Research Article

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Assessing crop performance in maize field trials using a vegetation index

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Abstract: New agronomic systems need scientific proof before being adapted by farmers. To increase the informative value of field trials, expensive samplings throughout the cropping season are required. In a series of trials where different application techniques and rates of liquid manure in maize were tested, a handheld sensor metering the red edge inflection point (REIP) was compared to conventional biomass sampling at different growth stages and in different environments. In a repeatedly measured trial during the 2014, 2015, and 2016 growing seasons, the coefficients of determination between REIP and biomass / nitrogen uptake (N_{upt}) ascended from 4 leaves stage to 8 leaves stage, followed by a decent towards tasseling. In a series of trials in 2014, and 2015, the mean coefficients of determination at 8 leaves stage were 0.65, and 0.67 for biomass and N_{upt} , respectively. The predictability of biomass or N_{upt} by REIP however, is limited to similar conditions (e.g. variety). In this study, REIP values of e.g. ~721, represent N_{upt} values from ~8 kg ha⁻¹ to ~38 kg ha⁻¹. Consequently, the handheld sensor derived REIP used in this series of experiments can show growth differences between treatments, but referential samples are necessary to assess growth parameters.

Keywords: nitrogen uptake, active crop sensor, red edge inflection point, crop development, field trials

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1 Introduction

Nitrogen (N) is a crucial nutrient for crop growth and below optimal plant N concentration can lead to significant yield losses (Mollier and Pellerin 1999, Plénet and Lemaire 2000). Excess N fertilization however, can have a negative impact on ecosystems (Sutton et al. 2011). To acquire reliable information on balanced N fertilization, researchers usually use multi-location and/or multi-annual field trials to acquire fundamental information (Gomez and Gomez 1984). Regarding maize (*Zea mays* L.) fertilization trials in areas with higher contents of soil organic matter, major differences in early-growth might become insignificant at harvest due to a high N mineralization potential (Federolf et al. 2017). If the harvest is the only sampling, it might be difficult to explain differences in yield and quality due to early-growth nutritional status (Clewer and Scarisbrick 2001). Retarded early-growth in maize however, might influence farmers decisions when it comes to adopting new strategies or they may stick to a known “safety first system” (Withers et al. 2000). Thus, detailed knowledge about nutrient interactions in the soil-plant system are crucial; therefore, sampling throughout the whole vegetation period is necessary (Clewer and Scarisbrick 2001).

Visual scoring is cheap and easy, but it is non-quantitative, difficult to standardize, and biased by human error (Montes et al. 2011; Olf et al. 2005). Soil and plant sampling is more accurate, but these practices are time consuming and costly. They lead to great quantities of samples, which require a workforce to obtain, process and analyze (Olf et al. 2005; Rambo et al. 2010). Furthermore, destructive sampling needs additional space in plots (Clewer and Scarisbrick 2001), increasing the area needed for each plot and thus, decreasing the chances of finding adequate and homogeneous sites for a field trial. In a series of trials, additional difficulties appear when crops (especially rapidly developing spring crops like maize; Birch et al. 2003) reach a planned sampling stage simultaneously at different sites. To

decrease the number of samples, researchers increasingly try to use chlorophyll-meters (Rashid et al. 2005), or measure vegetation indices via spectral nondestructive plant analysis to get information on biomass and crop nutritional status (Winterhalter et al. 2011; Osborne et al. 2002).

In the last decades several spectral indices have been evaluated for their ability to describe aboveground biomass. For example, leaf area index (LAI) and N uptake of broadacre crops such as maize, wheat (*Triticum aestivum* L.), or oilseed rape (*Brassica napus* L., Erdle et al. 2011; Thoren and Schmidhalter 2009) have been considered. Although a huge number of different indices exist, the normalized differenced vegetation index (NDVI, Rouse et al. 1974), and the red edge inflection point (REIP, Guyot and Baret 1988) have been used most frequently. The NDVI is very sensitive at low LAI values, but tends to saturate at moderate to high LAI (Baret and Guyot 1991). Thus, for crops with LAI values higher than 2, the REIP is a better predictor due to inclusion of red-edge information (Stickse et al. 2004). Mistele and Schmidhalter (2008a) showed a consistently useful correlation of the REIP and a NIR/NIR ratio (R780/R740) and the aboveground N uptake of maize crops, whereas limitations for the use of the NDVI and other single ratios, which combine the reflection in the red or green ranges with NIR, were found. However, they also experienced the need for a minimum of biomass for the REIP to return useful values. Compared to other vegetation indices, Stickse et al. (2004), found a high consistency of REIP values throughout different light conditions during a day.

The vegetation indices are mainly obtained by using multispectral cameras, which require significant post-processing, or ready to use sensors that measure, calculate and directly display obtained values. They are available as handheld devices (Tavakoli et al. 2014), mounted on purpose adjusted sensor platforms (Montes et al. 2011; Winterhalter et al. 2011), tractors (Mistele and Schmidhalter 2008b), or unmanned aerial vehicles (UAV, Rasmussen et al. 2016), or satellites (Thenkabail et al. 2000; Malenovsky et al. 2012). Satellite sensing, such as the ESAs Sentinel mission, provides several wavebands in the red edge and near infrared spectra with a spatial resolution of 10 m per pixel (Malenovsky et al. 2012), which is not sufficient for plot trials. UAVs, although offering certain opportunities and being available, require deep knowledge of image processing and data interpretation (Rasmussen et al. 2016). Thus, tractor or platform mounted sensing devices seem the most appropriate version for experimental stations, whereas handheld sensors can easily be transported from field to field, which makes

them convenient for diverse types of experimental series.

The purpose of our study was to evaluate the use of the REIP obtained with a handheld sensor to describe growth differences of maize stands in field trials, where different combinations of liquid manure (broadcast application, and subsurface injection without and with a nitrification inhibitor) and mineral starter fertilizers were tested in different environments (Federolf et al. 2016; Federolf et al. 2017). The research questions were: how soil-plant-fertilizer interactions influences on crop growth change sensor values obtained using REIP, and whether it is possible to link sensor measurements to crop parameters via regression analysis, independent of growth stage and crop management practices?

2 Material and Methods

2.1 Experimental sites, soil and weather conditions

To obtain a reasonable amount of data for the study, two different approaches were used. One experiment was conducted at Hollage, close to Osnabrück (Germany) in the 2014 - 2016 seasons, to gain in-depth insight into the possibilities of sensor use, especially during the early-growth development of maize. To check the usability of the sensor at different sites, a series of experiments was established in cooperation with the Chambers of Agriculture in North Rhine-Westphalia, Lower Saxony and Schleswig-Holstein at six sites in the years 2014, and 2015.

2.1.1 The Hollage experiment

In 2014, 2015 and 2016, field trials were conducted at Hollage, Lower Saxony, Germany (52°20' N, 07°58' E) on three adjacent fields. The soil types can be categorized as plaggic, or gleyic Podzols (IUSS Working Group WRB 2014) with sandy soil texture (>87% sand; for details see Table 1).

Maritime climate is dominating in northwestern Germany. Mean annual air temperature at the study site is 10.0°C and mean annual precipitation is 800 mm. On average, monthly precipitation increases from 41 mm in April to 79 mm in August. In 2014, a mild winter and above average temperatures in March and April led to higher soil temperatures compared to the long-term average. Higher air temperature throughout July, along with 129 mm of precipitation enabled very high growth rates of the maize plants. Thus, thermal time from planting to harvest in 2014

Table 1: Locations, climate and soil conditions, fertilization details and crop management practices

Location	Hollage	Haus Düsse	Merfeld	Sandkrug	Wehnen	Bovenau	Schuby
latitude	52°20' N	51°38' N	51°51' N	53°05' N	53°10' N	54°19' N	54°31' N
longitude	07°58' E	08°11' E	07°12' E	08°16' E	08°08' E	09°48' E	09°26' E
Climate							
mean an. precip. (mm)	860	836	880	841	823	847	844
mean an. air temp. (°C)	9.4	9.8	10.4	9.6	9.5	8.9	8.6
soil conditions							
soil type ¹	PZ-pi	LV-ha	PZ-pi	PZ-ha	PZ-gl	LV-gl	PZ-ha
texture ²	sand	silt	sand	Sand	sand	loam	sand
pH ³ (CaCl ₂)	2014 2015 2016	2014 2015	2014 2015	2014 2015	2014 2015	2014 2015	2014 2015
SMN ³ (kg ha ⁻¹)	5.3 5.5 5.4	6.8 6.8	5.0 5.0	5.3 5.4	4.8 5.3	6.4 6.2	5.4 5.3
P _{cal} ⁴ (mg 100 g ⁻¹)	35 45 20	15 40	15 10.0	23 23	13 41	68 31	23 29
nutrients applied via manure	8.0 7.8 7.2	7.0 5.7	9.2	11.0 8.0	11.0	15.0 8.6	14.0 11.2
total N (kg ha ⁻¹)	166 130 182	143 100	178 87	164 136	180 118	207 198	164 180
P (kg ha ⁻¹)	42 34 50	29 28	50 24	33 32	23 19	33 21	17 15
crop management practices							
variety	Ricardinio	LG30224	LG30254	LG30222	LG30222	LG30211 P7524	LG30211 P7524
planting	Apr 25 Apr 22 Apr 19	Apr 23 Apr 23	Apr 16 Apr 15	May 17 Apr 27	Apr 30 Apr 29	May 15 Apr 27	May 06 Apr 30
V8 sampling	- Jun 19 Jun 13	Jun 14	Jun 13 Jun 15	Jun 19 Jun 25	Jun 19 Jun 06	Jun 25 Jun 02	Jun 24 Jun 03
harvest	Oct 09 Sep 29 Sep 19	Oct 06 Oct 05	Sep 20 Sep 29	Oct 02 Oct 06	Oct 02 Oct 26	Oct 10 Oct 27	Oct 01 Oct 04

¹ Soil type with cm= cambic; g= gleyic; ha= haplic; pi= plaggic; LV= Luvisol; PZ= Podzol (IUSS Working Group WRB 2014)² Soil layer 0-30 cm³ SMN = Soil mineral nitrogen (NH₄-N + NO₃-N) prior to manure application (soil layer 0-60 cm)⁴ P_{cal} = Phosphorus extracted with calcium-acetate-lactate solution (soil layer 0-30 cm)

were above the long-term average (Figure 1). In 2015, May and June (i.e. the early growth period of maize) were cold and dry, but elevated temperatures in July and August 2015 led to reasonable crop growth rates. Finally, thermal time duration from planting to harvest was 1272°Cd, being close to the 2014 observation (1450°Cd). In 2016, April and May were extraordinarily dry. Most of the total precipitation during the season was due to a rainy June contributing 231 mm to a total of 424 mm of precipitation (Figure 2). A cold period after planting until the second week of May led to delayed emergence, but afterwards temperature was above average, especially in late August and September, leading to thermal time duration of 1401°Cd from planting to harvest.

Figure 1: Thermal time according to McMaster and Wilhelm (1997) from April to September at the Hollage experiment. Comparing long average (1994–2014) with 2014, 2015 and 2016 growing seasons

Figure 2: Cumulative precipitation (mm) from April to September at the Hollage experiment. Comparing long average (1994–2014) with 2014, 2015 and 2016 growing seasons

2.1.2 The experimental series

In 2014 and 2015, maize fertilization trials were conducted in cooperation with the extension service (i.e. the Chambers of Agriculture) in Lower Saxony, North Rhine-Westphalia and Schleswig-Holstein (see Table 1 for details). The five

sites were in regions with intensive animal farming on sandy soils. Due to long-term manure application, these soils are typically high to very high in soil P test levels (see Table 1). One site in a cash crop dominated area, with little livestock, showed a medium to high level of plant available soil P but this site did not receive considerable amounts of organic manure over the last decades.

Mean annual air temperature at the study sites varies from 8.6°C to 10.4°C from north to south, and mean air temperature from May to September ranges from 13.8°C to 15.4°C, respectively. Mean annual precipitation ranges from 742 mm to 880 mm (Ø 826 mm) with rainfall from May to September being from 323 mm to 376 mm (Ø 364 mm). In 2014, the air temperature was 1.3°C above the long-term average with May to September precipitation being rather close to the average, but with unusual rainfall distribution, having high rainfall in May and July. In 2015, air temperature and precipitation in the months of April, May and June were lower than in the 2014 season. There were major rainfall events, after the eight-leaf sampling (V8) in July; however, these led to similar total precipitation across both seasons.

2.2 Experimental designs and treatments

In the experimental series, a split-plot design with the two factors “liquid manure application” and “mineral starter fertilizer” was used with four replications. Main plots were randomized in each replication and subplots were randomized within main plots. Treatments in the factor “liquid manure application” were an unfertilized control (C), liquid manure broadcast (B), liquid manure injection (I) and liquid manure injection with nitrification inhibitor (I(N); see also Federolf et al. 2016). All plots were split into two 7 m long subplots (4 rows with 75 cm row spacing). Half of the plot received mineral starter fertilizer (“+”; 23 kg N ha⁻¹ and 10 kg P ha⁻¹) at planting, whereas the other half did not (“-”). The Hollage experiment was reduced to the four treatments of major interest, out of the experimental series. These were set up as a randomized complete block design with four replications. Besides an unfertilized control treatment (C-), a liquid manure broadcast treatment (B+) with mineral starter fertilizer (23 kg N ha⁻¹ and 10 kg P ha⁻¹) was compared to two liquid manure injection treatments with (I(N)-) and without nitrification inhibitor (I-), respectively. The plots consisted of four maize rows with 75 cm row spacing and were 25 m long to allow destructive analysis (see also Westerschulte et al. 2017). Nitrogen fertilization levels of all trials were adapted to local recommendations (Table 1).

2.3 Crop management practices

Crop management and crop protection in all experiments was done according to best management guidelines adapted to local needs for all treatments equally at each site. Different varieties were used in the experiments due to different soil and climate conditions. Planting dates, ranging from April 15 to May 17, were chosen individually for each site (see Table 1). Plant density was 9.2 plants m² at the Hollage experiment and 9 plants m² in the experimental series.

2.4 Measurements and samplings

2.4.1 Biomass samplings

Aboveground biomass was sampled at several vegetative stages (Vn stage when collar of nth leaf in B treatment was visible, details in Table 2) in the Hollage experiment, while only one sampling was done at V8 in the experimental series. At each sampling occasion, sensor measurements were conducted within two days before, or after biomass sampling, depending on weather conditions. To obtain biomass samples at Hollage, sixteen plants (20 plants at V3 and V4 to ensure sufficient material for lab analysis) per plot were cut at the stem base in the center rows. In the experimental series, ten plants were cut in the outer rows of the plots, as plot size did not allow destructive measurements in the growing season. All samples were dried at 80°C to constant weight. Nitrogen concentrations from representative subsamples of the dried material were then determined using the Kjeldahl method after fine grinding of the plant material (DIN 2005).

2.4.2 Sensor measurements

To determine the red edge inflection point (REIP) vegetation index a modified handheld device of the Fritzmeier ISARIA

sensor (Fritzmeier Umwelttechnik, Großhelfendorf, Germany) was used along with a smartphone to run the software (Haas 2013). The measuring device comprises of four LED elements, which emit monochrome light at predetermined wavelengths (670 nm, 700 nm, 740 nm, and 780 nm, respectively) in a measurement cycle, a light receiving element, and a control device to determine the intensity of light reflectance (Haas 2010). The software calculates the REIP index with the following equation: $REIP = 700 + 40 * ((R_{670} + R_{780}) / 2 - R_{700}) / (R_{740} - R_{700})$ (Haas 2010). The frequency of measurement cycles per second depends on light conditions and was always above 100 cycles per second in our trials. For each second, means of all measurement cycles are stored into a single file per plot, which then contains >10 obtained values.

The sensor head was walked along the two center rows of each plot in a round-trip, at a height of 60 cm above the whorl of the plants. While walking the sensor along the plots, the sensor head was always kept at nadir view to reduce the influence of the general anisotropy of spectral reflectance measurements (Casa et al. 2010). The field of view in this respective distance is a circle with approximately 25 cm diameter. The sensor measurements at the Hollage experiment were done on a weekly basis, depending on weather conditions, from roughly two-leaf stage to tasseling (only to 10 leaf stage in 2016, see Table 3). As the REIP shows little sensitivity to diurnal variations (Sticksel et al. 2004), the measurements were done at different respective ambient light conditions.

2.5 Calculations and data analysis

Based on dry matter accumulation and N concentrations, the plant N uptake was calculated for each plot. For further processing the obtained REIP values were averaged for each plot. For the Hollage experiment analysis of variance was performed with PROC MIXED in SAS, followed by a LSD post hoc test, if $P < 0.05$ (SAS Institute Inc. 2011).

Table 2: Biomass samplings at the Hollage experiment

	2014	2015	2016
V4 ¹ sampling	-	June 01	May 24
V6 sampling	June 10	June 08	June 03
V8 sampling	-	June 19	June 13
V10 sampling	June 30	June 29	June 24
VT sampling	July 22	July 24	-
harvest date	Oct. 09	Sep. 29	Sep. 19

¹Vn = vegetative leaf stage n, VT = tasseling

Table 3: Dates of sensor measurements the Hollage experiment

2014		2015		2016	
date ¹	DAP ²	date	DAP	date	DAP
May 22	27	May 20	28	May 24	35
May 27	32	May 26	34	May 31	42
June 03	39	June 01	40	June 03	45
-	-	June 08	47	June 08	50
June 10	46	June 11	50	-	-
June 18	54	June 16	55	June 16	58
June 26	62	June 19	58	June 23	65
June 28	64	June 24	63	-	-
July 04	70	June 29	68	-	-
July 10	76	July 09	78	-	-
July 15	81	July 16	85	-	-
July 24	90	July 24	93	-	-

¹ date = date of sensor measurement in the respective year

² DAP = days after planting

The following exponential regression was used (Thenkabail et al. 2000). $a = m \cdot e^{b \cdot \text{REIP}}$ (with “a” being either DM, or N_{upt} ; Eq. 1).

The respective correlations between aboveground biomass (DM), plant N uptake (N_{upt}) and the red edge inflection point (REIP) derived from the sensor measurements were computed using the “lm” function in the R software environment (R Core Team 2016) for each sampling occasion separately. Eq. 1 was linearized ($\ln(N_{\text{upt}}) = \ln(m) + b \cdot \text{REIP}$) and the Cook’s distance (Di) was used to identify outliers when $Di > 4/n$ (with n being the number of datapoints; Bollen and Jackman 1990). The regression was computed again after removal of outliers. For the nitrogen concentrations (N_{conc}), a linear regression was fitted ($N_{\text{conc}} = m + b \cdot \text{REIP}$), the Cook’s distance and removal of outliers were performed as with the linearized data.

Ethical approval: The conducted research is not related to either human or animals use.

3 Results

3.1 Devolution of sensor values

From the first (27, and 28 days after planting in 2014, and 2015, respectively) to the second (32, and 34 days after planting in 2014, and 2015, respectively) measurements, a sharp decline in REIP values was observed (-6.6 points from 723.3 to 716.7 in 2014, and -5.2 points from 713.8 to

708.6 in 2015, respectively; Figure 3; Supplementary Table 6). In the following measurements, the values increased until peaking at 76, and 78 days after planting in 2014, and 2015, respectively. In 2016 there was also a slight decline from the first to the second measurement (-1 point, Figure 3; Supplementary Table 6). However, the first and second measurements were later (35 DAP, and 42 DAP, respectively) than in the previous years and there were no measurements after V10 (65 DAP). The differences between treatments obtained by plant analysis go along with the REIP measurements.

3.2 Correlations between REIP and biomass, N concentrations and nitrogen uptake

3.2.1 The Hollage experiment

The coefficients of determination for the Hollage experiment are displayed in Table 4, and the raw data in Supplementary Table 6. In the 2014 season, for all measurements (V6, V10, and VT), the coefficients of determination for biomass and N_{upt} were above 0.7 and highly significant. In 2015, the coefficients of determination for biomass ($r^2 = 0.27^*$), as well as for N_{upt} ($r^2 = 0.23^{\text{n.s.}}$) were low at the V4 measurement. With ongoing crop development, the relationship between the values obtained by plant analysis and the sensor measurements increased, peaking at V8, when r^2 for the relation REIP / biomass was 0.82^{***} , and for REIP / N_{upt} at 0.92^{***} , respectively. At V10, and VT, the coefficients of

determination decreased again. In 2016, the coefficients of determination were above 0.8*** for biomass and N_{upt} at all sampling occasions.

The relationship between REIP and N_{conc} was variable throughout the study period. While in 2014, at V6, and V10, the coefficients of determination were high ($r^2 = 0.81^{***}$), for the VT measurement it was only $0.21^{\text{n.s.}}$. In 2015, only at the V4 measurement, a significant relationship was observed, while all other samplings did not show

significant relationships. In 2016, again the relationships were significant, but the coefficients of determination were lower (ranging from 0.37 to 0.7) than in 2014.

3.2.2 The experimental series

Although major differences in temperature and precipitation occurred between the two seasons, there was no obvious trend for a certain nutrient deficiency to be more expressed in one of the years. The Wehnen site, for example showed severe P deficiency symptoms in 2015 with a mean P concentration of only 2.32 g kg^{-1} in shoot biomass, whereas the neighboring site at Sandkrug showed high values (mean 4.60 g kg^{-1} in 2015). This P deficiency at the Wehnen site led to major differences in crop growth as the unfertilized control treatment (treatment C-) only produced $201 \text{ kg DM ha}^{-1}$ biomass until V8, whereas treatment I(N)+ produced $1475 \text{ kg DM ha}^{-1}$ at the same stage (Supplementary Table 7). For N concentrations, there was also no clear trend within the seasons. The coefficients of determination between REIP and N_{conc} were also low in this series (means for all sites are 0.24 and 0.12 in 2014 and 2015, respectively; Table 5). However, at some sites there were significant relations (Haus Düsse, Sandkrug and Wehnen in 2014, and Bovenau in 2015). The relations between REIP and biomass ranged from 0.47 and 0.53 (Merfeld 2014 and 2015, respectively) to 0.81 and 0.76 (Sandkrug 2014 and Wehnen 2015, respectively) with a mean of 0.65. Comparable results were observed for the coefficients of determination between REIP and N_{upt} . Lowest values were calculated for Schuby and Merfeld (0.49 and 0.50 in 2014, 0.62 and 0.52 in 2015, respectively) and highest for Sandkrug and Wehnen (both 0.81 in 2014, 0.82 and 0.81 in 2015, respectively).

4 Discussion

4.1 The Hollage experiment

The REIP values at the very early measurement dates in 2014 and 2015 were quite variable and the sharp decline to the second measurement five to six days later indicates a shift from mainly soil borne reflectance in the first, to a rather plant borne reflectance in the second measurement. Behrens et al. (2005) showed an influence of soil reflectance on the NDVI. As both NDVI wavebands are also used for the REIP, the influence of soil reflectance at low biomass levels most likely also affected the first measurements in our trials. For maize aboveground biomass significant

Figure 3: Development of REIP values for treatments in the Hollage experiment in 2014, 2015, and 2016. Means of treatments fertilized with manure and mineral starter fertilizer (MSF) [C = no manure, no MSF, B = manure broadcast with MSF, I = manure injection without MSF, I(N) = manure injection with nitrification inhibitor without MSF]

	2014					2015					2016									
	growth stage	r ²	p	N _{conc}	biomass	growth stage	r ²	p	N _{upt}	biomass	growth stage	r ²	p	N _{conc}	biomass	growth stage	r ²	p	N _{upt}	biomass
V4 ¹		no data																		
V6		0.75	***	0.81	***		0.87	***		0.60	***	*	0.30	*	0.27	***				
V8		no data								0.82	***	n.s.	0.23	n.s.	0.82	***				
V10		0.77	***	0.81	***		0.83	***		0.66	***	***	0.02	n.s.	0.66	***				
VT		0.80	***	0.21	n.s.		0.76	***		0.43	**	n.s.	0.17	n.s.	0.43	**				

Table 5: Coefficients of determination (r^2) and the results of the correlation analysis (p values) between biomass, N concentration (N_{conc}), N uptake (N_{upt}) and the red edge inflection point (REIP) at V8 stage of maize in the experimental series for the seasons 2014 and 2015

	2014						2015							
	biomass			N _{conc}	N _{upt}			biomass			N _{conc}	N _{upt}		
site	r ²	p	r ²	p	r ²	p	r ²	p	r ²	p	r ²	p	r ²	p
Bovenau	0.69	***	0.02	n.s.	0.61	***	0.59	***	0.38	***	0.66	***		
Haus Düsse	0.69	***	0.48	***	0.68	***	no data							
Merfeld	0.47	***	0.13	*	0.50	***	0.52	***	0.01	n.s.	0.54	***		
Sandkrug	0.81	***	0.44	***	0.81	***	0.73	***	0.12	n.s.	0.82	***		
Schuby	0.52	***	0.04	n.s.	0.49	***	0.57	***	0.10	n.s.	0.62	***		
Wehnen	0.80	***	0.36	***	0.81	***	0.76	***	0.01	n.s.	0.81	***		

differences between treatments at the Hollage experiment in 2015 occurred as early as V4 (Federolf *et al.* 2017). At this stage, the obtained values were lower than in the earlier measurement in both 2014 and 2015, but the differences between treatments became detectable. Thus, for sensing differences in agronomic maize trials with the device used, the plants should at least have four leaves to guarantee sufficient leaf area and biomass, as other studies also showed a need for minimum biomass for the REIP to give accurate readings (Mistele and Schmidhalter 2008a). At V6 the coefficients of determination were higher and the estimations of biomass, and N uptake were quite good throughout the three seasons. In 2015 however, the coefficient of determination was lower compared to 2014 and 2016 (0.60 versus 0.87, and 0.80 for REIP and N uptake in 2015, 2014 and 2016, respectively). Due to complex nutrient interactions when fertilizing with liquid manure, in the 2015 season the plants in some treatments showed P deficiency symptoms, low P concentrations and reduced biomass (Federolf *et al.* 2017). In contrast to N deficiency, P deficiency has no major effect on leaf chlorophyll content (Al-Abbas *et al.* 1974) and photosynthesis per unit leaf area, but leads to reduced leaf growth and LAI (Plénet *et al.* 2000). According to Osborne *et al.* (2002) early season P deficiency influences near infrared (NIR) reflectance, especially between V6 and V8 growth stages. In our trials, P deficiency symptoms decreased with ongoing development until V8 stage (Federolf *et al.* 2017). The coefficients of determination peaked between V6 and V10 in the Hollage experiment. Within these growth stages, also the growth differences between treatments were high, no matter whether N or P was limiting. Although under P deficiency, biomass and N uptake might be overestimated to a certain degree by reflectance measurements due to changing spectral properties.

At tasseling, the coefficients of determination were lower than at the previous stages. This might be due to difficulties concerning canopy architecture and to saturation effects in VI's (Rambo *et al.* 2010), as the field of view (FOV) of the tested sensor is only 25 cm in diameter and thus does not properly represent the field situation with 75 cm row distance. Drouet und Bonhomme (1999) investigated the leaf area density in row canopies of maize. In a stand like ours, leaf area density and leaf N per area was distributed very heterogeneously between intra- and inter-row spaces. They also found a positive correlation of irradiance interception of a certain leaf area and the respective laminal N content, indicating that N

translocation processes tend towards photosynthetically active leaf area (Drouet und Bonhomme 1999). Winterhalter *et al.* (2012) found a decrease in total N contents from the top to the bottom of the plants. Thus, at growth stages after stem elongation, as the field of view of any sensor above the canopy is not able to obtain data from lower leaves, the informative value of hyperspectral data is probably reduced. Furthermore at tasseling, plant height (>2.4 m) and tassels hamper the definition of the crop canopy and the sensors usually measure reflectance on leaves (Winterhalter *et al.* 2012).

Regarding the changes in REIP values and their respective coefficients of determination to biomass and N uptake from the Hollage experiment, the most appropriate timing of measurement seems from V6 until stem elongation. However, the sensor values might be influenced by other stress factors than N deficiency.

4.2 The experimental series

Due to small plots, the plant sampling had to be done in the outer rows of each plot whereas the sensor measurement was done in the center rows. Although root proliferation of the inter row space, and thus also in the "inter-plot" space, in maize stands of 75 cm row width is unlikely prior to V8 growth stage (Schröder *et al.* 1997), edge effects between treatments cannot be totally excluded. This might have influenced the coefficients of determination, which were mainly lower than in the Hollage experiment. Furthermore, there was only one sampling per site per year, which hinders the evaluation of crop development differences in this series of trials.

Especially in 2015, due to low temperatures at all sites, limited growth was observed. At the Bovenau site, frost damage was still visible on the plants during V8 sampling, leading to heterogeneous conditions throughout the trial. Additionally, as was also seen in the 2015 data from the Hollage experiment after a cold period, nutrient deficiency symptoms other than N may occur, hampering the precision of the sensing data. The most severe P deficiency symptoms occurred at Wehnen 2015, the least at Sandkrug 2015, though at these trials the highest coefficients of determination were found. Thus, as also stated by Osborne *et al.* (2002), for later growth stages, the influence of P on spectral measurements seems to decrease.

4.3 Repeatability of measurements

Regarding all combinations of measured REIP and biomass or N uptake of our experiments ($n = 448$), a simple regression cannot easily be drawn (see Figure 4), although there is a dependency between the factors. The interference of soil reflectance is a weakness of vegetation indices in early growth stages (Hatfield et al. 2008; Rambo et al. 2010). As our trials were performed on a range of soils (from sandy Podzols to silty Luvisols, see Table 1), in different regions, which themselves influence crop growth and canopy architecture, due to different photoperiods (Liu et al. 2013; Bonhomme et al. 1991). Growth differences were observed between the sites (Federolf et al. 2016) and also found in the sensor values. Furthermore, we also used different varieties with different traits like earliness, canopy architecture, or nutrient uptake dynamics, which might also lead to differences in the sensor values (Montes et al. 2011). Sellers (1985) reported a strong influence of leaf inclination on reflection of solar radiation when maize LAI was low, especially at higher solar angles. This influence however, might be lower when active sensors are used. Moreover, there is a general consent that other stress factors, such as herbicides, diseases or drought also reduce chlorophyll contents in plant leaves and hence reflection properties, leading to a “blue shift” of the red edge (Carter und Knapp 2001).

In the present study, when looking at REIP values of e.g. $\sim 721 (\pm 0.2)$, the respective N uptake varied from $\sim 8 \text{ kg ha}^{-1}$ to $\sim 38 \text{ kg ha}^{-1}$ and the biomass from 180 kg ha^{-1} to 1280 kg ha^{-1} . Thus, for any conversion of REIP values into biomass or N uptake (or for producing fertilizer application recommendation maps), reference samples for field specific calibration need to be taken (Hatfield et al. 2008, Olf et al. 2005). Compared to visual scoring however, the VI from a sensor is less prone to human bias, it can be standardized and is able to quantify and add value to visual impressions. As spectral sensors are relatively cheap and easy to use, such measurements might increase the explanatory power of field trials. Still, it must be kept in mind, that N deficiency is not the only influencing factor on sensor derived VI, thus these values always need to be regarded with respect to current and previous conditions.

The field of view of the sensor used in this study however is only 25 cm in diameter, which is insufficient for typical maize stands with 75 cm row spacing. When used as a handheld sensor, this is not an issue as one can easily center walk the sensor head on the row. One upside of this is the reduction of background noise, due to limited

Figure 4: Correlation between REIP and N uptake (N_{upt}) and the respective exponential regression (dotted line) from the Hollage experiment and the experimental series

inter-row reflectance interference at early growth stages. Increasing the field of view of the sensor by increasing the sensing diameter to at least covering one row width would reduce the necessity of accurate row detection. More robust oblique view systems (Schmidhalter et al. 2008) however, need larger plot sizes.

5 Conclusion

In a range of experiments, the used sensor could gather useful data describing biomass and nutritional status of maize. Although the coefficients of determination and a shift of the obtained red edge inflection point (REIP) due to different background noise or canopy architecture do not allow a direct calculation of crop parameters from the REIP values, the used handheld sensor is a viable tool to quantify growth differences within one field trial. Regarding the objectivity and easiness of spectral handheld sensors, they can be a powerful tool in field trials.

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Supplementary Table 6: Raw data means of four replications for aboveground dry matter biomass (DM), whole plant nitrogen concentrations (N_{conc}), plant nitrogen uptake (N_{upt}) and red edge inflection point (REIP) for the treatments in the Hollage experiment (control (C-), broadcast (B+), injection (I-), and injection with nitrification inhibitor ((N)-))

growth stage ¹	treatment ²	2014					2015					2016				
		DM	N_{conc}	N_{upt}	REIP	DM	N_{conc}	N_{upt}	REIP	DM	N_{conc}	N_{upt}	REIP	DM	N_{conc}	N_{upt}
		kg ha ⁻¹	g kg ⁻¹	kg ha ⁻¹	nm	kg ha ⁻¹	g kg ⁻¹	kg ha ⁻¹	nm	kg ha ⁻¹	g kg ⁻¹	kg ha ⁻¹	nm	kg ha ⁻¹	g kg ⁻¹	kg ha ⁻¹
V4	C-	no data														
	B+	33.5				1.74	41.21	0.92	712.3					13.4	42.7	0.57
	I-	50.60				1.14	41.21	29.1	52.9					717.2		
	I(N)-	23.3				1.50	41.21	16.8	48.3					716.0		
		47.68						21.2	52.5					716.1		
		29.0														
		50.77														
V6	C-	169	28.7	4.8	718.2											
	B+	466	28.0	13.1	719.3									71.2	39.9	2.84
	I-	523	35.6	18.6	721.0									172.6	44.6	7.69
	I(N)-	569	38.1	21.7	721.0									97.5	43.2	4.22
														138.6	46.2	6.42
V8	C-	no data														
	B+	457.3				19.47	43.05	9.41	716.5					328	33.0	10.84
	I-	41.67				14.55	43.05	1011	37.7					722.6		
	I(N)-	288.1				23.33	43.05	675	40.5					722.0		
		49.48						839	40.5					722.2		
		455.3														
		50.17														
V10	C-	1044	18.1	18.9	721.0											
	B+	1860	18.4	34.1	720.8									1217	21.4	32.43
	I-	2803	20.3	57.0	723.8									2812	25.5	89.21
	I(N)-	3055	22.7	69.5	724.1									2269	24.5	69.29
														2435	25.9	78.64
VT	C-	5880	11.1	65.1	721.6									no data		
	B+	8706	12.1	104.8	724.2											
	I-	12294	11.6	143.2	724.5											
	I(N)-	12835	12.0	155.0	724.7											

¹Vn = vegetative leaf stage n, VT = tasseling²treatments fertilized with liquid manure (C = Control without manure; B = Manure broadcast; I = Manure injection; I(N) = Manure injection with nitrification inhibitor) and without (-), or with (+) mineral starter fertilizer, as described in section 2.2

Supplementary Table 7: Raw data means of four replications for aboveground dry matter biomass (DM), whole plant nitrogen concentrations (N_{conc}), plant nitrogen uptake (N_{upt}) and red edge inflection point (REIP) for the treatments an V8 growth stage in the experimental series

	2014				2015			
treatment ¹	DM	N _{conc}	N _{upt}	REIP	DM	N _{conc}	N _{upt}	REIP
	kg ha ⁻¹	g kg ⁻¹	kg ha ⁻¹	nm	kg ha ⁻¹	g kg ⁻¹	kg ha ⁻¹	nm
Bovenau								
C-	752	42.88	32.32	720.9	275	36.44	10.10	719.3
C+	1015	41.83	42.22	721.6	331	37.20	12.40	720.6
B-	761	43.48	33.02	720.5	289	38.64	11.18	719.9
B+	999	41.87	41.84	721.9	391	38.87	15.21	721.3
I-	758	45.05	34.17	720.9	270	39.86	10.84	720.3
I+	964	43.17	41.67	721.7	320	38.81	12.66	720.2
I(N)-	943	45.47	42.87	721.6	255	38.65	9.95	720.2
I(N)+	1177	44.58	52.49	722.6	302	39.97	12.10	721.4
Haus Düsse								
C-	278	38.36	10.67	717.7	no data			
C+	442	39.43	17.44	720.1				
B-	352	38.46	13.63	718.3				
B+	542	39.19	21.29	720.2				
I-	602	43.29	26.06	720.2				
I+	745	42.74	31.84	721.0				
I(N)-	694	42.58	29.56	720.7				
I(N)+	695	42.98	29.85	722.0				
Merfeld								
C-	658	37.15	24.42	721.6	351	46.30	16.25	722.3
C+	973	36.39	35.40	722.6	669	44.04	29.38	723.2
B-	946	37.05	35.06	722.2	371	44.64	16.57	721.3
B+	1134	36.99	41.89	723.0	705	42.69	30.01	723.4
I-	1063	38.77	41.24	723.3	542	45.01	24.39	722.1
I+	1197	38.25	45.76	723.4	652	44.77	29.16	723.8
I(N)-	1087	39.34	42.63	723.3	582	47.66	27.75	722.8
I(N)+	1300	40.02	52.07	723.2	640	45.52	29.37	723.3
Sandkrug								
C-	387	42.17	16.32	720.5	388	41.06	15.91	718.0
C+	443	42.74	18.91	721.0	745	40.97	30.50	721.1
B-	486	45.18	21.93	721.3	461	43.47	20.09	718.4
B+	581	45.04	26.18	721.9	841	41.89	35.22	721.0
I-	574	46.53	26.73	721.5	511	49.65	25.34	719.7
I+	641	46.55	29.84	722.2	680	50.36	34.29	721.4
I(N)-	551	45.47	25.04	721.5	556	48.05	26.67	720.3
I(N)+	632	45.88	29.01	722.2	736	49.38	36.40	721.4
Schuby								
C-	567	37.15	21.15	721.2	186	41.36	7.72	720.0
C+	776	37.70	29.31	722.0	413	41.66	17.22	721.1
B-	772	40.31	31.14	721.7	259	44.10	11.47	721.2
B+	864	40.07	34.59	721.8	382	43.20	16.51	721.5
I-	773	42.33	32.70	722.0	240	44.72	10.72	720.6
I+	825	41.99	34.64	722.1	389	43.85	17.09	721.6
I(N)-	697	44.48	31.01	721.9	294	44.24	13.16	720.8
I(N)+	959	43.69	41.82	722.6	436	43.67	19.01	721.3
Wehnen								
C-	317	39.06	12.50	718.0	201	37.17	7.54	721.0
C+	542	40.58	22.04	721.5	1121	32.89	36.88	723.3
B-	289	37.98	10.98	718.1	264	36.86	9.74	720.9
B+	607	40.00	24.38	721.2	1240	32.35	40.26	723.1
I-	382	41.36	15.83	719.4	612	39.80	24.33	722.7
I+	699	41.37	28.97	722.0	1114	38.28	42.64	724.4
I(N)-	502	41.05	20.78	720.9	1145	37.58	43.06	725.1
I(N)+	682	41.50	28.22	722.5	1475	37.95	55.81	725.4

¹ treatments fertilized with liquid manure (C = Control without manure; B = Manure broadcast; I = Manure injection; I(N) = Manure injection with nitrification inhibitor) and without (-), or with (+) mineral starter fertilizer, as described in section 2.