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CORRELATION DEPENDENCES BETWEEN CROP REFLECTION INDICES, GRAIN YIELD AND OPTICAL CHARACTERISTICS OF WHEAT LEAVES AT DIFFERENT NITROGEN LEVEL AND SEEDING DENSITY

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Abstract

Improvement of crop remote sensing application in precision agriculture systems and development of algorithms for satellite and aerial imagery interpretation necessitate comparing remote sensing and ground-based survey data. This paper is the first to report data on spectral characteristics of the leaf diffuse reflection in spring wheat, their relationship with plant productivity, colorimetric characteristics and reflection indices of the crop vegetation cover, depending on the crop management technologies. The first research objective was to assess the dependence of crop canopy optical characteristics and productivity on seeding density and the rates of pre-treatment with nitrogen fertilizer. The second objective was to reveal correlations between the canopy remote sensing data and the leaf diffuse reflection parameters registered by a contact sensor (on the example of spring wheat *Triticum aestivum* L. cv. Daria). The plants were grown on the test plots (Menkovo experimental station of Agrophysical Research Institute, Leningrad Province, Gatchina District) in 2020-2021 years. In total, six test plots with an area of 100 m^2 were assigned. Nitrogen rates ranged from 0 (no fertilizers applied) to 200 kg/ha with increments of 40 kg/ha, and seeding rates of 500 and 600 seeds per m^2. The diffuse light reflection of leaves was registered in situ on stages BBCH 30-31 "booting" and BBCH 53-55 "earing" by a fiber-optical spectroradiometric system (Ocean Insight, USA) in the range from 350 to 1000 nm with a step size of 0.3 nm. After the reflectance spectra recording, the plants were dried to constant weight and each plant was weighed to assess correlations with the optical parameters of leaves. The light diffusion index R_{800} was determined from the spectra of reflected radiation. The reflectance indices calculated were the following: ChlRI (chlorophyll index), PRI (photochemical index), FRI (flavonoids index), WRI (water content index), ARI (anthocyanins content index) and FRI (flavonoids content index). These indices estimate the intensity of the photosynthetic apparatus function and the efficiency of light use in photosynthesis. The crop canopy remote sensing was performed at BBCH 25-27 ("tillering"), BBCH 30-31 ("booting"), BBCH 53-55 ("earing"), and BBCH 61-65 ("blossoming") stages using two synchronized digital cameras Canon G7X (Canon Inc., Japan) mounted on a Geoscan 401 quadcopter (Geoscan, Russia). From a height of 75-120 m, the digital images were obtain in the visible and near infrared spectral ranges. The vegetation indices NDVI (Normalized Difference Vegetation Index) and ARVI (Atmospherically Resistant Vegetation Index) were calculated based on the optical characteristics. For quantitative interpretation of the colorimetric characteristics of leaves and the crop canopy, we used a three-dimensional model of the CIELAB color space. Plants were weighted during the growing season, and, after harvesting, the grain productivity was estimated for the plants sampled from 0.25 m^2 reference plots. The obtained results indicate that at the early stages of plant development when the vegetation cover remains open, NDVI characterizes the degree of nitrogen supply rather exactly and identifies areas with underdeveloped plants. However, with the development of plants and the formation of a closed vegetation cover this index fails to provide reliable results. ADVI also fails to provide reliable information about the state of spring wheat plants and identifies areas that require additional fertilize application. A close linear correlation between the rate of applied nitrogen fertilizer, the net productivity of plants and spectral characteristics of leaves measured in situ occures until the late stages of development (BBCH 53-55 “earing”, BBCH 61-65 “blossoming”). Crop monitoring based on colorimetric characteristics made it possible to detect changes in the crop canopy associated not only with the plant development and crop density, but also with the spectral characteristics of the diffuse reflection of plant leaves. A comparison of the remote and contact sensing data allows us to conclude that the ChlRI, PRI, FRI and WRI indices can successfully identify areas in which nitrogen deficiency has developed during the closed canopy formation, when the commonly used indices, for example, NDVI, fail to be reliable.

Keywords: spring wheat, reflection indices, spectral characteristics, remote monitoring, nitrogen deficiency, precision agriculture

The spatial variability of the state of crops, which is determined remotely, is due to many factors (soil, climatic, biological, and technological) and depends on the optical properties of leaves, plant architectonics, crop structure. The main function of the vegetation cover is to intercept radiation for photosynthesis and other metabolic processes. This interception occurs with varying degrees of efficiency, which depends mainly on the area of the formed leaves and their orientation relative to the sun. Since the growth and yield of plants are determined mainly by photosynthesis, they are directly dependent on the amount of absorbed light and the efficiency of its conversion in chloroplasts. Agrophytocenosis, the structure of which provides greater absorption of light and its most efficient use, is usually characterized by a higher intensity of plant photosynthesis and yield. For the development of precision farming systems, a fundamentally new methodological, physical, technical and experimental base is currently needed, which will become the main structural component in assessing the variability of crop characteristics and monitoring their condition [1].

The radiation reflected by leaves and other phytoelements carries complete information about the biochemical composition, tissue architecture, physiological state of plants, and specific changes in their spectral characteristics and the amount of reflected radiation make it possible to evaluate the response to the action of various stressors [2-4] and make a yield forecast [5]. Deciphering the information embedded in the optical characteristics of plants underlies the remote and ground-based sensing of vegetation, but the complex nature of the optical systems of plants makes this process ineffective [3]. Over the past decades, approaches have been developed to gain insight into some aspects of plant structure and function from multi- and hyperspectral reflectance data. The content of photosynthetic pigments, the presence of anthocyanins, flavonoids and water in tissues determine the activity of photosynthesis processes, the spectral characteristics of diffuse scattering of leaves and plant cover as a whole. Previously, the most promising reflection indices were described and their use for diagnosing the physiological state of plants even in the absence of visible symptoms of growth inhibition and oppression was considered [6, 7].

The pattern of reflection of different wavelengths of light by leaves in the range of photosynthetically active radiation and near infrared radiation of the electromagnetic spectrum is very different from that for the soil [8]. Differences in the reflectance spectra of the soil and the vegetation cover formed by agrophytocenosis have a clear potential for remote diagnostics of the state of plants both in breeding programs when screening genotypes for efficiency [9] and in assessing the feasibility and volume of using various agricultural practices. The usually observed spatial heterogeneity of the optical characteristics of agrophytocenoses is associated with differences in the content of water, nutrients and other soil properties, as well as with the features of agricultural technologies used in the cultivation of crops (seeding rates of seeds, their quality, the degree of contamination of crops, etc.)

Insufficient use of remote sensing tools significantly limits the possibilities of precision farming technologies. Largely, this is due to the lack of problem-oriented databases and a significant backlog of methods for decrypting information received from sensors and devices that monitor crops. A simple metric for measuring reflectance differences is the normalized difference vegetation index (NDVI), which was proposed by J.W. Rose et al. [10] and was used to assess the state of vegetation using multispectral remote sensing [11, 12]. N. Oppelt et al. [13] used NDVI in hyperspectral studies to monitor the physiological parameters of wheat. They found that NDVI becomes insensitive to chlorophyll content below 0.3 g/m^2 and also above 1.5 g/m^2. It is probably the most commonly used index for analyzing crop health and provides indirect estimates for leaf area index, light absorption and potential photosynthetic activity [8, 14, 15]. Since NDVI responds to changes in vegetation cover during crop growth and development, it is often used to predict yields [16, 17].

Remote monitoring makes it possible to significantly improve the methods of crop forecasting and operational control over the state of crops both globally and locally [18, 19]. At the initial stages of development and improvement of remote diagnostic methods, it is necessary to compare the obtained data with the results of a ground survey of crops [20]. The data obtained during remote and ground survey of crops at the same time, in the same areas of the fields, are necessary to increase the accuracy of diagnostics and identify criteria and identification indicators of the state of crops [6, 7, 20]. In remote and ground monitoring methods are widely used to evaluate the optical characteristics of leaves, leaf area index and/or projective soil cover, characteristic of a particular crop.

In this work, for the first time, we submit data on the spectral characteristics of the diffuse reflectance of spring wheat leaves, the relationship of the spectral characteristics with plant productivity, colorimetric characteristics, and vegetation reflection indices of the vegetation cover formed by this crop, depending on the applied crop management technologies.

The purpose of the work is to evaluate the dependence of the optical characteristics and crop productivity on the seeding rate and the dose of nitrogen fertilizers applied before sowing on the example of spring wheat (*Triticum aestivum* L.). We also aimed to reveal correlations between the remotely measured optical characteristics of the vegetation cover and the spectral characteristics of diffuse leaves reflection using a contact sensor.

Materials and methods. The studies were carried out on crops of spring wheat (*Triticum aestivum* L.) cv. Daria. The plants were grown in the field of the Menkovsky branch of the Agrophysical Research Institute (AFI) (Leningrad Region, Gatchina District) in 2019-2020. The soil was soddy-weakly podzolic medium-cultivated light loamy with a humus content of 2.07%, exchangeable calcium of 8.38 mmol/100 g, magnesium of 2.88 mmol/100 g, mobile compounds of phosphorus and potassium of 565 and 140 mg/kg, ammonium and nitrate nitrogen of 12.37 and 8.21 mg/kg; pH$_{KCl}$ 5.7. The thickness of the arable layer is 22 cm.

Six test plots with an area of 100 m^2 were laid, on which the dose of evenly applied nitrogen (nitrogen rate, NR) varied from 0 (without fertilization) to 200 kg/ha with a step of 40 kg/ha. The azophoska fertilizer were applied before sowing ($^2/_3$ of nitrogen dosage) and ammonium nitrate was applied as top dressing at the stage BBCH 25-27 (“tillering”) (the remaining $^1/_3$ of nitrogen dosage). To create crops of different density, each of the test plots was divided into two 50 m^2 plots. The seeding density (SR) on one of them was equal to 6.0 million/ha, on the second to 5.0 million/ha, that is, 500 and 600 seeds/m^2 (SR500 and SR600, respectively).

The spectral characteristics of the diffuse reflectance of leaves were determined in situ at stages BBCH 30-31 (“booting”) and BBCH 53-55 (“earing”) using a fiber-optic spectroradiometric system (Ocean Insight, USA) in the range from 350 to 1000 nm with a step 0.3 nm. To select plants for the purpose of analyzing the spectral characteristics of the leaves, the sowing on the test plots was conventionally divided into 4 parts, and 5-6 plants (20-30 plants in total) were selected from the center of each part, which were transported with a clod of moist earth to the laboratory. To record diffuse reflectance spectra, leaves that had completely finished growing were used, placing the sensor in the middle part of the leaf blade, to the left and right of the central vein. The recorded spectra (at least 20 spectra for each variant) were processed in the Microsoft Excel 2013 program, where the average reflectance values were calculated for all wavelengths of the measured range of 350-1000 nm. After recording the reflectance spectra, the plants were dried to constant weight at a temperature of 85 °C and weighed individually. The resulting parameter (biomass of one plant, B_{1p}) was used to study correlations with the optical characteristics of leaves.

From the spectra of reflected radiation, we determined the measure of leaf light scattering by the R_{800} [21] and calculated the reflection indices characterizing the activity of the photosynthetic apparatus: the content of chlorophyll (ChlRI), the photochemical activity of the photosynthetic apparatus (PRI), the content of water (WRI), anthocyanins (ARI) and flavonoids (FRI).

We used the following formulas:

$$\mathrm{ChlRI} = (R_{750} - R_{705})/(R_{750} + R_{705} - 2R_{445}) \text{ [21]},$$
$$\mathrm{PRI} = (R_{570} - R_{531})/(R_{570} + R_{531}) \text{ [22]},$$
$$\mathrm{ARI} = R_{750}\,(1/R_{550} - 1/R_{700}) \text{ [23]},$$
$$\mathrm{FRI} = [(1/R_{410}) - (1/R_{460})] \times R_{800} \text{ [24]},$$
$$\mathrm{WRI} = (R_{970} - R_{920})/(R_{970} + R_{920}) \text{ [25]},$$

where R is the reflection value, the subscripts are the wavelengths reflected from the sheet surface.

For the convenience of presenting data and obtaining positive index values, the constant value C was used in the calculation formulas for PRI, ARI and FRI, from which the values of the listed indices were subtracted. The modified reflection indices were obtained: $\mathrm{PRI}_{mod} = C1 - \mathrm{PRI}$, $\mathrm{ARI}_{mod} = C2 - \mathrm{ARI}$, $\mathrm{FRI}_{mod} = C3 - \mathrm{FRI}$. The experimentally selected values of the constants C1, C2 and C3 were equal to 0.5, 0.7 and 0.7, respectively [26]. Correlation dependences between all listed reflection indices and net productivity under the action of various abiotic stressors on wheat [5] and barley [26] plants were considered earlier.

During the main development stages of spring wheat (tillering, BBCH 25-25; booting, BBCH 30-31; earing, BBCH 53-55; blossoming, BBCH 61-65), the crops were photographed remotely. Digital images in the visible and near infrared spectral ranges were obtained from a height of 75=120 m using two synchronized Canon G7X digital cameras (Canon, Inc., Japan) mounted on a Geoscan 401 quadrocopter (Geoscan, Russia). Aat least three images of crops were obtained at each of the test sites.

Analysis of digital images of crops and registration of their spectral characteristics were carried out using the Erdas Imagine program (Erdas, Inc., USA).

When processing the optical characteristics, the vegetation indices NDVI (normalized difference vegetation index) and ARVI (atmospheric resistant vegetation index) were calculated:

$$\mathrm{NDVI} = (\mathrm{NIR} - \mathrm{RED})/(\mathrm{NIR} + \mathrm{RED}),$$
$$\mathrm{ARVI} = (\mathrm{NIR} - R_b)/(\mathrm{NIR} + R_b),$$

where NIR and RED are reflections in the near infrared and red regions of the spectrum, respectively, Rb = RED – a × (RED – BLUE), as a rule, a = 1, with little vegetation cover and an unknown type of atmosphere a = 0.5 [27].

To quantify the colorimetric characteristics of leaves and plant cover formed by crops, the CIELAB 3D color space model adopted by the International Commission on Illumination was used [28].

During the growing season at the stages BBCH 25-27, BBCH 30-31, BBCH 53-55 and BBCH 61-65, the plant biomass (Bp) was determined by the weight method. For this purpose, record plots with an area of 0.25 m^2 were allocated on test plots. All plants from the record plot were cut at the level of the root collar, dried to constant weight at 85 °C, and weighed. At the end of the growing season, the grain productivity of plants was determined (Yg, grain weight, g/m^2). In this case, plants were collected from selected 0.25 m^2 plots. The repetition of the experiment in determining Bp and Yg was 3-fold.

Statistical processing was performed using Microsoft Excel 2013 and Statistica 8 software (StatSoft, Inc., USA). The average values of the studied parameters were determined. The significance of differences between the variants was assessed by parametric (Student's *t*-test) and non-parametric (Spearman's rank correlation coefficient and Wilcoxon's pairwise comparison test) statistics. Differences between the variants were considered statistically significant at $p \leq 0.05$. The strength of the factor effect (η^2, partial eta-squared) for the level of nitrogen nutrition and seeding rate was determined as a percentage as the ratio of the corresponding sum of squared deviations of the studied optical parameters from their average values to the total sum of squares.

Results. The data of the measurements are presented at http://www.agrobiology.ru.

Remote assessment of crops by vegetation indices (NDVI and ARVI) and analysis of their relationship with net productivity. It is shown that a yield forecast can be made based on the results of assessing the state of crops in the early stages of development. One of the main criteria for such an assessment is the amount of accumulated biomass, which can be determined both by the direct weight method and by non-invasive optical methods, including remote sensing.

The relationship between grain productivity (Yg, g/m^2) and the biomass of wheat plants formed at booting (B_{p1}, g/m^2), earing (B_{p2}, g/m^2), and blossoming (B_{p3}, g/m^2) was linear and credible:

$$Yg = 48.76 + (0.30 \times B_{p1})\ (r = 0.64;\ p = 0.024;\ R^2 = 0.42),$$

$$Yg = 36.06 + (0.15 \times B_{p2})\ (r = 0.82;\ p = 0.001;\ R^2 = 0.67),$$

$$Yg = 97.41 + (0.13 \times B_{p3})\ (r = 0.88;\ p = 0.0001;\ R^2 = 0.78).$$

Such a close correlation between grain yield and biomass values, even at the early stages of plant growth, allows us to conclude that biomass can serve as one of the main parameters in predicting grain yield. Its evaluation using remote methods creates good prospects for monitoring crops and developing new technologies to manage the production process of plants.

1. Relationships of plant biomass with normalized difference vegetation index (NDVI) **and atmospherically resistant vegetation index** (ARVI) **in spring wheat (*Triticum aestivum* L.) cv. Daria over plant growth stages** (Menkovsky branch of the Agrophysical Research Institute AFI, Leningrad Region, Gatchina District, 2019-2020)

Relationship equations	PC, %	*r*	p	R^2
Tillering (BBCH 25-27)				
Bp = –0.913 + (2.674 × NDVI)	43.7	0.927	0.008	0.86
Bp = –0.104 + (0.257 × ARVI)		0.801	0.056	0.54

Continued Table 1

Booting (BBCH 30-31)				
$Bp = -2.015 + (5.729 \times NDVI)$	62.4	0.810	0.009	0.66
$Bp = -0.526 + (0.703 \times ARVI)$		0.798	0.057	0.64
Earing (BBCH 53-55)				
$Bp = 0.711 + (3.75 \times NDVI)$	65.0	0.138	0.795	0.019
$Bp = 0.235 + (1.032 \times ARVI)$		0.545	0.263	0.297
Blossoming (BBCH 61-65)				
$Bp = -15.66 + (33.84 \times NDVI)$	48.1	0.730	0.097	0.533
$Bp = -0.707 + (2.044 \times ARVI)$		0.843	0.035	0.716

Note. Bp — plant biomass, kg/m^2, PC — projective cover of plants (maximum value for each of six 100-m^2 test plots at seeding density of 600 seeds per 1 m^2). Relationship equations between Bp and vegetation indices NDVI and ARVI are based on the average values of the parameters measured on three record plots allocated on each of the six test plots.

Analysis of remote monitoring data indicates the dependence of vegetation indices on sowing density and nitrogen nutrition. The values of the parameters were maximum at booting stage (0.56-0.63 on average for the options) and minimum at tillering stage (0.49-0.56). For SR500, the NDVI values increased up to the earing stage, while for SR600, the increase in the index occurred only up to the booting stage. This is probably due to the faster closing of the vegetation cover at SR600. A significant relationship between plant biomass and the vegetation index NDVI occurred only at the early stages of plant development, and there was no statistically significant relationship between the traits after the main stem extention (Table 1). There was no significant relationship between the biomass of plants formed per 1 m^2 of the test plot and ARVI for all survey periods, with the exception of blossoming stage. It can be concluded that this index is not suitable for monitoring spring wheat crops in order to detect field areas that require additional application of nitrogen fertilizers and adjustment of the nitrogen nutrition regime of plants.

At the late phases of growh (blossoming stage), the correlation coefficients between plant biomass and vegetation indices NDVI and ARVI were quite high (0.730 and 0.843, respectively). However, due to the strong variability of NDVI values for the vegetation cover formed on test plots, the relationship with NDVI was unreliable, whereas the relationship between Bp and ARVI was statistically significant.

The content of chlorophyll (chlorophyll index, ChlRI) in wheat leaves changed significantly depending on the dose of applied nitrogen and the seeding density (SR500 or SR600) (Table 2). The strength of the factorial influence of nitrogen nutrition on ChlRI was high and was equal to 35.1% of the total variability of the indicator in the experiment. The seeding rate had a small (3.7%) but significant effect on ChlRI (see Table 2). No statistically significant interaction between the dose of applied nitrogen fertilizers and the seeding density was found.

The introduction of nitrogen fertilizers, in addition to increasing the content of chlorophyll, also changed the photosynthetic efficiency. One of the parameter used in the work, the photochemical reflectance index (PRI_{mod}), made it possible to evaluate the efficiency of the photosynthetic apparatus of plants and the regulation of light flux in pigment-protein complexes [22]. The photochemical reflectance index, based on the results of testing on various crops [29, 30], provides a quick and non-destructive diagnostic of the effectiveness of photosynthetic processes in leaves or vegetation cover. This index does not depend on the leaf structure and is determined by the concentration of carotenoids and the activity of their conversion in the xanthophyll cycle, which ultimately determines the efficiency of the use of light energy and its conversion in photosystem II [22].

2. Reliability and the degree of influence of technological factors on leaf reflectance indices measured with a contact sensor in spring wheat (*Triticum aestivum* L.) cv. Daria plants at earing stage BBCH 53-55 (Menkovsky branch of the Agrophysical Research Institute AFI, Leningrad Region, Gatchina District, 2019-2020)

Reflectance indices	Factors					
	SR		NR		SR × NR	
	p	η^2	p	η^2	p	η^2
ChlRI	< 0.001	3.7	< 0.001	35.1	0.185	0.8
SIPI	0.051	0.9	< 0.001	11.8	0.108	1.1
R_{800}	0.001	2.5	0.111	1.1	0.001	3.1
PRI_{mod}	0.630	1.0	< 0.001	30.4	0.661	0.1
ARI_{mod}	0.872	0.006	0.97	0.01	< 0.001	3.3
FRI_{mod}	0.001	2.4	< 0.001	27.0	0.043	1.5
WRI	< 0.001	28.7	< 0.001	9.5	0.96	0.1

Note. SR — seeding rate (2 options), NR — nitrogen rate (N dosage, 6 options), SR × NR — interaction of the factors; η^2 — the effect size for the factor or factors' interaction, p — the significance level for reliable effects. Reflectance indeces for chlorophyll (ChlRI), for carotenoids to total chloorophylls (SIPI), for light scattering in leaf bades (R_{800}), photochemical reflectance index (PRI_{mod}), anthocyanin reflectance index (ARI_{mod}), flavonoid reflectance index (FRI_{mod}), and water reflectance index (WRI, water content) are submitted. Reliability and the degree of the influence of SR и NR parameters on the leaf reflectance indices were assessed by their averages for 2 options for SR and 6 options for NR, 12 options in total. The average value of reflection indices was determined in a sample of at least 20 plants.

Based on the relationship of PRI with leaf area and chlorophyll content in corn and soybeans, it was concluded that the dynamics of changes in PRI during plant growth results from a combination of the activity of xanthophyll cycle and the total pool of chlorophylls and carotenoids which are formed during adaptation to environmental conditions [31].

Usually, an increase in PRI values marks an increase in thermal dissipation due to a decrease in the amount of absorbed light energy, which is used by the plant in the photochemical processes of photosynthesis.

Nitrogen nutrition had the strongest effect on PRI_{mod} (see Table 2). This factor contribution exceeded 30% influence. With a deficiency of nitrogen nutrition (N_0), the photochemical reflectance index had the highest value, indicating the dissipation of light energy not used for photosynthesis, that is, the lowest efficiency of its use. The presowing application of nitrogen contributed to a more efficient light conversion in the photochemical photosynthetic reactions, as can be seen from the decrease in PRI_{mod} as the dose of nitrogen fertilizers applied before sowing was increased.

There was no statistically significant effect of the seeding rate (SR500 or SR600) on the photochemical activity index PRI_{mod}. However, the trend towards an increase in this indicator occurred under nitrogen deficiency (N_0) and higher seeding density (SR600).

The leaf structure-independent pigment index (SIPI) makes it possible to estimate the ratio of the carotenoid content to the amount of chlorophyll, Car/Chl [21]. G.A. Blackburn [32] confirmed that this index has a non-linear dependence on the Car/Chl ratio, which is best described using a logarithmic model ($R^2 = 0.86$). SIPI lacks sensitivity at low Car/Chl ratios, but becomes more sensitive at high Car/Chl ratios. In the present work, the SIPI values were 11.8% influenced by the rate of nitrogen fertilizers. There were no significant changes in SIPI depending on the seed density.

In addition to chlorophylls and carotenoids, the spectral characteristics of the diffuse reflection of leaves and other phytoelements are determined by the content of phenolic compounds (for example, anthocyanins and flavonoids), the presence of which changes the quality and quantity of light penetrating to chloroplasts. Usually, under oxidative stress, the content of anthocyanins and flavonoids increases, which contributes to the shielding of chloroplasts, preventing their destruction from absorbing excess light energy and reducing or eliminating the effects of oxidative stress.

We did not find significant changes in the anthocyanin index (ARI$_{mod}$), both depending on nitrogen nutrition and the seeding rate. The flavonoid index (FRI$_{mod}$) significantly increased with nitrogen deficiency ($\eta^2 = 27\%$, $p < 0.001$) and in denser sowing ($\eta^2 = 2.4\%$, $p = 0.001$). A small but significant interaction was observed between the dose of applied nitrogen and plant density (see Table 2).

In our experiment, there was no statistically significant effect of nitrogen nutrition on the R$_{800}$ index, the value of which depends on the leaf structure, mainly on the cell size and intercellular space. However, the plant density in the crop had a small, statistically significant effect on this index (see Table 2).

WRI has been tested in a number of studies to assess the water status of plants [25, 33]. M. Gutierrez et al. [34] concluded that WRI reveals genetic differences in drought tolerance at the crop cover level and that WRI can be used to quickly and inexpensively assess plant water status. We found that WRI depends both on the dose of applied nitrogen and on the plant density in the crop. Moreover, the sowing density had a stronger effect ($\eta^2 = 28.7\%$) than nitrogen deficiency ($\eta^2 = 9.5\%$) (see Table 2). It has previously been shown that WRI is related to water content, leaf water potential, stomatal conductance, and canopy temperature under water stress [35]. A strong negative linear relationship between grain productivity and WRI was found for various wheat genotypes, and the ability to predict plant yield using this water band index was shown [38].

Estimation of biomass and leaf reflectance indices (ChlRI, SIPI, PRI$_{mod}$, and FRI$_{mod}$) at different stages of plant growth made revelaed their close correlation with the nitrogen content in the soil (Table 3). Characteristically, the significant relationship between the listed traits observed at the early stages of plant development (booting) persisted at later stages (earing). As it was shown earlier, the use of NDVI did not provide detection of differences in the state of wheat plants at stages later than booting for a seeding rate of 500 and 600 pcs/m2 (see Table 1).

3. Spearman's rank correlation coefficients for soil nitrogen, plant biomass and leaf reflectance indexes measured with a contact sensor in spring wheat (*Triticum aestivum* L.) cv. Daria plants at booting and earing stages (Menkovsky branch of the Agrophysical Research Institute AFI, Leningrad Region, Gatchina District, 2019-2020)

Parameter	Bootin		Earing	
	NR	B$_{lp}$, g	NR	B$_{lp}$, g
B$_{lp}$	0.95*		0.83*	
ChlRI	0.98*	0.91*	0.93*	0.76*
SIPI	–0.93*	–0.86*	–0.83*	–0.81*
R$_{800}$	0.07	0.02	–0.59	–0.50
PRI$_{mod}$	–0.83*	–0.88*	–0.90*	–0.81*
ARI$_{mod}$	0.24	0.38	–0.28	–0.48
FRI$_{mod}$	–0.91*	–0.79*	–0.83*	–0.67*
WRI	0.63*	0.64*	0.72*	0.74*

Note. NR — the rate of nitrogen fertilizers from 0 to 200 kg/ha with a 40 kg/ha step, B$_{lp}$ — plant biomass. Reflectance indeces for chlorophyll (ChlRI), for carotenoids to total chloorophylls (SIPI), for light scattering in leaf bades (R$_{800}$), photochemical reflectance index (PRI$_{mod}$), anthocyanin reflectance index (ARI$_{mod}$), flavonoid reflectance index (FRI$_{mod}$), and water reflectance index (WRI, water content) are submitted. The Spearman correlation coefficient was calculated for the average values of the reflectance and biomass (B$_{lp}$) indices of plants, measured in 6 NR options in 2-fold repetition (12 options in total). The average values of the reflection indices and B$_{lp}$ for each option were determined in a sample of at least 20 plants.

* Correlation between traits is significant at $p < 0.05$.

The optical characteristics of wheat leaves depend on the content of chlorophyll (ChlRI), carotenoids (SIPI), some phenolic compounds (ARI and FRI), structure of leaves (R$_{800}$) and their water content (WRI). A change in each of these features is accompanied by a modification of the spectral characteristics of the diffuse reflectance of leaves and a change in their colorimetric characteristics. For example, with a decrease in the concentration of chlorophyll, which absorbs blue and red radiation, the proportion of yellow and blue-green radiation in the diffuse reflectance spectrum of a leaf increases. This inevitably leads to a change in the colorimetric characteristics (color) of leaves.

The results obtained indicate that at the early stages of plant development, while the formed cover remains open, NDVI provides accurate assessment of the degree of nitrogen supply of plants and identifies areas of the field where plants are less developed. According to the results of field tests [12, 17], NDVI is closely related to plant biomass and leaf area index. This allows us to consider this vegetation index as a reliable indicator of plant health which can be assessed remotely. However, as the plants develop and close canopy is formed, the results are no longer reliable. The close relationship between the colorimetric characteristics of the closed vegetation cover and the leaves of the upper layer of wheat plants (see Table 3) suggests that the ChlRI, PRI, FRI, and WRI indices can be successfully used for remote assessment of crop areas in which nitrogen nutrition is deficient.

Assessment of the crop state by colorimetric parmeters of their digital images. For a timely assessment of the nitrogen needs of plants, some researchers used images of crops obtained with digital cameras, followed by an assessment of the colorimetric characteristics of the vegetation cover [6, 7, 33, 36]. Digital imaging techniques have also been used to determine the degree of projective soil cover, which is closely related to leaf area index, aboveground biomass, and nitrogen content in the early stages of plant development [19, 37, 38].

According to the CIELAB 3D color space model [21], L denotes the lightness, A denotes the red/green component, and B denotes the yellow/blue component. The maximum value of 100 corresponds to an ideal reflective diffuser (white color), the minimum value of L is zero which corresponds to black color. The A and B axes are numerically unlimited. Positive values of A are inherent in the red object, negative values are green. A yellow object has positive B values, while a blue object has negative B values.

The results of our study showed that the colorimetric characteristics of the vegetation cover formed by wheat plants during the transition to earing changed significantly depending on nitrogen nutrition. A remote assessment of colorimetric characteristics showed that an increase in the dose of nitrogen applied before sowing from 40 to 200 kg/ha (or 5-fold), the L decreased on average from 48.3 to 33.2 (1.45 fold), A increased from –9.7 to –7.7 (1.26-fold), and B decreased from 17.2 to 10.7 (1.64-fold). Measurements done during the earing period with a contact sensor that was placed on the upper leaves also revealed the variability of their colorimetric characteristics depending on the pre-sowing nitrogen dosage. With an increase in the nitrogen dosage from 40 to 200 kg/ha, the parameter L changed from 49.2 to 41.7 (1.18-fold), A from –9.7 to –7.18 (1.35-fold), B from 21.4 to 10.9 (1.96-fold). The results obtained indicate that B is the most sensitive indicator characterizing plant provision with nitrogen.

A statistically significant correlations of the L, A, B values for the upper leaves with the plant nitrogen supply were found both when the colorimetric characteristics were measured contactly and by the remote assessment (Table 4).

The value of the parameter L of the CIELAB 3D color model increased as the dose of applied nitrogen increased, That is, with a nitrogen deficiency, the formed vegetation cover became lighter. Chromatic components A and B were also sensitive to changes in plant nitrogen nutrition. An increase in the B parameter is a marker of nitrogen deficiency and yellowing of the leaves. A shift towards large negative values along the A-axis as the dose of nitrogen nutrition increases indicates that wheat leaves have a more saturated green color. During the remote examination of crops at the beginning of booting when the vegetation cover had not yet closed, there was no close relationship between the NR applied before sowing and the chromatic components A and B ($p \le 0.05$). There was a statistically significant relationship between the L value and the nitrogen level in the soil ($r = -0.942$, $p = 0.0049$, $R^2 = 0.887$), apparently, due to an increased contribution of the colorimetric characteristics of the background soil in crops with less developed plants to the colorimetric characteristics of the formed vegetation cover.

4. Correlations of soil nitrogen concentration with colorimetric parameters of crop and leaves in spring wheat (*Triticum aestivum* L.) cv. Daria plants at earing stage (Menkovsky branch of the Agrophysical Research Institute AFI, Leningrad Region, Gatchina District, 2019-2020)

CIELAB 3D colorimetric parameters	r	p	R2
Ld	–0,933	0,0065	0,811
Ad	0,977	0,0007	0,956
Bd	–0,977	0,0002	0,955
Lc	–0,943	0,0015	0,889
Ac	0,964	0,0019	0,930
Bc	–0,984	0,0001	0,969

Note. Parameters of CIELAB 3D color model measured from digital images of crops obtained distantly (Ld, Ad, Bd) and of upper leaves obtained with a contact sensor (Lc, Ac, Bc). The correlation coefficients between the dose of nitrogen fertilizers NR and the average values of the remotely and contact measured parameters of the LAB model were calculated for 6 NR options in 2 repetitions (12 options in total). Mean Lc, Ac, and Bc values were determined for a sample of 20 plants in each option; mean Ld, Ad, and Bd values were determined for three crop images for each NR option.

The relationship between the parameters of the CIELAB 3D model, measured remotely from a height of 75-100 m (Ld, Ad and Bd) and in the upper leaves (Lc, Ac and Bc) was linear and especially significant between the chromatic components Ld and Lc, and also Bd and Bc (Table 5).

5. Relationship between CIELAB 3D parameters of crops measured distantly (Ld, Ad, Bd) **and of upper leaves measured with a contact sensor** (Lc, Ac, Bc) **in spring wheat (*Triticum aestivum* L.) cv. Daria** (Menkovsky branch of the Agrophysical Research Institute AFI, Leningrad Region, Gatchina District, 2019-2020)

Relationship equations	*r*	p	R^2
$Ld = -31.463 + (1.585 \times Lc)$	0.845	0.034	0.714
$Ad = -2.541 + (0.715 \times Ac)$	0.969	0.0014	0.939
$Bd = 5.598 + (0.527 \times Bc)$	0.959	0.0006	0.919

Note. For options and sample sizes, see Table 4.

Chromatic components A and B were closely associated with ChlRI, FRI_{mod}, and PRI_{mod} indeces ($p < 0.05$), while the L value correlated with WRI and, to a lesser extent, with ChlRI.

Our results indicate that at the early stages of plant growth, while the canopy cover remains open, NDVI makes it possible to accurately diagnose the degree of nitrogen supply to crops and identify field areas where plants are less developed. In numerous field trials [12, 17], NDVI is closely related to plant biomass and leaf area index. However, for a closed vegetation cover, the use of NDVI does not provide reliable assessment of the plant nitrogen status. The close relationship between the colorimetric characteristics of the closed vegetation cover and the leaves of the upper layer of wheat plant (see Table 5) suggests that the ChlRI, PRI, FRI, and WRI indices can be successfully used for remote assessment of crop areas where nitrogen nutrition is deficient.

Thus, on the example of spring wheat cv. Daria, the dependence has been established of the vegetation cover optical characteristics on the plant standing density and, to the greatest extent, on the dose of nitrogen fertilizers applied. It was found that the normalized difference vegetation index (NDVI), with a high degree of reliability ($R^2 = 0.85$, $p = 0.009$), reflects the accumulated plant biomass value in the first half of the growing season up to the BBCH 31 stage (booting). When the projective soil cover is 60% or more, it is impossible to reliably estimate the biomass value by NDVI. The use of the atmospheric resistant vegetation index (ADVI) also does not allow obtaining reliable information about the state of spring wheat plants and identifying planting areas that require additional fertilizers. It has been shown that with nitrogen deficiency and a high seeding rate, the intensity of photosynthesis decreases, as evidenced by the lower chlorophyll accumulation (ChlRI) in the leaves. In addition to reducing the intensity of photosynthesis, unfavorable vegetation conditions lead to a decrease in the efficiency of using light in photosynthesis. An increase in the photochemical reflectance index (PRI_{mod}) and flavonoid index (FRI_{mod}) indicates a decrease in the efficiency of light use and inhibition of plant growth. The pre-sowing application of nitrogen contributed to more efficient light conversion in the photosynthetic photochemical processes. The higher seeding rate (600 vs. 500 seeds per 1 m_2) had a negative effect on the efficiency of light use. The water index WRI was found to increase with denser seeding and nitrogen deficiency. Such changes indicate a lower water content and likely inducate leaf aging and a decrease in their photosynthetic activity. The increased seeding rate has the most significant negative impact on WRI. The negative impact of the increased seeding rate remained in the variants with pre-sowing application of nitrogen. Diagnostics of the state of crops by colorimetric characteristics (parameters L, A, and B of the three-dimensional CIELAB model) makes it possible to detect changes in the vegetation cover associated not only with plant growth stage and density, but also with the spectral characteristics of the diffuse reflection of leaves.

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