

## Article

# Intensive Production and Management of Marandu Palisadegrass (*Urochloa brizantha* 'Marandu') Accelerates Leaf Turnover but Does Not Change Herbage Mass

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**Abstract:** Pasture intensification through higher stocking rates, nitrogen fertilization and intensified grazing management in beef cattle production optimizes pasture use by increasing the forage harvested. We aimed to assess its effects on the morphogenesis and canopy structure of *Urochloa brizantha* 'Marandu' (marandu palisadegrass) pastures. The treatments consisted of marandu palisadegrass pastures managed under continuous stocking and a canopy height of 25 cm, with different levels of intensification: extensive, semi-intensive, and intensive systems N-fertilized with 0 kg, 75 kg, and 150 kg N ha<sup>-1</sup> year<sup>-1</sup>, respectively, as ammonium nitrate (32% of N), with four replicates (paddocks) in a completely randomized design. Phyllochron (9.8 days) and leaf lifespan (34.7 days) were shorter in intensified pastures, whereas herbage mass was similar among treatments. Extensive pastures had a higher proportion of senescent material; thus, more intensive systems showed higher proportions of leaves and stems, although the leaves-to-stem ratio remained similar across production systems. The defoliation interval was lower in intensive (14.4 days) and higher in extensive (18.7 days) treatments. Thus, pasture intensification accelerates leaf appearance, decreases leaf lifespan, shortens the tiller defoliation interval and increased herbage accumulation rate but does not change herbage mass. The extensive system produces excessive forage losses due to dead material.

**Keywords:** canopy; intensification; morphogenesis; ammonium nitrate; beef cattle; *Urochloa*

## 1. Introduction

The sustainable intensification of pastoral ecosystems is achieved in numerous ways, such as through a consortium of forage species, grazing by different herbivores, the strategic supplementation of beef cattle, irrigation, fertilization, or the integration of crop–livestock–forestry. All of these options yield high productivity levels, thereby contributing to a low C footprint [1–3]. To produce more food (meat) in the same area, pasture management is a fundamental factor, because it is from primary production (pasture) that secondary production (meat) occurs.

The success of pasture-based cattle production depends on optimizing the intake of grazing animals, which is significantly affected by the canopy structure [4]. The daily intake of grazing animals is dependent on tiller size and density, bulk density, canopy height, leaf-to-stem ratio, and stem length, which may limit grazing in the lower canopy layers [5].

Accordingly, structural characteristics are a result of the combination of morphogenic variables, such as leaf appearance rate, leaf elongation rate, and leaf lifespan [2]. Morphogenic variables show how tillers develop according to the conditions of the pastoral ecosystem. N fertilization accelerates the rate of leaf expansion [6] and decreases the lifespan of leaves [5] in response to increased canopy C and N cycling. Furthermore, N increases forage production and alters the canopy structure. Consequently, the application of the synthetic form is a management method that allows for an increase in pasture stocking rate [2,7,8].

Marandu palisadegrass (*Urochloa brizantha* 'Marandu'), which is managed under continuous grazing at different canopy heights, alters their tillering dynamics [7,8]. In general, pastures managed at higher canopies (>25 cm) showed a lower tiller density, lower tiller appearance rate, and higher tiller mortality, resulting in a lower leaf-to-stem ratio and higher proportion of dead material [7,8]. Thus, based on desirable tillering characteristics, forage mass, and animal performance, a canopy height of 25 cm has been recommended to manage marandu palisadegrass when subjected to continuous grazing [7,9].

Although the target height of canopy management is standard for all paddocks, the N supply alters the morphogenic variables. Consequently, structural components such as the percentage of leaf, stem, and dead material in the canopy will present different proportions, as noted by Ruggieri et al. [9], according to grazing intensity.

For instance, Delevatti et al. [10] observed linear increases in herbage mass and the accumulation of palisade grass in a pasture fertilized with increasing doses of N (as urea) and continuous stocking managed at a 25 cm canopy height. This allowed an increase of stocking rate from 3.3 AU (animal unit = 450 kg ha<sup>-1</sup>) in unfertilized pastures, to 6.5 AU in the 270 kg N ha<sup>-1</sup> fertilized pastures. However, the lack of N replacement and inadequate grazing management lead to a pasture structure that results in low productivity [2].

Pasture intensification can be achieved through the adoption of diverse technologies, such as fertilization and pasture management, to optimize the forage consumed by animals, and animal supplementation [10–12]. In this study, pasture intensification refers to intensive grazing management acquired through a combination of increased stocking rate, N fertilization, and grazing management practices. Considering the complex plant–animal interrelationship in grassland systems, the understanding of how pasture intensification alters the morphogenic and structural variables of marandu palisadegrass is pivotal to establish improved management practices for achieving the sustainable intensification of animal production.

We hypothesized that pasture intensification of marandu palisadegrass (acquired through a combination of increased stocking rate, N fertilization, and grazing management) will alter the morphogenic variables to increase the leaf-to-stem ratio and decrease the proportion of dead material within the canopy. Therefore, the objective of this study was to assess the effect of pasture intensification on the morphogenic and structural variables of marandu palisadegrass pastures that are fertilized with ammonium nitrate and managed under continuous stocking with a canopy height of 25 cm.

## 2. Materials and Methods

### 2.1. Study Site

The experiment was carried out at the Beef Cattle Center of Sao Paulo State University "Julio de Mesquita Filho" (UNESP), Jaboticabal, Sao Paulo, Brazil (21°15'22" S, 48°18'08" W; 595 m altitude). The climate is humid and subtropical, with dry winters and warm summers (Aw), according to Köppen's classification, and the soil is classified as a typical Hapludox with a clayey texture [13]. The pasture was established in 2005 with a monoculture of marandu palisadegrass. The experimental area comprised 24 ha, divided into 12 paddocks of approximately 2 ha each. The experimental period was from December to April of 2018/2019 and 2019/2020. Before the beginning of the experimental period, on November of 2018, all paddocks were controlled grazed to reach the target canopy height of 25 cm, and similar initial herbage mass. Between the end of the first and the beginning of the second year (i.e., from April to December 2019), all pastures were monitored and sporadically

grazed so that, at the beginning of the second year, on December of 2019, all paddocks have reached the target canopy height of 25 cm, and similar initial herbage mass.

Soil samples (0–20 cm) from each paddock were collected at the beginning of the experimental period in each year. The average values were pH (CaCl<sub>2</sub>) 5.0, organic matter 23.6 g dm<sup>-3</sup>, P-resin 7.4 mg dm<sup>-3</sup>, K<sup>+</sup> 1.9 mmol<sub>c</sub> dm<sup>-3</sup>; Ca<sup>2+</sup> 19.9 mmol<sub>c</sub> dm<sup>-3</sup>; Mg<sup>2+</sup> 10.2 mmol<sub>c</sub> dm<sup>-3</sup>; H+Al 24.7 mmol<sub>c</sub> dm<sup>-3</sup>; sum of bases 31.9 mmol<sub>c</sub> dm<sup>-3</sup>; cation exchange capacity 56.7 mmol<sub>c</sub> dm<sup>-3</sup>; and base saturation 55.8%.

## 2.2. Experimental Design

The treatments consisted of different levels of pasture intensification (a combination of increased stocking rate, N fertilizer, and management practices), which were extensive, semi-intensive, and intensive, with four replicates (paddocks) in a completely randomized design. The grazing method comprised continuous and put-and-take stocking [14] to maintain a canopy height of 25 cm. The adjustment of the number of animals in each paddock was made weekly, considering the maximum amplitude of 8% variation on canopy height (23–27 cm). After canopy height being measured, as described later (item 2.3.2), animals were put in the paddock whenever the canopy height reached the upper height limit and taken off when the height reached the lower limit.

Paddocks from extensive, semi-intensive, and intensive treatments were N-fertilized with 0 kg, 75 kg, and 150 kg of N ha<sup>-1</sup> year<sup>1</sup>, respectively, as ammonium nitrate (32% of N), only during the experimental periods. These were then fractioned into three identical doses per year, based on precipitation distribution according to the occurrence of rainfall, on the following dates: 19 December 2018; 23 January 2019; 23 February 2019; 10 December 2019; 30 January 2019; and 20 March 2020. The evaluations started when the canopy height reached 25 cm, and the initial herbage mass did not differ among treatments ( $p > 0.05$ ). Weather data were obtained from the Agrometeorological Station of the Department of Engineering and Exact Sciences at UNESP, located 500 m from the experimental area.

The mean values of temperature, rainfall, and number of rainy days in 2018/2019 and 2019/2020 were 24.8 °C and 24.5 °C, 731.7 mm and 798 mm, and 62 days and 58 days, respectively (Table 1). The analysis of variance comparing these variables for the 2018/2019 and 2019/2020 cycles was similar, with regard to mean temperature ( $p = 0.56$ ), rainfall ( $p = 0.93$ ), and number of rainy days ( $p = 0.99$ ). These environmental conditions were not restrictive to plant development.

**Table 1.** Monthly temperatures, rainfall and rain events during experimental seasons 2018/2019<sup>a</sup> and 2019/2020<sup>b</sup>.

	Mean Temp. (°C)	Max. Temp. (°C)	Min. Temp. (°C)	Rainfall (mm)	Rainy Days (Number)
<b>2018</b>					
December	25.2	30.0	20.2	88.2	16
<b>2019</b>					
January	26.1	32.7	20.9	148.1	11
February	24.4	30.9	20.4	282.6	17
March	24.5	31.0	20.1	115.2	12
April	23.9	30.6	19.0	97.6	6
December	25.2	30.3	20.1	230.0	14
<b>2020</b>					
January	25.1	30.9	20.1	266.3	19
February	24.5	29.2	19.9	185.0	18
March	24.7	31.2	18.1	84.1	6
April	22.9	29.4	16.5	32.6	1

<sup>a</sup> 10 December 2018–29 April 2019; <sup>b</sup> 9 December 2019–27 April 2020; Agrometeorological Station of the Department of Engineering and Exact Sciences at UNESP.

The animals used in this study were cared for according to the rules of the São Paulo State University Animal Care and Use Committee and the National Council of Animal Experimentation Control. The committee reviewed and approved the experiment as well as all procedures carried out in the study (certificate number 7979/18). In each year, 48 young Nellore bulls (*Bos indicus*), with an initial age of approximately 15 months and initial body weight (BW) of  $308 \pm 20$  kg, were used to manage pastures under continuous stocking, using the put-and-take method to maintain a pre-established grazing height of 25 cm.

The stocking rate differed ( $p < 0.05$ ; SEM =  $\pm 0.01$ ) among the levels of intensification, increasing from 1.9 AU to 2.8 AU and 3.8 AU for the extensive, semi-intensive, and intensive level of intensification, respectively. Note that 1 AU was considered to be an animal with a BW of 450 kg. Canopy height and stocking rate were included so that the level of intensification could be inferred without misinterpretation between treatment and management.

### 2.3. Data Collection

#### 2.3.1. Morphogenic Measurements

To evaluate morphogenesis, two representative points at the canopy average height per paddock were marked with a fixed wooden stake in the ground until the end of the evaluation period of the final year. At each point, four tillers (eight per paddock) were selected and tagged with colored plastic rings [15]. Tagged tillers were measured twice a week and changed every 28 days.

The length of the fully expanded, expanding, and senescent leaves, height of the pseudostem (stem + sheath), and height of the extended tiller were measured in the tagged tillers. The expanded leaves were measured from the ligule, while the expanding leaves were measured from the ligule of the last expanded leaf. In the senescent leaves, only the green portion of the leaf was measured (those with a maximum of 50% brown parts). Tillers were classified as dead when more than 50% of the parts were brown. The first leaf portion of each tagged tiller was identified by white liquid paper correction fluid, so that it could be identified in subsequent collections. The height of the pseudostem was measured from the base of the soil to the height of the ligule of the last expanded leaf. The height of the extended tiller was measured from the base of the soil to the apex of the highest leaf. The average weight per tiller was estimated by collecting a sample of 30 tillers per paddock every 28 days.

#### 2.3.2. Canopy Structure Measurements

The canopy height was measured weekly by using a centimeter-graduated ruler, at the curvature of the upper leaves, at 80 random points per paddock. The canopy heights among the levels of intensification were similar (25.3 cm;  $p > 0.12$ ; SEM =  $\pm 3.7$ ).

Herbage mass was estimated every 28 days. All plants within the perimeter of a metal circular rim (0.25 m<sup>2</sup>) were harvested at ground level at four representative sites per paddock to represent the mean herbage mass of the paddocks. Samples were then separated into dead material, stem + sheath, and green leaves before drying at 55 °C to a constant weight to estimate the forage dry matter per hectare.

Herbage accumulation (HA) was quantified using the paired cage method [16] every 28 days. The cubic exclusion cages (1 m<sup>3</sup>) were allocated to two random sites per paddock. Samples (initial herbage mass) from 1 m<sup>2</sup> inside the cage (paired samples) were clipped to ground level. After 28 days of cage placement, forage samples were collected again and dried at 55 °C in a forced-air dryer to a constant weight and weighed. The herbage accumulation rate (kg ha<sup>-1</sup> d<sup>-1</sup>) was calculated by dividing the cumulative forage dry mass by the number of days between evaluations. Cumulative forage dry mass was obtained from the difference between the forage dry mass of the samples collected from inside the cage on the date of sampling and the forage dry mass of the sample outside the cage (paired sample) on the date of the previous sampling.

## 2.4. Data Calculations

### 2.4.1. Morphogenic Variables

The leaf appearance rate (leaves day<sup>-1</sup>) was estimated as the ratio between the number of leaves produced and the evaluated interval, while the phyllochron (the time interval between the appearance of successive leaves) was considered to be the inverse value. The leaf lifespan (days) was calculated as the product of the average number of green leaves per tiller and the average phyllochron. The leaf elongation duration (days) was calculated as the product of the average number of expanding leaves and the average phyllochron. The elongation (mm per leaf day<sup>-1</sup>) and senescence (mm per leaf day<sup>-1</sup>) rates were calculated as the ratio between the elongation, or average senescence, of the leaf between two consecutive evaluations and the interval of evaluation. Senescence was identified by the yellowing of the leaf apex, and leaves that presented yellowing in more than 50% of the blade were considered as senescent [17].

The total number of leaves per tiller was determined by the number of expanding and expanded leaves of the tiller, not counting those that had more than 50% senescence, whereas the number of live expanded leaves per tiller was determined by discarding expanding leaves and those with more than 50% senescence.

### 2.4.2. Defoliation Intensity and Frequency

To calculate the defoliation intensity (INT), the grazed leaf blades were identified and used in the following formula:

$$\text{INT} = [(\text{initial length} - \text{final length}) / \text{initial length}], \quad (1)$$

where INT is the defoliation intensity of grazed leaf blades.

To calculate the removal intensity of the extended tiller, grazed tillers were identified and used in the following formula:

$$\text{EXT} = [(\text{initial length of extended tiller} - \text{final length extended tiller}) / \text{initial length of extended tiller}] \times 100, \quad (2)$$

where EXT is the removal intensity of the extended tiller.

The defoliation interval (days to return to the same tiller) was calculated using the following formula:

$$\text{Defoliation interval} = \text{No. of touches on pasture days} / \text{duration of evaluation}. \quad (3)$$

## 2.5. Statistical Analyses

The experiment was conducted in two consecutive years and within five periods of each year (28-day cycle, repeated measure), according to a randomized complete block design (block = year), with three treatments (levels of intensification) and four replicates (paddocks). All variables were analyzed as repeated measures in time (periods). Data were tested for normality and equal variance using the Shapiro–Wilk normality test and the Bartlett test of homogeneity of variances, respectively. The best covariance structure used for repeated-measures analyses was chosen as the one that achieved the lowest corrected Akaike information criterion. Comparisons between treatments were performed using Tukey's test, and significance was set at  $p \leq 0.05$ . Data were analyzed using the MIXED procedure of SAS (version 9.3; SAS Institute, Cary, NC, USA).

## 3. Results

### 3.1. Morphogenic Variables, Senescence Rate, and Elongation Duration

The leaf appearance rate increased, whereas the phyllochron decreased with increasing levels of pasture intensification from extensive to intensive (Table 2).

**Table 2.** Morphogenic variables, number of leaves, leaf elongation duration, and senescence rate of marandu palisadegrass (*Urochloa brizantha* 'Marandu') pastures under different levels of intensification.

Variable	Level of Intensification			p-Value	SEM *
	Extensive	Semi-Intensive	Intensive		
Phyllochron (days)	13.3 <sup>a</sup>	11.7 <sup>b</sup>	9.8 <sup>c</sup>	<0.0001	0.06
Leaf appearance rate (leaves day <sup>-1</sup> )	0.08 <sup>c</sup>	0.09 <sup>b</sup>	0.11 <sup>a</sup>	<0.0001	0.05
Leaf elongation rate (mm per leaves day <sup>-1</sup> )	0.10 <sup>b</sup>	0.11 <sup>a</sup>	0.12 <sup>a</sup>	<0.0001	0.06
Leaf senescence rate (mm per leaves day <sup>-1</sup> )	0.17	0.15	0.15	0.06	0.02
Leaf elongation duration (days)	15.9 <sup>a</sup>	14.7 <sup>a</sup>	12.8 <sup>b</sup>	<0.0001	0.0008
Leaf lifespan (days)	42.2 <sup>a</sup>	41.2 <sup>a</sup>	34.7 <sup>b</sup>	0.02	0.31
Number of total leaves	4.3 <sup>b</sup>	4.6 <sup>a</sup>	4.6 <sup>a</sup>	0.0005	0.005
Number of live leaves	3.8 <sup>b</sup>	4.0 <sup>a</sup>	4.1 <sup>a</sup>	0.001	0.004
Number of expanded leaves	2.6 <sup>b</sup>	2.8 <sup>a</sup>	2.9 <sup>a</sup>	0.0017	0.008
Number of leaves in elongation	1.20 <sup>c</sup>	1.25 <sup>b</sup>	1.30 <sup>a</sup>	<0.0001	0.004
Number of leaves in senescence	1.13	1.17	1.17	0.88	0.006

\* Standard error of the mean. <sup>a,b,c</sup>: Means in the same row followed by different letters differ at  $p \leq 0.05$  by Tukey's test.

The leaf elongation rates were higher in the intensive and semi-intensive treatments than in the extensive pasture intensification treatment, yet the leaf senescence rates did not differ (Table 2).

The leaf lifespan (average 41.7 days) and leaf elongation duration (average 15.3 days) were longer ( $p < 0.05$ ) in the extensive and semi-intensive treatments than in the intensive pasture intensification treatment (Table 2).

The number of expanded (average 2.8), live (average 4.1), and total leaves (average 4.6) was higher ( $p < 0.05$ ) in the semi-intensive and intensive treatments than in the extensive (2.6, 3.8, and 4.3, respectively) pasture intensification treatment. Moreover, the number of expanding leaves increased ( $p < 0.05$ ) from extensive to intensive systems.

### 3.2. Canopy Structural Variables

The herbage mass was similar (average 5400 kg DM ha<sup>-1</sup>) among pasture intensification systems; however, it tended to be smaller ( $p = 0.07$ ) for the extensive system (Table 3). The herbage accumulation rate (Table 3) was higher in the semi-intensive and intensive treatments than in the extensive pasture intensification treatment.

**Table 3.** Herbage mass; proportions of leaf, stem, and dead material; leaf-to-stem ratio; accumulation rate; tiller weight of marandu palisadegrass (*Urochloa brizantha* 'Marandu') pastures under different levels of pasture intensification.

Variable <sup>1</sup>	Level of Intensification			p-Value	SEM *
	Extensive	Semi-Intensive	Intensive		
Herbage mass (kg DM ha <sup>-1</sup> )	5150	5500	5550	0.07	0.44
Leaf (%)	21 <sup>c</sup>	27 <sup>b</sup>	33 <sup>a</sup>	<0.0001	0.01
Stem (%)	16 <sup>b</sup>	22 <sup>a</sup>	25 <sup>a</sup>	<0.0001	0.01
Herbage accumulation rate (kg DM ha <sup>-1</sup> day <sup>-1</sup> )	76.1 <sup>b</sup>	117.1 <sup>a</sup>	104.8 <sup>a</sup>	0.02	0.87
Leaf-to-Stem ratio	1.44	1.48	1.55	0.70	0.02
Dead material (%)	61 <sup>a</sup>	49 <sup>b</sup>	42 <sup>c</sup>	<0.0001	0.06
Tiller weight (g)	0.88 <sup>a</sup>	0.79 <sup>b</sup>	0.72 <sup>c</sup>	<0.0001	0.01

<sup>1</sup> DM = dry matter ha<sup>-1</sup>, \* standard error of the mean. <sup>a,b,c</sup>: Means in the same row followed by different letters differ at  $p \leq 0.05$  by Tukey's test.

The proportion of leaves and stems of semi-intensive and intensive pasture treatments was higher than that of extensive pastures, but the leaf-to-stem ratio did not differ among treatments (Table 3). The percentage of dead material decreased with the intensification of pastures (Table 3). Furthermore, the weight of the tillers differed among treatments (Table 3), and lighter tillers were observed in semi-intensive and intensive pasture treatments.

### 3.3. Defoliation Intensity and Interval

Individual extended tillers were longer (27.5 cm;  $p < 0.05$ ) in the semi-intensive, shorter (24.9 cm) in the extensive, and intermediate (26.8 cm) in the intensive pasture intensification treatment. The intensive treatment presented higher defoliation intensity of leaves and extended tillers than the semi-intensive treatment but did not differ from that of the extensive treatment (Table 4). Defoliation interval, which represents how long the animals take to return and graze the tiller again, was the longest for the extensive pasture intensification treatment (Table 4).

**Table 4.** Defoliation intensity of leaf blades, removal intensity of extended tillers, and defoliation interval of marandu palisadegrass (*Urochloa brizantha* ‘Marandu’) pastures under different levels of pasture intensification.

Variable	Level of Intensification			p-Value	SEM *
	Extensive	Semi-Intensive	Intensive		
INT <sup>1</sup> (%)	57 <sup>ab</sup>	53 <sup>b</sup>	58 <sup>a</sup>	0.002	0.01
EXT <sup>2</sup> (%)	23 <sup>ab</sup>	22 <sup>b</sup>	26 <sup>a</sup>	0.002	0.007
Defoliation interval (days)	18.7 <sup>a</sup>	15.5 <sup>b</sup>	14.4 <sup>b</sup>	<0.0001	0.47

<sup>1</sup> INT is the defoliation intensity of grazed leaf blades, <sup>2</sup> EXT is the removal intensity of the extended tiller, \* standard error of the mean.  
a, b: Means in the same row followed by different letters differ at  $p \leq 0.05$  by Tukey's test.

## 4. Discussion

The leaf appearance rate represents the displacement of the sheath within the pseudostem. The leaf appearance rate presented lengths that were similar among treatments (average of 12.6 cm;  $p > 0.05$ ), presumably owing to similarities in the maintenance of canopy height at  $\pm 25$  cm. Previous studies have shown that shorter pseudostems promoted a higher rate of appearance and lower phyllochron of tillers [18,19], however, this was not observed in this study. Previous studies have also shown that large inputs of N decrease the phyllochron of C4 grasses because N accelerates the sprouting of the tillers, thereby facilitating a rapid reconstitution of the leaf area [6,20]. The combination of N fertilization, canopy height of  $\pm 25$  cm, high stocking rate, and short defoliation interval probably reflected a low phyllochron (or faster leaf appearance rate) in the intensive treatment, while allowing tillers to maintain their leaf area index and photoassimilate production.

The high leaf elongation rates of intensive and semi-intensive pasture intensification treatments were presumably owing to N fertilization in the canopy. N is a part of the chlorophyll molecule, and its level determines a higher concentration of chloroplasts and higher efficiency in the capture of intercepted radiation. Numerous studies have reported that N promotes the growth of the foliar lamina [21,22]; consequently, an increased leaf growth rate allows higher grazing frequencies and, if forage productivity is also enhanced, it can lead to an increase in the stocking rate. Therefore, if this does not occur, the opportunity to graze foliar lamina is lost, owing to senescence.

The lack of difference in leaf senescence (average of 0.16 mm per leaves day<sup>-1</sup>) among the pasture intensification systems was expected because it is not affected by leaf defoliation but rather by environmental characteristics, especially water deficits [23], which were not observed during the two collection cycles (Table 1).

N accelerates the growth of leaf tissues by increasing leaf elongation rate and light competition [11,24–26]. The similarity in the leaf lifespan of semi-intensive and extensive pasture intensification treatments is not consistent with that in previous studies, which showed a decrease in leaf lifespan with N fertilization [3,26,27], presumably indicating that the semi-intensive system was under-grazed. Conversely, the similarities in the values for the duration of leaf elongation between semi-intensive and extensive pasture treatments were consistent with the results of Gastal and Lemaire [28], who reported that leaf elongation duration was not affected by N application in studies with C3 grasses [29] and

other plant species [30–33]. In the present study, there was a difference in leaf elongation duration between the semi-intensive and intensive pasture systems. Nevertheless, because we studied a gramineous photosynthetic C4 route, the discrepancy between our results and those of Gastal and Lemaire [28] might indicate the need for further studies to understand the minimum dose of N required to alter this variable in C4 grasses.

The number of leaves per tiller is a result of the interaction between the leaf appearance rate and leaf lifespan [34,35]. However, as the intensification of the system increased, these variables began to differ among treatments (Table 2), presenting a higher number of expanded, live, and total leaves in the more intensified systems. Gastal and Lemaire [5] explained that N supply alters cell division rates in expanding leaves, thereby stimulating cell production with increased N doses. Moreover, the number of senescent leaves was similar among pasture intensification systems (average 1.15;  $p = 0.87$ , SEM =  $\pm 0.006$ ). Similar to our findings, no difference in the number of senescent leaves was observed in a previous study between pastures that were unfertilized and fertilized with 150 kg N ha<sup>-1</sup>. Otherwise, N fertilization increased the number of senescent leaves at doses of 300 kg ha<sup>-1</sup> and 450 kg ha<sup>-1</sup> [36], which would result in higher intensification levels than those used here. This suggests that the cycling of N in the cellular metabolism of senescent leaves may only be affected by high doses of N [37,38].

Furthermore, the more intensified systems promoted an increase in the herbage accumulation rate due to the increased N fertilizer, which allowed higher stocking rates. However, the herbage mass was similar (average 5400 kg DM ha<sup>-1</sup>) among the pasture intensification systems. This is because of the combination of canopy height being maintained at  $\pm 25$  cm with N input and the increased stocking rate of the more intensified systems, where herbage mass production was controlled by animal consumption. Although N is the most limiting element for plant growth [5], and the application of this element promotes an increase in the herbage accumulation rate [10]; in this study, more animals were allocated to the area to maintain the canopy height at  $\pm 25$  cm. This resulted in similar amounts of herbage mass, which was an instantaneous measurement, among the different levels of pasture intensification.

Nonetheless, the lack of differences in herbage accumulation rate between semi-intensive and intensive systems, but with lower stocking rate in semi-intensive than intensive systems, might suggest an under grazing condition in semi-intensive levels of pasture intensification. On the other hand, a previous study discussed the limitations of enclosure cage techniques in continuously stocked, with variable stocking rate, pastures and concluded that the cage technique tended to overestimate forage accumulation and to result in high variability in forage accumulation rate calculations, depending on the number and choice of sampling sites, and the measurement of initial forage mass [39]. When the number of sampling sites is small, as in the present study, to avoid excessive destructive sampling, the accuracy of forage mass estimative tended to decrease [39]. Then, the lack of differences in herbage accumulation rates between semi-intensive and intensive systems might have resulted from the high variability and limitations inherent to the method.

The pseudostem is the leaf support structure and, thus, intensified pasture systems with greater leaf percentages also had greater pseudostem participation. Delevatti et al. [10] also observed a higher proportion of stems in N-fertilized pastures.

The highest proportion of dead material in the extensive system is probably due to the higher number of senescent tissues that had adhered to the tiller from the management practices of the dry season. Furthermore, the C:N ratio is known to act in the decomposition of dead material. In this process, the microorganisms use spare inorganic N in the soil to sustain their growth, which is pushed by the availability of C [40]. Thus, in pastoral production systems, soil N immobilization is predominant, explaining the decrease in the percentages of dead material with the intensification of the system in which N fertilizer was applied. In fact, a previous study observed that marandu palisadegrass pastures fertilized with N produced higher concentrations of N in the litter ( $7.9 \pm 0.4$  g kg<sup>-1</sup> organic matter, OM), resulting in a lower C:N ratio ( $34.0 \pm 2.8$ ) than in unfertilized pas-

tures ( $44.1 \pm 2.8$ ; [41]), which might have favored the decomposition of dead material by microorganisms.

The leaf-to-stem ratio exhibited compensation in the proportions of leaf, stem, and dead material that occurred in the canopy. In semi-intensive and intensive systems, the N inputs, together with the increased stocking rate, favored dead material decomposition and stimulated the generation of new tissues (stem and leaves). Although the proportions of leaf, stem, and dead material differed among treatments (Table 3), the leaf-to-stem ratio was similar. This indicated that compensation between the photosynthetic and the support/translocation tissues might have occurred, irrespective of the pasture intensification treatments.

Lighter tillers were observed in semi-intensive and intensive pasture systems where fertilization occurred. According to Gomide et al. [42] and Calvano et al. [43], as new leaves appear (Table 2), increasing competition is established between the tillers for light, nutrients (N), and water, thereby generating a greater number of tillers per area. In the case of unfertilized pastures, such as extensive systems, tillers tend to be heavier to preserve the scarce resources of the environment. Sbrissia and Silva [8] explained the presence of compensation between the size and density of marandu palisadegrass tillers to maintain the leaf area index and light interception ability of the canopy, which is driven by environmental factors (water, radiation, temperature, and nutrients) and management [5]. Although we have not measured tiller density, a rough estimate by dividing the herbage mass and the mean weight of tillers (Table 3) would result in a tiller density of 585, 696 and 770 tillers  $m^{-2}$  in extensive, semi-intensive and intensive systems, suggesting a compensation between the size and density of tillers. Furthermore, changes in the tiller dynamic supported changes in pasture structure, in which intensive and semi-intensive systems depicted more available green (leaves plus stem) herbage mass than extensive systems.

Intensification via N allowed the canopy structure to have a greater number and percentage of leaves, a consequence of the higher rate of leaf appearance. In the extensive system with stocking rate adjustments to maintain canopy height at 25 cm, cattle were allowed to have an average daily gain similar to that of the intensified levels, with an average of 0.880 kg ( $p = 0.67$ , SEM = 0.04, data not shown).

The extended tiller was longer in the semi-intensive systems when compared to the extensive and intensive systems because the canopy was N-fertilized, which promoted plant tissue growth, but the stocking rate was moderate. However, intensive systems presented a high stocking rate and defoliation intensity of the extended tiller, showing that N allows for the recovery of the tiller length, but defoliation regulates its height. In the extensive system, although the stocking rate was lower compared to the others, the defoliation intensity was high; nevertheless, without N input, shorter extended tillers were obtained. In fact, Boval et al. [44] reported that tillers are larger in N-fertilized canopies. Nonetheless, regarding pasture intensification levels, we observed that both N and defoliation intensity dictated the size of the extended tiller.

The semi-intensive system presented lower defoliation intensities of the leaf and extended tiller, but had similar defoliation intervals than the intensive systems (Table 4). This indicates that the lower proportion of leaf and extended tiller removal was presumably due to an increase in the nutritional value of the leaves [44], resulting from N fertilization [10] combined with the actual stocking rate (2.8 AU, intermediate among the systems; [45]), which allowed the animals to select only the most digestible portions of the leaf.

Conversely, the intensive system had a higher proportion of foliar blade, extended tiller removal, and defoliation interval. Although N fertilization of the intensive system might have increased the nutritional value of the pasture [10], the actual stocking rate (3.8 AU, the highest among pasture intensification systems) may have implied the maximum grazing of the more digestible tissues of the pasture [46]. The extensive system had a low defoliation interval due to the low stocking rate (1.9 AU), but the high intensity of

foliar blade and extended tiller removal, which was similar to the intensive systems, was probably a way to compensate for the low nutritional value of the pasture [47].

The pasture intensification treatments did not alter the canopy height due to target management. Meanwhile, a high rate of leaf appearance and a decrease in the leaf lifespan was observed, demonstrating the ability of the canopy to adapt to the imposed conditions. Recently, researchers have suggested flexibility in management based on target heights, proposing heights in the lower and/or upper limits [3,48]. These heights are within the resistance limits of tillers, that is, respecting their regenerative capacity, with care that the lower limit makes the plant more demanding with regard to fertilization and soil fertility. Therefore, it will soon be necessary to study the morphogenic and structural variables of the canopy within this flexibility, and with less volatile N sources, to increase the stocking rate and, consequently, create improved sustainability in the use of pastures.

### *Implications*

The morphogenic variables indicate that N accelerates the flow of appearance and expansion of leaf blades, meaning new leaves being promptly available for consumption by cattle, making it possible to place more animal units per area. If we observe the morphogenesis data and canopy structure of the extreme systems (extensive and intensive systems), we can infer that the intensive system provided more suitable grazing management practices because there was no difference in the herbage mass, with less phyllochron, a shorter lifespan of the leaf, more leaves, and less dead material. Therefore, the canopy renews itself in the face of intensification, as supported by the higher stocking rate. Conversely, the extensive system management did not receive the grazing frequency needed to utilize the available forage and avoid underutilization. Consequently, the extensive system produced excessive dead material resulting in a lower proportion of leaves and stems than in semi-intensive and intensive systems, yet, without differences in the leaf-to-stem ratio across intensification systems of marandu palisadegrass. Despite the leaf lifespan being longer in extensive systems, the infrequent defoliation led to more senescent and ungrazed herbage.

The extensive system, although named as such, has an inherent stocking rate adjustment control and sets the initial parameters for grazing management regarding future fertilization, supplementation, irrigation, and other management practices. The semi-intensive system may lead us to conclude that it was under-grazed because the phyllochron was intermediate. However, the leaf lifespan did not differ from that of the extensive system, as even with greater fertilization, it showed the lowest value of defoliation intensity (leaf blade and extended tiller).

Prior to this experiment, pastures were managed to mimic an extensive scene of production with low inputs of fertilizers or gypsum. The results obtained in this study can be improved in the future by adopting management strategies that improve soil structure. Delevatti et al. [10] observed that, in an adjacent area with high fertility, the stocking rate increased from 3.3 AU without N fertilization to 6.5 AU with 270 kg of N ha<sup>-1</sup>. Otherwise, the low soil fertility in the studied area is close to the scenario of livestock farming in Brazil, especially in the transition region from the Atlantic Forest to the Cerrado.

The management practices adopted here (height at ±25 cm, control of stocking rate, and N fertilization), supported by the data of morphogenesis and canopy structure, demonstrate the effect of pasture intensification as an alternative to increase productivity and decrease pasture areas. In this way, areas can be spared from deforestation in the face of criticism that pastoral activity should be intensified to preserve resources, according to Cardoso et al. [2].

## **5. Conclusions**

Pasture intensification alters both the morphogenic and structural variables of the marandu palisadegrass canopy. The ecosystem of the marandu palisadegrass pasture, managed under continuous and put-and-take stocking at a canopy height of ±25 cm and

fertilized with ammonium nitrate, is improved by the intensification of pasture usage. This is supported by the analysis of morphogenic and structural variables of the canopy, combined with the defoliation interval and intensity of defoliation.

Pasture intensification accelerates leaf appearance, decreases leaf lifespan, and shortens the tiller defoliation interval, but does not change the herbage mass. Furthermore, the extensive system produces excessive amounts of forage loss due to dead material.

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