

Original Article

Use of autochthonous microorganism-based biol to enhance agronomic responses and nutritional value of *Megathyrsus maximus* for sustainable ruminant feeding

Uso de biol à base de microrganismos autóctones para melhorar as respostas agrônômicas e o valor nutricional de *Megathyrsus maximus* para alimentação sustentável de ruminantes

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Abstract

This study investigates the potential of biol prepared from autochthonous microorganisms (AMs) as a sustainable biofertilizer to improve the agronomic performance and nutritional value of *Megathyrsus maximus* under tropical conditions. Conducted in the Ecuadorian Amazon, the experiment evaluated the effects of varying biol dosages (**T1**, 3; **T2**, 4, and **T3**, 5 L/ha⁻¹) on agronomic parameters such as plant height, basal cover, tiller density, and chemical composition. The results revealed significant improvements in plant growth and forage yield, particularly in the 5 L/ha⁻¹ treatment (**T3**), which recorded the highest crude protein (CP) content of 12.85%. The biol treatments also increased tiller density and enhanced basal cover, indicating a favourable impact on plant development and ground cover efficiency. Chemical analysis showed that CP contents surpassed those obtained with conventional fertilizers, while neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents remained consistent, ensuring the forage quality required for ruminant nutrition. Additionally, the economic evaluation showed that the biol application costs were significantly lower than those of synthetic fertilizers (192 vs. 225 \$), providing a cost-effective alternative for pasture management. This research highlights the multifaceted benefits of utilizing biol derived from AMs, including improved forage productivity, enhanced nutritional quality, and reduced environmental impact. The findings emphasize the potential of biol as a viable strategy to promote sustainable agricultural practices in tropical regions, contributing to the global demand for eco-friendly alternatives to chemical fertilizers. Further research is recommended to optimize application protocols and assess long-term ecological and economic impacts.

Keywords: agronomic characterize, biofertilizer, chemical composition, fertilizer, tropical conditions, microorganism autochthonous (AMs).

Resumo

Este estudo investiga o potencial do biol preparado a partir de microrganismos autóctones (MAs) como um biofertilizante sustentável para melhorar o desempenho agrônômico e o valor nutricional de *Megathyrsus maximus* em condições tropicais. Conduzido na Amazônia equatoriana, o experimento avaliou os efeitos de diferentes dosagens de biol (**T1**, 3; **T2**, 4 e **T3**, 5 L/ha⁻¹) em parâmetros agrônômicos como altura da planta, cobertura basal, densidade de perfilhos e composição química. Os resultados revelaram melhorias significativas no crescimento da planta e na produção de forragem, particularmente no tratamento de 5 L/ha⁻¹ (**T3**), que registrou o maior teor de proteína bruta (PB), atingindo 12,85%. Os tratamentos com biol também aumentaram a densidade de perfilhos e melhoraram a cobertura basal, indicando um impacto favorável no desenvolvimento da planta e na eficiência da cobertura do solo. A análise química mostrou que os teores de PB superaram os obtidos com fertilizantes convencionais, enquanto os teores de fibra em detergente neutro (FDN) e fibra em detergente ácido (FDA) permaneceram consistentes, garantindo a qualidade da forragem necessária para a nutrição dos ruminantes. Além disso, a avaliação econômica mostrou que os custos de aplicação do biol foram significativamente menores do que os dos fertilizantes sintéticos.

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(192 vs. 225 dólares), fornecendo uma alternativa econômica para o manejo de pastagens. Esta pesquisa destaca os múltiplos benefícios da utilização de biol derivado de MAs, incluindo maior produtividade de forragem, melhor qualidade nutricional e impacto ambiental reduzido. As descobertas enfatizam o potencial do biol como uma estratégia viável para promover práticas agrícolas sustentáveis em regiões tropicais, contribuindo para a demanda global por alternativas ecológicas aos fertilizantes químicos. Mais pesquisas são recomendadas para otimizar os protocolos de aplicação e avaliar os impactos ecológicos e econômicos de longo prazo.

Palavras-chave: caracterização agrônômica, biofertilizante, composição química, fertilizante, condições tropicais, microrganismos autóctones (MAS).

1. Introduction

The issue of long-term credibility in climate policy is a critical component in the successful implementation of measures aimed at mitigating the impacts of greenhouse gases (GHGs) (Boer et al., 2011; Schwarz et al., 2022; Beauchemin et al., 2022; Fonseca et al., 2023). This stems from two primary factors: the long atmospheric lifetimes of GHGs and the inertia inherent in energy capital stock. Greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) have varied atmospheric lifetimes, ranging from decades to centuries (Cornejo and Wilkie, 2010; Figueiredo et al., 2017; Sanz-Cobena et al., 2017). CO_2 , for instance, can remain in the atmosphere for over a century, while methane has a shorter lifespan but a much higher immediate warming potential. This persistence means that the effects of emissions are not transient but accumulate over time, leading to long-term changes in the Earth's climate system (Alonso and Guzmán 2010).

Agriculture is a significant source of GHGs, contributing to climate change through various processes and activities (World Bank, 2008; Schwarz et al., 2022). The sector is responsible for approximately 10-12% of total global anthropogenic GHG emissions, with key gases including methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2) (Beauchemin et al., 2022; De-Jongh et al., 2022). Therefore, the emissions from agriculture contribute significantly to global warming, with methane and nitrous oxide having much higher global warming potentials than carbon dioxide (Menardo et al., 2021). Methane is approximately 28-36 times more potent than CO_2 over a 100-year period, while nitrous oxide is about 298 times more potent (Tedeschi et al., 2020; Czubaszek et al., 2022; Soder and Brito, 2023). These emissions exacerbate climate change, leading to more extreme weather events, changes in precipitation patterns, and increased temperatures, which in turn affect agricultural productivity and food security (Gerber et al., 2013; Rojas-Downing et al., 2017; FAO, 2022a). The application of fertilizers in agriculture is a critical factor in enhancing crop yields and food production (Havlin et al., 2005; Vasco et al., 2021; Anuradha and Singh, 2021). However, it also significantly contributes to greenhouse gas (GHG) emissions, particularly nitrous oxide (N_2O), a potent greenhouse gas with a global warming potential approximately 298 times that of carbon dioxide (CO_2) over a 100-year period (Chadwick et al., 2000; Hyland et al., 2014; Hristov et al., 2019). Also, nitrification and denitrification are the primary microbial processes responsible for N_2O emissions in soils (Chadwick et al., 2000; Subbarao and Searchinger, 2021). In fact, nitrification is the aerobic conversion of ammonium (NH_4^+) to nitrate

(NO_3^-), while denitrification is the anaerobic reduction of nitrate to nitrogen gases, including N_2O (Schwarz et al., 2022). Fertilizer application increases the availability of nitrogen in the soil, enhancing these processes and leading to higher N_2O emissions (Menardo et al., 2021). Chemical fertilizers, primarily composed of nitrogen (N), phosphorus (P), and potassium (K), are applied to enhance plant growth by providing essential nutrients that might be lacking in the soil (Vasco et al., 2021; Ismael et al., 2021). These fertilizers have been a cornerstone of the green revolution, contributing to significant increases in agricultural productivity. However, evidence of the last ten years has reported that its use has raised concerns about environmental sustainability and biodiversity loss (Bernués et al., 2011; Eyhorn et al., 2019; Huera-Lucero et al., 2020). Chemical fertilizers can alter the composition and diversity of soil microbial communities (Vasco et al., 2021). High nitrogen inputs, for instance, can favor fast-growing, nitrogen-loving microbial species, leading to a decline in microbial diversity. In addition, runoff from fertilized fields can lead to nutrient enrichment in water bodies, causing eutrophication (Vasco et al., 2021). This process results in algal blooms, hypoxia, and the loss of aquatic biodiversity. Consequently, the impact of chemical fertilization on biodiversity is a growing concern in the context of sustainable agriculture and environmental conservation. Chemical fertilizers, while crucial for enhancing crop yields, can lead to nutrient imbalances, soil degradation, water pollution, and a decline in biodiversity (Ismael et al., 2021). Addressing these challenges requires a multi-faceted approach that integrates scientific, technological, and policy-driven strategies. The use of chemical fertilizers in the Amazon region of Ecuador poses significant challenges to biodiversity and ecosystem health (Vasco et al., 2021; Mena et al., 2006). However, by adopting sustainable agricultural practices and implementing effective policy measures, it is possible to mitigate these impacts and promote a more harmonious relationship between agriculture and the environment. Therefore, the growing concerns over the environmental impacts of chemical fertilizers have encouraged interest in sustainable alternatives, such as biofertilizers (Figueroa-Saavedra and Guamán-Rivera, 2023; Guerrero-Pincay et al., 2023). Biofertilizers are natural fertilizers that contain living microorganisms, which promote plant growth by increasing the availability of nutrients to plants (Sansinenea, 2021; Anuradha and Singh, 2021; Madawala, 2021). AMs biology is a fascinating and emerging field that explores the role of microorganisms in mountain ecosystems representing a key tool in the circular economy (Pandey and Yarzabal, 2018; Orjales et al., 2019). These microorganisms, which include

bacteria, fungi, archaea and viruses, are fundamental to the health and functioning of these ecosystems (Dhakar and Pandey, 2020; Armada et al., 2018). Therefore, by integrating biols into production systems, efficient resource management is promoted, decreasing dependence on chemical inputs and reducing costs, while improving crop productivity and quality (Kuwahara et al., 2018). Little scientific evidence has reported that AMs contribute significantly to the carbon and nitrogen cycle in pastures or forages. Aiming this, a study was performed to assess the efficacy and environmental impact of biol derived from AMs in improving the productivity, chemical composition and sustainability of pastures.

2. Materials and Methods

2.1. Location of the experiment and edaphic conditions

The present investigation was carried out in the parish of San José de Guayusa, which is geographically located at latitude south 0°15'34.9" and longitude west 77°03'49.1". The climate in the region is characterized by humid tropical rainforests (Inzunza, 2007; INAMHI, 2021). The average rainfall is 2942 mm annually, with an annual average temperature of 29.7 °C. The *M. maximus* was established in 2015 at 4 kg/ha seeding rate. The physical-chemical properties of the soil were 8% of organic matter, 5.5 ppm of phosphorus (P), 0.83 meq of potassium (K) and 55% base saturation. In addition, the soil texture is loamy clay loam and cationic relationships of (Ca + Mg) of 67.3 meq/100 mL. The experimental area was fertilized with 200 kg N/ha applied at seeding (Guerrero-Pincay et al., 2023).

The biol based on AMs was elaborated as follows: 4 bags of mountain litter were placed, that is to say, the soil from the collection of the AMs, adding the 2 bags of rice powder, the 4 liters of molasses and the 18 liters of whey. To this a mixture was made with the hands (kneading) to integrate all the ingredients previously mentioned to be placed in the tank of 200 ml and to create pressure on the mass to compact finally the tank was closed leaving it in a rest for a lapse of 20 days. The chemical composition is shown in Table 1.

Table 1. Chemical composition of Biol Prepared of AMs.

Parameter	Unit	Value
pH		3.6
Conductivity	mS / cm	16.7
Total nitrogen (TN)	mg/L	628.8
Phosphorus (P)		165.8
Potassium (K)		5712.5
Calcium (Ca)		893.1
Magnesium (Mg)		384.5
Iron (Fe)		70.0
Copper (Cu)		0.12
Zinc (Zn)		0.87
Manganese (Mn)		5.4

2.2. Experimental treatments

For this study, an area of 1250 m² was divided into four treatments with three replications each under a completely randomized block design, as detailed below; **Control**, not organic fertilization; **T1**, 3 litres of biol × 9.5 litres water ha⁻¹; **T2**, 4 litres × 8.5 litres of water ha⁻¹ and **T3**, 5 litres of biol × 7.5 litres water ha⁻¹.

2.3. Sampling and data determinations

2.3.1. Forage mass

Forage mass (FM) was assessed through the double sampling technique described by NRC (1962). Therefore, for direct measurements, we used one 0.25 m² square to collect random samples in each treatment prior every grazing throughout the experimental period. The FM was estimated by cutting and weight the forage. A visual scale was used as an indirect measurement method, where the scale ranged from 1 to 3, meaning that 1 is the lowest herbage mass and 3 is the highest (Haydock and Shaw, 1975). In addition, during the direct determination of forage, two samples of approximately 0.5 kg for treatment were collected. The first sample was dried in the forced air circulation oven at 60 °C for 72 h in order to determine dry matter (DM kg ha⁻¹), while one second sample of fresh grass was frozen at -20 °C for the determination of its chemical composition.

2.3.2. Plan cover score

Plant scoring of the plots was realized in order to determine the extent of cover by the forage. To this end, scoring was yielded using five-point grading score as suggested by Onyeonagu and Asiegbu (2013) for subjective evaluation, where degree of cover was assessed as, 1 (< 20%) labelled as very low; 2 (20 to 39%) labelled as low; 3 (40 to 59%) labelled as medium; 4 (60 to 79%) labelled as high and 5 (80 to 100%) labelled as very high.

2.3.3. Stem to leaf ratio

The density of tiller population (TPD; tiller/m²) was determined by directly counting the number of tillers within a 1 × 0.25 m² sampling frame. This frame was positioned at the average plot height for each treatment and during each harvest, as referenced by Grant et al. (1983), Sbrissia et al. (2001) and Sousa et al. (2019).

2.3.4. Pasture height

During the course of the study, the height of the canopy was assessed with a 1-meter ruler marked in centimeters at various randomly selected locations for each treatment before each harvest (Guamán-Rivera et al., 2023).

2.4. Chemical composition

Before the analysis, the grass samples that had been frozen were allowed to thaw at room temperature. Subsequently, they were treated at 60 °C for a duration of 24 hours, after which they were milled and blended using a cyclone mill (Retsch SM2000, Retsch, Haan, GE) equipped with a 1 mm sieve. The chemical analysis followed by AOAC

(2000) guidelines and was performed in duplicate, with results presented on a dry matter basis. To determine dry matter (DM), samples were dried to 103 °C for 24 hours, while ash content was determined by incineration at 550 °C for 5 hours. Using the Kjeldahl method, crude protein was calculated as a percentage of nitrogen multiplied by 6.25. Crude fiber (CF) analysis was carried out using the Weende method, involving acid hydrolysis with 1.25% H₂SO₄ and subsequent alkaline hydrolysis with 1.25% NaOH. In contrast, neutral detergent fiber (NDF), and acid detergent fiber (ADF) were measured sequentially on an ash-free basis, following the procedures of Van Soest et al. (1991), with the addition of sodium sulfite and thermostable α-amylase (Ankom Technology, Fairport, NY, USA).

2.5. Statistical analysis

All variables were analysed with the statistical software SAS version 9.4 (Institute Inc., Cary, NC, USA). Before conducting the analysis, the normality of the data was assessed using the PROC UNIVARIATE procedure. Following this, a linear mixed model was applied to process the data, treating the treatments, time, and their interaction as fixed effects, while blocks and residual error were considered as random effects. Means are presented as minimum squares (PDIF) and compared with a Dunnett test. Additionally, the PROC CORR procedure in SAS was employed to establish correlations between agronomic and compositional variables. The means were presented as separate least squares means, utilizing the PDIF option in SAS. Statistical differences are identified at a significance level of $P < 0.05$, with trends noted at $P < 0.10$.

3. Results

3.1. Biol prepared of AMs

The Table 1 shows the chemical properties of biol elaborated of AMs. The pH measurements reveal that the biol is highly acidic, averaging (3.6) due to its favourable fermentation process aimed at acquiring nutrients and suppressing soil and plant microorganisms. The electrical

conductivity reading of 16.7 mS/cm points to moderate salinity levels. Although the total nitrogen content stands at a satisfactory level of (628.8 TN), there is a higher concentration of ammonium compared to nitrates. To enhance fertilization, it is advisable to boost the total nitrogen content to approximately 1000 ppm, which can be achieved by incorporating organic matter or ammonium salts like ammonium phosphate. Furthermore, the findings show that calcium is present in an optimal range for the majority of crops, at (893.1 mg/L). However, micronutrients, particularly copper at (0.12 mg/L) and zinc at (0.87 mg/L), may necessitate adjustments through the foliar application of a concentrated micronutrient solution.

3.2. Agronomic responses

The agronomic responses by the effect of Biol Prepared of AMs are shown in Table 2. The height plant exhibited notable differences among treatments ($P < 0.002$). Specifically, treatments T2 and T3 resulted in taller plants (70 ± 1.7 cm) compared to the control group (69 ± 1.7 cm) or when 3 L of biol was applied (T1, 67 ± 1.7 cm). Similarly, the treatments influenced the basal cover of *M. maximus* ($P < 0.001$; Table 2). Higher doses of AMs (T2 and T3) led to a greater average cover ($25 \pm 2.6\%$) than the lower dose of AMs at 3 L (T1, $19 \pm 2.6\%$) and the control ($17 \pm 2.6\%$). These findings align with an improved response in the number of stems and tillers ($P = 0.003$ to 0.04 ; Table 2), which were more abundant in T1 and T3, respectively. Concerning the period effect, except for the number of tillers ($P = 0.25$), the other agronomic variables were significantly affected by the application of AMs ($P = 0.01$ to 0.001 ; Table 2). Furthermore, when examining the interaction between treatment and period (Table 2), plant height ($P < 0.001$), green and dry biomass ($P = 0.01$), and the number of tillers ($P = 0.04$) demonstrated substantial responses potentially attributable to the effects of the AMs.

3.3. Chemical composition

Regarding the chemical composition of *M. maximus* grass, numerical differences have been detected in the crude protein (CP) contents ($P = 0.07$), T3 which received 5 l/ha⁻¹

Table 2. Agronomic responses of *Megathyrus maximus* by effect of Biol Prepared of AMs.

Item	Treatment				Period								SEM	P value		
	Control	T1	T2	T3	1	2	3	4	5	6	7	8		Trat	P	T × P
Plant height, cm	69 ^a	67 ^b	70 ^a	70 ^a	58 ^E	64 ^D	80 ^A	83 ^A	57 ^E	70 ^C	68 ^C	75 ^B	1.7	0.002	0.001	0.001
Basal cover, %	17 ^{bc}	19 ^b	25 ^a	26 ^a	18 ^B	26 ^A	13 ^C	25 ^A	6 ^D	27 ^A	28 ^A	30 ^A	2.6	0.001	0.001	0.82
Stem, # m ²	14 ^{bc}	16 ^{ab}	18 ^a	17 ^a	17 ^B	24 ^A	19 ^B	17 ^B	9 ^D	18 ^B	13 ^C	13	1.1	0.04	0.001	0.7
Stem/Leaf Ratio	29	30	27	28	31 ^B	13 ^D	21 ^C	36 ^A	31 ^B	27 ^B	34 ^A	37 ^A	2.7	0.11	0.001	0.99
Tillers (#)	5 ^b	5 ^b	5 ^b	7 ^a	5	5	5	6	6	4	6	5	0.39	0.003	0.25	0.04
Green forage (kg/ha ⁻¹)	3863	3905	4413	4465				4967 ^A				3355 ^B	881	0.8	0.01	0.9
Dry matter (kg/ha ⁻¹)	618	884	882	937				991 ^A				669 ^B	170	0.19	0.01	0.94

SEM, error standard of the means. ^{a-b} Means with different letters in the same row differ at $P < 0.05$ by treatment effect. ^{A-B} Means with different letters in the same row differ at $P < 0.05$ by period effect.

of AMs biol, together with the control showed a higher CP content ($12.85 \pm 0.2\%$, on average) than those observed for T1 and T2 which did not differ between them ($12.17 \pm 0.2\%$, on average; $P > 0.05$). Similarly, the period influenced the CP contents during the experiment (Table 3), the second cut obtained +27% more PB than a first cut (14.38 vs. $10.45 \pm 0.2\%$). However, despite having obtained higher PB contents in the T3 treatment (Table 3), the NDF contents were higher than in the AMs ($55.62 \pm 0.16\%$, on average) and control ($55.0 \pm 0.16\%$) treatments. Consequently, a negative correlation was detected for PB and NDF ($r = -0.89$; $P < 0.001$), which could be explained by the cutting effect ($P < 0.0007$) whose contents increased by +3% with respect to initial values (54.87 vs. $56.62 \pm 0.16\%$). Corresponding to the above-mentioned, only a statistical trend has been obtained for ADF between treatments ($P = 0.07$; Table 3). In this sense, the T1 and T3 treatment ($32 \pm 0.37\%$, on average) and control ($32.1 \pm 0.37\%$) showed lower contents than those observed for T2 ($33.4 \pm 0.37\%$). In addition to this, and as expected, lignin contents did not exceed ($2.41 \pm 0.46\%$) with no differences between treatments ($P = 0.19$) or by cut-off effect ($P = 0.17$).

In economic terms, the present study evaluated the use of AMs as a biofertilizer. These microorganisms, purchased at a cost of \$0.15 per litre, proved to be a promising alternative to improve cattle pastures and thus tend to improve both milk and meat production. To complement the cost analysis of the most efficient treatment (T3), the total cost per hectare was determined to be \$192. Considering that 40 litres were applied per hectare, it can be inferred that the doses used in this treatment represent a fraction of the total recommended volume (100 litres/ha). In this analysis, the treatment (T3) reached the maximum dose of the study, thus achieving optimal results at a lower cost than potentially required for a full application with chemical fertilizers. This could indicate a high efficiency of the microorganisms used or synergies with other factors in the system.

4. Discussion

4.1. Biol of AMs

Agriculture faces a major challenge in the sustainable production of food to supply the world's population. The

Food and Agriculture Organisation of the United Nations (FAO) estimated a population growth of 13% by 2030 and 30% by 2050, so a 70% increase in agricultural production will be needed to meet the problems of malnutrition and ensure food security (FAO 2022b, 2021). Plant growth-promoting bacteria (PGPB) may play a decisive role in facilitating plant growth in soil (Armada et al., 2018) and might be especially relevant to recovery degraded pastures. There are not studies that have evaluated biols elaborated of AMs. Other experimental works used manure cattle (Gil-Ramírez et al., 2023) for prepared biols and it reported lower pH than our study (6.33 vs. 3.6). Whereas Nagler et al. (2019) using biol prepared of ruminal liquid observed similar values than those obtained in this study (3.3 pH, on average). So, the increase in the pH as a function of manure application has been attributed to the calcium carbonate and bicarbonate found in manure (Eghball et al., 1996; Whalen et al., 2000). In addition, the low concentrations of electrical conductivity are associated with the consumption of soluble compounds by microorganisms because they are in a stage of accelerated growth. Nevertheless, Rayne and Aula (2020) stated that farmers should use manure rather than completely replacing it with commercial fertilizers. In fact, fertilization is known to have a beneficial effect on soil fertility and many other soil properties, thereby contributing to the overall health of the soil. Consequently, organic nutrient sources are sources of carbon (C) which plays a role in improving soil quality and climate change mitigation. In this pioneer work, our research team showed that biol of AMs could be a fertilization potential for recovery of the soil health under tropical conditions instead of chemical synthetic fertilization.

4.2. Agronomic responses

The agricultural sector (crop and livestock farming) is a significant contributor to greenhouse gas emissions worldwide, accounting for approximately 14% of human-caused greenhouse gas emissions (Mazzetto et al., 2015; Figueiredo et al., 2017; Schwarz et al., 2022). Use of fertilizer to increase food production is accepted as a practice in developed countries and as a goal in developing countries. However, plants are sensitive to temperature and might

Table 3. Chemical composition of *M. maximus* by effect of Biol Prepared of AMs.

Item	Treatment				Cut		SEM	P value	
	Control	T1	T2	T3	I	II		Trat	Cut
Composition, %									
Crude protein	12.45 ^x	12.1 ^y	12.25 ^y	12.85 ^x	10.45 ^B	14.38 ^A	0.2	0.07	0.001
NDF	55.00 ^b	55.45 ^b	55.8 ^b	56.75 ^a	54.87 ^B	56.62 ^A	0.16	0.006	0.0007
ADF	32.1 ^y	31.9 ^y	33.4 ^x	32.1 ^y	32.65	32.12	0.37	0.07	0.15
Lignin	2.35	2.65	3.00	1.65	2.7	2.12	0.46	0.19	0.17
Ash	15.12	16.08	18.95	13.57	15.76	15.72	2.14	0.35	0.98
Organic matter	84.88 ^b	83.92 ^b	81.05 ^c	86.43 ^a	84.24	84.28	1.30	0.03	0.45

SEM, error standard of the means. ^{a-b} Means with different letters in the same row differ at $P < 0.05$ by treatment effect. ^{A-B} Means with different letters in the same row differ at $P < 0.05$ by period effect.

grow differently in the tropics (Scarnecchia and Kothmann, 1982; Gogerddan, 2001; Nnadi et al., 2015). This difference could be reflected in either the total nutritional needs, the kinds and proportions of nutrients needed, or a combination of quantity and quality of nutrients. Referential studies by Guerrero-Pincay et al. (2023) using biol + Urea in *M. maximus* have reported lower plant height than our study (62 vs. 70 cm). In the same way, Figueroa-Saavedra and Guamán-Rivera (2023) fertilized *Brachiaria decumbens* with manure biol + urea (50:50 proportion) reporting lower plant height than those in Biol of AMs (63 vs. 70 cm). AMs, are mixtures of beneficial microorganisms obtained from mountainous, biodiversity-rich soils. They include lactic acid bacteria, yeasts, actinobacteria, fungi and phototrophic microorganisms that work together to improve soil fertility and promote plant growth (Eghball et al., 1996; Nagler et al., 2019; Whalen et al., 2000; Puntillo et al., 2020). These microorganisms break down organic matter, produce essential nutrients and fight pathogens, strengthening plant resistance to disease. Some scientific evidence has shown that these microorganisms can release organic acids and enzymes that solubilise nutrients such as phosphorus and potassium, facilitating their uptake by roots (Anuradha and Singh, 2021). Therefore, beneficial microorganisms can stimulate the production of key enzymes in roots that facilitate the uptake of essential nutrients. Consequently, AMs can promote the growth of beneficial bacteria and fungi, which improves nutrient availability and transport (Madawala, 2021). In addition, their use reduces dependence on chemical fertilizers and pesticides, promoting sustainable agricultural practices. AMs are also used in composting and reclamation of degraded soils, contributing to efficient environmental management. In this study, the T2 y T3 treatments showed higher forage yields than those in control, which in part it could be explained yet AMs act as plant growth promoters, which have beneficial effects on plant growth and health (Vázquez-Conde, 2014). However, the lower yield at 12 months of experimentation on forage mass could be due to the high precipitation of the last months has caused that the biol of AMs is not properly absorbed to the cells (washout effect) with the corresponding low productive yield.

4.3. Chemical composition

Data from MAGAP (2022) indicates that cattle raising is an essential economic activity for families in the Amazon region. More recently, Peralta et al. (2024) reported that in Orellana Province the bovine population is high, but this does not necessarily mean greater productivity levels. In fact, meat and dairy production in arid, equatorial, and tropical regions is often less productive than in temperate regions due to lower nutritional quality of forage grasses, infertile soils, and adverse climatic conditions (Guamán-Rivera et al., 2023). Although, the level of nitrogen fertilization (i.e., chemical synthetic) is reflected in the nutritional quality and yield production of most forages (Perramon et al., 2017). This strong demand negatively affects cultivation as producers strive to boost productivity while facing challenges in achieving sustainable production with less reliance on chemical

inputs (Costa-Leite et al., 2019). Under this scenario, some studies have been performed to partially replace the use of chemicals for organic fertilizers. Referential data by Guerrero-Pincay et al. (2023) and Figueroa-Saavedra and Guamán-Rivera (2023) have reported CP contents ranged from (8 to 10%, on average) when urea + biol was combined (50:50, proportion). According to Givens et al. (2000) forages with CP contents less than 6% based on DM, are not suitable for feeding ruminants. However, our study showed higher CP contents ($12 \pm 0.2\%$, on average) using biol of AMs than referential works in *B. decumbens* as well as *M. maximus*. Results that would be explained by several synergistic mechanisms that improve nitrogen availability and the efficiency of plant metabolism. Considering that there are no studies on the use of AMs as an organic alternative instead of chemical fertilization in forages. There is into account that, organic fertilizers have lower absorption when compared to chemical nitrogen. Therefore, the accumulated effect of repeated of AMs applications is important, and it should be accounted for in fertilizer planning (Perramon et al., 2017).

On the other hand, in this first study, the fibre cell contents did not vary among treatments. Contrary to the observed by Guerrero-Pincay et al. (2023) where the combination of urea and manure biol resulted in lower NDF levels when compared to the control group, and slightly lower values when compared to the group that received chemical nitrogen fertilization. It can be linked to the stimulation of new tiller growth and a decrease in the proportion of plant biomass allocated to structural carbohydrates (Moore and Mott, 1973; Niwiska, 2012; Moore and Hatfield, 2015). Another study carried out by Figueroa-Saavedra and Guamán-Rivera (2023) reported that *Brachiaria brizantha* fertilized with 50% biol exhibited lower cell wall content compared to treatments using synthetic nitrogen fertilizers. Therefore, a priori this first investigation opens the window about the use of biol elaborated of AMs as a potential fertilization strategy. Finally, in economic terms, the use of AMs processed biol would allow us to save \$33 per hectare when compared to chemical nitrogen fertilisation (\$192 vs. \$225).

5. Conclusions

The present research demonstrates that the application of biol elaborated with AMs constitutes a promising agronomic strategy for the sustainable improvement of tropical pastures such as *M. maximus*. The results obtained showed significant increases in all agronomic responses and crude protein content (12.85% in treatment T3), exceeding the metrics associated with conventional chemical fertilizers. Furthermore, the use of biol not only maintains adequate fibre quality (NDF and ADF) for ruminant nutrition but also significantly reduces fertilizer costs per hectare, contributing to higher profitability. From an environmental perspective, biol of MMs minimises reliance on chemical inputs, promoting biodiversity and reducing ecological impact in tropical production systems. Despite that, more studies a long term should be performed to evidence the impact on soil microbiota.

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