

Article

Nitrogen Requirement of a Tropical Grass Hybrid Determined by Model Identity Analysis

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Highlights

What are the main findings?

- Model identity analysis successfully distinguished tropical forage grasses with different nitrogen requirements.
- The Mulato II grass exhibited a nitrogen response pattern more similar to *Urochloa brizantha* than to *Urochloa decumbens*.

What are the implications of the main findings?

- Model identity analysis provides a rapid and objective approach for generating preliminary nutrient recommendations for newly released forage cultivars.
- This approach may substantially reduce the time and resources required to develop preliminary fertilization recommendations before long-term field validation.

Abstract

Methods capable of inferring the nutritional requirements of forage cultivars from related genotypes may help generate preliminary fertilization recommendations when experimental information is limited. This study evaluated whether model identity analysis can be used to infer the nitrogen requirement of Mulato II hybrid grass from the response patterns of its progenitor species, *Urochloa brizantha* cv. Marandu and *Urochloa decumbens* cv. Basilisk. A greenhouse experiment using pots filled with an Oxisol was conducted using a completely randomized design in a 3×5 factorial arrangement, with three grasses and five nitrogen doses (0, 100, 200, 300, and 400 mg dm⁻³). Forage production, tiller density, and nutritive value were evaluated during establishment and regrowth phases. Linear and quadratic regressions were fitted, and significant models were compared using model identity tests. When model differences were detected, intercepts and parameters were further compared using F-tests. In both establishment and regrowth phases, Mulato II showed response patterns similar to *Urochloa brizantha* for most evaluated variables, with model identity observed except for tiller number and crude protein concentration. In contrast, Mulato II differed from *Urochloa decumbens* for all measured traits. Therefore, under the greenhouse conditions evaluated, the nitrogen requirement of Mulato II is more closely related to that of *Urochloa brizantha*, demonstrating the potential of model identity analysis



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as a tool for generating preliminary fertilization recommendations for forage cultivars from related genotypes before field validation.

Keywords: fertilization recommendation; genotype comparison; nitrogen fertilization; *Urochloa* spp.

1. Introduction

Nitrogen is the nutrient that most strongly influences forage production in tropical grasses because it stimulates leaf elongation, tillering, canopy development, and forage accumulation, resulting in greater pasture productivity and improved soil cover [1–3]. Adequate nitrogen fertilization is therefore essential to sustain forage production and prevent pasture degradation, particularly because nitrogen is one of the nutrients most intensively removed from pasture systems [4,5]. In addition to increasing forage production, appropriate nitrogen management can improve fertilizer use efficiency and reduce nutrient losses through volatilization and leaching [6–8].

Despite its importance, defining appropriate nitrogen fertilization rates remains challenging because plant responses are influenced by genotype, environmental conditions, soil fertility, water availability, management practices, and interactions with other nutrients [9,10]. Consequently, establishing nitrogen requirements requires extensive experimentation across different conditions, demanding substantial time and financial investment. In addition, the limited understanding of how soil and climatic factors affect nitrogen mineralization, immobilization, and plant nitrogen demand still restricts the development of optimal fertilization strategies and robust management recommendations for tropical forage systems [10].

This challenge becomes even greater when new forage cultivars are released. In many cases, specific fertilization recommendations are unavailable because the studies required to determine nutrient requirements have not yet been completed. The situation is particularly complex for hybrid cultivars, whose responses may differ from those of their parental species due to the combination of distinct genetic backgrounds [11,12].

Methods capable of inferring the nutritional requirements of forage cultivars from related genotypes may help generate preliminary fertilization recommendations when experimental information is limited. Among these methods, model identity analysis provides a statistical framework for comparing regression equations and determining whether different genotypes exhibit similar responses to a given factor [13–15]. When response models are statistically identical, management recommendations established for one genotype may potentially be extended to another.

Model identity analysis has been successfully applied in forestry, animal science, and agricultural research to compare response curves, reduce the number of equations required to represent biological processes, and identify similarities among models [15–19]. Such applications are particularly relevant to forage science because the response of tropical grasses to nitrogen fertilization is strongly influenced by genetic and management factors, resulting in considerable variation in forage production and nutritive value among genotypes and environmental conditions [20]. However, the application of model identity analysis in forage science remains limited. Evidence from a previous study suggests that this approach may be useful for comparing fertility responses among tropical forage cultivars and for inferring nutrient requirements when direct information is unavailable [11].

Model identity analysis may be particularly useful for newly released forage cultivars and hybrid grasses whose nutritional requirements have not yet been established. This

approach is especially relevant when hybrids are derived from parental species with contrasting fertility requirements, as similarities in their response patterns may provide a basis for preliminary fertilization recommendations.

Mulato II is a tropical forage hybrid developed from *Urochloa brizantha* and *Urochloa decumbens*. These are two of the most widely cultivated tropical forage species because of their high forage production, persistence, and adaptation to grazing systems [21]. However, they differ in their adaptation to soil fertility and responsiveness to nitrogen fertilization, with *U. brizantha* generally requiring higher soil fertility than *U. decumbens* [22].

Therefore, identifying which parental species exhibits a response pattern most similar to that of Mulato II may provide a basis for inferring its nitrogen requirement. We hypothesized that the nitrogen response of Mulato II would be statistically similar to that of one of its progenitor species, allowing its nutritional requirement to be inferred through model identity analysis. The objective of this study was to determine whether the nitrogen requirement of Mulato II hybrid grass is more similar to that of *Urochloa brizantha* or *Urochloa decumbens* using model identity analysis.

2. Materials and Methods

2.1. Experimental Site and Design

The experiment was conducted over 102 days, from 5 January to 17 April 2011, in a greenhouse at the Federal University of Rondonópolis, Brazil, using a completely randomized design in a 3×5 factorial arrangement comprising three forage grasses and five nitrogen rates, totaling 15 treatments with four replications. The grasses evaluated were *Urochloa brizantha* cv. Marandu, *Urochloa decumbens* cv. Basilisk, and Mulato II (*Urochloa ruziziensis* $\times$ *Urochloa decumbens* $\times$ *Urochloa brizantha*). Nitrogen was supplied at rates of 0, 100, 200, 300, and 400 mg dm⁻³.

2.2. Soil Preparation and Crop Establishment

Experimental units consisted of 5-dm³ pots containing five plants each. The soil was classified as a medium-textured Oxisol (Table 1), collected from the 0–20 cm layer under native Cerrado vegetation, and subjected to initial chemical and particle-size analyses [23] before liming and establishment fertilization to determine soil amendment and fertilizer requirements.

Table 1. Particle size and chemical characterization of the Oxisol before liming and establishment fertilization.

pH	P	K	Ca	Mg	Al	H	CEC	V	m	O.M.	Sand	Silt	Clay
CaCl ₂	mg dm ³				cmolc dm ³			%			g kg ⁻¹		
4.7	2.4	28	0.3	0.2	1.1	4.2	5.9	9.8	66.3	22.7	740	105	155

CEC: cation exchange capacity; O.M.: organic matter; V: base saturation; m: aluminum saturation.

After collection, the soil was sieved through a 4 mm mesh and placed in the pots. Base saturation was increased to 50% using dolomitic limestone (RTNP = 86%), according to the recommendations for tropical forage grasses [24,25], providing an adequate fertility condition for both *Urochloa brizantha* and *Urochloa decumbens*. This was followed by a 30-day incubation period under moisture conditions close to maximum water retention capacity. Briefly, pots were filled with air-dried soil, weighed, and placed in trays with water up to two-thirds their height. Soil became saturated by capillary action to remove oxygen. After saturation, pots were drained, weighed again, and the water retention capacity was calculated from the weight difference.

At the end of the incubation period, establishment fertilization was applied with phosphorus, potassium, and sulfur at rates of 200, 150, and 40 mg dm⁻³, respectively. Micronutrients were supplied using boric acid, copper chloride, zinc chloride, and sodium molybdate at rates of 1.5, 2.5, 2.0, and 0.25 mg dm⁻³, respectively [26]. All fertilizers were applied in solution.

Seeds were initially sown in trays containing washed sand. Seven days after emergence, ten seedlings were transplanted into each pot. Five days later, plants were thinned to five seedlings per pot, and nitrogen treatments were applied using urea as the nitrogen source. Nitrogen was applied as an aqueous urea solution to facilitate its incorporation into the soil, thereby reducing the potential for ammonia volatilization. During the establishment phase, nitrogen fertilization was split into five equal applications at 3-day intervals after transplanting to avoid excessive osmotic effects [27] and ensure a gradual nitrogen supply throughout plant establishment.

During the incubation period, soil moisture was maintained at 60% of maximum water retention capacity using the gravimetric method. After transplanting, soil moisture was monitored daily using tensiometers, and pots were irrigated whenever necessary to maintain soil moisture close to the maximum water retention capacity throughout the experimental period.

2.3. Measurements and Sampling

Thirty days after thinning, the first measurements were performed. Chlorophyll index was assessed on the middle portion of three recently fully expanded leaves using a chlorophyll meter (ClorofiLOG/CFL 1030; Falker, Porto Alegre, Brazil). Tillers and leaves were manually counted in each pot, and then the plants were harvested at 10 cm above the soil surface and placed in paper bags. For *Urochloa decumbens*, plants were positioned vertically because of their prostrate growth habit. After harvest, the forage mass was dried in a forced-air oven at 55 °C for 72 h and weighed. The number of leaves per tiller was estimated as the ratio between the number of leaves and the number of tillers. Leaf appearance rate was calculated by dividing the number of leaves per tiller by the regrowth interval between harvests. The inverse of leaf appearance rate was defined as the phyllochron, representing the time interval (days) required for the appearance of successive leaves. This harvest was considered the establishment phase.

Following the first harvest, nitrogen treatments were reapplied twice at 30-day intervals. Potassium was also supplied after each harvest at 200 mg dm⁻³ as potassium chloride. The same measurements were performed during both regrowth cycles. The mean of the two regrowth evaluations was considered the regrowth phase. Dried forage samples were ground using a knife mill (Wiley type) and analyzed for nitrogen concentration [28].

At the final harvest, roots were collected, washed under running water on 4 mm mesh sieves, dried at 55 °C for 72 h, and weighed. Root biomass was evaluated only at the final harvest because root sampling during the establishment phase would have destroyed the experimental units and prevented the subsequent regrowth evaluations.

2.4. Statistical Analysis

Data from the establishment and regrowth phases were analyzed separately. Linear and quadratic regression models were fitted as a function of nitrogen rate for each forage grass, and the model providing the best fit and biological interpretation was selected. For significant quadratic regressions, the nitrogen rate associated with the maximum or minimum response was estimated from the first derivative of the regression equation, whereas for significant linear responses, the highest nitrogen rate evaluated (400 mg dm⁻³) was considered the optimum value within the experimental range.

Whenever significant regression responses were detected, model identity analysis was performed according to the methodology proposed by Regazzi [15,29]. The procedure consisted of comparing complete and reduced regression models through the likelihood ratio test to determine whether a common equation could adequately describe the responses of different grasses to nitrogen fertilization.

Model identity tests were performed for all pairwise comparisons among grasses (*Urochloa brizantha* × *Urochloa decumbens*, *Urochloa brizantha* × Mulato II, and *Urochloa decumbens* × Mulato II). The comparison between *Urochloa brizantha* and *Urochloa decumbens* was included as a biological reference because these species differ in their adaptation to soil fertility conditions and nitrogen requirements. Thus, the ability of the model identity test to distinguish between these contrasting grasses provided additional support for its application in evaluating the nitrogen requirement of Mulato II.

When model identity was confirmed, a single equation was considered sufficient to represent the response of both grasses. When the null hypothesis was rejected, indicating a lack of model identity, additional tests were conducted to compare intercepts and regression coefficients in order to identify the source of divergence between equations. All statistical analyses were performed at the 5% significance level.

Principal component analysis (PCA) was performed separately for the establishment and regrowth phases using the correlation matrix of forage mass, leaf number, tiller number, chlorophyll index, phyllochron, and nitrogen concentration. Variables were standardized, and the first two principal components were used for graphical interpretation. Confidence ellipses (95%) were added to visualize group separation and similarity among grasses. All statistical analyses were performed using R software (version 4.6.0; R Foundation for Statistical Computing, Vienna, Austria) [30].

3. Results

3.1. Establishment Phase

The fitted regression curves (Figure 1) visually illustrate the response patterns that were subsequently evaluated by model identity analysis. All evaluated variables were described by quadratic regression models ($p < 0.05$), except tiller number in *Urochloa brizantha*, which exhibited a linear response to nitrogen fertilization (Table 2). Maximum forage mass, leaf number, and tiller number were generally obtained at nitrogen rates between 200 and 300 mg dm⁻³, whereas maximum chlorophyll index occurred between 253 and 355 mg dm⁻³, depending on the grass species (Table 2). The lowest coefficients of determination (R^2) were observed for forage mass and leaf number in *Urochloa decumbens* and for phyllochron in *Urochloa brizantha* (Table 2).

Table 2. Means, linear (L) and quadratic (Q) responses, regression models, and nitrogen rates associated with maximum or minimum predicted responses for forage mass, leaf number, tiller number, chlorophyll index, phyllochron, and nitrogen concentration of *Urochloa brizantha* (Briz), *Urochloa decumbens* (Dec), and Mulato II (Mul) grasses subjected to nitrogen fertilization during the establishment phase.

Grass	Nitrogen (mg dm ⁻³)					<i>p</i> -Value		Regression Model (R ²)	Dose * (mg dm ⁻³)
	0	100	200	300	400	L	Q		
Forage mass (g pot ⁻¹)									
Briz	7.8	9.9	11.0	12.2	9.1	0.112	0.007	y = 7.5 + 0.03397N – 0.000073N ² (0.86)	234
Dec	9.9	14.0	18.0	12.1	12.1	0.414	<0.001	y = 10.1 + 0.05391N – 0.000128N ² (0.65)	209
Mul	7.6	11.9	13.1	12.3	11.0	0.021	0.001	y = 7.8 + 0.04509N – 0.000094N ² (0.96)	239

Table 2. Cont.

Grass	Nitrogen (mg dm ⁻³)					<i>p</i> -Value		Regression Model (R ²)	Dose * (mg dm ⁻³)
	0	100	200	300	400	L	Q		
Leaves (number pot ⁻¹)									
Briz	43	56	68	66	56	0.033	0.006	y = 41.5 + 0.20752N – 0.000424N ² (0.98)	242
Dec	65	98	138	97	112	<0.001	<0.001	y = 66.5 + 0.43014N – 0.000843N ² (0.65)	255
Mul	40	68	73	71	63	0.007	<0.001	y = 42.0 + 0.27136N – 0.000557N ² (0.95)	243
Tillers (number pot ⁻¹)									
Briz	17	19	23	27	21	0.021	0.075	y = 18.3 + 0.015250N (0.47)	400
Dec	21	43	55	47	43	<0.001	<0.001	y = 21.5 + 0.25439N – 0.000505N ² (0.94)	247
Mul	18	25	28	29	26	0.003	0.007	y = 17.9 + 0.08143N – 0.000154N ² (0.99)	264
Chlorophyll index									
Briz	33.6	39.8	42.1	47.2	45.1	<0.001	<0.001	y = 33.4 + 0.07564N – 0.000113N ² (0.98)	354
Dec	35.7	43.8	48.1	46.9	44.3	<0.001	<0.001	y = 35.8 + 0.09719N – 0.000192N ² (0.99)	253
Mul	37.7	45.2	47.2	50.5	50.4	<0.001	0.001	y = 38.0 + 0.07049N – 0.000099N ² (0.98)	355
Phyllochron (days leaf ⁻¹)									
Briz	12.1	10.3	10.0	12.0	11.5	0.808	0.016	y = 11.8 – 0.01363N + 0.000035N ² (0.47)	193
Dec	9.5	13.1	12.1	14.7	11.9	<0.001	<0.001	y = 9.7 + 0.03276N – 0.000066N ² (0.70)	249
Mul	13.5	10.7	11.5	12.2	12.2	0.507	0.007	y = 13.0 – 0.01681N + 0.000039N ² (0.54)	214
Nitrogen concentration (g kg ⁻¹)									
Briz	12.6	21.4	23.7	24.5	24.0	<0.001	<0.001	y = 21.1 + 0.13347x – 0.000230x ² (0.97)	290
Dec	10.6	17.2	20.4	21.1	23.2	<0.001	<0.001	y = 17.7 + 0.09916x – 0.000132x ² (0.97)	377
Mul	12.1	21.7	24.9	25.7	26.2	<0.001	<0.001	y = 20.4 + 0.14591x – 0.000236x ² (0.97)	309

*: nitrogen rate associated with the maximum or minimum predicted response.

Model identity analysis revealed significant differences between *Urochloa brizantha* and *Urochloa decumbens* for all evaluated traits (Table 3). The only similarities detected were the regression parameters for leaf number and the intercepts of the chlorophyll index equations.

Table 3. Model identity, intercept, and parameter tests for forage mass, root mass, leaf number, tiller number, chlorophyll index, phyllochron, and nitrogen concentration of *Urochloa brizantha*, *Urochloa decumbens*, and Mulato II grasses subjected to nitrogen fertilization during the establishment phase.

Identity Test	Forage Mass	Leaves	Tiller	Chlorophyll Index	Phyllochron	Nitrogen
<i>Urochloa brizantha</i> × <i>Urochloa decumbens</i>						
Model	0.0005	<0.0001	<0.0001	0.0021	0.0001	<0.0001
Intercept	<0.0001	<0.0001	<0.0001	0.0879	0.0001	<0.0001
Parameter	0.0002	0.1139	<0.0001	0.0188	<0.0001	0.0033
Mulato II × <i>Urochloa brizantha</i>						
Model	0.1450	0.1612	0.0086	<0.0001	0.1745	0.0123
Intercept	-	-	<0.0001	0.0021	-	<0.0001
Parameter	-	-	<0.0001	0.9335	-	0.0590
Mulato II × <i>Urochloa decumbens</i>						
Model	0.0488	<0.0001	<0.0001	0.0001	0.0004	<0.0001
Intercept	0.0021	0.0038	0.0056	0.1016	0.0008	<0.0001
Parameter	0.0071	0.0477	0.0002	0.0059	<0.0001	0.0291

Mulato II exhibited model identity with *Urochloa brizantha* for forage mass, leaf number, phyllochron, and nitrogen concentration (Table 3). The lack of identity for tiller number was associated with the linear response observed in *Urochloa brizantha* and the quadratic response observed in Mulato II. For chlorophyll index, model identity was rejected; however, regression parameters were similar, with differences occurring only in the intercepts (Table 3).

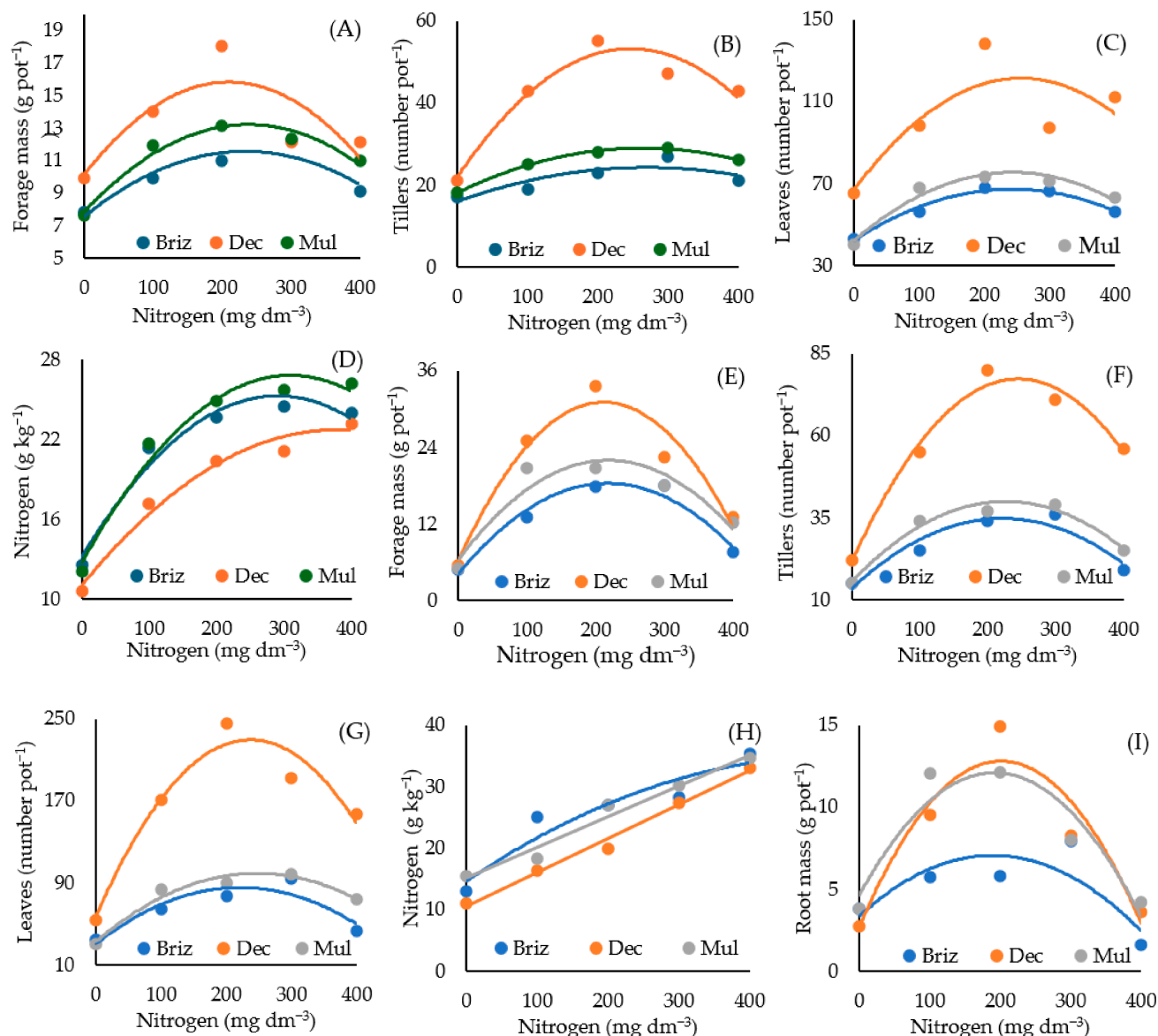


Figure 1. Regression curves for forage mass (A,E), tiller number (B,F), leaf number (C,G), nitrogen concentration in forage (D,H), and root mass (I) of *Urochloa brizantha* (Briz), *Urochloa decumbens* (Dec), and Mulato II (Mul) during establishment (A–D) and regrowth (E–H), with root mass evaluated at the final harvest (I).

Model identity analysis revealed significant differences between *Urochloa brizantha* and *Urochloa decumbens* for all evaluated traits (Table 3). Among the individual model components, similarity was observed only for the regression parameters of leaf number and the intercept of the chlorophyll index equation. Similarly, Mulato II differed from *Urochloa decumbens* for all evaluated variables (Table 3). For forage mass, leaf number, tiller number, and nitrogen concentration, Mulato II presented lower intercept values than *Urochloa decumbens*, whereas a higher intercept was observed for phyllochron. The only similarity detected between Mulato II and *Urochloa decumbens* was the intercept of the chlorophyll index equation.

In the establishment phase, the first two principal components explained 81.5% of the total variation, with PC1 and PC2 accounting for 59.3% and 22.2%, respectively (Figure 2). Forage mass, leaf number, tiller number, chlorophyll index, and nitrogen concentration were positively associated with PC1, whereas phyllochron was negatively associated with

this axis. *Urochloa decumbens* was separated from the other grasses along PC1, while Mulato II and *Urochloa brizantha* showed substantial overlap of their confidence ellipses (Figure 2).

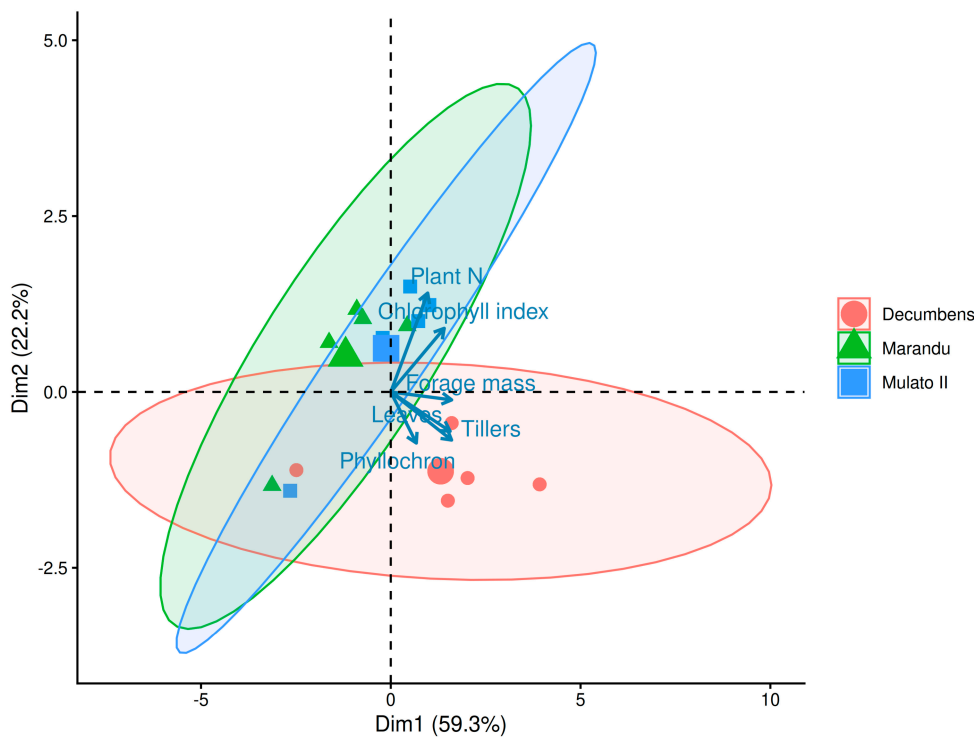


Figure 2. Principal component analysis (PCA) biplot of *Urochloa brizantha*, *Urochloa decumbens*, and Mulato II grasses subjected to nitrogen fertilization during the establishment phase. The first two principal components explained 81.5% of the total variation (PC1 = 59.3%; PC2 = 22.2%). Ellipses represent the 95% confidence interval for each grass.

3.2. Regrowth Phase

During regrowth, all variables were described by quadratic regression models, except nitrogen concentration in *Urochloa decumbens* and Mulato II, which exhibited linear responses, and phyllochron in *Urochloa brizantha*, for which no significant regression model was detected, resulting in an average value of 8.4 days leaf^{−1} (Table 4).

Table 4. Means, linear (L) and quadratic (Q) responses, regression models, and nitrogen rates associated with maximum or minimum predicted responses for forage mass, root mass, leaf number, tiller number, chlorophyll index, phyllochron, and nitrogen concentration of *Urochloa brizantha* (Briz), *Urochloa decumbens* (Dec), and Mulato II (Mul) grasses subjected to nitrogen fertilization during the regrowth phase.

Grass	Nitrogen (mg dm ⁻³)					<i>p</i> -Value		Regression Model (R ²)	Dose * (mg dm ⁻³)
	0	100	200	300	400	L	Q		
Forage mass (g pot ⁻¹)									
Briz	4.90	13.06	17.90	17.98	7.52	0.172	<0.001	y = 4.2 + 0.13014N – 0.000300N ² (R ² = 0.96)	216
Dec	5.53	25.09	33.49	22.48	13.10	0.093	<0.001	y = 6.3 + 0.23338N – 0.000552N ² (R ² = 0.94)	211
Mul	5.00	20.71	20.75	17.95	12.20	0.119	<0.001	y = 6.4 + 0.14238N – 0.000327N ² (R ² = 0.90)	212
Root mass (g pot ⁻¹)									
Briz	3.79	5.75	5.79	7.91	1.57	0.725	0.046	y = 3.3 + 0.03920N – 0.000104N ² (R ² = 0.68)	188
Dec	2.72	9.50	14.94	8.25	3.62	0.932	<0.001	y = 2.7 + 0.10040N – 0.000250N ² (R ² = 0.90)	201
Mul	3.79	12.06	12.12	7.98	4.18	0.610	0.001	y = 4.6 + 0.07769N – 0.000202N ² (R ² = 0.89)	192

Table 4. Cont.

Grass	Nitrogen (mg dm ⁻³)					<i>p</i> -Value		Regression Model (R ²)	Dose * (mg dm ⁻³)
	0	100	200	300	400	L	Q		
Leaves (number pot ⁻¹)									
Briz	34	64	77	94	43	0.214	0.001	y = 29.9 + 0.50060N – 0.001129N ² (R ² = 0.84)	222
Dec	54	171	245	192	157	<0.001	<0.001	y = 56.9 + 1.45829N – 0.003077N ² (R ² = 0.94)	237
Mul	30	83	90	98	74	0.012	0.002	y = 32.8 + 0.53568N – 0.001083N ² (R ² = 0.96)	247
Tillers (number pot ⁻¹)									
Briz	15	25	34	36	19	0.161	<0.001	y = –2.9 + 0.24714N – 0.000643N ² (R ² = 0.97)	192
Dec	22	55	80	71	56	<0.001	<0.001	y = –2.0 + 0.33639N – 0.000705N ² (R ² = 0.95)	239
Mul	15	34	37	39	25	0.001	0.001	y = –1.2 + 0.21839N – 0.000505N ² (R ² = 0.82)	216
Chlorophyll index									
Briz	28.5	42.7	45.5	49.4	46.0	<0.001	<0.001	y = 29.2 + 0.13924N – 0.000244N ² (R ² = 0.97)	285
Dec	26.4	39.2	43.5	44.7	42.6	<0.001	<0.001	y = 27.0 + 0.13184N – 0.000235N ² (R ² = 0.99)	281
Mul	28.4	44.2	46.0	46.9	48.8	<0.001	<0.001	y = 29.6 + 0.13694N – 0.000226N ² (R ² = 0.95)	303
Phyllochron (days leaf ⁻¹)									
Briz	8.9	7.9	8.9	7.7	8.5	0.320	0.249	-	-
Dec	8.2	6.5	6.5	7.5	7.2	0.300	0.006	y = 7.9 – 0.01190N + 0.0000267N ² (R ² = 0.52)	223
Mul	9.9	8.2	8.2	8.2	9.3	0.308	<0.001	y = 9.7 – 0.01638N + 0.0000410N ² (R ² = 0.94)	200
Nitrogen concentration (g kg ⁻¹)									
Briz	13.0	25.1	26.9	28.2	35.4	<0.001	0.003	y = 14.6 + 0.07792N – 0.000075N ² (R ² = 0.90)	519 **
Dec	11.0	16.4	19.8	27.3	33.1	<0.001	0.160	y = 10.5 + 0.05508N (R ² = 0.99)	400
Mul	15.5	18.3	26.9	30.1	34.6	<0.001	0.591	y = 15.0 + 0.05003N (R ² = 0.98)	400

*: nitrogen rate associated with the maximum or minimum predicted response; **: estimated optimum outside the range of nitrogen rates evaluated.

Maximum forage mass, leaf number, and tiller number were obtained at nitrogen rates ranging from approximately 190 to 250 mg dm⁻³, while maximum root mass occurred between 188 and 201 mg dm⁻³ (Table 4). The lowest coefficients of determination were observed for root mass in *Urochloa brizantha* and phyllochron in *Urochloa decumbens* (Table 4).

Model identity analysis revealed significant differences between *Urochloa brizantha* and *Urochloa decumbens* for all evaluated variables (Table 5). Similarity was observed only for the regression parameters of the chlorophyll index and root mass. Mulato II differed from *Urochloa decumbens* for all shoot-related traits evaluated (Table 5). However, root mass exhibited model identity between these grasses, indicating a similar root response pattern to nitrogen fertilization. For forage mass, leaf number, tiller number, and nitrogen concentration, Mulato II showed lower intercept values than *Urochloa decumbens*, whereas phyllochron presented a higher intercept.

Table 5. Model identity, intercept, and parameter tests for forage mass, root mass, leaf number, tiller number, chlorophyll index, phyllochron, and nitrogen concentration of *Urochloa brizantha*, *Urochloa decumbens*, and Mulato II grasses subjected to nitrogen fertilization during the regrowth phase.

Identity Test	Forage Mass	Root Mass	Leaves	Tiller	Chlorophyll	Phyllochron	Nitrogen
<i>Urochloa brizantha</i> × <i>Urochloa decumbens</i>							
Model	<0.0001	0.0243	<0.0001	<0.0001	0.0008	<0.0001	<0.0001
Intercept	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Parameter	0.0270	0.1157	0.0004	0.0002	0.7352	0.0003	0.0007
Mulato II × <i>Urochloa brizantha</i>							
Model	0.1932	0.0852	0.0621	0.0049	0.4728	0.1755	0.0122
Intercept	-	-	-	<0.0001	-	-	<0.0001
Parameter	-	-	-	0.1050	-	-	0.0590
Mulato II × <i>Urochloa decumbens</i>							
Model	0.0488	0.9185	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Intercept	0.0021	-	0.0022	0.0003	<0.0001	<0.0001	<0.0001
Parameter	0.0071	-	0.0012	0.0011	0.1609	0.6517	0.0291

Mulato II exhibited model identity with *Urochloa brizantha* for forage mass, root mass, leaf number, chlorophyll index, and phyllochron (Table 5). Differences were detected only for tiller number and nitrogen concentration, and in both cases the divergence was associated exclusively with differences in intercepts, whereas regression parameters remained similar.

During the regrowth phase, the first two principal components explained 91.0% of the total variation, with PC1 accounting for 64.4% and PC2 for 26.6% (Figure 3). As observed during establishment, forage mass, tiller number, chlorophyll index, crude protein concentration, and nitrogen concentration were positively associated with PC1, whereas phyllochron exhibited an opposite relationship. *Urochloa decumbens* remained clearly separated from the other grasses, while Mulato II and *Urochloa brizantha* continued to exhibit substantial overlap.

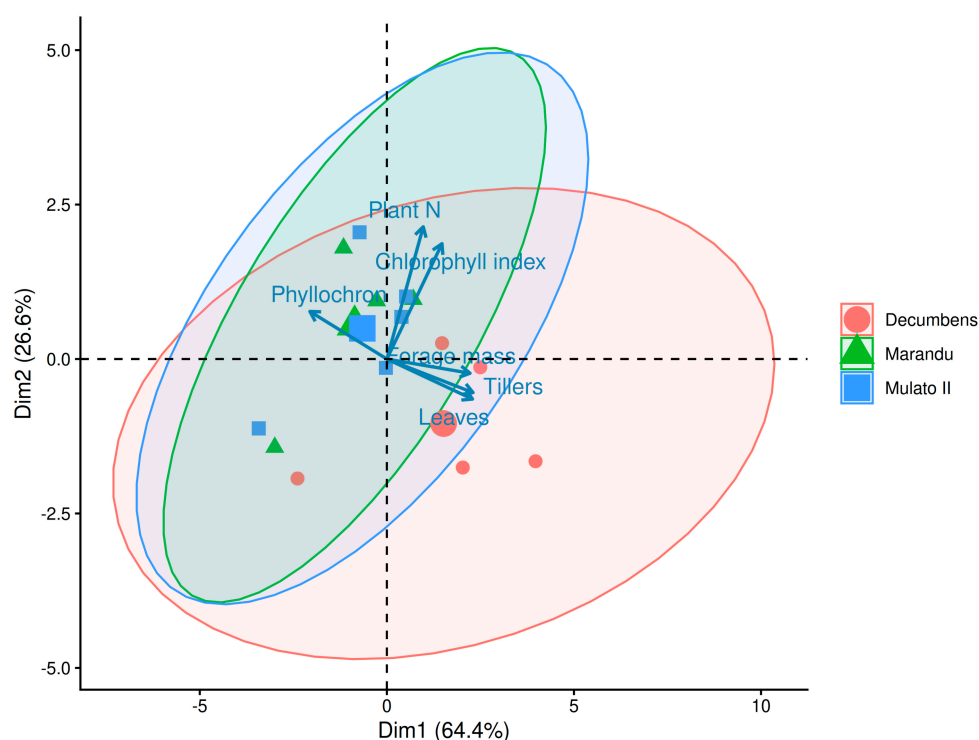


Figure 3. Principal component analysis (PCA) biplot of forage mass, root mass, leaf number, tiller number, chlorophyll index, phyllochron, and nitrogen concentration in *Urochloa brizantha*, *Urochloa decumbens*, and Mulato II grasses subjected to nitrogen fertilization during the regrowth phase. The first two principal components explained 91.0% of the total variation (PC1 = 64.4%; PC2 = 26.6%). Confidence ellipses represent the 95% confidence interval for each grass.

4. Discussion

The clear differences observed between *Urochloa brizantha* and *Urochloa decumbens* during both establishment and regrowth support the ability of model identity analysis to distinguish grasses with contrasting nitrogen responses. These species are widely recognized as differing in their adaptation to soil fertility, nutrient use efficiency, and responsiveness to fertilization [31]. Therefore, the rejection of model identity between these grasses demonstrates that the method was sufficiently sensitive to detect biologically meaningful differences in nutritional requirements. This conclusion was independently supported by PCA (Figures 2 and 3), which consistently separated *Urochloa decumbens* from the other grasses in both growth phases.

The similarity observed between Mulato II and *Urochloa brizantha* for most productive, morphogenic, and nutritional traits indicates that the nitrogen requirement of the

hybrid is more closely associated with *Urochloa brizantha* than with *Urochloa decumbens*. This result is consistent with the breeding history of Mulato II, which originated from interspecific hybridization involving *Urochloa ruziziensis*, *Urochloa decumbens*, and *Urochloa brizantha* [31,32]. Previous studies have classified Mulato II as a grass of intermediate to high productive potential and moderate fertility requirement [33], which agrees with the response patterns observed in the present study. The overlap between Mulato II and *Urochloa brizantha* in the PCA further reinforces this similarity (Figures 2 and 3), suggesting that both grasses share comparable responses to nitrogen fertilization and, consequently, similar responses to nitrogen fertilization, suggesting comparable nitrogen requirements under the evaluated experimental conditions (Figure 1).

In contrast, Mulato II differed from *Urochloa decumbens* for most evaluated traits during both establishment and regrowth. The lower intercepts observed for forage mass, leaf number, tiller number, and nitrogen concentration indicate reduced performance of the hybrid under nitrogen deficiency. This result suggests a greater dependence on nitrogen fertilization to express its productive potential. Under low nitrogen availability, reduced tillering may compromise canopy density, leaf area development, and pasture recovery after defoliation, ultimately increasing the risk of pasture degradation [1–4].

However, greater nitrogen dependence should not be interpreted as a requirement for excessive fertilizer inputs. Studies conducted under grazing conditions have shown that increasing nitrogen rates does not necessarily result in proportional gains in forage production and may even reduce nitrogen use efficiency [34,35]. Therefore, nitrogen fertilization strategies should be adjusted according to the nutritional requirements of each forage species and soil nutrient supply in order to maximize productivity while minimizing nutrient losses and environmental impacts. Such an approach is particularly important for cultivars with greater nitrogen responsiveness, such as Mulato II, because adequate fertilization is required to sustain productivity and pasture persistence without compromising resource-use efficiency [36].

Therefore, nitrogen fertilization is decisive not only for sustaining pasture productivity but also for maintaining pasture persistence and reducing the risk of degradation over time [37,38]. Furthermore, long-term evidence has shown that increased nitrogen fertilization can enhance forage production and support higher stocking rates without causing significant deterioration of soil physical quality, thereby enabling sustainable grazing intensification [39]. Consequently, although Mulato II may offer greater productive potential than *Urochloa decumbens*, its successful use is likely to depend on more intensive fertility management.

Root mass exhibited a distinct response pattern. Although model identity was observed with both parental species for root mass, the evidence supporting identity was stronger for the comparison with *Urochloa decumbens* ($p = 0.9185$) than for *Urochloa brizantha* ($p = 0.0852$). This result suggests that root responses of Mulato II may be more closely associated with *Urochloa decumbens*, as illustrated by the fitted regression curves (Figure 1), although this interpretation should be viewed cautiously because both comparisons satisfied the model identity criterion at the adopted significance level.

This finding may be agronomically relevant because *Urochloa decumbens* is commonly associated with a vigorous root system and adaptation to stressful environments [40]. Greater root biomass has been associated with improved water and nutrient acquisition, reserve accumulation, and greater tolerance to drought and grazing stress [41–43]. Accordingly, the results suggest that Mulato II may combine shoot responses more similar to *Urochloa brizantha* with a root biomass response more similar to *Urochloa decumbens*. However, because only root dry mass was evaluated, this inference is limited to root biomass and should not be extrapolated to root morphology or other functional root traits.

The agreement between PCA and model identity analysis strengthens the reliability of the conclusions. While PCA efficiently summarized multivariate relationships and visually demonstrated the clustering pattern among grasses, it does not quantify whether response equations are statistically equivalent. In contrast, model identity analysis directly evaluates similarities among regression models through comparisons of intercepts and parameters. Thus, PCA should be viewed as a complementary exploratory tool, whereas model identity analysis provides the statistical basis for inferring similarities in nutritional requirements.

The practical implications of this methodology extend beyond nitrogen fertilization. Newly released cultivars often lack information regarding nutrient requirements, grazing management targets, defoliation frequencies, and optimal canopy heights. Because model identity analysis compares overall biological response patterns rather than isolated measurements, the approach may also be applied to infer recommendations related to grazing management, cutting height, canopy structure, forage accumulation, and responses to other nutrients. An additional advantage is that all genotypes are evaluated under identical environmental and management conditions, allowing differences in response to be attributed primarily to genotype rather than environmental variation. When model identity is rejected, comparisons of intercepts and regression parameters help identify the source of divergence, distinguishing differences in performance under nutrient deficiency (intercepts) from differences in responsiveness to management or fertilization (regression parameters). Therefore, the method represents a rapid, low-cost, and accessible strategy for generating preliminary management recommendations that should subsequently be validated under field conditions.

The applicability of this approach depends on the availability of reference genotypes with well-characterized response patterns. Importantly, these reference genotypes do not necessarily need to be the parental species, as model identity analysis can also be applied using biologically relevant cultivars with experimentally characterized response patterns, as previously demonstrated for the *Brachiaria* hybrid cv. Ipyporã [11].

Although field validation remains essential, this approach may substantially reduce the time required to generate useful recommendations for forage producers and researchers. It should be noted, however, that the present study was conducted under greenhouse conditions, where environmental variability, grazing effects, and soil heterogeneity are substantially reduced. Moreover, although all treatments received the same liming and basal fertilization according to established recommendations for tropical forage grasses, soil chemical properties were not determined after fertilization to confirm that the target fertility levels for nutrients other than nitrogen had been achieved.

Nevertheless, the predominantly quadratic responses observed for most variables indicate that nitrogen was the primary limiting nutrient under experimental conditions. Furthermore, the restricted rooting volume of pots, together with the absence of nutrient leaching and grazing, may also have influenced plant responses compared with field conditions. Consequently, the magnitude of the responses observed may differ under practical production conditions. Despite these limitations, the relative similarities and differences identified among grasses are expected to remain biologically relevant because they are consistent with the contrasting nutritional requirements previously reported for *Urochloa brizantha* and *Urochloa decumbens*. Therefore, model identity analysis provides a promising framework for generating preliminary management recommendations that can subsequently be refined through long-term field experimentation.

5. Conclusions

Model identity analysis proved to be an effective statistical approach for distinguishing forage grasses with contrasting nitrogen requirements and for identifying similarities in

nitrogen response patterns among related genotypes. The method indicated that the nitrogen fertilization response of Mulato II is more similar to that of *Urochloa brizantha* than to *Urochloa decumbens*, suggesting comparable nitrogen requirements between the hybrid and *Urochloa brizantha*. The agreement between model identity analysis and principal component analysis further reinforces the robustness of this conclusion.

Beyond nitrogen fertilization, the results demonstrate the potential of model identity analysis as a rapid, low-cost, and accessible tool for generating preliminary management recommendations for newly released forage cultivars and hybrids when specific information is unavailable. This approach may contribute to accelerating the development of fertilization and grazing management recommendations while long-term field validation studies are still being conducted, supporting the use of model identity analysis as a complementary strategy for developing preliminary recommendations before extensive field information becomes available.

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