Evaluating the Impact of Vegetation Indices on Plant Nitrogen Uptake Prediction: A Comparative Study of Regression Models at Various Growth Stages

Binita Ghimire[1], Lorena N. Lacerda[1] and Thirimachos Bourlai[2]

[1]Department of Crop and Soil Sciences, University of Georgia, Athens, GA 30602.

[2]School of Electrical and Computer Engineering, University of Georgia, Athens, GA 30602.

A paper from the Proceedings of the
16th International Conference on Precision Agriculture
21-24 July 2024
Manhattan, Kansas, United States

Abstract. *Nitrogen and water play crucial roles in impacting both the health and yield of corn crops. However, their demands vary under different soil and weather conditions. Unfortunately, current nitrogen management practices in irrigated fields in the state of Georgia overlook this variability. Thus, this oversight may lead to insufficient nitrogen application, causing plant stress or excessive nitrogen application that can lead to environmental impact. To address this challenge, a precise assessment of plant nitrogen uptake (PNU) at various critical stages of corn is essential. Therefore, this study exploited remote sensing technology using a multispectral sensor with five bandwidths to predict PNU at the V6, R1, and R6 corn growth stages. The experimental design was a split-plot randomized complete block with three replications, featuring main plots under four irrigation rates (100%, 120%, 50%, and rainfed) and subplots under six nitrogen rates (0, 67, 136, 202, 269, and 336 kg/ha). Multispectral imaging started at the sixth vegetative stage and continued weekly. On the same days, ground-based physiological parameters were collected using an LI600 porometer/fluorometer. Plant samples were collected at three distinct growth stages, coinciding with image collection. Analysis showed that various vegetation indices (VIs) demonstrated strong correlations with PNU. These VIs were refined through correlation analysis and Random Forest feature selection and then evaluated using linear regression (LR), multiple linear regression (MLR), and Random Forest regression (RFR) models to analysis their predictive ability for PNU. The findings indicate that the RFR model, which explained as high as 0.78 of the PNU variability at the V6 stage, consistently outperformed other models by effectively addressing the overfitting issues seen in simpler regression analyses. However, LR models displayed higher consistency between training and validation stages, making it simple and the best model in most of the stages. To predict PNU, CIG at the V6 stage, a combination of GNDVI and NDRE at the R1 stage, and CVI at the R6 stage were found to be most effective. This research emphasizes that identifying stage-specific indices and selecting suitable models are essential for accurately predicting PNU and improving in-season nitrogen*

management strategies.

Keywords. *Nitrogen Stress, Plant Nitrogen Uptake, Vegetation Indices (VIs), Machine Learning, Remote Sensing.*

Introduction

Nitrogen is an essential nutrient that significantly impacts both yield and quality of corn. Corn demands nitrogen in high levels, necessitating substantial applications of synthetic nitrogen fertilizers. Research by Zhang et al. (2023) and Ogola et al. (2002) showed that application of nitrogen fertilizer can increase corn yield by 43 to 68%. However, excessive nitrogen use not only contributes to economic inefficiency, but can lead to environmental issues like leaching of nitrates into waterways and the release of nitrous oxide, a potent greenhouse gas as highlighted by Zhang et al. (2023). Current nitrogen management practices in various agricultural regions of the United States including the state of Georgia, follow a one-size-fits-all approach based on yield goals (Morris et al. 2018; Sawyer et al. 2006). This method often overlooks local variations in landscape, soil texture, and irrigation practices, leading to nitrogen overapplication and environmental degradation. Studies highlight that the nitrogen use efficiency (NUE) in corn remains low, with only 30-40% of the applied nitrogen being utilized by the crop, while remaining nitrogen risks leaching into the environment (Hammad et al. 2020; Sharma and Bali 2018). Therefore, the management of nitrogen fertilizer applications presents a significant challenge. Moreover, studies by Ashraf et al. (2016) and Wang et al. (2017) showed that nitrogen uptake and its efficiency are heavily dependent on soil moisture conditions, further complicating nitrogen management (Ashraf et al. 2016; Wang et al. 2017).

To mitigate the environmental risk, and enhance NUE, it is important to optimize nitrogen application based on crop agronomic demands of the corn. This necessitates accurate prediction of plant nitrogen uptake (PNU) that maximizes yield and minimizes environmental risk. However, the spatial and temporal dynamics of corn nitrogen demand under various irrigation levels complicate the prediction of PNU through traditional methods of tissue sampling (Hammad et al. 2017). Such methods will be not only costly, labor-intensive but also provide only localized information that may not represent the entire field (Meisinger et al. 2008). Nevertheless, recent advances in remote sensing technology offer a promising alternative (Becker et al. 2020; Zhang et al. 2019). Multispectral sensors, which capture data across various bands of the electromagnetic spectrum, including visible (VIS; 400–700 nm), near-infrared (NIR; 400–700 nm), and red-edge (RE; 690–730 nm) bands, can detect within-field variability in nitrogen levels (Thompson and Puntel 2020). This approach is critical for refining nitrogen management strategies to address deficiencies effectively and optimize agricultural outputs.

Notably, vegetation indices (VIs) that incorporate the red-edge band have been proven most effective in identifying in-season nitrogen stress in corn, as highlighted by Li et al. (2014). However, the effectiveness of VIs in detecting nitrogen stress can vary depending on the growth stage (Burns et al. 2022). Determining PNU at critical stages of corn development is crucial, particularly at the vegetative stage 6 (V6), where plants have fully developed six leaves and begin significant nitrogen uptake; reproductive stage one (R1), or the silking stage, where plants enter the reproductive phase and demand substantial nitrogen for kernel development; and reproductive stage 6 (R6), the maturity phase, which reflects the total nitrogen required to achieve the intended yield (Nleya et al. 2016).

Regression modeling serves as a foundational method in predictive analytics and is widely used to estimate agricultural outcomes based on multiple variables (Jeong et al. 2016; Maulud and Abdulazeez 2020). Simple linear regression (LR), while straightforward, often falls short in agricultural applications where the relationships between variables are complex and interdependent (Gaso et al. 2019; Wei and Molin 2020). In such scenarios, multiple linear regression (MLR) becomes crucial as it can incorporate numerous predictors and capture their collective influence on a target variable (Mehnatkesh et al. 2012). However, its effectiveness diminishes when the interactions among variables are non-linear (Rajković et al. 2022).To address these limitations, more sophisticated approaches such as Random Forest regression (RFR) have gained importance (Jeong et al. 2016). This machine learning technique, which utilizes multiple decision trees, is particularly well-suited for agricultural data sets characterized by high-dimensional and non-linear relationships (Shaikh et al. 2022). Therefore, this study aims to compare performance of different regression analysis and assess the predictive capabilities of different VIs in detecting nitrogen variability and predicting PNU at the V6, R1, and R6 growth stages of corn.

Materials and Methods

Experimental Sites and Treatments

The field experiment was conducted at Iron Horse Farm (IHF) located in Greene County, Georgia (GA) (33° 43′ 36.84″ N, 83° 18′ 3.31″ W) in 2023. The research trial occupied an area of 1.6 ha with predominantly sandy clay loam soil, and organic matter ranging from 2 to 4%. The field was designed in a split-plot randomized complete block design, with irrigation levels as the main plot factor and nitrogen rates as the sub-plot factor with three replications (r=3). The irrigation treatment comprises four levels: full irrigation (FI) at 100%, 120% FI, 50% FI, and rainfed (0% FI) based on SI CropFit application recommendations for 100%. The nitrogen levels include six different rates: 0 kg N/ha- control, 67 kg N/ha, 135 kg N/ha, 202 kg N/ha, 269 kg N/ha, and 336 kg N/ha. The highest rate of nitrogen is based on an expected yield goal for Georgia of 250 bu/ac. Urea – ammonium nitrate (liquid, 32%) was applied in a split dose, 30% at planting and 70% at the V6 stage.

Data Collection

Spectral reflectance of the entire field was collected weekly from the V6 growth stage until the crop reached maturity using a multispectral RedEdge-P (AgEagle Aerial Systems Inc., Wichita, KS) sensor mounted on a DJI Matrice 300 RTK UAV (Da Jiang Innovations (DJI), Shenzhen, China). Images were captured within two hours before or after solar noon on sunny and cloud-free days. On the same days as the UAV flights, ground data were also collected using an LI-600 fluorometer/porometer (LI-COR Biosciences, Lincoln, NE) between 10 am and 2 pm. Measurements were taken from three plants per plot from the fully developed uppermost leaf and averaged to obtain one measurement per plot. The LI-600 parameters analyzed in this study were the electron transfer rate (ETR), and the quantum efficiency of photosystem II (PhiPS2) at the leaf level.

Similarly, UAV flights were also conducted at the V6, R1, and R6 growth stages during plant sample data collection. Six plants per plot were collected at these three critical growth stages and sent to a laboratory for leaf tissue nitrogen concentration analysis. Results were used to calculate the total plant N concentration (PNC). PNC was further multiplied by above ground biomass (ABG) for each plot at each stage to calculate PNU (eq. 1).

$$PNU = PNC * AGB \tag{1}$$

Data Analysis

Analysis of remotely sensed data to observe its ability to detect nitrogen variability in the irrigated corn field conditions, validations with ground data and PNU predictions includes following steps:

1) Processing of multispectral images and VIs calculation
2) Spearman correlation analysis between ground-collected data and VIs
3) Random Forest feature selection
4) Regression analysis to predict PNU using selected VIs
    a. Linear regression model (LR)
    b. Multivariate linear model (MLR)
    c. Random Forest regression model

1. *Processing of Multispectral Images and Calculation of Vegetation Indices (VIs):*

Multispectral images collected weekly at various growth stages were stitched using Pix4D software (Pix4D, Lausanne, Switzerland). Subsequently, a random forest algorithm from the FieldimageR package in R (Matias et al. 2020) was used for soil pixel removal from each single band. Based on the literature, twelve VIs were selected and calculated (Table 1) based on their ability in detecting chlorophyll content and nitrogen variability in plants. The indices were extracted specifically from the central four rows of each plot, applying a 1.2-meter negative buffer from the plot boundaries to avoid edge effects.

Table 1: List of Vegetation Indices calculated in this study.

VIs	Name	Formula	Source
NDVI	Normalized Difference Vegetation Index	(NIR - R) / (NIR + R)	(Jw 1973; Tucker 1980)
NDRE	Normalized Difference Red Edge	(NIR -RE) / (NIR + RE)	(Gitelson and Merzlyak 1994)
GNDVI	Green Normalized Difference Vegetation Index	NIR-G/NIR + G	(Gitelson and Merzlyak 1997)
BNDVI	Blue Normalized Difference Vegetation Index	NIR-B/NIR + B	(Beisel et al. 2018)
CIG	Chlorophyll Index - Green	(NIR/G) – 1	(Gitelson, Gritz, et al. 2003)
CIRE	Chlorophyll Index-red edge	(NIR/RE) – 1	(Gitelson et al. 2005; Gitelson, Viña, et al. 2003)
MTCI	MERIS Terrestrial Chlorophyll Index	(NIR − RE)/(RE − R)	(Dash and Curran 2004)
CVI	Chlorophyll Vegetation Index	(NIR*R)/(G^2)	(Vincini et al. 2008)
DVI	Difference Vegetation Index	NIR-RE	(Tucker 1979)
EVI	Enhanced vegetation Index	2.5*(NIR-R)/(NIR + 6*R-7.5*B +1)	(Matsushita et al. 2007)
MSAVI	Modified Soil-Adjusted Vegetation Index	(2* NIR + 1 - sqrt((2 * NIR + 1)^2- 8 * (NIR - R))) /2	(Qi et al. 1994)
RDVI	Renormalized Difference Vegetation Index	(NIR - R) / sqrt(NIR + R)	(Roujean and Breon 1995)

NIR: Near Infra-red; R: Red; RE: Red-Edge; G: Green; B: Blue, bands

Spearman Correlation Analysis

A Spearman correlation analysis was conducted using R at a significance level of 0.05 between ETR and PhiPS2 and VIs in different days after planting (DAP). Similarly, correlation analysis of VIs and PNU was done at three growth stage V6, R1 and R6 separately to identify the most effective VIs for predicting PNU.

Feature Selection

VIs demonstrating a significant correlation above 0.5 with PNU at each stage were subjected to feature selection using the Random Forest algorithm, which is built on classification and regression trees using R. This algorithm iteratively selects and evaluates layers of features, discarding the least important ones and refining the selection to the most significant variables. This process helps in selecting the most predictive VIs for different growth stages for further regression analysis.

Regression Analysis

The selected VIs were used to build regression models for the stages V6, R1, and R6. The dataset, comprising data from 72 plots, was split into a training set (70%) and a validation set (30%) for each stage. Various models, including LR, MLR and RFR, were evaluated based on their predictive accuracy using metrics such as R^2 (Determination Coefficient) (Equation 2), which measures the proportion of variance in the dependent variable predictable from the independent variable(s), RMSE (Root Mean Square Error) (Equation 3) , which quantifies the average magnitude of the prediction errors and the Willmott's Agreement Index (WAI) (Equation 4), which ranges from 0 (no agreement) to 1 (perfect agreement), indicating the accuracy of the model predictions relative to observed data. Regressions were performed in R using the tidyverse package (Wickham and Wickham 2017).

$$R^2 = \frac{\sum_{t=1}^{N}(Yest - \bar{Y})}{(Yobs - \bar{Y})} \tag{1}$$

$$\text{RMSE} = \sqrt{\frac{\sum_{t=1}^{N}(Yobs\ -\ Yest)^2}{n}} \quad (2)$$

$$\text{d} = 1 - \frac{\sum_{t=1}^{N}(Yobs - Yest)^2}{\sum_{t=1}^{N}(|Yest - \bar{Y}| + (|Yobs - \bar{Y}|)} \quad (3)$$

where R^2 is the determination coefficient, RMSE is the root mean square of the average error and d is the Willmott index coefficient. Yobs indicates the observed values, Yest indicates estimated values by the models, n is the total number of data analyzed and $\bar{Y}$ is the average value of the estimated variable.

Linear Regression

Based on the random forest feature selection, the six most important VIs were selected for each stage. LR was run for each VI with PNU to identify which VIs were most significant at different stages to predict PNU.

Multiple Linear Regression

To determine if additional VIs could enhance predictive accuracy, we employed MLR models. Five models were sequentially run to evaluate the impact of systematically reducing the number of VIs based on their importance. The first model incorporated all six VIs that has high importance in feature selection analysis. In each subsequent model one input variable was removed based on the lowest importance value. This approach helped to identify the optimal number of VIs necessary for accurate predictions and to assess the impact of each VI's removal on model performance, providing insights into the relative importance and contribution of each VI.

Random Forest Regression

A more sophisticated technique, RFR, was utilized to refine prediction models further across the three growth stages. This method leverages multiple decision trees to improve prediction accuracy. It involved running five models, each analyzing different combinations of the top six VIs, mirroring the approach used in the multiple regression models. Given the small dataset, leave-one-out cross-validation was applied to increase accuracy. The optimal values for 'mtry' and 'tree' parameters were determined based on a grid search, selecting the configuration with the highest R^2 value. This parallel analysis between RFR and MLR models allowed for a robust comparison of methodologies in handling variable importance and model simplification.

Results and Discussion

The Spearman correlation analysis was conducted to evaluate the correlation between multispectral sensor-derived VIs and parameters that can indicate corn nitrogen stress including PNU at three distinct growth stages (V6, R1, and R6) and photosynthetic parameters (ETR and PhiPS2) at eight different dates after planting (36, 43, 54, 63, 69, 75, 82 and 97 DAP). The results shown in Figures 1 indicate significant correlations between most of the VIs and PNU across these stages. At the V6 stage, out of 12 VIs, 10 exhibited high and significant correlations ($r > 0.63$) with PNU, with the DVI showing the highest correlation ($r = 0.79$). DVI is the combination of NIR and Red-edge band and at an early stage when canopy is not fully closed, DVI can detect small difference in plants nitrogen conditions due to its strong relation with chlorophyll content (Hua and Zhao, 2021). However, this correlation decreases at the R1 stage when plants reach full development, indicating a decreased sensitivity of DVI during the mid-growth stage. The correlation again increases at the maturity stage, suggesting DVI’s sensitivity to detecting difference in chlorophyll content during the stage of leaf senescence, where total NIR reflectance decreased with increase in background exposure (Knipling, 1970). Similar pattern is seen in other indices that have NIR band such as NDVI, NDRE, GNDVI, CIG, CIRE, EVI, and MSAVI. Further, a study by Li et al. (2014) also found that red-edge-based indices are better for estimating PNU as they are more sensitive to the differences in chlorophyll. In contrast, indices such CVI and BNDVI, which rely on visible light, show lower correlations with PNU in early growth stages. This is likely because the photosynthetic pigments, which heavily influence these visible indices, have not yet fully developed to their potential, making them less sensitive to variations in plant conditions. As corn matures and reaches the R1 stage, the BNDVI index shows the highest correlation (r = 0.65), indicating sensitivity to the decrease in reflectance at the blue band due to increased absorption of blue light by the higher chlorophyll content and denser canopy. At the R6 stage, the CVI showed the highest correlation (r = 0.76) with PNU.

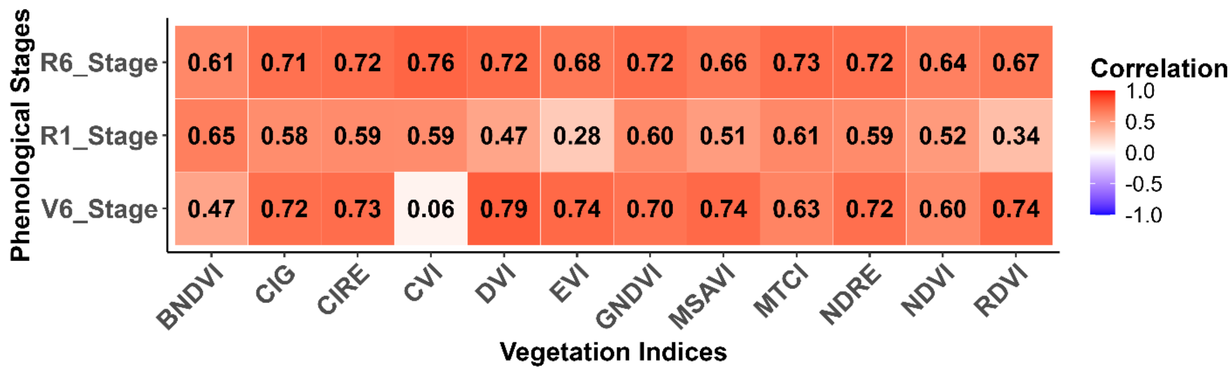

Fig. 1. Spearman correlation analysis between vegetation indices (VIs) and plant nitrogen Uptake (PNU) by growth stages

Correlation analysis between physiological parameters (ETR and PhiPS2) and multiple VIs are shown across multiple DAP (Fig. 3). At 36 DAP, an early growth stage, CIG show moderate correlations (r = 0.46) with PhiPS2, suggesting their potential predictive value during initial growth. However, at this stage, most VIs display weaker correlations with both ETR and PhiPS2. During the intermediate stages (43, 54, 63, 69, 75, and 82 DAP), lower correlation values are predominant. At 54 DAP, except for DVI and ETR, most VIs show negligible to no correlation with either physiological parameter, indicating a low chance of predictive capability during theses stages. Increased correlations occur at 97 DAP, a later growth stage among various indices with both ETR and PhiPS2. DVI and SAVI consistently demonstrated low correlations across most

DAPs, suggesting minimal predictive utility for both ETR and PhiPS2.These results highlight that VIs based on multispectral data may not effectively capture small changes in physiological parameters, which are highly dynamic with micro-climate conditions.

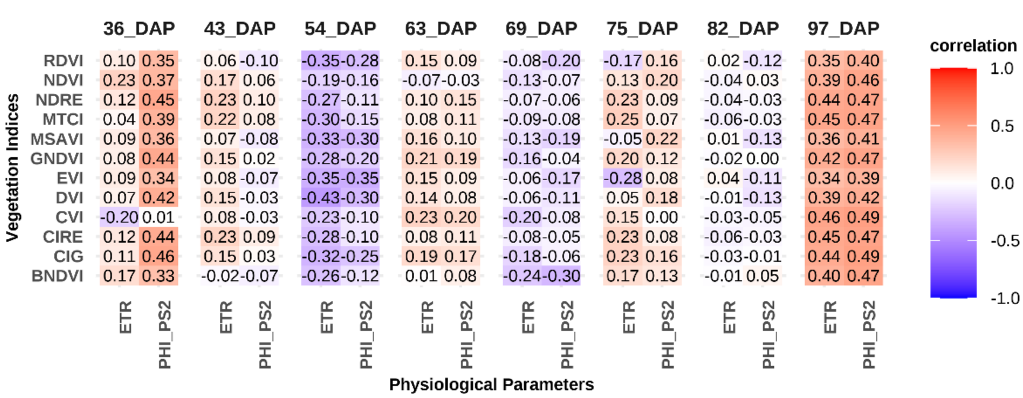

Fig. 2. Spearman correlation analysis between vegetation indices (VIs) and plant physiological parameters ETR (electron transfer rate) and PhiPS2 (quantum efficiency of photosystem II)

Random Forest Feature Selection Algorithm

The random forest feature selection algorithm applied to VIs with correlation values greater than 0.5 with PNU at different crop growth stages are presented in Figure 2. Results showed that at the V6 stage DVI, and CIG were identified as the most important, each achieving importance values greater than 12, suggesting their strong predictive power in early growth stages. These indices captured distinct aspects of plant health and vigor, potentially linked to the nitrogen status of the plants at this early vegetative stage (Burns et al. 2022; Li et al. 2014) . The MTCI was deemed the least important index, indicating lesser contribution in nitrogen uptake estimation at early stages.

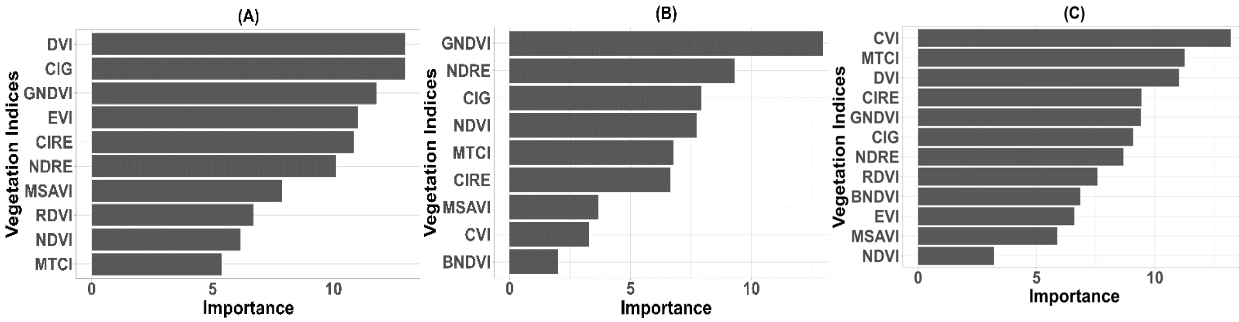

Fig. 3. Random Forest feature selection of highly correlated vegetation indices with plant nitrogen uptake at A) V6 B) R1 C) R6 Phenological Stage

At the R1 stage, GNDVI emerged as the most significant, exceeding an importance value of 10, and NDRE closely following at approximately 9, highlighting their utility in reflecting PNU variability during the onset of the reproductive stage. BNDVI, CVI and MSAVI are the least important variables in PNU estimation with importance values lower than 5. At the R6 stage CVI, MTCI, and DVI were highlighted as the top indices showing their potential effectiveness in capturing critical information on PNU at a mature stage of growth, each with importance values above 10, while NDVI was the least important with a value lower than 5.

The observed variability in the importance rankings of VIs across different growth stages highlights the dynamic nature of plant development and the consequential difference in the selection of these indices. This variability indicates the necessity for developing a stage-specific predictive model for PNU, which facilitates more precise and timely agricultural decision-making based on multispectral data. Similar results were observed by Barzin et al. (2020), which showed that the influence of VIs in the ability to predicting yield also varies with phenological stage of corn.

Regression Models for PNU

Linear Regression Model

Linear regression models were developed to analyze the relationship between VIs and their ability to explain the variation in PNU at different growth stages of corn (Table 2). At the V6 stage, CIG had the strongest relationship with PNU, achieving a consistent coefficient of determination (R^2) of 0.60 for both training and validation, closely followed by GNDVI with an R^2 of 0.58 for training and 0.59 for validation. Both have a high WAI of 0.86 for validation. Additionally, NDRE and CIRE also showed high R^2 values of 0.61. All VIs at R1 stage showed relatively lower R^2 compared to V6 and R6, ranging from 0.35 to 0.36 for training and up to 0.48 for validation. The WAI also shows a pattern of improvement from training to validation, suggesting that despite lower R^2 values, the models' predictions align reasonably well with actual data trends when tested against unseen data. In the R6 stage, CVI stood out as the strongest predictor, with an R^2 value of 0.61 for training and 0.72 for validation. MTCI also demonstrated high predictive strength with R^2 values of 0.71, followed closely by CIRE and GNDVI, each with an R^2 of 0.70. Similarly, high WAI values (0.84 to 0.90) across the board underscore strong agreement between the models' predictions and observed data, highlighting the models' reliability at the R6 stage. Moreover, the observed trends suggest that the predictive power of VIs increases with the maturity of the plants, with higher performance noted particularly at the R6 stage. The increased ability of models in explaining the variation in PNU in the validation dataset at R6 compared to the training might be a result of model overfitting due to the low number of validation data points. The models should be tested again with a bigger dataset to confirm the model's performance.

Table 2. Linear regression model performance metrics for plant nitrogen uptake (Kg/ha) with different vegetation indices at critical phenological stages (VIs).

Stage	Input Variables	R^2	RMSE	Willmott Agreement	R^2	RMSE	Willmott Agreement
		Training			Validation		
V6	DVI	0.50	3.53	0.81	0.56	4.02	0.83
	CIG	0.60	3.18	0.86	0.60	3.81	0.86
	GNDVI	0.58	3.23	0.85	0.59	3.87	0.86
	EVI	0.39	3.91	0.74	0.54	4.09	0.85
	CIRE	0.52	3.46	0.82	0.61	3.76	0.86
	NDRE	0.53	3.43	0.83	0.61	3.77	0.87
R1	GNDVI	0.35	31.28	0.71	0.47	29.58	0.83
	NDRE	0.36	31.11	0.71	0.47	29.64	0.82
	CIG	0.36	30.94	0.72	0.48	29.39	0.83
	NDVI	0.35	31.32	0.71	0.31	33.80	0.74
	MTCI	0.35	31.35	0.70	0.45	30.11	0.82
	CIRE	0.36	30.89	0.72	0.46	29.75	0.82
R6	CVI	0.61	34.18	0.87	0.72	39.38	0.90
	MTCI	0.57	35.67	0.85	0.71	40.13	0.90
	DVI	0.54	37.06	0.83	0.68	41.93	0.90
	CIRE	0.56	36.35	0.84	0.70	40.94	0.89
	GNDVI	0.56	36.32	0.84	0.70	40.53	0.90
	CIG	0.55	36.83	0.84	0.69	41.17	0.89

Multiple Linear Regression Model

Five different MLR models were analyzed to investigate the best combination of VIs to predict PNU at different stages of plant growth (Table 3). At the V6 stage, as the number of input variables decreases, the models show a general trend of improving R^2 values in validation, increasing from 0.45 to 0.59, which suggests that simpler models (using fewer VIs) maintain or enhance prediction accuracy.

Table 3: Multiple regression model performance metrics for plant nitrogen uptake (Kg/ha) with different vegetation indices (VIs) at different phenological stages

Stage	Input Variables	R^2	RMSE	Willmott Agreement	R^2	RMSE	Willmott Agreement
		Training			Validation		
V6	DVI, CIG, GNDVI, EVI, CIRE, NDRE	0.58	3.02	0.88	0.45	3.78	0.88
	DVI, CIG, GNDVI, EVI, CIRE	0.58	3.07	0.87	0.53	3.60	0.88
	DVI, CIG, GNDVI, EVI,	0.59	3.07	0.87	0.56	3.59	0.89
	DVI, CIG, GNDVI	0.60	3.08	0.87	0.57	3.68	0.88
	DVI, CIG	0.61	3.08	0.87	0.59	3.67	0.88
R1	GNDVI, NDRE, CIG, NDVI, MTCI, CIRE	0.30	30.29	0.74	0.21	30.54	0.80
	GNDVI, NDRE, CIG, NDVI, MTCI	0.32	30.30	0.74	0.26	30.44	0.80
	GNDVI, NDRE, CIG, NDVI	0.33	30.31	0.74	0.30	30.53	0.80
	GNDVI, NDRE, CIG	0.34	30.52	0.73	0.36	30.03	0.81
	GNDVI, NDRE	0.33	31.08	0.71	0.42	29.49	0.83
R6	CVI, MTCI, DVI, CIRE, GNDVI, CIG	0.67	29.43	0.91	0.38	49.53	0.87
	CVI, MTCI, DVI, CIRE, GNDVI	0.67	29.74	0.91	0.45	48.14	0.88
	CVI, MTCI, DVI, CIRE	0.68	29.75	0.91	0.49	47.96	0.88
	CVI, MTCI, DVI	0.59	33.88	0.87	0.66	40.00	0.90
	CVI, MTCI	0.60	33.92	0.87	0.66	41.13	0.89

The model using DVI and CIG as input variables showed the best performance. It has the highest R^2 and displays consistency between training and validation tests, with R^2 values of 0.61 for training and 0.59 for validation, demonstrating that it generalizes well to new data without overfitting. At the R1 stage, reducing the number of VIs consistently improved the validation R^2 from 0.21 to 0.42, suggesting that models with fewer variables are more effective at this stage. The reduction of VIs also resulted in lower RMSE for validation, decreasing to 29.49 kg/ha, which reflects improved model accuracy with simpler input configurations. WAI increases as the number of VIs decreases, peaking at 0.83, indicating that the model's predictions align better with the actual data as complexity is reduced. The model featuring GNDVI and NDRE as inputs showed the best validation performance. At R6 stage, the model using CVI, MTCI, and DVI provided the best balance between complexity and performance. It achieved R^2 values of 0.59 for training and 0.66 for validation, coupled with an RMSE of 40.00 kg/ha and WAI of 0.90. These metrics suggest high predictive accuracy and reliability. Despite a slight increase in RMSE from training to validation (33.88 to 40.00), this model maintains high agreement (WA).

At each stage, the models with fewer VIs tended to show a better overall performance, indicating that simpler models often provide better or comparable predictive power and generalization capabilities. This pattern is particularly evident at stages R1 and R6, where reducing the number of VIs significantly improved validation metrics.

Random Forest Regression Model

Results for RFRs showed that all models at V6 showed a strong fit, maintained a high R^2 for training (0.90). While all models at V6 maintained a high R^2 during training, validation R^2 values ranged from 0.74 to 0.78. Notably, there is a reduction in RMSE when the model complexity is decreased to four VIs (DVI, CIG, GNDVI, EVI), suggesting an optimal balance between model complexity and predictive power. This specific combination achieved the best validation performance, marked by the lowest RMSE (3.01 kg/ha) and the highest R^2 (0.78), effectively capturing the essential features required for accurate predictions at this stage. However, a further simplified model using just DVI and CIG also showed promising results, with the same RMSE (3.01 kg/ha) and a slightly lower R^2 (0.76), offering a good trade-off between model simplicity and accuracy.

Table 4. Random Forest regression model performance metrics for plant nitrogen uptake (kg/ha) with different vegetation indices (VIs) at different phenological stages

Stage	Input Variables	R^2	RMSE	Willmott Agreement	R^2	RMSE	Willmott Agreement
			Training			Validation	
V6	DVI, CIG, GNDVI, EVI , CIRE , NDRE	0.90	1.71	0.96	0.74	3.18	0.90
	DVI , CIG , GNDVI, EVI , CIRE	0.90	1.73	0.96	0.77	3.06	0.91
	DVI , CIG , GNDVI, EVI	0.90	1.74	0.96	0.78	3.01	0.91
	DVI , CIG , GNDVI	0.90	1.78	0.96	0.76	3.10	0.91
	DVI , CIG	0.90	1.72	0.96	0.77	3.01	0.92
R1	GNDVI, NDRE, CIG, NDVI, MTCI, CIRE	0.88	15.07	0.94	0.53	33.99	0.78
	GNDVI, NDRE, CIG, NDVI, MTCI	0.88	15.26	0.95	0.55	33.64	0.79
	GNDVI, NDRE, CIG, NDVI	0.88	15.21	0.95	0.54	32.87	0.78
	GNDVI, NDRE, CIG	0.86	16.02	0.94	0.57	31.03	0.82
	GNDVI, NDRE	0.87	15.47	0.95	0.54	30.96	0.82
R6	CVI, MTCI, DVI, CIRE, GNDVI, CIG	0.90	18.39	0.97	0.71	42.69	0.88
	CVI, MTCI, DVI, CIRE, GNDVI	0.90	18.39	0.97	0.73	41.62	0.88
	CVI, MTCI, DVI, CIRE	0.90	18.75	0.96	0.75	40.57	0.89
	CVI, MTCI, DVI	0.90	18.70	0.96	0.73	41.52	0.89
	CVI, MTCI	0.89	19.07	0.95	0.70	43.99	0.86

A general trend was observed at R1, where reducing the number of VIs from six to three improved the validation RMSE and maintained or slightly enhanced the R^2. The simplest model, using GNDVI and NDRE, had the best RMSE (30.96 kg/ha) for validation. However, the model that included GNDVI, NDRE, and CIG showed the best R^2 (0.57) for validation, with more consistent R^2 values between training and validation compared to other configurations. This model offered a better balance between consistency, complexity, and accuracy. At the R6 stage, all models showed similar R^2 values for training showing good model fit. While analyzing the validation data, the model featuring CVI, MTCI, DVI, and CIRE showed the best performance, with the lowest RMSE (40.57 kg/ha) and highest R^2 (0.75).

Across all stages, there is a clear trend where models with fewer input variables tend to perform equally well or better for validation than their more complex counterparts. Contrasting these observations, Jang et al. (2024) reported that multivariate analysis provided greater accuracy than univariate analysis in predicting yield variability under different nitrogen applications. This finding suggests that while incorporating additional VIs might seem beneficial, it does not necessarily enhance model performance and may contribute to overfitting. Importantly, each developmental stage of vegetation monitoring appears to have an optimal VI configuration that maximizes model efficacy, underscoring the importance of adopting stage-specific modeling strategies. Similarly, research conducted by Shrestha et al. (2023) on corn yield prediction aligns with these insights. Their study highlighted the dynamic nature of canopy reflectance and emphasized the critical role of accounting for growth stages and environmental conditions to achieve accurate predictions.

Moreover, although RFR models exhibit strong performance during training, the accuracy levels during validation varied significantly. This variation underscores the necessity of selecting model complexities judiciously to ensure both generalizability and high predictive performance across different plant growth stages.

Comparative Analysis of Linear Regression, Multiple Linear Regression, and Random Forest Regression Models

This study evaluated the performance of three different regression models—LR, MLR, and RFR—across various plant growth stages using selected VIs to predict physiological nitrogen uptake (PNU). Overall, the RFR models generally delivered superior predictive performance.

At the V6 stage, the LR model utilizing the CIG VI outperformed the simplest MLR model, which incorporated both DVI and CIG VIs. The addition of DVI did not significantly enhance PNU prediction, rendering the single VI LR model with CIG as the superior performer at this stage. Despite the strong performance of the LR model ($R^2 = 0.60$), the least effective RFR model still surpasses even the best LR model. Once again, at R1 stage the LR model performed better than the MLR models. The CIG VI, with an R^2 of 0.48 in validation, performed well. However, the RFR model, which includes GNDVI and NDRE, proved to be superior, achieving a higher R^2 of 0.57 in validation. At maturity, the RFR model, which uses CVI, MTCI, DVI, and CIRE, achieved the highest accuracy (R^2 of 0.90 in training and 0.75 in validation) and the lowest RMSE (40.57 in validation). Although the RFR model rates highly in terms of R^2 and RMSE, significant variation between these metrics in validation data suggests a decrease in model reliability under validation conditions.

Conclusion

The study utilized twelve multispectral sensor-based VIs calculated using different combination of green, blue, and red, NIR and red edge. Spearman correlation analysis of VIs with physiological parameters showed correlation can be seen at early and late stages. Higher number of VIs are correlated with physiological parameters and PNU at maturity. Especially with PNU, DVI is highly correlated at V6 stage, BNDVI at R1 stage and CVI at R6 stage. Further, correlation analysis and random forest feature selection underscore the importance of selecting stage-specific VIs for precise predictions, highlighting the distinct predictive capabilities of CIG at the V6 stage, GNDVI and NDRE at the R1 stage, and CVI at the R6 stage.

The findings revealed that while the RFR model generally provided superior predictive performance, its effectiveness was hindered by the relatively small dataset of just 72 samples for each stage. This small sample size was not sufficient to fully leverage the potential of machine learning models, which typically require larger datasets to capture and generalize complex interactions effectively. While the current study provides valuable insights into the specific VIs that are effective at different growth stages, it also highlights the challenges faced when working with limited datasets in complex predictive modeling scenarios. This studied in being repeat in two separate locations to test the robustness of the predictive models.

Acknowledgement

We would like to acknowledge and thank the University of Georgia Iron Horse Plant Science Farm staff for assisting with field management, and João Gabriel Santos and Sandesh Shrestha for their contributions with data collection.

References

Ashraf, U., Salim, M. N., Sher, A., Sabir, S., Khan, A., Pan, S., & Tang, X. (2016). MAIZE GROWTH, YIELD FORMATION AND WATER-NITROGEN USAGE IN RESPONSE TO VARIED IRRIGATION AND NITROGEN SUPPLY UNDER SEMI-ARID CLIMATE. *Turkish Journal Of Field Crops*, *21*(1), 88–96. https://doi.org/10.17557/tjfc.93898

Barzin, R., Pathak, R., Lotfi, H., Varco, J., & Bora, G. C. (2020). Use of UAS Multispectral Imagery at Different Physiological Stages for Yield Prediction and Input Resource Optimization in Corn. *Remote Sensing*, *12*(15), 2392. https://doi.org/10.3390/rs12152392

Becker, T., Nelsen, T. S., Leinfelder-Miles, M., & Lundy, M. E. (2020). Differentiating between Nitrogen and Water Deficiency in Irrigated Maize Using a UAV-Based Multi-Spectral Camera. *Agronomy*, *10*(11), 1671. https://doi.org/10.3390/agronomy10111671

Beisel, N. S., Callaham, J. B., Sng, N. J., Taylor, D. J., Paul, A.-L., & Ferl, R. J. (2018). Utilization of single-image normalized difference vegetation index (SI-NDVI) for early plant stress detection. *Applications in Plant Sciences*, *6*(10), e01186. https://doi.org/10.1002/aps3.1186

Burns, B. W., Green, V. S., Hashem, A. A., Massey, J. H., Shew, A. M., Adviento-Borbe, M. A. A., & Milad, M. (2022a). Determining nitrogen deficiencies for maize using various remote sensing indices. *Precision Agriculture*, *23*(3), 791–811. https://doi.org/10.1007/s11119-021-09861-4

Dash, J., & Curran, P. J. (2004). The MERIS terrestrial chlorophyll index.

Gaso, D. V., Berger, A. G., & Ciganda, V. S. (2019). Predicting wheat grain yield and spatial variability at field scale using a simple regression or a crop model in conjunction with Landsat images. *Computers and Electronics in Agriculture*, *159*, 75–83. https://doi.org/10.1016/j.compag.2019.02.026

Gitelson, A. A., Gritz, Y., & Merzlyak, M. N. (2003). Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *Journal of plant physiology*, *160*(3), 271–282.

Gitelson, A. A., & Merzlyak, M. N. (1997). Remote estimation of chlorophyll content in higher plant leaves. *International journal of remote sensing*, *18*(12), 2691–2697.

Gitelson, A. A., Viña, A., Arkebauer, T. J., Rundquist, D. C., Keydan, G., & Leavitt, B. (2003). Remote estimation of leaf area index and green leaf biomass in maize canopies. *Geophysical research letters*, *30*(5).

Gitelson, A. A., Viña, A., Ciganda, V., Rundquist, D. C., & Arkebauer, T. J. (2005). Remote estimation of canopy chlorophyll content in crops. *Geophysical research letters*, *32*(8).

Gitelson, & Merzlyak, M. N. (1994). Quantitative estimation of chlorophyll-a using reflectance spectra: Experiments with autumn chestnut and maple leaves. *Journal of Photochemistry and Photobiology B: Biology*, *22*(3), 247–252.

Hammad, H. M., Abbas, F., Ahmad, A., Bakhat, H. F., Farhad, W., Wilkerson, C. J., et al. (2020). Predicting Kernel Growth of Maize under Controlled Water and Nitrogen Applications. *International Journal of Plant Production*, *14*(4), 609–620. https://doi.org/10.1007/s42106-020-00110-8

Hammad, H. M., Farhad, W., Abbas, F., Fahad, S., Saeed, S., Nasim, W., & Bakhat, H. F. (2017). Maize plant nitrogen uptake dynamics at limited irrigation water and nitrogen. *Environmental Science and Pollution Research*, *24*(3), 2549–2557. https://doi.org/10.1007/s11356-016-8031-0

Hua, Y., & Zhao, X. (2021). Multi-Model Estimation of Forest Canopy Closure by Using Red Edge Bands Based on Sentinel-2 Images. *Forests*, *12*(12), 1768. https://doi.org/10.3390/f12121768

Jang, C., Namoi, N., Wolske, E., Wasonga, D., Behnke, G., Bowman, N. D., & Lee, D. K. (2024). Integrating plant morphological traits with remote-sensed multispectral imageries for accurate corn grain yield prediction. *PLOS ONE*, *19*(4), e0297027. https://doi.org/10.1371/journal.pone.0297027

Jeong, J. H., Resop, J. P., Mueller, N. D., Fleisher, D. H., Yun, K., Butler, E. E., et al. (2016). Random forests for global and regional crop yield predictions. *PloS one*, *11*(6), e0156571.

Jw, R. (1973). Monitoring vegetation systems in the great plains with ERTS. In *Third NASA Earth Resources Technology Satellite Symposium, 1973* (Vol. 1, pp. 309–317).

Knipling, E. B. (1970). Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. *Remote Sensing of Environment*, *1*(3), 155–159. https://doi.org/10.1016/S0034-4257(70)80021-9

Li, F., Miao, Y., Feng, G., Yuan, F., Yue, S., Gao, X., et al. (2014). Improving estimation of summer maize nitrogen status with red edge-based spectral vegetation indices. *Field Crops Research*, *157*. https://doi.org/10.1016/j.fcr.2013.12.018

Matias, F. I., Caraza-Harter, M. V., & Endelman, J. B. (2020). FIELDimageR: An R package to analyze orthomosaic images from agricultural field trials. *The Plant Phenome Journal*, *3*(1), e20005.

Matsushita, B., Yang, W., Chen, J., Onda, Y., & Qiu, G. (2007). Sensitivity of the enhanced vegetation index (EVI) and normalized difference vegetation index (NDVI) to topographic effects: a case study in high-density cypress forest. *Sensors*, *7*(11), 2636–2651.

Maulud, D., & Abdulazeez, A. M. (2020). A review on linear regression comprehensive in machine learning. *Journal of Applied Science and Technology Trends*, *1*(2), 140–147.

Mehnatkesh, A., Ayoubi, S., Jalalian, A., & Dehghani, A. A. (2012). Prediction of rainfed wheat grain yield and biomass using artificial neural networks and multiple linear regressions and determination the most factors by sensitivity analysis.

Meisinger, J. J., Schepers, J. S., & Raun, W. R. (2008). Crop Nitrogen Requirement and Fertilization. In *Nitrogen in Agricultural Systems* (pp. 563–612). John Wiley & Sons, Ltd. https://doi.org/10.2134/agronmonogr49.c14

Morris, T. F., Murrell, T. S., Beegle, D. B., Camberato, J. J., Ferguson, R. B., Grove, J., et al. (2018). Strengths and Limitations of Nitrogen Rate Recommendations for Corn and Opportunities for Improvement. *Agronomy Journal*, *110*(1), 1–37. https://doi.org/10.2134/agronj2017.02.0112

Nleya, T., Chung, C., and Kleinjan, J. (2016). Chapter 5: Corn Growth and Development. In Clay, D.E., Carlson, C.G., Clay, S.A. and Byamukama, E. (eds). iGrow Corn: best Management Practices. South Dakota State University.

Ogola, J. B. O., Wheeler, T. R., & Harris, P. M. (2002). Effects of nitrogen and irrigation on water use of maize crops. *Field Crops Research*, *78*(2), 105–117. https://doi.org/10.1016/S0378-

4290(02)00116-8

Qi, J., Chehbouni, A., Huete, A. R., Kerr, Y. H., & Sorooshian, S. (1994). A modified soil adjusted vegetation index. *Remote sensing of environment*, *48*(2), 119–126.

Rajković, D., Marjanović Jeromela, A., Pezo, L., Lončar, B., Zanetti, F., Monti, A., & Kondić Špika, A. (2022). Yield and Quality Prediction of Winter Rapeseed—Artificial Neural Network and Random Forest Models. *Agronomy*, *12*(1), 58. https://doi.org/10.3390/agronomy12010058

Roujean, J.-L., & Breon, F.-M. (1995). Estimating PAR absorbed by vegetation from bidirectional reflectance measurements. *Remote sensing of Environment*, *51*(3), 375–384.

Sawyer, J., Nafziger, E., Randall, G., Bundy, L., Rehm, G., Joern, B., et al. (2006). Concepts and Rationale for Regional Nitrogen Rate Guidelines for Corn.

Shaikh, T.A., Mir, W.A., Rasool, T. *et al.* (2022). Machine Learning for Smart Agriculture and Precision Farming: Towards Making the Fields Talk. *Arch Computat Methods Eng* **29**, 4557–4597. https://doi.org/10.1007/s11831-022-09761-4

Sharma, L. K., & Bali, S. K. (2018). A Review of Methods to Improve Nitrogen Use Efficiency in Agriculture. *Sustainability*, *10*(1), 51. https://doi.org/10.3390/su10010051

Shrestha, A., Bheemanahalli, R., Adeli, A., Samiappan, S., Czarnecki, J. M. P., McCraine, C. D., et al. (2023). Phenological stage and vegetation index for predicting corn yield under rainfed environments. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1168732

Thompson, L. J., & Puntel, L. A. (2020). Transforming Unmanned Aerial Vehicle (UAV) and Multispectral Sensor into a Practical Decision Support System for Precision Nitrogen Management in Corn. *Remote Sensing*, *12*(10), 1597. https://doi.org/10.3390/rs12101597

Tucker, C. J. (1979). Red and photographic infrared linear combinations for monitoring vegetation. *Remote Sensing of Environment*, *8*(2), 127–150. https://doi.org/10.1016/0034-4257(79)90013-0

Tucker, C. J. (1980). Remote sensing of leaf water content in the near infrared. *Remote sensing of Environment*, *10*(1), 23–32.

Vincini, M., Frazzi, E., & D’Alessio, P. (2008). A broad-band leaf chlorophyll vegetation index at the canopy scale. *Precision Agriculture*, *9*, 303–319.

Wang, Y., Janz, B., Engedal, T., & Neergaard, A. de. (2017). Effect of irrigation regimes and nitrogen rates on water use efficiency and nitrogen uptake in maize. *Agricultural Water Management*, *179*, 271–276. https://doi.org/10.1016/j.agwat.2016.06.007

Wei, M. C. F., & Molin, J. P. (2020). Soybean Yield Estimation and Its Components: A Linear Regression Approach. *Agriculture*, *10*(8), 348. https://doi.org/10.3390/agriculture10080348

Wickham, H., & Wickham, M. H. (2017). Package tidyverse. *Easily install and load the ‘Tidyverse*.

Zhang, Lingchun, Zhang, X., Gao, Q., & Yan, L. (2023). Nitrogen Application Effect on Maize Yield, NH3, and N2O Emissions in Northeast China by Meta-Analysis. *Agronomy*, *13*(6), 1479. https://doi.org/10.3390/agronomy13061479

Zhang, Liyuan, Zhang, H., Niu, Y., & Han, W. (2019). Mapping Maize Water Stress Based on UAV Multispectral Remote Sensing. *Remote Sensing*, *11*(6), 605. https://doi.org/10.3390/rs11060605