Estimation of leaf nitrogen concentration on winter wheat by multispectral imaging

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ABSTRACT

Precision agriculture can be considered as one of the solutions to optimize agricultural practice such as nitrogen fertilization. Nitrogen deficiency is a major limitation to crop production worldwide whereas excess leads to environmental pollution. In this context, some devices were developed as reflectance spot sensors for on-the-go applications to detect leaves nitrogen concentration deduced from chlorophyll concentration. However, such measurements suffer from interferences with the crop growth stage and the water content of plants. The aim of this contribution is to evaluate the nitrogen status in winter wheat by using multispectral imaging. The proposed system is composed of a CMOS camera and a set of filters ranged from 450 nm to 950 nm and mounted on a wheel which moves due to a stepper motor. To avoid the natural irradiance variability, a white reference is used to adjust the integration time. The segmentation of Photosynthetically Active Leaves is performed by using Bayes theorem to extract their mean reflectance. In order to introduce information related to the canopy architecture, i.e. the crop growth stage, textural attributes are also extracted from raw images at different wavelength ranges. N_c was estimated by partial least squares regression (R^2 = 0.94). The best attribute was homogeneity extracted from the gray level co-occurrence matrix (R^2 = 0.91). In order to select in limited number of filters, best subset selection was performed. N_c could be estimated by four filters (450 ± 40 nm, 500 ± 20 nm, 650 ± 40 nm, 800 ± 50 nm) (R^2 = 0.91).

Keywords: leaves nitrogen concentration, multispectral imaging, reflectance and textural attributes, wavelength selection

1. INTRODUCTION

Precision agriculture is a recent concept to increase land productivity by reducing environmental impacts and economical costs while maintaining crop quality and yield[1]. In this context, one of the main challenges in agriculture requires the knowledge of nitrogen (*N*) plant deficiencies in real time and by taking into account spatial variabilities to provide only the necessary amount of nitrogen fertilizer. Indeed, plant growth is hampered when N is lack while an excess leads to a risk of environmental contamination or economic losses[2]. In this context, new technologies are developed to acquire a large number of data in real time and in a non-destructive way[3].

A large number of these new devices are based on optical plant properties. Under different nitrogen stresses, the physiological state of plants changes and influences spectral responses of crops[4]. On the field, two scales are studied by specific devices.

At the leaf scale, hand-held chlorophyll meters are used to improve nitrogen input fertilizer managements. These devices estimate chlorophyll concentration in leaf which can be related to nitrogen concentration since leaf chloroplasts contain 70% of the leaf nitrogen concentration[5]. Two kinds of measurement system exist. Measurement can be based on the difference of light absorption through the leaf at 650 nm and 940 nm. The first wavelength is selected due to absorption of red light by chlorophyll while the second wavelength, in the near infrared, serves as a reference[6]. An example of device is the SPAD-502. In durum wheat, the use of SPAD index had showed a medium nonlinear relationship (R^2 of 0.68) with nitrogen nutrition index (NNI)[9]. In a second approach, the reflectance of light emitting at 660 nm and 740 nm is used to estimate nitrogen concentration in leaf. An example of such device is the Plant Pen NDVI-300 [7]. Chlorophyll meters present however drawbacks. The measurement area is small (6 mm²) and the spectral response interact with other parameters such as the leaf thickness[8].

At the canopy scale, spectral-optical spot sensors are used for estimating N_c by means of vegetation index computation. However, interferences can be observed due to the influence of other factors such as the water supply, diseases or other nutrient deficiencies. Commercial devices such as GreenSeeker and Crop Circle ACS-470 can be mentioned. The first one measures reflectance in two spectral ranges giving the NDVI index whereas Crop Circle ACS-470 is configured to provide reflectance measurement from six narrow spectral bands which supply a large set of vegetation index. Hyperspectral data can be gained with spectroradiometer device and provide large vegetation indices. Canopy Chlorophyll Content Index (CCCI) computed from hyperspectral reflectance had a correlation of 0.76 with nitrogen stress index[10].

Images acquired by camera are studied to replace spectral-optical spot sensors. Conventional color cameras offer the possibility to compute color indices and link them with plant physiological characteristics. It was found a correlation of 0.88 between normalized red computed on image acquired with active camera and nitrogen content in leaves[11]. Hyperspectral cameras can study a spectral range from 400 nm to 1000 nm with a spectral resolution reaching 3.7 nm [12]. These devices acquire a large volume of data which need to be reduced due to redundancy of information between similar wavelengths[13].

Studies describe previously were based on the reflectance measurement in several spectral bands to predict N_c. Nevertheless, other visual characteristics such as textural features computed on images could be related to nutrient content[14]. Indeed, nitrogen deficiency influences the architecture of canopy by decreasing leaf area and plant size[15]. Imaging has is promising in the development of measurement systems of nitrogen leaves concentration since it allows visualizing large scene and providing both spectral and textural information.

The objective of this study was (i) to evaluate the potential of reflectance attribute and the importance of new information brought by textural attributes computed on multispectral images in order to estimate nitrogen leaves concentration of winter wheat and (ii) to select a limited number of filters in order to develop a dedicated instrument.

2. MATERIALS AND METHODS

2.1 Experimental field

Field experiments were conducted at University of Liège, Gembloux Agro-Bio Tech (Belgium) during the 2013-2014 growing season (Bordia field, 50.56° N, 4.69°E) and during the 2015-2016 growing season (Lonzée field, 50.55°N, 4.73°E). Seven different nitrogen input treatments were applied for both growing seasons (Table 1). These strategies were designed around the Belgian farmers' current practice, which consists in applying 60 kg N ha^{-1} respectively at tillering, redress and last-leaf stages. The winter wheat seeds (*Triticum aestivum*, cv. Edgar) were sown on the 24th of October 2013 at a grain density of 350 grains/m². The residual nitrogen concentration in soil was 122 kg N ha^{-1} (14/03/2014). Two repetitions were made for each treatment which means that eight plots were studied. During the second growing season in 2015-2016, winter wheat (*Triticum aestivum,* cv. Anapolis) was sown on the 29th of October 2015 at a grain density of 300 grain/m². The residual nitrogen concentration in soil was 10 kg N ha^{-1} (01/03/2016). Four repetitions were made for each treatment which means that twenty plots were studied.

2.2 Nitrogen leaves concentration

Wheat plants were destructively sampled on five dates: the 11 April 2014, 7 June 2014, 23 May 2016, 30 May 2016 and 6 June 2016. Plants within two 0.5 m lengths of row per plot were cut and placed in coolers. Reference measurement of nitrogen concentration in leaf tissues were obtained by Kjeldahl method. This method implied sampling of green leaves, separation of stems, and oven-drying at 70°C. The N concentration is expressed on the basis of unit dry weight (mg N g^{-1}DW).

Table 1: Fertilization strategies during both growing seasons [kg N ha^{-1}].

Treatment	2103-2014			
	12/03/2014	07/04/2014	27/05/2014	Total
0-0-0	0	0	0	
60-60-60	60	60	60	180
50-40-65	50	40	65	155
30-30-90	30	30	90	150
Treatment	2015-2016			
	21/03/2016	12/04/2016	18/05/2016	Total
0-0-0	0	0	0	0
60-60-60	60	60	60	180
30-60-90	30	60	90	180
30-90-60	30	90	60	180
90-30-60	90	30	60	180

2.3 Multispectral acquisition system

A multispectral vision system was designed to acquire top-down images of the scene (covered area of approximately 0.25 m²) in the visible and the near infrared spectra (Figure 1). The acquisition system included a monochrome 12 bits (4096 gray levels) 1.3 megapixels camera (BCI-5, C-Cam Technologies, Belgium) with a filter wheel equipped with 22 band pass interference filters (Table 1). The filters were selected to cover the sensitivity range of the camera sensor and had a central wavelength (LW) ranging from 450 nm (blue) to 950 nm (NIR) (Table 2). They were relatively wide (40-100 nm FWHM). The rotation of the filter wheel was controlled by a stepper motor.

In-field spectral measurements made under natural ambient illumination were significantly influenced by solar radiation changes from cloudy to sunny situations, which affects spectral responses at all stages of plant growth. To solve that problem, a white reference plate was used and the integration time was automatically adjusted in order to acquire images through each filter with the white reference radiance at about 3800 grey levels and a precision of ± 5%.

The image acquisition and the motor rotation were controlled by a program written in C++.

Figure 1: Multispectral vision system, computer and structure (left), camera and wheel filters (right).

Table 2: Interference filters

Color	Central wavelength	Narrow bandwidth		Broad bandwidth	
		ID	FWHM	ID	FWHM

Blue	450	S	50	U	80
Green	500	V	40	O	80
Green	550	T	50	K	80
Red	600	R	50	C	80
Red	650	F	40	J	80
Red edge	700	Q	50	L	80
Red edge	750	E	40	X	80
NIR	800	W	50	N	100
NIR	850	H	40	G	100
NIR	900	I	40	M	100
NIR	950	D	40	P	100

2.4 Images pretreatment

Image pre-processing was divided into three main algorithms which were used for computing the leaves mean reflectance in an image and extracting textural attributes from an image.

The first algorithm aims to compute the mean white reference. This includes (i) the application of a mask on the image to select the white reference; (ii) the search of the maximum pixel radiance R_{max}; (iii) the application of a threshold value (0.87 of R_{max} was chosen for obtaining acceptable results in both visible and NIR images); (iv) the calculation of the mean white radiance value.

The second algorithm (Fig. 2, left) aims to discriminate the Photosynthetically Active Leaves (PAL) from the rest of the image by using the Bayes' theorem[16]. This theorem aims to calculate for each pixel the probability to be assigned in different classes. The number of classes was set at two (PAL and not PAL) (Figure 2).

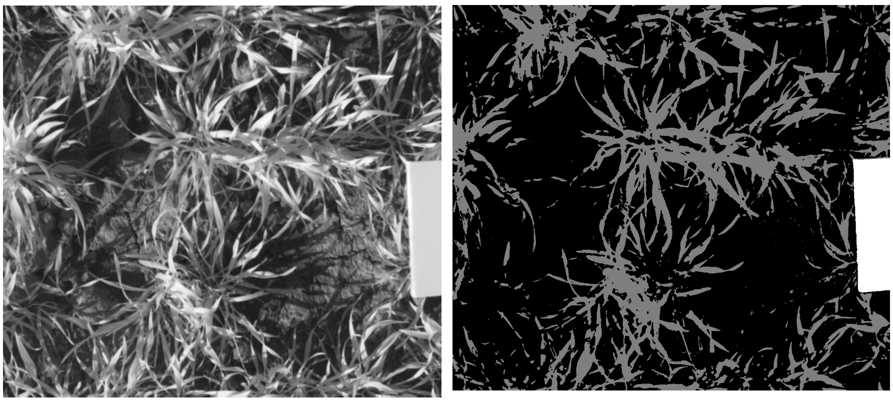

Figure 2: canopy image acquired with filter W at 800 nm (left) and corresponding segmented image (right)

The third algorithm comprised several steps (i) image background correction; (ii) image normalization by mean white reference radiance; (iii) mask application to only select PAL; (iv) calculation of mean reflectance of PAL at each filter wavelength.

2.5 Images attributes

Seven different attributes were calculated on each filter of multispectral images. The mean reflectance of leaves was obtained by extracting the PAL from the segmented image and normalizing with respect to the white reference reflectance. Gray level co-occurrence matrix (GLCM), which explains the distribution of gray scale transition between adjacent pixels (Figure)[17], were computed for each corrected image. Contrast (Eq. 5), correlation (Eq.6), energy (Eq. 7) and homogeneity (Eq. 8) were computed from this matrix giving information about the texture of the image[18,19]. $P_{(i,j)}$ represents the number of times a pixel with gray level i is adjacent to a pixel with a gray level j [20].

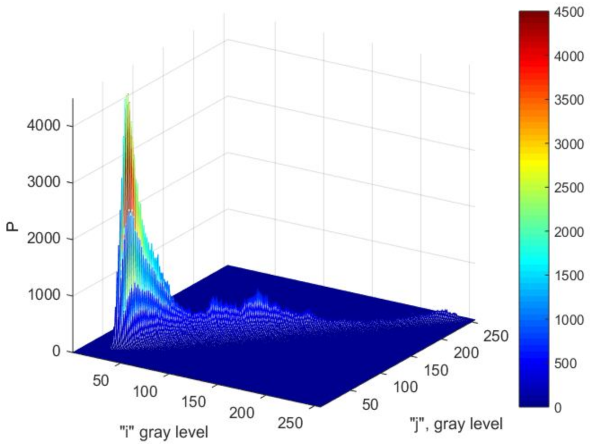

Figure 3**:** 3D representation of the gray level co-occurrence matrix computed on Figure 2 **(**left**)** with MATLAB R2015.

$$\sum_{ij} |i \quad j|^2 P_{(i,j)} \tag{5}$$

$$\sum_{ij} \frac{(i \quad \mu_i)(j \quad \mu_j) P_{(i,j)}}{\sigma_i \sigma_j} \tag{6}$$

$$\frac{\sum_{ij} P_{(i,j)}^2}{n} \tag{7}$$

$$\sum_{ij} \frac{P_{(i,j)}}{1 + |i - j|} \tag{8}$$

Two last attributes were the standardized dispersion coefficient on X axis (SDCX) and Y axis (SDCY) from magnitude of Fourier transforms 2D (Figure 4). Normalization was made by dividing results with maximum value.

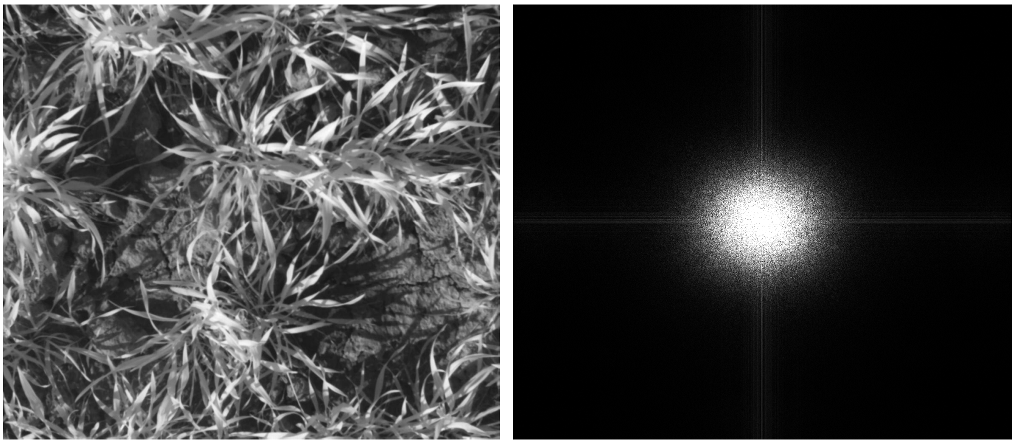

Figure 4: Canopy image acquired with filter W at 800 nm (left), and amplitude of 2D FFT computed (right).

A total of 154 attributes, obtained on base of 7 attributes computed on multispectral images composed of 22 filters, was used to predict N_c. All these attributes were computed with MATLAB® R2015a (the Mathworks,USA).

2.6 Data analysis

An analysis of variance (ANOVA) considering two factors (dates and fertilizer input level) was carried out to assess N_c. Three different statistical methods were used in order to create model to estimate N_c based on variables. Partial least squares regression (PLS) is a statistical modelling method used to predict one or several responses (Y) when the number of variable (X) is higher than the number of observation or when there is an high correlation between variables. *PLS* reduces the large number of initial variables into a smaller set of uncorrelated principal components (PC)[21,13]. Optimal number of components chosen for predictive model was fixed with cross-validation method. Sequential replacement is a statistical method suitable to select relevant variables into a large set of variables in order to explain a response[22]. Best subset regression is a statistical method which generates all possible combination of variables to predict a response and selects the best subset on the basis of adjusted coefficient determination.

3. RESULTS

3.1 Nitrogen leaves concentration

Table 3 presents the wheat leaf N concentration measured during the both growing seasons. The mean N_c was 28.9 mg N g^{-1}DW the standard deviation was 8.8 mg N g^{-1}DW, and the minimum and maximum were equal to 15.2 mg N g^{-1}DW and 47.2 mg N g^{-1}DW, respectively. The range was therefore 32 mg N g^{-1}DW.

Table 3: Nitrogen leaves concentration for 2014 and 2016 campaigns.

Date	Modality [kg/ha]	n	N_c [%]			
			min	max	mean	standard deviation
2016-04-11	0-0-0	4	1.5177	1.6149	1.5523	0.0438
	60-60-60	4	2.0664	2.2123	2.1268	0.0658
	30-60-90	4	1.5763	1.7977	1.7181	0.0972
	30-90-60	4	1.5377	1.9044	1.7848	0.1679
	90-30-60	4	2.2178	2.3078	2.2685	0.0377
2014-05-23	0-0-0	2	2.918	3.519	3.219	0.425
	60-60-60	2	3.592	3.917	3.755	0.23

	50-40-65	2	3.358	3.699	3.529	0.241
	30-30-90	2	3.206	3.349	3.2775	0.1011
2014-05-30	0-0-0	2	2.7	3.081	2.891	0.269
	60-60-60	2	2.839	3.402	3.12	0.398
	50-40-65	2	3.321	3.356	3.3385	0.0247
	30-30-90	2	3.325	3.341	3.333	0.0113
2014-06-06	0-0-0	2	3.263	3.433	3.348	0.1202
	60-60-60	2	3.732	3.875	3.8035	0.1011
	50-40-65	2	3.778	3.825	3.8015	0.0332
	30-30-90	2	3.555	3.842	3.699	0.203
2016-06-07	0-0-0	2	2.4571	2.5982	2.5277	0.0998
	60-60-60	2	3.9768	4.0309	4.0039	0.0383
	30-60-90	1	3.6838	3.6838	3.6838	*
	30-90-60	2	3.909	4.716	4.312	0.571
	90-30-60	2	3.9269	3.9517	3.9393	0.0175

3.2 Partial least squares regression

The partial least square (PLS) regression aimed to reduce the 22 filter responses to a smaller set of uncorrelated components. Table 4 shows the results of the PLS regression taking into account all the attributes together and each of them separately. The PLS method gave the best model by considering all the attributes ($R^2 = 0.94$).

Table 4: Results of prediction of N_c with different image attributes extracted from corrected images.

Attributes	Optimal components	R^2	Adjusted R^2	RMSE [mg N g-1DW]
All attributes	9	0.94	0.94	2.03
Reflectance	10	0.91	0.91	2.54
Contrast	6	0.87	0.87	3.01
Correlation	5	0.79	0.79	3.56
Energy	9	0.83	0.83	3.30
Homogeneity	10	0.91	0.91	2.48
SDCX	9	0.87	0.87	2.92
SDCY	11	0.88	0.88	2.86

3.3 Filters selection

Using best subset regression with acceptable Mallow's Cp, four filters were selected for each attributes separately (Table 5). The number of filters was fixed at four in order to put them in a dedicated device. Determination coefficients R^2 varied between 0.78 and 0.89 for textural attribute of homogeneity. Adjusted R^2 were similar to corresponding R^2. It is noted that for three attributes (reflectance, energy and homogeneity), all selected filters were in the visible range of light spectrum.

Table 5: Selection of filters by the best subset method.

	Selected filters	R^2
Reflectance	J,K,O,U	0.88

Contrast	F,J,V,X	0.83
Correlation	J,N,P,U	0.78
Energy	C,J,O,V	0.78
Homogeneity	C,J,O,V	0.89
SDCX	D,F,N,X	0.78
SDCY	N,S,T,V	0.83

Considering the filters selected by the best subset regression method for each attribute separately (Table 5), the four following filters were extracted due to their repetitive occurrence: J (650 ± 40 nm), N (800 ± 50 nm), O (500 ± 40 nm) and V (500 ± 20 nm). Based on all the attributes of these four filters, a multiple linear regression was performed to estimate N_c (R^2 = 0.91). Due to the redundancy of central wavelength between filters O and V, filter O was replaced by filter U (450 ± 40 nm) which appears only two times in the best subset regression analyses. Despite that fact, a multiple linear regression performed on these four filters led to a determination coefficient R^2 equal to 0.93. The most relevant attributes of the four filters J (650 ± 40 nm), N (800 ± 50 nm), U (450 ± 40 nm) and V (500 ± 20 nm) was extracted by means of a best subset regression. The number of attribute was fixed once R^2 was greater than 0.9. Six attributes could be efficient for evaluating N_c (R^2 = 0.91, adjusted R^2 = 0.90 and RMSE = 2.74 mg N g^{-1}DW) (Table 6).

Table 6: Multiple linear regression based on the four filters J,N,U and V.

Term	**Coefficients regression**
Constant	3.1829
Reflectance_J	-25.7103
Reflectance_U	48.0889
Energy_J	1.1134
Energy_N	-25.9770
Homogeneity_N	4.0304
Homogeneity_V	-5.0758

4. DISCUSSION CONCLUSION

In this study, the nitrogen leaves concentration is estimated by means of multispectral imaging using both reflectance and textural attributes and focusing on a limited number of relevant filters. The best results are obtained with the full-multispectral approach, including all attributes of all filters (R^2 = 0.94).

The results presented in this contribution showed that a model based on reflectance and textural attributes extracted from only four filters could be considered as efficient to estimate N_c (R^2 = 0.91). One selected filter had central wavelength in the red (J) spectral region corresponding to the radiation absorption by plant chlorophyll, linked to the N concentration. Two other filters (U, V) had a central wavelength at 450 and 500 nm (blue-green) which probably correspond to radiation absorption of both carotenoids and chlorophyll. The last filter had a central wavelength in the NIR with a quite large bandwidth including a part of the red-edge.

The reflectance attribute is usually used for estimating the nitrogen leaves concentration. Textural attributes are promising to enhance the estimation model since they could be correlated to the canopy architecture which is also influenced by the nitrogen leaves concentration.

The sources of uncertainties in the multispectral vision system are numerous and are mainly related to the image treatment of the canopy which reveals complex. In further studies, the multispectral approach could be extended by considering wider ranges of N leaves concentration, different water content, several cultivars, …

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