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Vegetation Indices as Rapid, Non-Destructive Tool to Assess Nitrogen Status in Irrigated Rice

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ABSTRACT

The use of optical radiation sensors is a promising strategy for nitrogen management as it reduces the costs of chemical analyses and allows quick decision-making in the supplementary application of N to irrigated rice. By combining three spectral reflectance bands (red, far-red, and near-infrared), 22 vegetation indices (VIs) were computed and assessed for their effectiveness in estimating the nitrogen status of rice crops. The results indicated that the selected VIs considerably underestimated dry leaf biomass (DLB) and did not efficiently estimate N status parameters, such as leaf N concentration (LNC) and leaf N uptake, at the vegetative stage. The large variations in these N status parameters can be explained by the VI in subsequent stages. The VI selected in the parametrisation process was promising for explaining variation in DLB and leaf area index at the reproductive and grain-filling stages. However, the VI showed low performance in estimating LNC at the reproductive stage. The modified red-edge soil-adjusted VI and normalised difference red-edge index showed high performance in estimating the N nutrition index in the growth stage and across the whole crop cycle. These results show the importance of using active sensors for effective crop N status estimation.

1 | Introduction

The excessive use of nitrogen fertilisers in rice production areas is a critical problem. Nitrogen (N) overuse results not only in low N use efficiency, but also causes environmental disturbances, increases the susceptibility of crops to pests and diseases, reduces cooking and nutritional qualities and consequently reduces the economic return. The monitoring of nitrogen status in irrigated rice through indices derived from field plant sampling and laboratory analyses—such as dry leaf biomass (DLB), leaf area index (LAI), leaf nitrogen content (LNC), and leaf nitrogen uptake (LNU)—has limited practical application in large-scale crop production due to the difficulty of obtaining real-time measurements. The nitrogen status of rice (*Oryza sativa* L.) can be assessed by calculating the nitrogen nutrition index (NNI), obtained by the ratio of the plant's nitrogen concentration to the critical nitrogen concentration in the crop [1]. Although chlorophyll metres have been used for real-time determination of crop nitrogen status, they typically rely on measurements from specific leaves. However, these leaves may exhibit varying metabolic activities even within the same plant, making it challenging to apply this tool effectively on a large scale [1–3]. Rationalising nitrogen supply in both space and time presents a significant challenge in large-scale rice production. This highlights the need for the development of technologies that enable rapid, real-time diagnosis of nitrogen status throughout the entire growth period [3–5].

Summary

- This paper highlights the systematic evaluation of 22 vegetation index (VI) for their efficiency in estimating N in rice.
- The M_RESAVI and NDRE indices showed efficiently in estimating N nutrition index in irrigated rice.
- The use VI in linear models is effective for estimating the N status of the crop.

The use of optical radiation sensors is a promising strategy for nitrogen management, as it reduces the costs of chemical analyzes and allows quick decision-making in the supplementary application of N to irrigated rice [6, 7] compared to plant N monitoring based on indices derived from laboratory analyses or chlorophyll metres. Another advantage of using canopy sensors is that, as they are artificially activated, they are not dependent on the availability of ambient light [6].

The literature highlights the use of various sensors, such as the GreenSeeker sensor (Trimble Navigation Limited, Sunnyvale, California, USA), Crop Circle ACS-210 (Holland Scientific Inc. Lincoln, Nebraska, USA), and Crop Spec (Topcon Positioning Systems Inc., Livermore, California, USA), for obtaining vegetation indices (VIs) and estimating nitrogen status in rice [8, 9]. While these sensors are widely used, they are limited in their ability to capture radiation across a narrow range of bands—typically only two fixed bands (e.g., red and near-infrared (NIR), NIR and green, or NIR and far red). This limitation restricts the number of indices that can be derived, with the most commonly used being the normalised difference vegetation index (NDVI), green difference vegetation index (GDVI), red edge difference vegetation index (REDVI), and vegetation ratio index (RVI, NIR/Red). This may be a limitation because optimal wavelengths and vegetation indices vary for different biophysical crop parameters and growth stages. In this context, sensors capable of capturing more than two spectral bands may enhance the prediction of nitrogen status by generating multiple spectral VIs [3, 10–12]. Growing maise under different N and water management conditions Shirastsuchi et al. [13] determined two VI calculated from three spectral bands capable of differentiating the nitrogen state of the crop. Cao, Miao, Wang et al. [14] found that the VI MCARI1 calculated from three spectral bands (green, far red and NIR) of Crop Circle ACS 470 was consistent correlations with rice biomass and plant N uptake in different years, varieties and growth stages. In this study, four additional indices demonstrated comparable performance in estimating the rice nitrogen nutrition index (NNI).

The aim of this study was to investigate whether radiation bands and their respective combinations, known as vegetation indices, can be used to estimate the nitrogen status of irrigated rice at different growth stages. This was achieved using linear models, which offer lower mathematical complexity [15], allowing for faster estimates and more efficient decision-making in diagnosing the nitrogen status of irrigated rice plants. Linear regression is commonly used to establish relationships between vegetation indices and nitrogen status parameters [2, 6, 7, 11].

2 | Materials and Methods

2.1 | Description of Experiments

We conducted six field experiments at the Brazilian Agricultural Research Corporation (Embrapa) Rice and Beans, Goiás, from 2014 to 2017. According to Köppen's classifications [16], the region is a tropical savanna and the soil is type Dystrophic Haplic Gleysol. The experiments were designated as follows: GO_14/15 (1, P), GO_14/15 (2, V), GO_15/16 (1, V), GO_16/17 (1, V), GO_16/17 (2, V), and GO_17/18 (1, P), where numbers separated by slash (14/15, 15/16, 16/17, and 17/18) represent the crop season and the number (1 or 2) and letter (P or V) inside the parentheses are the number of experiments per crop season (1 or 2) and model parametrisation (P) and model validation (V), respectively. GO_14/15 (1, P) and GO_17/18 (1, P) were selected for parametrisation because data collection in these experiments was performed at shorter intervals. More details about these experiments can be found at Santos, Zanon et al. [17], Santos, Heinemann et al. [3] and Table 1.

2.2 | Sensor, Data Collection and Vegetation Indices (VI)

The Crop Circle ACS-430 active sensor (Holland Scientific Inc., Lincoln, NE, USA) that simultaneously incorporates red, near-red, and infrared spectral bands was used to collect canopy reflectance across growth stages. Details about the sensor used in this study and your operation have been reported by Santos, Heinemann et al. [3]. Using the three reflectance measurement bands incorporated in the Crop Circle ACS-430 sensor it is possible to calculate various VI [18]. We selected 22 spectral VIs for this study listed in Table 2 and also in Santos, Heinemann et al. [3].

2.3 | Crop Periods and N Status Parameters

We divided the rice crop cycle into four periods: beginning of vegetative stage (V1 to V9, around 40 days after emergence), end of vegetative stage (V10 to R1), reproductive stage (R2 to R4), and grain-filling stage (R5 to R8). We also analysed all crop cycles, V1 to R8. Five N status parameters were used to measure the VI efficiency in rice crops: dry leaf biomass (DLB), leaf area index (LAI), leaf N content (LNC), leaf N uptake (LNU), and NNI.

To calculate N status parameters, samples at 0.5-m depth were collected from each plot after obtaining readings using the ACS-430 sensor. Collections were performed weekly for parametrisation and fortnightly for the validation experiments. Subsamples of fresh plants (50% of the collected material) were separated into green leaf blades (leaf), dry leaf blades (dead leaf), culm + sheath (tiller), and panicles. Leaf area (LA, m^2) was measured using the LI-3100 (LI-COR) photoelectric area metre, and the LA index (LAI) was calculated using Equation (1).

$$LAI = LA/SA \tag{1}$$

TABLE 1 | Description of experiments and data set used in this study for parametrisation and validation.

Experiment ID	Crop season (year)	Cultivars ID[a]	Sowing/ Transplant date (day/month)	Date of phenological stages	Harvested date
Parametrisation					
GO_14/15(1, P)	14/15	1, 2, 3, 4	18/12	05/02[b], 21/02[c], 20/03[d]	30/03/2015
GO_17/18(1, P)	17/18	1, 5, 6, 7	27/10	05/01[b] 03/02[c], 01/03[d]	06/03/2018
Validation					
GO_14/15(2, V)	14/15	1,2, 3, 4	10/11	03/01[b], 24/01[c], 17/02[d]	26/02/2015
GO_15/16(1, V)	15/16	1, 2, 3, 4, 5	28/09	27/11[b], 20/12[c], 16/01[d]	24/01/2016
GO_16/17(1, V)	16/17	1, 5	27/09	04/12[b], 30/12[c], 25/01[d]	30/01/2017
GO_16/17(2, V)	16/17	1, 5	18/10	16/12[b], 16/01[c], 10/02[d]	19/02/2017

[a]Cultivar ID: 1 = BRS Catiana, 2 = BRS Jaçanã, 3 = BRS Pampa, 4 = BRS Taim, 5 = IRGA 424, 6 = BRS Pampeira, and 7 = A 702 - CL.
[b]Correspond to panicle initiation.
[c]Flowering.
[d]Mature stages.

where LA is the sampled leaf area, and SA is the soil area corresponding to the LA sample collected.

After collection, fresh samples were dried in a forced ventilation oven at 75°C to a constant mass. A part of DLB (~250 g) was used to determine the LNC using the standard Kjeldahl-N method [28]. LNU was calculated by multiplying N concentration with DLB. The NNI was calculated as described by Lemaire et al. [1]:

$$\mathrm{NNI} = \mathrm{Na}/\mathrm{Nc} \tag{2}$$

where Na is the actual N concentration measured as a percentage of DLB, and Nc is the critical N concentration as a percentage of DLB (%), as described by Sheehy et al. [29]:

$$\mathrm{Nc} = 5.18 * \mathrm{W}^{-0.52} \tag{3}$$

where W is the DLB in Mg ha^{-1}. The nitrogen nutrition index (NNI) reflects the nitrogen status of the crop, with values greater than 1 suggesting a non-limiting nitrogen status, while values less than 1 indicate nitrogen deficiency. It serves as a valuable tool for improving the diagnosis of nitrogen status in crops [3, 30].

2.4 | Statistical Analysis

Data collected from parametrisation experiments (GO_14/15(1, P)) and (GO_17/18(1, P)) were mainly used to develop linear, quadratic, logarithmic, exponential, and power regression models, and data collected from validation field experiments were subsequently used to validate the selected regression models. The coefficients of determination (R^2) for the relationships between VI and the N status parameters in rice crops (DLB, LNC, LAI, LNU, and NNI) were calculated using R software [31]. R^2 was used for parametrisation as it correlates with the percentage of variability of the indicator that can be explained by an index, and indices with a high percentage of explanation of this variability were sought (> R^2). Few studies [2, 27, 32] applied this statistical index for model parametrisation. Only the nine best models (highest R^2) that explained the variability between N status parameters and VI were selected for the validation process.

The models' performance for the validation process was evaluated by comparing the root mean square error (RMSE, Equation (4)), percent bias (PBIAS %, Equation (5)), and Willmott's index of agreement (d, between $0 < d < 1$, Equation (6)). These statistical indices measure the model's ability to predict the response of interest with independent data and are more effective for evaluating the models' performance than R^2.

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(Y_i - O_i)^2} \tag{4}$$

where P and O are, respectively, the predicted and observed values.

$$\mathrm{PBIAS} = \left[\frac{\sum_{i=1}^{n}\left(Yi^{obs} - Yi^{\mathrm{sim}}\right) * 100}{\sum_{i=1}^{n}\left(Yi^{\mathrm{obs}}\right)}\right] \tag{5}$$

Percent bias (PBIAS) measures the average tendency of the simulated data (sim) to be larger or smaller than their observed counterparts (obs).

$$d = 1 - \frac{\sum_{i=1}^{n}(P_i - O_i)^2}{\sum_{i=1}^{n}\left(\left|P_i - \overline{O}\right| + \left|O_i - \overline{O}\right|\right)^2} \tag{6}$$

where P and O are, respectively, the predicted and observed values.

They measure the difference (error) between predicted and observed values (RMSE), the percent deviation between the predicted and observed values (PBIAS), and the agreement (d) of the model, that is, to what extent the model can adapt to a situation that was not targeted by the parametrisation [27, 33].

TABLE 2 | Calculated spectral vegetation indices (VI) selected for this study.

ID	VI	Equation	References
1	Normalised difference vegetation index (NDVI)	(NIR − R)/(NIR + R)	Holland scientific [18]
2	Ratio vegetation index (RVI)	NIR/R	Holland scientific [18]
3	Chlorophyll index red-edge (CHL)	(NIR/RE) − 1	Holland scientific [18]
4	Simple ratio index red (SRI)	NIR/R	Holland scientific [18]
5	Non-linear vegetation index (NLI)	$(((NIR^2) - R)/((NIR^2) + R))$	Holland scientific [18]
6	Re-normalised difference vegetation index (RDVI)	$((NIR - R)/(NIR + R)^{1/2})$	Holland scientific [18]
7	Modified simple ratio (MSR)	$((NIR/R) - 1)/(((NIR/R)^{1/2}) + 1)$	Holland scientific [18]
8	Water invariant chlorophyll index 1 (WICI1)	(NIR − RE)/(NIR + R)	Holland scientific [18]
9	Water invariant chlorophyll index 2 (WICI2)	(NIR − RE)/(RE + R)	Holland scientific [18]
10	Near infra-red band reflectance (NIR)	NIR	Holland scientific [18]
11	Red-edge ratio vegetation index (RERVI)	NIR/RE	Jasper et al. [19]
12	Red-edge difference vegetation index (REDVI)	NIR − RE	Cao, Miao, Wang et al. [14]
13	Normalised difference red-edge (NDRE)	(NIR − RE)/(NIR + RE)	Barnes et al. [20]
14	Red-edge wide dynamic range vegetation index (REWDRVI)	(a × NIR − RE)/(a × NIR + RE) (a = 0.12)	Cao, Miao, Wang et al. [14]
15	Optimised vegetation index 1 (Vplot 1)	100 × (lnNIR − lnRE)	Jasper et al. [19]
16	Red-edge chlorophyll index (CIRE)	NIR/RE − 1	Gitelson et al. [21]
17	Modified red-edge simple ratio (MSR_RE)	(NIR/RE − 1)/ROOT(NIR/RE + 1)	Cao, Miao, Wang et al. [14] modified of Chen [22]
18	Red-edge soil adjusted vegetation index (RESAVI)	1.5 × [(NIR − RE)/(NIR + RE + 0.5)]	Cao, Miao, Wang et al. [14] modified of Sripada et al. [23]
19	Modified RESAVI (M_RESAVI)	$0.5 \times [2 \times NIR + 1 - ROOT((2 \times NIR + 1)^2 - 8 \times (NIR - RE))]$	Cao, Miao, Wang et al. [14] modified of Qi et al. [24]
20	Red-edge optimal soil adjusted vegetation index (REOSAVI)	(1 + 0.16) × (NIR − RE)/(NIR + RE + 0.16)	Cao, Miao, Wang et al. [14] modified of Rondeaux et al. [25]
21	Red-edge Re-normalised different vegetation index (RERDVI)	(NIR − RE)/ROOT(NIR + RE)	Cao, Miao, Wang et al. [14] modified of Roujean and Breon [26]
22	Modified REWDRI (REWDRVI2)	(a × (NIR − RE))/(a × (NIR + RE)) (a = 0.12)	Adapted of Cao, Miao, Feng et al. [27]

Source: Santos, Heinemann et al. [3].

However, if the best fit function is non-linear, the sensitivity of the VI to the N status indicator is not constant and, therefore, d and RMSE may be misleading [27, 34]. In this case, PBIAS is considered a better indicator of the performance of VI in estimating plant N status. The higher the d values in the performance and the lower the RMSE and PBIAS values (absolute value), the greater the precision and accuracy of the model in predicting the crop N status.

3 | Results

3.1 | Variability of Rice N Status Parameters

The nitrogen status parameters showed significant variation across the analysed periods (indicated by high coefficient of variation (CV) values, Table 3). The variability of N status parameters across seasons and cultivars was fundamentally related to climate variation in seasons and cultivars used in this study. Throughout the entire crop cycle, the LAI exhibited the highest coefficient of variability. In the beginning of vegetative stage (V1–V10) DLB and LNC exhibited greatest variability, whereas LNU, LAI, and NNI displayed the highest variability at the grain-filling stage (R5 to R8). LNCs decreased across crop cycle. In contrast, the average values of N status parameters (DLB, LNU, LAI, and NNI) increased from the beginning of vegetative to the reproductive stage and decreased again at the grain-filling stage (Table 3). The values NNI for all crop stages was bellow 1, which indicates N deficiency in production environments [35].

3.2 | Parametrisation of VI and N Status Parameters

At the beginning of the vegetative stage, the calculated VI proved efficient ($R^2 \geq 0.60$) in estimating N status parameters in rice plants (Table S1). However, at the end of the vegetative stage, the VI was not efficient ($R^2 \leq 0.20$). In the other periods,

TABLE 3 | Statistical description of N status parameters (dry leaf biomass (DLB), leaf area index (LAI), leaf N content (LNC), leaf N uptake (LNU), and N nutrition index (NNI)) across growth stages (beginning of vegetative: V1–V9; end of vegetative: V10–R1; reproductive: R2–R4 and grain filling: R5–R8), whole crop cycle (V1–R8), cultivars and years.

	Growth stages															
	Beginning of vegetative stage (n = 40)				End of vegetative stage (n = 52)				Reproductive stage (n = 105)				Grain filling (n = 83)			
N status	Range	Mean	SD	CV (%)	Range	Mean	SD	CV (%)	Range	Mean	SD	CV (%)	Range	Mean	SD	CV (%)
DLB (kg ha^{-1})	185.5–2683.1	1238.4	647.4	52.3	774–3305	1877.4	657.5	35	1581–5156	2524	704	27.9	599–3838	1936	850	44
LNC (g kg^{-1})	20.4–53.3	35.7	10.03	28.1	19.5–36.6	29.2	4.1	14	19.1–36.3	26.6	2.86	10.7	14.36–32.1	23.5	4.5	19.3
LNU (kg ha^{-1})	9.6–68.2	40.2	17.5	43.5	22–87.2	54.2	19.2	35.4	39.1–161	67.6	23	34.1	8.6–106	47	24	51
LAI (m^2 m^2)	0.29–5.47	2.27	1.41	62.2	1.22–5.74	3.07	1.16	37.8	1.69–8.3	3.74	1.51	40.4	0.46–7.69	2.6	1.85	71.1
NNI	0.41–1.05	0.70	0.18	26.1	0.48–1.06	0.76	0.17	21.7	0.53–1.45	0.83	0.17	20	0.21–1.07	0.64	0.22	34.5

	V1 – R8 (n = 280)			
N status	Range	Mean	SD	CV (%)
DLB (kg ha^{-1})	185–5156	2046	850	41.6
LNC (g kg^{-1})	14.3–53.3	27.5	6.4	23.4
LNU (kg ha^{-1})	8.6–161	55.1	24.2	44
LAI (m^2 m^2)	0.29–8.32	3.07	1.64	53.6
NNI	0.21–1.46	0.74	0.20	27.2

Note: n represents the sample numbers.

the VI also efficiently explained the variation in N status parameters, except for LNC, which had the lowest capacity of variation explanation by the VI, compared with that at the beginning of vegetative stage. The ranking of the best VI varied with N status parameters among the crop periods, and these VI were generally higher (> R^2) than NDVI (Normalised Difference Vegetation Index) and RVI (Ratio Vegetation Index) to explain the N indicator variability (Table S1).

Saturated effects were observed regarding the relationship of DLB and LAI with NDVI equal to 0.70 (Figure 1). The variability in leaf N concentration was best explained at the beginning of the vegetative stage, with a sharp drop in its measurement using models selected for other growth periods (Table S1).

3.3 | Validation of VI and N Status Parameters

The performances of the selected models in the calibration process are listed in Table S2. Considering the crop cycle, the most promising VI for BFS were CIRE (Red-Edge Chlorophyll Index) and MSR_RE (Modified Red-Edge Simple Ratio), which presented values closer to 1 for the statistical index d in comparison with the other indices (Table S2). Both VI were linearly adjusted. These indices also provided the lowest RMSE values (Table S2). For LNC, the performance of the selected indices was similar (Figure 2). For LAI and LNU estimations, NDVI and RVI showed a worse performance than the other VI selected. This was also observed for NNI (Table S2), which was estimated most efficiently by the M_RESAVI (Modified Red-Edge Soil Adjusted Vegetation Index) index (d = 0.62).

The selected models considerably underestimated the DLB at the beginning (−63.8 < PBIAS < −74.1) and end (−22.4 < PBIAS < −23.4) of the vegetative stage (Table S2). Better convergences (low bias) between simulated and observed DLB values were obtained using NDVI and RVI during the reproductive stage. However, for the grain-filling stage and all crop cycles, these indices were lower than the others (Table S2). LAI estimated by the selected models was only effective (−0.8 < PBIAS < 4.6) in the reproductive stage, suggesting that the N status indicator is not likely to be determined by VI in all growth periods or even using a generalist model for the entire cycle.

LNC and LNU were not estimated efficiently (−53.5 < PBIAS < 79.6 and −22.8 < PBIAS < −50.1, respectively) at the initial vegetative stage. For the other stages and all crop cycles, there was an improvement in the convergence between the simulations from the models adjusted with the validation data (Table S2). For these indicators, the performance of VI contrasted between growth periods and all crop cycles, and the estimation performance for LNU using NDVI and RVI was lower than that for other indices.

From the N status parameter in rice, NNI could be best estimated by the VI selected in the crop growth periods as well as in all crop cycles. NDVI and RVI performed better than the other VI at the beginning of the vegetative stage (accuracy ≥ 97.5%

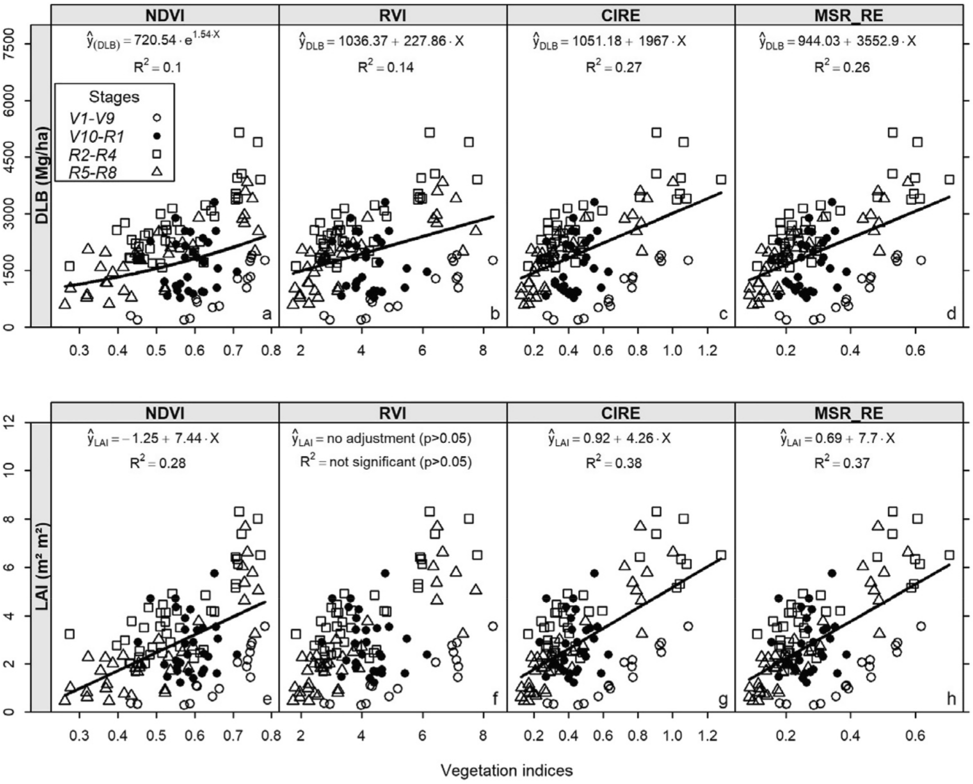

FIGURE 1 | Scatterplot for dry leaf biomass (DLB, upper panel) and leaf area index (LAI, bottom panel) and vegetation index (NVDI, RVI, CIRE, MSR_RE). Empty and filled black circles and squares represent the beginning of vegetative stage (V1–V9), end of vegetative stage (V10–R1), reproductive stage (R2–R4), and grain filling stage (R5–R8) across cultivars and crop season.

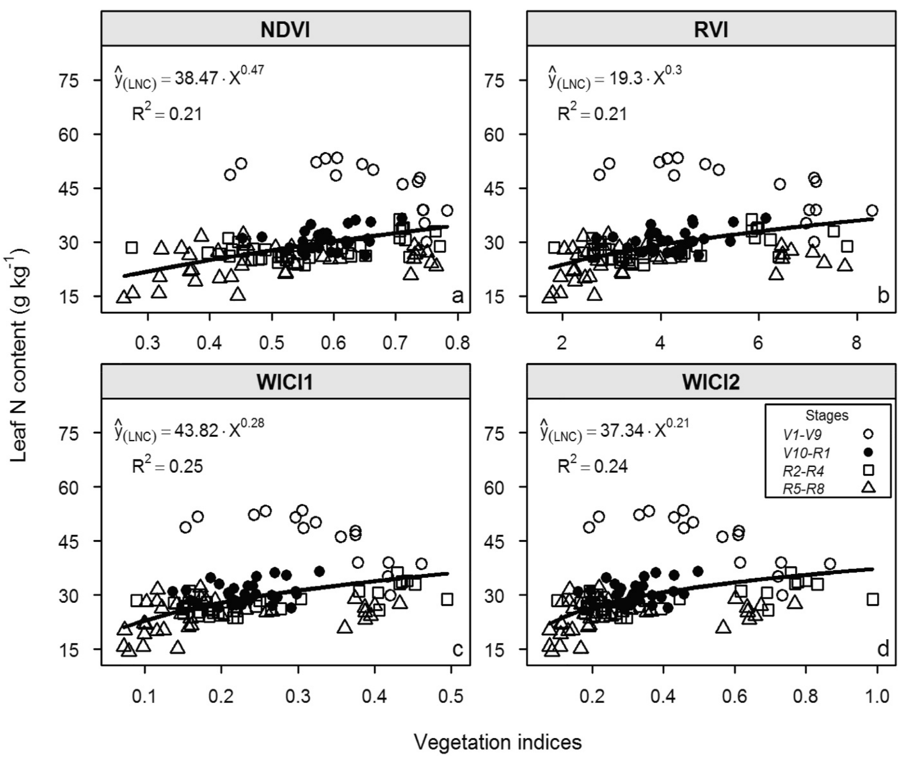

FIGURE 2 | Scatterplot for leaf N content and the vegetation indices: (a) NVDI, (b) RVI, (c) WICI1, and (d) WICI2. Empty and filled black circles and squares represent the beginning of vegetative stage (V1–V9), end of vegetative stage (V10–R1), reproductive stage (R2–R4), and grain filling stage (R5–R8) across cultivars and crop season.

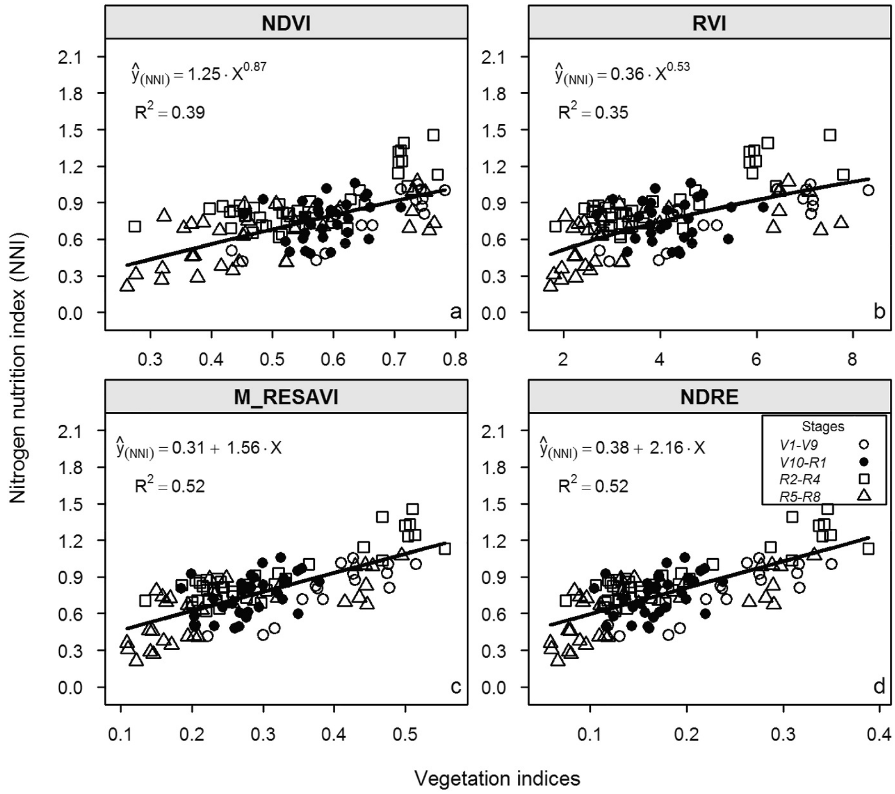

FIGURE 3 | Scatterplot for nitrogen nutrition index (NNI) against vegetation indices: (a) NVDI, (b) RVI, (c) M_RESAVI, and (d) NDRE. Empty and filled black circles and squares represent the beginning of vegetative stage (V1–V9), end of vegetative stage (V10–R1), reproductive stage (R2–R4), and grain filling stage (R5–R8) across cultivars and crop season.

and $d > 55$). These indices were not promising at the end of the vegetative stage (they did not even adjust to explain the variability), and in the other stages, although they presented considerable precision (accuracy ≥ 90), they showed low aggregation to the models selected for estimating NNI, revealing inconsistency in performance with the increase in VI variation (Figure 3a and 3b). In this sense, it is worth highlighting M_RESAVI and NDRE indices, which showed an accuracy of ≥ 85% when used in exponential and power models, respectively, at the beginning of vegetative stage and the reproductive stage or in 1st degree linear models at the end of vegetative stage and throughout the crop cycle (Table S2).

4 | Discussion

The use of the NDVI for estimating of the DLB and LAI is less effective because of the saturation [14, 36] (Figure 1 and Table S2). The saturation effect is influenced by the choice of spectral bands and the normalisation applied in the VI equation [37]. The relationship between VI and N status parameters is affected by growth stages [4, 6, 27, 38]. Figures 2 and 4 show that the vegetative stage considerably differs from the reproductive and grain-filling stages, forming two clusters when LNC and LNU were plotted against the VI.

VI explained the variation in the N status parameters at the beginning of the vegetative stage (V1 to V9). However, for the validation process, all VI selected were inefficient in estimating the N status parameters in irrigated rice at the beginning and the end of the vegetative stage, except for NNI. Probably, the reflectance effect of the water depth on the ground surface was higher at this stage and has a higher impact on spectral band sensors. The estimation performance of the N status parameters by the selected VI improved considerably at the reproductive and grain-filling stages, where the canopy partially covered the field (Table S2). At these stages, we observed an improvement in LNC estimation based on the VI selected in the validation process. Cao, Miao, Wang et al. [14] also reported that the variation in N content in irrigated rice is more explained by the VI at the reproductive stage ($R^2 = 0.34$) than at the vegetative stage ($R^2 = 0.08$). Despite the improvement in performance compared with the vegetative stage (beginning and end of vegetative stage), the explanation for the variation in LNC by the VI selected in the validation process was weak ($R^2 = 0.26$ and 0.45 in the reproductive and grain-filling stages, respectively). Biomass accumulation is not accompanied by N absorption, resulting in canopy-dominant biomass, which affects the reflectance of small leaves and, thus, the N content estimation [38].

Overcoming the saturation effect of NDVI under moderate to high biomass is a great motivation to search for VI that may be more effective in assisting in the precision management of N. In this study, RVI had lower saturation than NDVI in estimating DLB for the whole cycle (Table S2). Similar to RVI, the indices MSR_RE and CIRE were less sensitive to the saturation effect than NDVI in DLB estimation. The saturation effect is related to the selected spectral bands and the effect of normalisation incorporated in the VI equation [37]. The authors suggest that

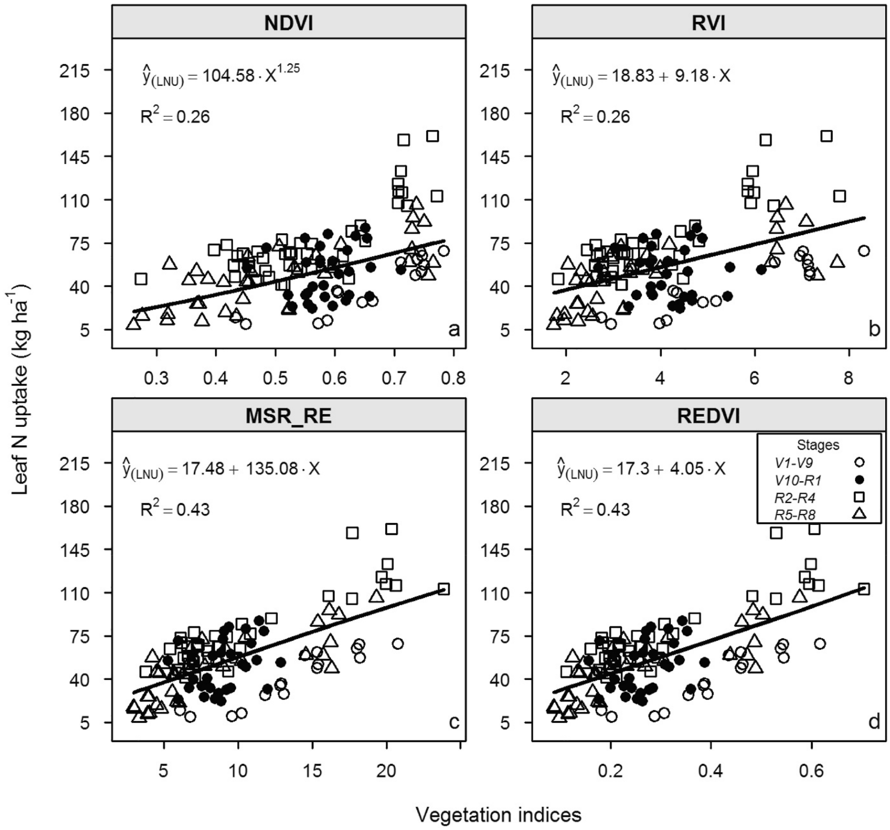

FIGURE 4 | Scatterplot for leaf N uptake against vegetation indices (a) NVDI, (b) RVI, (c) MSR_RE, and (d) REDVI. Empty and filled black circles and squares represent the beginning of vegetative stage (V1–V9), end of vegetative stage (V10–R1), reproductive stage (R2–R4), and grain filling stage (R5–R8) across cultivars and crop season.

saturation can be minimised by utilising near wavelengths, which have a greater ability to penetrate the plant canopy. The results obtained in this study for DLB estimation based on VI are similar to those obtained by Gnyp et al. [37] showing that the normalisation equation applied (RVI vs. NDVI) and near spectral bands used (NIR and FR, see MSR-RE and CIRE in Table 2) can reduce the NDVI saturation effect.

VI showed similar performance for LNC estimation over the crop cycle and reproductive stage. The NDVI showed the worst performance among the selected VI at the end vegetative and grain-filling stages (Table S2). Li, Miao et al. [39] and Cao, Miao, Feng et al. [27] found the same results for winter wheat. According to Yao, Miao, Huang et al. [6], for rice, VI explained less than 40% of the N variation in the plant, which is in agreement with the results obtained in this study. The performance of VI selected in the parameterisation and validation process and their explanatory capacity for estimating the agronomic parameter LNC showed that they were efficient only at the reproductive stage. Identification of VI for estimating LNC in the reproductive stage (R2-R4: before flowering), when there is still any plant response to N supplementation, can lead to efficient N management, especially of cultivars with high demand for this nutrient.

All VI showed similar performance for estimating the agronomic parameter LNU at the beginning and end vegetative stages. However, none of the VI effectively explained LNU variation in the rice plant. In the reproductive and grain-filling stages and the whole crop cycle, NDVI and RVI were less efficient than other indices in both the parametrisation and validation processes (Figure 4, Tables S1 and S2). This information allows us to estimate the LNU per unit area for several VI, allowing a timely supply of N, especially when deficiency is identified at the beginning of the reproductive stage. Better performance by NDVI and RVI for the whole crop cycle was observed in this study for estimating LNU. Cao, Miao, Feng et al. [27] and Yao, Miao, Cao et al. [40] also reported that the variation in LNU was 78%–82% and 70%–73% in winter wheat and rice, respectively. This shows that these VI are more sensitive to variations across sites, cultivars, growth stages, and crop seasons than other VI. Another limitation of NDVI is that this index can saturate with high biomass and become even more inefficient in the estimation of N status parameters.

Although DLB, LAI, LNC, LNU, and NNI are all N status parameters, NNI is considered the best indicator for the assessment of N nutrition in crops [1, 14]. NNI is related to a critical N amount that ensures plant growth. The need for destructive sampling and chemical analysis for N determination has raised the interest in remote sensing technology to estimate NNI in a non-destructive way [39, 41]. Three main approaches can be applied to estimate NNI in a non-destructive manner using detection technologies. The first approach is to estimate the biomass accumulation and N content of the plant. It is possible to determine the critical N concentration using the estimated biomass, where NNI is calculated as the ratio between the actual N concentration and the predicted critical N concentration for the accumulated biomass [14]. The results of this study suggest that rice DLB can be estimated at the reproductive and grain-filling stages (R^2 = 0.69 and 0.74, respectively) based on VI. However, the variation in LNC was only moderately explained by VI (R^2 = 0.37–0.45), and this parameter had the lowest percent bias (PBIAS ≤ 6.6 for the best indices, Table S2) at the reproductive stage. Except in the reproductive stage, this approach would not be efficient in estimating NNI. The estimation of crop N status using remote sensing technologies has been a challenge, especially at the vegetative stage [9, 14]. The second approach refers to the use of canopy detection technologies to estimate biomass and N content in plants. Based on the estimated biomass, critical N absorption can be calculated using the critical N absorption curve [1]. In this method, the NNI is determined as the ratio of the plant's actual nitrogen uptake (specific to the cultivar and known in advance for the given growth stage) to the estimated critical uptake. The results of this study (Tables S1 and S2) show that LNU was well estimated by the VI, except at the vegetative stage. This finding indicates that this approach may work well to estimate INN indirectly at key growth stages (stem elongation, panicle initiation, and grain filling). The actual and critical N concentrations can be used to measure excess or deficiency of N and help determine the amount of N topdressing. The third approach involves using sensing technology to directly estimate the NNI, similar to how chlorophyll metres operate [41, 42] and active canopy sensors [43].

The performance of VI for estimating NNI was satisfactory for all growth stages (d = 0.37–0.77) and the whole crop cycle (d = 0.51–0.62). NDVI and RVI showed better performance for estimating NNI at the beginning of the vegetative stage. At the end of the vegetative stage, M_RESAVI showed better performance for estimating NNI. Among the VI applied in this study, M_RESAVI and NDRE stood out from the 22 VI for estimating NNI (Tables S1 and S2). At the reproductive and grain-filling stages and the whole crop cycle, these VI explained on average NNI variation of 7%, 13%, and 13% greater using adjusted models 8%, 15%, and 8% more aggregated to the independent validation data compared with the NDVI and RVI, respectively. Yao, Miao, Cao et al. [40] also found that the VI, NDVI, and RVI are inefficient for estimating NNI in rice. Our results are similar to the results of Cao, Miao, Feng et al. [27], who estimated NNI satisfactorily using M_RESAVI and demonstrated encouraging performance results for rice crop (R^2 = 0.78).

Our results infer that VI adequately estimated NNI, with R^2 equal to 0.52 in the parametrisation process for the whole crop cycle, achieving 0.80 at the reproductive stage. The validation results in data from independent experiments were also promising (d = 0.62) for the whole crop cycle. This demonstrates that active sensors with more than two fixed bands, especially incorporating the RE spectral band, are better for estimating the crop N status. In Brazil, for irrigated rice areas with high yields, indices such as M_RESAVI and NDRE may be more suitable for managing precision applications and optimising the cost of chemical analysis to determine crop N status as well as for topdressing, which may be reduced over the cycle. More studies are needed to further evaluate the performance of these VI in estimating NNI under different environmental conditions and management.

There is growing interest in strategies that optimise the use of synthetic and natural resources such as N in rice production environments, especially under the irrigated system, which allows for higher yields than rainfed cultivation. The results obtained with this research allow us to infer that the management of N status in tropical irrigated rice can be done based on the NNI estimated by the M_RESAVI and NDRE indices. Recent technologies have suggested the combined use of remote sensing information with handheld remote-controlled devices. This combination presents itself as a promising approach for quantitative and spatially distributed diagnostics to support the application of N at variable rates, enabling the creation of NNI maps. In Italy, the application of N at variable rates through this approach made it possible to reduce the environmental impact of nitrogen fertilisation from 13.6% to 11% in rice cultivation [44]. The possibility of estimating the NNI of irrigated rice by spectral technologies has also been reported in China [4]. Once the NNI of the crop is accurately estimated, the established models become useful to provide theoretical bases for the precise management of nitrogen fertilisers contributing to an increase in crop yield. Therefore, interest in technologies that allow the non-destructive estimation of NNI in large areas is growing and promising.

5 | Conclusions

The VI calculated in this study considerably underestimated DLB (−74.1 < PBIAS < −63.8 and −23.4 < PBIAS < −22.4) at the beginning and end vegetative stages, respectively. In addition to DLB, LNC and LNU were not efficiently estimated at the vegetative stage (PBIAS > 10%). Larger variations in these indicators are explained by the VI in the subsequent growth stages. The selected VI were promising for explaining DLB and LAI variability at the reproductive and grain-filling stages. The lowest performance was achieved by the VI for estimating LNC at the reproductive stage. NDVI and RVI were less efficient for estimating LNU at the reproductive and grain-filling stages, and the whole crop cycle than the other VI. M_RESAVI and NDRE had the best performance for estimating NNI.

Author Contributions

Marcos Paulo dos Santos: conceptualization (lead), data curation (lead), formal analysis (lead), investigation (equal), methodology (lead), project administration (equal), resources (lead), software (lead), supervision (equal), validation (lead), visualization (lead), writing – original draft (equal), writing – review and editing (equal). **Nívea Patrícia Ribeiro Reges:** data curation (equal), formal analysis (equal), writing – review and editing (supporting). **Alberto Baêta dos Santos:** investigation (equal), methodology (equal), visualization (equal), writing – original draft (equal), writing – review and editing (equal). **Luís Fernando Stone:** data curation (equal), investigation (equal), methodology (equal), supervision (equal), validation (equal), writing – original draft (equal), writing – review and editing (equal). **Alexandre Bryan Heinemann:** conceptualization (equal), formal analysis (equal), funding acquisition (lead), methodology (equal), resources (lead),

software (equal), supervision (equal), validation (equal), visualization (equal), writing – original draft (equal), writing – review and editing (lead).

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are contained within the article and Supplementary Materials.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.