Nitrogen Sensing by Using Spectral Reflectance Measurements in Cereal Rye Canopy

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Abstract. *Cereal rye (cereale secale L.) is a winter crop well suited for cultivation especially besides high yield areas because of its relatively low demands on the soil and on the climate as well. In 2016 about 4.9% of arable land in Germany was cultivated with cereal rye (Statistisches Bundesamt, 2017).*

Unlike other crops such as wheat, there is little research on cereal rye for site specific farming. Furthermore, also in a cereal rye cultivation it is necessary to minimize nitrogen loss. This is especially important in low yield areas to avoid any lodging of the plants because of an overdose of mineral nitrogen (N). Therefore, an efficient fertilization strategy becomes important. To achieve this goal, some essential crop parameters such as above-ground plant biomass, N-content and N-uptake are necessary. The objective of this work was to evaluate the suitability of various vegetation indices to describe the N-uptake of cereal rye.

Over a period of nine years, from 2007 to 2016 two different kinds of plot experiments were conducted. The first one was a fertilization experiment, which consisted of only one variety of cereal rye treated with nine different N-rates from 0 up to 250 kg N per hectare (ha) which were applied at different growth stages. The second experiment consisted of four different varieties (population varieties as well as hybrids) and was treated with two different N-rates (100 and 160 kg N per ha). Each of the two experiments was designed as a double plot with four replications, in which one was for non-conducting reflectance measurements of the canopy during the *vegetation period, and at the end harvesting with a plot combine; whereas the neighboring plot was for biomass sampling. Biomass samples for nitrogen analysis and sensor measurements have been performed four times during the growing season at certain growth stages.*

The results indicated that certain vegetation indices (VI) calculated from reflectance measurements represented well the N-uptake of the crops at different growth stages. Especially simple ratios, calculated from two different wavelengths, as well as the Red Edge Inflection Point (REIP) represent best the N-uptake and the influence of the different cultivars was negligible. In comparison, the coefficients of determination of the widely known NDVI were only good at an early growth stage and, in addition, the higher the N-uptake was, the more NDVI showed a saturation effect at later growth stages. By using the NDVI-formula with other spectral areas to describe crop parameters the results were different. The saturation effect almost disappeared and the coefficient of determination increased significantly.

Keywords. *Cereal Rye, Fertilization, Reflectance measurements, Vegetation Indices*

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Introduction

In cereal production, the aim of the farmer is to optimize the yield in combination with a minimum of usage of mineral fertilizer. For this purpose and in order to avoid a N deficiency, it is necessary to know which amount of mineral N has to be given.

The challenge of the farmer is the derivation of an optimal N supply for the plants. An increasing amount of mineral N results in higher grain yield, as well as plant height. And, in addition in higher number of plants per m^{-2}, number of grains per spike, number of spike per m^{-2}, spike weight and grain protein content (Hussein et al., 2006). To get a maximum grain yield with high protein content, farmers often apply an excessive amount of mineral N-fertilizer. These very high doses of mineral N lead to nitrate (NO_3) leaching into groundwater, as well as nitrous oxide (N_2O) and methane (CH_4) emissions from the soil (Jacinthe and Lal, 2003; Köhler et al., 2006). However, the N supply of the canopy is one of the most important factors. It is a result of soil-borne N mineralization and supplemental applied N fertilizer (Moraghan et al., 2003). The usual practice is to split the whole amount of mineral N fertilizer into several parts and to apply it at certain growth stages (BBCH, Meier, 2001) to get a maximum of yield and quality.

N mineralization depends on soil as well as on weather conditions. N fertilizer applications, only based on information of soil analyses may result in in over- or undersupply of mineral N, because weather induced variations are not considered. In addition, the release of soil-borne N shows no homogeneity within a heterogeneous field (Baxter et al., 2003). For an estimate of N requirement only based on soil analyses, a high number, as well as a high areal resolution of soil tests becomes necessary. This approach is time-consuming and not economic.

As growth and development of plants are depending on the actual nutrient status, plants itself are the best indicators for estimating the N requirement. By using reflectance measurements, farmers can fertilize as needed to maximize yield while keeping their environmental footprint to a minimum. The current N-status can be determined easily by using optical sensors which detect the light of different wavelengths reflected by the plants. These sensors have already proved their ability to guide nitrogen fertilization in winter wheat (Link et al., 2002). Furthermore, Philips et al. (2004) showed that sensor values are well correlated with the current N-uptake of the plants. Sensors which detect the reflectance show their results usually as vegetation indices. The aim of this study was to compare different vegetation indices regarding their suitability to indicate the actual N-uptake in relation to the growth stage and current fertilization in combination with the lowest possible influence of different varieties.

Material and methods

Cereal rye trials were conducted in the years 2007 to 2016 in southern Bavaria near Freising in the northeast of Munich at two different trial sites of the Technical University of Munich. The soil was a silty and sandy loam, respectively, with a good fertility for grain production.

The cereal rye field trials consisted of two separate trial systems. One was a N-enrichment trial (N-optimization) and the other was a trial of cereal rye varieties. The randomized double plot design of both trials was composed of four replicates. One plot was for the sensor measurements and the other one was for biomass sampling at different growth stages.

In the years 2007 to 2016, the trial factor ‘N-treatment’ on the trial site ‘N-optimization’ consisted of 16 factor levels (Table 1). N fertilizer doses amounted from 0 to 250 kg N ha^{-1}, split and applied at different growth stages. The trial of cereal rye varieties consisted of five different hybrid-, as well as population-varieties, treated with four different application rates of mineral N, split and applied at different growth stages (Table 2). Calcium ammonium nitrate (27% N) was used as N fertilizer.

Table 1. N treatments of the N-optimization field trials from 2007 to 2016

BBCH	Application rate [kg N ha^{-1}]															
25	0	40	40	40	40	80	80	80	80	60	60	60	60	60	60	80
32	0	30	30	50	50	30	30	50	50	30	30	50	50	50	80	90
49	0	30	50	30	50	30	50	30	50	30	50	30	50	0	0	80
∑	0	100	120	120	140	140	160	160	180	120	140	140	160	110	140	250

Table 2. Cultivars and N treatments of the cereal rye cultivars trials from 2007 to 2016

Hybrid- (H) and population- (P) cultivars Askari (H), Brasetto (H), Matador (P), Recrut (P), Visello (H)				
BBCH	Application rate [kg N ha^{-1}]			
25	0	30	60	40
32	0	40	60	90
49	0	30	40	40
∑	0	100	160	170

Four times during the vegetation period, at the growth stages BBCH 30, BBCH 37, BBCH 49 and BBCH 65, biomass samples above ground were gathered. For each biomass sampling, one row of a plot was harvested. The samples were afterwards weighed and chopped. Then a sub-sample was oven-dried and re-weighed to calculate above-ground dry matter yield, followed by a conversion of the values into mass per ha. A part of the sample was grounded and afterwards the N content was analyzed by using a vario MAX auto-analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Dry matter yield and N concentration in dry matter were multiplied to compute N uptake in above-ground biomass.

The sensor device for detecting reflectance spectra was a HandySpec two channel field spectrometer (tec5 AG, Oberursel, Germany). It recorded simultaneously irradiation and reflection with a spectral resolution of 3 nm in a range from 360 nm to 1050 nm. An optical fiber receiver within the device with a viewing angle of 25° was used to determine reflection. A diffuser plate on top of the device detected irradiance within a 180° viewing angle. To calculate spectral reflectance, emission was divided by irradiance and corrected with an internal standard spectrum. Reflectance spectra were measured at the same time biomass samples were gathered. The sensor device was held about 50 cm above the canopy in a horizontal position. Each measurement covered an area of 0.04 m^2. In each plot seven readings were recorded and merged into an average value. The measurements were made between about 11 AM and 1 PM, to make sure the sun is approximately in zenith.

Various wavelengths of the spectra were used to calculate eleven vegetation indices (VI). An overview over the calculated VIs, as well as their mathematical formulas and the references is given in Table 3.

Table 3. Formulas and references of the vegetation indices

Vegetation index	Formula	Reference
REIP	$700 + 40 \frac{0.5(R_{670} + R_{780}) - 700}{R_{740} - R_{700}}$	Guyot and Baret (1988)
NDVI	$(R_{780} - R_{670})/(R_{780} + R_{670})$	Rouse et al. (1974)
IR/R	R_{780}/R_{670}	Pearson and Miller (1972)
IR/G	R_{780}/R_{550}	Tabeke et al. (1990)
IRI 1	R_{740}/R_{730}	Reusch (1997)
IRI 2	R_{740}/R_{720}	Reusch (1997)
SAVI	$\frac{1.5(R_{780} + R_{670})}{R_{780} - R_{670} + 0.5}$	Huete (1988)
NDI 1	$(R_{750} - R_{780})/(R_{750} + R_{780})$	Müller (2008)
NDI 2	$(R_{780} - R_{740})/(R_{780} + R_{740})$	Müller (2008)
SR 1	R_{740}/R_{780}	Müller (2008)
SR 2	R_{780}/R_{740}	Müller (2008)

Analysis of variance and regression were calculated with the statistical software package SPSS 22.0 (IBM Inc.). Tests for significance were made by using the Tukey-test.

Results

In the ten-years trial period of cereal rye N-optimization, the quadratic regression analysis of the different vegetation indices showed that the coefficient of determination was heterogeneous (Table 4). This was not only between the VIs, but also within the different growth stages. Table 4 also shows that SAVI had always the lowest coefficients of determination. For the VIs REIP, NDI 2, SR 1 and SR 2 the highest coefficients of determination could be observed at growth stage BBCH 30. In comparison to growth stage BBCH 30 the R^2-values of the VIs IR/R and IR/G dropped at BBCH 37, rose at BBCH 49 and dropped again at BBCH 65. The coefficients of determination of NDI 1 were nearly at the same high level from BBCH 30 to BBCH 49 and then decreased slightly to BBCH 65. The highest R^2-Values at all growth stages were found for the VIs REIP, NDI 2, SR 1 and SR 2.

Table 4. Comparison of the coefficients of determination of the vegetation indices (quadratic regression, year 2007 - 2016)

	Vegetation index [coefficients of determination]										
BBCH	REIP	NDVI	IR/R	IR/G	IRI 1	IRI 2	SAVI	NDI 1	NDI 2	SR 1	SR 2
30	0.95	0.63	0.64	0.88	0.72	0.68	0.40	0.88	0.97	0.97	0.97
37	0.94	0.70	0.46	0.77	0.86	0.83	0.36	0.89	0.94	0.94	0.93
49	0.89	0.57	0.70	0.87	0.85	0.81	0.32	0.87	0.89	0.89	0.89
65	0.76	0.55	0.38	0.61	0.61	0.72	0.73	0.73	0.76	0.77	0.75

The analysis of variance of the eleven VIs demonstrates, whether and how exactly the various N-treatment levels could be differentiated at for the fertilization important growth stages. Only if the N levels can be well differentiated by using a vegetation index, a precise site specific fertilization can be made. Then this covers best the actual fertilizer needs of the plants. Table 5 shows the results of this analysis at growth stage BBCH 37. The field 'N-quantity' describes the total amount of mineral N given during the vegetation period till growth stage BBCH 37. In comparison with the average N-uptake at BBCH 37, the fields of the VIs show the letter which describes the ability of an index to differentiate the N-fertilization.

Table 5. Comparison of the results of the ANOVA of the VIs according to the N-quantity at BBCH 37 (2007 - 2016)

N-quantity [kg N ha-1]	Average N-uptake [kg N ha-1]	Vegetation index										
		REIP	NDVI	IR/R	IR/G	IRI 1	IRI 2	SAVI	NDI 1	NDI 2	SR 1	SR 2
0	45.8	a	a	a	a	a	a	a	a	a	a	a
40	81.0	b	b	a	ab	b	ab	b	b	b	b	b
70	98.5	c	b	a	bc	bc	bc	b	bc	bc	bc	bc
80	110.4	c	b	b	c	c	c	b	cd	cd	c	c
90	110.4	c	b	b	c	c	c	b	cd	cd	c	c
110	123.5	d	b	b	c	c	c	b	d	d	d	d
130	129.0	de	b	b	c	c	c	b	de	de	de	de
140	130.7	de	b	b	c	c	c	b	de	de	de	de
170	154.4	e	b	b	c	d	d	b	e	e	e	e

The results show that NDVI and SAVI could reliable differentiate only between unfertilized and fertilized plants. Therefore, the average N-uptakes resulting from the various N-quantities could not be distinguished. IR/R, IR/G, IRI 1 and IRI2 are only able to differentiate the canopy between unfertilized, slightly and highly fertilized. Even with these VIs, an exact differentiation of the canopy at higher levels of N-fertilization is not possible. The VIs REIP, NDI 1, NDI 2, SR 1 and SR 2 represent the average N-uptake best and therefore a good differentiation of the various amounts of available N is possible. These effects are shown exemplary for the VIs REIP and NDVI in the Figures 1 and 2.

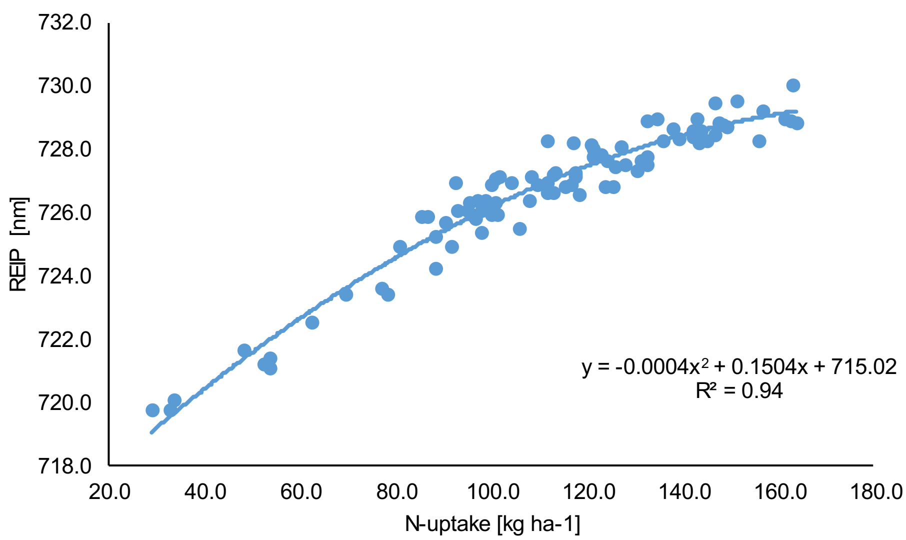

Figure 1. Regression analysis of N-uptake to REIP at BBCH 37 (all N-levels, 2007 - 2016)

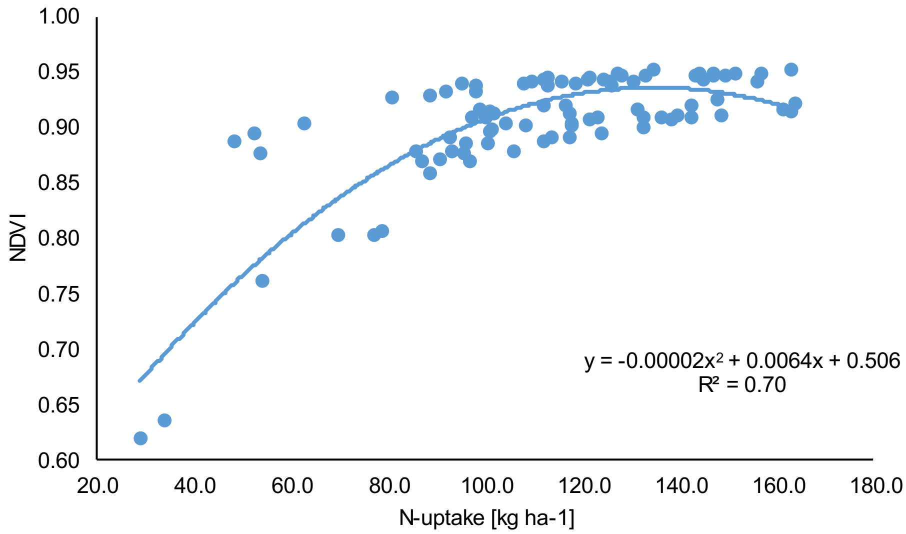

Figure 2. Regression analysis of N-uptake to NDVI at BBCH 37 (all N levels, 2007 - 2016)

The regression analysis of the NDVI, shown in Figure 2, shows that regardless the increasing N-uptake, even of 50 to 80 kg N ha^{-1}, the NDVI-values vary between 0.75 and 0.95. Therefore, a precise differentiation of the actual N-uptake is no longer possible. This is a sign for a significant saturation effect. From Figure 1 and Table 4 it can be seen that at the VIs, which have the highest coefficients of determination at all growth stages, the R^2-values decrease the higher the growth stage was. This results in development-dependent regressions of the various vegetation indices and is exemplary shown for the VI REIP in Figure 3. For a better illustration of the change of the estimation-equations, the ordinate contains the N-uptake, whilst the VI is on the abscissa.

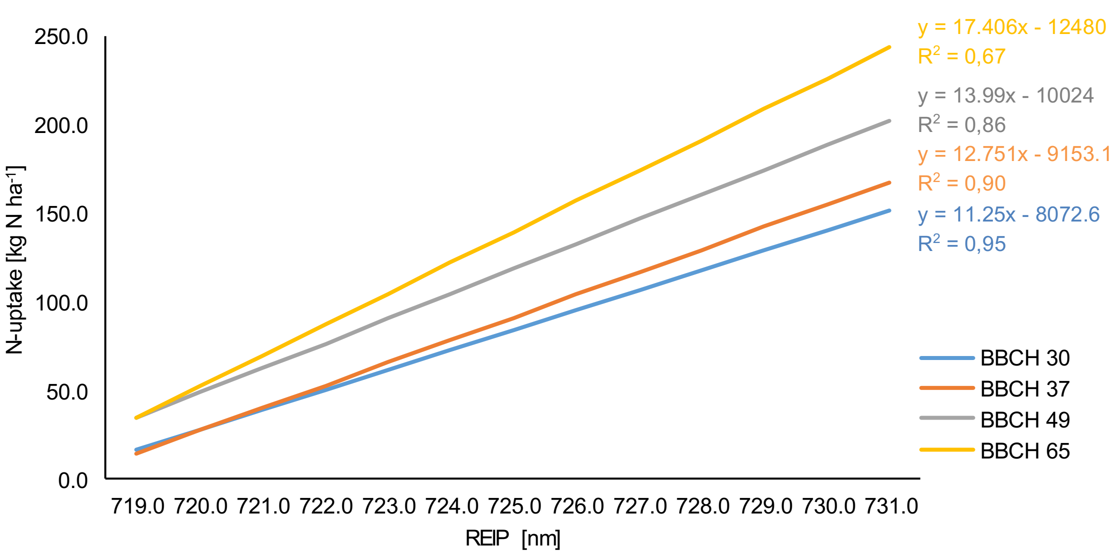

Figure 3. Growth stage-dependent courses of the linear regressions of the VI REIP to N-uptake (all N-levels, 2007 - 2016)

Figure 3 shows that the VI REIP has distinct growth stage-specific differences that allow a more detailed differentiation of the N-uptake of the different growth stages by using the REIP values. Thus, based on the REIP value in combination with the growth stage specific regression equation, the actual N-uptake can be estimated with a very high quality. The other examined VIs, except for NDVI, IR/R and SAVI show a comparable behavior to the REIP. As already shown in Table 4, IR/R, NDVI and SAVI have low coefficients of determination at all growth stages. Additionally, growth stage-specific differences of these vegetation indices were much more pronounced. This behavior is shown exemplary for the NDVI in Figure 4.

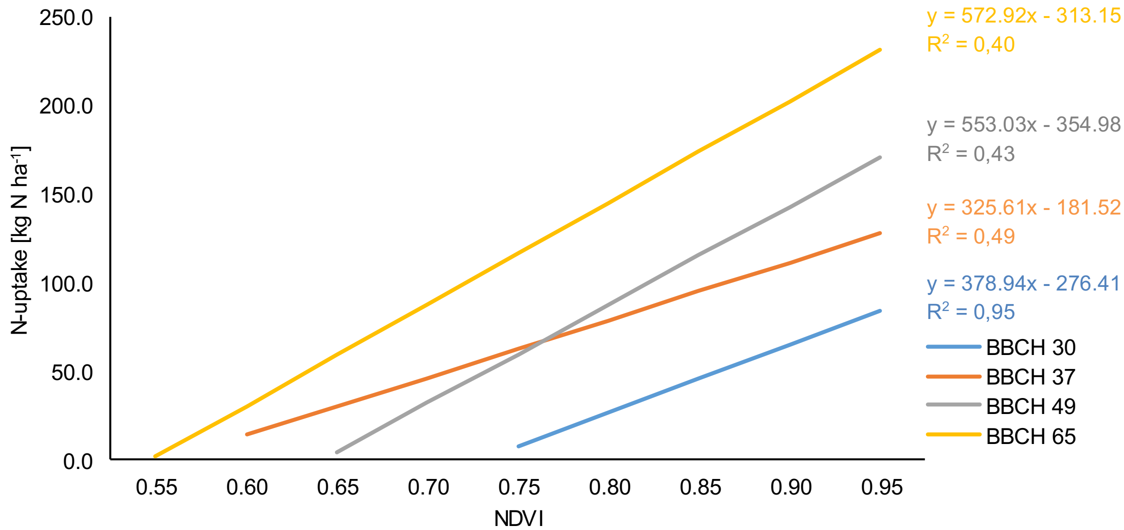

Figure 4. Growth stage-specific courses of the linear regressions of the NDVI to N-uptake (all N-levels, 2007 - 2016)

Therefore, and because of additional saturation effects, as shown in Figure 2, these VIs are not suitable to ensure accurate estimations of the N-uptake of the canopy.

Besides the aspect of the differentiation of the N-treatments, a possible influence of the varieties is also important. The influence of the various varieties on the results of the measurements, at for the fertilization important growth stages should be as small as possible. To analyze this topic, an analysis of variance of the various varieties was performed.

Table 6. Influence of the varieties on the VIs (p-values, all cultivars, 2007 - 2016)

	Vegetation index										
BBCH	REIP	NDVI	IR/R	IR/G	IRI 1	IRI 2	SAVI	NDI 1	NDI 2	SR 1	SR 2
30	0.740	0.194	0.318	0.055	0.196	0.295	0.007	0.230	0.589	0.586	0.571
37	0.248	0.751	0.751	0.321	0.002	0.976	0.031	0.038	0.217	0.234	0.186
49	0.014	0.297	0.297	0.019	0.009	0.278	0.000	0.213	0.021	0.028	0.014
65	0.060	0.019	0.019	0.019	0.109	0.009	0.002	0.038	0.023	0.028	0.021

Levels of significance			
p > 0.05	p > 0.01-0.05	p <= 0.01	p <= 0.001
not significant	signifcant	very significant	most significant

Table 6 shows the levels of significance of the vegetation indices (p-values). The lower the influence of the cereal rye varieties on the VIs is, the higher the levels of significance are. The VI REIP was not sensitive to an influence of the varieties at for fertilization important growth stages BBCH 30 and BBCH 37. At BBCH 49, however, with p = 0.014 the varieties caused a significant influence on the VI REIP, which returned to non-significant values at BBCH 65. The measurement results of IRI 2, IR/R and NDVI were not affected by the varieties, except for growth stage BBCH 65. But shown in Table 4, these VIs have lower coefficients of determination for N-uptake in the N-optimization trials, and they are only able to differentiate between unfertilized and fertilized plants. Furthermore, SAVI was strongly influenced by the varieties at all growth stages. The VI IR/G shows, with higher coefficients of determination to the N-uptake, a similar behavior as SAVI, except at growth stage BBCH 37. As shown in Table 4 and in comparison with other VIs, both of them have generally low coefficients of determination to the N-uptake. At for the fertilization important growth stages BBCH 30 and BBCH 37, the other examined VIs did not show any relevant influence of the varieties and showed at the same time high coefficients of determination. To the N-uptake. Nevertheless, an increasing influence of the varieties on the results of the measurements of the VIs at later growth stages could be observed. These relationship of the influence of the varieties and the VIs are exemplified for the VIs REIP and NDVI at BBCH 30 in the Figures 5 and 6.

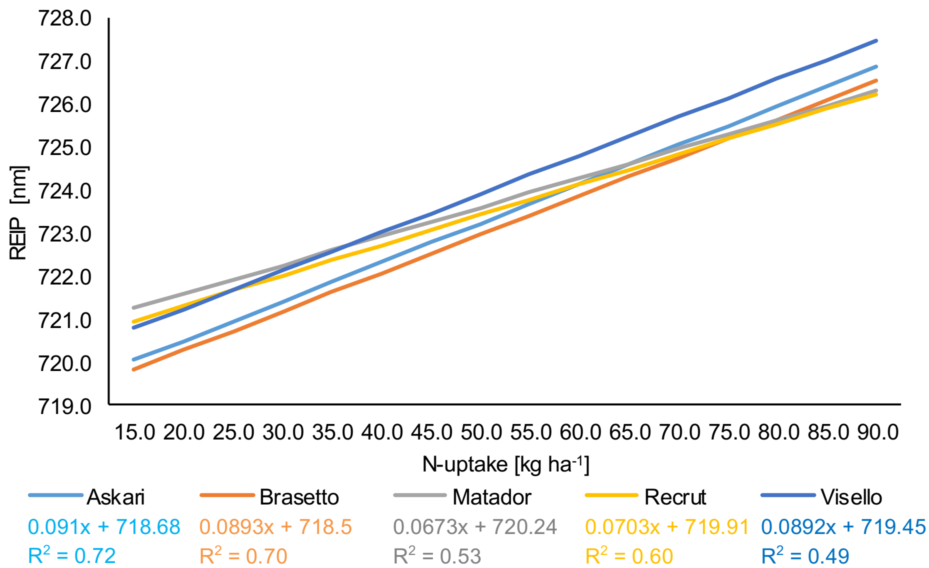

Figure 5. Influence of the varieties on the results of the measurements of REIP at BBCH 30 (2007 – 2016)

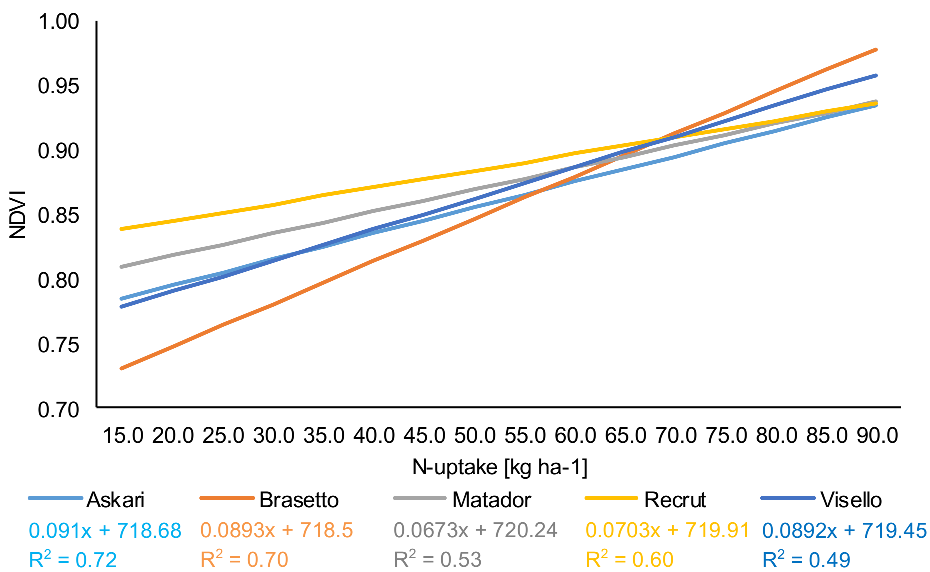

Figure 6. Influence of the varieties on the results of the measurements of NDVI at BBCH 30 (2007 - 2016)

Summary

Even in growth stage BBCH 30 with a relatively low soil cover, very high coefficients of determination over all years up to R^2 = 0.97 could be determined. The SAVI, which is a further development of the NDVI to minimize the influence of the soil (Huete, 1988), showed even at BBCH 30 with R^2 = 0.40 the lowest coefficients of determination. Furthermore, the coefficients of determination of the SAVI were getting worse during the later vegetation period, although the soil was completely covered by the canopy and an influence of the ground was no longer present. As shown in Table 3, the VIs NDVI and IR/R use the same wavelengths of 670 nm and 780 nm as the SAVI. Table 4 shows that they have significantly higher coefficients of determination at all examined growth stages than the SAVI. The difference is that SAVI uses a correction factor. The fact that the research of SAVI was with done cotton and lovegrass (eragrostis lehmanniana), suggests that the correction factor is unsuitable to use it in cereal rye canopy. Despite higher coefficients of determination of the NDVI than the SAVI, with a maximum of $R^2 = 0.63$ at BBCH 30 it is considered to be an unsuitable indicator for determining the N-uptake of cereal rye. It also seems to be confirmed that the combination of red- as well as infrared wavelengths is influenced by various background effects, such as soil reflection and beam path within the leafs. For the NDVI these effects have already been described by Huete and Jackson (1988), Vogelmann et al. (1993) and Elvidge and Chen (1995). The VI IR/G clearly showed that wavelengths other than those used in NDVI and IR/R were significantly less affected by background effects. The wavelengths of 780 nm and 550 nm used in this VI have the effect that the coefficient of determination at BBCH 30 increased higher than NDVI and IR/R up to $R^2 = 0.88$. The VIs REIP and NDI 2 and in a slightly weaker form also the VIs NDI 1, SR 1 and SR 2, showed the highest coefficients of determination at all growth stages. For the REIP this is because of the use of four different wavelengths of red (670 nm and 700 nm) and infrared (740 nm and 780 nm). Obviously by using the wavelength 740 nm background effects were minimized. This is confirmed by the high coefficients of determination of the VIs NDI 2, SR 1 and SR 2, regardless their mathematical formulae. This showed a clear correlation between these VIs and the N-uptake. Also an effect of decreasing coefficients of determination at all examined growth stages during the growing season could be observed. This may be because of a saturation effect as shown for the NDVI at BBCH 37. Thus, above a certain N-uptake a vegetation index cannot differentiate anymore.

Furthermore, an influence of the varieties on the result of the VIs could be observed. But this influence varied depending on the vegetation index and the growth stage. The effects of the varieties on the results of the VI REIP were not significant, except growth stage BBCH 49, where it was significant over all years. The ANOVA showed for all vegetation indices no significant influence of the varieties at for fertilization important growth stages, except for the VIs IR/G, IRI 1, NDI 1 and SAVI. However, a slightly influence could still be observed, the remission of light could be the reason, because of a different structure of the varieties within the canopy.

Because these assumptions could not be clarified completely, further investigations and trails are necessary.

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