

Article

Impact of Standing Water Level and Observation Time on Remote-Sensed Canopy Indices for Rice Nitrogen Status Monitoring

Gonzalo Carracelas ^{1,2,*} , John Hornbuckle ¹  and Carlos Ballester ¹ 

¹ Deakin University, Centre for Regional and Rural Futures, Faculty of Science Engineering & Built Environment, Hanwood, NSW 2680, Australia; carlos.ballesterlurbe@deakin.edu.au (C.B.); j.hornbuckle@deakin.edu.au (J.H.)

² National Institute for Agricultural Research (INIA), Tacuarembó 45000, Uruguay

* Correspondence: gcarracelas@inia.org.uy

Abstract: The observation time and water background can affect the remote sensing estimates of the nitrogen (N) content in rice crops. This makes the use of vegetation indices (VIs) for N status monitoring and topdressing recommendations challenging, as the timing of panicle initiation and the water level in bays usually differ between farms even when managed using the same irrigation technique. This study aimed to investigate the influence of standing water levels (from 0 to 20 cm) and the time of image acquisition on a set of N-sensitive VIs to identify those less affected by these factors. The experiment was conducted using a split-plot experimental design with two side-by-side bays (main plots) where rice was grown ponded for most of the growing season and aerobically (not permanently ponded), each with four fertilization N rates. The SCCCI and SCCCI² were the only indices that did not vary depending on the time of the day when the multispectral images were collected. These indices showed the lowest variation among water layer treatments (5%), while the Clg index showed the highest (20%). All VIs were significantly correlated with N uptake (average $R^2 = 0.73$). However, the SCCCI² was the index that showed the lowest variation in N-uptake estimates resulting in equal N-fertilizer recommendations across water level treatments. The consistent performance of SCCCI² across different water levels makes this index of interest for different irrigation strategies, including aerobic management, which is gaining increasing attention to improve the sustainability of the rice industry.



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Keywords: vegetation indices; unmanned aerial vehicle; irrigation; N uptake; remote sensing

1. Introduction

Rice is the major staple food crop worldwide with an average rice consumption per capita of 53 kg per year and a total production of milled rice of 530 million tons (Mt) in the 2023/2024 rice-growing season [1]. Rice production is forecast to increase to ~548 Mt by 2033, which will be lower than the projected global rice consumption of 552 Mt for that year, with such a rise in consumption mainly driven by population growth [2]. Future rice systems will face the challenge of increasing grain production, closing yield gaps and improving input use efficiency while reducing environmental impacts [3]. Research focused on optimizing nitrogen (N) fertilization and irrigation water management in rice is crucial for addressing the main limiting factors of crop production [4,5] and therefore is of significant importance to the agriculture sector and society.

In Australia, rice is mainly cultivated in the Murrumbidgee and Murray valleys of New South Wales (the Riverina) where the highest grain yields are generally achieved compared to other rice-producing countries worldwide [3]. In the 2023/2024 rice growing season, the average rice grain yield was 11.5 t ha⁻¹, ranging from 9.3 to 13.5 t ha⁻¹,

with a total average N application of 207 kg N ha^{-1} , ranging from 184 up to 224 between the different rice growing regions [6]. A yield gap of 4.2 t ha^{-1} exists between high- and low-yielding farmers in Australia [6], as it was also reported for other rice producing countries like, USA [7], Uruguay [8], Brazil [9], Argentina [10] and African countries [11]. This highlights the potential to increase grain yields and close the observed gaps between producers. Rice is the largest irrigated crop worldwide, using more water than other cereal crops [12]. Traditional irrigation management worldwide has been continuously flooding during most of the crop cycle. Water scarcity could potentially limit the water availability for rice production, leading to non-flooded irrigation management and more aerobic soil conditions in the future [13]. Like in many other rice-producing regions of the world [14], rice production in Australia is very susceptible to water availability for irrigation, and increasing water productivity has become a major objective for the rice industry [15,16]. The interest in alternative irrigation techniques to the traditional ponded systems has grown within the rice industry in recent years. One technique that is receiving attention lately due to recent advancements in irrigation automation technology is aerobic rice production [17]. In aerobic rice production, the crop is irrigated intermittently, with the soil allowed to dry to a certain deficit before being irrigated again. Recently, this technique has been reported to significantly improve water productivity on a commercial scale by reducing total irrigation water with respect to the traditional ponded management [18].

Attaining high grain yields regardless of the water management strategy followed is essential to boost water productivity in rice farming systems. For this to occur, soil nitrogen availability for the crop must not be deficient. Nitrogenous fertilizers are crucial for global rice production and food security [19,20], as nitrogen is the most important nutrient for achieving high yields [21,22]. However, an excessive nitrogen fertilizer supply can lead to lodging and yield reductions, as well as an increase in production costs and a negative impact on the environment [23,24]. In direct drilled rice, as the preferred rice-sowing method when conditions are favorable in the Riverina, N management consists of an initial fertilizer application at sowing, followed by the application of urea at tillering before the crop is ponded (pre-permanent water) and a subsequent top-dressing application at panicle initiation. The paddock history and grain yield expectation determine the rate of N to be applied to the crop pre-permanent water at tillering [25]. The second application of urea is commonly guided by plant N uptake measurements conducted at panicle initiation (PI) using the PI tissue test service [26,27]. However, this approach is labor intensive, provides limited information on spatial variability and very often is not representative of the field due to the reduced number of samples usually collected [28]. Remote sensing technology has been shown to be useful to monitor the N status of crops and better capture the spatial variability within fields [29–32]. This is because N deficiency in plants leads to a reduction in the leaf chlorophyll content and an increase in reflectance in specific regions of the spectrum, such as the green, red and particularly the red edge regions [29,31]. Crop reflectance can be measured with a range of sensors from ground-based, drone, plane and satellite platforms [22]. Current research on rice N status monitoring in the Australian rice industry is focused on providing growers with remote-sensing-based tools from satellite platforms to estimate N uptake, aiming to optimize N management [33]. Nitrogen uptake information obtained from models developed on ponded rice fields is made available to growers in a dashboard as an N uptake map for them to be able to make decisions based on that information and fertilization guidelines to adjust the top-dressing N rates at PI [34].

Several factors have been identified as influencing canopy light reflectance that make accurate plant N uptake estimations from remote sensing measurements a challenging task. Research has shown that canopy light reflectance is affected by the time of image acquisition [22,35–39], shade from upper leaves in dense crops at PI [40,41] and the existence of biotic or abiotic stress (water deficits, N deficiency, pests and diseases) [22,42]. The canopy light reflectance is also influenced by standing water, the water depth and the turbidity [5,22,43–45]. All of this research implies that the N-estimation performance of models developed for a rice crop with a certain water level and water quality or from images collected at a specific time of the day will be different when used for another rice crop with a varied level of water and/or quality or even the same crop if images are collected at a different time of the day. In a recent study, Carracelas et al. [46] showed that the relationship between N uptake and the chlorophyll sensitive vegetation indices (VIs) used for tracking N deficiency in rice differed between a ponded rice crop and a crop grown aerobically due to the standing water. These authors [46] identified that the simplified canopy chlorophyll content index (SCCCI) and red-edge chlorophyll (Clre) index were the VIs for which the relationship with N uptake was less affected by the presence or absence of water when the multispectral images were collected; further research was encouraged on the subject to identify indices with similar N-uptake-estimation performance regardless of the water background conditions. Since the water level in rice fields at PI can vary largely within a rice-growing region depending on a number of factors (water management strategy, water availability, crop height, field layout) and the water background influences canopy reflectance measurements, identifying indices that could be minimally or not affected by the water background would allow for more generalized and accurate models to estimate N uptake and optimize N management.

This study assessed the N-status-monitoring performance of VIs sensitive to the nitrogen content at rice PI, across different times through the day and across a range of five water levels (0–20 cm), with the main objective of identifying the N-sensitive indices least influenced by standing water and the time of image acquisition. The study was conducted using a split plot experimental design with two side-by-side bays (main plots) where rice was grown ponded for most of the growing season and aerobically (not permanently ponded) and with four fertilization N-rates (split plot). This enabled the assessment of grain yields and N-uptake responses to different crop management (water and nitrogen) strategies and their comparison as a secondary objective of the study.

2. Materials and Methods

2.1. Study Site Description

The study was conducted in a ~2 ha area at the experimental site of Rice Research Australia Pty LTD (RRAPL) located near Jerilderie in New South Wales, Australia (Lat: 35°19'30"S, Long: 145°31'32"E) during the 2023/2024 rice growing season (Figure 1).

A total of 32 soil samples (0–30 cm depth) were collected prior to sowing on the 11 October 2023/2024 with a soil corer sample machine (Christie Engineering, Delaware Road, Horsley Park, NSW Australia) to determine soil chemical and physical properties at the site. Soil samples were sent to a professional laboratory (EAL Environmental Analysis Laboratory, Lismore, NSW, Australia) for analysis (Table 1). The soil type was clay brownish based on the texture and basic color, respectively, and was classified as a transitional red brown earth [47]. The soil presented, in general, a low organic matter percentage, low P and low N levels and a lower Ca/Mg ratio than the indicative guidelines for a clay heavy soil [48,49] (Table 1), the latter most likely associated with compaction and reduced water infiltration.

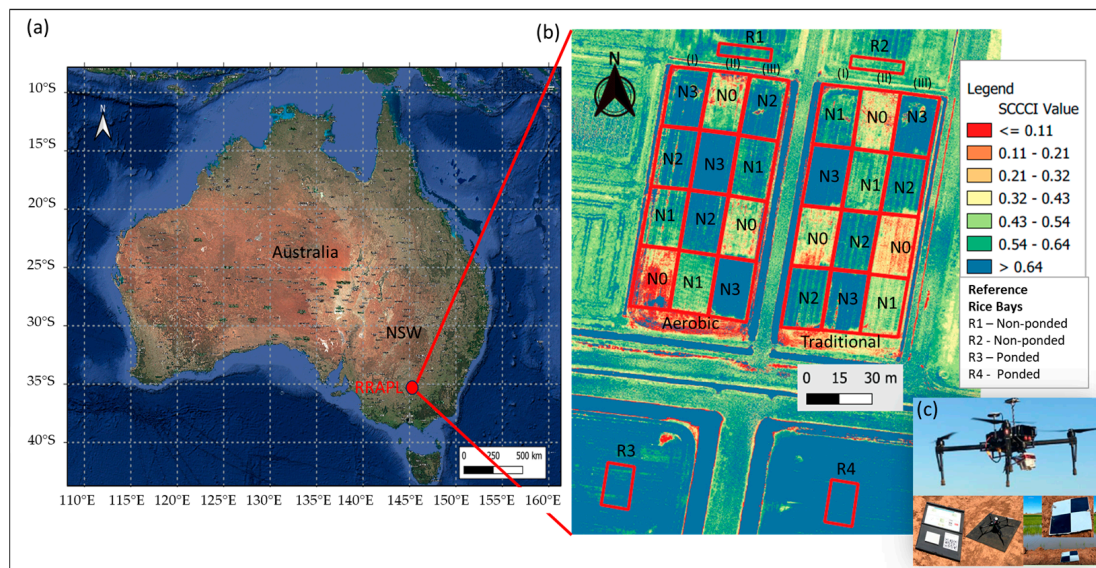


Figure 1. (a) Location of the experimental site at RRAPL (Rice Research Australia Pty Ltd.) in Jerilderie, New South Wales, Australia. (b) Simplified canopy chlorophyll content index (SCCCI) map at rice panicle initiation (11 January 2024), with the N-rate (N0, N1, N2 and N3) plots, replicates (I, II, III) and location of the bays used as a reference for the background conditions indicated (R1, R2, R3 and R4), and (c) illustration of the UAV and multispectral camera, calibration panel and ground control points used in the study.

Table 1. Chemical and physical parameters of the soil at the experimental site. Results obtained at the Environmental Analysis Laboratory—EAL. Available online: www.scu.edu.au/eal (accessed on 5 January 2025).

Soil Parameters	Average	Soil Parameters	Average
pH (water)	7.0	Zn-Zinc (mg/kg)	0.7
pH (CaCl ₂)	6.2	Mn-Manganese (mg/kg)	19.0
Estimated Organic Matter (% OM)	1.2	Fe-Iron (mg/kg)	47.1
Phosphorus (mg/kg P) (Colwell)	30.9	Cu-Copper (mg/kg)	1.8
Nitrate Nitrogen (mg/kg N)	8.1	B-Boron (mg/kg)	1.3
Ammonium Nitrogen (mg/kg N)	1.0	Si-Silicon (mg/kg Si)	104.8
Sulfur (mg/kg S)	26.3	Total Nitrogen (%)	0.1
Electrical Conductivity (dS/m)	0.1	Carbon/Nitrogen Ratio	6.5
Ca—Exchangeable Calcium (mg/kg)	1902.1	Texture (ISSS classification)	Clay
Mg—Exchangeable Magnesium (mg/kg)	896.4	Sand > 20 µm	40.5%
K—Exchangeable Potassium (mg/kg)	258.0	Silt (2–20 µm)	11.6%
Na—Exchangeable Sodium (mg/kg)	168.8	Clay (<2 µm)	47.8%
Al—Exchangeable Aluminium (mg/kg)	3.0	Gravel > 2 mm	0.1%
ECEC—Effective Cation Exchange Capacity (cmol+/kg)	18.3	Basic Soil Color	Brownish

2.2. Field Management

Land preparation consisted of minimum tillage on laser-leveled bays. All tillage operations and pre- and post-emergence herbicide applications were conducted on dry soil.

The Japonica medium grain variety Sherpa was direct-drilled on the 14 October 2023 leaving a row spacing of 20 cm that resulted in a seeding rate of 130 kg per ha. After planting, both bays were irrigated and drained (flush irrigation) six times from the 16 October until 21 November to promote emergence. Plant establishment measured on 16 November was similar in all bays with an average of 173 plants per m² and values ranging from 138 to 233 plants per m² ($n = 144$). On 28 November, one bay was flooded to grow ponded rice, and the other one was flush-irrigated the whole season to grow rice aerobically.

2.3. Water Management

Irrigation water was managed to grow permanently ponded rice in one plot of 60 m × 140 m (8400 m²) and aerobic rice in another plot of the same size (Figure 1). In the

traditional ponded management, a water layer (defined as a level of water above the soil surface) of about 15 cm was maintained for most of the crop cycle except for the microsporogenesis stage when the water layer was increased to 25 cm to buffer the crop against possible low air temperatures that could cause pollen sterility [50,51]. The plot was drained at the end of the growing season when the grain moisture was 23% to allow the soil to dry for harvest.

In the plot with aerobic rice, irrigation events were followed by drainage of the bay to establish aerobic soil conditions. The soil water tension at a 15 cm depth was left to dry to about -20 kPa, although it occasionally dropped to a minimum value of -30 kPa (Figure 2). That corresponded to irrigation events occurring at an interval of 3–5 days.

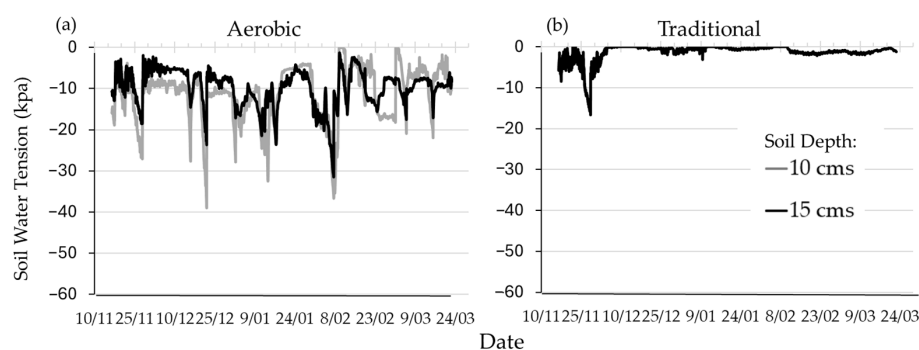


Figure 2. Soil water tension measured with watermark sensors at 10 cm (grey line) and 15 cm (black line) in the (a) aerobically and (b) traditionally managed bays.

2.4. Nitrogen Management

The main plots were both fertilized with mono-ammonium phosphate (MAP) at 150 kg ha^{-1} . The first urea application occurred on 27 November at the early pre-tillering (V3–V4) stage [52]. Urea was top dressed in both main plots using a tractor with an automatic variable rate spreader to apply four N rates replicated three times in a split plot design with each subplot having a size of 20 m by 35 m (700 m^2) (Figure 1). The N rates applied were a control with no N (N0), the recommended N rate (N2) according to the maximum attainable grain yield expectation and bay history and a dose 50% below the recommended N rate (N1) and 50% above the recommended N-rate (N3). In the ponded plot, 100% of the N was applied up front at early tillering before permanent water (Figure 3). In the plot where rice was grown aerobically, 40% of the N was applied at pre-tillering (V3–V4), with the remaining 60% at tillering, on 22 December before the start of the reproductive period PI [52] (Figure 3). After each urea application, water was held in the plots for one week for N stabilization. Totals of 0, 75, 150 and 225 kg N ha^{-1} were applied in the N0, N1, N2 and N3 treatments, respectively.

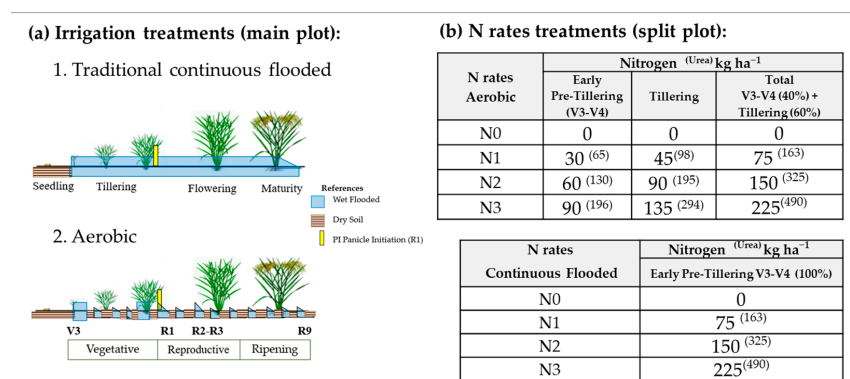


Figure 3. (a) Schematic of the irrigation treatments (traditional continuous flooded and aerobic), and (b) details of the fertilization treatments applied in the study.

2.5. Water Layer Treatments

At rice panicle initiation on 11 January 2024, water from the ponded plot was drained in the morning to ground level (WL1 = 0 cm) to have the same water level in both main plots. Six ground control points were deployed to determine the boundaries of the study site, and multispectral images were taken around midday with a six-band multispectral camera (Altum, MicaSense Inc., Seattle, WA, USA) installed on an unmanned aerial vehicle (UAV) system (Matrice 100, DJI) (Figure 1). After this, water was allowed to enter both bays for about 1 h, and images were collected again. This process was repeated five times with the last set of images collected at 4:30 pm with a water layer in both main plots of ~20 cm (WL5). The time when images were taken and the water level at the main plots at the moment of the measurements is provided in Table 2. To account for the effect that the time of the day when images were captured had on canopy reflectance [38], images were collected in contiguous bays planted with rice at the north and south sides of the study site where the crop remained either ponded or in absence of surface water during all flights. Two areas in each of these two bays were selected as reference plots under non-ponded (R1 and R2 plots) and ponded conditions (R3 and R4 plots) (Figure 1).

Table 2. Water levels at both main plots when multispectral images were collected along with the time at which each UAV flight took place at panicle initiation.

Water Layer Treatment	Water Height Average (cm)	UAV Flight Time
WL1	0	12:18
WL2	8	13:35
WL3	12	14:30
WL4	16	15:32
WL5	20	16:30

2.6. Remote Sensing Measurements

Flights were automated using the Pix4Dcapture app (available online: <https://www.pix4d.com/product/pix4dcapture>, accessed on 20 December 2024) on an iPad to capture images with an 80% overlap at 60 m above ground level, which provided a ground sampling distance of 2.64 cm. The six bands of the camera corresponded to the spectral reflectance in the blue, green, red, red edge, near infrared (NIR) and long-wave infrared regions centered at 475, 560, 668, 717, 842 and 1100 nm, respectively. Only the green, red, red edge and NIR bands were used to compute the selected vegetation indices for this study, and they are presented in Table 3. Pictures of a reflectance calibration panel were taken before each flight to remove the effects of sunlight variation and reflectance characteristics. The multispectral images were processed with Agisoft software (version 1.8.0, Agisoft Metashape, Saint Petersburg, Russia). Images of the ground control points were used during the image processing to geo-reference the images. The Geo-TIFF files generated for each set of images with information of the multispectral bands were post-processed in QGIS (version 3.26). Vector grids were created to delimit the reference plots and subplots leaving a one-meter buffer gap between subplots at each main (irrigation) plot. The QGIS tool zonal statistics was used to obtain the average reflectance at each subplot and reference plots for each flight and calculate the VIs in Excel according to the formulas shown in Table 3. All the VIs assessed have been previously reported in the literature as sensitive to the crop N status except the squared simplified canopy chlorophyll content index (SCCCI²). The SCCCI² was included to assess whether this index could improve the performance of the simplified canopy chlorophyll content index (SCCCI), as previously observed for the normalized difference red edge index (NDRE) and NDRE² indices [28].

Table 3. Vegetation Indices with their formulation computed from multispectral images.

Vegetation Index	Formulation	References
Squared of normalized difference red edge	$NDRE^2 = \left(\frac{NIR-RE}{NIR+RE} \right)^2$	[28]
Chlorophyll green	$Clg = \left(\frac{NIR}{G} \right) - 1$	[53–55]
Chlorophyll red-edge	$Clre = \left(\frac{NIR}{RE} \right) - 1$	[53,55]
Simplified canopy chlorophyll content index	$SCCCI = \left(\frac{NDRE}{NDVI} \right) = \frac{\left(\frac{NIR-RE}{NIR+RE} \right)}{\left(\frac{NIR-R}{NIR+R} \right)}$	[56]
Squared of simplified canopy chlorophyll content index	$SCCCI^2 = \left(\frac{NDRE}{NDVI} \right)^2 = \left(\frac{\left(\frac{NIR-RE}{NIR+RE} \right)}{\left(\frac{NIR-R}{NIR+R} \right)} \right)^2$	Current study

G, R, RE and NIR correspond to reflectance in the green, red, red edge and near infrared regions of the spectrum. NDVI: Normalized difference vegetation index.

2.7. Crop Parameters

Plant samples were collected at PI for above-ground plant biomass determinations. This parameter was used to calculate the N uptake at PI and assess possible differences in canopy development between irrigation treatments at PI. Panicle initiation occurred when 30% of main tillers had a developing panicle 1–3 mm long [57]. Plants from each subplot were cut at the ground level within an area of $1 \times 1 \text{ m}^2$, which included 5 plant rows. Three samples were collected per subplot (N-rate treatments) in both irrigation plots (72 samples in total). Plants were dried in the oven at 60°C until a constant weight was observed.

For the NIR tissue test, at least 5 whole plants, including leaves and tillers, were randomly separated per sample (~100 g) at PI to determine the N concentration (N%) at the Australian grain storage facility (AGS—Sunrice group) [26]. Nitrogen uptake was estimated multiplying N% by the dry matter weight.

The rice grain yield was determined on 27 March 2024 by harvesting a total area of $\sim 180 \text{ m}^2$ per plot with an experimental header (Yanmar, YH7115, Osaka, Japan). Three strips of 25 m long by 2.1 m (header's width) were harvested in each plot when the grain moisture was below 21%. The rice grain yield was normalized to 14% moisture.

Grain moisture was measured with FOSS (Infratec) equipment (FOSS Analytical, Hilleroed, Denmark) [58]. The samples were treshed with a grain sample cleaner (Pfeuffer) [59] and then visually inspected for any contaminants. A total of 373 individual grains per sample (~9000 grains) of 24 samples collected at harvest were scanned to determine potential grain milling quality with PaddyVision® [60,61], a new instrument specifically designed for the rapid analysis of dry or wet paddy rice at the Australian Grain Storage laboratory (Sunrice Group, Leeton, Australia). The main parameters measured were the percentage of fissured or total cracked and immature grain in paddy samples, the grain milling quality score and the pearled percentage.

2.8. Data Analysis

The statistical analysis was performed using R software (R version 4.2.2) [62]. An analysis of variance (ANOVA) was performed to evaluate the influence of water layer management and N-rate application on the VIs (SCCCI, SCCCI², NDRE², Clre, Clg). Means were compared using Tukey's tests. The relationship between the VI and N uptake was assessed for each water layer treatment at panicle initiation. Pearson correlation analysis was performed using the cor.test function [62]. Different models were tested to describe the relationship between VIs and N uptake at PI. The best fitting models were selected based on the coefficient of determination (R^2) and Akaike information criterion (AIC) values. A linear regression model was fitted on the log-transformed dependent variable N-uptake data for the exponential model. Linear regression models were fitted using the lm function in R [63]. A linear–plateau model was fitted for the relationship between SCCCI² and N uptake using the nls function in R [64,65].

3. Results

3.1. Biomass, N% and N Uptake

The N treatments had a statistically significant effect on aboveground biomass, N% and N uptake. The treatment that received the highest N rate (N3) had the highest plant N uptake and N% followed by the N2 treatment. Both N3 and N2 treatments had a significantly higher N% and N uptake than the N1 and N0 treatments and produced significantly more biomass than the no-fertilized treatment N0 (Table 4, visually illustrated in Figure 4). No statistically significant differences in aboveground biomass, N% and N uptake were observed at PI when the multispectral images were collected between the irrigation plots managed traditionally and aerobically (Table 4).

Table 4. Above-ground plant biomass, nitrogen percentage (N%) and nitrogen uptake by irrigation and nitrogen rate (N rate) at panicle initiation.

Treatments	Biomass DM Kg ha ⁻¹	N %	N Uptake Kg N ha ⁻¹
Irrigation			
Aerobic	8094	1.62	142
Traditional	9741	1.55	163
Irrigation effect	ns	ns	ns
N rate			
N0	5575 ^c	0.96 ^c	54.2 ^c
N1	8202 ^{bc}	1.18 ^c	98.0 ^c
N2	10,993 ^a	1.81 ^b	197.3 ^b
N3	10,898 ^{ab}	2.39 ^a	260.5 ^a
N rate effect	***	***	***
Irrigation × N rate effect	ns	ns	ns
Mean	8917	1.59	152.5

Means followed by different letters are significantly different. Asterisks indicate statistical significance at $p < 0.001$ '***'. 'ns' indicates non-significant differences.

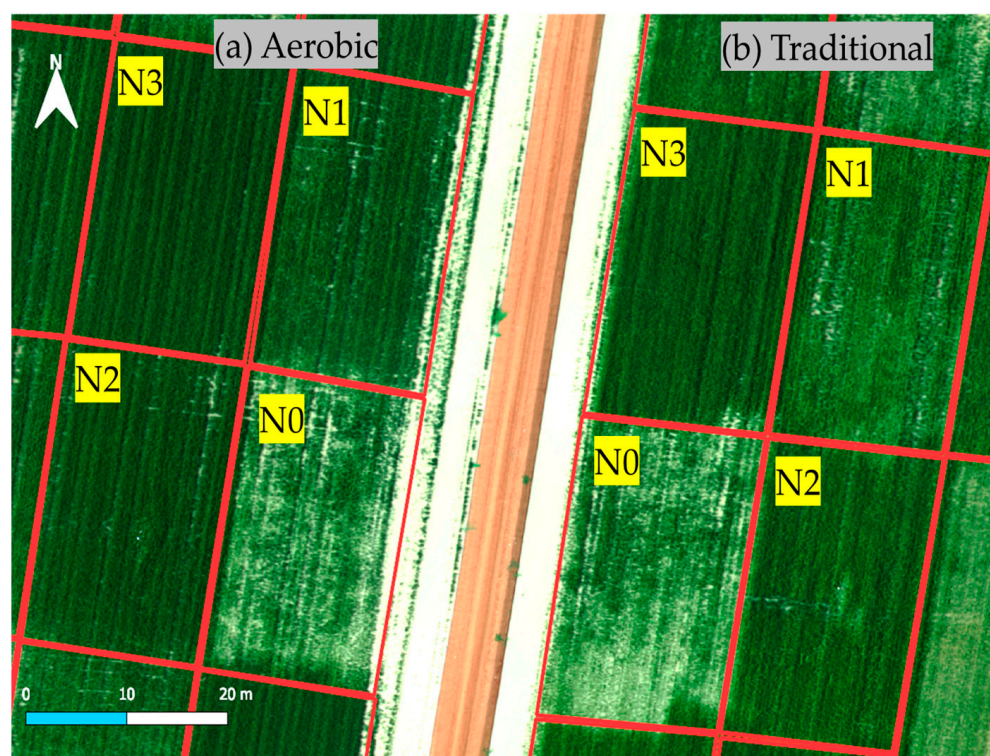


Figure 4. Red–green–blue image of the trial site at panicle initiation (11 January 2024), when the water layer was 16 cm (WL4; 15:32 pm), highlighting the canopy coverage of a few plots of the N rate N0, N1, N2 and N3 treatments in both (a) the aerobic rice and (b) traditionally managed plot.

3.2. Spectral Measurements

Figure 5 illustrates the N-rate effect results of the statistical analysis for the VIs when data from the five sets of images were assessed together. All the VIs significantly increased with the N rate. The SCCCI, SCCCI^2 , Clre, NDRE^2 and Clg ranged from 0.4 to 0.7, 0.2 to 0.5, 0.7 to 3.8, 0.07 to 0.43 and 1.8 to 9.3, respectively.

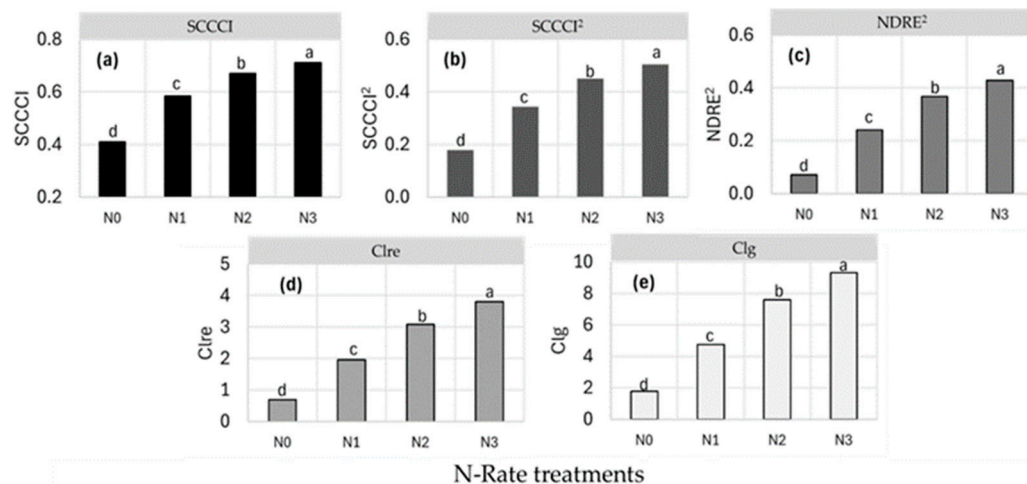


Figure 5. Nitrogen rate (N rate) treatments effect on (a) SCCCI, (b) SCCCI^2 , (c) NDRE^2 , (d) Clre and (e) Clg at panicle initiation. Different letters within N rate treatments for each vegetation index indicate statistically significant differences with a probability of less than 5%.

When evaluating the response of the VIs to the water level and time of image acquisition, the NDRE^2 , Clre and Clg indices were significantly affected, but not the SCCCI and SCCCI^2 indices (Figure 6). No significant interactions were observed between the N rate and water layer treatments for the VIs except for the Clg index, in which, contrary to what was observed for the N1, N2 and N3 treatments, no statistically significant differences were observed among the second, third, fourth and fifth set of images (WL2, WL3, WL4 and WL5 treatments, respectively) in the N0 treatment.

To better discern between the effect that the time of the day at which images were collected and level of water in the main plots had on the VIs, the relative variation of the indices with respect to the first remote sensing measurement (at 12:18 h) was assessed for the experimental plots and reference plots (ponded and not ponded), where the water background remained unaltered during the study (Figure 7). On average, for the non-ponded reference plots, variations of up to 19% were observed for the NDRE^2 , Clre and Clg indices with respect to the first set of multispectral images. The variation observed for the NDRE^2 , Clre and Clg indices with respect to the first set of multispectral images in the ponded reference plots was up to 17%, 26% and 38%, respectively (Figure 7). Most of the variation in these indices was observed in the measurements taken at 13:35 h and 14:30 h in the non-ponded plots and at 15:32 h and 16:30 h in the ponded reference plots. Contrary to that observed for the NDRE^2 , Clre and Clg indices, a lower variation (up to 1.5%) was observed, on average, for the SCCCI and SCCCI^2 with respect to the first set of images in the non-ponded and up to 6% in the ponded reference plots (Figure 7). The SCCCI and SCCCI^2 were also the indices with the lowest variation with respect to the first set of images (up to 5%) in the experimental plots, where the water layer background was higher every time images were collected. The variation observed in the NDRE^2 and Clre indices was identical (up to 13%), while the Clg index reported the highest variation, which was up to 22%.

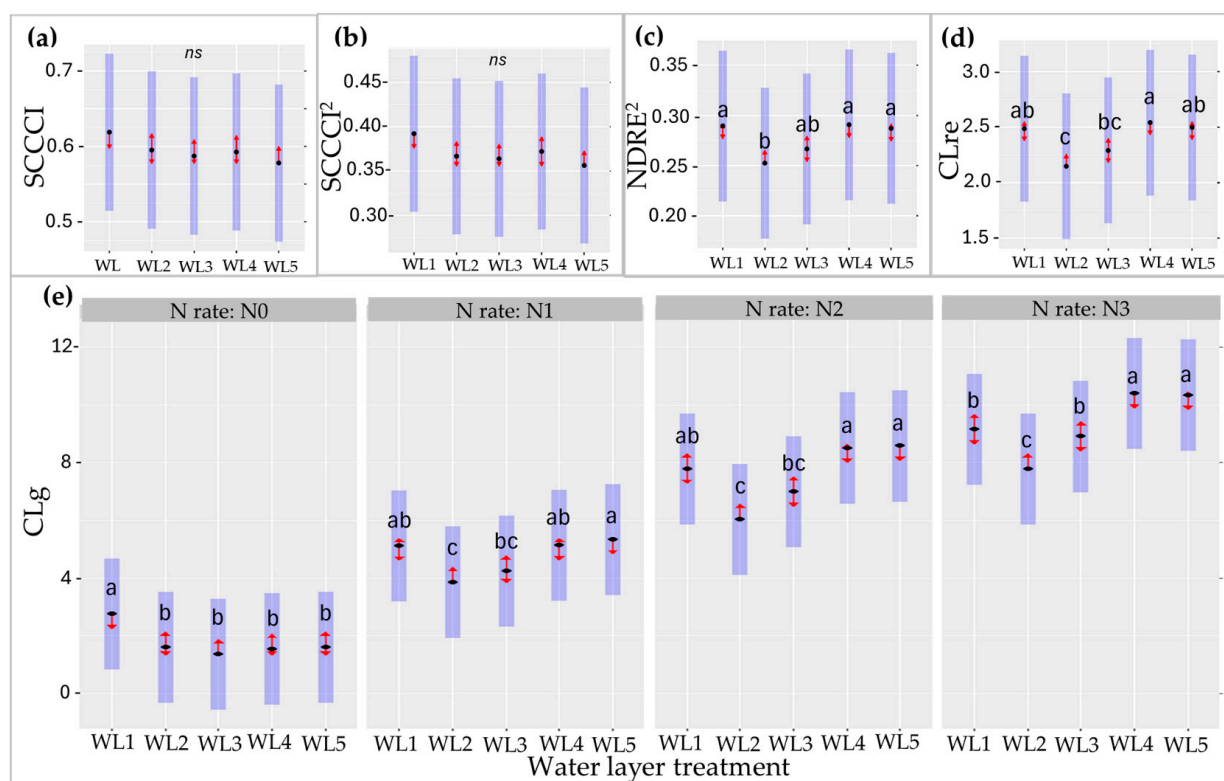


Figure 6. Water layer effect on (a) SCCCI, (b) $SCCCI^2$, (c) $NDRE^2$, (d) Cl_{re} , and (e) Cl_g spectral indices at panicle initiation. For the Cl_g index, in which a significant interaction ($p < 0.001$) between the N rate and water layer treatment was observed, data are reported for each N-rate treatment. Black dots represent the mean values, blue bars indicate the standard error and red arrows indicate the confidence interval. Different letters within water layer treatments for each vegetation index indicate statistically significant differences with a probability of less than 5%, while “ns” indicates non-significant differences.

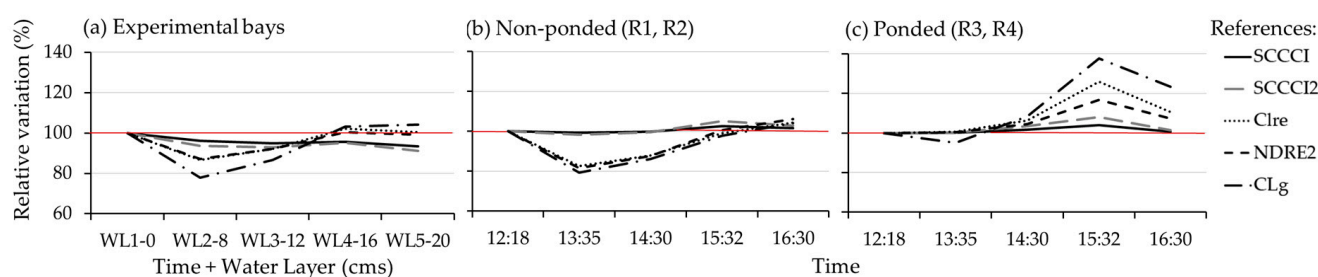


Figure 7. Relative variation for the SCCCI, $SCCCI^2$, $NDRE^2$, Cl_{re} and Cl_g in relation to the first set of images acquired at 12:18 h at panicle initiation (red line) for the (a) experimental plots, (b) non-ponded (average of R1 and R2 plots) and (c) ponded (average of R3 and R4 plots) reference plots.

3.3. Nitrogen Uptake Relationship with Vegetation Indices

All of the VIs were significantly correlated with N uptake at PI, with the coefficient of determination ranging from 0.70 to 0.78 (Figure 8). The relationship found between N uptake and SCCCI was best fitted by an exponential curve in all set of images and water layer treatments (Figure 8). That was also the case for the $SCCCI^2$ and $NDRE^2$ index in most cases, except for the first set of images and first two sets of images, respectively, in which a linear relationship fitted better (Figure 8). Both the Cl_g and Cl_{re} indices were linearly correlated with N uptake under all evaluated water layer treatments (Figure 8).

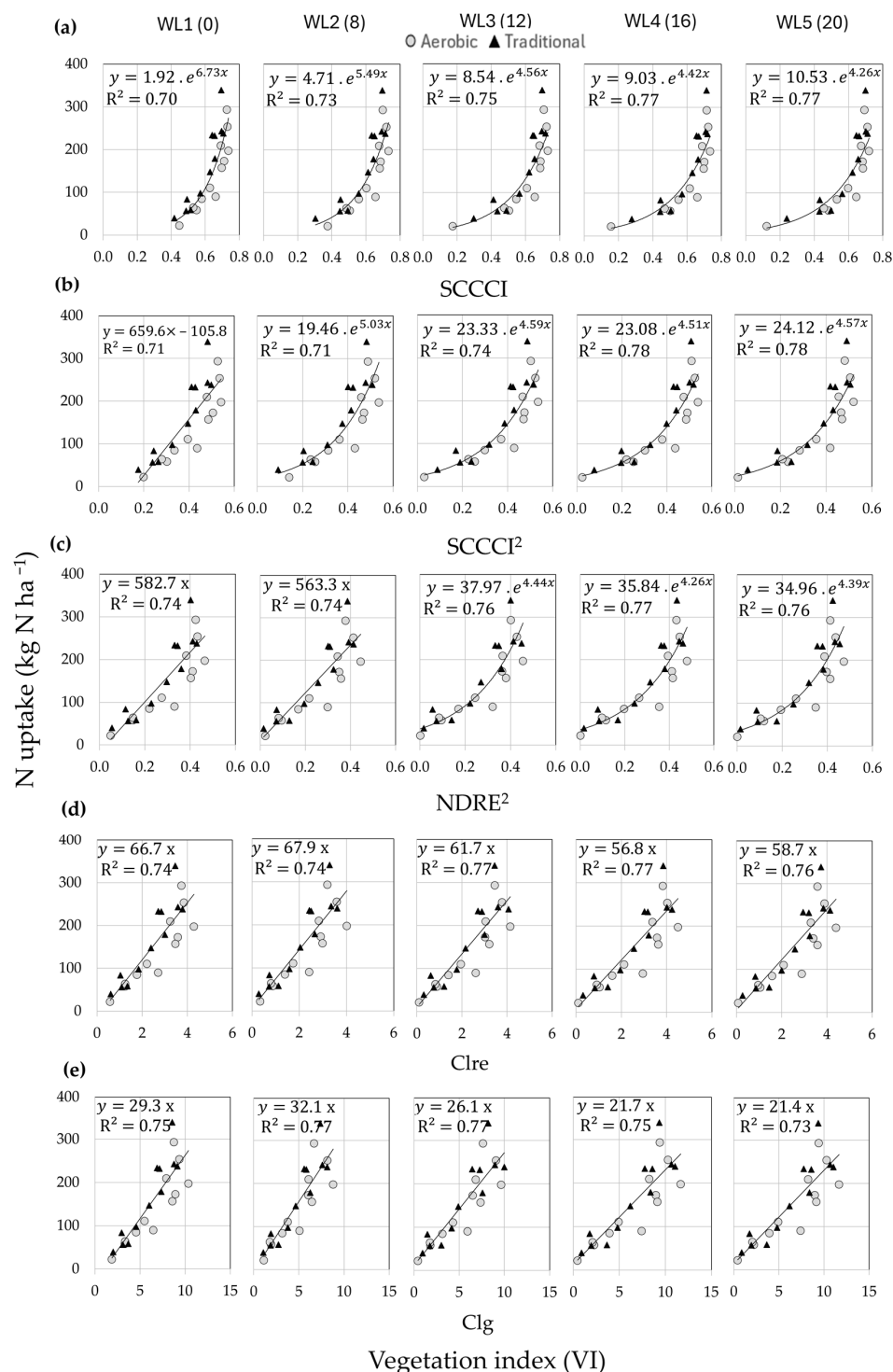


Figure 8. Relationship between N uptake at PI and (a) SCCC I, (b) SCCC I² (c) NDRE², (d) Clre and (e) Clg for each water layer (WL) treatment. Model parameters are shown when statistically significantly different ($p < 0.001$).

When data for all sets of images, including all water layer treatments (from 12:18 h to 16:30 h), were pooled and analyzed together for each vegetation index, the relationship between N uptake and SCCC I, SCCC I² and NDRE² was best fitted by an exponential curve, while the Clre and Clg indices showed a linear relationship with N uptake (Figure 9). The coefficient of determination for the relationship with N uptake was similar among the VIs (0.70 to 0.74). However, a higher dispersion was observed for the Clg, Clre and NDRE² indices than for the SCCC I and SCCC I² (Figure 9).

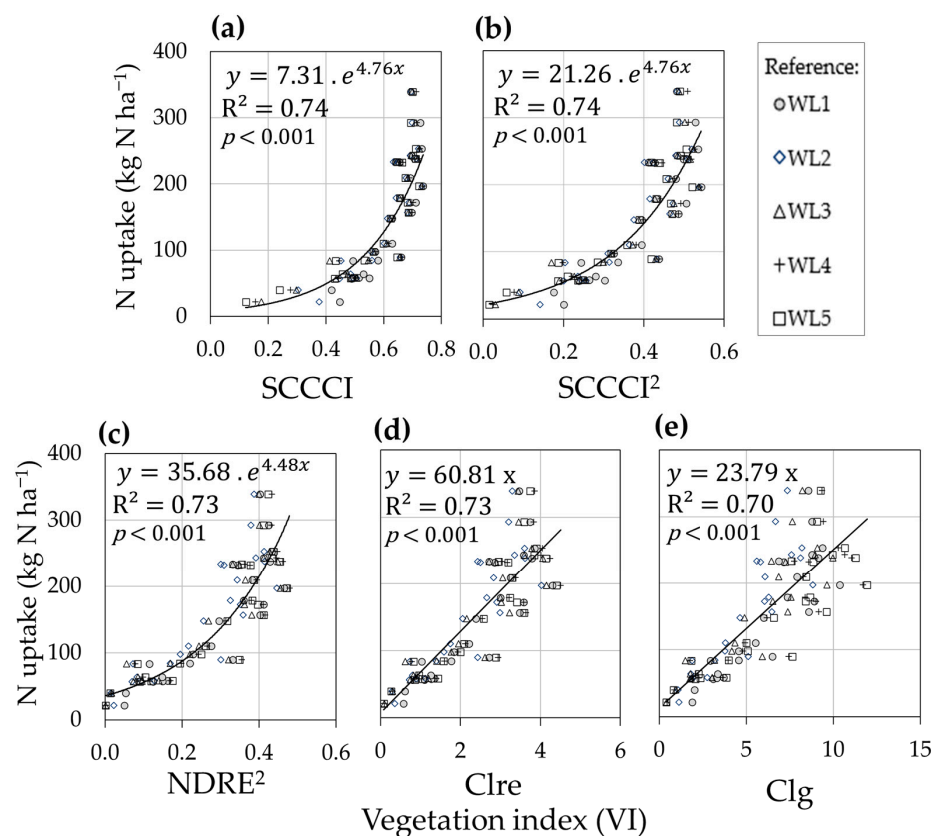


Figure 9. Relationship between N uptake and (a) SCCCI, (b) SCCCI² (c) NDRE², (d) Clre and (e) Clg indices at PI for all the image data sets (from 12:18 h to 16:30 h) assessed together. WL: water layer treatments. Model parameters are shown only when they were statistically significantly different.

A more detailed analysis of the general model obtained for the SCCCI² showed that there is a lineal relationship between this index and N uptake up to 156 kg N ha⁻¹ (Figure 10a). This could have positive practical applications when using this index for top dressing recommendations because the N fertilizer amendments in the rice-growing region where the study was conducted are only recommended for N uptake values at PI within the range in which the relationship was lineal [34] (Figure 10b).

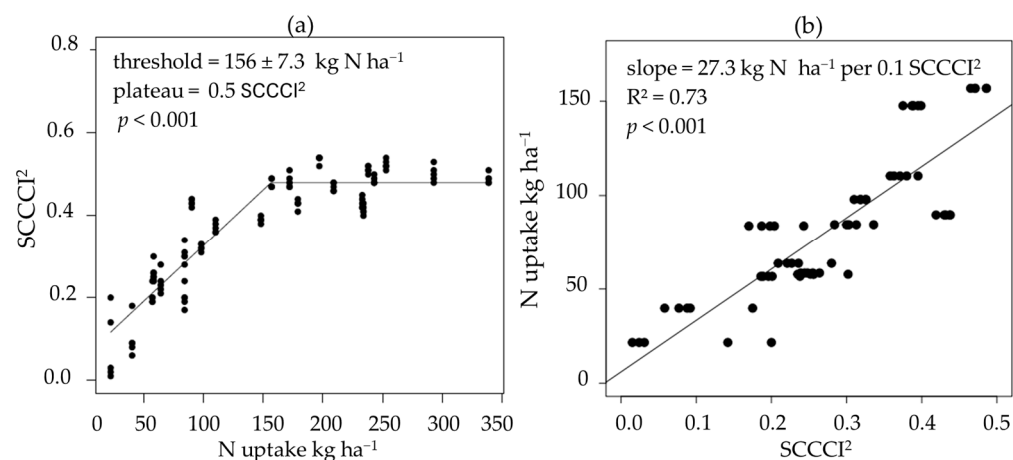


Figure 10. (a) Relationship between SCCCI² and N-uptake levels at panicle initiation from the fitted linear–plateau model (black line) to determine the VI value at which N uptake plateaus. (b) Lineal relationship model between N uptake and SCCCI² up to the plateau threshold value. All parameters (±standard error) were statistically significant ($p < 0.001$). R^2 : coefficient of determination. Model parameters are shown only when they were statistically significantly different.

3.4. Grain Yield and Quality

No differences in grain yield and quality were observed between the plots managed traditionally and aerobically at harvest (Table 5). No interactions were observed between the irrigation and N-rate treatments. Regarding the N-rate treatments, the highest yield was achieved with the N3 treatment, although no statistically significant differences were observed between this and the N2 treatment (Table 5). The lowest yield was obtained with the N0 treatment. The nitrogen rate also had a significant effect on the percentage of cracked and immature grains, as well as the quality score (Table 5). The pearled percentage was similar between all N-rate treatments (Table 5).

Table 5. Rice grain yields (Mg ha^{-1}) and grain milling quality parameters measured with PaddyVision® [60,61], for the irrigation and N-rate treatments.

Treatments	Rice Yield (14%) Mg ha^{-1}	Cracked %	Grain Milling Quality		Score
			Pearled %	Immature %	
Irrigation					
Aerobic	10.4	21.0	0.18	8.9	7.0
Traditional	10.3	32.1	0.20	3.8	6.4
Irrigation effect	ns	ns	ns	ns	ns
N rate					
N0	6.4 ^c	31.4 ^a	0.23	2.5 ^b	6.6 ^{ab}
N1	10.0 ^b	40.4 ^a	0.14	3.6 ^b	5.6 ^b
N2	12.0 ^{ab}	23.8 ^{ab}	0.05	7.6 ^{ab}	6.9 ^a
N3	13.1 ^a	10.7 ^b	0.36	11.7 ^a	7.8 ^a
N-rate effect	***	***	ns	***	*
Irrigation × N-rate effect	ns	ns	ns	ns	ns
Mean	10.4	26.6	0.19	6.4	6.7

Means followed by different letters are significantly different. Asterisks indicate statistical significance at $p < 0.001$, '***', $p < 0.05$ '**' and 'ns' indicates non-significant differences.

4. Discussion

The SCCCI^2 , SCCCI , NDRE^2 , Clre and Clg indices were all able to distinguish the N-rate treatments at PI, which ranged from 0 to 225 Kg N ha^{-1} , with all indices increasing with a higher N rate. This result was expected because all of these indices have been reported in the literature as sensitive to the plant N content [28,33,46,53–55,66–70]. That was the reason why they were selected for this study, aiming to assess how they are affected by the water layer background and observation time. Having accurate plant N uptake estimations in rice crops at PI is important to determine the optimal top-dressing N-rate recommendations and to achieve the maximum attainable crop yield. We showed, in this study, that vegetation indices recommended for N status monitoring in rice can vary notably depending on the time of the day when the images are taken and the water level background at the moment of image acquisition. This variation in the vegetation indices can lead to inaccurate N recommendations when a model is used in different conditions from those in which it was originally developed [28,66,70].

4.1. Effect of Standing Water Layer and Time of Image Acquisition on the VIs

Among the indices studied, the SCCCI and its squared version were the least affected VIs by the observation time (Figure 7). The reference plots (ponded and not ponded), in which water background was unaltered during the day of measurements, showed that the NDRE^2 , Clre and Clg indices can experience a variation equal or higher than 20% due only to the time of the day when the spectral images are collected. Other authors have also reported variations in chlorophyll-sensitive indices, like NDRE , through the day due to the observation time, particularly in under-fertilized treatments where the canopy is not fully developed and surface water is exposed [38]. Background removal approaches have been recommended to minimize

the effect of the observation time on leaf N content estimates [39]. Our study showed that under the conditions in which this study was conducted, implementing background removal approaches could not be necessary when using the SCCCI and its variant $SCCCI^2$ because of the low variation they experienced among observation times. However, background removal methods could be of interest in other conditions and particularly in crops with low canopy ground cover and bare soil exposure.

In the experimental plots, the water layer was 4–8 cm higher every time images were collected and thus, variations in the VIs throughout the day were attributed to both the water layer and observation time. The SCCCI and $SCCCI^2$ were the only indices that did not significantly differ between water layer treatments (Figure 6) and the ones that show the least variation with respect to the first observation time (Figure 7). The variation observed for the SCCCI and $SCCCI^2$, although low (up to 5.0%), was higher than the variation observed for these indices in the non-ponded reference plots (1.5%) when only the observation time was the parameter to consider (Figure 7). This result would suggest that the water layer had an impact on the SCCCI and $SCCCI^2$, although notably lower than the impact observed on the rest of the VIs assessed. Some authors have also observed the impact of standing water on reflectance bands commonly used to compute VIs used for monitoring the N status in rice [22,45]. Hoshi et al. [43] observed a higher water radiant absorption with higher water layers and a reduction in the spectral reflectance. Other authors observed that increases in the water depth led to a decrease in absorption in the visible region of the spectrum and reflectance in the NIR [5,44,45]. The lower effect that the water layer and observation time had on the SCCCI and $SCCCI^2$, compared to the other VIs, may be attributed to the fact they are the result of normalizing the NDRE by the NDVI.

In our study, no interactions were found between the water layer and N-rate treatments for all the VIs except for the Clg. These results would suggest that canopy coverage that was higher in the N2 and N3 treatments according to the biomass measurements (visually illustrated in Figure 4) did not influence the effect of the water layer and observation time on the vegetation indices. This result would agree with other authors reporting differences in reflectance measurements in fully closed canopy rice plots due to the presence of surface water [71]. However, there is also research reporting no effects of water background on rice canopy reflectance when the leaf area index is higher than 5 [45].

4.2. Relationship Between N Uptake and the Vegetation Indices

The relationship between N uptake and the SCCCI was most accurately represented by an exponential model. While the SCCCI has not been tested on rice crops, in other crops where the SCCCI has been tested for N-status determinations, like cotton, the relationship was lineal [68]. The Clre and Clg relationship with N uptake adjusted better to a linear model as also reported by Brinkhoff et al. [33]. The $NDRE^2$ and $SCCCI^2$ exhibited both linear and exponential models, depending on the observation time and water level (Figure 8). A linear relationship was reported for the $NDRE^2$ index in [28,33]. When data from all the remote sensing measurements were analyzed together for each VI, all of them were significantly correlated with N uptake. However, the lowest impact of the observation time and standing water level on the SCCCI and $SCCCI^2$ led to a lower dispersion in comparison with the $NDRE^2$, Clre and Clg indices (Figure 9).

The R^2 values observed for the relationship between the VIs and N uptake when data from all the measurement times were combined together ranged from 0.70 (for the Clg index) to 0.74 (SCCCI, $SCCCI^2$ and Clre). These R^2 values obtained in our study are slightly lower than those reported by Carracelas et al. [46] for the same VIs in a study with the rice cultivar INIA Merin in Uruguay and higher than the R^2 values reported in previous drone-based studies that ranged from 0.64 (for the NDRE) [72] to 0.70 (for the

Clre and Clg) [70]. Figure 11 is shown to better discuss the possible impact of using the relationships obtained between the VIs and N uptake with each water layer treatment for N fertilizer recommendations in the under-fertilized N1 treatment. The N-rate recommendations were obtained from the remotely sensed estimated N uptake and guidelines provided by AGS Sunrice based on targeted N-uptake values at PI [34]. According to these guidelines, N fertilizer applications of 120, 90, 60, 45 and 30 kg N ha⁻¹ are suggested for N uptake range levels of 0–75, 76–105, 106–125, 126–135 and 136–145 kg N uptake ha⁻¹, respectively (Figure 11a). No nitrogen fertilizer is recommended for N-uptake levels above 146 kg N ha⁻¹ at PI. Estimated N uptake for all the VIs varied between water layer treatments (Figure 11a). The observed variability, however, was notably lower for the SCCCI² (estimated N uptake ranged from 112 to 125 Kg ha⁻¹) than for the other indices. The low variability in estimated N uptake obtained with the SCCCI² led to no differences in the recommended N rate (60 Kg ha⁻¹) among water layer treatments. The variability observed in the estimated N uptake using relationships obtained for the other VIs at each water layer treatment, on the other hand, led to differences in N fertilizer recommendations up to 15, 45, 60 and 90 Kg ha⁻¹ for the Clre, SCCCI, NDRE² and Clg indices, respectively (Figure 11b).

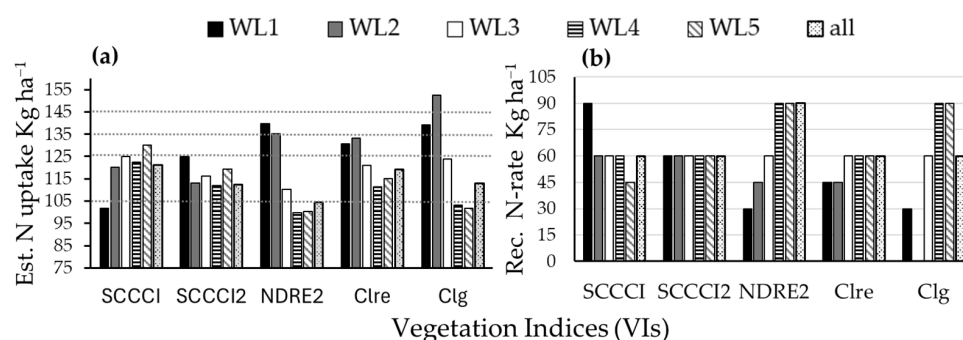


Figure 11. (a) Estimated N uptake in the under-fertilized N1 treatment for each of the vegetation indices (mean value of SCCCI = 0.59, SCCCI² = 0.35, NDRE² = 0.24, Clre = 1.96 and Clg = 4.75) at PI using the models obtained for each water layer (WL) treatment and the general model in which data from all the water layer treatments are combined. The horizontal grey grid lines separate the N-uptake range levels at which the N fertilizer recommendations change. (b) N rate topdressing recommendation based on the estimated N uptake at PI and the table guidelines developed for Australia [34] for N topdressing recommendations.

The fact that the SCCCI² was minimally affected by the observation time and water layer background and that its relationship with N uptake led to no variation in N fertilizer recommendations suggests that this index, which has not been reported elsewhere, could be of great interest for rice N-uptake monitoring at PI regardless of the irrigation management. That means that it could be used in crops under traditional ponded management regardless of the level of water in bays, as well as in aerobic rice management with no surface water at the moment of the spectral measurements. Providing tools that could assist in optimizing rice crop management has been suggested to be positive for boosting the adoption of water-saving strategies, like aerobic rice management by growers [17,18,46]. Such strategies have been shown, in this study and others that also evaluated alternative irrigation techniques [13,18,73,74], to not reduce the grain yield and milling quality in comparison to ponded rice when the water and nutrient management is optimum.

Although the current study shows clear evidence of the better performance of the SCCCI² in predicting N uptake compared to the other VIs studied and commonly reported for N status monitoring [5,33,66,67,70], more research is required at commercial scales on current and newly released cold-tolerant varieties under varied canopy coverage conditions to obtain a general model for optimizing N management in rice.

5. Conclusions

This study showed that the N-sensitive vegetation indices NDRE², Clre and Clg that have been reported in the literature as useful for rice N status monitoring can vary depending on the observation time and water layer background. This implies that different N fertilizer recommendations could be proposed when using these indices to monitor the rice N status under different conditions from those in which the models were originally developed.

The SCCCI and SCCCI² that are the result of normalizing the NDRE by the NDVI were identified as indices that are not influenced by the observation time and that are minimally affected by the water background. Particularly, the SCCCI² was the index with the lowest variation in the N uptake and N topdressing recommendations among water layer treatments. The linear relationship observed for the SCCCI² and N uptake for the range of crop N uptakes in which N fertilizer adjustments are recommended makes this a potential practical index to be used by rice growers. Its consistent performance across various water layers makes this index of interest for optimizing N fertilization under diverse irrigation-management strategies, including aerobic rice that, in this study, did not impair the grain yield and milling quality compared to traditionally ponded crops. Aerobic rice management is currently gaining attention as a strategy for improving water productivity and ensuring the sustainability of the rice industry.

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