

## Short Communication

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# Effect of pruning height and organic fertilization on the morphological and productive characteristics of *Moringa oleifera* Lam. in the Peruvian dry tropics

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**Abstract:** The objective of the study was to evaluate the effect of pruning height (PH) and organic fertilization dose (FD) on the morphology and productive characteristics of *Moringa oleifera* Lam. We germinated seeds collected from 10-year-old shrubs, and 2-month-old seedlings were transplanted in the final field. We used a two-factor design of PH (PH1:0.4, PH2:0.8, and PH3:1.2 m from the ground) and FD (FD0:0, FD1:500, FD2:750, and FD3:1,000 g of decomposing goat manure). We carried out an initial pruning 4 months after transplanting and the harvests every 45 days. After three consecutive harvests, PH3 improved  $N^{\circ}$  branches ( $12.53 \pm 3.09$ ) and dry matter ( $21.98 \pm 1.30\%$ ), but PH1 showed greater stem lengths ( $1.65 \pm 0.24$  m) ( $p < 0.01$ ). There was no difference in the stems and leaf weights between PH2 and PH3, and no trait varied according to FD ( $p > 0.05$ ). The PH  $\times$  FD interaction can improve the plant diameter ( $p < 0.01$ ) and dry matter ( $p < 0.05$ ) with PH2 ( $56.79 \pm 3.71$  mm) and PH3 ( $23.20 \pm 1.04\%$ ) from FD1. We found an increasing trend in  $N^{\circ}$  branches, plant diameter ( $p < 0.01$ ), and the leaf–stem ratio. However, in the third harvest, the biomass production trend was downward for

a short period for an adequate replacement of nutrients from the incorporated organic fertilizer. It is recommended to prune *M. oleifera* at 1.2 m from the ground to stimulate greater biomass and maintain the leaf–stem ratio throughout the evaluated harvests and apply more than 500 g of goat manure after each harvest to restore the nutrients extracted from the soil.

**Keywords:** tropical livestock, fodder shrubs, goat manure, sprouts, leaf-stem ratio, biomass yield

## 1 Introduction

*Moringa oleifera* Lam. is a perennial deciduous tropical shrub belonging to the Moringaceae family, with great nutritional and medicinal qualities due to its content of bioactive compounds [1]. It is a multifunctional and fast-growing crop with a great ability to adapt to various environmental conditions [2]. *M. oleifera* has been widely used for food, agricultural, medicinal, and industrial purposes, purification of drinking water, antibacterial efficacy, and cosmetics, among others, and as an unconventional feed in animal production, as a protein source [3–7].

In several tropical regions of South America, livestock farming plays an important role in the economic development and food security of the population [8–10]. However, this economic activity frequently faces problems of scarcity of forage resources, which makes it difficult to achieve long-term sustainability, especially in the context of climate change [11,12]. Problems related to the low availability of forage and its poor nutritional quality occur throughout the year, mainly in the dry season, making this production deficient [13]. Therefore, there is a need to look for sources of feed and forage for livestock that increase the volume of biomass and contain better nutritional content to increase productive efficiency in livestock herds [14,15].

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*M. oleifera* can be used as forage for ruminants due to its nutritional qualities and biomass volume [16]. In goats, including 9% *M. oleifera* leaves in the diet allowed dry matter intake to increase from 258 to 335 g/day [17]. Greater daily weight gains (from 55 to 86 g/day) were also observed when supplemented with 20% of the diet [18]. In sheep, weight gains from 31 to 118 g/day were found when providing *ad libitum* lambing and 500 g of *M. oleifera* dry matter [19]. By including 40–50% *M. oleifera* forage in the diet, cattle achieved daily gains from 900 to 1,200 g/day [20], and by including 20% dry matter in the diet of dairy cows, milk production was similar to that of cows fed with 20% soybean meal in their diet [14]. In grazing systems, the inclusion of 2–3 kg/day of *M. oleifera* forage in dairy cows grazing on *Brachiaria brizanta* pastures improved dry matter intake and milk production from 3.1 to 5.1 kg/day [21], as well as in a study with *Bos indicus* × *Bos taurus* in Peru [22].

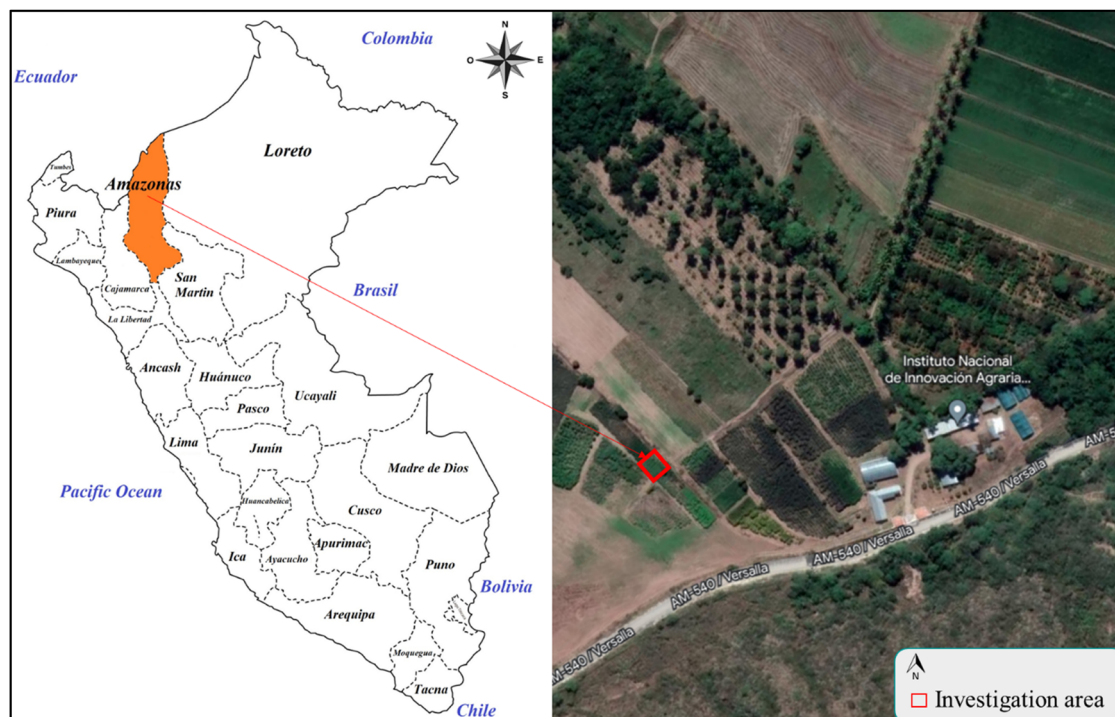
The *M. oleifera* bush develops well without the application of large amounts of fertilizers, although it achieves better development in clay loam soils, with a slightly acidic to neutral pH and an adequate supply of organic or mineral fertilizer. However, among the aspects that also influence the *M. oleifera* forage productivity are the age of the crop, irrigation, cutting frequency, and formative pruning [23]. For example, the application of organic fertilization based

on bovine manure improves the *M. oleifera* forage production up to 6.61 t dry matter/ha [24]; pruning can influence shoot growth, biomass production, and dry matter availability [25]. Under irrigation and fertilization conditions, *M. oleifera* can increase stem height after 60 days, but dry matter yield can be higher than after 45 days [26]. Under conditions of the dry tropics of Peru, there are still no studies on the productive behavior of *M. oleifera*; therefore, the objective of this study was to evaluate the effect of pruning and organic fertilization dose on the morphological and productive characteristics of *M. oleifera* under dry tropical conditions in Utcubamba, Peru.

## 2 Materials and methods

### 2.1 Location

The study was carried out from February to September 2021 in the Agrarian Experimental Station “Amazonas,” located 7 km from the “El Milagro-Versalla” highway, El Milagro district, Utcubamba province, Amazonas Department, Peru. The area is located at coordinates 5°39'48.1"S 78°32'08.9"W (Figure 1). The zone is classified as a tropical Dry Forest (Aw)



**Figure 1:** Location of the experiment at the Agrarian Experimental Station “Amazonas,” Utcubamba province, Amazonas Department, Peru. Source: Modified Google Maps. Accessed on May 28, 2023.

according to the Köppen–Geiger system, where the climate is warm with temperatures ranging from 16 to 31°C and relative humidity of around 69.9% as an annual average.

## 2.2 Experimental design

The study was developed under a 3 × 4 factorial arrangement in a randomized complete block design, with three levels of pruning height (PH) of *M. oleifera* shrubs (0.4, 0.8, and 1.2 m from the ground) and four levels of doses of organic fertilization (0, 500, 750, and 1,000 g of decomposing goat manure). In total, 12 combinations or treatments were obtained, and each one was made up of 10 shrubs or repetitions, making a total of 120 shrubs in an area of 180 m<sup>2</sup> for the entire experiment. Subplots of every ten shrubs were installed in consecutive rows (blocks) and were randomly assigned to each treatment, with a distance of 1 m × 1 m between plants. In the area, the physicochemical characteristics of the soil before installing the experiment were the following: pH 7.5, electrical conductivity 4 mS/m, organic matter 1.88%, C 1.09%, N 0.09%, P 7.35 ppm, and K 219.81 ppm, with slope, lighting, temperature, and precipitation conditions uniform for all plants.

## 2.3 Forest nursery

Bags were prepared and filled with agricultural soil substrate, river sand, and rice husks at a 2:2:1 ratio, respectively. Shrubs of *M. oleifera* of approximately 10 years of age were selected to collect seeds at physiological maturity in November 2020. On December 1, 2020, the collected seeds were sown directly in each bag with the substrate. After 2 months in the nursery, when the shrubs reached 30 cm in height, they were transplanted to the final field for their establishment.

## 2.4 Installation of *M. oleifera* in the plots

The area was mechanized by a tractor with a disc plow, and after 15 days, the area was crossed by a tractor with a harrow. On February 5, 2021, 2-month-old seedlings were transplanted in holes of 0.3 m × 0.3 m × 0.3 m, with a distance of 1 m × 1 m between plants and between rows, in a total area of 180 m<sup>2</sup>. The initial pruning was carried out after 4 months of transplant with a bow-type saw, according to

three PHs and the dose of organic fertilization (FD) based on decomposing goat manure (approximate content of 7% N, 2% P, and 10% K).

## 2.5 Morphological evaluation

After 45 days of pruning, the first harvest was made. The measurements were made at three harvests every 45 days after the initial pruning. For each treatment, five shrubs were taken from the central row, making a total of 60 shrubs. The following morphological and productive characteristics were recorded:

- Number of branches per shrub.
- Stem height (m) measured from the base of the stem shoot to the apical part of the largest shoot [26].
- Plant diameter (mm) measured at 10 cm from the ground, with a digital Vernier.
- Stem weight per shrub (kg). The stems were cut at the base of the stem and weighed.
- Leaf weight per shrub (kg). The weight of the leaves separated from the stems.
- Leaf–stem ratio. Leaf weight was divided by the stem weight of each shrub.
- Dry matter content (%) consisted of weighing a sample of green forage (100 g). The sample was placed inside an oven at 60°C for 72 h until a constant weight was obtained [27].
- Dry matter yield (t DM/ha) was calculated based on the biomass produced in each treatment per hectare (t/ha), and the dry matter content previously determined in the samples.

## 2.6 Statistical analysis

The normal distribution and homogeneity of variances of the data were evaluated with the Shapiro–Wilk ( $p > 0.05$ ) and Levene ( $p > 0.05$ ) tests, respectively. Then, an ANOVA of a complete block design was performed with the main effects of PH, FD, blocks, and PH × FD interaction for N° branches, stem height, plant diameter, stem weight, leaf weight, leaf–stem ratio, dry matter, and dry matter yield. In addition, the independent effect of harvest and its interaction with PH and FD was analyzed using ANOVA and Student–Newman–Keuls test ( $p < 0.05$ ) and the repeated measures ANOVA with the Greenhouse–Geisser test ( $p < 0.05$ ). The comparison of means of the interactions was determined using the Bonferroni adjustment test ( $p <$

0.05). Correlations were analyzed using Pearson coefficients ( $p < 0.05$ ), and a principal component analysis was performed with a KMO test value of 0.431 and a significance level of Bartlett's sphericity test of  $p < 0.001$  using the IBM SPSS Statistics 25 software.

### 3 Results

No differences were found in any morphological and productive characteristics of *M. oleifera* according to the dose of organic fertilization (FD) ( $p > 0.05$ ). The average values were the following:  $N^\circ$  branches,  $10.36 \pm 3.33$ ; stem height,  $1.53 \pm 0.24$  m; plant diameter,  $47.86 \pm 5.49$  mm; stem weight,  $0.97 \pm 0.30$  kg; leaf weight,  $1.17 \pm 0.35$  kg; leaf–stem ratio,  $1.23 \pm 0.29$ ; dry matter,  $20.89 \pm 1.34\%$ ; and dry matter yield,  $5.11 \pm 1.55$  t DM/ha (Table 1). However, there was significant variation according to PH, where PH3 stood out in  $N^\circ$  branches and dry matter, but the stem height was greater in PH1; however, the stem weight, leaf weight, and dry matter yield did not vary significantly between PH2 and PH3 ( $p < 0.01$ ). The plant diameter was greater in PH2 ( $p < 0.01$ ), but there was no significant difference between PH1 and PH3. Furthermore, a significant variation according to

harvest was found, where  $N^\circ$  branches and plant diameter were significantly higher in the third harvest of forage, followed by the second and first harvest ( $p < 0.01$ ). Stem height and stem weight were higher at the first harvest and then tended to lower in the next cuts ( $p < 0.01$ ). There were no differences in the leaf weight between the first and third harvests, and there was no difference in the leaf–stem ratio between the second and third harvests.

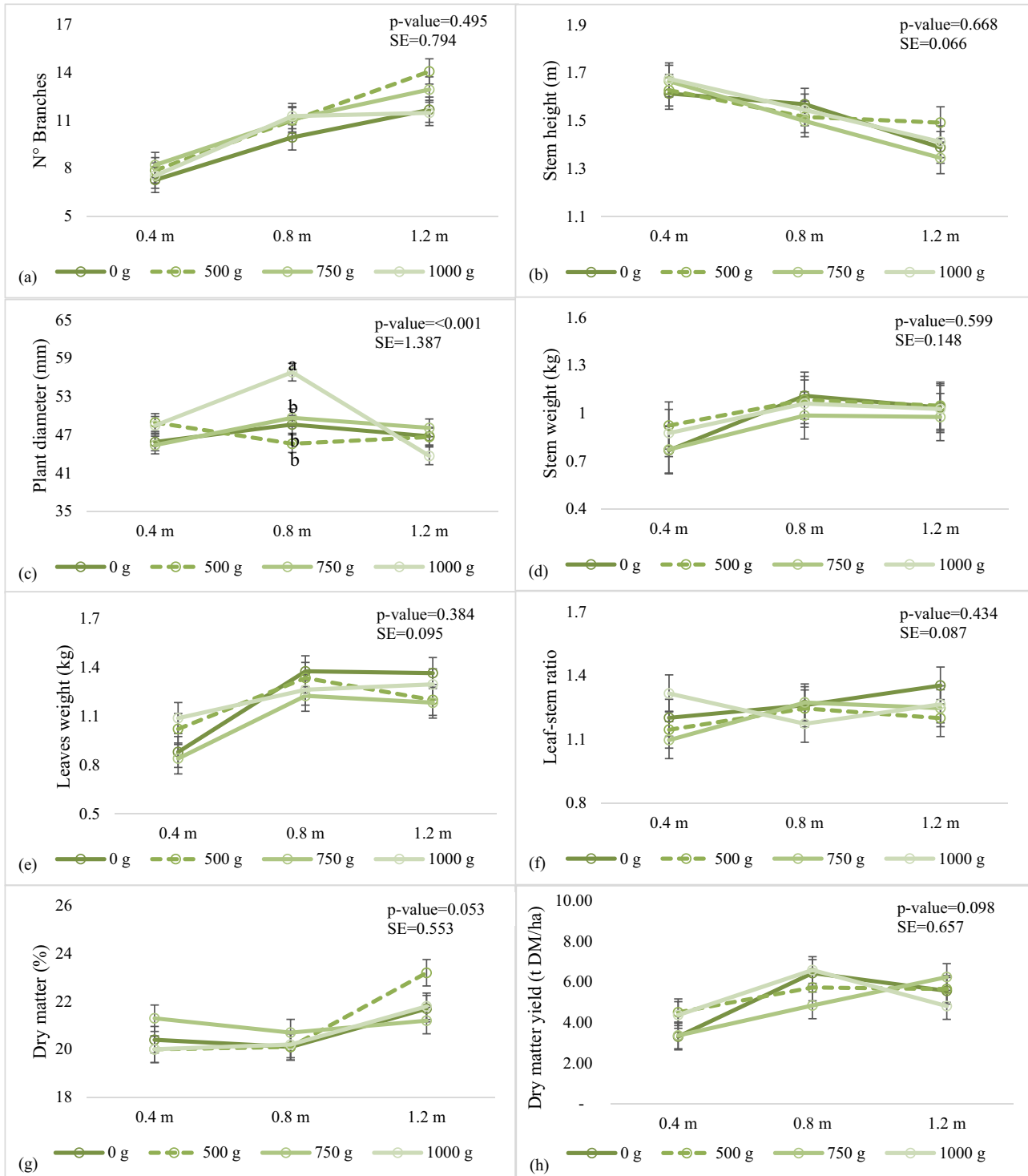
An interactive effect was found between PH  $\times$  FD for the plant diameter ( $p < 0.01$ ). As shown in Figure 2c, PH2 (0.8 m) with FD3 (1,000 g) improved the plant diameter to  $56.79 \pm 3.71$  mm compared to the use of 0, 500, and 750 g of decomposing goat manure at the same PH2 ( $48.60 \pm 4.75$ ,  $45.58 \pm 4.77$ , and  $49.65 \pm 4.80$  mm, respectively). On the other hand, as shown in Figure 2g, the dry matter content at PH3 (1.2 m) was higher with FD1 (500 g) ( $23.20 \pm 1.04\%$ ) than with FD2 ( $21.20 \pm 1.35\%$ ), but the difference was not significant ( $p > 0.05$ ). No interaction effect was found in the  $N^\circ$  branches, stem height, stem weight, leaf weight, leaf–stem ratio, and dry matter yield ( $p > 0.05$ ).

Due to the high variability of harvest morphological and productive characteristics, the interaction between PH and FD was analyzed. As shown in Table 2, no effect was found in the  $N^\circ$  branches, stem height, plant diameter, stem weight, and leaf–stem ratio ( $p > 0.05$ ) for the

**Table 1:** Morphological and productive characteristics of *M. oleifera* according to the PH, fertilization dose, and harvest

	$N^\circ$ branches	Stem height (m)	Plant diameter (mm)	Stem weight (kg)	Leaf weight (kg)	Leaf–stem ratio	Dry matter (%)	Dry matter yield (t DM/ha)
<b>PH</b>								
PH1 (0.4 m)	7.72 <sup>c</sup>	1.65 <sup>a</sup>	47.15 <sup>b</sup>	0.84 <sup>b</sup>	0.96 <sup>b</sup>	1.19	20.43 <sup>b</sup>	3.88 <sup>b</sup>
PH2 (0.8 m)	10.82 <sup>b</sup>	1.53 <sup>b</sup>	50.16 <sup>a</sup>	1.16 <sup>a</sup>	1.30 <sup>a</sup>	1.24	20.28 <sup>b</sup>	5.89 <sup>a</sup>
PH3 (1.2 m)	12.53 <sup>a</sup>	1.41 <sup>c</sup>	46.28 <sup>b</sup>	1.02 <sup>a</sup>	1.26 <sup>a</sup>	1.27	21.98 <sup>a</sup>	5.56 <sup>a</sup>
<i>p</i> -value	<0.001	<0.001	<0.001	0.002	<0.001	0.346	<0.001	<0.001
SE	0.53	0.04	1.05	0.10	0.06	0.06	0.39	0.45
<b>Fertilization dose (FD)</b>								
FD0 (0 g)	9.62	1.52	47.07	0.97	1.21	1.27	20.73	5.09
FD1 (500 g)	10.98	1.55	47.04	1.02	1.19	1.20	21.10	5.29
FD2 (750 g)	10.73	1.50	47.70	0.91	1.08	1.20	21.07	4.81
FD3 (1,000 g)	10.09	1.54	49.64	0.99	1.22	1.25	20.67	5.25
<i>p</i> -value	0.21	0.82	0.09	0.28	0.26	0.56	0.77	0.85
SE	0.70	0.05	1.15	0.11	0.08	0.06	0.51	0.59
<b>Harvest</b>								
First	8.48 <sup>c</sup>	1.77 <sup>a</sup>	46.38 <sup>b</sup>	1.14 <sup>a</sup>	1.26 <sup>a</sup>	1.11 <sup>b</sup>		
Second	10.35 <sup>b</sup>	1.35 <sup>c</sup>	47.12 <sup>b</sup>	0.80 <sup>b</sup>	1.00 <sup>b</sup>	1.28 <sup>a</sup>		
Third	12.23 <sup>a</sup>	1.47 <sup>b</sup>	50.08 <sup>a</sup>	1.07 <sup>a</sup>	1.26 <sup>a</sup>	1.31 <sup>a</sup>		
<i>p</i> -value	<0.001	<0.001	<0.001	0.001	<0.001	<0.001		
SE	0.59	0.03	1.05	0.10	0.07	0.06		
Total	10.36	1.53	47.86	0.97	1.17	1.23	20.89	5.11

SE: Standard error. Different superscript letters in columns represent significant differences at the  $p < 0.01$  level by the Student–Newman–Keuls test.



**Figure 2:** Interaction between the PH × organic fertilization dose on the morphological and productive characteristics of *M. oleifera*. N° Branches (a), stem height (b), plant diameter (c), stem weight (d), leaf weight (e), leaf-stem ratio (f), dry matter (g), and dry matter yield (h). SE: standard error.



**Table 2:** Interaction between the PH × harvest on morphological and productive characteristics of *M. oleifera*

Harvest	PH	N° branches	Stem height (m)	Plant diameter (mm)	Stem weight (kg)	Leaf weight (kg)	Leaf-stem ratio
First	PH1	5.85	1.92	45.67	0.92	0.94 <sup>b</sup>	1.02
	PH2	9.15	1.75	48.37	1.32	1.53 <sup>a</sup>	1.17
	PH3	10.45	1.63	45.10	1.18	1.31 <sup>ab</sup>	1.12
Second	PH1	7.75	1.44	46.72	0.72	0.80 <sup>c</sup>	1.17
	PH2	10.40	1.39	50.05	0.88	1.09 <sup>b</sup>	1.28
	PH3	12.90	1.22	44.60	0.81	1.10 <sup>b</sup>	1.39
Third	PH1	9.55	1.58	49.05	0.87	1.13 <sup>b</sup>	1.37
	PH2	12.90	1.46	52.05	0.98	1.28 <sup>ab</sup>	1.26
	PH3	14.25	1.38	49.15	1.08	1.37 <sup>a</sup>	1.29
SE		0.60	0.03	1.37	0.13	0.08	0.07
Harvest × PH <i>p</i> -value		0.755	0.121	0.796	0.781	0.016	0.071
(*)Repeated measures <i>p</i> -value		0.682	0.060	0.198	0.680	0.007	0.138

SE: standard error. Different superscript letters in columns represent significant differences at the  $p < 0.05$  level by the Bonferroni adjustment test. PH1: 0.4 m, PH2: 0.8 m, PH3: 1.2 m of PH. (\*)Repeated measures ANOVA with the Greenhouse–Geisser test.

interaction between PH × harvest. Significant interaction ( $p < 0.05$ ) and effect of repeated measures ( $p < 0.01$ ) on the leaf weight were found, where a greater leaf weight was found with PH2 in the first harvest ( $1.53 \pm 0.36$  kg) and with PH3 in the third harvest ( $1.37 \pm 0.33$  kg); however, with PH1 in the second harvest, the lowest leaf weight was obtained ( $0.80 \pm 0.21$  kg) ( $p < 0.05$ ). Although PH3 did not have the best leaf weight performance in the first harvest, in the second harvest, it was similar to PH2, and in the third harvest, it surpassed PH2, although not significantly.

As shown in Table 3, no effect was found in any morphological and productive characteristics of *M. oleifera*

( $p > 0.05$ ) for the interaction between FD × harvest. In both interaction analyses, a similar behavior of the variables was observed for all three harvests. The values of N° branches, plant diameter, and leaf–stem ratio are incremental according to the evaluated harvest; on the other hand, the values of stem height, stem weight, and leaf weight experienced a dramatic decrease in the second harvest, and in the third harvest they did not reach the values of the first harvest, except for the leaf weight. However, a significant effect of repeated measures was found on the number of branches, which was greater in the third harvest and lower in the first harvest ( $p < 0.05$ ).

**Table 3:** Interaction between the organic fertilization dose × harvest on the morphological and productive characteristics of *M. oleifera*

Harvest	Fertilization dose	N° branches	Stem height (m)	Plant diameter (mm)	Stem weight (g)	Leaf weight (g)	Leaf-stem ratio
First	FD0	7.73 <sup>d</sup>	1.74	45.31	1.13	1.28	1.12
	FD1	8.20 <sup>d</sup>	1.75	44.83	1.16	1.29	1.13
	FD2	9.40 <sup>c</sup>	1.76	47.39	1.09	1.15	1.04
	FD3	8.60 <sup>d</sup>	1.82	47.10	1.18	1.32	1.13
Second	FD0	9.80 <sup>c</sup>	1.34	47.17	0.75	1.04	1.42
	FD1	10.40 <sup>b</sup>	1.37	46.17	0.91	1.02	1.17
	FD2	11.13 <sup>b</sup>	1.31	45.76	0.70	0.91	1.31
	FD3	10.07 <sup>c</sup>	1.37	49.39	0.86	1.03	1.23
Third	FD0	11.33 <sup>b</sup>	1.50	48.73	1.04	1.31	1.28
	FD1	14.33 <sup>a</sup>	1.51	50.13	0.99	1.25	1.29
	FD2	11.67 <sup>b</sup>	1.44	49.93	0.94	1.19	1.27
	FD3	11.60 <sup>b</sup>	1.44	51.53	0.93	1.30	1.39
SE		0.88	0.05	1.58	0.15	0.10	0.08
Harvest × FD <i>p</i> -value		0.198	0.635	0.810	0.831	0.999	0.273
(*)Repeated measures <i>p</i> -value		0.024	0.194	0.067	0.720	0.997	0.371

SE: standard error. FD0: 0 g, FD1: 500 g, FD2: 750 g, FD3: 1,000 g of organic fertilizer (goat manure). (\*)Repeated measures ANOVA with the Greenhouse–Geisser test.

**Table 4:** Correlations of morphological and productive characteristics of *M. oleifera*

Variable	Stem height (m)	Plant diameter (mm)	Stem weight (g)	Leaf weight (g)	Leaf–stem ratio	Dry matter
$N^{\circ}$ branches	–0.394**	0.301**	0.223**	0.402**	0.243**	0.254*
	<0.01	<0.01	<0.01	<0.01	<0.01	0.05
Stem height (m)		0.070	0.438**	0.228**	–0.293**	–0.111
		0.35	<0.01	<0.01	<0.01	0.40
Plant diameter (mm)			0.344**	0.338**	0.019	–0.102
			<0.01	<0.01	0.796	0.44
Stem weight (g)				0.816**	–0.313**	0.085
				<0.01	<0.01	0.52
Leaf weight (g)					0.224**	–0.035
					<0.01	0.79
Leaf–stem ratio						–0.305*
						0.02

Significant correlation at the  $p < 0.05$  level (\*) and at the  $p < 0.01$  level (\*\*).

Significant and direct correlations were found among the plant diameter, stem height, stem weight, and leaf weight ( $p < 0.01$ ), except for  $N^{\circ}$  branches with stem height, which was inverse (Table 4). The leaf–stem ratio directly correlated with  $N^{\circ}$  branches and leaf weight but inversely with stem height and stem weight ( $p < 0.01$ ). The dry matter content directly correlated with  $N^{\circ}$  branches but inversely with the leaf–stem ratio ( $p < 0.01$ ).

Using principal component analysis, three components were obtained that explained 82.83% of the information of the morphological and productive variables evaluated in this study in a general way (Table 5). As shown in Table 6, component 1 strongly and positively correlated with  $N^{\circ}$  branches, plant diameter, stem weight, and leaf weight and explained 41.54% of the variance. Component 2 strongly and positively correlated with stem height and negatively with leaf–stem ratio, which explained 64.14% of the accumulated variance. Component 3 strongly and positively correlated with dry matter, which explained 82.83% of the total accumulated variance.

## 4 Discussion

The effect of PH and organic fertilization dose (FD) on the morphological and productive characteristics of *M. oleifera* forage was evaluated. Stem pruning is a cultural practice that stimulates stem branching and biomass production and facilitates harvest [25]. In this study, after 45 days of growth, pruning at 0.4 m from the ground (PH1) stimulated a greater length of new stems, but pruning at 0.8 m (PH2) and 1.2 m (PH3) increased the number of sprouting branches, leaf weight, stem weight, leaf–stem ratio, and dry matter content. In PH2 and PH3, the number of active buds for regrowth was greater than those in PH1 due to the greater length of its main stem, which would explain its greater production of harvested biomass. However, supported by the negative correlation between the number of branches and stem height, the lower number of new branches in PH1 offers an advantage for the growth of new stems in length and a disadvantage for PH2 and PH3, probably due to the ability to the nutritional reserves to sustain all of the shoots, although the low leaf

**Table 5:** Total explained variance of morphological and productive variables of *M. oleifera* subjected to different PHs and organic fertilization dose

Component	Initial eigenvalues			Sum of the squared saturation of extraction		
	Total	Variance percentage	Accumulated percentage	Total	Variance percentage	Accumulated percentage
1	2.91	41.54	41.54	2.91	41.54	41.54
2	1.58	22.60	64.14	1.58	22.60	64.14
3	1.31	18.69	82.83	1.31	18.69	82.83
4	0.57	8.12	90.95			
5	0.42	5.94	96.89			
6	0.21	3.06	99.94			
7	0.00	0.06	100.00			

Extraction method: principal component analysis.

**Table 6:** Principal component matrix of morphological and productive variables of *M. oleifera* subjected to different PHs and organic fertilization dose

Variables	Component		
	1	2	3
N° branches	0.783	-0.244	0.350
Stem height (m)	-0.062	0.854	-0.320
Plant diameter (mm)	0.725	0.401	-0.177
Stem weight (g)	0.917	0.205	0.019
Leaf weight (g)	0.932	-0.068	-0.143
Leaf-stem ratio	0.231	-0.759	-0.438
Dry matter	0.056	0.091	0.916

stem ratio in PH1 suggests applying PHs that increase biomass in weight rather than length, as in PH2 and PH3. There are various reports on the effect of PH on the morphology of *M. oleifera*. du Toit et al. [28] found that moderate and severe pruning at 2 m and 1 m from the ground, respectively, are able to stimulate the growth of the main stem circumference and biomass production, similar to this study, but not for the production of flowers and seeds. Likewise, Cauch-Cauch et al. [29] also reported higher biomass yield in pruning at 0.7 m than at 0.5 m from the ground. Ruiz-Hernández et al. [25] obtained greater stem length and survival of new shoots in two consecutive prunings at 0.75 and 1.00 m compared to 1.50 m from the ground, although they did not find differences in biomass production or protein content in the leaves. However, among the discordant reports, Ramírez et al. [30] found higher biomass yield, greater number of shoots, leaf weight, stem weight, and leaf-stem ratio, with pruning at 0.5 m than at 0.75 and 1.00 m from the ground. Truong et al. [31] reported a greater number of shoots, number of leaves per shoot, and biomass yield at 0.55 m than at 0.45, 0.65, 0.75, and 0.85 m from the ground. Regarding the leaf-stem ratio, forage species for animal feed require leaf-stem ratio values above 1 since a greater proportion of leaves can provide a greater quantity and quality of nutritional content than the proportion of stems. In this study, higher values of leaf-stem ratio were correlated with lower values of stem height, stem weight, and dry matter content (inverse correlation). Furthermore, the mean leaf-stem ratio (1.23) was lower than the finding by Ledea et al. [32] (1.40) at 45 days after pruning, although they reported that the cut was made 10 cm from the ground. In the branches of woody shrub species, such as *M. oleifera*, the proportion of structural carbon components is more predominant than in herbaceous species [33], so the leaf-stem ratio values could be relatively lower.

The organic fertilization dose (FD) did not influence any morphological or productive characteristics of *M. oleifera*. Furthermore, no trend of increase or decrease in the

evaluated variables was found, so we hypothesize that the evaluation period, up to 135 days after the application of FD, was not enough for the mineralization of the organic fertilizer by soil microorganisms to be used by plants [34]. Organic fertilizers based on animal manure and composting are slow to release nutrients and require periods of up to 3 years for their mineralization, making them available to plants compared to inorganic fertilizers [35,36]. Mota-Fernández et al. [37] and Sol-Quintas et al. [38] did not find a significant effect of the application of compost, vermicompost, and bovine manure bocashi on the number of new branches, stem height, number of leaves, and leaf area of *M. oleifera* during several harvests, although they were positively correlated with the content of N, P, K and MO. However, the average stem height and leaf weight in this study (1.53 m and 1.17 kg per bush, respectively) were higher than those reported by García and Quevedo [39], who obtained 0.43 m and 914.83 g when incorporating inorganic N fertilization (1.52 g), P (1.52 g), K (1.52 g), and poultry manure (62 g) per plant, after 45 days of cutting. In addition, dry matter content values lower than those of this study were reported ( $20.89 \pm 1.34\%$ ) at 90 days of cutting with bovine manure [40]. Although *M. oleifera* has good biomass production capacity over a wide range of planting densities, its productivity depends on the good availability of nutrients and soils in the pH range of 4.5–8.0 [41,42]. The edaphoclimatic conditions and the content of nutrients applied in fertilization in each study area can influence the growth rate, generating variation in the reports of morphology and productivity of this crop [24].

The significant interaction PH  $\times$  FD suggests that the performance of *M. oleifera* subjected to PH of 0.8–1.2 m can improve the plant diameter and dry matter content when applying FD of 500–1,000 g. The greater production of new branches and leaf-stem ratio in high pruning can stimulate a greater photosynthetic rate and absorption of nutrients from fertilizers for greater production of biomass useful as forage; however, according to the absence of the significant independent effect of FD, a longer evaluation period is required to reinforce the hypothesis of this interactive effect. PHs of 2 and 1 m from the ground could stimulate the growth of the main stem circumference [28], and the application of bovine manure to *M. oleifera* can improve stem diameter values [43]; however, the age at the uniformization cut and planting density could influence the morphology of the plant. The greater the distance between plants, the greater the diameter of plants, stem heights, and number of new branches [31,44].

The effect of harvest increased the number of branches and plant diameter and decreased the height and weight of stems. Although in the third harvest, an increase in the



number of branches was observed, their length decreased throughout the harvests, probably due to the extraction of nutrients from the soil and the insufficient period for the use of the organic fertilizer supplied, resulting in smaller new branches. However, there was no difference in the leaf weight of the first and third harvests and the leaf-stem ratio of the second and third harvests. The healing effect of the shrub subjected to initial pruning could stimulate the growth of new shoots, stems, and leaves and, consequently, an increased production of biomass [25]. Furthermore, Ojiako et al. [45] reported that growth periods greater than 40 days between cuts promote the accumulation of reserves for greater production of new branches after pruning. Thus, Ledea-Rodríguez et al. [26] found a greater yield of leaves, stems, and dry matter content after 60 days than after 45 days of cutting. In a silvopastoral system or forage-cutting shrubs, it is expected that productive characteristics will be conserved or improved through harvests; however, the incremental decrease in productive variables may indicate an imbalance in nutritional replenishment to the soil or failures in cultural management. Pruning plants with stem diameters between 5 and 10 mm, as well as poorly performed pruning, can reduce the potential for regrowth and production of new branches of *M. oleifera* for greater biomass production in several harvests per year [46].

An increasing trend in leaf weight was found in the PH  $\times$  harvest interaction, where initially PH2 showed the highest leaf weight in the first harvest, but in the second and third harvest, PH3 was similar and even surpassed PH2, probably because greater biomass production promoted a greater photosynthetic rate and accumulation of carbon and nitrogen in the root portion [47]. The longer root development time in advanced harvests, independently of foliage growth, could be related to a greater accumulation of reserves for robust regrowth in subsequent harvests, as observed in longer cutting frequencies of *M. oleifera* [26]. PH1 remained with the lowest leaf weight values, suggesting that PH  $<$  0.4 m may have difficulties for forage production in this species. No interactive effect was found between FD  $\times$  harvest; however, two clear trends were observed in the variables evaluated according to the interaction graphs. The number of new branches, plant diameter, and leaf-stem ratio increased throughout the harvests due to the accumulation of nutrients in the roots and the production of structural elements of older plant tissues. On the other hand, the stem height, stem weight, and leaf weight showed a dramatic decrease in the second harvest and a recovery in the third harvest, probably due to adverse environmental conditions during the development period. This observation suggests the high dependence of some productivity variables of *M. oleifera* on the environment and cultural management, where the seasonality of

rainfall, irrigation, effective fertilization, and pruning can maintain high forage production throughout the year.

The KMO test value was less than 0.6 (0.431), but Bartlett's sphericity test showed a significance value  $p <$  0.001, which indicates that principal component analysis is appropriate in this group of variables. The measured variables were grouped into three components to reduce their dimensionality, which is supported by the correlation values found between the variables. Component 1 grouped four variables ( $N^\circ$  branches, plant diameter, stem weight, and leaf weight), while Component 2 grouped two morphological and productive variables (leaf-stem ratio and stem height); however, Component 3 grouped only the dry matter content of *M. oleifera* forage, contributing the largest percentage of the variance by a single variable (approximately 20%).

## 5 Conclusions

Under the study conditions, PH of 1.2 m from the ground can stimulate the greatest production of new branches, biomass yield, and dry matter of *M. oleifera*. Although the independent effect of FD in the study period was not significant, the use of more than 500 g of decomposing goat manure could improve the plant diameter and dry matter content in PH of 0.8–1.2 m. In the third harvest, the number of new branches and plant diameter improved, but the biomass production trend was downward for a short period for an adequate replacement of nutrients from the organic fertilizer. However, PH of 1.2 m maintained leaf production throughout the evaluated harvests. The morphological and productive variables of *M. oleifera* subjected to different PHs and doses of organic fertilization can be grouped into three components, which explains 82.83% of the accumulated variance, where only the dry matter content contributed approximately 20% of the total variance. Finally, the effect of pruning and fertilization on the physiological and nutritional variables of *M. oleifera* shows a greater impact on the variation in future studies with a focus on animal feeding.

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