RESPONSE OF SUGARCANE TO NITROGEN RATES: INSIGHTS FROM SPECTRAL BIOINDICATORS, BIOPHYSICAL MEASUREMENTS, AND PHOTOCHEMICAL VARIABLES

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HIGHLIGHTS

The vegetation indices should be used according to the phenological stage of the sugarcane crop to monitor foliar N.

The spectral sensors with bands close to the red edge are more sensitive to the foliar N concentration.

The vegetation indices are more efficient for monitoring sugarcane cultivation than the foliar chlorophyll measurement.

ABSTRACT

Sugarcane (*Saccharum spp. L*) is a crop with a high nitrogen demand, making it essential to utilise tools that facilitate nutritional diagnosis at each phenological stage, enabling timely corrective measures.This work aimed to evaluate the effect of nitrogen application rates (0, 80, 160, and 240 kg N Hm^{-2}) on the spectral response, photochemical properties, and biophysical characteristics of the sugarcane crop. A randomised complete block design was implemented, comprising four treatments, five replications, and biannual measurements taken from 60 to 210 days after emergence within the planting square. The nitrogen (N) doses had significant effects on stem height, the number of fully expanded green leaves, leaf area index, and foliar N content. Spectral indices near the red edge, such as Red Edge Normalized Difference Vegetation Index and Enhanced Vegetation Index were more sensitive than biophysical variables in detecting differences between N doses. Additionally, the Normalized Difference Vegetation Index and Green Normalized Difference Vegetation Index successfully identified variations in leaf nitrogen concentration associated with phenological development, particularly during the early stages of crop growth. The aforementioned spectral indices can be effectively incorporated as diagnostic tools in the operational management of nitrogen fertilisation in sugarcane crops.

KEY WORDS

Vegetation index, chlorophyll, phenological development

INTRODUCTION

Sugarcane (*Saccharum spp. L.*) is a semi-evergreen crop with a high nitrogen (N) demand, requiring up to 500 kg N hm^{-2}. It is the most important crop in tropical and subtropical regions (FAO, 2017), driven by the growing demand for sugar and ethanol in emerging economies (Zhong et al., 2021), as well as for other biomass-derived by-products such as bagasse, fertilisers, cogeneration energy, and bioplastics. Colombia ranks as the second-largest consumer of fertilisers in Latin America, with usage 5.8 times higher than the regional average of 84.2 kg hm^{-2} of arable land, exceeding countries such as Chile, Brazil, Mexico, and Argentina (Rodríguez et al., 2019).

Monitoring the health status and physiological condition of crops is essential in any agricultural production system, particularly for identifying symptoms associated with deficits or excesses in the fertilisation of key elements such as nitrogen (N). This element, required in large quantities, is a limiting factor for plant growth in both natural and agricultural environments. In this context, there is a global need to develop economical, non-destructive, rapid, reproducible, and repeatable alternatives for agricultural monitoring, using reliable data acquisition methods. Among these tools, spectral bioindicators stand out as valuable alternative inputs for crop modelling and management.

The management of nitrogen fertilisation in traditional crops such as sugarcane is crucial due to the environmental costs, including pollution by NO_3^- and N_2O, as well as the economic costs it entails for this agroecosystem. Consequently, diagnosing the crop's nutritional status, particularly its response to nitrogen fertilisation, requires precise and reliable alternatives. Nitrogen fertilisation in sugarcane poses a challenge (Amaral et al., 2015), as there are few early and reliable tools to determine the availability of this element, especially in tropical regions. This is primarily due to the complexity of the nitrogen (N) cycle, soil variability, and the lack of methods for real-time determination of N availability. In the pursuit of optimising this practice, it has been found that foliar N concentration can be related to spectral response, measured using optical spectroscopy techniques such as diffuse reflectance at the crop canopy scale (Amaral et al., 2015; Andrade et al., 2015).

Studies have reported experiments under various soil and climatic conditions, using different fertiliser sources, nitrogen dosages, and sugarcane varieties. However, in most cases, the number of samplings conducted is limited, typically involving no more than two measurement points for spectral response, biophysical, and photochemical characteristics, across different nitrogen fertilisation rates during the crop's vegetative cycle. In light of this, there is a need for specific studies aimed at developing standardised measurement protocols to determine the spectral response of particular sugarcane varieties throughout the complete vegetative cycle and under specific soil and climatic conditions. Collecting such information would facilitate the creation of databases containing spectral bioindicators and enable periodic monitoring to evaluate the effects of varying nitrogen fertilisation rates. This is particularly important given the high variability of nitrogen forms available in the soil and their critical role in the optimal development of the crop.

According to the above and the importance of managing nitrogen fertilisation due to the environmental and economic costs it imposes on agroecosystems, it is essential to diagnose the nutritional status of crops using accurate and reliable technologies in alluvial areas such as the Cauca River Valley. These areas are particularly vulnerable to nitrate contamination resulting from the inefficient application of nitrogen fertilisation in agriculture (Arauzo et al., 2011). Among these technologies, optical spectroscopy stands out as it allows the acquisition of reflectance spectra and vegetation indices such as VOGRE and DVI, which adequately represent nitrogen concentrations in sugarcane crops (Reyes Trujillo et al., 2021). However, our hypothesis suggests that no single vegetation index should be relied upon to assess the nutritional status of plants. Instead, the effectiveness of specific indices may vary depending on the phenological stage of the crop, making some indices more suitable than others for nutritional diagnosis.

The global relevance of our work lies in its contribution to the development and application of advanced, accessible technologies adapted to the agroecological conditions of tropical regions, for the monitoring and management of agricultural crops. The main purposes of this research include the development of methods to assess crop health, the acquisition of spectral data as an analytical tool, and the creation of light interaction models.

The general objective was to evaluate the spectral bioindicators, biophysical, and photochemical variables under different doses of nitrogen fertilization and phenological stages in sugarcane cultivation. The specific objectives of this study are: 1) To identify and apply techniques based on optical spectroscopy of diffuse reflectance and transmittance in the visible and near-infrared (VIS-NIR) range. These methods will enable a non-destructive, rapid, cost-effective, reproducible, and repeatable evaluation of the health and physiological status of the crops. 2) To utilise the spectral properties of the crop, determined by its biochemistry, leaf structure, and biophysical characteristics, to infer its metabolism and physiology. This will facilitate the study of how environmental stresses, such as nutrient deficiencies (particularly nitrogen), impact the overall spectrum or specific bands. 3) To establish models for analysing light interaction with the crop at the leaf and canopy level in the variety (CC-01 194).

MATERIALS AND METHODS

The experiment was conducted between April 2018 and August 2019 at the Experimental Station of the Agricultural Soil and Water Laboratory (LASA) (3°22'22.58" N; 76°31'47.57" W) at the Meléndez campus of Universidad del Valle, Cali, Colombia (Figure 1). The experimental site is situated at an altitude of 995 metres above sea level (MASL) within a tropical dry forest climate, characterised by two rainy seasons and two dry seasons alternating throughout the year. Figure 2 illustrates the monthly total rainfall and average temperature during two cycles of the rapid growth stage of sugarcane. The total rainfall during the planting cycle was 575.4 mm, with an average temperature of 24.1 °C. The predominant soil type is Udertic Haplustoll, classified as part of the Arroyo (AY) consociation. This soil is characterised by slow internal drainage, very slow external drainage, and a moderately deep to deep effective rooting depth.

Figure 1. Location of the experiment: Experimental Station of the Agricultural Soil and Water Laboratory - LASA. Universidad del Valle.

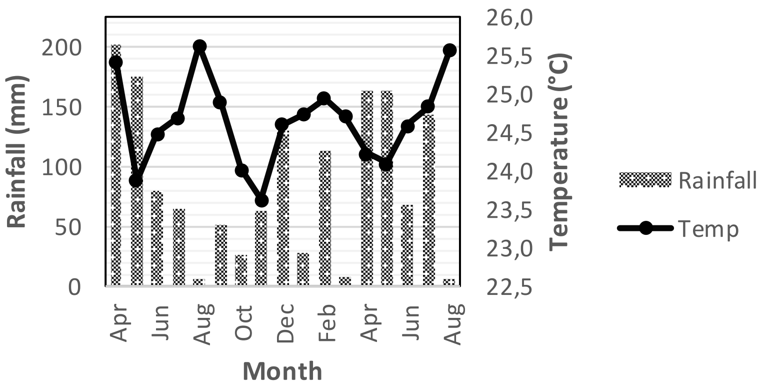

Figure 2. Total monthly rainfall and mean temperature during the two cycles of the rapid growth stage of sugar cane variety CC 01-1940 in 2018-2019.

A randomised complete block design with five replications was implemented for the two cycles. The experimental layout was determined based on the soil's organic matter (OM) content to minimise variability. Four nitrogen (N) levels, using UAN-32 solution, were applied, resulting in a total of 20 experimental units (EUs), each measuring 33 m². Each EU consisted of four furrows, 5 metres in length and spaced 1.65 metres apart, planted with the sugarcane variety CC 01-1940. To prevent border effects between treatments, a furrow was left as a buffer between plots.

The N levels applied during the rapid growth stage were: (i) 0 kg N hm^{-2}, (ii) 80 kg N hm^{-2}, (iii) 160 kg N hm^{-2}, and (iv) 240 kg N hm^{-2}. High-frequency localised fertigation was scheduled to deliver the N doses and meet the requirements for other primary nutrients. This was done according to the dosage for each EU and aligned with the nutrient absorption rates (kg hm^{-2} $month^{-1}$) proposed by Muñoz Arboleda (2018). Additional agricultural practices, including tillage, pest and disease control, and herbicide application, were carried out following the recommendations of Vivero Valens (2018).

The seeding square was sown on 20 April 2018, with seed placement at the bottom of the furrow at a rate of 7 to 10 buds per linear metre. During this cycle, six monthly measurement campaigns were conducted, beginning at 60 days after emergence (DAE) and continuing until 210 DAE. It is important to note that in the Cauca River Valley, the sugarcane growth cycle typically spans 12 to 13 months; however, this study focused solely on the tillering and rapid growth phases, covering a period of up to seven months. The response variables, sampling protocol, and methods employed are summarised in Table 1.

Table 1. Spectral, photochemical, biophysical variables and measurement method.

Variable		Method
Spectral	Reflectance (R)	*In situ* radiometry
Photochemical	Nitrogen (N)	Kjeldahl in the laboratory
	Chlorophyll a y b (Chl)	Sonication - Spectrophotometry in the laboratory
	Spad index (SPAD)	*In situ* chlorophyll meter
Biophysical	Number of stems (SN), stem height (SH), fully expanded green leaves (GLFE), and TCH	*In situ* measurement.
	Leaf area index (LAI)	Gap fraction. LAI-2200C In Situ Canopy Analyzer

The following vegetation indices were calculated from the spectral data (Table 2)

Table 2. Calculated vegetation indices

Vegetation Index	Vegetation Index
(1) Cirededge = (750/710) - 1	(16) CVI = (820*668)/(560^2)
(2) $NDVI_{750/650}$= (750 - 650)/(750 + 650)	(17) CVIRE1 = (717*668)/(560^2)
(3) NDVIg = (750 - 550)/(750 + 550)	(18) CVIRE2 = (820*717)/(560^2)
(4) CIgreen = (820/550)-1	(19) CIRE1 = 717/(668-1)
(5) CCCI = ((820 - 717)/(820 + 717))/((820 - 668)/(820 + 668))	(20) CIRE2 = 820/(717-1)
(6) NDVI = (820 -668)/(820 +668)	(21) CIG = 820/(560-1)
(7) NDVIRE = (717-668)/(717+668)	(22) CIGRE = 717/(560-1)
(8) NDERE = (820-717)/(820+717)	(23) NGRDI = (560-668)/(560+668)
(9) GNDVI = (820-560)/(820+560)	(24) ENDVI = ((820+560)-(2*475))/((820+560)+(2*475))
(10) GNDRE = (717-560)/(717+560)	(25) ENDRE = ((717+560)-(2*475))/((717+560)+(2*475))
(11) BNDVI=(820-475)/(820+475)	(26) NDRE = ((780-730)/(780+730))
(12) BNDRE=(717-475)/(717+475)	(27) VOGRE = (740)/(720)
(13) EVI = 2.5*(820-668)/(820+6*668-7.5*475+1)	(28) DVI = ((780-668)
(14) EVIRE1 = 2.5*(717-668)/(717+6*668-7.5*475+1)	(29) MNDVI8=((755-730)/(755+730))
(15) EVIRE2 = 2.5*(820-717)/(820+6*717-7.5*475+1)	

(Adapted from Henrich et al., 2012)

In this study, the controlled design was specifically an experiment involving a repeated measurement factor (time – six campaigns) and two additional factors. The first is a block effect or control factor with five levels, determined by the spatial variability of the organic matter content. The second is the treatment factor, comprising four levels of nitrogen (N) dosage.

According to the behavior of the different variables (photochemical, biophysical, and spectral in terms of bio-indicators) measured in the controlled experiment, the application of repeated measures and randomized complete block designs were required. The time factor was measured at different time points, within a block effect in which all levels of the treatment factor have been applied. The general linear model representing this design is given by equation 1:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \tau_k + (a\tau)_{i/k} + (\beta\tau)_{j/k} + \varepsilon_{ijk} \quad \text{(Equation 1)}$$

i= 1,2,3,4,5 j= 1,2,3,4 k=1,2,3,4,5,6

Where μ is the overall average. α_i is the effect of the *i*-th level of the block effect (plots). β_j is the effect of the treatments (fertilizer dosage). τ_k is the effect of the repeated measurement factor (time); $(a\tau)_{i/k}$ is the interaction between the block effect and the repeated measure. $(\beta\tau)_{j/k}$ is the interaction between the treatments and the repeated measure, and ε_{ijk} is the random error.

Repeated measures designs offer the advantage of requiring fewer experimental units compared to other designs, as they account for the residual effect arising from measuring the response variable on the same unit over time. In this study, the assumption of sphericity was validated using Mauchly's W test. In cases where sphericity was not met, a univariate analysis of variance (ANOVA) was conducted. This approach incorporates various test statistics for non-sphericity adjustments, including Greenhouse-Geisser, Huynh-Feldt, and the upper bound correction, with the Greenhouse-Geisser adjustment often regarded as the most robust under such conditions. When sphericity is satisfied, it is possible to perform either multivariate or univariate contrasts. However, univariate contrasts using the epsilon-corrected lower bound statistic are generally preferred due to their reliability in such scenarios.

The other assumption to be tested is the normality of the errors, for which the Shapiro-Will test was applied. Once overcoming these assumptions, univariate or multivariate contrasts were analyzed to make comparisons of the significance of the repeated measurement variable and the treatments.

A randomized complete block design was used for the variables for which the repeated measures design does not apply to determine the effect of the treatments. The model describing this type of experiment is given by equation 2:

$$y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} \quad \text{(Equation 2)}$$

$$i=1, 2,3,4,5 \qquad j= 1,2,3,4$$

Where μ is the overall average, α_i represents the effect of the blocking factor, β_j represents the effect of the treatment factor and ε_{ij} represents the random error.

The assumptions of the model are: $\varepsilon_{ij} \sim N(0, \sigma^2)$, with σ^2 constant y Cov (ε_{ij}, $\varepsilon_{i'j''}$)=0 $\forall i \neq i'; j \neq j'$. The hypotheses to be tested for this model are:

$$H_0: \tau_1 = \tau_2 = \tau_3 = \tau_4 = \tau \qquad \text{vs.} \qquad H_1: \tau_\iota \neq \tau_{\iota'} \qquad \forall\, i \neq i'$$

Calculations of univariate and multivariate analysis of variance, in the case of the repeated measures model and analysis of variance of the randomized complete block design, as well as profile plots and assumption validation test, were performed in the statistical package, IBM SPSS Statistics 28.

RESULTS AND DISCUSSION

In this section, data from the experimentation on the seeding square cycle, during the tillering and rapid growth stages, were analyzed.

- Biophysical variables: SN, the hypothesis of sphericity is not rejected at a significance level of 0.124, while for the variables SH, GLFE, and LAI it is rejected at a significance level of 0.000.
- For the photochemical variables: SPAD, Chla, and Chlb the hypothesis of sphericity is not rejected at significance levels of 0.089, 0.509, and 0.834

respectively, while for the variable N, it is rejected at significance levels of 0.019.

- For the spectral variables: NDVI750650C1, NDVIg, NDVI, NDVI, NDVIRE, GNDVI, BNDRE, EVIRE1, ENDRE, the hypothesis of sphericity is not rejected at significance levels between 0.066 and 0.456, while for the variables CIrededge710C1, CVI, Cigreen, CCCI, NDERE, GNDRE, EVI, CVIRE2, CIG, CIGRE, NDRE, VOGRE, DVI, MNDVI8 it is rejected at significance levels below 0.038.
- In all cases, the variables are analyzed through univariate contrasts, differentiating the test statistics. If they comply with sphericity, the Greenhouse-Geisser test is applied. For the variables that do not comply with it, the lower bound test is applied.

The analysis of variance performed for the repeated measures design of the variables SN, SH, GLFE, and LAI, for which the validation of assumptions about the error was initially performed, fulfilled both for normality and homogeneity of variances, with significance levels above 0.10. Regarding the analysis of the interaction effect between fertilizer doses and time, it was found that at p-value > 0.39, there is no interaction between them, indicating that the treatments can be analyzed independently for the four biophysical variables. The time block effect interaction (plots) was significant for the variables SN, SH, and LAI at a significance of less than 0.08, indicating that the time is dependent on the block effect. Therefore, the time independent of block and treatments is only analyzed for the variable GLFE, this time being significant at a level of 0.000.

Given the independence between fertilizer rates and time, it was possible to perform another ANOVA to compare fertilizer rates within blocks. It was found that there are significant differences (p-value 0.059) between some pairs of doses only for the variable LAI, for which a Tukey post-ANOVA analysis was carried out and it was found that, at a significance level of 0.05 (Figure 3), doses of 0, 80 and 160 kg N Hm^{-2} have statistically equal effects on the LAI variable. The same is true for the second group 80, 160, and 240 kg N Hm^{-2}. Therefore, the difference between 0 kg N Hm^{-2} and 240 kg N Hm^{-2} is the variable with the best results among the biophysical variables.

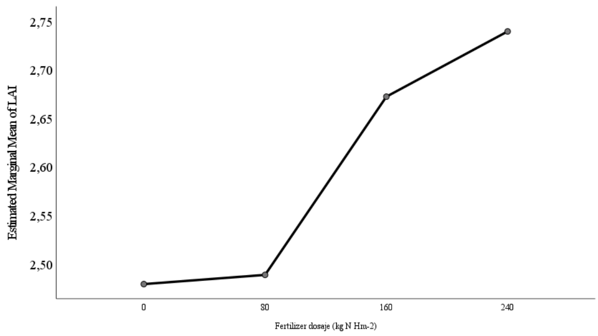

Figure 3. The average behavior of the LAI variable

For the variables SN, SH, and GLFE, there were no significant differences between fertilizer doses at significance levels greater than 0.24. Figure 4 shows the average behavior of these variables, finding similar marginal mean values for each treatment during the six sampling campaigns.

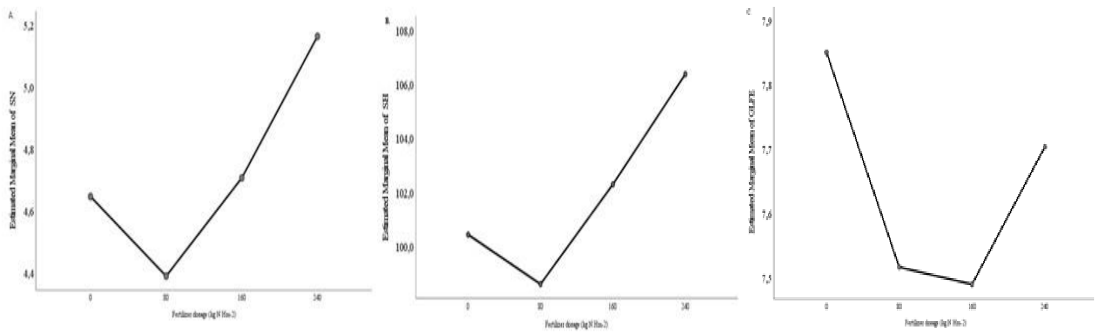

Figure 4. The average behavior of the variables SN (a), SH (b), and GLFE (c)

Figure 5 shows the time-independent behavior of the treatments for the marginal means of the biophysical variables. The observed trends are in line with the results reported by CENICAÑA (2018) for the variety CC 01-1940. A decrease in the population has been found up to six months after emergence (MDE), the greatest height of stems is reached between 5-6 MDE and the variables GLFE and LAI show an increase between emergence and 7 MDE.

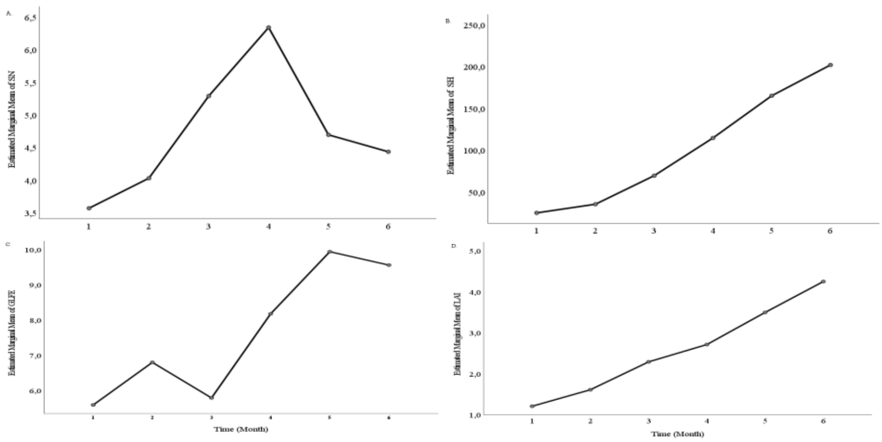

Figure 5. The behavior of time is independent of treatments

In the randomized complete block design for the variable TCH, their models meet the model error assumptions for both normality and homogeneity of variances at significance levels greater than 10%. The analysis of the variance of this design, to compare the treatments for the variable TCH, found that the variation is explained by the effects of fertilizers and the block effect (TCH: SC= 24383). Significant differences between any pair of treatments were found to exist at a significant level of less than 0.001 (p-value) for TCH.

To establish differences between treatments for the variable TCH, a Tukey's post-ANOVA test was carried out, for which, at a significant level of 5%, it was established that: Fertilizer doses 0 and 80 exert the same effect on TCH. Fertilizer doses 160 and 240 also have the same effect (Figure 6). Therefore, N doses below 160 kg N Hm^{-2} (commercial dose) affect the decrease of TCH. Meanwhile, doses above this value have no significant effect in terms of increasing TCH. The above confirms what was reported by CENICAÑA, 2018 that the variety CC01-1940 is sensitive to environmental stress by N, both by deficit and excess.

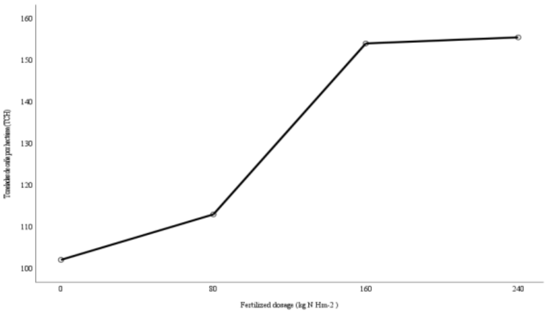

Figure 6. Tukey's test profile graph for the variable TCH

For the analysis of the variables SPAD, Chla, Chlb, and N, given the result of the sphericity test, univariate analysis with assumed sphericity test statistic was examined. Meanwhile, for the variable N, we used within-subjects effects tests, univariate with the lower bound test statistic.

For these variables (SPAD, Chla, Chlb, and N), it was obtained that there is no interaction between time and fertilizer doses with significance levels greater than 0.146. Therefore, fertilizer doses were analyzed independently of time. An ANOVA was used to compare fertilizer doses and found that there are significant differences between fertilizer doses at significant levels less than 0.04. At a significant level of 5%, it was found that:

SPAD: Doses 0 and 80 exert the same effect on this variable, and fertilizer doses 80 and 160 exert the same effect on the variable. The same occurs with treatments 160 and 240, which are also statistically equal, while dose 0 has a different effect to doses 160 and 240; dose 80 has a different effect to dose 240.

Chla: Doses 0 and 80 exert the same effect on this variable, and fertilizer amounts 80, 160, and 240 exert the same effect on the variable, while dose 0 is different from 160 and 240. The same is true for the variable Chlb.

N: Doses 0 and 160 exert the same effect on this response variable, and fertilizer doses 80, 160, and 240 exert the same effect on the variable, while dose 0 is different from 80 and 240.

Figure 7 illustrates that the variables effectively differentiate the effect of fertiliser doses over time, particularly in the case of the extreme treatments. However, for variable N, the doses of 0 and 160 kg N hm^{-2} exhibit a similar effect on the response. This phenomenon can be attributed to the mineralisation of the labile fraction of organic matter, which, under the soil and environmental conditions typical of Mollisols, may stimulate biological nitrogen fixation. This process can supply a significant portion of nitrogen that is available for crop uptake (Vivero Valens, 2018).

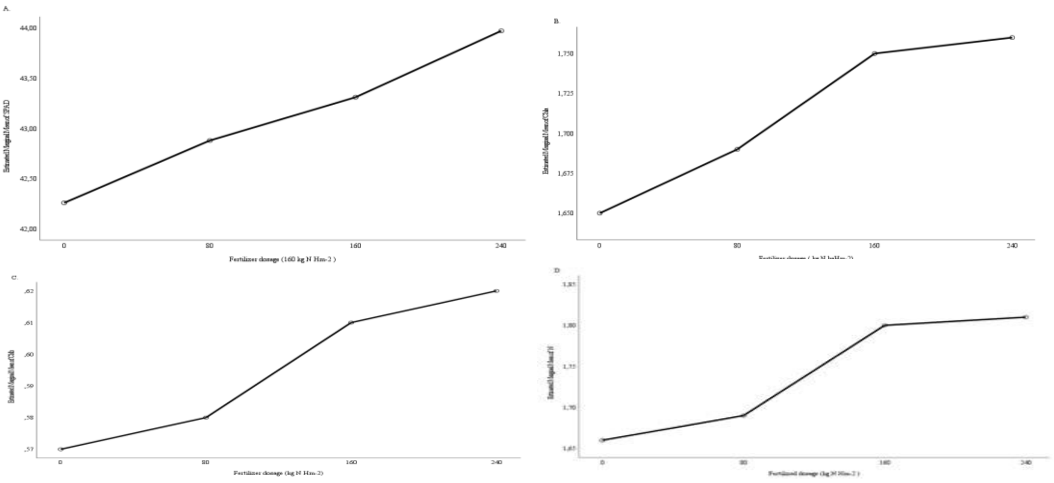

Figure 7. Profile graph for the variables SPAD, Chla, Chlb, and N

Additionally, once the plant absorbs nitrogen in its various molecular forms, it can be incorporated into young tissues as proteins or simpler compounds. It is also present in leaves, stems, and molecules such as purines, pyrimidines, vitamins, alkaloids, and enzymes (Melino et al., 2022). This high functionality of nitrogen within the plant, combined with the factors influencing its availability in the soil and its uptake by the plant, underscores the complexity of achieving significant differences in experiments where nitrogen fertilisation is a key factor.

However, Chla and Chlb, despite the close relationship in their molecular composition with N, can differentiate between fertilizer doses of 0 and 160 kg Hm^{-2}, and this is an indication of the effectiveness of spectral bioindicators using specific bands of the electromagnetic spectrum related to Chl absorption. In addition, nitrogen fertilization has been reported to increase the content of Chl

(Latsague et al., 2014; Sánchez et al., 2018; Seneweera et al., 2011). It indicates the efficiency of nitrogen fertilization (Kopsell et al., 2004) as N favors Mg uptake, which influences Chl synthesis (Shi et al., 2021) and the photosynthetic capacity of leaves because Calvin cycle and thylakoid proteins account for the major part of leaf N (Gibson, 2005).

The SPAD results are consistent with those reported by several authors, when used as an indicator of N nutritional deficiency, and incorporated in models to estimate the spatial distribution of N and improve agronomic fertilization practices (Lin et al., 2010; Markwell et al., 1995; Ribeiro et al., 2015; Zhang et al., 2019; Zhao et al., 2016). However, it has limitations associated with the large number of samples that must be taken to obtain acceptable results and the field operation in a crop with the morphological characteristics of sugar cane.

For the analysis of variance of the repeated measures design for the spectral variables, initially, these met the error assumptions of both normality and homogeneity at significance levels greater than 10%. In the analysis of variance, in general, it was found that at significance levels greater than 0.167 (p-value), no interaction effect between time and fertilizer rates was evident, specifically for the variables NDVI750/650, NDVI, CVI, ENDRE, Cirededge, Cigreen, CCCI, NDERE, GNDRE, BNDRE, EVI, CVIRE2, CIG, CIGRE, NDRE, VOGRE, DVI, MNDVI8. This indicates that there is no dependency between time and fertilizer doses. Therefore, time will be analyzed independently of fertilizer doses.

The analysis of variance to compare treatments under a randomized complete block design without the effect of time showed that for the variables CVI, CCCI, GNDRE, BNDRE, CIGRE, ENDRE, and DVI at significance levels greater than 0.188 (p-value) the doses are not significantly different (Tabla 3). Meanwhile, for the variables NDVI750/650, NDVI, Cigreen, NDERE, EVI, CVIRE2, CIG, NDRE, VOGRE, MNDVI8, and CIrededge710, significant differences were found with p-values less than 0.082 (Table 4).

Table 3. Estimated marginal mean the variables CVI, CCCI, GNDRE, BNDRE, CIGRE, ENDRE, and DVI.

	Estimated Marginal Mean						
Time (Month)	**CVI**	**CCCI**	**GNDRE**	**BNDRE**	**CIGRE**	**ENDRE**	**DVI**
1	2.25	0.46	0.29	0.59	2.10	0.50	23.8
2	2.83	0.43	0.35	0.61	2.50	0.51	28.3
3	3.86	0.57	0.33	0.50	2.20	0.40	33.8
4	3.43	0.58	0.26	0.49	2.10	0.42	28.8
5	4.66	0.75	0.28	0.56	2.30	0.48	55.6
6	5.15	0.7	0.34	0.58	2.80	0.50	49.1

Table 4. Estimated Marginal Mean for the variables NDVI750/650, NDVI, Cigreen, NDERE, EVI, CVIRE2, CIG, NDRE, VOGRE, MNDVI8 and CIrededge710

	Estimated Marginal Mean					
Time (Month)	NDVI750650	NDVIg	Cigreen	NDERE	EVI	CVIRE 2
1	0.66	0.55	2.60	0.31	1.74	6.60
2	0.64	0.57	2.90	0.28	1.69	8.50
3	0.64	0.57	3.50	0.37	1.78	9.00
4	0.68	0.6	4.30	0.48	2.09	9.60
5	0.78	0.69	6.80	0.60	2.30	15.20
6	0.82	0.71	8.00	0.60	2.43	20.00

	Estimated Marginal Mean				
Time (Month)	CIG	NDRE	VOGRE	MNDVI8	Clrededge710
1	4.20	0.19	1.70	0.18	1.60
2	4.60	0.15	1.50	0.13	1.30
3	5.10	0.20	1.60	0.18	1.70
4	6.60	0.29	1.90	0.25	2.40
5	9.50	0.38	2.30	0.33	3.50
6	12.40	0.40	2.50	0.34	3.90

For the latter results, a Tukey post-ANOVA test was performed to establish the differences at a significant level of 0.05. In these profile graphs, the variables' trend can be observed showing the sensitivity to identify effects and at what age of the crop they can achieve the highest efficiency. For each response variable, it was found that:

- NDVI750/650: Fertilizer rates 0, 80, and 160 exert the same effect on this response variable, as well as fertilizer rates 160 and 240 exert the same effect on this response variable. Differences were found between doses 0, 80, and 240 kg Hm^{-2}. The same is true for the bio-indicators NDVI, Cigreen, CVIRE2, CIG, NDRE, VOGRE, MNDVI8, and CIrededge710. Therefore, the efficiency of these bio-indicators would lie in identifying extreme N stress conditions and confirming with some bio-indicators what is found in the MFA under uncontrolled conditions.

- NDERE: Fertilizer rates 0 and 80 kg Hm^{-2} exert the same effect on this response variable, and fertilizer rates 80 and 160 kg Hm^{-2} exert the same effect on this response variable. The same is true for the 160 and 240 kg Hm^{-2} treatments, which are also statistically equal, while the 0 dose is different from 160 and 240 kg Hm^{-2}. The dose of 80 kg Hm^{-2} is different from the 240 kg Hm^{-2} dose. The same applies to EVI. These vegetation indices are more effective in identifying the effect of different N doses, which was not possible with the biophysical variables. EVI is also appropriate for retrieving critical crop biophysical variables and changes in the leaf area index (LAI), debido a que está directamente relacionado con la transpiración del cultivo y el proceso de fotosíntesis y a que incorpora la franja azul lo que lo hace más susceptible a la variabilidad especial del cultivo (Woldemariam et al., 2024)

In the repeated measures model, the interaction between time and treatments was significant for the variables NDVI, NDVIRE, GNDVI, and EVIRE1; therefore, they are analyzed by taking the dependence between them into account. Figure 8 shows the behavior of the treatments for each of the times of these response variables. The behavior of the NDVI and GNDVI bioindicators can be highlighted from the trend presented. It can identify variations generated by phenological development, mainly from times 1 and 2 compared to times 5 and 6; they are probably associated with changes in the canopy structure, but not with the effect of fertilizer doses, except for time 1 when the vegetation indices tend to increase in magnitude. Therefore, vegetation indices tend to be more efficient at advanced crop stages. However, the NDVI may saturate when the canopy reaches its maximum development, limiting its ability to provide accurate biomass information. Soil moisture can influence the spectral signature of crops during their early stages. As the canopy closes, vegetation indices become more effective due to the reduced red reflectance caused by chlorophyll presence and the increased near-infrared (NIR) reflectance driven by leaf growth and canopy expansion (Woldemariam et al., 2024).

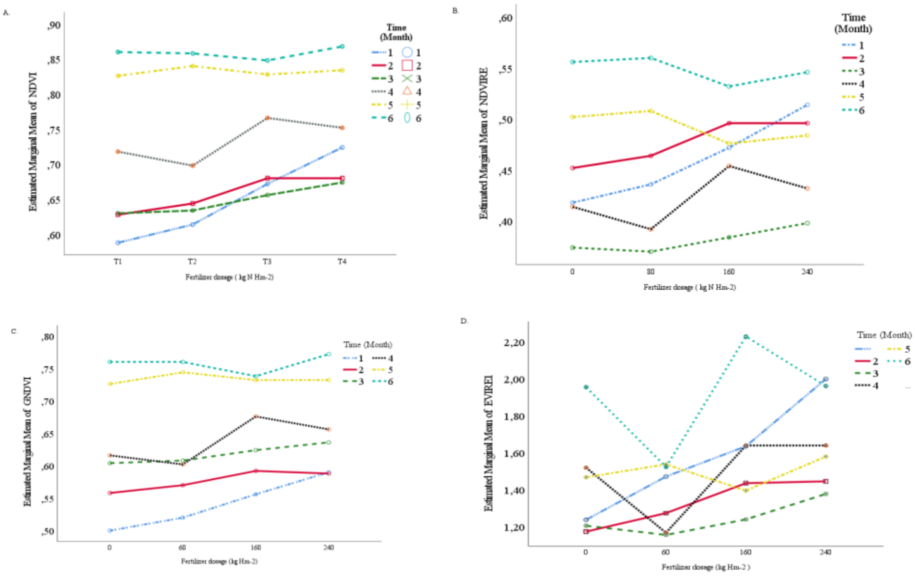

Figure 8. Interaction graphs between time and fertilizer rates for the variables NDVI, NDVIRE, GNDVI, and EVIRE1

The findings of this research align with previous studies that have highlighted the utility of the red edge (761 nm) in estimating biophysical and photochemical variables across various agricultural crops (Nolasco M., 2015; Odi-Lara et al., 2010). Similarly, recent studies on sugarcane have confirmed the importance of integrating spectral information with soil-climatic variables and the crop's physiological characteristics, underscoring the need for integrated approaches to sustainable agricultural management (Inigarro, 2014; Rueda et al., 2015).

CONCLUSIONS

Spectral bioindicators in the visible and near-infrared range (VIS-NIR) provide valuable information for inferring photochemical and biophysical variables of crops. They enable the determination and monitoring of crop health and physiological status in a non-destructive, rapid, cost-effective, reproducible, and repeatable manner.

The results of the analysis of the efficiency of the response variables allowed us to determine their temporal dependence, which is linked to variations arising from phenological development, particularly changes in canopy structure (biophysical) caused by fertilisation. Understanding the temporal variability of spectral variables is crucial for interpretation, their application in sugarcane crop monitoring, and the selection of sensors with specific bands, especially in the red-edge region. In this spectral range, vegetation indices such as Ciredge, Chlorophyll Vegetation Index Red Edge, Enhanced Vegetation Index, Red Edge Normalized Difference Vegetation Index, Vogelmann Red Edge Index, and Mean Normalized Difference Vegetation Index Landsat 8 were identified. These indices proved capable of differentiating between the various nitrogen doses and the commercial dose of 160 kg N hm^{-2}, making them suitable for incorporation into the operational management of fertilisation under commercial crop conditions. Among them, the Normalized Difference Vegetation Index and Green Normalized Difference Vegetation Index were the most sensitive in detecting variations in nitrogen concentration according to the plant's phenological development.

This research is globally significant due to its focus on using advanced and precise tools, such as spectral indices, for efficient nitrogen management in a key crop like sugarcane. Its global implications include:

1. **Optimization of nitrogen use**: Sugarcane is a nitrogen-intensive crop, and its efficient management contributes to more sustainable agriculture by reducing costs and minimising environmental impacts such as nitrate pollution.

2. **Global relevance of the crop**: Sugarcane is a strategic crop in many regions worldwide, being a primary source of sugar and biofuels. This makes research of this nature of global interest.

3. **Application of advanced technologies**: The use of spectral indices as diagnostic tools allows for precise, real-time monitoring of the crop's nutritional status, a practice that can be replicated in various agricultural contexts and adapted to other species.

4. **Promoting agricultural sustainability**: These techniques support precision agriculture, fostering practices that maximise yield while conserving natural resources.

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