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Interspecies Prediction of Nitrogen Content in Processed Plant Samples Using Spectroscopic Modeling and Transfer Learning

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ABSTRACT

Employing machine learning models on preprocessed samples is an effective alternative for leaf nitrogen quantification, reducing analysis time and improving fertilizer efficiency. This study evaluates predictive performance and transfer learning of models for nitrogen (N) concentration across different plant species, along with visual analysis of spectral patterns. A spectral dataset was developed using pre-processed samples from crops (coffee, pear, sugarcane, bean, and maize), forage (Brachiaria), and ornamental plants (e.g., Gypsophila). Leaf samples were collected from field-grown plants, oven-dried at 60°C with forced air circulation, and ground to 2.0 mm. Spectra were measured with a FieldSpec spectroradiometer (350–2500 nm). Visual analysis compared plants of distinct photosynthetic cycles (C3 and C4) and among species of the same cycle. Nitrogen quantification was performed using Partial Least Squares Regression (PLSR) and Random Forest (RF). Transfer learning was assessed in three ways: (i) between species; (ii) temporal stability; (iii) evaluation with an independent dataset comprising multiple agricultural species. Results showed C3 plants had lower reflectance in the 400–670 nm bands and higher N levels compared to C4 crops. Regardless of crop type or photosynthetic cycle, characteristic absorption features were detected at 530 nm and 615 nm, absent in fresh samples. PLSR achieved superior performance ($R^2 = 0.95$, RMSE = 2.16 g kg^{-1}, MAPE = 10.70%) compared to RF ($R^2 = 0.88$, RMSE = 3.4 g kg^{-1}, MAPE = 13.48%). Edaphoclimatic and physiological conditions influenced transfer learning, highlighting the potential and limitations of applying spectral models across species and environments.

1 | Introduction

Nitrogen (N) is one of the most important nutrients in agriculture, directly influencing crop growth (Fageria and Baligar 2005; Ye et al. 2020), production, and quality (Berger et al. 2020; Lemaire et al. 2008). Nitrogen acts as a primary regulator of plant physiological processes and the ecosystem (Abdalla et al. 2021). N is incorporated into proteins and chlorophyll from leaf cells to support carbon fixation, biosynthetic processes, Rubisco, bioenergetics, and light capture (Liu et al. 2021). Therefore, determining the N levels in crops has been widely discussed and studied (Berger et al. 2020; Fu et al. 2021), aiming to provide adequate amounts of nitrogen for plant growth and development while avoiding high costs, environmental risks, and negative effects from both excess and deficiency in nitrogen fertilization (Ye et al. 2020).

In recent years, spectroradiometry has been widely studied as an innovative and nondestructive technique (Azadnia et al. 2023) for estimating nutrients in plants (Amaral et al. 2022; Johnson et al. 2021; Shi et al. 2022). Spectroscopy combined with multivariate calibration models has proven to be an efficient approach (Ely et al. 2019; Wang et al. 2024) for estimating leaf traits through spectral reflectance in the visible (VIS), near-infrared (NIR), and short-wave infrared (SWIR) wavelengths (Couture et al. 2013; Zhang et al. 2021). Plant nutrients have been successfully predicted by spectroscopy, which reflects the potential of combining full-spectrum methods and machine learning techniques, such as partial least squares regression (Lima et al. 2024; Misbah et al. 2024; Pullanagari et al. 2021) and Random Forest regression (RFR) (Perich et al. 2021; Pourreza et al. 2025), respectively.

Many studies have demonstrated that fresh leaf spectroscopy, whether measured at the canopy level or using intact leaves in the laboratory, can be used to detect nitrogen deficiencies in various crops, including sugarcane (Fiorio et al. 2024), maize (Oliveira et al. 2024), apple trees (Chen et al. 2020), and oranges (Osco et al. 2020). The ability to quantify nutrients based on fresh leaf spectral data has shown promising results, but inconsistencies in model calibration have been reported in the literature (Rotbart et al. 2013). These studies indicate that using preprocessed leaves (dried and ground) significantly improves calibration performance. This is partly due to the effect of moisture, which influences the absorption intensity of specific spectral features (Prananto et al. 2020).

Another important challenge in using fresh leaf samples is the spatiotemporal generalization of predictive models across environments with varying soil and climate conditions (Silva et al. 2024). Some studies have applied transfer learning to extend models trained on one dataset to those collected under different environmental conditions (Ma et al. 2019; Tian et al. 2019). However, the impact of spectral variability on model performance across datasets remains insufficiently understood, partly due to the influence of leaf traits' heterogeneity on predictive accuracy (Wang et al. 2024). Additionally, the transferability of models between plant species or cultivars is hindered by spectral differences among crops and species-specific interactions with environmental factors, which significantly limit cross-species model performance (Berger et al. 2020; Tian et al. 2019; Wang et al. 2024). One alternative to overcome these limitations is to explore the potential of predictive models using preprocessed samples (Silva et al. 2024). Although sample preprocessing requires additional time due to oven drying and grinding, it offers significant advantages by reducing leaf water content, which would otherwise strongly affect spectral features and increase dispersion between replicates (Prananto et al. 2020). A second alternative would be the application of the spiking method, which consists of adding a small number of representative samples to the calibration set to optimize model transferability. This procedure tends to increase the accuracy of predictions for local samples, adapt to new conditions while preserving independent dataset evaluation (Wijewardane et al. 2023).

In this study, we hypothesize that spectral models developed from preprocessed samples can predict leaf nitrogen content with greater accuracy. However, it remains unclear whether the efficiency of model generalization is compromised by the use of different crops, species, or photosynthetic pathways (C3 and C4), even when samples have undergone a homogenization process. To improve model transfer adjustments, the spiking method was employed, which contributes to increased prediction accuracy for local samples. Furthermore, there are few studies that thoroughly assess how nitrogen levels affect the shape, intensity, and other aspects of spectra, identifying specific features associated with nitrogen, such as peaks, absorption features, or reflectance intensity. Thus, the aim of this study was to evaluate spectral models and their generalization for predicting nitrogen concentrations in preprocessed samples from multiple agricultural crops, pastures, and ornamental plants. The results may offer benefits for both producers and the industry, including reduced time requirements for crop nutritional assessment and improving nitrogen fertilizer use efficiency.

2 | Material and Methods

The method employed in this study consists of nine steps: (i) selection of crops for laboratory analysis of leaf nitrogen content (LNC); (ii) leaf sampling; (iii) sample preprocessing; (iv) spectral measurements; (v) LNC determination in the laboratory; (vi) spectral preprocessing; (vii) visual characterization of spectra; (viii) application of machine learning models; and (ix) transfer learning of the models (Figure 1). Each of these steps is described in detail in the following sections.

2.1 | Sample Collection, Processing, and Spectral Signature Acquisition

Sugarcane leaf sampling was conducted in an experiment established on soil classified as a Red-Yellow Ultisol (Argissolo Vermelho-Amarelo—PVA) with a very clayey texture. The regional climate is classified as humid subtropical (Cwa), with an average annual precipitation below 1400 mm, characterized by a rainy summer and a dry winter (Alvares et al. 2013). Forage sampling was carried out in a Cerrado area on soil classified as an Orthic Quartzarenic Neosol (Neossolo Quartzarênico Órtico—RQo) with a sandy texture. The regional climate is characterized as a transitional zone between humid subtropical (Cfa) and tropical (Aw), with mean temperatures ranging from 22°C to 24°C and annual precipitation between 1200 and 1400 mm (SEPLAN-MS 1990). The remaining samples used in this study were provided by reference laboratories specializing in foliar chemical analysis. All foliar sampling was performed during the peak of vegetative development of the crops.

The sampling strategy encompassed 555 samples from different agricultural species, associated with distinct soil and climatic conditions, resulting in a highly variable dataset suitable to support robust analyses. Samples were obtained from coffee (4 samples), pear (8 samples), sugarcane (240 samples), bean (16 samples), maize (7 samples), forage (190 samples), and *Gypsophila* (18 samples). Additionally, an independent dataset was established for model validation, consisting of 72 samples previously collected from different agricultural species, such as tomato and orange, which were identified in this study as "other crops".

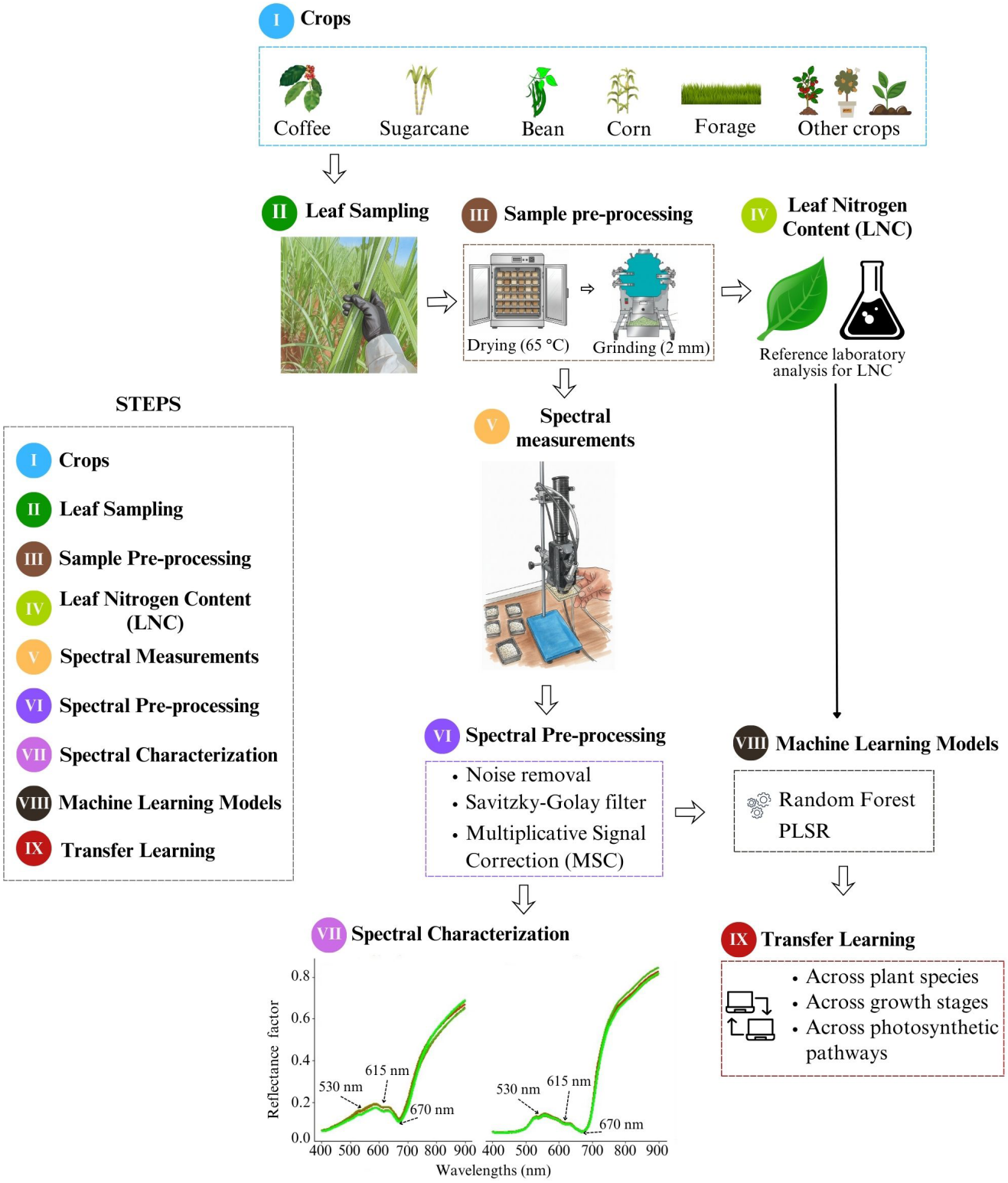

FIGURE 1 | Workflow for quantifying N concentrations from homogeneous samples.

All samples were dried in a forced-air circulation oven at 60°C, ground using a mill, and passed through a 2.0 mm sieve. Subsequently, spectral measurements were performed in the laboratory with a FieldSpec 3 ASD FieldSpec FR Spectroradiometer (ASD—Analytical Spectral Devices Inc., Boulder, CO, USA), which covers from 350 to 2500 nm, with spectral resolutions of 1.4 nm (350–1050 nm) and 2 nm (1050–2500 nm). A Leaf Clip probe connected via optical fiber was used to maintain constant light intensity and orthogonal incidence during all measurements, ensuring fully controlled acquisition conditions. The instrument was calibrated every 20 readings using the Lambertian surface integrated into the Leaf Clip (Fiorio et al. 2024). Each sample was measured in triplicate, and the values were subsequently averaged to correct for possible inconsistencies in individual readings.

2.2 | Spectral Preprocessing

The spectra were initially corrected for measurement inconsistencies caused by external factors such as environmental variations and light scattering (Fiorio et al. 2024; Silva et al. 2024). The data preprocessing consisted of the following steps: (i) removal of spectral bands strongly affected by noise (350–400 nm); (ii) spectral correction using the Multiplicative Signal Correction (MSC) method (Lu et al. 2020); (iii) filtering using the Savitzky–Golay (SG) algorithm (Savitzky and Golay 1964), with a smoothing window of 3 points and a second-order polynomial (Shen et al. 2020).

2.3 | Visual Spectra Characterization

Spectral characterization aimed to identify the shape and distinct patterns of reflectance spectra in relation to crop type, photosynthetic pathway, and edaphoclimatic conditions. The analysis was structured around two key comparisons: (i) Between species with different photosynthetic pathways (C3 vs. C4), in which we aimed to evaluate whether reflectance behavior differs between plants with distinct photosynthetic metabolism types; and (ii) Between species sharing the same photosynthetic pathway, where the goal was to assess whether these plants exhibit similar spectral patterns or present notable differences.

To support this analysis, the spectra were grouped into seven categories based on a histogram analysis. Reflectance values were divided into frequency intervals to discriminate distribution characteristics and to detect key features such as outliers and trends.

2.4 | Machine Learning Models

2.4.1 | Partial Least Squares Regression (PLSR)

PLSR is a multivariate statistical technique well suited for handling multicollinear variables, such as the spectral bands used in this study, which exhibit high inter-band correlation. In addition, PLSR performs well with datasets containing a limited number of independent observations (Wold et al. 2001). In this study, the model was implemented using the Nonlinear Iterative Partial Least Squares (NIPALS) algorithm, which efficiently extracts latent variables from high-dimensional data with relatively few observations (Martens and Naes 1992). PLSR has been widely adopted for calibrating nutrient prediction models based on hyperspectral data (Flynn et al. 2023; Rodrigues et al. 2020; Silva et al. 2024).

During the calibration stage, PLSR uses the information from the independent variables (spectra) and dependent variables (N concentrations) to generate new variables, called factors. Model fitting aims to find the smallest possible number of PLS factors that explain the dependent variables. In this study, K-Fold cross-validation ($k = 10$) was applied to select the best number of factors that minimize the Root Mean PRESS statistic. Additionally, the maximum number of factors was set to 15, avoiding the excessive use of components and minimizing the risk of overfitting while still ensuring a good predictive capacity (Thomson et al. 2020).

The relevance of wavelengths for predicting LNC was assessed using the Variable Importance in Projection (VIP) score. The VIP index quantifies the contribution of each spectral band to the latent variables in the model (Chong and Jun 2005). Wavelengths with VIP values greater than 0.8 were considered significant for the construction of the PLSR model. The PLSR algorithm was implemented using the "pls" package (Mevik and Wehrens 2024) in the R software environment.

2.4.2 | Random Forest (RF)

RF is a machine learning algorithm commonly used for classification and regression tasks (Breiman 2001). This model works in the form of trees based on bootstrap or bagging, which generates multiple independent models. For each tree, a random subset of independent variables (spectra) is selected at each split node. The division of nodes is performed to minimize the mean squared error (MSE) in the data (Hwang et al. 2023). The algorithm seeks to divide the data in such a way that the resulting groups are as homogeneous as possible concerning the dependent variable. The greater the reduction in error (MSE) associated with a variable, the more important it was in the model. Thus, the importance of the variables is determined by the amount of error reduction (MSE) achieved throughout the trees.

In the prediction phase, RF produces the final prediction for regression tasks based on the average of all trees (Tang et al. 2024). In this study, the optimization of the model's hyperparameters considered the number of trees ($ntree = 500$) and number of variables for each tree ($mtry = 10$).

2.5 | Modeling and Generalization Assessment

In the generalization process, three distinct scenarios were defined to assess predictive performance. First, models were applied across species, in which models trained on a set of species were tested on species not included in the calibration. This strategy corresponds to a Leave-One-Group-Out validation approach and aimed to assess spectral similarity and the predictive robustness of models when applied to new plant species. Second, the temporal stability of the models was tested by training them with data from two different seasons and evaluating their performance on data from a third season of the same crop. This scenario allowed for the analysis of spectral pattern consistency in response to physiological and structural changes during crop development. Finally, models were trained using data from all crop species included in the study and then tested on a completely independent dataset containing various agricultural species grouped under the category "other crops."

To account for domain shift effects during model generalization, a supervised domain adaptation (fine-tuning) procedure was adopted. In this approach, the original test set was partitioned into two subsets: a fine-tuning subset comprising 30% of the samples and a held-out evaluation subset comprising the remaining 70%. The base model, previously trained on the source domain, was fine-tuned using only the adaptation subset, allowing partial adjustment to local spectral characteristics. The performance of the adapted model was then evaluated

exclusively on the held-out subset, which was not used during the fine-tuning process. This strategy aims to improve prediction accuracy under varying spectral conditions associated with crop type, species, phenological stage, photosynthetic pathway, and seasonal variability, while maintaining a clear separation between the adaptation and evaluation phases. Similar supervised adaptation approaches have been reported in previous studies (Wijewardane et al. 2023).

2.6 | Validation

For each model (PLSR and RF), a global analysis was generated, using the entire dataset. Models were trained using 10-fold cross-validation and the predictive potential was evaluated using the coefficient of determination (R^2), root mean squared error (RMSE), mean absolute percentage error (MAPE), and interquartile performance ratio (RPIQ), as described in Equations (1–4), respectively.

$$R^2 = \frac{\left[\sum(\gamma_p - \overline{\gamma}_p)\cdot(\gamma_o - \overline{\gamma}_o)\right]^2}{\sum(\gamma_p - \overline{\gamma}_p)^2 \cdot \sum(\gamma_o - \overline{\gamma}_o)^2} \quad (1)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}} \quad (2)$$

$$MAPE = \frac{1}{n}\sum_{i=1}^{n}\frac{|y_i - \hat{y}_i|}{y_i} \quad (3)$$

$$RPIQ = \frac{Q3 - Q1}{RMSE} \quad (4)$$

where γ_p and $\hat{y}_i$ are the predicted values, γ_o and y_i are the observed values, $\overline{\gamma}_p$ and $\overline{\gamma}_o$ are the means of the predicted and observed values, respectively.

Spectral preprocessing was performed using the ParLes software (version 3.1). Machine learning model training, as well as spectral analysis and graphical visualization, were conducted using the R statistical computing environment, employing the *caret*, *pls*, *randomForest*, and *ggplot2* packages.

3 | Results and Discussion

3.1 | Descriptive Statistics of Leaf Nitrogen Contents

Table 1 presents the descriptive statistics of leaf nitrogen content ($g{\cdot}kg^{-1}$) for each crop evaluated in the study, as well as for the aggregated group of samples not individually identified, referred to as "other crops". The mean LNC values showed marked variation among crops, reflecting physiological differences and distinct patterns of nitrogen accumulation. In general, higher mean LNC values were observed in crops with C3 photosynthetic metabolism when compared to C4 crops. The "other crops" group exhibited the greatest variability in LNC values, with a standard deviation of $6.97\,g{\cdot}kg^{-1}$. This high variability is associated with the fact that this group comprises different plant species with distinct physiological characteristics and patterns of nitrogen absorption and assimilation, which increases data dispersion.

3.2 | Visual Spectral Characterization

Comparisons between photosynthetic pathways included Gypsophila and bean as representatives of the C3 cycle (Figure 2A), and forage and sugarcane for the C4 cycle (Figure 2B). Crop selection was based on high variability of nitrogen concentration and the number of available samples. Visual spectral characterization focused on the 400–900 nm range to facilitate the identification of distinct reflectance patterns. LNC was classified into five ascending categories, from the lowest to the highest values. Although LNC variation was relatively small across all crops (Figure 2), the sensor was still able to distinguish these differences based on the spectra. Leaves with lower LNC exhibited higher reflectance in the visible range (400–680 nm), whereas those with higher LNC showed reduced reflectance.

Remarkably, Gypsophila and bean (C3 species) exhibited lower reflectance in the 400–670 nm range than C4 plants, despite significantly higher nitrogen levels. This is related to the greater light absorption by photosynthetic pigments, especially chlorophyll, which tends to be more concentrated in C3 plants. This higher pigment concentration is often associated

TABLE 1 | Descriptive statistics of leaf nitrogen content ($g{\cdot}kg^{-1}$) by crop, including number of samples (*n*), mean, median, standard deviation (SD), minimum (Min) and maximum (Max) values.

Crop	*n*	Mean	Median	SD	Min	Max
Sugarcane	240	14.11	13.68	2.31	9.36	20.16
Forage	190	12.16	12.24	2.92	4.48	20.32
Gypsophila	18	42.88	43.15	2.25	37.30	45.60
Bean	16	45.55	46.20	3.35	39.00	50.40
Pear	8	24.12	23.40	2.37	22.32	28.80
Maize	7	28.08	28.80	2.49	23.04	30.96
Coffee	4	26.68	26.36	1.56	25.20	28.80
Other crops	72	31.42	31.68	6.97	12.96	53.28

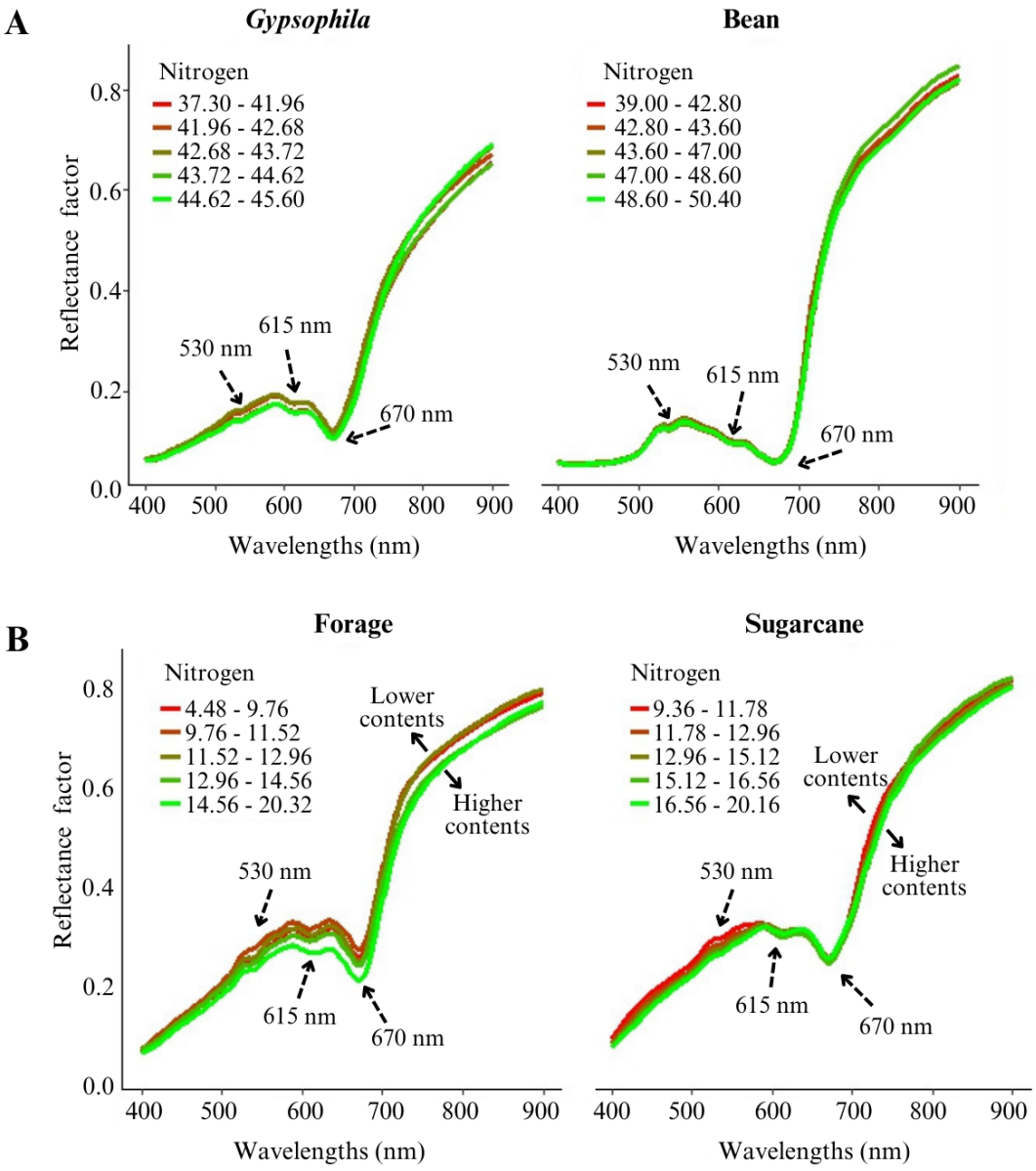

FIGURE 2 | Spectral curves in the 400–900 nm wavelength range, classified from the lowest to highest LNC for crops with different photosynthetic pathways: (A) C3 (Gypsophila and Bean) and (B) C4 (Forage and Sugarcane).

with the elevated Rubisco content in C3 species, an enzyme that is essential for photosynthesis and is nitrogen-dependent (Mu and Chen 2021). On the other hand, C4 plants contain less Rubisco but are more efficient, requiring less nitrogen (Mu and Chen 2021; Wang et al. 2021). These plants have lower concentrations of photosynthetic pigments, resulting in higher spectral reflectance.

C4 plants exhibited distinct patterns in the red-edge region (680–750 nm) depending on nitrogen concentration. Lower nitrogen levels caused a red-edge shift toward shorter wavelengths (blue range), whereas higher nitrogen levels shifted the response toward the near-infrared (longer wavelengths). This behavior suggests that reduced foliar nitrogen leads to an earlier inflection point in the red-edge spectral region and demonstrates its sensitivity to LNC variations (Silva et al. 2024).

Regardless of crop type or photosynthetic cycle, characteristic absorption features were identified at wavelengths around 530 nm, 615 nm, and 670 nm (±5 nm). Notably, absorption features at 530 nm and 615 nm are typically absent in fresh samples (Fiorio et al. 2024; Silva et al. 2024), likely masked by leaf water content. This highlights one advantage of using preprocessed samples, which enable a more accurate characterization of the spectral signature.

Nitrogen is a significant element both in the photosynthesis process and in chlorophyll concentration (Mu and Chen 2021). In this context, it is well-established in the literature that the feature at 670 nm (red region) is influenced by the content of chlorophylls "a" and "b" (Moriwaki et al. 2023; Terashima et al. 2009; Zhu et al. 2014). Although few studies have demonstrated this relationship, absorptions at 530 nm (green-edge region) are described as highly sensitive to chlorophyll variations. Similarly,

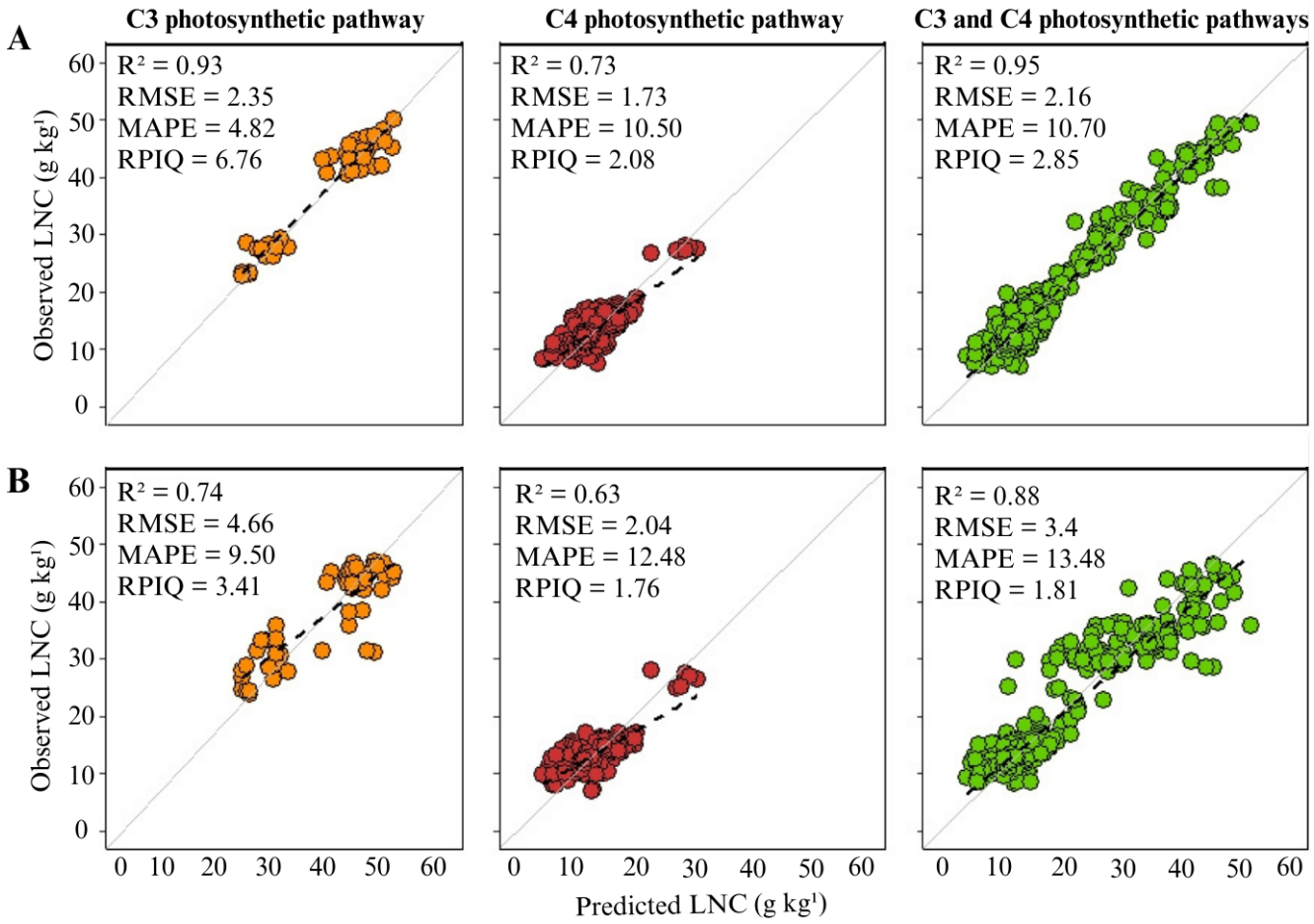

FIGURE 3 | Nitrogen prediction for C3, C4 and Global photosynthetic pathway crops using the algorithms: (A) Partial Least Squares Regression (PLSR); and (B) Random Forest (RF). Model performance was evaluated using 10-fold cross-validation.

the region centered on 615 nm is effective for estimating chlorophyll content (Gitelson et al. 2003). Therefore, our findings may contribute to the development of new technologies for estimating chlorophyll content, such as vegetation indices based on the 530, 615, and 670 nm bands.

3.3 | PLSR and RF Models for Nitrogen Prediction

Prediction models based on PLSR (Figure 3A) and RF (Figure 3B) algorithms were calibrated using the global dataset, as well as subsets for C3 and C4 crops. Calibration included only coffee, pear, sugarcane, bean, maize, and forage crops. The PLSR models showed the most promising results in all three tested conditions, with R^2 values of 0.93, 0.73, and 0.95, and RMSE of 2.35 g kg^{-1}, 1.73 g kg^{-1}, and 2.16 g kg^{-1} for the C3, C4, and global datasets, respectively. PLSR performs well because it handles correlated hyperspectral variables effectively (Fiorio et al. 2024; Reyes-Trujillo et al. 2021; Silva et al. 2024). For this reason, it is widely used in nutrient prediction models based on hyperspectral data (Flynn et al. 2023; Reyes-Trujillo et al. 2021; Rodrigues et al. 2020; Santos et al. 2023). On the other hand, although the RF models showed slightly lower performance compared to PLSR, they also demonstrated good results, with R^2 values of 0.74, 0.63, and 0.88, and RMSE values of 4.66 g kg^{-1}, 2.04 g kg^{-1}, and 3.4 g kg^{-1} for the C3, C4, and global datasets, respectively.

Models calibrated with the global dataset had the best performance, with $R^2 > 0.88$, RMSE < 3.4 g kg^{-1}, and MAPE < 13.48%. This can be attributed to the larger volume of data available for the models, which favored the learning process, especially for decision tree-based techniques (Zhao et al. 2022). Furthermore, in the global models, the highest RMSE was 3.4 g kg^{-1}, corresponding to an error of 13.48% (Figure 3B). On the other hand, the lowest RMSE was 2.16 g kg^{-1}, with an error of 10.70% (Figure 3A). These are excellent results, especially considering the diversity of the analyzed crops (coffee, pear, sugarcane, bean, maize, and forage), which have differences in their leaf characteristics, nitrogen contents, photosynthetic cycles, and vegetative stages. Additionally, we believe that a greater number of samples will improve the model learning process, resulting in more accurate predictions.

3.4 | Variables of Highest Importance in the Global Models

Most important bands in the PLSR (Figure 4A) and RF (Figure 4B) predictions were calculated considering only the global models. The analysis identified which wavelengths and features were the most relevant for nitrogen content prediction. In general, spectral ranges in the visible (450–680 nm) and red-edge (680–750 nm) were the most important ones. All

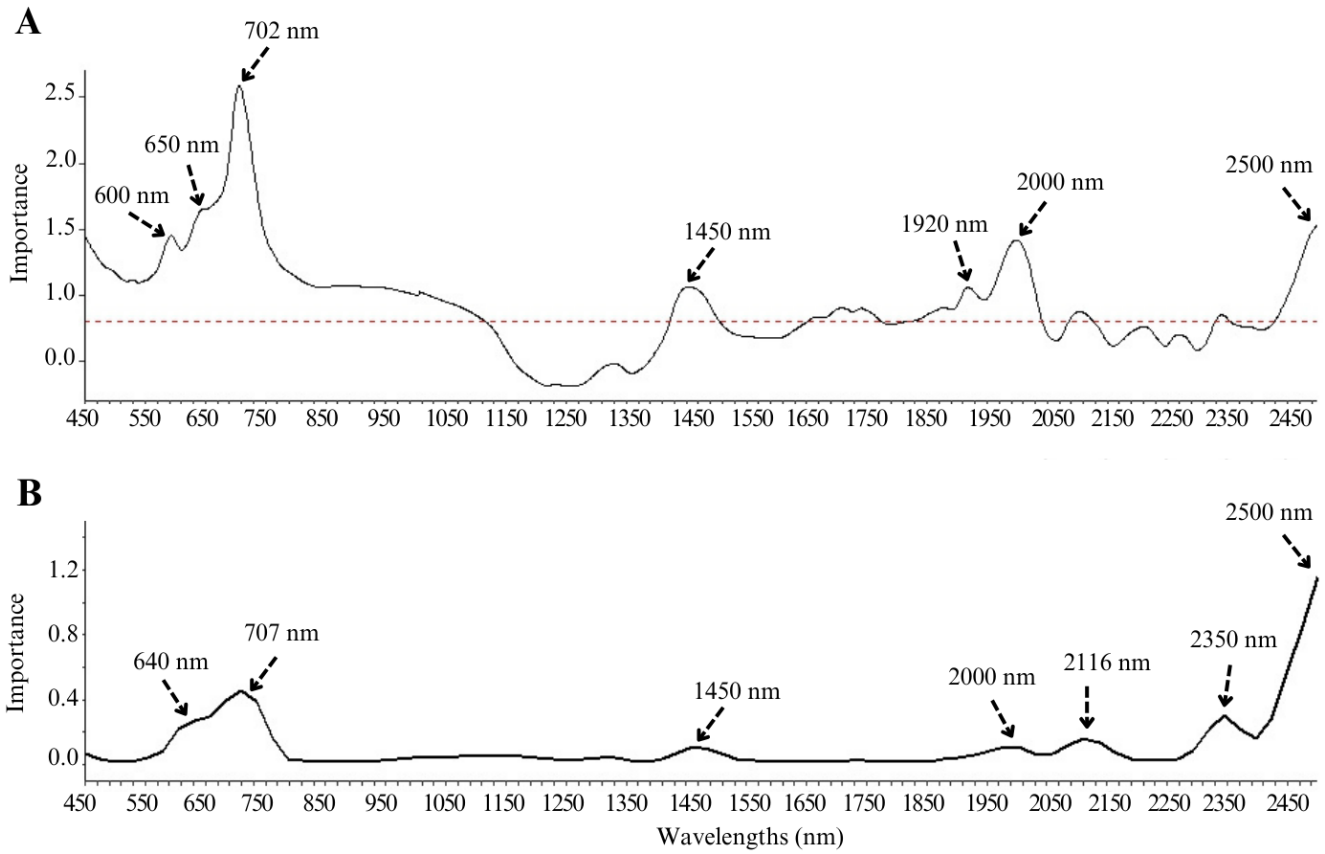

FIGURE 4 | Wavelengths of highest importance in regression models during the prediction stage. (A) For the PLSR models, only wavelengths above 0.8 (dashed line) were considered; and (B) Random Forest. The calculation of the most important bands was considered only for the global models.

visible and red-edge bands showed some degree of contribution to the model, but the most pronounced peaks were observed in the 702 nm (PLSR) and 707 nm (RF) bands. The short-wave infrared range had important peaks around 1450 nm (PLSR and RF), 1920 nm (PLSR), 2000 nm (PLSR and RF), 2116 nm (RF), 2350 nm (RF), and 2500 nm (PLSR and RF).

Although the data used in this study came from preprocessed leaf samples, it was observed that the visible and red-edge bands showed the greatest importance in constructing the nitrogen predictive models. This trend has also been identified in previous studies with fresh leaves, such as in sugarcane (Fiorio et al. 2024; Reyes-Trujillo et al. 2021), maize (Oliveira et al. 2024), and canola (Li et al. 2016). These results indicate the robustness of these spectral ranges for estimating nitrogen content, regardless of the physiological state of the sample. Furthermore, wavelengths in the visible and red-edge regions are widely recognized for their association with the absorption of photosynthetic pigments in plants, such as chlorophyll (Li et al. 2016). Chlorophyll plays a key role in light absorption and energy transfer to the photosynthetic apparatus (Sims and Gamon 2002). In conditions of nitrogen deficiency, there may be structural and functional changes in chloroplasts, given that nitrogen is an essential nutrient for both the photosynthetic process and chlorophyll synthesis. It is estimated that about 75% of the nitrogen present in the leaf is allocated in chloroplasts, primarily used in the formation of proteins related to the photosynthetic apparatus (Alharbi et al. 2022; Mu and Chen 2021). Given the importance of these spectral regions for nitrogen prediction, future studies may explore the use of the most informative wavelengths identified in this work to develop less complex spectral models, as well as to evaluate the balance between dimensionality reduction, computational efficiency, and predictive performance.

3.5 | Generalization of Models Between Crops

Model generalization was applied to the sugarcane, forage, and "other crops" categories using the PLSR (Figure 5B) and RF (Figure 5A) algorithms. For sugarcane, the RF model delivered the best performance, with an RMSE of 1.82 g kg^{-1}, MAPE of 10.15%, and RPIQ of 1.98, outperforming PLSR (RMSE = 2.05 g kg^{-1}, MAPE = 11.38%, RPIQ = 1.76). In contrast, PLSR showed better accuracy for forage, with an RMSE of 2.59 g kg^{-1}, MAPE of 17.40%, and RPIQ of 1.42. For the "other crops" category, PLSR again outperformed RF, achieving an R^2 of 0.77, RMSE of 3.66 g kg^{-1}, MAPE of 10.20%, and RPIQ of 2.95, indicating better predictive performance.

The low predictive performance observed for forage models can be attributed to two main factors. First, data were collected in three different seasons (summer, autumn, and spring), resulting in a dataset with high variability concerning the photosynthetic rates, biomass, vegetative stages, and LNC concentrations. These seasonal variations directly contribute

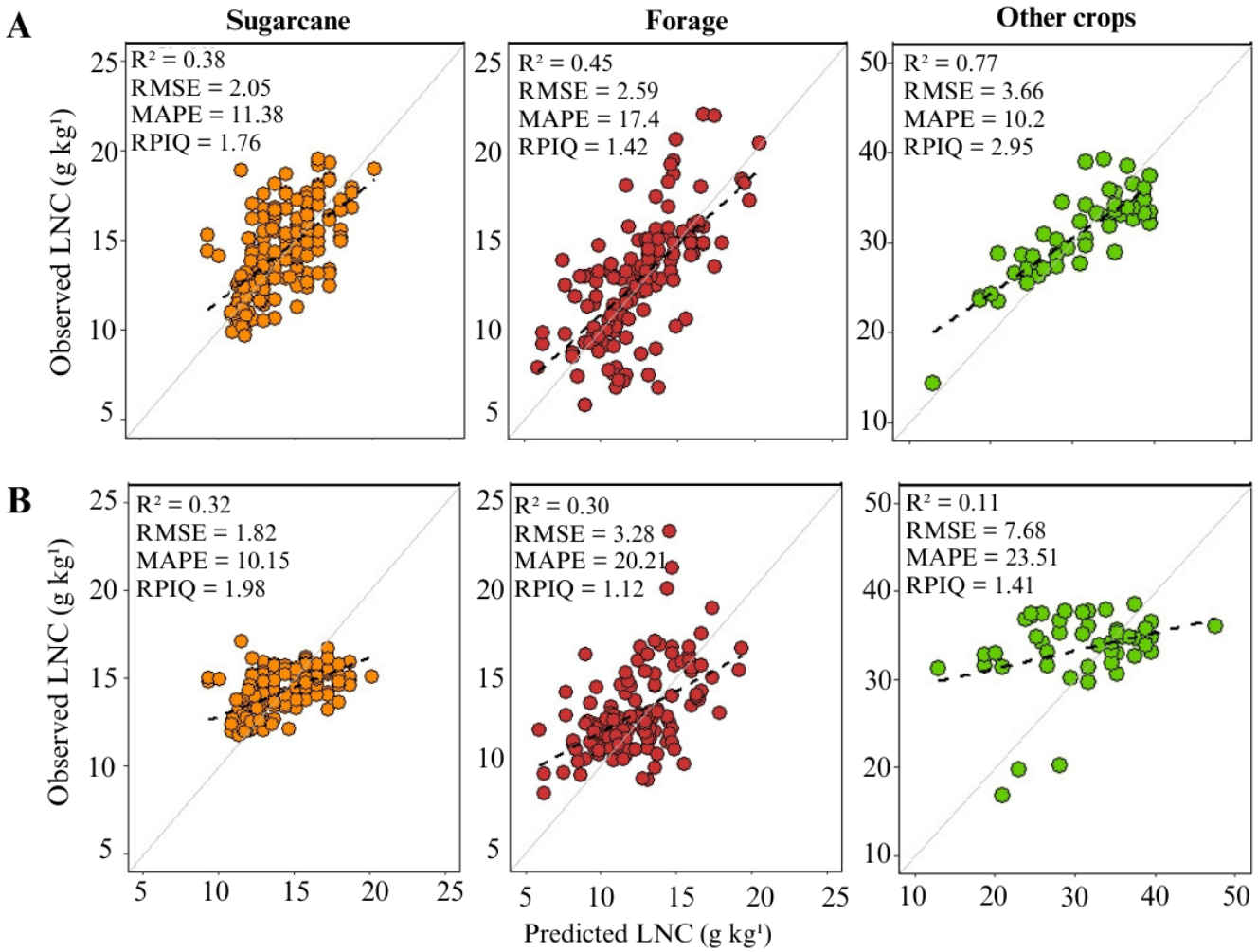

FIGURE 5 | Predictive performance of the models (A) PLSR and (B) RF in the generalization of leaf nitrogen content prediction for different crops: Sugarcane, forage, and the aggregated category "other crops".

to data heterogeneity and hinder model generalizations (Silva et al. 2024). Another important factor is the amount and types of data used in the model training process. In this case, the data used in the model calibration phase were from different crops with distinct spectral characteristics and nitrogen concentrations, even though 30% of the forage data were used to partially adjust the model to the new spectral conditions. Similar results have been found in previous studies, where the relationship between LNC varied between plant species and environmental conditions, resulting in low model generalization (Wan et al. 2022). However, we believe that as training data sets expand and more data from the same crop are obtained, model performance in terms of accuracy and generalization will improve (Silva et al. 2024; Wan et al. 2022; Wang et al. 2020). Given that the match between model complexity and training data volume is a crucial factor influencing model generalization (Huang et al. 2025).

3.6 | Generalization of Models Across Different Seasons

Figure 6 shows the generalization capacity of the RF model (Figure 6A) and PLSR model (Figure 6B) in predicting leaf nitrogen content in the forage crop, with evaluation conducted in three seasons: summer (February), autumn (April), and spring (October). The best predictive performance of the RF model was identified in April ($R^2 = 0.64$; RMSE = 2.09 g·kg^{-1}; MAPE = 11.25%; RPIQ = 1.84) and February ($R^2 = 0.50$; RMSE = 1.74 g·kg^{-1}; MAPE = 12.74%; RPIQ = 2.12). However, in spring (October), the performance of the model reduced significantly (RMSE = 3.50 g·kg^{-1}; MAPE = 41.07%; RPIQ = 1.46). For the PLSR model, in February and April, the model demonstrated good accuracy (RMSE = 1.70–1.87 g·kg^{-1}; MAPE = 9.93%–12.61%; RPIQ = 2.05–2.17), indicating good temporal robustness. However, in spring (October), the model's performance also reduced significantly (RMSE = 3.49 g·kg^{-1}; MAPE = 38.40%; RPIQ = 1.47), suggesting limitations in its generalization capacity for that time of year.

Seasonal dynamics significantly influenced model generalization for forage in both PLSR and RF, with model performance declining markedly during the October evaluation. This was likely due to shifts in canopy structure and physiological status, as the spectrum is sensitive to changes in biomass accumulation, vegetative tissue flow, and photosynthetic rates across seasons (Sbrissia et al. 2020). Models trained with data from periods of high vegetative activity (February and April) struggled

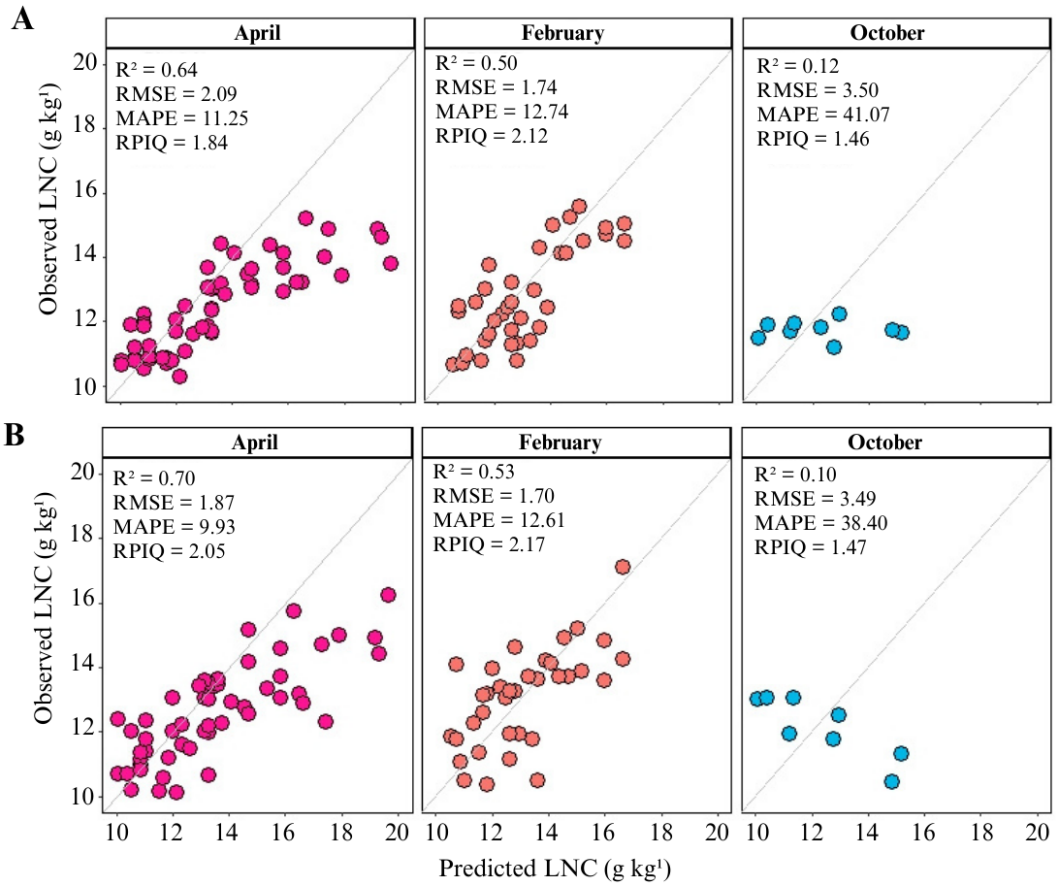

FIGURE 6 | Performance of the model (A) RF and (B) PLSR in generalization leaf nitrogen prediction for forage across different seasons (February, April, and October).

to predict spectral responses in October, when forage exhibited signs of senescence, including biomass reduction and chlorophyll degradation.

4 | Considerations

The spectra of preprocessed samples exhibited a similar pattern across all crops, with strong absorption in the blue and red regions and high reflectance in the red-edge and near-infrared bands. Unlike fresh leaves, the spectra from processed samples revealed consistent absorption features at 530 nm, 615 nm, and 670 nm, regardless of crop type or photosynthetic pathway. Future work should examine more crops to confirm if the patterns observed in this study are consistent in other species.

Our findings demonstrate that spectroscopic modeling combined with transfer learning is a promising approach for estimating foliar nitrogen concentration across multiple crop species. Nevertheless, even when using preprocessed samples, aspects such as phenological stage, crop species, and seasonal conditions must be considered to ensure model generalization. Based on these insights, we propose three alternatives for future research: (i) exploring other machine learning approaches, including deep learning, to improve predictive accuracy; (ii) enhancing the variability of calibration datasets and applying hybrid strategies, such as incorporating global samples and a small number of related observations or implementing spiking methods for model adjustment (Wijewardane et al. 2023); and (iii) developing crop-specific models, which may be more feasible when a wide range of spectral responses is available under different environmental and nutritional conditions.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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