivera et al. 2021). These differences may be attributed to variations in environmental conditions, agronomic practices, and growth stage at harvest. Rainfall during the final weeks may also have contributed to modest variation in nutrient concentrations through dilution effects.

It is well established that DM concentrations tend to decline during the rainy season (Cardona et al. 2020; Astúa et al. 2021; Rivera et al. 2021), which may explain the relatively low values observed in our study. During the 60-day trial period, cumulative rainfall totaled 558.3 mm across 38 rainy days. Additionally, DM concentration in *T. diversifolia* is known to be influenced by factors such as growth stage (Astúa et al. 2021), fertilization (Botero et al. 2019; Arias et al. 2023), cutting height (Canto et al. 2023), and planting density (Paniagua et al. 2020).

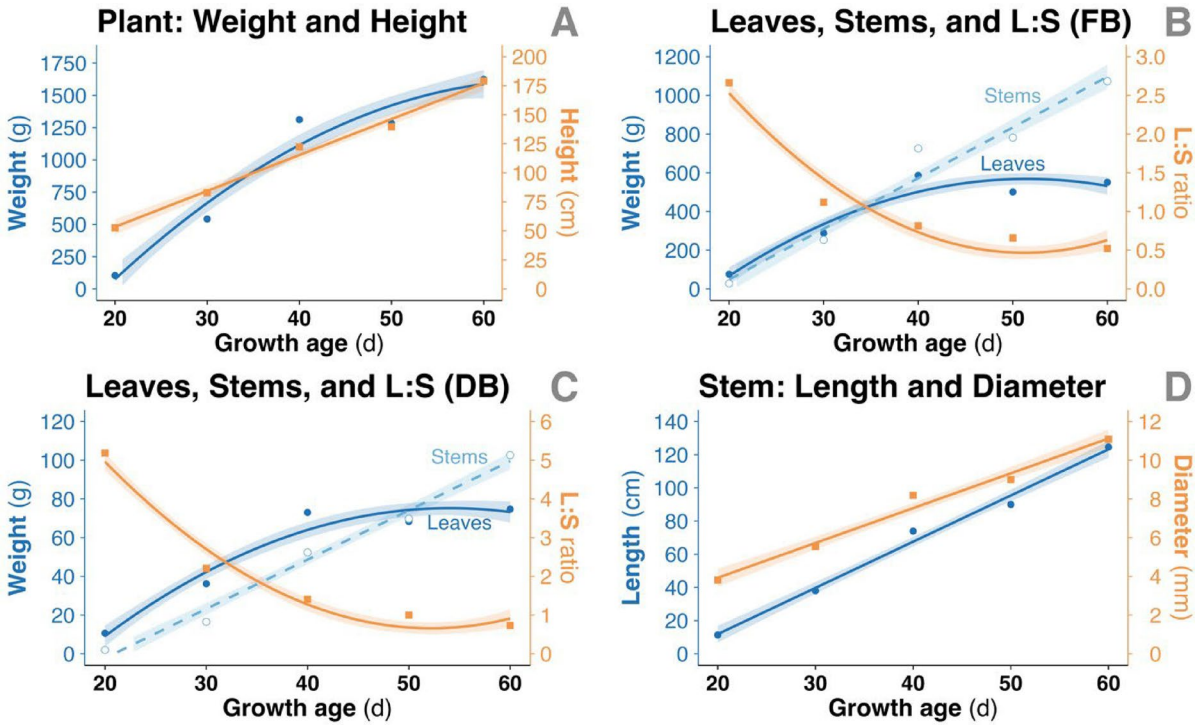


Fig. 6 Observed means and fitted curves describing changes plant height, plant weight (whole, leaves, and stems), and stems size of *Tithonia diversifolia* (Hemsl.) A. Gray across growth ages. Solid and dash lines represent the fitted; ● and ■ represent observed means at each growth age, and shaded bands represent the 95% confidence interval of the fitted curves. The selected models were all statistically significant ($P < 0.001$). Model equations were: (**A**) Plant Weight: $y = -1531.5 + 94.49 \cdot x - 0.7084 \cdot x^2$; Plant Height: $y = -8.51 + 3.10 \cdot x$. (**B**) Leaves Weight (FB): $y = -771.7 + 52.04 \cdot x - 0.505 \cdot x^2$; Stems Weight (FB): $y = -475.2 + 26.19 \cdot x$; L:S (FB): $y = 6.01 - 0.217 \cdot x + 0.0021 \cdot x^2$. (**C**) Leaves Weight (DB): $y = -91.4 + 6.17 \cdot x - 0.057 \cdot x^2$; Stems Weight (DB): $y = -53.2 + 2.55 \cdot x$; L:S (DB): $y = 11.97 - 0.43 \cdot x + 0.0042 \cdot x^2$. (D) Stem Length: $y = -43.9 + 2.79 \cdot x$; Stem Diameter: $y = 0.33 + 0.18 \cdot x$. DB, dry basis; FB, fresh basis; L:S, leaf-to-stem ratio

Several studies have documented an increase in DM concentration with advancing plant age (Cardona et al. 2020; Paniagua et al. 2020; Ruiz et al. 2021; Arias et al. 2023)—a common trend among forage species (Paniagua et al. 2020)—as water content decreases due to increased lignification (Arias et al. 2023). These findings reflect a typical forage maturation pattern, in which nutrient density declines and fiber content increases as plant age advances. As commonly observed in forages, harvesting at shorter harvest intervals may therefore favor nutritional quality.

However, in the present study, DM concentration peaked at 20 days and remained relatively stable through 30 days. This early peak may be explained to the high L:S ratio observed at 20 days (2.66, fresh basis), given that leaves consistently showed higher DM concentrations (93.4 – 119.2 $g \cdot kg^{-1}$) than stems (65.1 – 98.5 $g \cdot kg^{-1}$). Similar trends have been reported in other studies (Arias et al. 2023; Lezcano et al. 2012). Since the evaluation began at 20 days post-regrowth, limited stem development at this early stage may have contributed to the observed pattern.

Although both whole-plant and stem DM concentrations followed a quadratic pattern in our study, whole-plant DM at the final evaluated stage remained slightly lower than at the initial stage, whereas stem DM increased. Verdecia et al. (2018) reported a quadratic increase in whole-plant DM concentration with advancing regrowth age, which contrasts with the pattern observed here. As discussed earlier, these differences may be explained by the consistently higher DM concentration of leaves compared with stems, the progressive reduction in the L:S ratio with age,

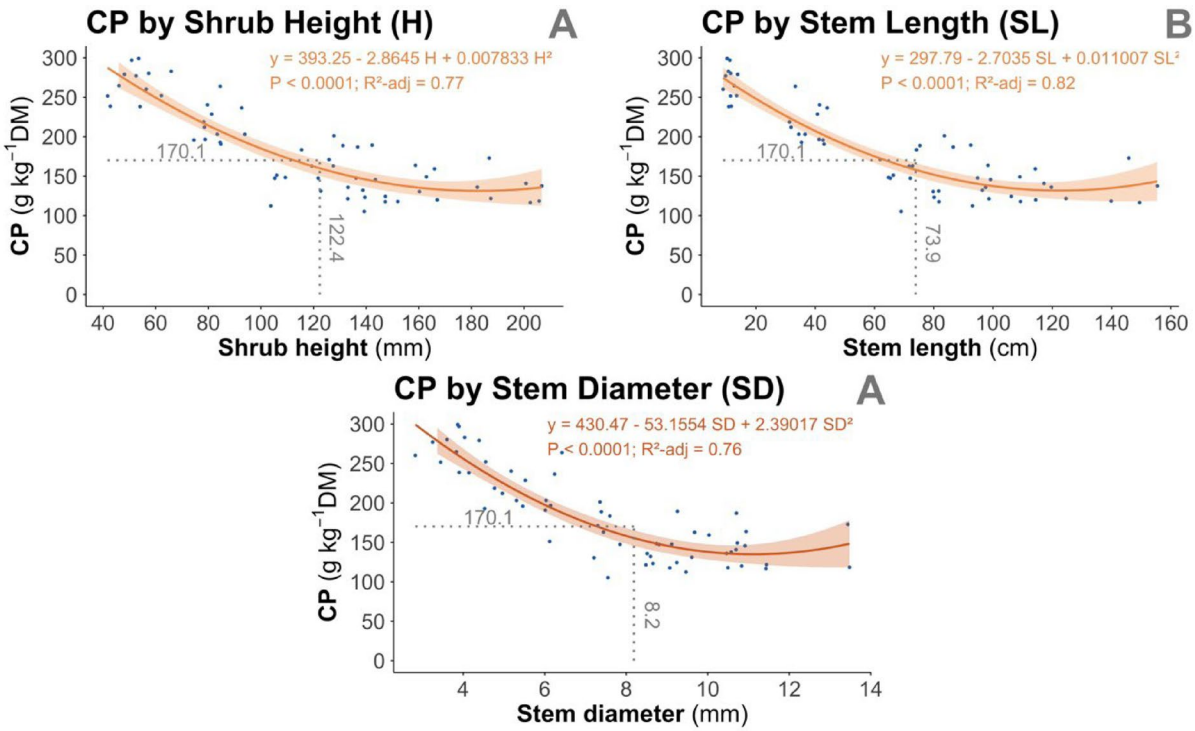


Fig. 7 Selected models describing the relationship between morphological traits and crude protein concentration in *Tithonia diversifolia* (Hemsl.). Relationship between CP concentration and (**A**) plant height, (**B**) stem length, and (**C**) stem diameter. The solid orange lines represent the fitted models, and the shaded bands indicate the 95% confidence intervals. Gray dashed lines represent the average CP concentration at 40 days (horizontal) and the corresponding mean values of plant height, stem length, and stem diameter (vertical). Gray numeric labels indicate the respective average values used as reference points. Model selection was based on the highest adjusted R^2_{adj} and the lowest values of the Akaike and Bayesian information criteria. All selected models were statistically significant ($P < 0.001$). CP, crude protein; H, height; R^2_{adj}, adjusted R-squared; SL, stem length; SD, stem diameter

and the fact that our evaluation covered only early regrowth stages.

Although DM concentration provides useful information on forage maturity, its practical relevance is limited when considered in isolation, as farmers base harvest decisions primarily on the amount of dry matter harvested per unit area. When DM concentration is evaluated together with DM yield, a more agronomically meaningful interpretation emerges. In the present study, DM concentration showed relatively modest age-related variation, whereas DM yield increased substantially with regrowth age. This indicates that increases in total DM production were driven mainly by biomass accumulation rather than changes in DM concentration *per se*. Therefore, optimal harvest timing should be interpreted based on the combined trajectory of DM concentration and DM yield, rather than on either variable alone.

Chemical composition

Protein content. *T. diversifolia* is widely recognized as a protein-rich forage (Botero et al. 2019; Guatusmal et al. 2020), making the monitoring of CP concentration critical for determining the optimal harvest time. In the present study, CP values ranged from 120 to 298 g·kg^{-1} DM, consistent with those reported by other authors (Guatusmal et al. 2020; Vargas et al. 2022; Uu et al. 2023). As noted in previous studies (Cardona et al. 2020; Guatusmal et al. 2020; Paniagua et al. 2020; Arias et al. 2023), CP concentration decreased with advancing plant age and followed a quadratic trend, with the lowest values observed at 50 and 60 days. These results suggest that *T. diversifolia* should be harvested before 50 days to optimize CP content. Notably, the CP value at 40 days (170.1 g·kg^{-1} DM) was comparable to those reported

by some authors (Cardona et al. 2017; Elizondo 2021) and substantially higher than typical values for tropical grasses (72 – 91 g·kg^{-1} DM) (Juárez et al. 2018).

The quadratic behavior of CP observed in this study also aligns with the well-known dilution of protein as structural tissues expand. Herrera et al. (2017) reported a linear decrease in nitrogen (N), whereas Ramírez et al. (2023) described a quadratic decline—both patterns consistent with the reduction in CP, given that N is directly proportional to CP content. Verdecia et al. (2018) likewise found a quadratic decline in CP at later regrowth ages. Together, these findings support the age-related reduction in protein concentration, although the specific shape of the relationship may vary depending on the regrowth range evaluated.

Fiber content. High fiber concentrations can limit DM intake and digestibility in ruminants (NRC 2001). Neutral detergent fiber represents the total fibrous fraction (Van Soest et al. 1991), while ADFn comprises the more lignified and less digestible components (Lemaire and Belanger 2019). Lignin further reduces digestibility and energy availability (Arias et al. 2023). Compared to tropical grasses, *T. diversifolia* typically presents lower aNDFn (215.7 – 392.6 g·kg^{-1} DM) and ADFn (212.7 – 460.6 g·kg^{-1} DM) concentrations, which enhances its nutritional quality (Juárez et al. 2018; Uu et al. 2023). In our study, all fiber fractions—including lignin—increased with plant age, consistent with earlier findings (Cardona et al. 2020; Paniagua et al. 2020; Arias et al. 2023). Peak values were recorded at 60 days for aNDFn, 50 days for ADFn, and 40 days for ADL.

We found a linear increase in aNDFn and ADFn, as reported by other authors (Herrera et al. 2017), which is consistent with physiological maturation and the progressive accumulation of structural carbohydrates. Verdecia et al. (2018) also documented an increase in these fiber fractions, but with a quadratic pattern. This difference may be attributed to the limited number of regrowth ages in their study (only three points), which can artificially favor higher-order models. In contrast, our more frequent sampling during early regrowth allowed the identification of a clear linear trend.

ADL showed a quadratic response, which is consistent with the expected increase in lignification as tissues mature. Herrera (2018) found linear increase of lignin. Although Verdecia et al. (2018) likewise reported a quadratic pattern for lignin, the decline observed after the peak in our results is difficult to explain biologically and may be related to the early regrowth ages evaluated.

Energy content. Net energy is a key nutritional factor affecting dairy cow performance (NRC 2001). In this study, NE_L values ranged from 748 to 1292 cal·kg^{-1} DM, which were lower than those reported by previous studies (1,330–1,700 cal·kg^{-1} DM) (Cardona et al. 2020; Guatusmal et al. 2020; Arias et al. 2023). These lower values may be explained by the higher ADFn concentrations in our samples, which are known to reduce energy availability (Phuong et al. 2013). Interestingly, NE_L was lowest at 30 days and increased up to 50 days, with no significant gains beyond that point.

Non-structural carbohydrates. This fraction is an important energy source for high-producing dairy cows (NRC 2001), as it is fermented into propionate (Maldini et al. 2019)—a key precursor for milk synthesis (McKay et al. 2019)—In our study, NSCn concentrations ranged from 300.5 to 391.2 g·kg^{-1} DM, comparable to those reported by Cardona et al. (2017) and higher than those found in other studies (Arias et al. 2023). The high NSCn levels observed here may be attributed to the relatively low aNDFn concentrations, as NSCn values often increase when fiber content declines (NRC 2001). Furthermore, our values were corrected for nitrogen content, which may explain discrepancies with studies that did not apply this adjustment.

Fat content. Its concentrations in fresh forages typically range from 30 to 80 g·kg^{-1} DM, primarily in the form of galactolipids (Schroeder et al. 2004). In our study, EE values ranged from 12.3 to 18.9 g·kg^{-1} DM, which are lower than those reported by some authors (29.7–35.9 g·kg^{-1} DM) (Botero et al. 2019), but comparable to values found by others (14.9–27.6 g·kg^{-1} DM) (Rivera et al. 2021).

Organic matter. The highest OM concentrations were observed at 60 days. As noted by Paniagua et al. (2020), OM content tends to increase with plant age due to the faster accumulation of DM relative to mineral uptake, which results in a proportional decline in ash content.

Overall, the observed changes in CP, fiber fractions, NSCn, EE, and OM across regrowth ages reflect the combined effects of physiological maturation and

site conditions. Previous studies have shown that environmental and management factors— such as temperature, rainfall, fertilization (Botero et al. 2019), cutting height (Uu et al. 2023), and genotype (Rivera et al. 2021)—can influence the nutritional composition of *T. diversifolia*. These factors help explain the variability among studies and highlight the importance of considering both plant age and local conditions when defining optimal harvest timing.

Biomass yield

Dry matter yield per cut increased with plant age, although the trend was not linear throughout the evaluation period. Between 41 and 50 days, plants gained only 13 g of DM per shrub, compared to 68.6 g during the preceding interval (31–40 days) and 46.1 g in the subsequent interval (51–60 days). Biomass production in *T. diversifolia* is influenced by environmental conditions such as temperature, rainfall (Rivera et al. 2021; Uu et al. 2023), light intensity (Chukwuka et al. 2007), and fertilization (dos Santos et al. 2021). Importantly, the increase in DM yield with regrowth age occurred despite relatively stable or declining DM concentrations, highlighting that biomass accumulation, rather than DM concentration, was the primary driver of total DM production.

The reduced biomass accumulation between 41 and 50 days, coincident with lower bright-sunshine duration (22.9 h) and a cooler mean temperature (18.8 °C), relative to other intervals (25.2–81 h; 19.3–21.1 °C; Fig. 6). These results underscore the role of solar radiation and temperature in modulating biomass accumulation in *T. diversifolia*, consistent with evidence that cumulative thermal time is a genetically influenced driver of plant development (Bernardon et al. 2021).

In our study, the growth stage associated with optimal forage value —defined as the regrowth stage that best balances forage nutritional quality (CP, fiber fractions, and energy) with biomass and annual dry matter yield— occurred at approximately 458 °C·d, whereas Angulo et al. (2024) reported a much lower requirement of 300–340 °C·d for high-altitude environments. This difference suggests that *T. diversifolia* may require different GDD accumulations across elevations and/or reflect differences in the assumed base temperature. Indeed, Paredes et al. (2025) showed that base temperature tends to be lower in cool-season than in warm-season species, supporting the need to estimate a species- and site-specific base temperature for *T. diversifolia* in tropical mid-altitudes.

Although per-cut yields of most components increased almost linearly with regrowth age, the corresponding annual yields showed a clear quadratic pattern. This indicates that beyond approximately 40 days, the additional biomass gained per cut does not translate into higher annual production because the longer regrowth interval reduces the number of cuts achievable per year. Thus, even when yield per cut continues to rise with age, annual yield tends to plateau—or even decline—once regrowth becomes too slow to compensate for the reduced cutting frequency. These quadratic responses highlight the trade-off between maximizing yield per cut and optimizing total annual production.

These patterns suggest that harvesting around 35–40 days may provide the best compromise between yield per cut and total annual production. At this interval, nutrient yields per cut are already high, while regrowth is still rapid enough to maintain a favorable number of cuts per year, preventing the decline in annual yield observed at longer regrowth periods.

Morphological structure and growth dynamics

The weights of the whole plant, leaves, and stems increased with growth age following a quadratic trend, while the L:S ratio declined according to a cubic pattern (Fig. 6A–C). Maximum leaf yields were observed at 40 and 60 days, whereas the highest stem biomass (both fresh and dry) was recorded at 60 days. At 20 days, leaves accounted for 73% of total plant biomass, but this proportion declined to 33% by day 60. These results align with findings by Uu et al. (2023), who reported similar reductions in the leaf proportion—from 70.3% to 28.2%—depending on harvest date.

Notably, the number of stems began to decline after 40 days of growth, suggesting that biomass gains beyond this point were primarily driven by increased stem mass. This shift toward greater stem biomass reflects increased allocation to structural components as the plant grows larger (Lemaire and Belanger 2019). These results indicate that as *T.* diversifolia matures, structural development is

increasingly prioritized over leaf production, resulting in a progressive decline in the L:S ratio. The decline in the L:S ratio with advancing plant age, also noted by Vargas et al. (2022), indicates reduced metabolic tissue and increased structural investment. At 40, 50, and 60 days, L:S ratios on a DM basis were 1.42, 1.00, and 0.73, respectively, showing that leaf biomass exceeded stem biomass only up to 40 days. This ratio is commonly used to estimate the balance between metabolic and structural tissues and is positively associated with forage digestibility. Higher L:S ratios typically indicate more digestible forages, while lower values reflect increased lignification and reduced nutritional quality (Lemaire and Belanger 2019).

Plant height, stem length, and stem diameter also increased with growth age, following trends similar to those reported in previous studies (Ruiz et al. 2021). Plant height generally followed a linear pattern, while stem length exhibited a quadratic pattern. These allometric variables explained a substantial proportion of the observed variation and may serve as practical field indicators of plant maturity.

Morphological and nutritional variables were strongly correlated, but these associations were largely driven by their shared dependence on regrowth age rather than by direct physiological linkages. Consequently, morphological traits cannot be interpreted as independent predictors of forage quality. Even so, because plant height, stem length, and stem diameter follow consistent age-driven trajectories, they may still serve as practical field indicators of plant maturity and approximate harvest stage. Under the conditions evaluated, approximately 40 days represented the point at which nutritional quality and annual biomass yield were jointly optimized.

The relationships observed between CP concentration and morphological traits reflect coordinated changes associated with plant maturation rather than independent predictive effects of morphology. As shown in the Results, apparent morphology–CP associations disappeared once regrowth age was accounted for, indicating that both nutritional and structural traits are primarily driven by developmental stage. In this context, morphological traits such as plant height, stem length, and stem diameter can be interpreted as descriptors of plant maturity and its associated nutritional changes, rather than causal determinants of CP concentration. Although these traits coincided with higher nutrient yields at intermediate regrowth stages, the present dataset does not allow validation of morphology as a robust indicator of the optimal harvest stage, because each regrowth age was sampled only once and morphological variability under different growth rates or environmental conditions was not assessed.

In addition to regrowth age, planting density is known to influence the morphological development of *T. diversifolia*. Higher densities can increase competition for light and nutrients, potentially resulting in thinner stems and reduced branching, as commonly observed in other shrub species (Ramkumar and Anuja 2017). In *T. diversifolia,* plant density has been shown to affect forage yield and chemical composition (Akinola et al. 2025), suggesting that morphological responses such as stem diameter may also vary with spacing. Therefore, the relatively high density used in our study could have contributed to the smaller stem diameters observed. Although the planting density applied here was defined at the time of field establishment (2015), it falls within the range of high-density treatments reported in more recent studies. For example, Akinola et al. (2025) evaluated a 60 × 20 cm spacing (83,333 plants·ha^{-1}), indicating that the density used in the present study is consistent with later agronomic recommendations for intensive biomass production.

Optimal harvest time

The findings of this study indicate that the optimal harvest time for *T. diversifolia* under mid-altitude tropical conditions is 40 days of regrowth, based on the L:S ratio and the concentrations of CP and fiber fractions. At this stage, the plant maintains moderate levels of aNDFn and ADFn, despite a relatively high ADL content.

Nutritional composition is a critical determinant of forage harvest timing, as it directly influences animal performance. However, both biomass and nutrient yield must also be considered, as they affect production costs and the sustainability of livestock systems. Therefore, the optimal harvest time should reflect a balance between nutritional quality and total yield (Paniagua et al. 2020).

In this study, the highest per-cut yields of DM, CP, EE, and NE_L were observed at 60 days, while NSCn yield peaked at both 50 and 60 days. Nevertheless,

the highest annual yields of DM, CP, NSCn, and OM were achieved with a 40-day harvest interval. These results suggest that although harvesting at 60 days maximizes nutrient yield per cut, harvesting every 40-day optimizes total annual biomass and nutrient production—except for NE_L.

The study also aimed to identify phenological traits associated with optimal cutting time. Although strong correlations were observed between morphology and both CP concentration and nutrient yields, these relationships were driven by their shared dependence on regrowth age rather than by direct physiological linkages. Therefore, morphology cannot be used as an independent predictor of forage quality. Nonetheless, because morphological traits follow consistent and predictable age-related trajectories, they offer practical reference points for describing plant maturity at the optimal harvesting stage. In this study, the harvest interval that best balanced nutritional quality and annual biomass yield was 40 days of regrowth. At this age, plants reached approximately 122 cm in height, with a stem measuring about 8.2 mm in diameter and 73 cm in length (Fig. 7).

It is important to note that the present study evaluated a single cutting event per harvest interval, providing a snapshot of the morphological and nutritional responses of *T. diversifolia* at different regrowth stages. Therefore, the results should not be interpreted as indicative of long-term persistence or cumulative yield performance across multiple harvest cycles. Future studies incorporating repeated cuts, regrowth dynamics, and stand longevity under varying harvest intervals would strengthen the understanding of sustainable productivity and management of *T. diversifolia* stands.

Conclusions

This study identified key phenological traits that serve as reliable indicators for determining the optimal harvest time for *T. diversifolia* under mid-altitude tropical conditions. The optimal cutting interval occurs at 40 days of regrowth, when the plant achieves a favorable balance between forage quality and biomass productivity.

Although morphology was correlated with both quality and yield, these relationships were driven by regrowth age. Therefore, morphological traits cannot be used as independent predictors of forage quality but may still serve as descriptive indicators of plant maturity.

Since plant height showed a consistent relationship with forage quality, it may represent a more reliable criterion than fixed harvest intervals, which are affected by seasonal variations in growth rate. Under the conditions evaluated, harvesting when plants reached approximately 122 cm in height (at around 40 days of regrowth), with a stem diameter of 8.2 mm and a stem length of 73 cm, optimized forage value by balancing nutritional quality and biomass accumulation. This height-based approach could serve as a practical guideline to improve the sustainability and productivity of forage-based livestock systems in tropical regions.

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Data availability Research data are available from the corresponding author by email upon reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

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