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Biological nitrification inhibition and forage productivity of *Megathyrsus maximus* in Colombian dry tropics

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Abstract: Agronomic, nutritional, and environmental aspects are integrated to promote sustainable tropical grassland production. Biological nitrification inhibition (BNI) is a plant-based strategy to improve nitrogen use efficiency by grasses in which they suppress the pace of soil nitrification *via* exudation of inhibitory compounds. To evaluate the effect of BNI on the productive performance of *Megathyrsus maximus* under field conditions, we evaluated a collection of 27 germplasm accessions and commercial cultivars of the forage grass in the dry tropics of Colombia. We measured plant yield dry matter, nutrition quality parameters, and nitrification rates of soil at 22 months after pasture establishment. Our results highlighted germplasm accessions of superior agronomic performance (for dry matter production and nutrition quality) and high capacity to decrease nitrification. Although no relation was observed between agronomic aspects, nutritional aspects, and nitrification rates, we conclude that there is no agronomic or nutritional penalty on environmentally friendly grasses, and BNI could be adopted as a target trait in plant breeding programs toward the development of eco-efficient forages and contribute to the sustainable intensification of livestock systems.

Keywords: Guinea grass; tropical agroecosystem; tropical forages; N uptake; environmental pollution

In tropical agroecosystems, livestock production is based on the use of grasslands. Given their geographic position, there is wide variability of forage species, which are used under different management. However, inadequate agricultural practices have generated negative impacts for the environment (e.g., greenhouse gas emissions, deforestation, water overconsumption, and soil contamination) and global human security (excessive use of antibiotics and outbreak of zoonotic diseases, among others) (Portador Garcia 2020).

Accordingly, sustainable intensification is an alternative for tropical livestock production systems based on forages (Zu Ermgassen et al. 2018) that aim to increase productivity (animal carrying capacity per unit of area) and at the same time provide different ecosystem services that contribute to soil fertility, erosion reduction, and climate regulation through CO_2 sequestration and improvement in carbon (C) and nitrogen (N) cycle balances, and therefore mitigate the risk of nitrate leaching and nitrous oxide emissions to the atmosphere (Lemaire et al. 2014, Yang et al. 2020).

Several alternatives have been proposed around environmental management and sustainability in livestock production. Aspects related to ruminants' digestive physiology, improvement of diets, and grasslands management have been associated with eco-efficient livestock production (Rao et al. 2015).

Species of the genus *Urochloa* (formerly *Brachiaria*) and *Megathyrsus* (formerly *Panicum*) exhibit properties to suppress the nitrification process provided by the release of inhibiting substances through the roots, known as biological nitrification inhibition (BNI) (Subbarao et al. 2006, 2013). BNI is a mechanism that contributes to increasing N use efficiency

(NUE) by the plant, decreasing N losses in the form of nitrate (NO_3^-) and nitrous oxide (N_2O), and mitigating environmental contamination (Coskun et al. 2017). This BNI ability by plants could be considered a regulatory ecosystem service that mitigates the negative impact of N_2O emitted to the atmosphere.

Megathyrsus maximus (Jacq.) B. K. Simon & S. W. L. Jacobs is widely distributed among lowland regions in Colombia. In livestock production systems, it stands out for its yield of dry matter and nutritional characteristics for dry tropics (Vivas-Quila et al. 2015, Morales-Velasco et al. 2016, Matínez-Mamian et al. 2020). It is also adequate for forming forage associations in grasslands and silvopastoral systems (Santiago-Hernández et al. 2016, Barragán-Hernández and Cajas-Girón 2019). These characteristics support the idea of extending research under an agronomic, environmental, and/or genetic framework with the aim of identifying genotypes adapted to diverse edaphoclimatic conditions in tropical livestock production systems that are also drivers of eco-efficient livestock production.

With the aim of identifying tropical forage material for plant breeding programs and germplasm selection, the productive and environmental behavior of a collection of *M. maximus* accessions was evaluated through the measurement of nitrification rates, N uptake, and agronomic and nutritional aspects under field conditions in dry tropical forest agroecosystem of Colombia.

MATERIAL AND METHODS

Location. This research was conducted in a dry tropical forest agroecosystem located in the Patía valley of southwestern Colombia, at 625 m a.s.l., with an average temperature of 27.9 °C and annual precipitation of 1 414 mm with two rainy seasons per year. Soils in the experimental location are of Order Mollisols. The soil chemical characteristics (0–20 cm depth) were as follows: pH – 6.26 (1:1 water); C_{ox} – 18.14 g/kg; N-total – 2.20 g/kg; P – 6.3 mg/kg; Ca 14.58 mg/kg; Mg 6.91 mg/kg; K 0.59 mg/kg; Na 0.10 mg/kg; cation exchange capacity – 27.10 $mmol_+$/kg; and B – 83 mg/kg.

Experimental design. In December 2015, we established in the field a collection of 27 *Megathyrsus maximus* (tillers) accessions provided by the germplasm bank of the International Center for Tropical Agriculture (CIAT) and control treatments for the measurement of nitrification rates such as bare soil plots, *Stylosanthes guianensis* (Aubl.) Sw. and *Urochloa humidicola* (Rendle) Morrone & Zuloaga, the latter as the material with low nitrification rate potential (i.e. high BNI) (Table 1). The experimental units (plots) were 4 m^2, separated by 1 m, and grouped in three blocks, each separated by 2 m. To guarantee the process of establishing plots, we used water irrigation and mechanical weed control. One year after establishment, we applied fertiliser only once at a rate of 150 kg N/ha and 95 kg P/ha. Soil samples to determine nitrification rates were taken from 27 accessions in October 2017 during the rainy season, with monthly precipitation of 190 mm, minimum temperature of 19 °C, and maximum temperature of 38 °C.

Determination of soil nitrification rate. After 42 days of regrowth in the plots, we collected soil samples (0–10 cm) using a tubular soil auger. Nitrification rate (NR) was determined using the microcosm incubation method (Núñez et al. 2018, Villegas et al. 2020). Samples were air-dried for 48 h, sieved (2 mm), and ground manually. The concentration of NH_4^+ and NO_3^- was determined in extracts of mineral N using 5 g of soil in KCl 1 mol/L (1:10 *w/v*), shaken for 30 min and filtered in Whatman paper No. 2. For the colorimetric determination of NH_4^+ and NO_3^-, we followed Borrero et al. (2017). In addition, we incubated soil subsamples of 3 g at 4, 8, and 20 days after supplementing with $(NH_4)_2SO_4$

Table 1. International Center for Tropical Agriculture (CIAT) identification numbers of different accessions of *Megathyrsus maximus* and control treatment evaluated

Treatment	CIAT accessions
Megathyrsus maximus	688, 693, 6500, 6571, 6658, 6796, 6897, 6948, 16005, 16027, 16034, 16035, 16036, 16038, 16039, 16044, 16049, 16051, 16055, 16057, 16061, 16062, 16068, 16069, and 26939
	comercial: 6299 var. Tobiata and 6962 var. Mombasa
Stylosanthes guianenesis	11995
Urochloa humidicola	679 var. Humidicola or Tully
Bare soil per plot	

(27 mmol) and measured NH_4^+ and NO_3^- production in each subsample using the mentioned methodology. We calculated nitrification rate as the slope of NO_3^- concentration as a function of incubation time.

Agronomic evaluation. The data resulted from an average of five evaluations conducted during the first semester of 2017 (January to June) during the rainy season, with 42 days of regrowth and 8 harvests a year approximately. We evaluated plant height according to Toledo and Schultze-Kraft (1982). For dry matter yield (DMY), we estimated the availability of green forage (GF) after cutting at the height of 30 cm from the ground and measuring the weight per plot in the field. Out of all GF, we weighed subsamples of approximately 200 g. They were dried in an oven with controlled ventilation at a temperature of 60 °C until reaching constant weight (48 h to 72 h). With the final weight of the subsample, we estimated dry matter. We also calculated the current percentage of flowering through observation of the experimental plot using the range 0–100%.

Nutritional evaluation. The analyses were conducted using near-infrared spectroscopy (NIRS) for tropical grasses (Mazabel et al. 2020) handled in the forage quality and animal nutrition laboratory of CIAT. Subsamples from the agronomic evaluation were used. The material was dried in an oven at a temperature of 60 °C for approximately 72 h until reaching constant weight. Then, the samples were ground using the Retsch SM 100 mill (Retsch GmbH, Haan, Germany) with a 1-mm sieve. We determined the following parameters: crude protein (CP); *in vitro* dry matter digestibility (IVDMD); acid detergent fiber (ADF), and neutral detergent fiber (NDF) in FOSS NIRS 6500 equipment, with software ISIScan (IS-2250) version 2.71 (FOSS NIRSystems Inc) (FOSS and Infrasoft International, USA). We took duplicates of the spectrums of each sample, using separate cells of quartz of inner diameter of 3.5 mm and 1 cm of diameter, in a wavelength range between 400 nm and 2 500 nm.

The results of the reference chemical analysis and the spectral signals of each sample were processed using WinISI version 4.9 (FOSS Analytical software, 2012). Then, the results were incorporated in equations generated in the CIAT laboratory as follows: R^2 of 0.93, 0.98, 0.85, and 0.98 and standard error for cross-validation (SECV) of 2.11, 1.22, 2.78, and 0.61 for NDF, ADF, IVDMD, and CP, respectively (Molano et al. 2016). This increases the action range and accuracy of the model.

We calculated total N dividing CP by the factor 6.25. N uptake was calculated as the product of DMY and total N.

Statistical analysis. Statistical differences among treatments were evaluated. For the variable NR, we conducted an analysis of variance and a multiple comparison test (Tukey's *HSD* (honestly significant difference)) using the packages rcompanion, multcompview, and emmeans in R v.4.0 (New Jersey, USA). We conducted a cluster analysis (cl) using the package HCPC and principal components using the package FactoMineR with the variables DMY, flowering, nutritional quality, and NR. Figures were created in R using the package ggplot2.

RESULTS AND DISCUSSION

Nitrification rate. In the *M. maximus* collection, 74.1% of the evaluated genotypes showed potential for BNI capacity since they decreased soil NR from 3.4% to 66.3% compared to the bare soil control. Plots with accessions 6962, 688, 16044, 6500, 16036, 16035, 6658, and 16049 showed lower NR than plots with *U. humidicola* (0.69 mg N-NO_3/kg soil/day). The values observed were slightly higher than those recorded by Vivas-Quila et al. (2017), who indicated a NR of 0.26 mg N-NO_3/kg soil/day in livestock production systems using improved forages (*Urochloa* hybrid and *M. maximus*) (Figure 1). As expected, *S. guianensis* had the highest NR among the evaluated materials, possibly because of its ability to fix N symbiotically (Subbarao et al. 2007). As legumes rely to a lesser extent on soil mineral N, keeping N in the NH_4^+ form in the soil may not represent an ecological advantage.

Previous studies have used bare soil plots as a control for high nitrification, mainly in greenhouse experiments with controlled conditions (e.g., Subbarao et al. 2009, Nuñez et al. 2018, Villegas et al. 2020). However, in our field study, six *M. maximus* accessions showed higher NR than the bare soil plots. According to Subbarao et al. (2015), plant root exudates may either stimulate or suppress nitrifier activity. The range of NR observed in the collection evaluated suggests that the interaction of *M. maximus* with soil nitrifiers may exhibit a variable response, and this can be more evident in the field where other N pools and actors intervene in N cycling.

Since 2007, research in *M. maximus* regarding its BNI capacity has been dynamic and recent studies have shown a greater BNI capacity of *Megathyrsus* than in previous research. This discrepancy might have resulted from the exploration of a higher genetic diversity (more genotypes) under different edaphic and environmental conditions. While Subbarao et al.

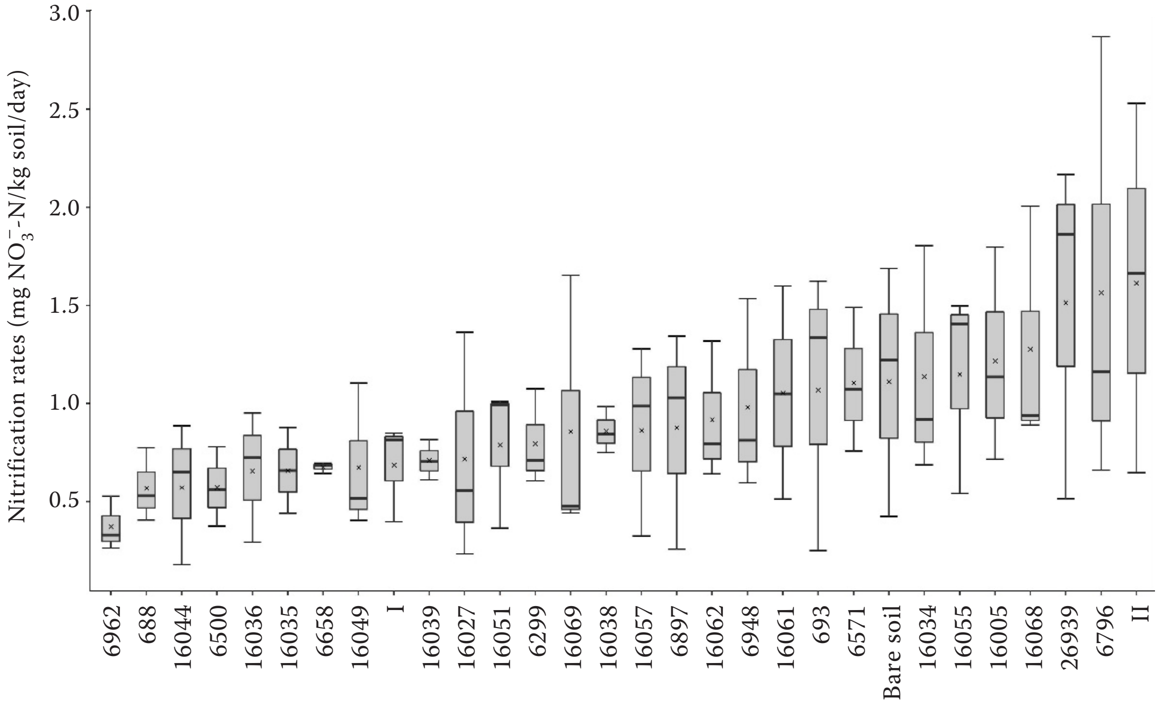

Figure 1. Nitrification rates of the soil of 27 germplasm accessions of *Megathyrsus maximus*. Nitrification rates are expressed as the slope of a linear regression between the concentration of NO$_3^-$ overtime after 20 days of incubation (n = 3). *Urochloa humidicola* (I), *Stylosanthes guianensis* (II), and bare soil were used as controls. For each treatment, crosses and bold horizontal lines inside each box denote the mean and median nitrification rate, respectively

(2007) evaluated only one accession of *M. maximus*, Villegas et al. (2020) found large variability in BNI for 119 germplasm accessions under greenhouse-controlled conditions. In this study, we evaluated 27 accessions under field conditions in a dry tropical forest of Colombia and observed accessions with high BNI capacity. However, most of the studies concur that the identification of BNI capacity for a given genotype should be complemented with other methodologies besides NR (Nuñez et al. 2018) to really understand how different diverse genetic and environmental elements control the potential for BNI (Villegas et al. 2020).

Low NR in soils of the Patía valley is possibly associated with the sustained management of extensive livestock production with null or poor application of N fertilisers in pastures (Subbarao et al. 2013). In addition, the soil pH has an effect on chemical forms, concentration, and availability of substrates that influence cellular growth and microorganism activity in the soil. This is reflected in different contributions to the oxidant activity of ammonia (Nicol et al. 2008, Li et al. 2018).

The above can be confirmed with findings in the Patía valley of Colombia, with reports of higher NR (2.5 mg N-NO$_3$/kg soil/day) in naturalised grasslands with Mollisols (Vivas-Quila et al. 2017) and even 10.7 mg N-NO$_3$/kg soil/day in greenhouse experiments with Vertisol: Typic Pellustert (Villegas et al. 2020). This broad range of NRs could be evidence of the influence of soil characteristics and environmental as well as the forage grass species used conditions on responsible elements of BNI. Therefore, it is important to explore other parameters, such as the amount and rate of root turnover, which may contribute to BNI in grasses (Nakamura et al. 2020).

Crops with BNI potential could be used in plant breeding programs oriented to environmentally sustainable food production (Butterbach-Bahl et al. 2013). Our results highlight a comparable performance of accession 688 evaluated here with commercial cultivars such as 6962 cv. Mombasa and 6299 cv. Tobiatá reported by Villegas et al. (2020) at greenhouse level as promising genotypes with high BNI potential probably due to their high capacity to uptake N and low soil NR even superior to that previously reported for *U. humidicola*.

If such genotypes of *Megathyrsus* are generated, they could decrease the quantity of synthetic N applied to the soil and diminish N-driven emissions to the atmosphere, which could be in the range of 2% to 13% of the total N applied (Gerber et al. 2013).

The field validation under farmers' conditions of the previously evaluated genotypes of *M. maximus* under greenhouse conditions is a step forward toward the selection of germplasm for plant breeding processes aimed at generating improved forages with the potential for mitigating negative environmental effects of tropical livestock production systems.

Nevertheless, we recognise the need for new studies that provide a deeper, more specific, and more complete understanding of the BNI phenomenon in *M. maximus*.

Agronomic and forage nutrition evaluation. Average and maximum values of DMY in the *M. maximus* collection observed in the Patía valley in Colombia ranged from 5.09 to 7.82 t/ha (Table 2) and were higher than those recorded in Brazil (Macedo et al. 2017) and Thailand (Hare et al. 2015). Nevertheless, in Vietnam (Asia), there are reports of up to 12.8 t/ha with 42 days' regrowth (Van Man and Wiktorsson 2003). Values of CP, NDF, and ADF observed in several accessions of *M. maximus* were higher than those reported by Dele et al. (2017) in tropical agroecosystems and by Villegas et al. (2020) under greenhouse (controlled) conditions. The nutrition quality of forage diets influences ruminal functionality and digestibility. Therefore, understanding the bromatological profile of tropical grasses makes it easier to develop strategies that promote the reduction of enteric emissions (Rivera-Herrera et al. 2017) and increase milk/meat productivity by area unit in livestock systems (Sakita et al. 2020). The minimum protein requirement to keep a functional rumen in bovines, according to Gaviria et al. (2015), is 8% CP. In our study, the average CP observed in the collection of *M. maximus* was 10%, with top values reaching 11.3%.

The agronomic and nutritional response of *M. maximus* mentioned above makes consider the species as an alternative for dry tropics to face the uncertainties that climate change may affect in livestock regions with the presence of extreme periods of drought and rain (Ashworth et al. 2016).

In the evaluation of forages for animal production, it is important to integrate different agronomic parameters for adoption by farmers (Mwendia et al. 2019) with nutritional composition and N concentration dynamics in relation to biomass accumulation (Lemaire and Belanger 2020) to predict the performance of livestock systems and the contribution to the environment.

Forage grasses classified in cluster2 (Cl2) were associated with high CP content and N uptake, DMY greater than 6.5 t/ha, and better nutritional quality (IVDMD ≥ 58% and ADF ≤ 37.5%). *Megathyrsus* accessions within Cl3 standed out for their height, adequate DMY, and low nutritional quality, with both groups showing low flowering and lower NR than bare soils.

The accessions located in Cl1 had the lowest DMY production and height, adequate nutritional quality, and high NR, with the highest flowering percentage vis-à-vis other accessions located in the other two clusters.

The cluster classification suggested that the NR values recorded for the evaluated genotypes are normally in an inverse relation to N uptake and DMY. Therefore, it is possible to infer that accessions of *M. maximus* with high yield and superior N uptake had the greatest ability inhibiting soil nitrification (Figure 2, Table 3).

When comparing the response of NR in the collection of *M. maximus* under field and greenhouse conditions, similar accessions have been highlighted by their low NR (e.g., 688, 6299 Tobiata, and 6962 Mombasa), such as those in Cl2 and 3 in this investigation and Cl3 in the research by Villegas et al. (2020); therefore, it can be estimated that a large part of the response can be consistently generated by the genotype.

Forage germplasm with an adequate relation between agronomic and nutritional aspects, N uptake, and NR might contribute to the sustainable intensification of livestock production since variables such as forage quality, and biomass accumulation would allow an increase of animal stocking rates, harvesting times, or grazing and rotation in grasslands (Zhang et al.

Table 2. Descriptive statistics of agronomic and nutrtional characteristics of a collection of 27 accessions of *Megathyrsus maximus* in the dry tropics of Colombia

Variable	Mean	SD	Min	Max
Height (cm)	130.48	9.66	112.27	149.40
Flowering (%)	32.19	26.25	0	83.00
DMY (t/ha)	6.50	0.75	5.09	7.82
NDF (%)	66.41	1.20	64.03	68.29
ADF (%)	38.88	1.27	37.05	41.24
CP (%)	10.07	0.81	8.48	11.30
IVDDM (%)	58.65	1.61	55.29	61.00

DMY – dry matter yield; NDF – neutral detergent fiber; ADF – acid detergent fiber; CP – crude protein; IVDMD – *in vitro* dry matter digestibility

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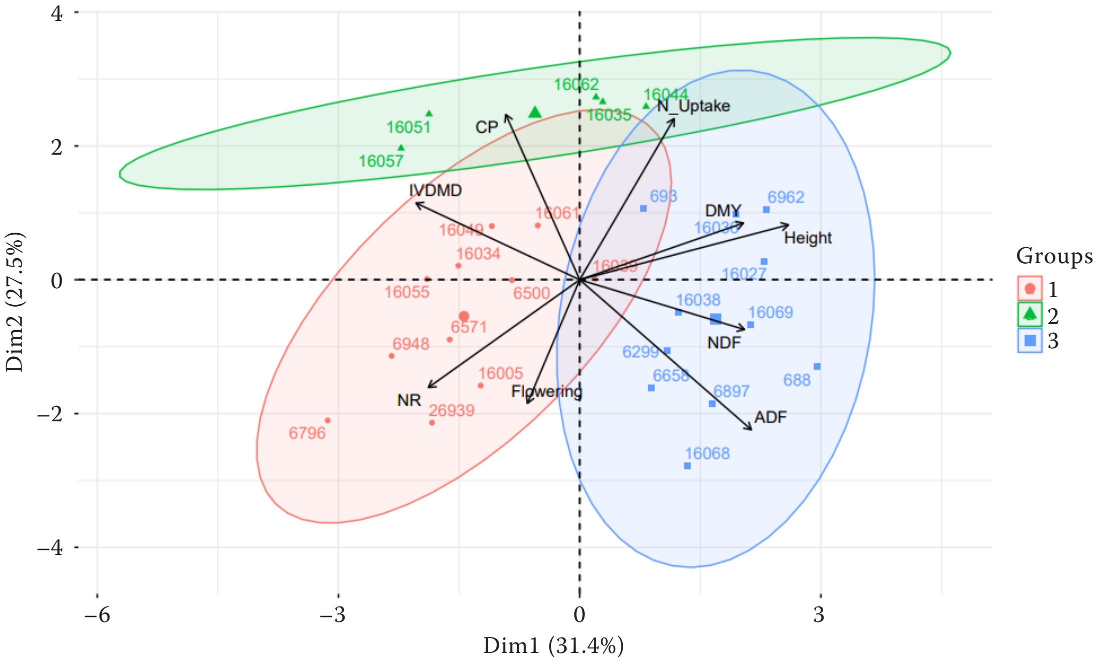

Figure 2. Cluster analysis based on principal components of the germplasm collection of *Megathyrsus maximus*. Cumulative variance accounts for 59%. NR – nitrification rate; CP – crude protein; IVDMD – *in vitro* dry matter digestibility; ADF – acid detergent fiber; NDF – neutral detergent fiber; DMY – dry matter yield

2018). These points are critical to support the land-sparing principle of releasing areas currently occupied by livestock for more sustainable practices such as conservation, reforestation, or agroforestry. This transition might count on external financial support to allow technology transfer networks that increase grasslands' productivity (Zu Ermgassen et al. 2018).

A comparison of NR and agronomic and nutritional characteristics suggests that *M. maximus* is a forage grass with superior dry matter yield and forage quality. However, it varies in the capacity of roots to interact with soil microbes and alter the NR (Pariasca et al. 2010, Villegas et al. 2020).

Nitrogen assimilation obtained along with the DMY of accessions within Cl2 and Cl3 possibly explain the low NR found and the efficiency that *M. maximus* can exhibit in the use of N-based fertilisers, which may contribute to a decrease in N_2O emissions (Abalos et al. 2018, Bowatte et al. 2018). Consequently, this observation strengthens the logic to intensify efforts to find germplasm accessions or develop through breeding forage species for the eco-efficient production of high-quality animal-sourced products. Although N uptake by plants is an important factor that influences the amount of N_2O emissions, several researchers report that other factors are also

Table 3. Characteristics of each of the clusters of accessions of *Megathyrsus maximus*

Cluster	Number of accessions	DMY (t/ha)	Height (cm)	N uptake (kg N/ha)	NR (mg NO_3-N/kg soil/day)	CP	IVDMD	NDF	ADF	Flowering
						(%)				
1	11	5.8 ± 0.3^{a}	124.7 ± 6.9^{a}	93.9 ± 6.3^{a}	1.0 ± 0.3	10.0 ± 0.6^{ab}	59.4 ± 0.5^{a}	66.1 ± 0.8^{ab}	38.4 ± 0.6^{a}	36.6 ± 21.4
2	5	7.1 ± 0.4^{b}	127.1 ± 6.5^{a}	124.2 ± 8^{b}	0.7 ± 0.1	10.9 ± 0.4^{a}	60.0 ± 1.1^{a}	65.5 ± 1.4^{a}	37.3 ± 0.1^{b}	19.1 ± 26.7
3	11	6.8 ± 0.6^{b}	137.6 ± 8.8^{b}	105.9 ± 8.9^{c}	0.7 ± 0.2	9.6 ± 0.7^{b}	57.2 ± 1.4^{b}	67 ± 1.0^{b}	40 ± 0.9^{c}	33.6 ± 30.6

Values are mean ± standard deviation. DMY – dry matter yield; NR – nitrification rate; CP – crude protein; IVDMD – *in vitro* dry matter digestibility; NDF – neutral detergent fiber; ADF – acid detergent fiber. Different letters in one column denote statistical differences according to the Tukey *HSD* (honestly significant difference) test ($\alpha = 0.05$)

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influential, such as soil characteristics, environment (rainfall and temperature), and forage management (Bowatte et al. 2018).

At a general level, we observed lower NR, high assimilation of N, and adequate agronomic and nutritional values in some of the 27 accessions of *M. maximus* evaluated under farmer field conditions in the Patía valley in Cauca, Colombia.

Altogether, this observation allows us to highlight the potential of this grass for climate-smart livestock production systems since there are various genotypes that stand out for their agronomic, nutritional, or environmental expression. At a broader scale, this type of study provides elements to select productive fodder material with efficient use of nitrogen-based fertilisers, and at the same time, may contribute to decreasing N_2O emissions to the atmosphere and N losses from leaching.

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