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EFFECT OF DIFFERENT NITROGEN FERTILIZATION RATES ON THE SPECTRAL RESPONSE OF *Brachiaria brizantha* cv. MARANDÚ LEAVES

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KEYWORDS

leaf nitrogen content, nitrogen content prediction, hyperspectral data, pastures, fertilization.

ABSTRACT

Hyperspectral sensors and regression analysis have been used to analyze the most important spectral ranges for biophysical parameters of target crops, aiding in management decision-making. This study aimed to analyze the spectral response of *Brachiaria brizantha* cv. Marandú leaves to increasing rates of urea fertilization and predict leaf nitrogen content (LNC). Four rates of urea fertilization (0, 25, 50, and 75 kg of N ha^{-1}) were applied. Eight leaves were collected per plot seven times at monthly intervals and subjected to hyperspectral analysis. Leaf spectral responses differed statistically within the visible region, particularly at 550 nm (green). The regression models achieved moderate to good R^2 values (0.53 to 0.83) for predicting LNC and identified important wavelengths in the red edge region (715 to 720 nm). These findings demonstrate the potential of spectral analysis to detect changes and forecast leaf nitrogen content in *B. brizantha* cv. Marandú crops at different fertilization levels.

INTRODUCTION

The Brazilian cattle herd ranks among the world's largest, accounting for 14.3% of the global herd in 2020, with 217 million heads (EMBRAPA, 2021). The success of animal production in Brazil hinges on the high-quality pastures provided to the livestock. *Brachiaria brizantha* cv. Marandú plays a significant role in this context, as it is extensively cultivated and covers a substantial portion, approximately 50%, of the introduced pastures in the Cerrado biome (EMBRAPA, 2018).

Understanding the physiology of grasses is crucial, especially regarding nitrogen fertilization. This element plays a vital role in vegetative growth, photosynthetic mechanisms, and synthesis of essential compounds such as chlorophyll, amino acids, cytochromes, enzymes, and coenzymes (Pires et al., 2021). Thus, achieving a balanced nitrogen fertilization is essential for biomass production and enhancing the quality of forage.

Urea, with its high nitrogen concentration and ease of commercialization, is a widely used nitrogen fertilizer. However, improper surface application can lead to nitrogen losses through volatilization (Guelfi, 2017). Monitoring and controlling nitrogen losses in the soil pose challenges due to the dynamic nature of this element, constantly undergoing transformations through chemical and biological processes (Reetz, 2017). Traditional nutritional diagnostic analyses for nitrogen in crops often involve expensive, time-consuming, and environmentally concerning methods (Dias et al., 2019).

Remote sensing is a valuable tool for monitoring nitrogen quantity and pasture quality without the need for sample destruction. The technique allows for data acquisition about an object without direct contact. It involves measuring and reading amounts of reflected or emitted electromagnetic energy from objects, which are then captured by sensors. In sum, this tool enables acquisition of information without physical sampling, providing a non-invasive and efficient approach for monitoring nitrogen levels and assessing pasture quality (Jensen, 2009).

Hyperspectral sensors are a type of sensor commonly used in remote sensing. They utilize light, whether natural or artificial, with numerous narrow and contiguous bands that overlap each other. This

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configuration enables the measurement of the continuous spectrum of the target being observed. Hyperspectral sensors cover a wide spectral range, typically from 350 to 2500 nm, and offer a potential spectral resolution as fine as 1 nm (Steiner et al., 2007; Formaggio and Sanches, 2017). In contrast, multispectral sensors capture spectral information using relatively broad wavelength bands. They provide less complex data and information content compared to hyperspectral sensors. However, multispectral sensors have the advantage of being lighter and more affordable (Mahlein et al, 2018) These sensors are still capable of capturing valuable spectral information, although with less spectral detail, making them a practical option for many remote sensing applications.

Hyperspectral sensor data acquisition involves reading the leaves or the plant canopy without causing damage to the plant tissue. By utilizing hyperspectral data, it becomes possible to apply various statistical tools to identify the specific information within the spectral curve that is relevant for detecting the plant's nitrogen requirement and determining its quantity.

Several studies have examined the spectral reflectance of forage grasses. Dias et al. (2019) found that the normalized difference vegetation index (NDVI) using multispectral bands effectively discriminated spectral changes in the canopy of Urochloa brizantha cv. Marandú in response to nitrogen fertilization. However, further research using hyperspectral sensors is necessary to determine the specific spectral ranges and bands that are most relevant for estimating important biophysical and biochemical parameters.

Based on the above, this study aimed to analyze the spectral response of *B. brizantha* cv. Marandú leaves to increasing urea fertilization rates in terms of differentiation and prediction of leaf nitrogen contents (LNC).

MATERIAL AND METHODS

The experiment was conducted at the College of Agriculture *Luiz de Queiroz* (ESALQ/USP) in Piracicaba city, São Paulo State, Brazil (22°42'16"S; 47°37'23"W, and 532-m altitude) (KOTTEK et al., 2006). The region has a *Cwa*-type climate according to Köppen’s classification, which stands for humid subtropical with dry winters and hot summers, and an average annual rainfall of 1280 mm and average temperature of 22°C. The hottest and coldest months have average temperatures of 25°C and 18°C, respectively (data obtained from a weather station within 100 meters of the study area).

Brachiaria brizantha cv. Marandú, sown by broadcasting, was used as the forage grass. The experimental design was a randomized complete block design (RCBD) with four blocks and four treatments, totaling 16 plots. Treatments consisted of different nitrogen fertilization rates using urea: B1 – 0 kg ha^{-1}, B2 – 25 kg ha^{-1}, B3 – 50 kg ha^{-1}, and B4 – 75 kg ha^{-1}, applied in each of the eight fertilizations, resulting in a total of B1 – 0 kg ha^{-1}, B2 – 200 kg ha^{-1}, B3 – 400 kg ha^{-1}, and B4 – 600 kg ha^{-1} at the end of the cycle.

Seven collections of *B. brizantha* cv. Marandú leaves were conducted throughout the experiment to perform spectral readings and nitrogen content measures. The leaves were collected one day before the scheduled forage cuts. The management practices followed the recommendations of Mesquita et al. (2010), where pasture was cut to a height of 10 cm using a backpack brush cutter, with an interval of approximately 30 days between cuts (when plots receiving the highest nitrogen rates reached their peak yields before senescence). After each cut, nitrogen fertilization was applied to the plots at different rates.

To ensure standardization, "+1" leaves were obtained and kept in refrigerated containers for transportation until the spectral readings to maintain leaf turgidity (Batista; Monteiro, 2007). Eight fixed collection points were established within each plot, resulting in a total of 128 leaves, with two spectral readings per leaf.

The spectral readings were performed using the FieldSpec™ 3 spectroradiometer (ASD Inc., Boulder, Colorado, USA), which covers a spectral range of 350 to 2500 nm at a 10-nm resolution. Readings on the adaxial surface of leaves were facilitated by using a leaf-clip accessory (ASD Inc., Boulder, Colorado, USA), which reduced the influence of external factors and maintained a constant sensor-leaf distance. Calibration with a white ceramic tile was conducted every 10 minutes to ensure data consistency. Two spectral readings were performed on the adaxial surface of each leaf, resulting in a total of 256 spectral readings per collection. Each reading represents an average of 30 consecutive readings (pre-determined average in the device's software).

The manufacturer's software, ViewSpec™ Pro (ASD Inc., Boulder, Colorado, USA), was used and generated a file with the 256 spectral signatures for subsequent analysis. First, the wavelength range to be used (400 to 2350 nm) for statistical analyses was selected, excluding the remaining ranges (350 to 399 nm and 2351 to 2500 nm) due to the presence of noise. Then, a principal component analysis (PCA) (Groot et al., 2001) was performed using The Unscrambler 9.7 software (CAMO Software AS, Oslo, Norway) to detect outliers among the spectral curves of the treatments. To complement the analysis, the pairwise comparison results (Tukey's test at 1% probability) for each wavelength and leaf nitrogen content (LNC) that showed significant differences among treatments (0, 25, 50, and 75 kg N ha^{-1}) were incorporated into the spectral signature graphs in Figures 1, 2, 3, and 4.

Partial least squares regression (PLSR) analyses were then conducted to identify the wavelengths most correlated with leaf nitrogen content (LNC) using LNC values obtained from chemical analyses and reflectance values. The model accuracy in predicting leaf nitrogen content was evaluated using Root Mean Square Error (RMSE) and the highest R^2 values for the predicted model.

RESULTS AND DISCUSSION

Spectral response of *Brachiaria brizantha* cv. Marandú leaves and mean comparison test

Table 1 shows significant differences ($P<0.01$) in leaf nitrogen content (LNC). In general, the control treatment (0 kg N ha^{-1} cut) had lower LNC compared to the treatment with 75 kg N ha^{-1} cut, except for the second collection where no significant difference ($P>0.01$) in LNC was observed.

In the fifth collection, the B1 application (25 kg N ha^{-1} cut) had a higher LNC than the B2 application (50 kg N ha^{-1} cut). Another difference between the treatments was observed in the sixth collection, with the B2 application (50 kg N ha^{-1} cut) surpassing the B3 application (70 kg N ha^{-1} cut).

These differences can be attributed to nitrogen losses when urea is used as a fertilizer. Viero et al. (2015) reported nitrogen losses through volatilization in urea, even after irrigation. Additionally, factors such as denitrification, surface runoff, and microbial immobilization (Lara Cabezas et al., 2000) can influence nitrogen fixation in the soil and contribute to these losses.

TABLE 1. Averages of nitrogen content in *Brachiaria brizantha* cv. Marandú leaves per collection.

Collection	B0(0)	B1(25)	B2(50)	B3(75)
1	21.85 B	24.50 AB	25.31 AB	26.37 A
2	22.49	23.06	23.45	24.35
3	23.45 B	25.37 AB	27.38 AB	28.57 A
4	21.57 C	23.34 BC	26.35 AB	29.73 A
5	22.84 B	23.55 B	22.01 B	25.87 A
6	21.22 B	25.14 AB	28.47 A	26.98 A
7	19.02 B	22.04 AB	23.91 A	25.42 A

Means followed by different letters in the row differ from each other by Tukey's test (α=0.01)

Figure 1 illustrates the spectral response for each collection within the visible region (400 to 720 nm). No significant differences were observed in the spectral response during the first collection, which is consistent with findings reported by Amaral & Molin (2014) where nitrogen fertilization only showed differences in the spectral response after 120 days.

According to the same authors, the lack of conclusive results in the visible region regarding fertilizer rate during the early collections was expected, as the nitrogen supply may not have reached the soil saturation necessary for optimal nutrient absorption by the plants, leading to reduced pigment production.

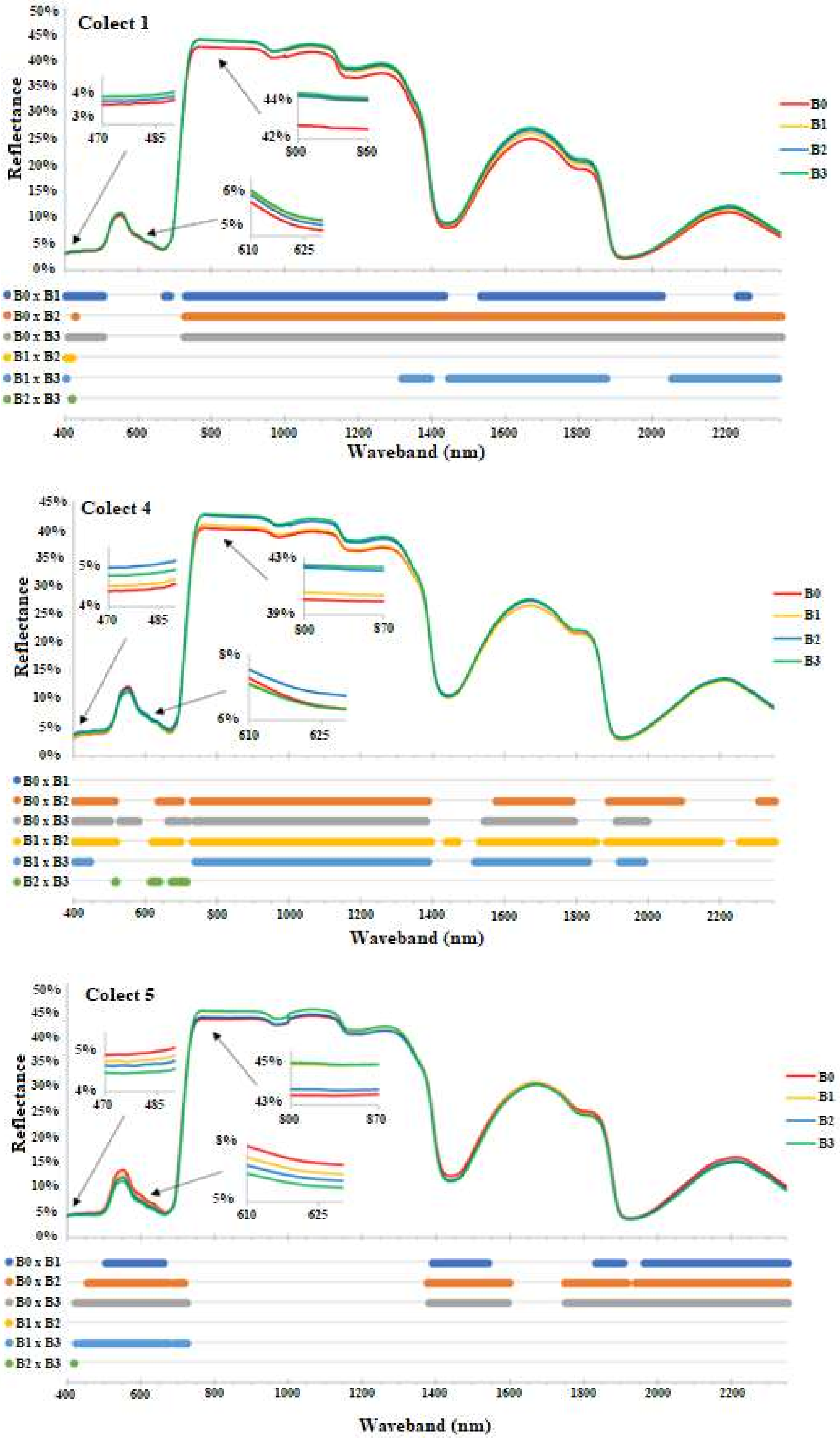
Chlorophyll, the primary pigment enhancing spectral characteristics in the visible and red-edge regions, contains 6% nitrogen (Asner, 2008). Consequently, the spectral properties of chlorophyll are closely related to nitrogen. The second and third collections' spectral curves, obtained before 120 days, did not exhibit differences in the visible region similar to the first collection. Due to the importance of chlorophyll in leaf nitrogen quantification and its influence on spectral response, the second and third collections were excluded from the study.

Except for the first, second, and third collections, where no differences were observed in the spectral curves within the visible region as the doses increased, the fourth to seventh collections showed higher reflectance in the curve of the control treatment (0 kg N ha^{-1}) compared to the curve with the maximum applied dose of 75 kg N ha^{-1}.

Spectral response amplitude is influenced by leaf pigments such as carotenoids, xanthophylls, and chlorophylls '*a*' and '*b*', which absorb radiation in the 480- and 620-nm regions, respectively, resulting in reflectance below 20%. The perception of green color in plants occurs at a wavelength of 560 nm in the electromagnetic spectrum, with a slight increase in reflectance observed. Additionally, there is an increase in spectral response in the red region (620 nm) related to the decrease in pigments and water in senescent leaves (Sims & Gamon, 2002).

Since nitrogen is a component of various molecules, including chlorophyll, it directly or indirectly participates in numerous biochemical processes, ultimately affecting crop development and yield (Kant & Rothstein, 2011). Therefore, increased nitrogen absorption leads to higher chlorophyll concentration, making plants greener and enabling them to absorb more energy, consequently reflecting less within the visible green region.

All collections exhibited differences in the electromagnetic spectrum corresponding to the red edge (705 to 750 nm) when comparing the leaf reflectance of the highest fertilization rate (75 kg N ha^{-1}) with that of the lowest fertilization rate (0 kg N ha^{-1}). The red edge is a specific region in the electromagnetic spectrum occurring between the red and near-infrared ranges and is considered the boundary between chlorophyll absorption in the red and near-infrared scattering caused by the internal leaf structure (Curran, 1991).


Colect 1
Reflectance
Waveband (nm)
B0
B1
B2
B3
B0 x B1
B0 x B2
B0 x B3
B1 x B2
B1 x B3
B2 x B3
Colect 4
Reflectance
Waveband (nm)
B0
B1
B2
B3
B0 x B1
B0 x B2
B0 x B3
B1 x B2
B1 x B3
B2 x B3
Colect 5
Reflectance
Waveband (nm)
B0
B1
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B3
B0 x B1
B0 x B2
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B1 x B3
B2 x B3

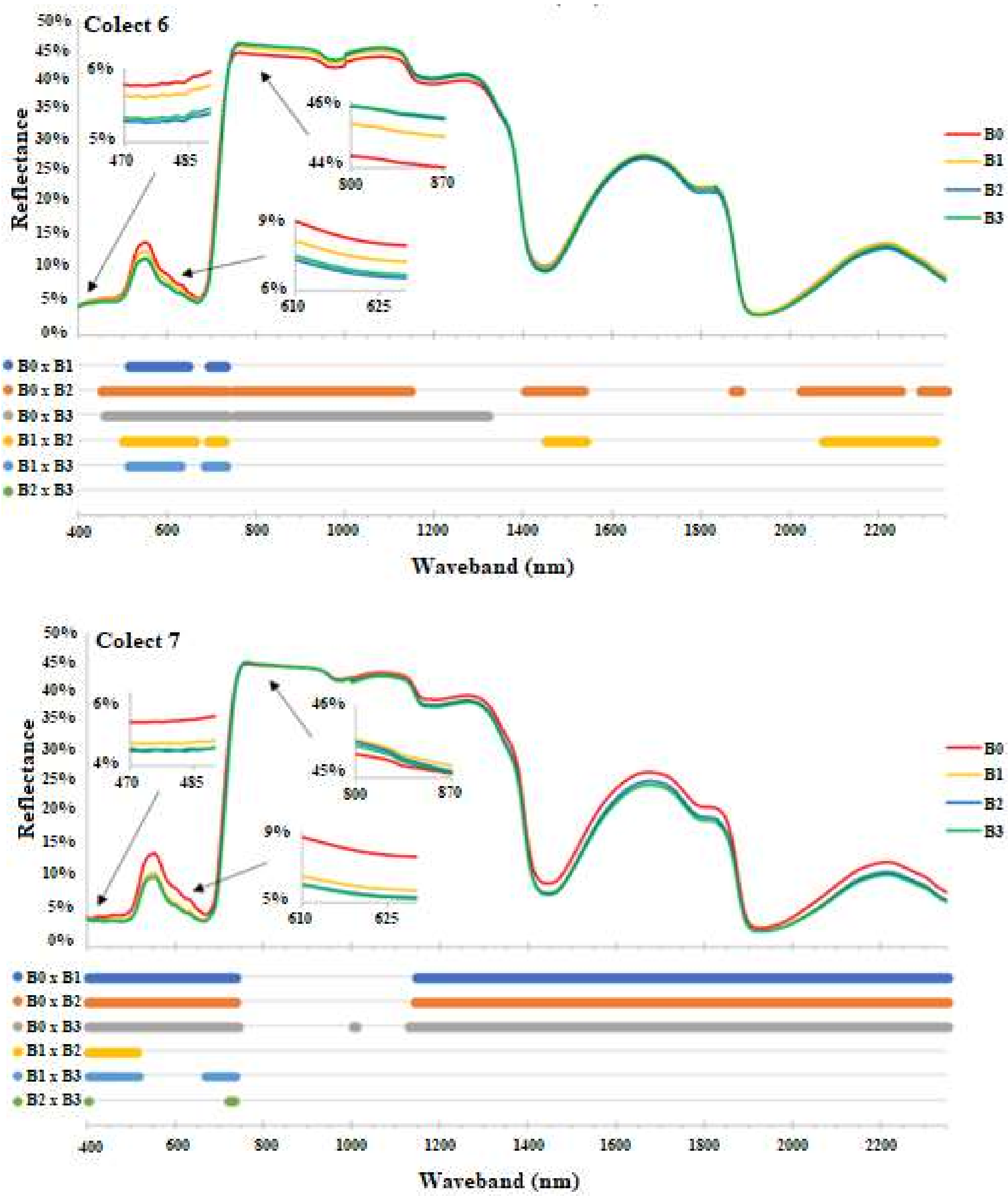


FIGURE 1. Spectral responses of *Brachiaria brizantha* cv. Marandú leaves and means comparison test for collection 7.
B0: 0 kg N ha^{-1} cut; B1: 25 kg N ha^{-1} cut; B2: 50 kg N ha^{-1} cut; B3: 75 kg N ha^{-1} cut. Each colored dot indicates bands that were significantly different according to Tukey's test (α=0.01).

In collections 1, 4, and 6, there was a noticeable difference in the near-infrared region (720 to 1100 nm), where the applications of 75 kg N ha^{-1} showed higher reflectance compared to the applications of 0 kg N ha^{-1}. These findings align with the results reported by Li et al. (2016), who studied the effect of urea fertilization on leaf nitrogen content in rapeseed (*Brassica napus* L.) and observed a decrease in reflectance with decreasing fertilization rates within the near-infrared plateau (750-1300 nm).

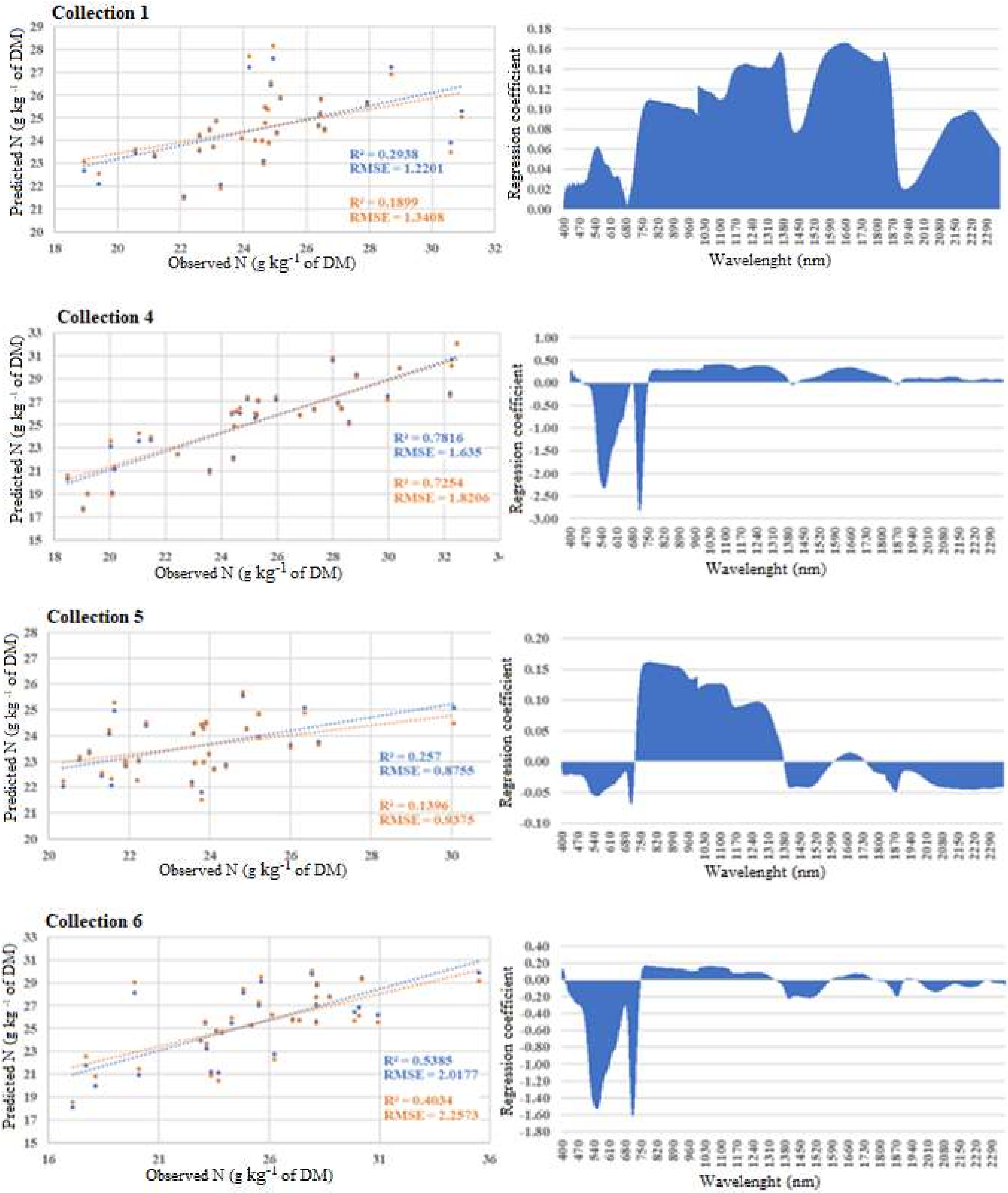
According to Li et al. (2016), reflectance tends to increase to around 40% and is associated with the internal cellular structure, which plays a crucial role in maintaining energy balance, preventing overheating, and protecting chlorophyll from destruction. These characteristics are linked to the biomass accumulation of forage crops in response to nitrogen fertilization.

Regression for nitrogen content forecast in *Brachiaria brizantha* cv. Marandú leaves

In the first collection (Figure 5), the wavelengths in the mid-infrared range, specifically between 1590 and 1700 nm, demonstrated higher prediction and validation coefficients (R^2), indicating their significant influence in the regression model. This region is highly sensitive to water presence, with absorption peaks around 1400 and 1900 nm, as reported by Jong et al. (2014), FANG et al. (2017), and Rodriguaz-Perez et al. (2018). The resulting R^2 value was 0.18 with an RMSE of 1.34.

The low R^2 values observed in collections 1, 2, and 3 can be attributed to incomplete soil saturation, leading to suboptimal nutrient absorption by the plants and subsequent reduction in pigment production, as highlighted by Amaral & Molin (2014). Due to their low pigment content, the results from these collections were not included in the analysis such as in section 3.1.

Validation yielded higher R² values for collections 4, 6, and 7. The visible regions, especially the green (550 nm) and red-edge (705 - 720 nm), exhibited a stronger influence on the prediction of leaf nitrogen content.



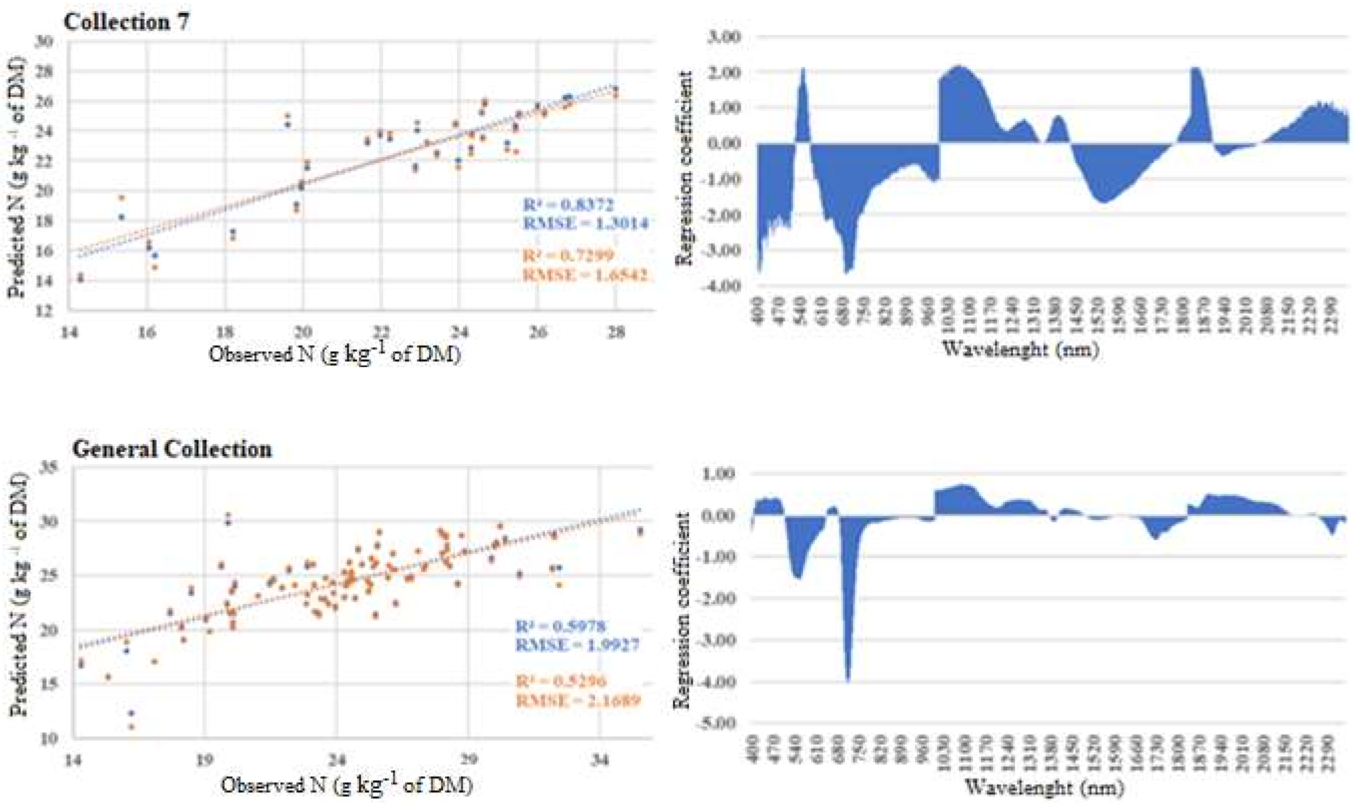


FIGURE 2. Partial Least Squares Regression of Collections 1, 4, 5, 6, and 7 for Estimation of Leaf Nitrogen Content in *Brachiaria brizantha* cv. Marandú.

R^2: coefficient of determination; RMSE: root mean square error; DM: dry matter. (Blue: calibration; Orange: validation).

Several studies have highlighted significant differences in leaf nitrogen content within the green and red-edge regions. Singh et al. (2017) concluded that wavelengths around 595 nm (green) and 701 nm (red-edge) are highly sensitive to nitrogen levels in sorghum varieties. Asner Martin (2008), using partial least squares (PLS) analysis, identified the region between 510 and 730 nm as having a major influence on chlorophyll determination.

In the fifth collection (Figure 2), the near-infrared wavelengths demonstrated a similar influence as in Collection 2. Consequently, there was a limited predictive power for nitrogen content in Collection 5, resulting in an R^2 of 0.26 in the calibration, with an RMSE of 0.88. During validation, the R^2 value decreased by 54% to 0.14, with an RMSE of 0.94. Issues with nitrogen application in this collection resulted in overdosing in some plots, impeding accurate prediction of leaf nitrogen content in the model.

As mentioned earlier, Collections 1, 2, 3, and 5 exhibited unfavorable characteristics for constructing a reliable model for forecasting nitrogen content in Marandú grass leaves. However, Collections 4, 6, and 7 possess similar characteristics in terms of spectral regions with the greatest influence, and most of them achieved R^2 values above 0.5. Consequently, a general regression model was generated based on these three collections.

The model derived from Collections 4, 6, and 7 (Figure 2) yielded reasonably satisfactory results (Saeys et al., 2005). Notably, the model places significant importance on wavelengths in the red-edge region. In a study by Barros (2022) examining hyperspectral responses sensitive to nitrogen variation, the red-edge spectral region emerged as one of the most crucial for predicting nitrogen content in sugarcane leaf nitrogen models.

CONCLUSIONS

The application of increasing urea rates to *Brachiaria brizantha* cv. Marandú pasture resulted in higher leaf nitrogen contents (LNC). These doses had a notable impact on the spectral response after 120 days, particularly in the visible region (400 to 700 nm), specifically at 550 nm (green), and the red-edge region (715-720 nm).

A general regression model was successfully calibrated to predict leaf nitrogen content in *B. brizantha* cv. Marandú plants, achieving an R^2 of 0.52 and an RMSE of 2.16 g kg^{-1}, using data from three collections. This model provides a valuable tool for estimating leaf nitrogen content in *B. brizantha* cv. Marandú forage crops.

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