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Biol, nitrogen fertilization, and topological arrangement effects on the yield and composition of maralfalfa grass (*Pennisetum* spp.) grown under subhumid conditions

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ABSTRACT

Objective: To evaluate the effect of planting arrangements and different fertilization treatments on biomass production and composition of maralfalfa grass (*Pennisetum* spp.) grown under subhumid conditions in Veracruz, Mexico.

Design/Methodology/Approach: A randomized complete block design with split plots was used. Planting density (40, 60, and 80 cm) was the main factor. Three biol formulas and two doses of urea fertilization (50 and 100 kg ha^{-1}) were the secondary factor. The following variables were measured: plant height, biomass yield, and leaf and stem weight. Data were analyzed through an ANOVA and Tukey's test (α=0.05).

Results: The 40-cm planting arrangement and Biol 1 (manure+water) treatment combination reached the highest biomass yield (123.11 t ha^{-1}) and plant height (2.44 m). Denser planting improved biomass production, while chemical fertilization increased leaf ratio. The interaction between planting arrangement and fertilization only had a significant impact on stem weight.

Study Limitations/Implications: The study was conducted under specific climatic conditions, which limited the generalizability of the results. Further research is recommended to assess long-term economic viability and sustainability.

Findings/Conclusions: High planting density combined with organic fertilization optimizes biomass yield and the efficient use of resources. Biols are a sustainable alternative that enhances forage productivity and soil health.

Keywords: Maralfalfa grass, organic fertilization, planting density, biomass, sustainable agriculture.

INTRODUCTION

The growing demand for high quality forages in intensive livestock systems has driven the search for sustainable alternatives to optimize biomass production, without compromising soil or environmental health (Karasawa *et al.*, 2023). Maralfalfa grass (*Pennisetum* spp.) is one of the most promising foraging species, given its fast growth, high biomass yield, and nutritional value (Fuller *et al.*, 2021; Condori *et al.*, 2018). Consequently, maralfalfa could become a strategic resource to improve livestock productivity in tropical and subtropical regions. In addition, the climate in these regions favors its development (Kebede *et al.*, 2023).

Biomass production and composition of tropical grasses are directly influenced by several factors, including planting density and fertilization type (Syam *et al.*, 2021; Ledea-Rodríguez *et al.*, 2023). Planting density impacts the competition for light, water, and nutrients, modifying the growth and morphology of crops (Tilus *et al.*, 2022). In addition, fertilization plays a key role, supplying essential nutrients for plant development. Although chemical fertilizers are widely applied, due to their fast availability, their excessive use have negatively impacted soil health and water quality (Pereira *et al.*, 2022; Bacarrillo-López *et al.*, 2021). Consequently, sustainable alternatives, such as liquid biofertilizers (biols), have been developed. Organic waste is fermented to prepare biols (Jara-Samaniego *et al.*, 2021). These products not only gradually provide nutrients, but also improve the physical, chemical, and biological structure of soils (Mupambwa and Mnkeni, 2018). Recent studies have proven that biols significantly increase forage production and the resistance of agricultural systems to climate change (Moreno-Sandoval *et al.*, 2022). Biol application promotes sustainability in production systems, reducing their dependency on chemical fertilizers and favoring beneficial biological processes in soils (Mupambwa and Mnkeni, 2018). The appropriate planting density combined with organic fertilization can enhance the efficient use of resources, sustainably increasing biomass yield (Gaudio *et al.*, 2019; Wang *et al.*, 2023). Meanwhile, other studies about foraging systems have shown the positive effects of planting geometry (Umesh *et al.*, 2022) and spacing between the plants on key morphological variables (Wang *et al.*, 2025). For their part, Oliveira *et al.* (2025) reported that nitrogen biological fixation significantly contributed to the biomass production of elephant grass grown in low-fertility soils, without synthetic nitrogen fertilizers. Nevertheless, information about the interaction between topological planting arrangements and biols in *Pennisetum* spp. crops is very limited, particularly in the case of maralfala grown under subhumid conditions. This lack of information provides an opportunity to further research these factors. Understanding the impact of these agronomic elements on the yield and morphological composition of the biomass is fundamental to design more efficient and sustainable strategies, aimed to improve forage productivity in tropical systems (Fang *et al.*, 2021). Therefore, the objective of this study was to evaluate the effect of different topological planting arrangements and fertilization treatments, as well as their interaction, on the production and composition of maralfalfa (*Pennisetum* spp.) biomass grown under subhumid conditions, in the state of Veracruz, Mexico.

MATERIALS AND METHODS

Location

This study was conducted at the Instituto Tecnológico Superior de Juan Rodríguez Clara (ITSJRC), located in the municipality of Juan Rodríguez Clara, Veracruz, Mexico, at 133 m.a.s.l. (INEGI, 2010). Based on the Mexican version of the Köppen system, this region is classified as AW_0 —*i.e.*, a subhumid warm climate. It has a 1,100 mm mean annual precipitation and a 24.5 °C mean annual temperature (García, 2004). The region mainly has a dystric cambisol soil, with a strongly acid pH. This condition directly impacts the development of foraging crops (Morales Rivera *et al.*, 2015; Tosquy-Valle *et al.*, 2020).

Establishment of the crop

The evaluation was conducted in a 525 m^2 area. The crops were established on July 5, 2023. The plant material chosen for the experiment was maralfalfa grass (*Pennisetum* spp.). Land preparation included a double harrowing and 80 cm separation between furrows. The grass was sown by hand during the early spring-summer season, following the method for tropical forages suggested by Cerdas-Ramírez *et al.* (2021). A randomized complete block design with an arrangement of split plots was used. The main factor was topological planting arrangement (PTA) with a 40, 60, and 80 cm distance between furrows. The secondary factor was the nitrogen fertilization systems (NFS) with three biol formulas and two doses of urea chemical fertilization (50 and 100 kg N ha^{-1}) (Cerdas-Ramírez *et al.*, 2021). Three repetitions per treatment were established. In order to minimize environmental variability, each block was randomized, guaranteeing the accuracy and reliability of the results (Villanueva-Avalos *et al.*, 2022).

Preparation of the biols

Biols were prepared through the anaerobic fermentation of organic wastes. This technique improves nutrient availability and promotes plant growth (Cabos *et al.*, 2019; Jara-Samaniego *et al.*, 2021). Table 1 shows the three formulated types of biols.

Measurement of morphological and physiological variables

Following the research protocols established by Botero-Londoño *et al.* (2021) and Mengistu *et al.* (2023), the morphological and physiological variables were evaluated to

Table 1. Composition and characteristics of the fertilization systems applied in the experiment.

Treatment	Main Composition	Objective
BIOL 1	Bovine manure + water	Provide a balanced supply of N, P, and K (anaerobic fermentation).
BIOL 2	Bovine manure + whey + molasses + yeast	Increase microbial activity and micronutrient availability
BIOL 3	Whey + molasses + mountain microorganisms + minerals (Ca, Mg, Zn)	Optimize nutrient absorption with a comprehensive profile
	Chemical Fertilization Treatments	
CF1	Urea (50 kg N ha^{-1})	Compare efficiency with biols (low nitrogen dose)
CF2	Urea (100 kg N ha^{-1})	Compare efficiency with biols (high nitrogen dose)

* BIOL=biol; CF (FQ)=chemical fertilization, N=nitrogen.

quantify maralfalfa grass yield. A Just Home MKZ-BAS-ACS209 digital scale was used to estimate weight.

Total biomass yield (TBY)

Three lineal meters of useful plots were harvested and weighted. Subsequently, leaves and stems were manually divided according to their morphology. Green leaf weight (GLW) was evaluated to determine the biomass of photosynthetic tissue.

Stem weight (SW)

Stems were weighted to determine structural biomass.

Plant height (PH)

Plant height was measured from the base of the stem to the flag leaf, using a 5-m aluminum topographic sight (Nedo).

Leaf percentage (LP) $LP(\%) = \left(\frac{GLW}{TBY}\right) \times 100$

Stem percentage (SP) $SP(\%) = \left(\frac{SW}{TBY}\right) \times 100$

Climatic variables

In order to evaluate the direct effect of weather on crop development, the experiment was conducted under rainfed conditions. Temperature and humidity were recorded with an RCW-800-TH Datalogger, while precipitation was measured with a Tenite 110672 pluviometer. Base temperature (BaseT) was determined with the following equation:

$$MediumT = \frac{(MaxT + MinT)}{2}$$

Meanwhile, two focuses were used to calculate BaseT.

Calculation of accumulated degrees-day:

Degrees-day (DeD): $DeD = MediumT - BaseT$

In addition, the methodology proposed by Dawson *et al.* (2004) and Huang *et al.* (2019) for forage grasses was used.

Statistical analysis

A two-way analysis of variance (ANOVA) was used to analyze the data in SAS v. 9.4 (SAS, 2009). The differences between means were evaluated with the Tukey's Test (α=0.05), guaranteeing the statistical rigor between treatments (Gaudio *et al.*, 2019; Cerdas-Ramírez *et al.*, 2021).

RESULTS AND DISCUSSION

Influence of climatic conditions

The humidity and warm conditions during the June-July crop cycle (153- and 176.7-mm rainfall, with 23-30 °C temperatures) favored the establishment and initial growth of maralfala grass. These results matched the reports of Calzada-Marín *et al.* (2014) and Morales Rivera *et al.* (2015). Meanwhile, the decrease of rainfall during August and September (34 and 43 mm) resulted in a moderated water stress that mainly impacted low density plantings. Similar results were registered by Mengistu *et al.* (2023). High density plantings were better at maintaining soil moisture and showed a higher physiological activity. The recovery of rainfall levels during October and November (195 and 140 mm) enabled a vigorous final growth. The gradual accumulation off degrees-days^{-1} (Figure 1) indicated favorable temperature conditions for continuous plant development, particularly for plants grown under an organic fertilization system (Dawson *et al.*, 2004; Huang *et al.*, 2019). Meanwhile, the highest yields obtained with a high planting density and the application of boils (Figure 2) confirm the influence of climatic factors on the agronomic development of the crop.

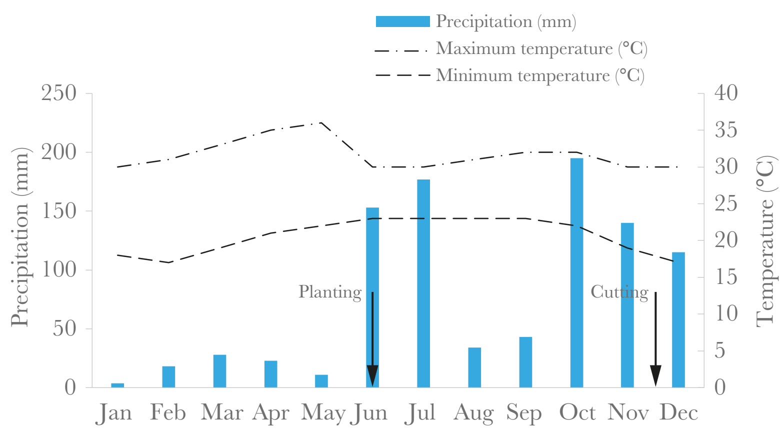

Figure 1. Environmental conditions associated with the growth of maralfalfa grass, under subhumid conditions.

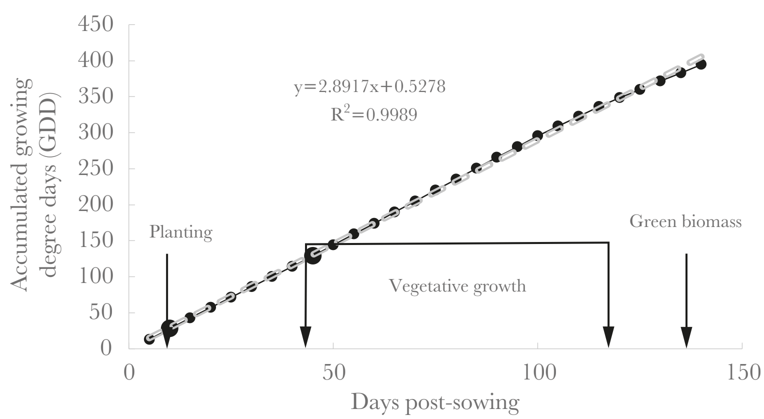

Figure 2. Ratio between days after sowing and the accumulated heat units in maralfalfa grass crops established in the Instituto Tecnológico Superior de Juan Rodríguez Clara.

Effect of the topological planting arrangements

The ANOVA showed statistically significant differences ($P<0.05$) in the main effects of the topological planting arrangements on plant height (PH), total biomass yield (TBY), green leaf weight (GLW), and stem weight (SW).

Table 2 shows that planting topology at 40 cm (T40) recorded the highest plant height (2.4 m), biomass yield (123.1 t ha^{-1}), green leaf weight (39.3 t ha^{-1}), and stem weight (83.7 t ha^{-1}). In addition, T40 recorded statistical differences from T60 and T80. Although leaf percentage (38.8%) was higher in T60, this treatment recorded a lower total yield. In contrast, T80 obtained the lowest values in all the production variables.

These results indicate that the narrower planting topology (40 cm) favors plant development and biomass accumulation, while larger distances significantly reduce forage yield under subhumid conditions.

Effect of the fertilization treatments

Table 3 shows that the fertilization treatments significantly influence plant height (PH) ($P<0.0001$), stem weight (SW), and the morphological composition of maralfalfa. The Biol 1 (manure+water) treatment recorded the greatest height (2.7 m) and the highest stem weight (SW) (65.2 t ha^{-1}), suggesting a gradual release of nutrients. Meanwhile, total biomass yield (TBY) and green leaf weight (GLW) did not record statistical differences. However, leaf percentage obtained higher results with CF2 (40.3%). High nitrogen doses had a positive effect on leaf development.

Table 2. Effect of topological planting arrangement and nitrogen fertilization on the yield and composition of maralfalfa grass (*Pennisetum* spp.) grown under subhumid conditions in Veracruz.

Topologías	PH	TBY	GLW	SW	LP	SP
T40 cm	2.4^{a}*	123.1^{a}	39.3^{a}	83.7^{a}	31.9^{b}	68.0^{a}
T60 cm	2.1^{a}	66.7 ^{b}	25.7^{b}	41.0^{b}	38.8^{a}	62.7^{b}
T80 cm	2.0^{a}	51.7^{b}	19.0 ^{b}	32.6^{b}	37.2^{a}	61.1^{b}

*Means with different letter in a column are statistically different (Tukey P=0.05). T40=planting topology at 40 cm; T60=planting topology at 60 cm; T80=planting topology at 80 cm; PH (ALTP)=plant height (cm); TBY (REN)=total biomass yield (t ha^{-1}); GLW (PVH)=green leaf weight (t ha^{-1}); SW=stem weight (t ha^{-1}); LP (PHOJ)=leaf percentage (%); and SP=stem percentage (%).

Table 3. Effect of nitrogen fertilization and biols on the yield and composition of maralfalfa grass (*Pennisetum* spp.) grown under subhumid conditions.

Fertilization Treatment	PH	TBY	GLW	SW	LP	SP
Biol 1	2.7^{a}	95.8	30.5	65.2 ^{a}	33.4^{b}	66.5^{a}
Biol 2	2.3^{ab}	74.4	25.0	49.4^{ab}	33.7^{b}	66.2^{a}
Biol 3	2.1^{b}	70.9	24.8	46.1^{b}	35.7^{b}	64.2^{a}
CF1	1.9 ^{b}	86.5	30.6	55.8^{ab}	36.7^{ab}	63.2^{ab}
CF2	1.8^{b}	74.8	29.2	45.6^{b}	40.3^{a}	59.6^{b}

*Means with different letter in a column are statistically different (Tukey P=0.05). Biol 1: manure+water; Biol 2: manure, serum, treacle, yeast, and micronutrients; Biol 3: serum, treacle, microorganisms, and minerals; CF1 (FQ1) (urea): 50 kg N ha^{-1}; CF2 (FQ2) (urea): 100 kg N ha^{-1}.

Interaction between planting arrangements and fertilization

Table 4 shows the interaction between topological arrangements and fertilization treatments. Biol 1 combined with T40 recorded the highest biomass yield (123.1 t ha^{-1}), followed by Biol 2 at the same density. These combinations also stood out in green leaf weight (GLW) and stem weight (SW). In contrast, T80 and CF2 recorded the lowest values, particularly regarding SW (32.6 t ha^{-1}).

Influence of the climatic conditions on the development of maralfalfa grass

Climatic conditions are important for the establishment and development of maralfalfa grass. Abundant rainfall and warm temperatures during the first months of the crop (June and July) favored a fast rooting and a strong plant growth. These results match the findings of Calzada-Marín (2014), who pointed out that maralfalfa grass has a positive response to subhumid warm climates, as a result of its high-water demand during the initial stages. Nevertheless, the reduction of rainfall during August and September had a higher impact on low density plantings (T60 and T80), perhaps due to the lower competition for resources. Similar results were reported by Morales Rivera *et al.* (2015) in their analysis of crops under water stress.

Table 4. Effect of the planting topology combination, nitrogen fertilization, and biols on the yield and composition of maralfala grass (*Pennisetum* spp.) grown under subhumid conditions.

Topological Arrangement	Fertilization Treatment	PH	TBY	GLW	SW	LP	SP
T40 cm	Biol 1	2.4	123.1	39.3	83.7 a	31.9	68.0
	Biol 2	2.3	95.8	30.5	65.2 a	33.7	66.5
	Biol 3	2.1	86.5	25.0	55.8 b	35.7	64.2
	CF1	1.9	86.5	30.6	55.8 b	36.7	63.2
	CF2	1.8	74.8	29.2	45.6 b	40.3	59.6
T60 cm	Biol 1	2.1	74.4	25.7	49.4 b	33.7	66.2
	Biol 2	2.3	70.9	24.8	46.1 b	35.7	64.2
	Biol 3	2.1	86.5	30.6	55.8 b	36.7	63.2
	CF1	1.9	74.8	29.2	45.6 b	40.3	59.6
	CF2	1.8	70.9	30.5	49.4 b	35.7	64.2
T80 cm	Biol 1	2.0	74.8	19.0	32.6 b	40.3	59.6
	Biol 2	1.9	70.9	25.7	41.0 b	38.8	62.7
	Biol 3	1.8	74.8	19.0	32.6 b	37.2	62.7
	CF1	1.8	70.9	25.7	41.0 b	38.8	62.7
	CF2	1.8	70.9	19.0	32.6 b	37.2	62.7

* Means with different letters in a column are statistically different (Tukey P=0.05). T40=planting topology at 40 cm; T60=planting topology at 60 cm; T80=planting topology at 80 cm; Biol 1: manure+water; Biol 2: manure, serum, treacle, yeast, and micronutrients; Biol 3: serum, treacle, microorganisms, and minerals; CF1 (FQ1) (urea): 50 kg N ha^{-1}; CF2 (FQ2) (urea): 100 kg N ha^{-1}; PH (ALTP)=plant height (cm); TBY (REN)=total biomass yield (t ha^{-1}); GLW (PVH)=green leaf weight (t ha^{-1}); SW=stem weight (t ha^{-1}); LP (PHOJ)=leaf percentage (%), and SP=stem percentage (%).

Effect of topological planting arrangements

Planting density had a significant effect on total biomass yield and morphological composition of maralfalfa grass. The treatments where furrows were located closer to each other (40 cm) recorded higher yields than those where furrows were 60 and 80 cm apart. These results prove that a higher plant population per area unit favors plant development and biomass accumulation. In physiological terms, denser plantings promote a higher competition for light, promoting longer stems and wider canopy area. This competition also intensifies root exploration, improving water and nutrient absorption and resulting in a more efficient use of resources. In addition, these results match the reports of Botero-Londoño *et al.* (2021), who recorded a higher biomass production and nutrient uptake in *Pennisetum purpureum* grown at higher densities. For their part, Villanueva-Ávalos *et al.* (2022) indicated that a wider canopy cover in dense planting enables a more efficient interception of active photosynthetic radiation, reducing evapotranspiration and favoring the microclimate of the crops. Meanwhile, more separation between furrows (T60 and T80) reduced the competition for light and promoted a higher leaf ratio in the biomass. These results are similar to the findings of Mengistu *et al.* (2023), who pointed out that low densities increased leaf development in *Pennisetum* spp. The highest stem development took place in denser plantings. This phenomenon reveals the preference for biomass assignation in the support structures required to maintain a more competitive architecture. According to Dawson *et al.* (2003), who studied the competition among perennial grasses, this response is associated with a higher meristematic activity and transport of photo-assimilates towards structural tissues.

Impact of the fertilization treatments

The application of organic biofertilizers, particularly Biol 1 (manure+water), promoted taller plants and a significant accumulation of structural biomass of *Pennisetum* spp. This effect could be the result of a gradual release of essential nutrients during the crop cycle, enabling a constant improvement of plant nutrition. According to Dubey (2019), this type of formulas favors photosynthetic activity and nutrient absorption, maintaining a stable soil fertility. Likewise, Rosabal *et al.* (2021) reported that the use of biols increases yield without dependence on synthetic fertilizers. In addition, Monge-Pérez *et al.* (2022) proved that biols improve soil moisture retention, which is a key factor for an ongoing physiological activity under subhumid conditions. These benefits show how organic treatments have a better performance than chemical treatments, particularly regarding plant height and stem weight.

Interaction between topological arrangements and fertilization

The combination of a high-density planting (40 cm between furrows) and the application of Biol 1 significantly favored the production of structural biomass of maralfalfa grass, particularly regarding stem development. This synergy suggests that the topological arrangement influences the efficient uptake of nutrients from biofertilizers, because a higher competition for light and resources promotes a preferred assignation towards support tissues. Hermitaño *et al.* (2022) reported similar results with *Pennisetum* spp. In their experiment, the appropriate management of organic fertilization in dense planting increased structural biomass. For their part, Caballero *et al.* (2023) reported that the adjustment of density and fertilization type improved nutrient uptake. Finally, Harish *et al.* (2022) and Kiruba and Saeid (2022) pointed out that the combination of biofertilizers and appropriate agricultural practices significantly increases the productivity of foraging crops. Under subhumid conditions, this joint strategy was more efficient than the use of chemical fertilization. Although treatments such as CF2 (100 kg N ha^{-1}) increased leaf percentage (Pereira *et al.*, 2022), they did not reach the total biomass yield recorded by Biol 1. These results are similar to those reported by Dubey (2019) and Hermitaño *et al.* (2022), who pointed out that biofertilizers gradually release nutrients, maintaining a steady growth of the crop, even under water stress. In addition, Maldonado-Quiñones *et al.* (2021) and Fayos-Fever *et al.* (2023) pointed out that organic fertilization increases yield in warm and subhumid environments. Finally, Rosabal *et al.* (2021) and Pereira *et al.* (2022) mentioned that the use of biols not only improves the efficient use of nutrient and water, but also contributes to the productive stability of the system and reduces the dependency on synthetic fertilizers, proving its value as a sustainable practice in tropical livestock systems.

CONCLUSIONS

In order to achieve a better morphological development and to improve yield under the subhumid conditions of Veracruz, the use of topological arrangements (high density plantings and the application of fertilizers) to provide nutrients for *Pennisetum* spp. is fundamental.

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