43. Potential of the dark green color index for dynamic monitoring of N requirements in wheat crop

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Potential of the dark green color index for dynamic monitoring of N requirements in wheat crop

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Abstract

Alternative methods are proposed for dynamic fertilization management based on different optical measurements to estimate the nitrogen (N) status of winter wheat (Nitrogen Nutrition Index -NNI). In the literature, the Dark Green Color Index (DGCI) has been proposed to assess NNI in rice or maize crops with visible images taken at the leaf scale. The objective of this work was to explore the potential of a relative DGCI to estimate NNI in wheat crops at the canopy scale, with visible images from different optical devices. Experiments conducted in 2022 on winter wheat revealed a high correlation between NNI based on relative DGCI measurements and conventional methods. Due to the configuration of the optical devices, the results are significantly similar when using multiple cameras, including smartphones.

Keywords: Nitrogen fertilization, N-Tester, RGB image, DGCI, proximal sensing.

Introduction

In France, fertilization of cereal crops is mainly carried out by applying mineral nitrogen (N) in the form of solid fertilizers using centrifugal spreaders. Although this equipment can apply variable-rate prescription to supply the right dose at the right place and at the right time, it is also necessary to consider the different growth stages of the crop to define the right times for this application. In most cases, instead of applying the complete seasonal N supplement all at once, the applications are divided up during the vegetative cycle according to the N requirements of the plant at those growth stages. In cereal crops, this application is generally divided into three to four applications. In order to determine the total amount of N fertilizer to supply to field crops, the balance sheet method (Comifer, 2011) has been widely used for decades as reference method. With this method, the amount of N fertilizer is calculated at the end of the winter to support the N requirement for an estimated yield level. However, this method lacks precision because it may be difficult to predict yields. Indeed, climate change and the increased presence of pests in agro-ecological cropping systems lead to greater uncertainty about the yield achieved compared to conventional systems of the past. For this reason, alternative methods, such as the APPI-N method (Ravier et al., 2018), have been developed to measure and assess the N requirements of plant in real-time using the N nutrition index, NNI (Lemaire et al., 1989). This dynamic monitoring of the crop N status allows to adjust N fertilization to plant need across its growth cycle NNI is defined as the ratio of the measured nitrogen concentration (%N) to the critical nitrogen concentration (%Nc; Justes et al., 1994). To determine the value of NNI, destructive analysis of aboveground biomass are needed. In addition, indirect measurement methods of NNI have

been developed, e.g. by measuring the leaf chlorophyll content using non-destructive infra-red proxi-detection devices such as chlorophyll meters (i.e., N-Tester, SPAD). Indeed, the literature reports that, depending on the type of crop and the growth stage, the N status of plant is strongly linked to the chlorophyll content of the leaves and thus to their color (Tracy et al, 1992). Remote sensing has provided dense hyperspectral information that can help us to predict the N requirements of crop. However, currently, visible imaging via smartphones is also being investigated as a simple proximity solution based on a dark green color index (DGCI; Karcher and Richardson, 2003). This index allows the estimation of N content by photographing plant leaves using a color chart to calibrate the method and correct for differences in lighting conditions during experiments. This index has been explored only on maize, soybean and rice crops (Rorie et al., 2011). In the end, all these proxi-techniques remain very tedious because they are punctual and therefore time-consuming.
This study proposed to explore the potential of a new index, relative DGCI, as derived from DGCI using visible imagery in winter wheat crop to estimate NNI at the canopy scale. The correlation of this index with those deduced from conventional methods is presented. To validate the canopy-scale image acquisition protocol, the impact of different factors was tested: 1) DGCI *vs.* relative DGCI to assess the need for standardization of image data; 2) different optical systems to assess the impact of white balance adjustment on image quality; 3) a pure wheat cultivar *vs.* a mixture of varieties to assess the robustness of the new indicator, DGCI.

Materials and methods

Field experimental site: Measurements were carried out in 2022 in 24 microplots (9 m ×1.5 m) at the experimental site of the Institut National de Recherche pour l'agriculture, l'alimentation et l'environnement (INRAE) center, located in Bretenière (Eastern France, 47°13' 25" N; 5°5' 57" E, altitude = 206 m). The main characteristics of the soil are a high content of silt and clay (respectively 50 and 45%) and little sand (5%) (INRAE, 2015), with an organic carbon content of 21 g/kg at and organic nitrogen of 2g/kg. The experiments were tilled and mechanically weeded. Half of the microplots were sown in November 2021 with a winter wheat (*Triticum aestivum* L.) cultivar LG LG Absalon and the other half with a mixture of cultivars (LG LG Absalon, Lipari, Rubisko and Tenor), designated thereafter as "Cultivar Mixture". The general methodology consisted of four steps.
1-Production of an N gradient in the experiments. A wide range of N rates were used to obtain a wide range of wheat NNI values, using several applications of N input doses of 50 kg N ha^{-1} in the form of NH_4NO_3. Five N fertilization rates ranging from 0 (T0) to 320 (Tmax) kg N ha^{-1} were thus carried out with different splitting of the N inputs (T1=1 input to T3: 3 inputs) from the end of winter to heading. Over-fertilized microplots (Tmax) were used as controls for the maximal levels of NNI (Table 1). To take into account local variations through replications, each of the modalities was doubled in a random design.

Table 1. Levels and dates of N fertilizer inputs (in kg N/ha^{-1}) for the 5 treatments (T0, T1, T2, T3, Tmax)

Fertilizer N applications through 50 kg N.ha^{-1} doses		14 February	7 March	28 march	18 April	Total
T0	0 dose	0	0	0	0	0
T1	1 dose	0	50	0	0	50
T2	2 doses	0	50	50	0	100
T3	3 doses	0	50	50	50	150
Tmax	Over-N fertilization	80	80	80	80	320

2- Sampling and measurements. The measurements were carried out on three dates (22 April, 3 May and 17 May) corresponding to two key fertilization stages: two nodes and heading. On each observation date, three types of measurements were performed simultaneously on a single observation area of 0.25 m² (four rows) of the microplots: aerial biomass samples and N content destructive measurements, N-tester measurements using chlorophyll meters and photographic images for the calculation of DGCI. This observation area corresponds to the area covered by a downward-facing camera located 1m from the ground.
Within each observation area, biomass samples were taken by sampling the entire aboveground biomass of the wheat plants over a length of 50 cm in three rows. The dry biomass was then deduced from weighing oven drying (80°C) for 48 hours. The NNI was then calculated using the equations of the N dilution critical curve for wheat.
Measurements with the N-tester (YARA France, 2022) were carried out according to the protocol indicated on the "Manual" of the device, via the clamp of a series of 30 leaves, considering the last spread leaf of the wheat plants. The device provides an average value per series of 30 measurements. It is repeated twice for each observation area. The average of these two values were used in the following. In order to associate the N-tester measurements with an NNI value, values were normalized to the maximal value obtained on overfertilized plot (Ravier et al., 2018).
The photographs were taken with three optical system (Canon EOS 760D; Realme smartphone; Galaxy Samsung 10+ smartphone) to test the effect of the camera type on the DGCI data collected. In order to ensure that all the leaves are clearly visible and to avoid saturation of the photo sensor at certain points, the photograph was taken under homogeneous light conditions. Furthermore, among the shooting parameters, the white balance (Manual *vs*. Automatic) is likely to have an influence on the dark green color index (DGCI) and several set-ups were tested. The modalities and number of images taken during the three measurement dates are shown in Table 2.
Inferential statistics were applied on crop and weed cover using an analysis of variance (ANOVA) and a non-parametric test (Kruskal Wallis Test) to assess the influence of four factors: cultivar, Growth stage, White Balance (BW) and Optical sensor. Statistical analyses were performed in R (version 4.0.3, R Core Team, 2020) and RStudio (2020), an integrated development environment for R.

Table 2. Description of the characteristics of the optical sensors for taking the dataset of images used at each observation date.

Growth Stage	Date	Camera type	Camera set-up	Datset	
			White balance	Number of images	
two-nodes	April 22nd	Canon	Automatic	40	60
		Samsung	Automatic	20	
	May 3rd	Canon	Automatic	48	96
		Realme	Automatic	24	
		Samsung	Manual	24	
heading	May 17th	Canon	Manual	48	95
		Realme	Manual	24	
		Samsung	Manual	23	

3-Image processing-DGCI indicator calculation. From the initial image (RGB), a vegetation image is deduced using an appropriate thresholding to extract only the vegetation pixels. The procedure is described in Gée and Denimal (2020). Then, by changing the color space from RGB (Red, green, Blue) to TSB (Hue, saturation, Brightness), the DGCI of each vegetation pixel is calculated from Rhezali and Lahlali (2017) defined as follow:

$$DGCI = \frac{\frac{H-60}{60}+(1-S)+(1-B)}{3} \quad (1)$$

Finally, the DGCI value of the image is calculated as the average of the DGCI of the vegetation pixels. The DGCI limits are between 0 (yellow hue, potentially reflecting low N content) and 1 (dark green hue, potentially reflecting high N content).
A ratio_DGCI was also calculated as the ratio between the DGCI measurement of the observed plot image and that of the image of an over-fertilised microplot (Tmax). The purpose of this new indicator is to avoid the calibration of images from color chart. The use of the ratio (ratio_DGCI), assuming that the same shooting parameters are used, makes it possible to get rid of the solar illumination conditions.

4-Study of the DGCI/NNI relationship. In order to explore the potential of the DGCI index as a proxy to estimate the NNI deduced from conventional methods (biomass, N-Tester), the DGCI and ratio-DGCI/NNI relationship is studied by examining the influence of different factors. Plant-related factors were studied through variation of wheat cultivar (pure one or a mixture of four) and the growth stage (two-nodes or heading). In addition, factors related to the optical sensors were explored: white balance (Manual vs. Automatic) and camera type (Canon EOS 760D; Realme smartphone; Galaxy Samsung 10+ smartphone).

Results and Discussion

Result presentation is structured in three parts with the validation of the NNI measurements by the classical methods (Biomass and N-Tester), then the validation of the NNI measurements via the image method. Finally, the influence of different factors related to the plant (variety, growth stage) and to the optical systems (white balance, camera types) will be discussed.

Validation of the relationship between NNI_Biomass and NNI_NTester: influence of wheat cultivar.

First, it was necessary to verify the quality of the linear relationship classically observed between the NNI_NTester and the NNI_Biomass. Measurements over the entire dataset over on all three observation dates revealed a significant correlation between the NNI_NTester and the NNI_Biomass with a better coefficient of determination in the case of the cultivar LG Absalon (R^2=0.52, p-value = 5.766e^{-12}) compared to the Cultivar Mixture (R^2=0.37, p-value = 6.528e^{-10}). In addition, better correlation was observed for measurements taken at the "two-nodes" growth stage. In the case of the cultivar LG Absalon the R^2 was 0.52 considering the whole data set (both stages) vs 0.82 for data issued of the "two-nodes" stage. For the Cultivar Mixture, R^2 was 0.37 (with the whole data set) vs 0.52 (data of "two-nodes" stage) (Fig. 1). Both for LG Absalon and Cultivar Mixture, the quality of the relationship was lower at the "heading" growth stage (0.24 and 0.45 respectively) than at the "two-nodes" stage (0.82 and 0.52 respectively).

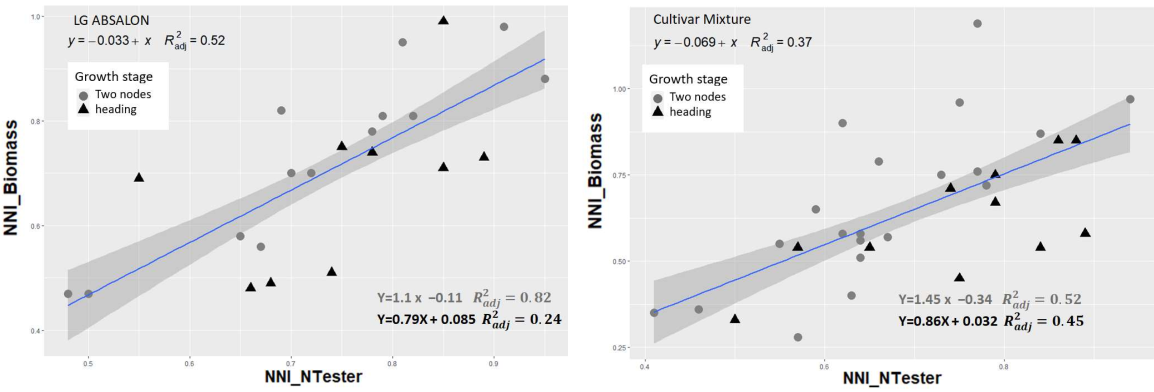

Figure 1. Effect of wheat cultivar (pure LG Absalon on left panel and mixture of four cultivars on right panel) and growth stage (two nodes and heading) and on the relationship between NNI_Biomass and NNI_NTester.

Validation of the relationship between ratio_DGCI and NNI_NTester. Before investigating the relationship between ratio_DGCI and NNI_NTester, the relation with DGCI has been explored. Regardless of the cultivar, the results show that DGCI is sensitive to light and therefore the relationship with NNI_NTester is degraded. Considering the measurements over the entire dataset over on all three dates the correlation between NNI_NTester and ratio_DGCI was then analyzed. It is considered statistically significant (p-value = 1.406e^{-7} and 1.565e^{-9} respectively) even though it is of low quality (R^2=0.35 for LG Absalon and R^2=0.42 Cultivar Mixture). The quality of the last relationship may be dependent on certain camera setup parameters. In particular, attention should be paid to the white balance (WB) setting where two options are available, "Manual" or "Automatic". Figure 2 presents the effect of the white balance in the case of the cultivar LG Absalon. Setting the white balance to the 'manual' option, improved was the correlation quality (R^2=0.72) as opposed to the 'automatic' option (R^2=0.24) which presents an increase in the dispersion of the data. Similar results were observed with the Cultivar Mixture. These results indicate the need to control the illumination between two shots by using the "Manual" mode rather than the automatic mode which performs a global average of the light sources without allowing to understand the interpretation which becomes different from human visual perception.

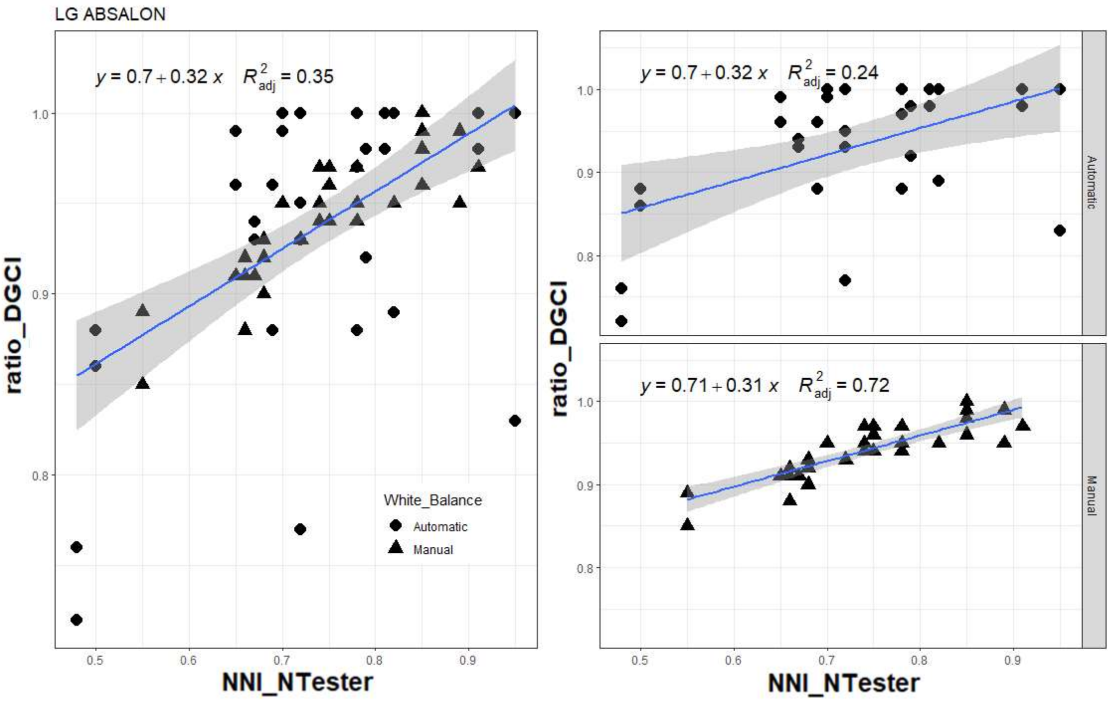

Figure 2. Relationship of ratio_DGCI ∝ NNI_NTester for LG Absalon cultivar (left panel) and evolution of the relationship depending on the white balance set up on the right panel (with "Automatic" option on the top and "Manual" option on the bottom).

<u>Influence of optical sensors on the relationship between ratio_DGCI and NNI_NTester.</u>
The top panel of Figure 3 allows us obsservation of how the imaging devices affect the ratio_DGCI values, deduced from the images. Considering the Canon camera as the reference system and, using the dataset from LG Absalon cultivar with "Manual" option of WB, it can be seen that, there is no significant difference (ns) on the ratio_DGCI values when obtained with the two smartphones (Realme and Galaxy Samsung S10+) or the Canon camera. This result reinforces the idea that the ratio_DGCI value obtained with the "Manual" option of WB is a robust indicator. Then, the relationship between ratio_DGCI and NNI_NTester can be evaluated by device type. This evaluation was carried out considering a specific case: "Manual" option for white balance with the LG Absalon cultivar at the "heading" growth stage due to the lack of data for the "Two nodes" stage. The effect of imaging devices on the ratio_DGCI values, deduced from the images from the smartphones, seem to give better results (R^2=0.92 for Realme and R^2=0.87 for Galaxy Samsung S10+) than those obtained with the Canon camera (R^2=0.76).
Other measurement campaigns are to be carried out to verify the robustness of the method over time and on other wheat cultivars. In the longer term, the use of this new indicator on smartphone or drone imagery should be envisaged, making the monitoring of wheat N status simpler and less restrictive for farmers and other users who are keen to use new, easy-to-use technologies at the scale of a plant cover.

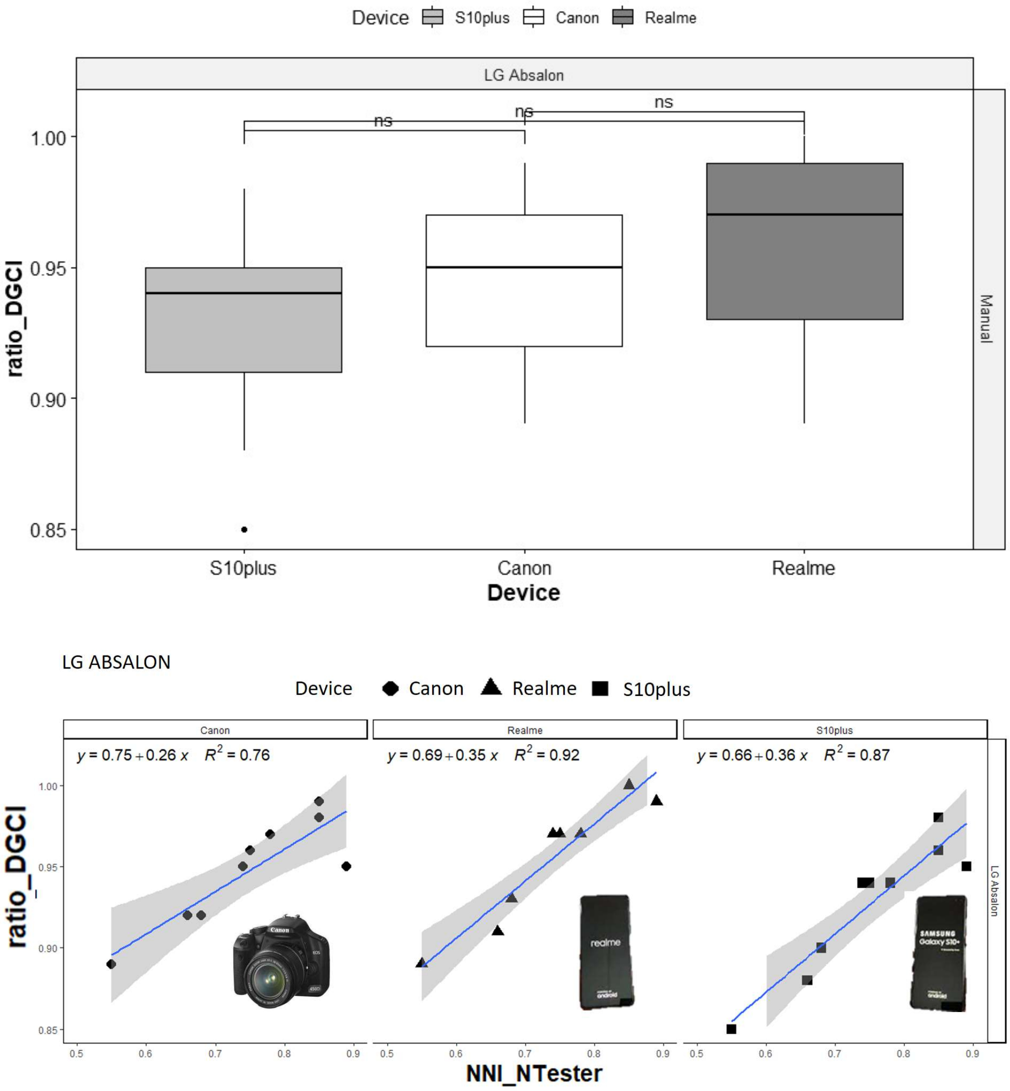

Figure 3. The top panel indicates that whatever the devices, it has no significant effect on the ratio_DGCI values. The bottom panel describes the variation of the R^2 of the relationship between ratio_DGCI and NNI_NTester for the three imaging devices in the case of LG Absalon cultivar at the "heading" growth stage and with a "Manual" option of white balance.

Conclusion

During the measurement campaign, provided that the white balance was used in "Manual" mode, the ratio_DGCI indicator deduced from visible imagery at canopy level was found to be relevant as an N-tester measurement for wheat NNI evaluation. The relationship between ratio_DGCI with NNI indicates an R^2 greater than 0.76 in the worst cases. Relevant results were obtained for 'Two nodes' growth stage. Finally, even if the relationship between the NNI and the ratio_DGCI is significant, the effectiveness of this measure still needs to be verified by applying it to the APPI-N method, an innovative method for the dynamic management of fertilisation in wheat crops.

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