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Boosting Biomass and Leaf Area with Biol: Morphological and Yield Responses of *Pennisetum* in the Peruvian Highlands

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Abstract

The intensive use of chemical fertilizers in agriculture contributes to environmental pollution, which has driven the search for sustainable alternatives such as organic fertilizers. Among these, biofertilizer has garnered interest due to its potential to improve crop growth and yield. The objective of this study was to evaluate the effect of two types of biofertilizer: Bio Chumbinia (standardized) and traditional biofertilizer, as well as a control treatment (water), on the morphology, growth, yield, and leaf area of Maralfalfa (*Pennisetum* sp.). Morphological and growth variables were measured every 14 days, while yield and leaf area were evaluated in two successive periods corresponding to 42 days of growth. The results indicated that most morphological and growth parameters were significantly influenced by treatment, time, and evaluation ($p < 0.05$), except for tiller number, blade number, and the blade emergence rate ($p > 0.05$). Bio Chumbinia showed superior values compared with the control at 6.0 cm for plant height, 0.1 cm/day in the growth rate, 4.1 cm for blade length, and 1.2 mm for blade width; when compared with the traditional biol, the values were similar. The growth rate and leaf emergence peaked on day 14 and subsequently declined. The fresh and dry matter yields were consistently higher on Bio Chumbinia treatment than others ($p < 0.05$). Although no differences were found for blade weight and leaf area between Bio Chumbinia and the control, the leaf area in Bio Chumbinia was 1400 cm^2 more than the control. The second evaluation showed improved productivity, which is consistent with the higher values on the morphological characteristics. No differences were observed in the leaf-to-stem + sheath dry matter ratio. These results demonstrate the potential of Bio Chumbinia to improve the productive performance of Maralfalfa as a foliar fertilizer in sustainable agricultural systems in Peru.

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1. Introduction

Agricultural pollution is a growing global concern, particularly in developing countries, where the excessive use of synthetic fertilizers and pesticides contributes significantly to environmental degradation [1]. In many developing regions, population growth and the intensification of production systems have increased the demand for synthetic fertilizers; however, these practices have led to soil and water contamination, posing risks to human health, ecosystems, and biodiversity [2–4].

High nitrogen inputs promote shifts in soil microbial communities, leading to reduced biodiversity, while nutrient runoff from fertilized fields can cause eutrophication in aquatic ecosystems [1]. Consequently, identifying sustainable alternatives to chemical fertilization has become a critical priority in the pursuit of resilient and environmentally responsible agriculture [5,6]. Therefore, different alternative technologies are being used for sustainable agriculture. In China, black biodegradable mulching is a sustainable choice for wheat production in rainfed conditions [7]; in other regions, anaerobic digestion is an environmentally friendly alternative.

Anaerobic digestion offers a promising solution by simultaneously treating organic waste and generating biogas along with nutrient-rich by-products, namely digestate and biol [8]. Biol, the liquid fraction, functions as a low-cost biofertilizer and has demonstrated potential to improve plant growth, the nutritional quality of forage, soil fertility, and crop productivity in low-input systems [9–16]. Moreover, its benefits extend to soil health by enhancing chemical, physical, and biological properties [17].

Nevertheless, the agronomic efficiency of biol varies depending on its composition, crop type, and environmental conditions, underscoring the need for localized studies [18]. In Peru, biol is produced at both industrial and traditional scales. The use of traditional biodigesters among smallholder farmers has expanded due to support from government programs and non-governmental organizations; however, scientific evaluations under Andean highland conditions remain scarce. This knowledge gap limits the informed adoption of biol-based fertilization strategies.

In response to this challenge, Peru's National Institute of Agrarian Innovation (INIA) has developed a patented product, "Bio Chumbinia", tailored for a wide range of crops and forage systems [19]. Among the most promising candidate crops, *Pennisetum* sp. stands out for its high biomass yield, rapid growth, and adaptability to tropical and subtropical climates, including high-altitude Andean regions [20–23].

Although the agronomic performance of Maralfalfa (*Pennisetum* sp.) under chemical fertilization is well documented, research on its response to organic amendments under highland conditions remains limited [20,24,25]. Therefore, this study aimed to evaluate the effects of different biol-based foliar fertilizers on the morphological traits, growth, yield, and leaf area of this forage crop, thereby contributing to the development of sustainable and climate-resilient forage production systems in the Peruvian Andes.

2. Materials and Methods

2.1. Description of the Research Area

This research was conducted at the Choccepuquio Research Center, affiliated with the Professional School of Agricultural Engineering at the Universidad Nacional de San Antonio Abad del Cusco. The center is located in the province of Andahuaylas, Apurímac Region, Peru, at an altitude of 2853 m above sea level (13°40′07.4″ S, 73°24′28.5″ W, Figure 1).

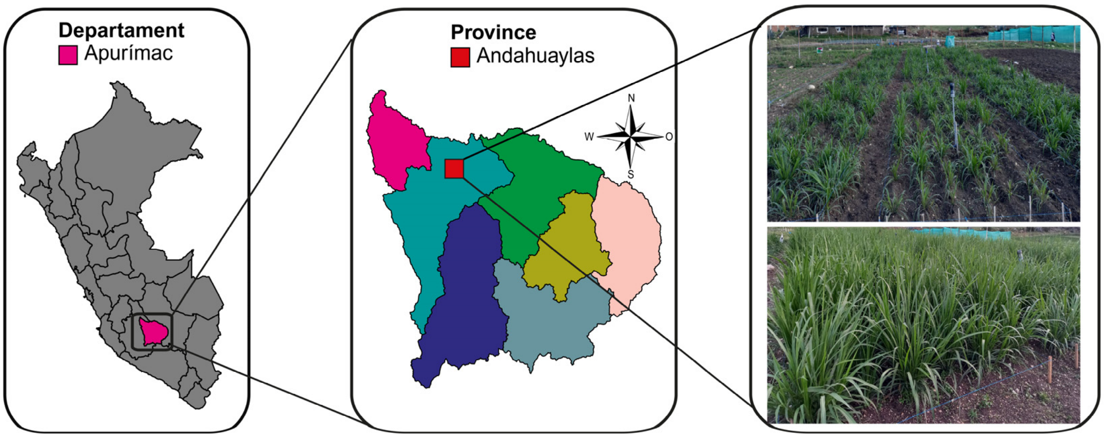

Figure 1. Geographic location of the study and experimental plot.

Figure 2 summarizes daily weather records (November 2023 to April 2024). The maximum temperature ranged from 12.6 to 26.4 °C, and the minimum from −5.0 to 13.6 °C (Figure 2A). Relative humidity was high and stable (≈77–98%). Precipitation was generally low, with isolated heavy events (up to 39 mm) (Figure 2B). The temperature, relative humidity, and precipitation values were taken from SENAMHI [26].

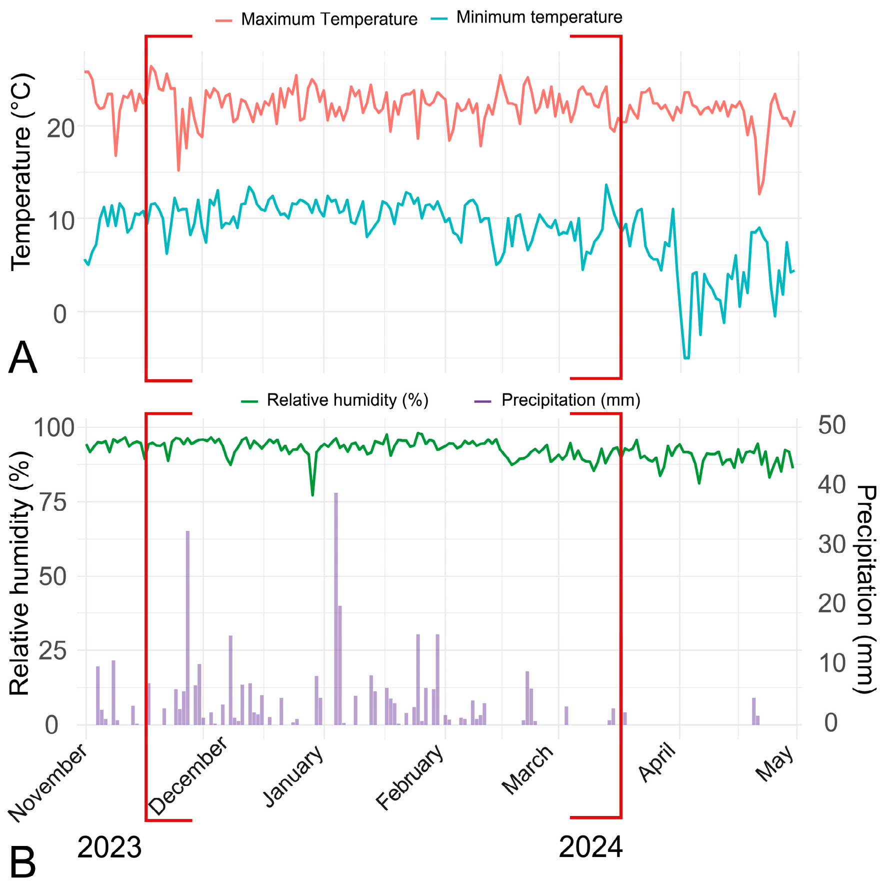

Figure 2. Meteorological conditions during the experimental period. (**A**) Maximum temperature; minimum temperature. (**B**) Precipitation; relative humidity.

2.2. Plot Design and Planting

Nine plots measuring 18.75 m^2 each (7.5 m × 2.5 m) were established, spaced 1.0 m apart, on uniform, slightly sloped soil. A randomized complete block design was used, with three blocks arranged according to the slope of the land. Treatments consisted of three types of biol: Bio Chumbinia (INIA-biol) (INIA, Andahuaylas, Peru), traditional biol, and a control (water), which were randomly assigned to the plots.

The soil was uniform and had a silty loam texture (36% sand, 57% silt, and 7% clay) with the following average (± standard error) values: pH 7.80 (± 0.00), organic matter 4.27% (± 0.03), total nitrogen 0.22% (± 0.00), total phosphorus 10.17 mg/kg (± 0.42), and extractable potassium 291.29 mg/kg (± 5.75). Soil fertilization was not carried out, as the soil did not present deficiencies for *Pennisetum* cultivation. Soil preparation was performed manually with pickaxes to ensure a uniform surface.

Vegetative cuttings of Maralfalfa (*Pennisetum* sp.) were sourced from a local producer. Each cutting measured approximately 20 cm in length and 2–3 cm in diameter and included two nodes. Cuttings were disinfected by immersion in a 1% sodium hypochlorite solution for one minute and then planted in furrows at 5.0 cm depth, with 0.5 m spacing between both plants and rows. Each plot was planted with 96 cuttings.

2.3. Plot Handling and Uniformization Cutting

Sprinkler irrigation was applied during prolonged dry periods to ensure uniform moisture distribution and was limited to the establishment period. Manual weeding was performed every two weeks until the sixth week, and subsequently as needed, once Maralfalfa (*Pennisetum* sp.) began to naturally suppress weed growth (Figure 3).

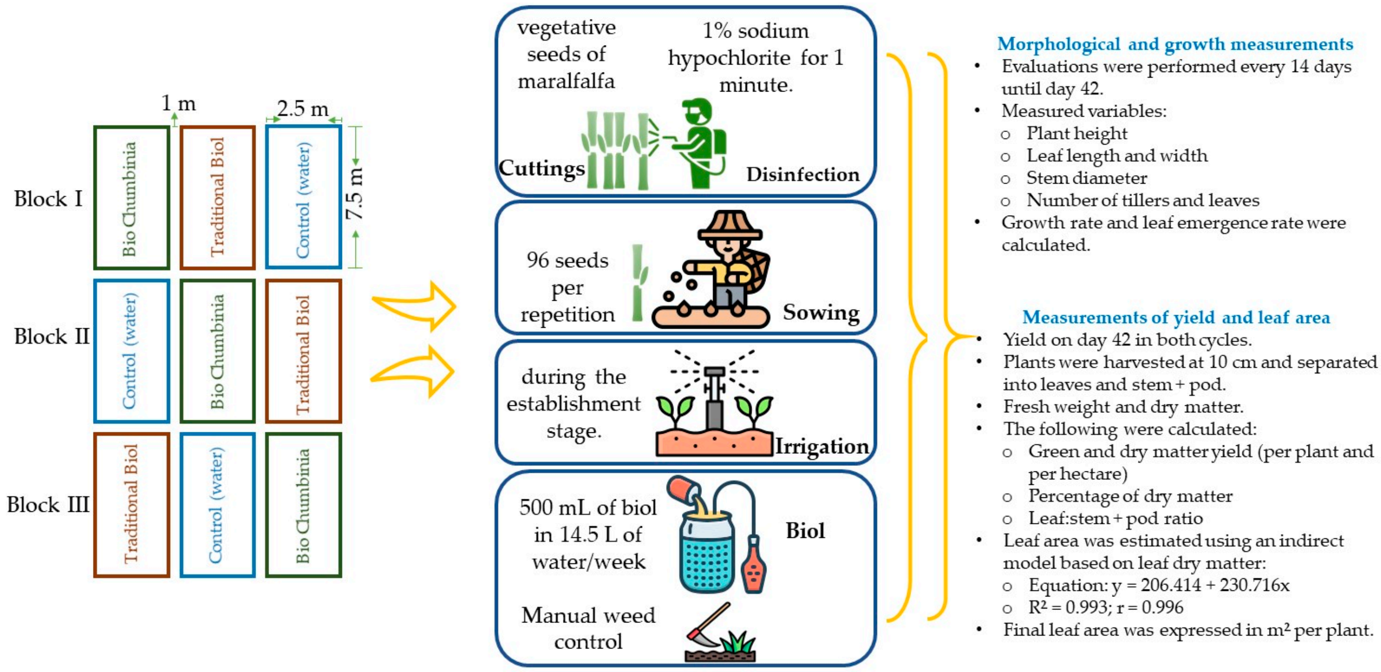

Figure 3. Experimental design, field layout, and variable measurements.

After an 18-week establishment period, a uniform cut was performed at a height of 10 cm using pruning shears. Eight centrally located plants per plot were randomly selected and tagged for subsequent evaluations of morphological traits, growth, yield, and leaf area. In total, 72 plants (8 per plot × 9 plots) were evaluated per cycle.

2.4. Treatments and Application

Three treatments were evaluated: Bio Chumbinia, traditional biol, and water (the control). Bio Chumbinia was obtained from the Chumbibamba Agricultural Experimental Station (INIA–Andahuaylas), whereas the traditional biol was prepared on site at the Choccepuquio Research Center using a mixture of organic inputs (Table 1), following the methodology described by Álvarez [27].

Table 1. Composition of traditional biol.

Ingredients	Unit of Measurement	Amount
Cow manure (fresh)	Kg	5
Chicken manure	Kg	5
Guinea pig manure	Kg	3
Sugar	Kg	2
Ash	Kg	0.7
Eggshell (ground)	Kg	0.4
Cow's milk whey	L	3
Cow urine	L	2.5
Chicha de jora [1]	L	2.4
Alfalfa juice	L	1.5
Water	L	50

[1] Chicha de jora is an ancestral Andean beverage obtained by fermenting germinated corn (jora). Chicha has a composition of 70–90% water, 2–6% fermentable carbohydrates, 0.5–1.5% protein, and an approximate alcohol content of 1–3% v/v. It also provides minerals such as calcium (5–20 mg/100 mL), phosphorus (15–40 mg/100 mL), and iron (0.2–0.6 mg/100 mL) [28].

Table 2 shows that both biofertilizers have balanced nutritional profiles, with the potential to provide essential nutrients and promote plant growth.

Table 2. Chemical composition of standardized and traditional biol.

Nutrient	Unit of Measurement	Bio Chumbinia	Traditional
Nitrogen (N) [1]	%	0.11	0.5
Phosphorus (P) [2]	%	1.05	0.65
Potassium (K) [3]	%	0.22	0.74
Calcium (Ca) [3]	%	0.13	0.63
Magnesium (Mg) [3]	%	0.07	0.11
Zinc (Zn) [3]	mg/L	9.72	13.50
Copper (Cu) [3]	mg/L	2.27	7.45
Iron (Fe) [3]	mg/L	118.75	97.50
Manganese (Mn) [3]	mg/L	7.82	175
Sodium (Na) [3]	mg/L	677	42.80

[1] AOAC 955.04 [29], [2] ASTM 2974-20 [30], [3] AOAC 999.11 [31].

Treatments were applied weekly starting the day of the uniformization cut. Each application consisted of 500 mL biol diluted in 14.5 L of water, sprayed uniformly over the foliage using a backpack sprayer at a rate of 0.27 L/m^2, as recommended by [32]. The control plots received only water. Applications were performed in the morning at 8:00 a.m.

The climatic conditions between the first and second evaluations showed slight differences in precipitation, relative humidity, and temperature. However, it is important to note that all treatments were exposed to both evaluation periods.

The climatic conditions recorded at the study site during both evaluation periods, following NASA recordings [33], are presented below:

- First evaluation: Precipitation (173.91 mm), relative humidity (76.74%), and temperature (13.71 °C).

- Second evaluation: Precipitation (186.99 mm), relative humidity (79.33%), and temperature (13.33 °C).

2.5. Morphology and Growth Measurements

After the uniformization cut, morphological traits were evaluated in two consecutive periods. In each period, the characteristics were evaluated every 14 days, until day 42 (harvest). Evaluations of the morphological and growth characteristics were conducted on eight plants per plot, yielding a total of 72 plants in each evaluation (8 plants/plot × 9 plots). Plant height was measured from the base to the apex of the flag leaf, while blade length was measured from the ligule to the apex using a ruler. Stem diameter was recorded at the second internode of the tiller bearing the flag leaf using a digital caliper, and blade width was measured at the middle third of the blade. Tiller and blade counts were also recorded to calculate the growth rate and blade emergence rate.

2.6. Yield and Leaf Area

Yield and leaf area were evaluated on day 42, and measurements were repeated over two cycles. Plants were harvested at a height of 10.0 cm; leaf blades and stem + sheath fractions were separated and weighed using a precision balance. Samples were oven-dried at 105 °C for 24 h to determine dry matter content. Based on these measurements, the fresh and dry matter yields (per plant and per hectare), dry matter percentage, and the blade-to-stem + sheath dry matter ratio were all calculated.

Leaf area was estimated indirectly from blade dry matter. To develop the estimation model, leaf blades from plants not included in the morphological and yield evaluations were used. For each blade, leaf area and dry matter were measured, yielding the following regression equation (Equation (1)):

$$y = 206.414 + 230.716x \quad (1)$$

where y represents leaf area (cm^2) and x represents blade dry matter (g). The model was constructed with 141 data points; it showed a coefficient of determination (R^2) of 0.993 and a Pearson correlation coefficient of 0.996. Leaf area of the experimental plants was calculated using Equation (1) and expressed in m^2.

2.7. Data Analysis

Exploratory data analysis was performed to identify potential outliers. The assumptions of normality and homoscedasticity were evaluated using the Shapiro–Wilk and Levene tests, respectively; the results of these tests showed *p*-values greater than 0.05 ($p > 0.05$), indicating that the assumptions were met. Although the initial analysis of variance (ANOVA) included the block, this was excluded from the model because it was not statistically significant. The interaction between treatment and evaluation was not significant and was also excluded from the final model.

A mixed linear model was applied to the morphological, growth, yield, and leaf area variables. For the morphology and growth, the model included the treatment, the day, and the evaluation as fixed effects, while individual plants were considered as the random effect. The yield and leaf area included the treatment and the evaluation as fixed effects, while the plant was considered as the random effect. When significant differences were detected, mean comparisons were performed using the Tukey test at a 0.05 significance level. All statistical analyses were conducted using the open-source software R (version 4.5.1).

3. Results

3.1. Morphology and Growth

Morphological and growth characteristics were significantly influenced by treatment, day, and evaluation period ($p < 0.05$), except for tiller number, blade number, and the blade emergence rate, which were not affected by treatment ($p > 0.05$). Among the treatments, Bio Chumbinia produced the highest values for plant height, growth rate, blade length, and blade width in comparison with the control (water); however, these values were similar when compared with the traditional biol. The stem diameter of Bio Chumbinia and traditional biol was similar, and both treatments were superior to the control. Meanwhile, tiller number, blade number, and the blade emergence rate were similar between treatments (Table 3).

Table 3. Means ± standard error of morphological and growth measurements of Maralfalfa (*Pennisetum* sp.) according to treatment, day, and evaluation *.

Factors	PH (cm)	GR (cm/Day)	TN (*n*/Plant)	BN (*n*/Plant)	BER (*n*/Day)	SD (mm)	BL (cm)	BW (mm)
Treatment								
Bio Chumbinia	83.9 ± 2.2 a	2.3 ± 0.1 a	37.5 ± 1.1 a	263.7 ± 11.6 a	8.3 ± 0.5 a	13.0 ± 0.3 a	62.1 ± 1.5 a	20.0 ± 0.3 a
Traditional	81.0 ± 2.1 ab	2.2 ± 0.1 ab	34.9 ± 1.1 a	239.7 ± 11.0 a	7.5 ± 0.5 a	12.4 ± 0.2 a	60.2 ± 1.4 ab	19.5 ± 0.3 ab
Water	77.9 ± 2.0 b	2.2 ± 0.1 b	35.1 ± 1.0 a	231.7 ± 9.5 a	7.2 ± 0.4 a	11.6 ± 0.3 b	58.0 ± 1.4 b	18.8 ± 0.3 b
p-value	0.00178	0.01109	0.42140	0.12790	0.09192	<0.001	0.00136	0.03086
Day								
14	57.3 ± 0.8 c	3.4 ± 0.1 a	30.8 ± 1.1 c	170.0 ± 7.5 c	12.1 ± 0.5 a	11.0 ± 0.2 c	40.9 ± 0.8 c	18.7 ± 0.4 b
28	81.4 ± 1.5 b	1.72 ± 0.1 b	35.2 ± 1.1 b	242.2 ± 10.1 b	5.2 ± 0.3 b	12.4 ± 0.2 b	65.0 ± 1.0 b	19.8 ± 0.3 a
42	104.0 ± 1.8 a	1.61 ± 0.0 b	41.5 ± 0.9 a	322.8 ± 10.5 a	5.8 ± 0.3 b	13.6 ± 0.2 a	74.4 ± 0.8 a	19.8 ± 0.2 a
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Evaluation								
First	67.1 ± 1.1 b	1.78 ± 0.1 b	27.5 ± 0.7 b	155.9 ± 4.7 b	5.51 ± 0.2 b	10.24 ± 0.1 b	52.4 ± 1.0 b	17.0 ± 0.2 b
Second	94.7 ± 1.8 a	2.69 ± 0.1 a	44.2 ± 0.7 a	334.1 ± 7.7 a	9.82 ± 0.5 a	14.43 ± 0.2 a	67.8 ± 1.1 a	21.9 ± 0.2 a
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

* Different letters in the columns for each factor indicate significant differences according to the Tukey test ($p < 0.05$). PH, plant height; GR, growth rate; TN, tiller number; BN, blade number; BER, blade emergence rate; SD, stem diameter; BL, blade length; BW, blade width.

All morphological parameters (plant height, tiller number, blade number, stem diameter, and blade length) increased significantly over time ($p < 0.05$). In contrast, the growth rate and blade emergence rate reached their maximum at day 14 and subsequently declined at days 28 and 42, which exhibited similar values. The blade width in days 28 and 42 we similar, and both were superior compared with day 14. When comparing evaluation periods, all measured traits were significantly higher in the second evaluation than in the first ($p < 0.05$), indicating cumulative growth throughout the experimental period.

3.2. Yield and Leaf Area

The fresh and dry weights per plant, as well as the yield (kg/ha), were significantly affected by treatment ($p < 0.05$), with Bio Chumbinia outperforming the other treatments. In contrast, the dry matter percentage was lower in the Bio Chumbinia treatment than in the traditional biol treatment ($p < 0.05$) but did not differ significantly from the control (Figure 4).

The stem + sheath dry matter of Bio Chumbinia treatment was higher than the traditional biol and the control ($p < 0.05$). The blade dry matter and leaf area of Bio Chumbinia were only higher when compared with the traditional biol ($p < 0.05$) but were statistically similar to the control ($p > 0.05$). The blade-to-stem + sheath dry matter ratio did not differ significantly among treatments ($p > 0.05$; Figure 5).

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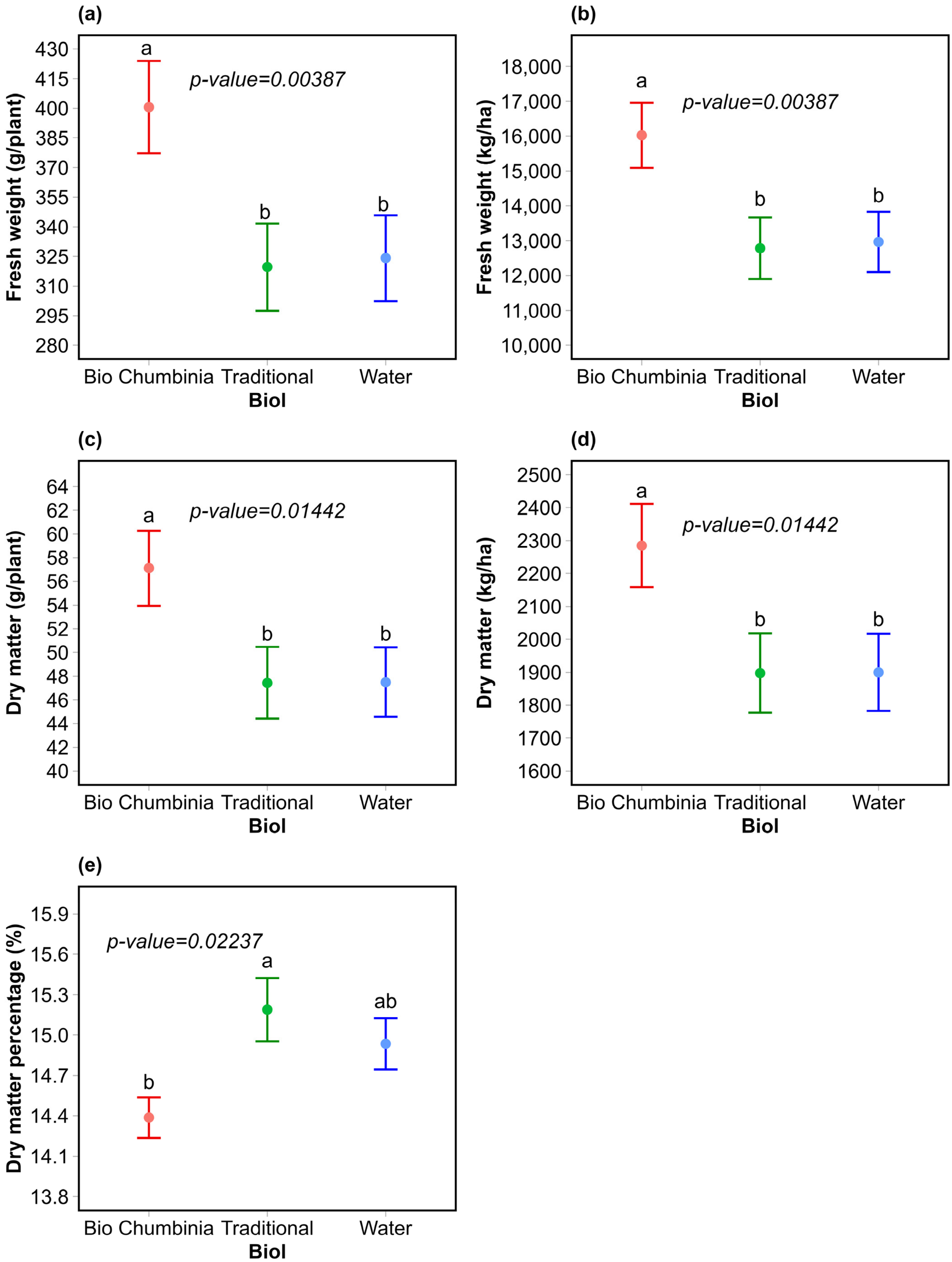

Figure 4. Yield and dry matter percentage of Maralfalfa (*Pennisetum* sp.) according to biol types. (**a**) fresh weight of individual plants; (**b**) fresh weight yield; (**c**) dry matter weight of individual plants;

(**d**) dry matter yield; (**e**) dry matter percentage. Different letters indicate significant differences between biol types according to the Tukey test ($p < 0.05$); the bars represent the standard error.

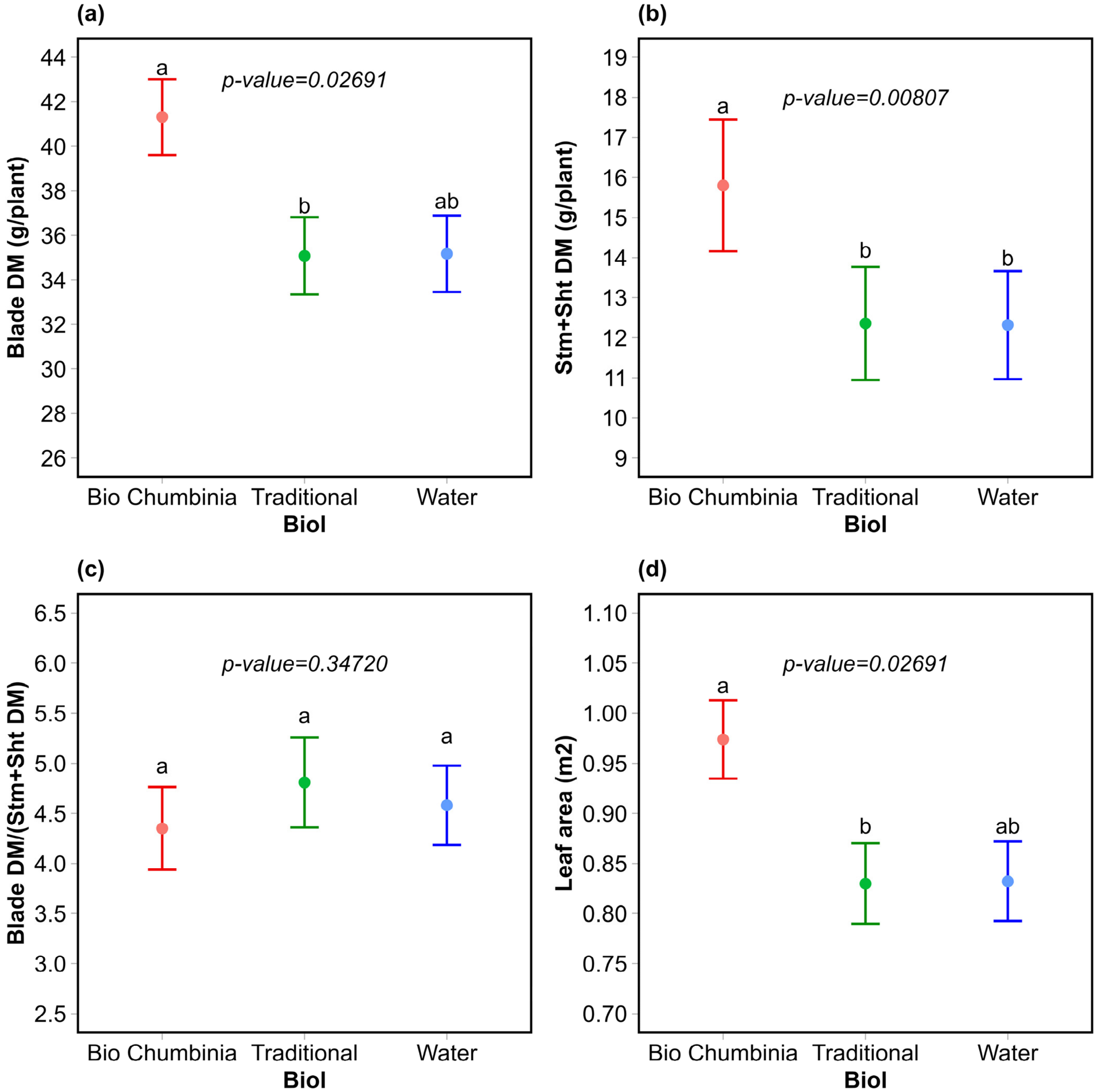

Figure 5. Dry matter yield of blade, stem + sheath, and leaf area of Maralfalfa (*Pennisetum* sp.) according to biol type. (**a**) blade dry matter; (**b**) stem+sheath dry matter; (**c**) blade-to-stem + sheath dry matter ratio; (**d**) leaf area. DM, dry matter; Stm + Sht, stem + sheath. Different letters indicate significant differences between biol types according to the Tukey test ($p < 0.05$).

All yield and leaf area parameters varied significantly with evaluation period ($p < 0.05$). Plants assessed during the second evaluation period exhibited higher fresh and dry weights per plant, blade and stem + sheath biomass, fresh and dry yields (kg/ha), and total leaf area compared with those evaluated during the first period. In contrast, the dry matter percentage and the blade-to-stem + sheath ratio were significantly lower in the second evaluation (Table 4).

Table 4. Means ± standard error of yield characteristics of Maralfalfa (*Pennisetum* sp.) according to the evaluation period.

Characteristics	*p*-Value	Evaluation I	Evaluation II
FW (g/plant)	<0.001	232.5 ± 9.2 b	463.7 ± 15.6 a
DM (g/plant)	<0.001	35.2 ± 1.4 b	66.2 ± 2.1 a
BDM (g/plant)	<0.001	30.3 ± 1.1 b	44.1 ± 1.3 a
Stm + Sht DM (g/plant)	<0.001	4.9 ± 0.3 b	22.1 ± 0.9 a
BDM/(Stm + Sht DM)	<0.001	7.1 ± 0.2 a	2.1 ± 0.1 b
DMP (%)	<0.001	15.2 ± 0.2 a	14.4 ± 0.2 b
FW (kg/ha)	<0.001	9300.9 ± 366.9 b	18,548.9 ± 623.3 a
DM (kg/ha)	<0.001	1408.7 ± 55.4 b	2646.0 ± 81.9 a
LA (m^2)	<0.001	0.7 ± 0.0 b	1.0 ± 0.0 a

Different letters in the rows for each characteristic indicate significant differences according to the Tukey test ($p < 0.05$). FW, fresh weight; DM, dry matter; BDM, blade dry matter; Stm + Sht DM, stem + sheath dry matter; DMP, dry matter percentage; LA, leaf area.

4. Discussion

4.1. Morphology and Growth

The use of Bio Chumbinia as a foliar fertilizer resulted in superior performance for most morphological and growth characteristics compared with the control (water) treatment, except for tiller number, blade number, and the blade emergence rate, which all remained similar across treatments. The positive effects of biol application on forage growth have also been reported in *Medicago sativa* [14,34], *Megathyrsus maximus* cv. Tanzania [15], *Pennisetum purpureum* [10], and *Pennisetum glaucum* L. [12]. In contrast, Condori et al. [34] observed differences in tiller number in *Pennisetum* sp. in response to bovine biol levels in Bolivia; however, they did not find significant differences in plant height, leaf number, or leaf length and width. Our findings indicate that the application of biol as foliar fertilizer would not promote the tillering and blade emergence rate.

The superior performance observed in the present study may be mainly related to the higher phosphorus (P) content and its synergistic effect with other nutrients in Bio Chumbinia, which was nearly twice that of the traditional biol (Table 2), considering that soil characteristics were uniform across treatments. Phosphorus is an essential macronutrient involved in fundamental processes such as energy transfer, photosynthesis, respiration, and cell growth, as it is a structural component of ATP, nucleic acids, and membrane phospholipids [35,36]. Adequate P availability promotes shoot biomass accumulation and vegetative growth due to its central role in carbon assimilation and in the regeneration of energy compounds during photosynthesis [37,38]. In contrast, phosphorus deficiency reduces photosynthetic activity, limits ATP synthesis, and restricts shoot development [39]. Although synergistic effects of other nutrients present in Bio Chumbinia cannot be ruled out, enhanced phosphorus availability represents a well-documented physiological mechanism that supports the growth response observed in grasses such as *Pennisetum* sp.

As expected, the morphological traits of Maralfalfa (*Pennisetum* sp.) increased over time (days), consistent with the findings of Calzada-Marín et al. [40], who reported continuous increases in morphological components up to 151 days. Similarly, studies evaluating *Pennisetum* sp. at different cutting ages have reported greater plant height at later stages, although tillering remained unaffected [24]. These variations depend on specific species characteristics and experimental conditions. In contrast, the growth rate and blade emergence rate were higher during the early stages of development (day 14), likely because plants initially prioritize rapid growth and leaf appearance before transitioning to a phase dominated by structural biomass accumulation [41].

Notably, the higher values of morphological and growth characteristics observed during the second evaluation period likely reflect cumulative plant development, as maturing plants tend to increase their tiller number, thereby enhancing yield, particularly under biofertilizer application. Climatic factors such as rainfall, temperature, and relative humidity, which are known to affect forage performance [42], would have had little effect on those characteristics, because their little differences between evaluation periods. In the present study, the second evaluation period had slightly higher rainfall (13 mm) and relative humidity (3%) than the first [28]. In *Pennisetum* sp., differences according to the season were found, with greater plant height observed during the rainy season [23]. Likewise, the morphological traits of Desho grass (*Pennisetum glaucifolium* cv. Areka-DZF-590) under rainfed conditions were significantly affected by the interaction between fertilizer application and year [43].

4.2. Yield and Leaf Area

Bio Chumbinia significantly outperformed traditional biol and the control in terms of fresh and dry matter yields per plant and per hectare (kg/ha); similar trends were observed for stem + sheath dry matter content. These findings are consistent with previous studies reporting improved yield and productivity in *Pennisetum purpureum* [11], *Pennisetum glaucum* L. [12], *Medicago sativa* [14], and hybrids of *Zea mays* [44] following biol application. In the latter study, slurry treatments were associated with higher net photosynthetic rates and stomatal conductance, resulting in biomass increases of up to 63% due to enhanced photosynthetic performance [44]. Bio Chumbinia significantly outperformed the traditional biofertilizer in blade dry matter and leaf area; despite no differences being found with the water treatment (Figure 5a,d), Bio Chumbinia showed higher values for blade dry matter (6.14 g/plant) and leaf area (0.14 m^2 = 1400 cm^2) compared with the water treatment. As for [34,35], the P content is key; the high amount of P in Bio Chumbinia would have improved the leaf development, as this element is needed for ATP synthesis, and its adequate availability promotes leaf expansion. However, the blade-to-stem + sheath dry matter ratio was not affected by treatment, suggesting a proportional distribution of biomass among structural components.

Dry matter yield and total leaf area were higher during the second evaluation period than during the first (Table 4), reflecting cumulative plant growth as biomass increases with plant maturity [41]. Since the climatic variations between the first and second evaluation were minimal, the higher yield in the last evaluation would be mainly due to the increase in its morphological characteristics, such as plant size, tillers, blade size, and stem diameter [33] (Table 3). In other studies, with marked climatic differences between seasons, variation was found for *Pennisetum* sp., with greater biomass production occurring during the rainy season [25]. Likewise, dry matter yield in Desho grass (*Pennisetum glaucifolium* cv. Areka-DZF-590) under rainfed conditions was significantly influenced by the interaction between fertilizer application and year [43].

Biomass production in *Pennisetum* sp. depends largely on photosynthetic CO_2 uptake and the synthesis of photoassimilates [40]. These processes are strongly influenced by nutrient availability, which is enhanced by biol application and, in turn, promotes plant growth. The allocation and distribution of photoassimilates not only determine the efficiency of resource use but also govern the magnitude of biomass accumulation [45]. In this study, the larger leaf area of Bio Chumbinia implies a greater number of photosynthetic structures, such as chloroplasts and stomata, which would have contributed to high photosynthetic activity.

Beyond nutrient supply, the superior performance of Bio Chumbinia likely reflects its positive effects on key physiological processes, including chlorophyll synthesis, enzymatic activity, and stomatal regulation, all of which enhance photosynthetic efficiency and overall plant development [46]. The observed increase in leaf area indicates improved light interception and greater biomass production [47,48], underscoring the potential of standardized biol not only as a nutrient source but also as a metabolic enhancer—particularly under highland conditions, where fluctuating temperatures and elevated UV radiation can constrain plant growth.

Leaf area is a critical determinant of a plant's photosynthetic capacity, as it governs light interception and photoassimilate production. In this study, plants treated with Bio Chumbinia exhibited the greatest growth in leaf area, which likely contributed to enhanced light capture and, consequently, increased nutrient accumulation across plant organs. This result is consistent with the findings of Condori et al. [34], who reported variation in leaf area of *Pennisetum* sp. in response to different levels of bovine biol in Bolivia.

Although traditional biol contains essential nutrients, Bio Chumbinia demonstrated consistent superiority for yield and leaf area parameters—under the same highland environmental conditions. While seasonal climatic patterns are known to influence forage yield [24], the consistent performance of Bio Chumbinia suggests that its standardized production process ensures a more stable and readily available nutrient composition compared with traditional biol, which is often produced using non-standardized methods that result in variable nutrient availability for plant uptake. Furthermore, given that plant performance is closely linked to morphological development [49], further research is warranted to elucidate the specific compositional differences between biol types and their physiological effects on forage species.

Fertilizer management is a critical factor influencing forage growth and yield [12]. The use of Bio Chumbinia represents a promising strategy for recycling livestock waste into high-value inputs for forage production. Biol serves as a source of nutrients and beneficial microorganisms that stimulate plant development and improve soil quality [16]. In this study, foliar application of Bio Chumbinia to Maralfalfa (*Pennisetum* sp.) demonstrated its strong potential to enhance yield traits and leaf area under highland environmental conditions.

Our study shows certain limitations that are described below. First, the experiment was conducted at a single site and during one growing season under Andean highland conditions free of synthetic fertilizer, which may limit the extrapolation of the findings to other agroecological zones or years with different climatic patterns. Second, the chemical characterization of biol did not include microbial composition or nutrient release dynamics. Furthermore, it is necessary to clarify the effects that the long-term application of biofertilizer may have on soil fertility. We encourage researchers in this field to conduct studies to understand these knowledge gaps.

5. Conclusions

The use of standardized biol (Bio Chumbinia) as a foliar fertilizer positively affected the yield and leaf area parameters of Maralfalfa (*Pennisetum* sp.). Although performance was similar to that of traditional biofertilizer in several variables (morphology and growth), the standardized formulation showed more consistent responses, suggesting a better balance and nutrient availability. This highlights its potential as a sustainable and eco-friendly alternative for utilizing livestock waste to improve forage productivity under agroecological systems, without synthetic fertilization. Foliar application of Bio Chumbinia (0.5 L of biol in 14.5 L of water) at a dose of 0.27 L/m^2 is recommended under the Peruvian highland conditions. However, due to the variability in the nutritional composition of biofertilizers, future research should focus on nutrient bioavailability, long-term soil–plant interactions, and economic viability to support its wider adoption.

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Abbreviations

The following abbreviations are used in this manuscript:

FW	Fresh weight
DM	Dry matter
BDM	Blade dry matter
Stm + Sht DM	Dry matter of stem + sheath
DMP	Dry matter percentage
LA	Leaf area
PH	Plant height
GR	Growth rate
TN	Tiller number
BN	Blade number
BER	Blade emergence rate
SD	Stem diameter
BL	Blade length
BW	Blade width
INIA	National Institute of Agrarian Innovation (Peru)
NASA	National Aeronautics and Space Administration

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