Technische Universität München
Lehrstuhl für Pflanzenernährung

Tractor based spectral reflectance measurements using an oligo view optic to detect biomass, nitrogen content and nitrogen uptake of wheat and maize and the nitrogen nutrition index of wheat

Bodo Mistele

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Agrarwissenschaften (Dr.agr.)

genehmigten Dissertation.

Vorsitzender: Univ.-Prof. Dr.agr., Dr.agr.habil. Hermann Auernhammer
Prüfer der Dissertation: 1. Univ.-Prof. Dr.sc.techn.(ETH Zürich) Urs Schmidhalter
2. Univ.-Prof. Dr.sc.techn.(ETH Zürich) Johannes Schnyder

Die Dissertation wurde am 21.12.2005 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München am 06.04.2006 angenommen.

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CRI: Canopy reflectance intensity
DM: Dry matter
EC_a: Apparent electrical conductivity
GPS: Global positioning system
LAI: Leaf area index
LED: Light emitting diode
N%: Nitrogen content
N_{act}: Actual nitrogen content
N_c: Critical nitrogen content
NDVI: Normalized difference vegetation index
NIR/G: Reflectance ratio between near infrared and green light
NIR/NIR: Reflectance ratio between near infrared and near infrared light
NIR/R: Reflectance ratio between near infrared and far red light
NIR/RR: Reflectance ratio between near infrared and red light
NIR: Near infrared
NNI: Nitrogen nutrition index
NRMSE: Normalized root mean square error
NSI: Nitrogen sufficiency index
R: Reflectance
REIP: Red edge inflection point
RMSE: Root mean square error
W: Dry biomass

1. General introduction

High yield, economical success and low environmental impact are targets of crop production. The nitrogen fertilizer application strategy influences yield, plant health, sustainability and economical success. Precision farming techniques make it possible to adjust the fertilizing rate to the site specific need of the crop within heterogeneous fields. Measuring soil nitrate is not an adequate method to depict the local differences because the range of the spatial pattern is short and the spatial pattern of soil nitrate may not be constant across time (Cahn et al., 1994). Another concept is to use the plant vigour as indicator for its N status (Mistele et al., 2004). Interesting canopy parameters as indicators for crop nitrogen requirements are nitrogen concentration, nitrogen uptake and above ground biomass. The spatial and temporal variations of these variables in the field must be determined in order to match the crop requirements as closely as possible (Schmidhalter et al., 2003).

Canopy reflectance data have been proofed to be a potential source to estimate several canopy variables related to physiological characteristics. Previous research has shown that spectral measurements can indirectly depict biomass (Hansen and Schjoerring, 2003; Serrano et al., 2000), N content (Cartelat et al., 2005; Link et al., 2005) and N uptake (Mistele et al., 2004; Vouillot et al., 1998) of plants. Spectrometers had been tested in crop plot experiments with the intention to acquire data related to the N status of plants. Most of the spectral measurements that had been conducted in the past used handheld sensors on the leaf level (Cartelat et al., 2005; Schepers et al., 1998) or on canopies grown under controlled conditions in small areas with small plots and under rather homogeneous conditions (Broge and Mortensen, 2002; Reusch, 1997; Sembiring et al., 1998).

Measurements in the nadir are the commonly used method for all canopy reflectance measurements but this optical geometry is highly sensitive to changes of the zenith angle since the canopy is no lambertian reflector. To gather constant data, it is important to measure around midday and always at the same zenith angle (Major et al., 2003).

In this work we measured with an oblique oligo view field spectrometer in compliance to the above mentioned restrictions. This oblique oligo view promised to measure nearly independent of the solar zenith angle because one optic always measured on the sunlight exposed side of the plants and another the shade side of the plants. Two other optics measured in two other directions. So the average signal is nearly constant at any solar zenith angle. Another effect of the oblique view is an increased field of view. It is possible to mount the sensor on the tractor for field scaled measurements (Reusch, 2003;

Schmidhalter et al., 2003). A further practical advantage of this geometrical setup is that the measured field does not lie in the shadow of the tractor and the spectrometer.

Unfortunately spectral measurements on the canopy level are not always constant between different site measurements. Different disturbances are known like the soil background (Daughtry et al., 2000; Huete et al., 1985) row direction (Pinter et al., 1987), cultivar (Sticksel et al., 2004), water stress (Curran et al., 2001) or annual influence (Osborne et al., 2002). These researchers demonstrated that spectral measurements on the canopy level are not always comparable.

The differences in spectral reflectance intensity of leaves with different N status are mainly related to differences in leaf pigments. Several studies have evaluated relationships between leaf chlorophyll concentration and leaf reflectance (Gitelson et al., 2003). Chlorophyll gives an indirect estimation of the nutrition status because much of the leaf nitrogen is integrated in chlorophyll (Lawlor, 2002). The relation between chlorophyll content and N content is contradictory discussed. Read (2002) found weak correlations with $R^2 = 0.32$ whereas others found very close correlation with $R^2 = 0.97$ (Cartelat et al., 2005). In addition, chlorophyll content is also closely related to plant stress like drought (Bahrun et al., 2003) or other nutrient deficiencies (Carter and Knapp, 2001). Some studies indicate that the strongest relationships with chlorophyll occur in the green spectrum near 550 nm or the far red spectrum near 700 nm (Carter and Spiering, 2002; Gitelson et al., 2003; Read et al., 2002). To estimate the dry weight of biomass per area reflectance measurements in the near infrared (NIR) waveband are useful. Reflectance in this area is mainly characterized by the amount of different optical densities between water saturated cell walls and intercellulars and in this way it is chiefly determined by biomass (Gates et al., 1965; Major et al., 2003).

The best relation between chlorophyll and reflectance measurements have been achieved on the leaf level (Cartelat et al., 2005; Gitelson et al., 2003). On the canopy level however spectral measurements correlate best with N uptake (Lukina et al., 2001; Schmidhalter et al., 2003).

Spectral canopy reflectance in our experiments was related to biomass, N content, and N uptake. Additionally we tested the relationship to the nitrogen nutrition index (NNI). This index is based on the critical N content. The critical N content indicates whether the N content of the canopy is high enough to develop maximum biomass (Greenwood et al., 1991; Lemaire et al., 2005). If a canopy with a certain amount of biomass has a lower N content than the critical N content, it needs fertilizer application. So the nitrogen nutrition index (NNI) was calculated as the ratio between the measured N content and the critical N content. If the NNI is higher then 1 the canopy is in an ample N state, if it is lower then one nitrogen lacks (Lemaire and Gastal, 1997).

Concerning crops many scientists described that there exists a relation between biomass production and nitrogen content in plants (Devienne-Barret et al., 2000; Farruggia et al., 2004; Gastal and Lemaire, 2002; Lemaire et al., 2005). The N content is very high at the beginning of crop growth. With increasing plant biomass the N content decreases until senescence. This decrease is mainly described as a dilution effect. Metabolic tissue as in leaf lamina is characterized by a constant high N content of 6.5 % whereas structural tissue as in veins is characterized by N content of 0.8 % (Lemaire and Gastal, 1997). Young leaves contain low structural tissue and when they grow up the relative portion of structural tissue increases. Therefore the N content in young leaves is high and when leaves grow up, their N content decreases.

The knowledge on the relationship between NNI and spectral canopy reflectance is rather limited. Link and Jasper (2003) used the optimum N rate for maximising the yield and related this to spectral measurements. This method works only in the backward direction and is therefore not suited to estimate an accurate N status of a canopy.

In another research the NNI was estimated and the leaf reflectance was measured with a NIR/red ratio. The relation between both was R^2=0.78. Additionally N uptake was calculated as foliar N content (leaf N content per m^2 x LAI) and related to spectral reflectance measurements with R^2=0.93, but these investigations were based on leaf level measurements (Vouillot et al., 1998).

Schepers et al. (1998) defined a nitrogen sufficiency index, or NSI. It is a canopy reflectance or transmittance based index. The optical canopy information is referenced with those from plants where N is not limited. Nitrogen fertilizer application is applied in the stress-based N treatment when reflectance of corn reached 96% of the reflectance of well-fertilized corn. This index excludes years and site effects but the system requires always reference plots.

The objective of the present study was (1) to validate reflectance measurements from a newly developed sensor under field conditions in large plots of about 800 m^2 and with calibration areas of about 25 m^2. We wanted to test and validate this new sensor device to improve the efficiency of N fertiliser management and (2) to determine how winter wheat spectral reflectance is related to the nitrogen nutrition index and whether this index is closer related to explain spectral measurements in comparison to N uptake.

2. Validation of tractor-based spectral reflectance measurements using an oligo view optic to detect the nitrogen status in winter wheat

2.1. Abstract

Fields with spatial differences in soil conditions require a variable, locally adjusted nitrogen fertiliser application during the growing season. Spectral measurements can be used to detect the canopy N status. The present study investigates the potential of a tractor-based field spectrometer with an oligo view optical setup to detect biomass, N content and N uptake in winter wheat in a three years´ field experiment. Ground truth validation were performed on large calibration areas of 25 m² varying in nitrogen supply. The results obtained show that strong correlations exist between reflectance indices and N uptake from the end of tillering to flowering ($R^2 = 0.90$). Thus, the tractor based passive sensor is a fast and suitable means to measure canopy N status. The optical setup allows for measurements being highly independent of day time, azimuth angle and cloudiness.

2.2. Introduction

It is well known that the N demand of a crop varies within single fields due to spatial differences in soil conditions (LaRuffa et al., 2001; McBratney and Pringle, 1999). Existing methods of soil and plant analysis are costly and time consuming in delivering information on the actual and spatially resolved N demand. However, previous research has shown that spectral measurements can indirectly describe biomass (Hansen and Schjoerring, 2003; Serrano et al., 2000), N content (Cartelat et al., 2005; Link et al., 2005) and N uptake (Mistele et al., 2004; Vouillot et al., 1998) of plants. Spectrometers had been tested in crop plot experiments with the vision to acquire data related to the N status of plants in order to improve the nitrogen fertilizer management. However most of the spectral measurements that had been conducted in the past used handheld sensors (Broge and Mortensen, 2002; Reusch, 1997; Sembiring et al., 1998) in small areas with small plots and under rather homogeneous conditions. Experiments were frequently performed on homogenous soil conditions with comparable water supply and rather homogenous soil nutrient status. Nitrogen application was then varied to create controlled differences in nitrogen supply. We conducted our experiments on heterogeneous fields within large plots of about 800 m² and with calibration areas of about 25 m².

Spectral measurements in the nadir are frequently influenced by the zenith angle. Mayor et al. (2003) reported that consistency in data requires reflectance to be measured only when the solar zenith angle provides sufficient irradiance, when sky conditions are uniform and bright and when the sensor view angle is close to nadir. To avoid the error, associated with the zenith angle different principles are available. Active sensor systems like the GreenSeeker (Ntech Industries, USA) (Lukina et al., 2001; Raun et al., 2002) and the laser sensors (Planto, Germany or Fritzmeier, Germany) (Bredemeier, 2005) use their own light source. We used a passive system with the sun as light source with an oligo view measuring optic which averages the optical signal from four directions. This oblique oligo view geometry provides advantages over the conventional nadir measurements which is based on the principle of vertical measuring. In our experiments one optical system continuously measures the sunlight exposed side of the plants and another the shade side of the plants. The resulting averaged signal is nearly constant even with changing zenith angle (Reusch, 2003). Another practical advantage of this geometrical setup is that the measured field is outside of the shadow of the tractor and the spectrometer. Finally, the oblique view increases the amount of biomass in the field of view (Mistele, 2004). Spectral reflectance of a canopy in the field is always a mixed signal of soil reflectance and plant reflectance (Guyot, 1990; Major et al., 2003). Thus, for our investigations we expected two advantages: 1. less interference from soil reflectance and 2. possible measurements during earlier development stages due to the improved signal stability, reduced signal/noise ratio and improved overall sensitivity of the method (improved limit of detection).

To our knowledge spectral measurements of the tractor based reflectance sensor having an oligo view optic were first time validated on the field scale. The investigated system is also commercially available as an operative instrument to control fertilizer application in real time with unknown index and algorithm. However spectral indices used to detect the nitrogen status with this system have not been described.

The objective of the present study was to validate reflectance measurements from a newly developed sensor with an oblique oligo view optic under field conditions. The aim of our study was to test and validate this new sensor device to improve the efficacy of N fertiliser management.

2.3. Material and methods

2.3.1. Experimental fields

The investigations were conducted at the research station of the Technical University of Munich in Bavaria in the south west of Germany in 2002, 2003 and 2004. Winter wheat (*Triticum aestivum* cv. Ludwig and in 2004 cropped with 50 % Tommi) was used as experimental plant. In this geographical area the yearly average precipitation is around 800 mm m^{-2} and the yearly average temperature is 7.5 °C. Yields typically vary between 6 and 10 Mg per hectare.

The experimental fields provided heterogeneous growth conditions as indicated in Table 2-1. The differences in the elevation within each site were between 10 m and 15 m and strong slopes were partly found on each field. Soil classification differs on and within the fields and the large range of the apparent electrical conductivity values indicate marked differences in soil texture in the upper 2 m of the soil.

Table 2-1: Description of field sites with size of experiment, elevation, row direction, apparent electrical conductivity (EC_a) and soil classification.
(Site specific soil classification according to Bayerisches Geologisches Landesamt, 1960; Bayerisches Geologisches Landesamt, 1962; Bayerisches Geologisches Landesamt, 1980; FAO et al., 1998).

Year	**2002**	**2003**	**2004**
Location	D 13	D 4	Haunerfeld
Size of experiment	2.0 ha	3.0 ha	3.6 ha
Elevation (m)	468 – 483	457 – 470	488 - 498
Direction	EES - WWN	EES - WWN	N - S
EC_a (mS m^{-1})	18 - 55	20 - 63	18 - 52
Soil classification	Cambisol: Silty clay loam Silty loam	Cambisol: Silty clay loam Silty loam Loam Sandy loam Skeletic Cambisol Cumulic Cambisol Cumulic Anthrosol	Cambisol: Silty loam Loam Clay loam

The experimental design included five N treatments with five (2002, 2003) and eleven (2004) replications respectively. Fertilizer application rates were 0, 90, 130, 170, 210 kg nitrogen per hectare, and additionally 250 kg ha^{-1} in 2003, respectively. Each plot was 15 m wide and 50 – 60 m long. The fertilizer was applied at four growth stages, in BBCH 22 (Meier, 1997) to enhance tillering, in BBCH 30 to increase the number of spikelets, in BBCH 40 to increase the number of glumes and in BBCH 50 for high protein content.

2.3.2. Spectral reflectance measurements

A tractor based radiometer similar to the Yara sensor (Yara GmbH & Co. KG, Dülmen, Germany) was used to measure the reflectance. It contained two units of a Zeiss MMS1 silicon diode array spectrometer with a spectral detection range from 400 to 1000 nm and a pixel distance of 3.3 nm. One unit was linked with a diffuser and measured the sun radiation as a reference signal. Simultaneously the other unit measured the canopy reflectance with an oligo view optic (Lammel et al., 2001). The spectrometer was connected with a four in one light fibre and the signal was optically averaged. The optical inputs were positioned with an azimuth angle of 80° between the front and rear side and 100° between the right and left side of the tractor. The zenith angle was set at 58°, +/- 6° to minimize the influence of the shadow of the tractor (Reusch, 2003; Schmidhalter et al., 2003). The signal intensity was not homogeneous in the measuring ellipse. It was rather twice as high at the spectrometer side as on the opposite end because light intensity decreases reciprocally with distance to its source. In addition, plant canopies do not represent a Lambertian reflector, implying an always remaining influence of the solar azimuth angle on light intensity. However, with the new oligo view optic it is possible to eliminate this bi directional influence nearly completely (Reusch, 2003). With the readings from the spectrometer units the canopy reflectance was calculated and corrected with a calibration factor, estimated with a $BaSO_4$ reflectance standard. To smooth the spectral readings a weighted average over 5 pixels was used in 2002 and 2003. In 2004 we calculated the fit of the fourth order over 5 pixels to get more accurate results.
In front of the tractor the sensor system was mounted 1.90 m above the canopy. The field of view consisted of four ellipsoids with 1.23 m in length, together around 4.5 m². With some exceptions (2002 and the last measurement each year) the measurements were conducted just before fertilizer application. The reflectance was measured at five wavelengths, which were at 550, 670, 700, 740 and 780 nm. The reflectance spectra can be divided into two parts. The first part is the visible range from 400 to 700 nm which is mainly influenced by plant pigments, first of all chlorophyll (Buschmann et al., 2000). The second part is the near infrared area from 700 through 1000 nm, which is mainly characterized by the amount of different optical densities between water saturated cell

walls and intercellular air spaces and chiefly corresponds with the biomass (Gates et al., 1965; Guyot, 1990). So we calculated some simple indices between the reflectance intensity at wavelengths in the visible area and in the NIR area.

In this study we calculated the normalized difference vegetation index (NDVI). The wavelength of 670 nm is used as the minimum reflectance reference because at this wavelength the chlorophyll has its maximum absorbance resulting in minimum reflectance.

$$NDVI = \frac{R_{780} - R_{670}}{R_{780} + R_{670}}$$

We calculated as well the reflectance intensity ratio between near infrared (NIR) and red (RR).

$$NIR / RR = \frac{R_{780}}{R_{670}}$$

The NIR to green reflectance intensity ratio was estimated, with the reflectance at 550 nm (green) being influenced by both, chlorophyll and carotenoid pigments.

$$NIR / G = \frac{R_{780}}{R_{550}}$$

The red edge of the reflectance intensity signature is an interesting area, because it contains both areas, the chlorophyll absorption, the cell wall reflectance and the alteration between those main effects. The reflectance at 700 nm (R) is beyond the chlorophyll maximum absorbance and the beginning of the red edge but the absorbance is still high.

$$NIR / R = \frac{R_{780}}{R_{700}}$$

With increasing N contents, not only the intensity of the reflectance in this areas changes, but also the inflection point. In the study we used a linear fitting for the red edge and a simplified formula to calculate the red edge inflection point (REIP) (Guyot et al., 1988).

$$REIP = 700 + 40 \frac{(R_{670} + R_{780}) / 2 - R_{700}}{R_{740} - R_{700}}$$

This formula of the REIP is mainly determined by the reflectance at the wavelength 780 nm and 740 nm because of the low arithmetic values of R_{670} and R_{700}. So a new index was calculated as the ratio between two closely related wavelengths in the NIR.

$$NIR/NIR = \frac{R_{780}}{R_{740}}$$

The spectral measurements had been conducted as described in Table 2-2.

Table 2-2: Spectral measurement conditions for the different samplings in the years 2002, 2003 and 2004. Growth stage (BBCH), average biomass, date and time, zenith angle, global radiation and weather conditions are indicated.

Year	**2002**			
Sampling	1st		2nd	
BBCH	32	32	51	55
Date	May 15	May 16	May 29	June 3
Time (hours, minutes)	11.40±8	9.45±15	12.35±5	13.10±10
Zenith angle (°)	55±1	37.5±2.5	62.5±0.5	62±0
Solar radiation (W m^{-2})	844	730	340	790
Weather conditions	sunny	sunny	sunny/ cloudy	sunny

Year	**2003**					
Sampling	1st		2nd		3rd	
BBCH	29	30	37	39	65	65
Date	May 4	May 5	May 16	May 19	June 4	June 5
Time (hours, minutes)	15.10±20	10.30±30	17.15±15	10.30±30	18.25±15	12.50±20
Zenith angle (°)	48.5±3.5	44±4	33±3	45.5±4.5	24.5±1.5	62.5±0.5
Solar radiation (W m^{-2})	680	750	830	100	170	840
Weather conditions	sunny/ cloudy	sunny	sunny	sunny, hazy	sunny	sunny

Year	**2004**							
Sampling	1st		2nd		3rd		4th	
BBCH	27	28	31	32	43	53	71	71
Date	Apr 26	Apr 27	May 5	May 12	May 29	June 6	July 5	July 6
Time (hours, minutes)	15.20±20	11.10±20	12.50±20	11.30±20	11.15±25	12.00±15	13.00±20	16.10±25
Zenith angle (°)	46±4	46±3	57±1	53.5±2.5	53.5±3.5	60±2	62±0	46.5±3.5
Solar radiation (W m^{-2})	580	800	100	710	320	880	410	560
Weather conditions	sunny/ cloudy	sunny/ cloudy	cloudy	sunny, hazy	cloudy	sunny	sunny/ cloudy	sunny

The canopy was measured two times with the spectrometer before each destructive biomass sampling. The measuring time and the weather conditions were carefully chosen, to evaluate the influence of low zenith angle or cloudy weather. So one measurement for each biomass sampling has been conducted at optimal conditions and the other under less optimal conditions. In this way the sensor was tested, not only during midday at full sun but also at low zenith angle and cloudy sky representing realistic use under farmers conditions.

2.3.3. Biomass and nitrogen measurements

Shortly after the spectral measurements plants were destructively harvested with a green forage chopper having a weighing unit to determine the above ground biomass. Small plots on both sides of the tractor were harvested, 1.5 m in width and around 8 m in length, matching exactly the sensed area. Spectral measurements were averaged over this area. A representative sub sample was removed and dried after weighing to estimate the total dry matter. The dried samples were milled and analysed for total N content with an elementary analyser according to Dumas (Macro-N, Foss Heraeus, Hanau, Germany). The N uptake was calculated as biomass x N content.

For the statistical analysis we used SPSS 11. Simple regressions were mainly calculated. We used linear or curvilinear models and calculated the coefficient of determination for all plots in one field. In this way the different soil conditions, fertilizer application rates, slopes and water resources were not considered. We did not focus on the mean value analysis as we expected big differences from plot to plot, independent of the fertilizer rate due to heterogeneous soil and field conditions.

2.4. Results

2.4.1. Destructively measured parameter of the crop canopy

Mean, minimum and maximum values and standard deviations of destructively measured parameters biomass, N content and the calculated nitrogen uptake of plants, grown at different nitrogen supplies are shown in Table 2-3.

Highly significant differences between N content, biomass and N uptake with exception of biomass in the second sampling in 2002 and the second sampling in 2003 were found within the N treatments for all three destructively measured parameters.

At the first sampling in 2002 and 2003, N uptake (determined as biomass times N content) was stronger correlated to biomass (R^2= 0.92 and 0.91) as well as in 2004 for the first two samplings (0.97 and 0.90) than to N content (R^2=0.74, 0.58 in 2002 and 2003; 0.19 and 0.64 in the first two destructive samplings in 2004, respectively).

Table 2-3: Range of destructively measured parameters N content, biomass and N uptake in 2002, 2003 and 2004.

2002												
Growth stage	**N content (%)**				**Biomass (kg ha^{-1})**				**N uptake (kg ha^{-1})**			
(BBCH)	min.	max.	ave.	Stddv	min.	max.	ave.	Stddv	min.	max.	ave.	Stddv
32	1.95	3.31	2.59	0.38	924	4208	2970	850	18	131	79	30
55	1.33	2.83	1.95	0.35	3340	8739	6390	1346	45	247	128	44

2003												
Growth stage	**N content (%)**				**Biomass (kg ha^{-1})**				**N uptake (kg ha^{-1})**			
(BBCH)	min.	max.	ave.	Stddv	min.	max.	ave.	Stddv	min.	max.	ave.	Stddv
30	1.92	3.37	2.61	0.33	209	1551	1022	307	6	47	27	10
39	1.56	2.99	2.46	0.44	1311	3949	2674	641	23	108	67	22
65	1.13	2.44	1.87	0.38	3609	9229	6621	1420	41	194	128	45

2004												
Growth stage	**N content (%)**				**Biomass (kg ha^{-1})**				**N uptake (kg ha^{-1})**			
(BBCH)	min.	max.	ave.	Stddv	min.	max.	ave.	Stddv	min.	max.	ave.	Stddv
27	3.01	5.42	4.30	0.48	121	1691	582	320	4	78	25	15
32	2.17	4.70	3.31	0.56	398	3088	1791	625	9	127	61	28
39	1.24	3.05	2.26	0.41	2687	9171	5731	1554	43	270	133	52
71	0.96	2.02	1.46	0.27	4341	15959	11367	2659	46	305	173	63

In the early growth stage the biomass per square meter differs much more than the N content in the leaves because all leaves were sun exposed, had fewer stems and plants were sufficiently provided with N. For plants with ample N availability, N uptake largely depends on the growth rate and varies with canopy biomass (Gastal and Lemaire, 2002). For the second, third and fourth samplings the influence of biomass decreases (R^2=0,83 in 2002, R^2= 0,80 and 0,86 in 2003 and 0.86 and 0.88 in 2004) whereas the influence of N content increases (R^2=0,84 in 2002, 0,59 and 0,80 in 2003 and 0.67 and 0.86 in 2004). This shows that in later growth stages large differences still exist in the biomass per square meter but also important differences in the N content of plant samples. Similar responses were observed by others (Baret and Fourty, 1997). The relationship between N content and biomass was stable in 2002 with an R^2 of 0,50 and varied in 2003 with 0,32, 0,17 and 0,49, and in 2004 with 0,10, 0,37, 0,34 and 0,58 at the respective samplings. A positive correlation between biomass and N content was partly observed mainly at later growth stages. That means that sometimes strong crop stands with much biomass contain higher N content than thin crop stands.

2.4.2. Spectral measurements

Four spectral readings in 2002, six in 2003 and eight in 2004 were conducted. In the Figures 2-1 to 2-3 coefficients of determination (adj. R^2-values) of the relationships between reflectance indices and canopy parameters are indicated. Results from nearly all spectral measurements were highly significant (P<=0.001) for biomass, N content and N uptake.

Still significant results were obtained for the NDVI versus N content at both measurements for the first sampling in 2003 and the NIR/RR index for the measurements at May 29, 2002. Normalized root mean square errors (NRMSE) of the REIP are indicated in Table 2-4. Good correlations were obtained for N uptake, except early measurements in 2004 at BBCH 27 – 28.

Table 2-4: Normalized root mean square errors (NRMSE) for the REIP, calculated as root mean square errors normalized with the range and measured at different growth stages (BBCH) for three years and depicted for biomass, nitrogen content and nitrogen uptake.

Year	**2002**				**2003**						**2004**							
Sampling	1st		2nd		1st		2nd		3rd		1st		2nd		3rd		4th	
BBCH	32	32	51	55	29	30	37	39	65	65	27	28	31	32	43	53	71	71
Date	15.5.	16.5.	29.5.	3.6.	4.5.	5.5.	16.5.	19.5.	4.6.	5.6.	26.4.	27.4.	5.5.	12.5.	29.5.	7.6.	5.7.	6.7.
Biomass	0,11	0,09	0,13	0,15	0,09	0,09	0,18	0,26	0,14	0,12	0,15	0,14	0,06	0,08	0,19	0,27	0,18	0,18
N content	0,08	0,06	0,18	0,08	0,11	0,12	0,11	0,11	0,06	0,05	0,14	0,12	0,16	0,14	0,25	0,26	0,08	0,07
N uptake	0,05	0,04	0,09	0,06	0,03	0,03	0,03	0,04	0,05	0,01	0,11	0,10	0,03	0,02	0,09	0,11	0,07	0,06

A comparison of the indices with the NRMSE was not possible. The NRMSE values differ between the indices because of different values of the indices. To eliminate the error caused by the different values of the indices we normalized the NRMSE with the range of the indices. But in spite of the normalisation the values of the REIP are 20 times as high as the values of NIR/NIR, but the same pattern is observed except that the scale is different. Weather conditions and zenith angle up to 30° did not influence the quality of the results as indicated by high R^2-values in Figures 2-1 to 2-3. Whereas these factors caused a shift of the reflectance values, the influence on the R^2 was low. A noticeable decrease in the R^2-values was observed at zenith angles less than 24° and changes between direct sun light (blue sky) and indirect sunlight (clouds). These factors influence mainly spectral measurements of the N content and the NIR red indices.

All indices correlated best with N uptake except the NDVI. N uptake was best described by REIP and NIR/NIR with R^2-values being constantly around 0.90 across all growth stages later than BBCH 29 and across all years. The indices NIR/R and NIR/G were as well good predictors for N uptake but they indicated a stronger saturation effect, which is also pointed out by the larger differences between R^2-values in Figure 2-4 resulting from linear and quadratic regressions. Both NIR red based indices were not particularly suited to describe N uptake except at the very early stages of BBCH 27 - 29.

Especially the NIR/RR index revealed to be a useful index to detect N uptake before BBCH 30 indicating a more linear relationship with a higher R^2 than the NDVI. The NIR/RR index is a particularly suitable biomass index and in the very early growth stages N uptake is mainly determined by biomass.

Biomass was well described in BBCH 30 by all indices, but for later measurements and earlier growth stages the quality decreases. Best descriptor for the relationship between biomass and the NDVI or NIR/RR indices was a quadratic regression. The comparison with the linear model in Figure 2-4 demonstrates that the NDVI showed a flattened relationship at higher biomass. Especially in 2002, the upper 60% of the regression between biomass and NDVI were totally plain, whereas for the NIR/RR this relationship was curvilinear. The REIP and the NIR/NIR relationships were relatively constant at all readings but at a lower level. They show nearly linear relationships for all measurements except the last one in 2002 and the first one in 2004.

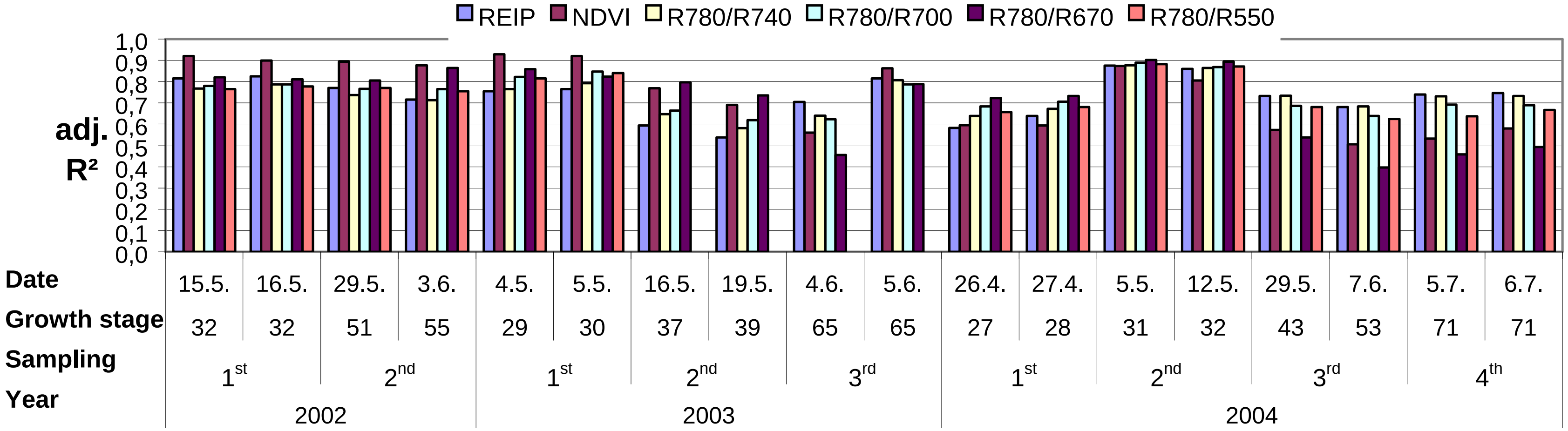

Figure 2-1: Coefficient of determination between biomass and reflectance indices for different indices and growth stages (BBCH) for the years 2002 to 2004.

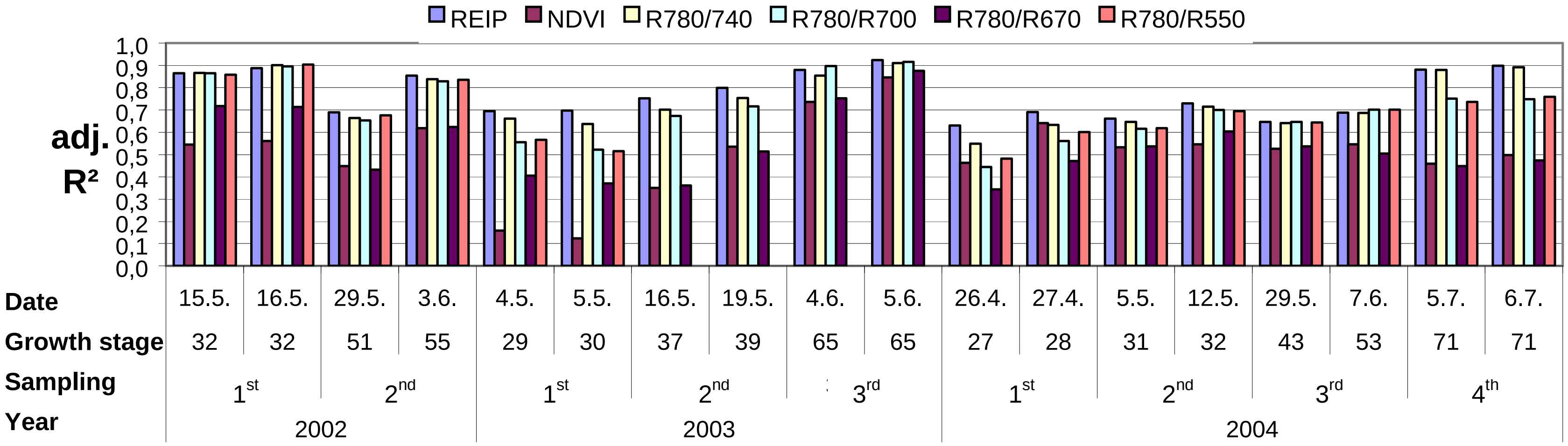

Figure 2-2: Coefficient of determination between N content and reflectance indices for different indices and growth stages (BBCH) for the years 2002 to 2004.

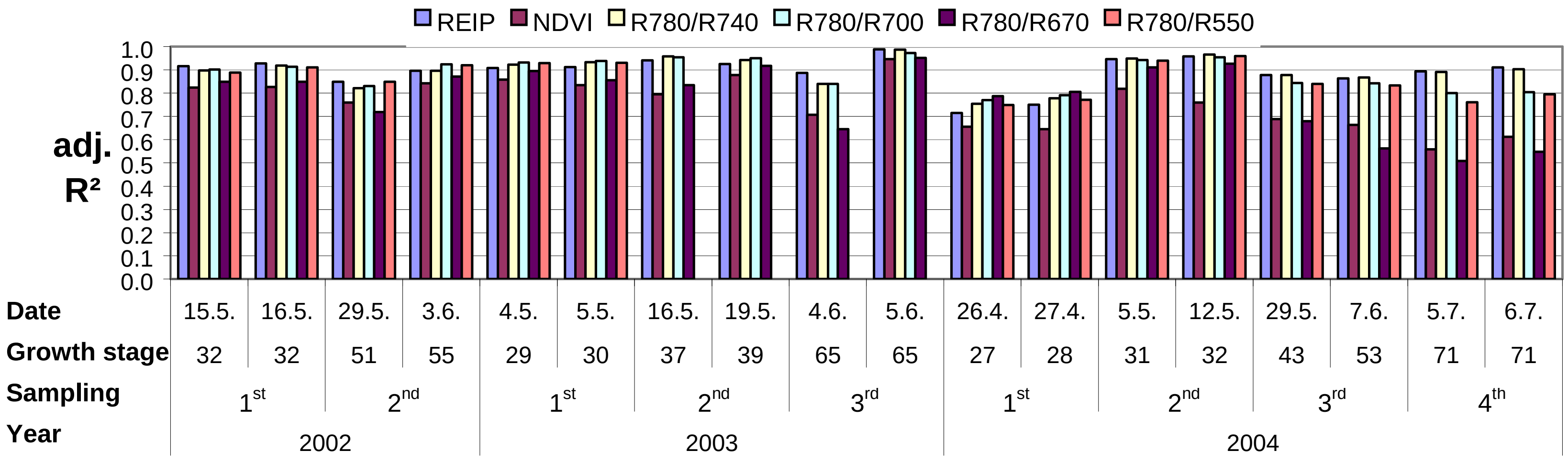

Figure 2-3: Coefficient of determination between N uptake and reflectance indices for different indices and growth stages (BBCH) for the years 2002 to 2004.

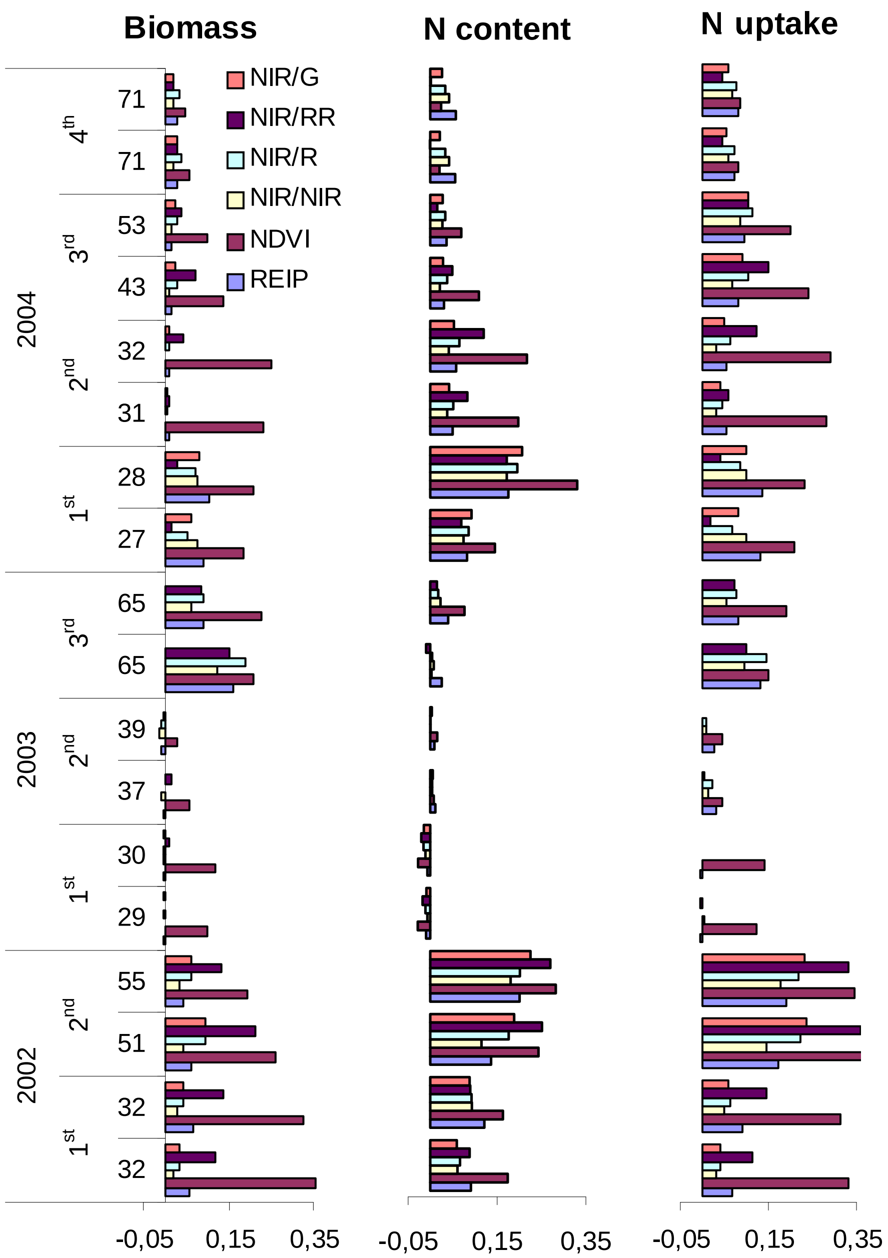

Figure 2-4: Differences between the coefficient of determination for linear and quadratic models depicting the relationship between N uptake, N content or biomass, and reflectance indices for different growth stages (BBCH) from three years.

The N content was best described by the indices REIP and NIR/NIR. The NIR red indices such as the NDVI and NIR/RR were not useful to predict the N content. The average R^2 was 30 % worse than for the other indices. Especially at the first sampling and also at the second sampling in 2003 the correlation was weak. One reason for this might be the low biomass. For all indices the R^2 increased with time in 2003 and 2004. In early stages the N uptake of canopies depends more on the biomass and in late stages more on the N content

(Gastal and Lemaire, 2002). Such a relationship could however not be derived from the 2002 data.

2.5. Discussion

The comparison of reflectance indices to canopy parameters demonstrates that it is possible to obtain good estimates of the N status in intact canopies with a tractor based spectrometer under field conditions. This scanning method is rapid, easy, non-destructive, tractor based and applicable to field scaled dimensions. The sensor showed no influence of different soil conditions. The N status was estimated in one single curve from canopies grown on sandy soil or clay soil.

The results demonstrate that early measurements at BBCH 30 give a reliable estimation of the N status. In comparison to the results from measurements in the nadir direction (Liebler et al., 2001) a positive effect of the oblique view for measurements at early growth stages could be observed.

Another positive effect of the oblique view was that the LAI (leaf area index) increases theoretically with a factor of 1.87. Some varieties may have more planar leaves so that the factor is not as high but there is still an increased amount of biomass in the field of view to be expected. In this way there are less interferences of soil reflectance with canopy reflectance. Soil and canopy reflectance is one single signal and it is not possible to differentiate between both (Major et al., 2003), but in spite of the increased amount of biomass in the field of view the signal intensity of the canopy reflectance is increased due to the improved signal stability, reduced signal/noise ratio and improved overall sensitivity of the method (improved limit of detection).

A consequence of increased biomass in the field of view is a stronger saturation effect compared to nadir measurements (Broge and Mortensen, 2002), especially in the red area, because of the high absorption. The differences between the reflectance values in this area are low in spite of the technical high contrast of the spectrometer because of the high sensitivity of the silicon array in this area. Also in nadir measurements saturation effects are known at LAI higher than 3, mainly by LAI but also by LAI*chlorophyll whereas the NDVI shows a perfect saturation and the NIR/R relationship is curvilinear (Aparicio et al., 2000; Serrano et al., 2000). This observation is in compliance with the results obtained in 2002 and 2004; in 2003 however the saturation effect was less pronounced. As an effect of this saturation the NIR/RR index is only useful to describe the N uptake up to BBCH 29. So far this behaviour had only been described by a model (Broge and Leblanc, 2001) whereas other scientists, measuring in the nadir, got best results up to senescence (Broge and Mortensen, 2002; Heege and Thiessen, 2002; Serrano et al., 2000). A reason for the saturation effect could be found in the competition for light among plants. It is reported to start at about LAI 2.5 and 1.6 t dry biomass per hectare (Justes et al., 1997).

For all non linear regressions the quadratic function was used. The expected situation for light reflectance in a high absorbance canopy is a saturation effect which is best described by a power function. But most of the regressions were best fit with quadratic functions which were also used by other researchers (Carter and Spiering, 2002; Read et al., 2002). This peculiarity could be caused by a particular feature which increases in a quadratic relationship.

The results of our field studies are in line with other studies obtained under well controlled experimental plot conditions with hand held spectrometers (Reusch, 1997). However the R^2 of the regressions for N uptake in our results were constantly high whereas the results of others were sometimes comparable and sometimes less good. This may be due to the measuring geometry, caused by the mixed signal obtained from the oligo view optic and enhanced by the large area used for the ground truth calibration.

Another effect related to the measuring geometry is the independency from weather conditions, because canopy and sun irradiance were measured simultaneously. Only at changes between direct sun light (blue sky) and indirect sunlight (clouds) a noticeable decrease in R^2 was observed.

The effect of the low zenith angle was eliminated by the four optics, so always one optic is facing the sun exposed side while another one is directed to the shady side of the plants. The results indicate that when using this measuring geometry one is no longer limited to measure in the 10 am – 2 pm window as commonly done with nadir measurements. A visible decrease in the R^2-values was observed at zenith angles less than 24°. Changing weather conditions and zenith angles up to 30° caused a shift in the reflectance values, but had low influence on the quality of the results as indicated by high R^2-values. The areal performance in fertilizer application is often 20 ha per hour and more, so the shift of the values can probably be neglected in an operative system, because fertilizing a field needs normally no longer than two hours and at the next field the system has to be calibrated again. Further investigations are necessary to describe this shift of the reflectance values caused by the zenith angle and weather conditions.

A further limitation of the system is the need of sufficient sun light. Very recently a system has been developed with halogen flash light and photodiodes with interference filters. This enables also measurements in the night and at dawn (Reusch, 2005).

For the detection of the N status of crops on the field level, also active systems available. These sensors have their own light source. One system, the GreenSeeker (Ntech Industries, USA) is equipped with a pulsed LED as light source and measures the canopy reflectance as a modulated signal. Probably because of the limited light energy from the LED the maximum distance between sensor and canopy is 0.8 m and the field of view is also only 0.8 m. The sensor is less sensitive to variable irradiance conditions than the sensor we used

and may be used also at dawn (Lukina et al., 2001; Raun et al., 2002). The sensor measures only the NDVI and therefore it is probably less suited to detect the N status in western European agriculture, because the NDVI is rather suited to detect the biomass than the N status, the quality of which was least as indicated in Figure 2-1. This observation was commonly made by others (Elwadie et al., 2005; Lukina et al., 2000).
Another active sensor concept is the laser induced chlorophyll fluorescence principle. This sensor uses a pulsed laser as light source and measures the chlorophyll fluorescence as a modulated signal. Two sensors are on testing phase, the laser sensor from Planto (Planto GmbH, Leipzig, Germany) (Bredemeier and Schmidhalter, 2003) and the MiniVeg N from Fritzmeier (Fritzmeier GmbH & Co. KG, Grosshelfendorf, Germany) (Schächtel et al., 2005). Both sensors are using point measurements, 5 mm in diameter. The Planto sensor uses a scanning mode that allows a better areal representation. This principle allows to detect independently chlorophyll density and biomass (Bredemeier and Schmidhalter, 2005). These sensors are rather weakly sensitive to variable irradiance conditions and they also perform at dawn and likely in the night (Bredemeier, 2005).

2.6. Conclusions

The investigated, tractor mounted spectrometer allows easy and accurate measurements of the N status and biomass in wheat crops. All tested indices showed good results. NDVI and NIR/RR were only useful for the detection of biomass and very early measurements of N uptake whereas REIP, NIR/NIR, NIR/G and NIR/R turned out to be useful indices to estimate the N status in the field. The measuring geometry showed several advantages in comparison to results obtained with a nadir optic. With this study we could show that the investigated type of field spectrometer is a useful tool to measure the N status of crop canopies and to deliver the information required for proper nitrogen management on farms. The system reliably detects nitrogen depleted and well supplied plants in heterogeneous fields.

Acknowledgment
The authors acknowledge and thank the German Federal Research Foundation for funding the research project FOR 473 TP10.

3. Validation of field-scaled spectral measurements of the nitrogen status in maize using an oligo view optic

3.1. Abstract

Heterogeneous crop stands require locally adapted nitrogen fertilizer application. Spectral measurements allow to measure crop nitrogen status. In the present study, we validated a tractor mounted field spectrometer with an oblique oligo view measuring optic measuring simultaneously sun radiation and canopy reflectance in four directions. Such measurements were found to be nearly independent of solar zenith angle and weather conditions. Biomass, nitrogen content and nitrogen uptake were determined in 10 m² calibration areas in 60 plots within a three-years´ maize field study, containing different N treatments and seeding densities. Strong correlations were obtained between reflectance indices and nitrogen uptake from the four leaf stage up to flowering. Weather conditions and zenith angle up to 30° hardly influenced the results. The tractor based oblique oligo view optic reflectance sensor represents a fast and suitable instrument to non-destructively sense crop nitrogen status and allows for site-specific fertilizer application when coupled with a fertilizer algorithm and variable rate applicator.

3.2. Introduction

Soil conditions e.g. water and nutrient supply, exposition and inclination may vary on heterogeneous field sites leading to local differences in plant growth (Auerswald et al., 1997). Differences in soil organic matter content, soil water availability and soil temperature influence nitrogen supply (Cahn et al., 1994; Cambardella et al., 1994). Warm and humid soil conditions enhance nitrogen supply, whereas dry periods decrease it. Under rainfed conditions both situations may occur (Kolberg et al., 1999). For economical and ecological reasons targeted spatially and temporally optimized nitrogen fertilizer application is required. Generally current fertilizing management sometimes uses soil analysis, but these practices disregard the locally variable nitrogen demand. Plant analysis is important to control the N status; nevertheless destructive methods are not appropriate.

Spectral measurements allow determining the crop nitrogen status. Several studies have evaluated relationships between leaf chlorophyll content and leaf reflectance (Cartelat et al., 2005; Gitelson et al., 2003). Chlorophyll gives an indirect estimation of the nutrition status because much of the leaf nitrogen is integrated in chlorophyll (Cartelat et al., 2005; Read et al., 2002). In addition, chlorophyll content is also closely related to plant stress like drought or other nutrient deficiencies. These studies indicate that the strongest relationships with chlorophyll occur in the green spectrum near 550 nm or the far red spectrum near 700 nm (Carter and Spiering, 2002; Gitelson et al., 2003; Read et al., 2002). To estimate the dry weight of biomass per area, reflectance measurements in the near infrared (NIR) waveband are useful. Reflectance in this area is mainly characterized by the amount of different optical densities between water saturated cell walls and intercellulars and in this way it is chiefly determined by biomass (Gates et al., 1965; Major et al., 2003).

Most of these spectral measurements have been conducted with handheld sensors, measuring in the nadir with small calibration areas used on homogeneous fields (Reusch, 1997). Regarding maize handheld sensors usually measure one row or less, representing small areas which represent a source of errors and creating a high variability. At least 2.5 rows in the field of view should be used in order to overcome the variability caused by interrow differences in the leaf area (Major et al., 2003). Particularly when the maize canopy height approaches 2 to 3 m, it is difficult to focus on a single row with a handheld sensor.

Measurements in the nadir are the commonly used method but this optical geometry is highly sensitive to changes in the zenith angle since the canopy is no Lambertian reflector. To gather constant data, it is important to measure around midday and always at the same zenith angle (Major et al., 2003). To overcome such limitations we measured with an oblique oligo view field spectrometer mounted on a tractor. From this oblique oligo view one can expect nearly independent measurements from the solar zenith angle because one optic always measures on the side of the plants exposed to the sun and another the side in the shadow (Mistele et al., 2004; Schmidhalter et al., 2003). Two other optics measured in two other directions. So the average signal may be nearly constant at any solar zenith angle. Another positive effect of the oblique view is an increased field of view (Reusch, 2003). Thus, for our investigations we expected less interference from soil reflectance (Daughtry et al., 2000; Huete et al., 1985) and possible measurements during earlier development stages due to the improved signal stability, reduced signal/noise ratio and improved overall sensitivity of the method (improved limit of detection).

This sensor was mounted on the tractor for field scaled measurements (Reusch, 2003; Schmidhalter et al., 2003). The aim of this investigation was to validate spectral measuring with an oblique oligo view optical setup in maize on the field scale with different soil and growth conditions.

3.3. Material and methods

3.3.1. Experimental fields

The investigation was conducted on the research station Dürnast belonging to the Chair of Plant Nutrition from the Technical University of Munich located near Freising. Maize (*Zea mays cv. Banguy*) was grown in three-years´ field experiments in the north of Munich. Different seeding densities and nitrogen treatments along with spatial variable soils leaded to a broad range in growth, development and N status of maize plants. These differences provided an ideal opportunity to validate relationships between canopy spectral reflectance measurements and maize crop parameters. A brief description of the experimental conditions is given in Table 3-1. The focus here is on the method and procedures used to measure maize performance and to obtain radiometric measurements.

Table 3-1: Experimental fields and field conditions in different years from 2002 to 2004. Location, field size, elevation, and field orientation, as apparent electrical conductivity obtained with EM38 (EC_a = apparent electrical conductivity). The relevant soil classification according to FAO nomenclature and soil texture are indicated
(Bayerisches Geologisches Landesamt 1960; Bayerisches Geologisches Landesamt 1962; Bayerisches Geologisches Landesamt 1980; FAO et al., 1998).

Year	**2002**	**2003**	**2004**
Location	D 1	D 4	Haunerfeld
Size of experiment	3.6 ha	3.8 ha	3.8 ha
Elevation (m)	462 - 474	458 - 475	486 - 498
Direction	EES - WWN	EES - WWN	N - S
EC_a (mS m^{-1})	n.m.	20 – 72	17 – 52
Soil classification	Cambisol: Silty clay loam Silty loam Loam	Cambisol: Silty clay loam Silty loam Loam Sandy loam Skeletic Cambisol Cumulic Cambisol Cumulic Anthrosol	Cambisol: Silty loam Loam Clay loam

For the three experimental years, different fields were chosen, each with heterogeneous soil conditions as indicated in Table 3-1. The differences in the elevation within each site were between 12 m and 18 m and in 2002 the experimental field had a gentle slope; in 2003 and 2004 fields had partly strong slopes. Different soil classifications and the large range in the apparent electrical conductivity indicate sizeable differences in soil texture in the upper 1-2 m of the soil profile. The average annual temperature in Dürnast is 7.5 C° with annual precipitation averaging 800 mm m^{-2} with maximum rainfall in summer.

In the field experiments five nitrogen treatments and three seeding densities were evaluated. The experimental design was a split-plot with seeding density as main plot and N treatments as subplots with four replications (total of 60 plots).

Each plot was 15 m in width and around 50 m in length, covering an area of 700 to 800 m². The three seeding densities were 6, 10 and 14 plants per m² to create differences in biomass and in N content; 5 N treatments with Alzon (46% N) (SKW, Wittenberg, Germany) were applied with 0, 70, 120, 170 and 220 kg per hectare nitrogen at plant emergence. Alzon is a long term urea-N fertilizer with ammonification inhibitor. Additionally at sowing 18 kg N per hectare was applied and placed below the seeds.

3.3.2. Spectral measurements

A multispectral tractor mounted radiometer (tec5, Oberursel, Germany) similar to the Yara N-Sensor (Yara GmbH & Co. KG, Dülmen, Germany) was used for the spectral measurements. This Sensor contains two spectrometer units measuring simultaneously reflectance and incident radiation. One unit is linked to a cosines corrected diffuser and measures the global radiation to compensate the error resulting from different light conditions. The other unit is linked to a four in one light fiber to create an optical mixed signal from four fields of view, measuring the canopy at the four edges of the sensor. The optical inputs were positioned with an azimuth angle of 80° between the front and rear side and 100° between the right and left side of the tractor. The zenith angle was set at 58° +/- 6° to minimize the influence of the shadow from the tractor (Reusch, 2003). The sensor was mounted in front of the tractor, two meters above the canopy. This enabled the sensor to measure four ellipses, each 1.25 m in length, together around 5 m². One effect of the oblique view is that the biomass in the field of view increases theoretically with a factor F of 1.9, calculated as $F = (\sin 32°)^{-1}$, compared to measurements in the nadir. Within spectral measurements, it is not possible to differentiate between soil reflectance and plant reflectance (Guyot, 1990). The oblique view increases the soil plant reflectance ratio and thus allows for earlier measurements with low soil coverage. A third effect of the oblique oligo view is a reduction of the effect caused by the zenith angle, because one optical system continuously measured the sunlight exposed side of the plants and another the shady side of the plants.

The sensor measures within a spectral detection range from 400 to 1000 nm and a pixel distance of 3.3 nm. To smooth the spectral readings, a weighted average of over 5 pixels of about 16.5 nm was used in 2002. In 2003 and 2004 a fit of the fourth order of over 5 pixels was calculated to get more accurate results. The reflectance was measured at five wavelengths at 550, 670, 700, 740 and 780 nm. These wavelengths contain information from the visible part (550, 670 and 700 nm) and from the near infrared (NIR) part (740 and 780 nm). The reflectance intensity at visible wavelengths is mainly influenced by plant pigments, first of all chlorophyll (Buschmann et al., 2000).

The reflectance intensity in the NIR is essentially characterized by the amount of different optical densities between water saturated cell walls and intercellular air spaces and corresponds mostly with the biomass (Gates et al., 1965; Guyot, 1990). Selected indices between the reflectance intensity at wavelengths in the visible area and the NIR area were calculated and are described below.

Normalized difference vegetation index (NDVI).

$$NDVI = \frac{R_{780} - R_{670}}{R_{780} + R_{670}}$$

Reflectance intensity ratio between NIR and red (RR).

$$NIR / RR = \frac{R_{780}}{R_{670}}$$

The NIR to green reflectance intensity ratio was estimated with the reflectance at 550 nm (green) being influenced by both, chlorophyll and carotenoid pigments.

$$NIR / G = \frac{R_{780}}{R_{550}}$$

The red edge of the reflection intensity signature is an interesting area, because it contains both areas, the chlorophyll absorption and the cell wall reflection, as well as the alteration between these main effects. The reflectance at 700 nm (R) is beyond the chlorophyll maximum absorbance and the beginning of the red edge; nevertheless, the absorbance is still high.

$$NIR / R = \frac{R_{780}}{R_{700}}$$

With increasing N contents, not only the intensity of the reflection in this areas changes, but also the inflection point. In the study, we used a linear fitting for the red edge and a simplified formula to calculate the red edge inflection point (REIP) (Guyot et al., 1988).

$$REIP = 700 + 40\frac{(R_{670} + R_{780})/2 - R_{700}}{R_{740} - R_{700}}$$

This formula of the REIP is mainly determined by the reflectance at a wavelength of 780 nm and 740 nm because of the low arithmetic values of R_{670} and R_{700}. An index was calculated as a ratio between two closely related wavelengths in the NIR.

$$NIR / NIR = \frac{R_{780}}{R_{740}}$$

Spectral measurements were conducted two times just before the biomass was sampled. Conditions prevailing throughout the measurements are described in Table 3-2. To validate the benefit of the new optical setup the measuring time and the weather conditions were carefully chosen, to evaluate the influence of low zenith angle or cloudy or rainy weather conditions. Opposite to the measurements in the nadir with a single spectrometer, the sensor does not need full sunshine during midday, it can also operate at a low zenith angle and cloudy sky. Consequently, we tested the sensor under field conditions that a farmer may experience.

3.3.3. Biomass and nitrogen measurements

Shortly after the spectral measurements plants were destructively harvested with a green forage chopper having a weighing unit to determine the above ground biomass (Mistele et al., 2004). Small plots of 1.5 m in width and 2 to 6 m in length on both sides of the tractor were harvested, matching exactly the sensed area. Spectral measurements were averaged in this area. A representative sub sample was removed and dried after weighing to estimate the total dry matter. The dried samples were milled and analyzed for total N content with an elementary analyzer according to Dumas (Macro-N, Foss Heraeus, Hanau, Germany). The N uptake was calculated as biomass x N content.
For the statistical analysis, we used SPSS 11 (SPSS Inc., Chicago, USA). Simple regressions were mainly calculated. We used linear or curvilinear models and calculated coefficients of determination averaged over all plots in one field. In this way, differences in soil conditions, fertilizer application rates, slopes and water supply were not considered.

Mean values were not included in the analysis, as we expected big differences from plot to plot due to heterogeneous soil and field conditions being independent of the fertilizer rate.

Table 3-2: Spectral measurement conditions for the different biomass samplings in the years from 2002, 2003 and 2004. Growth stage, average biomass, date and time, zenith angle, global radiation and weather conditions are indicated.

Year	**2002**					
Biomass sampling	1st		2nd		3rd	
Growth stage (BBCH)	14		32		67	
Biomass (kg ha^{-1})	516		2956		6051	
Date	June 18	June 20	June 26	July 1	July 17	July 22
Time (hours, minutes)	12:00	8:50	13:00	15:20	13:40	8:45
Zenith angle (°)	61	32 ± 2	63 ± 1	54 ± 2	61 ± 1	29 ± 3
Global radiation (W m^{-2})	921	619	870	565	475	597
Weather conditions	sunny	sunny	sunny	hazy	cloudy	sunny

Year	**2003**					
Biomass sampling	1st		2nd		3rd	
Growth stage (BBCH)	15		34		69	
Biomass (kg ha^{-1})	522		3494		8656	
Date	June 10	June 11	June 25	June 26	July 8	July 11
Time (hours, minutes)	16:40	10:20	12:10	10:55	16:25	16:30
Zenith angle (°)	42 ± 5	48 ±3	60 ± 2	51 ± 3	44 ± 4	43 ± 5
Global radiation (W m^{-2})	310	627	528	748	524	490
Weather conditions	cloudy	sunny	cloudy	sunny hazy	sunny/ cloudy	cloudy

Year	**2004**					
Biomass sampling	1st		2nd		3rd	
Growth stage (BBCH)	13		15		36	
Biomass (kg ha^{-1})	317		1232		4292	
Date	June 23	June 23	July 5	July 6	July 19	July 20
Time (hours, minutes)	12:25	17:00	10:55	13:15	15:30	12:40
Zenith angle (°)	62 ± 1	40 ± 3	52 ± 4	63 ± 1	50 ± 4	61 ± 1
Global radiation (W m^{-2})	327	111	289	647	555	804
Weather conditions	hazy	rainy	sunny/ cloudy	hazy	cloudy	sunny

3.4. Results

3.4.1. Biomass, nitrogen content and nitrogen uptake

For the calibration of spectral measurements, plants sampled were analyzed for dry weight, N content and nitrogen uptake. Mean, minimum and maximum values and standard deviations of destructively measured parameters of plants grown, at different nitrogen supplies, are shown in Table 3-3.

Table 3-3: Range, minimum and maximum values and standard deviation of the destructively sampled plant parameters biomass and nitrogen content as well as for the calculated N uptake, indicated for three years and three sampling times each.

Year	Biomass sampling	Biomass					N content					N uptake				
		Range	Min.	Max.	Avg.	StdDev	Range	Min.	Max.	Avg.	StdDev	Range	Min.	Max.	Avg.	StdDev
2002	1st	703	195	898	516	169	1.54	2.11	3.65	2.99	0.42	23	7	29	15.6	5.9
	2nd	4293	1414	5706	2956	841	1.37	1.65	3.02	2.35	0.36	138	25	163	70	27
	3rd	6141	2275	8416	6051	1294	1.2	0.93	2.13	1.46	0.28	116	23	139	90	28
2003	1st	701	198	898	512	177	1.04	2.75	3.79	3.39	0.18	23	7	30	17	5.7
	2nd	2746	2138	4884	3494	686	1.17	1.58	2.75	2.25	0.22	69	47	116	78	16
	3rd	4973	5855	10828	8656	1142	0.84	1.09	1.93	1.5	0.17	99	75	173	129	19
2004	1st	680	65	745	317	148	1.34	2.85	4.19	3.78	0.24	28	2	30	12	5.8
	2nd	2009	317	2327	1232	446	0.97	2.02	2.99	2.47	0.2	50	7	57	30	10
	3rd	4321	1818	6139	4292	1083	0.96	1.64	2.6	2.22	0.25	100	42	142	94	20

In 2002 for the second and the third biomass sampling, the range and standard deviation for all parameters was higher than in the two preceding years. At the first sampling, the range of the N content was also higher. These results correspond with the visual inspection of the plants, as partly weakly developed, light green crop stands and partly strongly developed, dark green crop stands were found in 2002.
This is also indirectly visible from results given in Table 3-4. The correlation between N uptake and N content was higher in 2002 than in 2003 and 2004. In 2004, there was no correlation between N content and N uptake but a strong correlation between N uptake and biomass. This demonstrates that the three years were different in their canopy development.

The N response in Table 3-4 gives further information about the differences between the three years. 2002 shows the expected N response. With increasing plant development, the differences between N treatments in biomass, N content and N uptake increased. On the contrary, the dry biomass shows no N response in 2003 and 2004. But in 2003, the N content shows a N response, which was probably affected by drought.

Table 3-4: Correlation between seeding density, N application, N uptake and destructively harvested plant parameters: dry biomass (DM), N content (N %) as well as the calculated N uptake.

Year	Sampling	correlation coefficients between							
		Seeding density and			N application and			N uptake and	
		DM	N%	N uptake	DM	N%	N uptake	DM	N%
2002	1st	0.47**	-0.32*	0.31*	0.26	0.74**	0.48**	0.95**	0.50**
	2nd	0.44**	-0.45**	0.20**	0.44**	0.61**	0.60**	0.91**	0.52**
	3rd	0.34*	-0.30*	0.06	0.60**	0.78**	0.82**	0.84**	0.79**
2003	1st	0.67**	-0.28*	0.65**	0.02	0.67**	0.14	0.98**	-0.08
	2nd	0.80**	-0.28*	0.66**	0.04	0.66**	0.37**	0.86**	0.25
	3rd	0.68**	-0.20	0.45**	0.12	0.56**	0.54**	0.69**	0.51**
2004	1st	0.46**	-0.10	0.43**	0.03	0.36**	0.08	0.99**	0.36**
	2nd	0.41**	-0.18	0.38**	0.17	0.26*	0.25	0.98**	-0.09
	3rd	0.58**	-0.37*	0.53**	0.19	0.25	0.39**	0.87**	-0.14

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.

3.4.2. Spectral measurements

Spectral measurements also showed clear differences between the years as indicated in Figure 3-1. Spectral measurements describe either biomass or N uptake best. In 2003 and 2004, spectral measurements described biomass best, except the measurements for the third sampling in 2003 and N uptake was also best described at later sampling times in 2002. The reason for this probably could be seen in the relation between the crop stand parameters N uptake and N content as indicated in Table 3-4. The N uptake correlates with

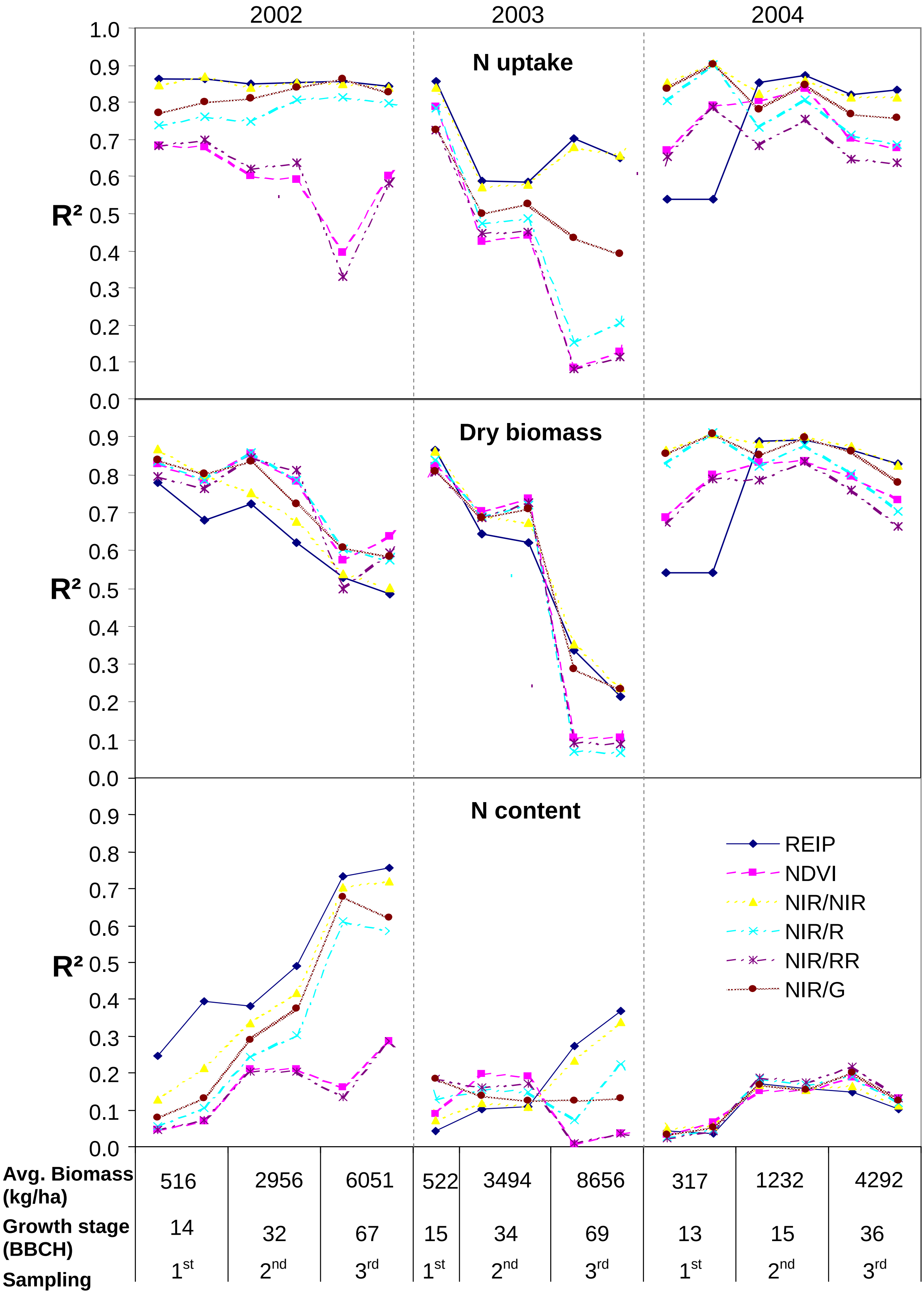

Figure 3-1: Coefficients of determination for the relationship between reflectance indices and canopy parameters biomass, N uptake and N content for three experimental years.

the N content only in 2002 and in the third sampling in 2003. In all the other samplings, N uptake correlates only with biomass.

Biomass is best described at early samplings across all years and indices. Regarding the detection of dry biomass in 2002 and 2003, all indices show best results at the earlier measurements and a decrease in their predictability with increasing biomass. Only at the earliest measurement in 2004, the amount of biomass was obviously too small and the predictability increased. Similarly the biomass was described by all indices.

N uptake was well described by the REIP and NIR/NIR with constant high R^2 in 2002. In 2003, the first measurement was on the same high level but measurements for the second and third sampling were much worse, whereas these two indices were again on a high level in 2004, except for measurements at the earliest sampling. Obviously the REIP was not useful for crop stands with only 300 kg ha^{-1} dry biomass. For all other measurements both indices detected N uptake, with the same quality. The NIR/G and NIR/R are also useful indices to describe N uptake except for the measurements of the last sampling in 2003. At this growth stage, the soil was very dry and NIR/G was a little bit better than NIR/R, but not as good as NIR/NIR and REIP. NIR/RR and NDVI were worst among the indices to describe N uptake. They were not useful to estimate N uptake with spectral measurements, especially after flowering as found for the last measurements in 2003 and 2004.
N content was hardly related to spectral measurements in 2003 and 2004. Only in the last measurements in 2003, the indices REIP and NIR/NIR correlated weakly with N content. But in 2002, spectral measurements described N content with increasing goodness as biomass increased. Best results could be achieved with the indices REIP and NIR/NIR. The indices NIR/R and NIR/G presented equally good results, whereas NIR/RR and NDVI were not useful to describe the N content.
Weather conditions and zenith angle up to 30° did not influence the quality of the results as indicated by high R^2-values in Figure 3-1. Even measurements under rainy conditions did not reduce the R^2. Whereas these factors caused a shift in the reflectance values, the influence on the R^2 was low. A noticeable decrease in the R^2-values was observed at changes between direct sun light (blue sky) and indirect sunlight (clouds).

3.5. Discussion

The comparison of reflectance indices to crop parameters demonstrates that it was possible to obtain good estimates of the N status with a tractor based spectrometer under field conditions. This scanning method is rapid, easy, non-destructive, tractor based and applicable to field-scaled dimensions. Spectral detection was not influenced by different soil conditions. The relationship between N uptake and canopy reflectance could be estimated with one curve from crop stands grown on sandy soil or clay soil. This is in contrast to others (Broge and Leblanc, 2001; Daughtry et al., 2000; Huete et al., 1985). They described an influence on the canopy reflectance signal by the soil colour.

In maize, the annual development of the crop stands in biomass and N content was very different, because of the different N supply of the soil during summer. This was also reported by others for maize (Osborne et al., 2002). The summer in 2003 was fairly dry with total rainfall being 200 mm from April to August. In June plants were partly wilting that probably negatively influenced nitrogen translocation and biomass production. In 2004, there was no nitrogen response at all observed with yield being at maximum. This could have been caused by high N supply from the soil eventually also resulting from high residual nitrogen contents remaining from the drought in 2003. The seeding density was slightly negatively correlated with the N content but positively with the dry biomass and the N uptake. In 2003, the biomass was clearly influenced by the seeding density. This could be also a consequence of drought.

If plants are well supplied with nitrogen and water they differ mainly in biomass and less in N content and for nitrogen deficiency in an opposite manner (Gastal and Lemaire, 2002; Olesen et al., 2002). Spectral measurements were sometimes better correlated with biomass and sometimes better with N uptake. But to support a fertilizer application, it is still a useful method to detect nitrogen deficiency and nitrogen oversupply. Even under drought this system could detect N deficiency. But the results illustrate also that spectral measurements were strongly influenced by plant water stress. This agrees with the results of others (Curran et al., 2001; Schlemmer et al., 2005; Serrano et al., 2000). Further activities have to be done to investigate the influence of water deficit on reflectance measurements with this system.

The results of our field studies are in line with other studies obtained under well controlled experimental plot conditions with hand-held spectrometers (Osborne et al., 2002). However, the R^2 of the regressions for N uptake were on a constant high level in those years with soil nitrogen deficiency, whereas the results of others were sometimes comparable and sometimes much worse. This may be due to the measuring geometry leading to mixed signals from the four corners of the tractor, together with the big areas used for the ground truth evaluations. Another positive effect of the oblique view is that the LAI (leaf area index) increases theoretically with a factor F of 1.9, because the rays of light pass along a longer way across the canopy. This effect is particularly interesting in maize, since the intercepted radiation per LAI is not as high as in other crops (Ehlert, 1996). Earlier measurements are therefore possible. The results demonstrate that early measurements at BBCH 14 give a reliable estimation of the N status. In comparison to results from early measurements in nadir direction (Osborne et al., 2002) higher R^2 values, influenced also by a positive effect of the oblique view, could be observed.

A consequence of increased biomass in the field of view is a stronger saturation effect compared to nadir measurements, especially in the red area, because of the high absorption. In this area, the differences are low, in spite of the high technical contrast of the spectrometer because of the high sensitivity of the silicon array. Also in nadir measurements, saturation effects are known (Aparicio et al., 2000; Schlemmer et al., 2005). As an effect of this saturation, the NIR/RR index was only useful to describe the dry biomass up to 3000 kg ha^{-1}.

A further positive effect related to the measuring geometry is the independence from weather conditions, as canopy reflectance and sun irradiance were measured simultaneously. The effect of the low zenith angle was eliminated by the four optics, causing always one optic to face the side exposed to the sun, while another one faced the shady side of the plants. The results indicate that using this measuring geometry, one is no longer limited to measure in the 10 am – 2 pm window as commonly done in nadir measurements. Weather conditions and zenith angle up to 30° caused a shift of the reflectance values, but had little influence on the quality of the results as indicated by high R^2-values. The area performance in fertilizer application is often 20 ha per hour and more, so the shift of the values can probably be neglected for the use in an operative system, because fertilizing a field needs normally no longer than two hours and on the next field the system has to be calibrated again. Further investigations are necessary to describe the shift in reflectance values caused by the zenith angle and weather conditions.

For the detection of the N status in crops on the field level, also active systems are available having their own light source. The GreenSeeker (Ntech Industries, Ukiah, California, USA) is equipped with a pulsed LED as light source and measures the canopy reflectance as a modulated signal (Beck and Vyse, 1994; Inman et al., 2005). Because of the limited light energy from the LED the maximum distance between the sensor and the canopy is 0.8 m and the field of view is only 0.8 m. However the sensor is less sensitive to variable irradiance conditions (Lukina et al., 2001). Probably the sensor may also be used at dawn and in the night (Raun et al., 2002). The sensor measures only the NDVI and therefore it is probably less suited to detect the N status for the agriculture in western Europe, because the NDVI detects the biomass best and the N status only less good as indicated in Figure 3-1. This observation was commonly made by others (Elwadie et al., 2005; Lukina et al., 2000).

Another active sensor concept is the laser induced chlorophyll fluorescence principle. This sensor uses a pulsed laser as light source and measures the chlorophyll fluorescence as a modulated signal. Two sensors are in the testing phase, the laser sensor from Planto (Planto GmbH, Leipzig, Germany) (Bredemeier and Schmidhalter, 2003) and the MiniVeg N from Fritzmeier (Fritzmeier GmbH & Co. KG, Grosshelfendorf, Germany) (Schächtel et al., 2005). Both sensors use point measurements, 5 mm in diameter. The Planto sensor uses a

scanning mode that allows a better areal representation. This principle allows to detect independently chlorophyll density and biomass (Bredemeier and Schmidhalter, 2005). These sensors are rather weakly sensitive to variable irradiance conditions and they also perform at dawn and likely in the night (Bredemeier, 2005).
The investigated spectrometer system proofed to measure reliable and precise the N uptake. Therefore this system further allows developing site-specific variable rate fertilizer application for maize crops. Fertilizer applications can be split either by applying the second rate at growth stage BBCH 14 with liquid fertilizer or with a high clearance tractor up to BBCH 36. In this way it is possible to detect the site-specific N status of the canopy and the applied N rate can be adjusted to the site-specific biomass development and nitrogen uptake for maize.

3.6. Conclusions

The investigated, tractor mounted spectrometer allows fast and accurate measurements of the N status and biomass in maize. The system reliably detects nitrogen in deficient and well-supplied plants in heterogeneous fields. All tested indices showed good results. In maize, the annual development of biomass and N content was very different, so spectral measurements sometimes detected biomass best and sometimes N uptake best. Even under drought, this system can detect N deficiency. The measuring geometry shows several advantages in comparison to results obtained with a nadir optic. With this study we could show that this type of field spectrometer is a useful tool to measure the N status to deliver the information required for optimized nitrogen management on farms.

Acknowledgment
The authors acknowledge and thank the German Federal Research Foundation for funding the research project FOR 473 TP10.

4. Estimating nitrogen nutrition index with spectral canopy reflectance measurements

4.1. Abstract

Spectral measurements are useful to estimate the nitrogen status of crops. Farmers use this information for site-specific fertilizing in precision farming systems. Up to now this spectral information was primarily related to nitrogen uptake or to chlorophyll content per area, but N uptake increases with canopy development and the effective N need of a crop needs to be estimated with an additional method.

The nitrogen nutrition index (NNI) describes directly the nitrogen status of a crop, similar as the SPAD meter does on the leaf level. This index is the ratio between the actual N content and the critical nitrogen content, which indicates the minimum N content for maximum biomass production of a canopy. The index indicates whether the N content is higher or lower than the optimum for a specific crop biomass. Relating the NNI to the canopy reflectance intensity (CRI) makes it possible to utilize it for site-specific farming. This parameter matches the needs of farmers as it directly indicates whether the plants are well supplied or nitrogen deficient. The NNI/CRI correlation was validated in a three-years´ field experiment with winter wheat with an R^2 of 0.95. The NNI was closer correlated to the CRI than to N uptake, N content and biomass.

4.2. Introduction

In middle Europe crop growth is mainly limited by nitrogen supply as long as water is not limited. Many scientists describe the biomass production of crops as a function of nitrogen content in plants (Devienne-Barret et al., 2000; Farruggia et al., 2004; Gastal and Lemaire, 2002; Lemaire et al., 2005). The N content of plants is rather high at early growth stages and continually decreases with plant growth up to the stage of senescence. This reduction in N content is typically interpreted as a dilution effect. Metabolic tissues as leaf lamina are known to be rich in N (6.5 %) whereas structural tissues such as veins are low in N (0.8 %) (Lemaire and Gastal, 1997). During leaf growth the proportion of structural tissue increases relative to the metabolic tissue. Therefore the N content in young leaves is high and when the leaf area increases, the N content decreases in leaves of isolated plants without shadowing effects. This phenomenon affects not only the leaves but the entire plant. This demonstrates that the N content depends on the plant biomass. If two different plants have the same N content but differ in biomass, it is possible that the plant with higher biomass is well supplied with N, but the plant with lower biomass may suffer from N shortage (Lemaire et al., 1995; Lemaire et al., 1992).

For plants in crop stands N distribution follows the same rules, but there is a further gradient of N distribution within the canopy from the top to the bottom. In upper leaf layers the N content is high and decreases in lower leaf layers. This gradient is directly correlated to light intensity in the canopy (Eichelmann et al., 2005; Gastal and Lemaire, 2002; Grindlay et al., 1995). On the top, plants show the expected N content, corresponding to the proportion between metabolic and structural tissue. N content for underneath leaf layers decreases linear with decreasing light intensity up to the light compensation point, where leaves become senescent. This plant material consists solely of structural tissue and the N content is as low as 0.8 % (Lemaire and Gastal, 1997).

To support the nitrogen fertilizer application regime in crop production it is important to know the adequate N content for a certain amount of biomass. For winter wheat several studies examined the minimum N content for maximum biomass production (critical N content) from tillering up to flowering (Devienne-Barret et al., 2000; Greenwood et al., 1991; Lemaire et al., 2005). So the nitrogen nutrition index (NNI) is calculated as the ratio between the measured N content and the critical N content. If the NNI is higher than one the canopy is in an ample N state, if it is lower then one it lacks nitrogen.

To assist farmers in N fertilising fast and simple methods are needed to estimate the N demand of their crop as well as the soil N-supply (Schmidhalter, 2005). Destructive methods to estimate the N content and calculate the NNI are very accurate but time consuming and expensive. Also optical transmission measurements with a handheld chlorophyll meter (SPAD) are time consuming however accurate on the measured spot (Blackmer et al., 1994; Cartelat et al., 2005). This accuracy is not sufficient on the canopy level because of the measurement position in the canopy. Such a device measures only one point of the last fully developed leaf, but the N-content is neither homogeneous within the flag leaf (Bredemeier, 2005; Cartelat et al., 2005) nor within the canopy (Eichelmann et al., 2005; Lemaire and Gastal, 1997; Pons et al., 1993). Additionally the values are influenced by the water content of leaves (Schlemmer et al., 2005).

Spectral measurements represent a fast and accurate means to estimate the N status, with chlorophyll having the predominant impact on the measurement. The best relation between chlorophyll and reflectance measurements have been obtained on the leaf level (Cartelat et al., 2005; Gitelson et al., 2003). The relation between chlorophyll content and N content is contradictorily discussed. Read et al. (2002) found weak correlations with $R^2 = 0.32$ whereas others found very close correlations with R^2 of 0.97 (Cartelat et al., 2005). On the canopy level however spectral measurements correlate best with N uptake (Lukina et al., 2001; Schmidhalter et al., 2003).

Regarding the similarity of reflectance and light distribution within the canopy spectral measurements may be well suited to record the N distribution within the canopy (Vouillot et al., 1998). In the upper leaf layer the N content is high and illumination is also high (Gastal and Lemaire, 2002). Lower leaf layers are less illuminated and a lower portion of the reflection signal is to be expected. So the mixed canopy reflectance promises to give a weighted signal from all leaf layers which represent possibly also the N content from different canopy layers.

The knowledge on the relationship between NNI and spectral canopy reflectance is rather limited. Link and Jasper (2003) used the optimum N rate for maximum yield and related this to spectral measurements. This method worked only in the backward direction and was not useful to estimate accurately the crop canopy N status.

Schepers et al. (1998) defined a nitrogen sufficiency index (NSI). It is a canopy reflectance or transmittance based index. The optical canopy information is referenced with that from plants where N is not limited. Nitrogen fertilizer application is applied in the stress-based N treatment when reflectance of corn reached 96% of the reflectance of well-fertilized corn. This index excludes years and sites effects but the system requires reference plots.

In another report the NNI was estimated and the leaf reflectance was measured with a NIR/red ratio and was related to each other with R^2=0.78 (Vouillot et al., 1998). Additionally N uptake was calculated as foliar N content (leaf N content per m² x LAI) and related to spectral reflectance measurements with R^2=0.93. However these investigations were based on leaf level measurements. -

The objective of this study was to determine the relation between winter wheat spectral reflectance and the nitrogen nutrition index and whether this index is better related to spectral measurements compared to N uptake.

4.3. Material and methods

4.3.1. Experimental fields

The experiments were conducted at the Technical University Munich in the south east of Germany. Winter wheat (*Triticum aestivum* cv. Ludwig, in 2004 cropped with 50 % Tommi) was examined in a three-years´ field experiment. The fields were at the research station Dürnast in Freising in the north of Munich. The average temperature was 7.5 C° and the average precipitation 800 mm per year with the maximum in summer. The experiments were conducted between 2002 - 2004 on different fields about 3 ha in size.

Heterogeneous fields had been chosen to obtain differences in the N status and in the biomass. Soil characteristics differed within and among the fields and the difference in soil heterogeneity was indicated in apparent electrical conductivities varying between 20-50 mS m^{-1}. This resulted in variable growth conditions within the fields. The experimental design consisted of five fertilizer rates with 0, 90, 130, 170 and 210 kg nitrogen per hectare and 5 replications. Altogether the experimental design contained 25 plots. Each plot was 15 m in width and 50 – 60 m in length. The fertilizer was applied at four different growth stages at BBCH 22, 30, 40 and 50 (Meier, 1997). The row direction was EES to WWN in 2002 and 2003 and S to N in 2004. Measurements were made just before fertilizer application in 2003 and 2004 and were obtained between the fertilizer applications in 2002.

4.3.2. Spectral measurements

To get spectral information of the crop canopy reflectance a tractor mounted field spectrometer was used, similar to the Yara sensor (Yara GmbH & Co. KG, Dülmen, Germany). This spectrometer contained two measuring units with pixel distances of 3.3 nm and measured simultaneously canopy reflectance and incident radiation. The unit, measuring the global radiation, was linked with a cosine corrected diffuser and the second unit was linked with a four in one light fibre, which measured with an oblique oligo view optic at all four edges of the tractor. The advantage of this optical setup was that always one optic measured the sunlight exposed side of the plants and another the shady side, so that there was hardly any effect of the zenith angle (Reusch, 2003).
A further effect was that the measured area was out of the shadow of the tractor. The resultingly measurement area were four ellipses at the four edges of the tractor. Each ellipse was 1.8 m², altogether 5 m² in size, resulting in a field area of 5 m². When the tractor moved along the track, strips on both sides of the tractor were measured, 1.5 m in width.
The canopy reflectance intensity was measured at 550, 670, 700, 740 and 780 nm as illustrated in Table 4-1. To depict the spectral canopy information we calculated the red edge inflection point (REIP) (Guyot et al., 1988):

$$REIP = 700 + 40 \frac{(R_{670} + R_{780})/2 - R_{700}}{R_{740} - R_{700}}$$

This index has been shown to describe the N uptake best (Mistele et al., 2004; Plenet and Cruz, 1997). For the measurements in 2002 and 2003 the same sensor was used but in 2004 a sensor identical in construction was used, equipped with an other electronic software and calibration algorithm.

Table 4-1: Spectral measurement conditions for the different samplings in the years 2002, 2003 and 2004. Growth stage, average biomass, date and time, zenith angle, global radiation and weather conditions are indicated.

Year	2002		2003			2004			
Sampling	1st	2nd	1st	2nd	3rd	1st	2nd	3rd	4th
Growth Stage (BBCH)	32	55	30	39	65	27	32	53	71
Date	May 15	June 3	May 5	May 19	June 5	April 26	May 12	June 6	July 5
Time (hour, minutes)	11.40±8	13.10±10	10.30±30	10.30±30	12.50±20	15.20±20	11.30±20	12.00±15	13.00±20
Zenith angle (°)	55±1	62±0	44±4	45.5±4.5	62.5±0.5	46±4	53.5±2.5	60±2	62±0
Solar radiation ($W\ m^{-2}$)	844	790	750	100	840	580	710	880	410
Weather conditions	sunny	sunny	sunny	sunny hazy	sunny	sunny-cloudy	sunny hazy	sunny	sunny-cloudy

4.3.3. Biomass sampling

To get information about the biomass and the N status of the crops within the plots the biomass was sampled exactly in the measured area by the sensor. The biomass was cut with a green forage chopper, equipped with a weighing unit to record the biomass immediately after cutting. The sampled area was 1.5 m in width and around 8 m in length on both sides of the tramline. A representative subsample was oven dried to estimate the dry matter. Then the subsamples were milled and analysed in the laboratory to estimate the total N content with an elementary analyser according to Dumas (Macro-N, Foss Heraeus, Hanau, Germany). The N uptake was calculated as biomass x N content.

4.3.4. Determination of the nitrogen nutrition index

The critical N content (N_c) of winter wheat was described by the following equation, based on several investigations in France (Justes et al., 1997):

$$N_c=5.35*W^{-0.442}$$

where N_c is the critical N content as a percentage of dry matter and W is the dry weight of aboveground biomass in t ha^{-1}. This equation can be applied when shoot dry matter is in the range 1.55 – 12 t ha^{-1} and for growth stages ranging from BBCH 30 to 65. In the range 0.2 – 1.55 t ha^{-1} the critical N content may be constant at a mean value of 4.4 % (Justes et al., 1997). For our experiments we did not pay attention to this restriction because the relation between spectral measurements and plant parameters is a continual function and it does not fit to a discontinual function like the critical N function with the limitation of minimal biomass. To indicate the N status for each plot with different biomass, independent of the growth stage, the nitrogen nutrition index was calculated as follows:

$$NNI = \frac{N_{act}}{N_c}$$

where N_{act} is the actual measured N content in percent of the dry matter of the canopy biomass and N_c the critical N content for the crops of each plot and its particular amount of dry matter (Lemaire and Gastal, 1997).

For the statistical analysis, we used SPSS 11 (SPSS Inc., Chicago, USA). Simple regressions were mainly calculated. Further curvilinear models were used and coefficients of determination averaged over all plots in one field calculated. In this way differences in soil conditions, fertilizer application rates, slopes and water supply were not considered. Mean values were not included in the analysis, as we expected large differences from plot to plot due to heterogeneous soil and field conditions being independent of the fertilizer rate.

4.4. Results

The results of the destructive analysis of the biomass samples are indicated in Figure 4-1. The N status during crop growth differentiates between the three experimental years. In 2002 at the first sampling 10 % of the plots were in a sufficient N status and 20 % at the second sampling. Considering, that the measurements took place between the fertilizer applications (10 days and 4 days after the last fertilizer application, respectively) a higher number of plots in an ample N status could be expected. In 2003 in the first two samplings all plots depicted N deficiency and in the third sampling 20 % were in an optimum N status and a clear improvement in the N status from the first to the third sampling could be seen. The results in 2004 were different compared to the other two years.
In the very early growth stage 4 % of the plots indicated a luxury N status of the crops, 22 % in the second, 39 % in the third and 11 % in the fourth sampling. For all samplings the N status was considerable improved compared to the previous years.

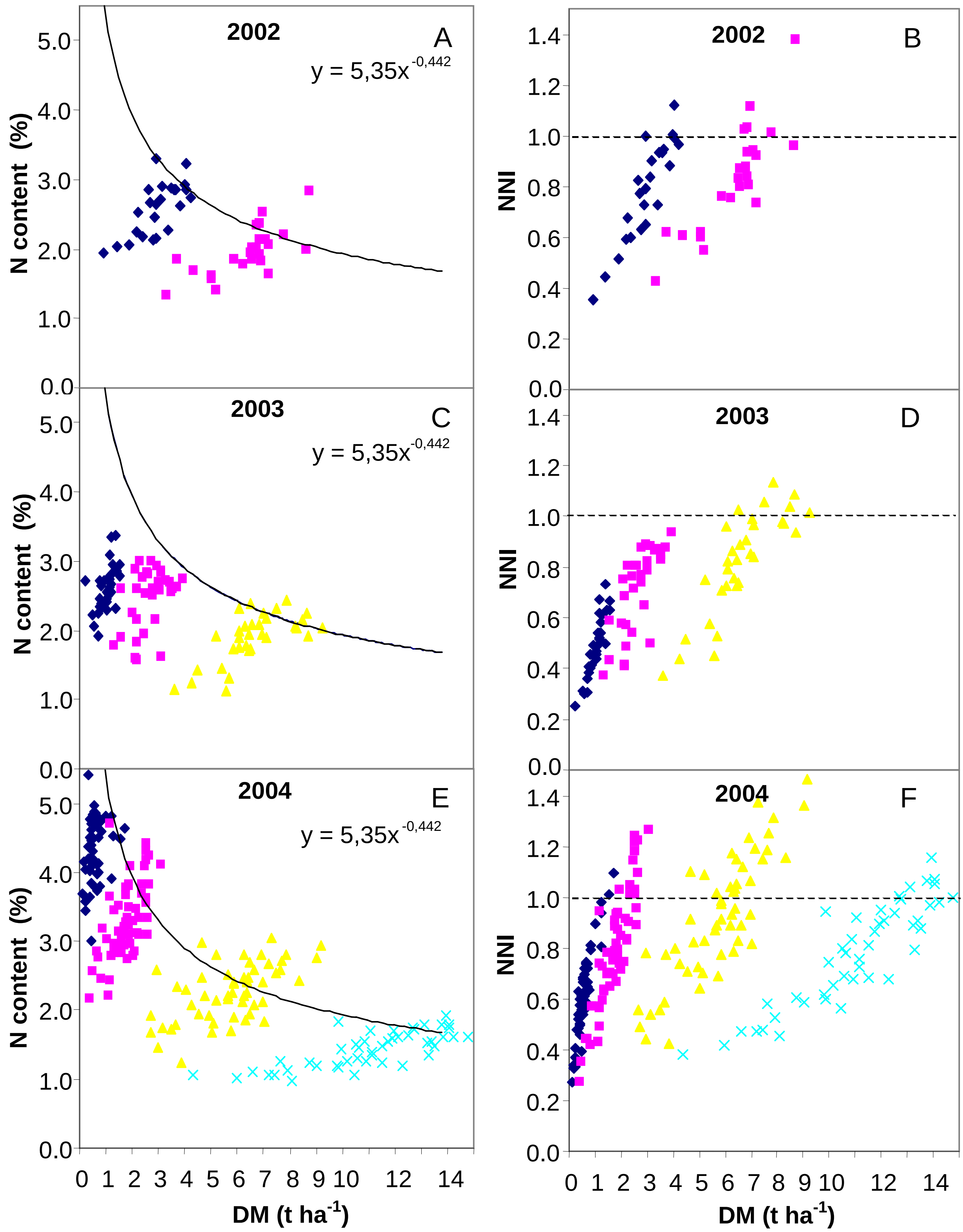

Figure 4-1: Destructive analysis of the biomass samples. A, C, E: Relationships between dry matter (DM) and N content with critical N curves (Justes et al., 1997) and B, D, F: Relationships between dry matter and nitrogen nutrition index (NNI) in the years 2002 to 2004 first sampling◆, second sampling■, third sampling▲ and fourth sampling✕.

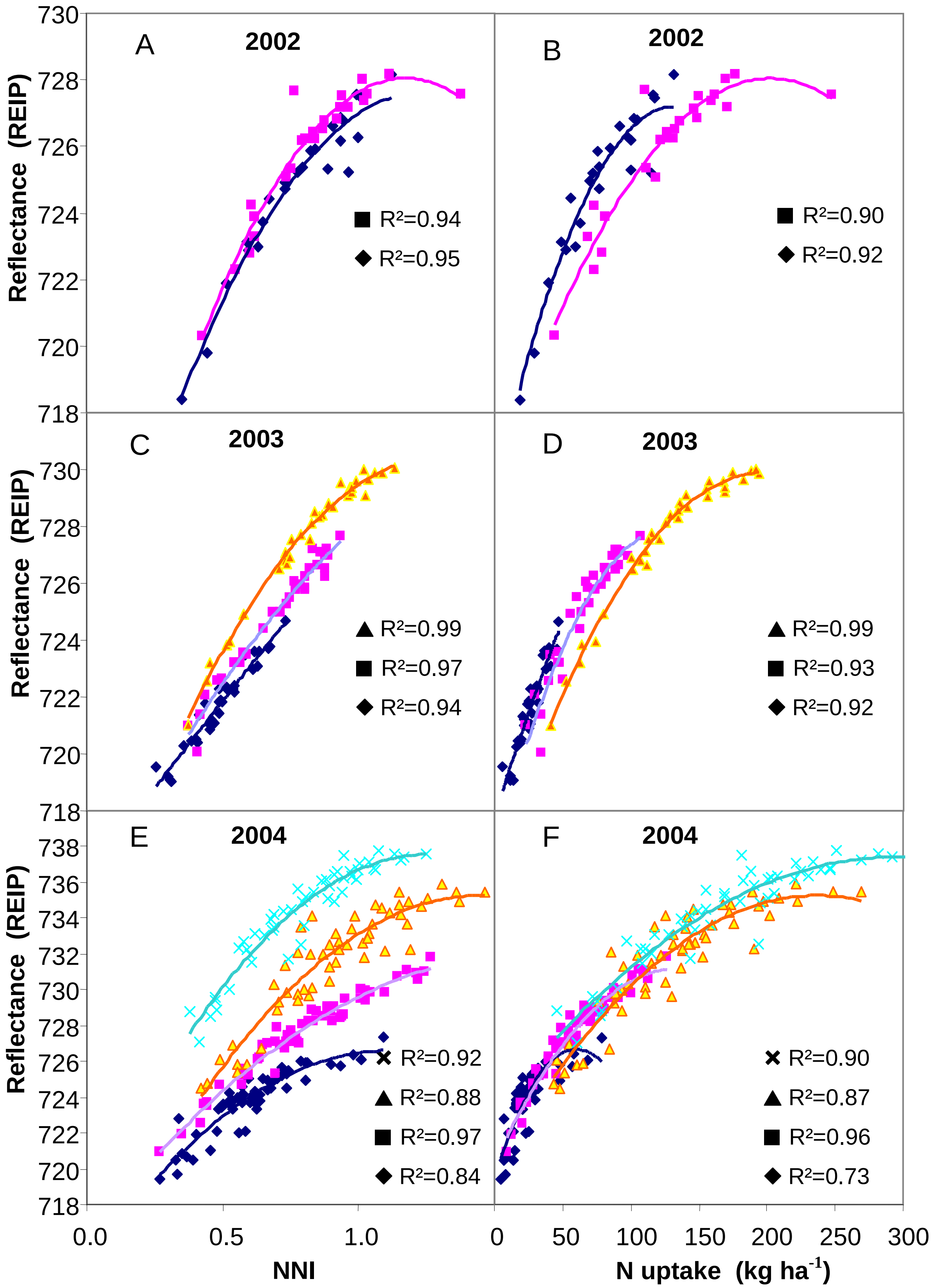

Figure 4-2: Spectral detection of the N status with: A, C, E: Relationships between nitrogen nutrition index (NNI) and reflectance intensity of the crop canopy calculated as REIP; B, D, F: Relationships between N uptake (kg ha^{-1}) and crop canopy reflectance calculated as REIP for the years 2002 to 2004 first sampling◆, second sampling■, third sampling▲ and fourth sampling✖.

For all three experimental years an increase in the N status could be observed from tillering to flowering as indicated in Figure 4-1 B, D and F. The fourth sampling in 2004 made after flowering showed a decreased N status compared to the third sampling.
The relationship between NNI and CRI is indicated in Figure 4-2. The R^2 between NNI and CRI was higher than for the relationship between N uptake and CRI for all measurements. The average R^2 was higher than 0.94 and even higher than 0.96, except for three invalid measurements in 2004.
The relationship between NNI and CRI was curvilinear, except for some of the early measurements in 2003 where all plots had a NNI lower than 1. For early measurements Figure 4-2 shows, that the lower the average NNI, the more linear is the relationship between NNI and CRI. This includes also the relationship between N uptake and canopy reflectance.

The curves of the fitted relationship between NNI and canopy reflectance within 2002 and 2003 paralleled each other. In 2002 the curves were closer to each other than the curves between N uptake and canopy reflectance. The pattern of the curves between N uptake and CRI and NNI respectively is similar to each other, only the positions of the curves were interchanged. For the relationships between NNI and CRI the slope of the curves increased as the biomass increased and the values increased, too. For the relationships between N uptake and CRI the opposite was observed. With increasing biomass the slopes of the curves decreased and the values decreased, too. In 2004 contrasting observations were found. The curves of the relationship between N uptake and canopy reflectance paralleled each other but the curves of the relationship between NNI and CRI differed strongly in their slopes and values.
The REIP values differed in their range from year to year. Each year measurements started at a REIP value of 719 nm but the maximum REIP value increased from 728 to 738 nm from 2002 to 2004.

4.5. Discussion

The results demonstrate that spectral measurements are useful to describe the N status of wheat canopies. Spectral measurements were closely related to both NNI and N uptake, being somehow closer related to NNI. The NNI seems to be particularly useful to describe the N status of crop canopies because it reflects whether they have an optimal N concentration for maximum biomass production in relation to their actual biomass or if they are nitrogen deficient. Information about the N uptake or the N content contains no direct information about the specific need for the actual amount of biomass, because the N uptake and N content do not consider the amount of biomass in the field. For example in a sandy area within the field, where plants are suffering because of insufficient water, the N uptake and the biomass may be low, but the N content may be similar as for other parts of the field with good growth conditions enabeling higher biomass and N uptake (Eck, 1988; Mirschel et al., 2005). The NNI however indicates for the drought affected crop stand a lower value than for plants amply supplied with water and nitrogen because it takes the biomass in consideration and not the growth stage. Therefore the NNI can deliver valuable information for fertilizer application decisions.

With a tractor mounted spectrometer this information can be achieved fast, easily and non-destructively. Canopy reflectance measurements based on the REIP showed no big differences in the relationships to the NNI or the N uptake, however with the NNI being ever closer related. This differs from results reported in other papers that found closer relationships between N uptake and spectral measurements than between spectral measurements and other crop canopy parameters (Link et al., 2005; Mistele et al., 2004). In this work closer relationships (R^2=0.94) were found as compared to Vouillot et al. (1998) in their attempt to estimate the NNI with spectral measurements (R^2=0.78). N uptake per area encompasses the total nitrogen of all parts of the plants, structural N and metabolic N. The concept of the NNI assumes that the structural N is constant at N deficiency or amply supplied crop stands, whereas the N content of metabolic tissue decreases with decreased N supply (Evans, 1972; Lemaire and Gastal, 1997). Not all N in metabolic tissue is contained in chlorophyll, but the ratio between chlorophyll and other components of the photosynthetic machinery increased linearly with increasing leaf N content (Lawlor, 2002). These observations support the hypothesis that the canopy reflectance signal is directly related to the gradient of N content in the metabolic tissue within the canopy. N content decreases in lower leaf layers and is directly correlated to the light intensity in these lower leaf layers (Gastal and Lemaire, 2002; Grindlay et al., 1995; Vouillot et al., 1998).

The critical N content is the basis of the NNI. This relationship is based on many experiments with increasing N rates in France and it is reported, that it is little affected by the growth rate, density, cultivar and pedoclimatic conditions (Justes et al., 1997), but the model has to our knowledge not yet been validated in Germany. For our investigations we also tested different other parameters, but modifications of the parameter within the model for critical N content did not improve the relationship between NNI and REIP.

The curves of the relationships between the NNI and the REIP measurements of the different biomass samples showed a shift in the values and different slopes. This was remarkable in 2004. But in 2004 the first measurement was done too early because the average biomass was only 580 kg ha^{-1} and the model described by Justes et al., (1997) starts from a biomass higher than 1500 kg ha^{-1} biomass. This is because in earlier growth stages less structural tissue is found and the same relationship between structural tissue und metabolic tissue applies. So it is difficult to differentiate crop canopies. The last measurement was too late because after flowering nitrogen becomes accumulated in the kernels. This stored nitrogen is not from structural or metabolic tissue and so it distorts the NNI (Lemaire and Gastal, 1997). A number of factors might explain the shift of the values between the curves of the second and third biomass samplings in 2004. Firstly, the precrop on the experimental field in 2004 was maize in 2003, and secondly in 2003 the summer was very hot and dry. Maize plants could not take up substantial amounts of fertilized nitrogen from the soil and residual N in the soil was high. A second reason for this different behaviour in 2004 could be seen in the two different cultivars used (Sticksel et al., 2004). This was not planned initially. However due to their resemblance we could not distinguish between the two varieties. And thirdly we could not rule out that the row direction caused differences in the measurements (Pinter et al., 1987). It was orientated S to N in 2004 and EES to WWN in the two years before. But also the soil colour (Daughtry et al., 2000) could influence the measurements. The use of a modified sensor with an other electronic, software and calibration could as well be responsible for this different behaviour.

But there was also a shift between the measurements in the years 2002 and 2003. These differences can not be explained easily. Probably other additional plant physiological factors influence canopy reflectance requiring further investigations.

The relationships between NNI and CRI were weaker in three measurements in 2004 than for the other measurements. Reasons for the lower R^2 values as found for the first and the last sampling were eventually due to the fact that they were out of the validity of the NNI curve. Within the third sampling in 2004 some possible mistakes occurred with the plot numbers and sample numbers.

Because CRI values cannot be related absolutely to NNI or N uptake a field calibration is necessary. This needs destructive methods to estimate the N content and biomass. For farmers this is not practical because it is time-consuming and costly. To use aids like the SPAD meter may include new sources of error. The SPAD meter measures also accurately the N status as the NNI, but generally only the last fully developed leaf is measured and this may perhaps not represent the entire canopy. Additionally SPAD measurements are influenced by the leaf water content (Schlemmer et al., 2005). Probably the best calibration would be to define absolute curves for different amounts of biomass. For this calibration further investigations have to be done to know the main factors, which are responsible for the strong shift of the curves between NNI and REIP in 2004. Different factors could be responsible for this shift like the use of another sensor, different cultivars, precrops, row direction or soil colour. All these factors have to be quantified to create a generalised calibration curve.

4.6 Conclusions

Spectral measurements were useful to describe the NNI in winter wheat. With a tractor mounted field spectrometer the N status could be estimated fast and non-destructively. The NNI delivered information whether the canopy was deprived of N or in luxury N status. The results showed high R^2-values between canopy reflectance (REIP) and NNI, slightly better than between canopy reflectance and N uptake. This information about the N status of crop stands by using spectral reflectance measurements is useful to support nitrogen fertilizer application within Precision Farming. Because there was still a small shift in the REIP values between the different biomass samplings, the field spectrometer has to be calibrated on the field. To explain this shift, factors, which caused this shift, have to be further investigated.

Acknowledgment

The authors acknowledge and thank the German Federal Research Foundation for funding the research project FOR 473 TP10.

5. Synthesis

The comparison of reflectance indices to canopy parameters demonstrates that it is possible to obtain good estimates of the N status in intact canopies with a tractor based spectrometer under field conditions in wheat and maize. This scanning method is rapid, easy, non-destructive, tractor based and applicable to field-scaled dimensions.

The results of our field studies are in line with other studies obtained under well controlled experimental plot conditions with hand-held spectrometers in wheat (Reusch, 1997) or in maize (Liebler, 2003). However the R^2 of the regressions for N uptake were on a constant high level in wheat and in maize, whereas results of other researchers were sometimes comparable and sometimes less good. This may be due to the measuring geometry, caused by the mixed signal obtained from the oligo view optic and enhanced by the large area used for the ground truth calibration.

5.1. Technical setup

For the spectral measurements a passive spectrometer system with an oblique oligo view was used. The system used the sun irradiance as light source.

5.1.1. Simultaneous measurement of incident radiation and canopy reflectance

Sun irradiance and canopy reflectance were measured simultaneously to be independent from irradiance conditions. This technique enables measurements on sunny and at cloudy days. Even measurements during rainy periods were possible. Only at sudden changes between direct sun light (blue sky) and diffuse sunlight (clouds) a noticeable decrease in the R^2 was observed. For all the other measurements the close relationship between canopy reflectance and the N uptake showed, that different irradiance conditions did not influence the R^2 of the results, but caused a shift of the values.

For measurings in the nadir, scientists often used a standard reflector like Spectralon to measure the sun radiation instead of simultaneous measurements. There is always a time shift between sun radiation measurements and canopy reflectance measurements. If the irradiation conditions were not totally stable, they may result in an error within the measurements (Duggin and Cunia, 1983; Major et al., 2003).

A limitation of the system is the need of sufficient sun light because the passive sensor system has no own light source. A modified, recent developed sensor system is equipped with halogen flash light and photodiodes with interference filters. This might enable measurements also in the night and at dawn (Reusch, 2005). Probably there are limitations for measurements under full sunshine.

5.1.2. Technical calibration

Technical calibration is necessary to make data comparable to measurements with other devices with the same technical setup and to other systems (Duggin and Cunia, 1983). Because of the simultaneous measurements the sensor contains two spectrometers, one measures the canopy reflectance and the second the sun radiation. These spectrometers have to be calibrated. For this calibration a reflectance standard like Spectralon is not useful, because it is not a Lambertian reflector and it delivers only correct result close to the hot spot near 90° angle (unpublished results). There would be an error because of the oblique view of 58 ° and a second error because of the changing zenith angle of the sun. A correct calibration of such a sensor system is only possible in an optical laboratory with standardized light sources at right angles, otherwise sensor systems will not deliver comparable results.

A further problem of the sensor is the incorrect cosine corrected diffuser for the optics measuring the sun radiation. A calibration curve is necessary to correct this error for each possible zenith angle (unpublished results). Therefore measurements could be conducted at different zenith angles to calculate a calibration factor for each relevant wavelength because the error is different for each wavelength. To use these calibration factors the zenith angle must be known. It can be calculated from the GPS position and GPS time.

5.1.3. Oblique oligo view optic

The effect of the changing zenith angle was eliminated by an oblique oligo view setup with four optics, so always one optic is facing the sun exposed side while another one is directed to the shadow side of the plants. The results demonstrate that when using this measuring geometry one is no longer limited to measure in the 10 am – 2 pm window as commonly done in nadir measurements. A visible decrease in the R^2-values was observed at zenith angles less than 24°. Weather conditions and zenith angle up to 24° caused a shift of the reflectance values, but had little influence on the quality of the results as indicated by high R^2-values. Further investigations are necessary to describe the shift of the reflectance values caused by the zenith angle and weather conditions.

One part of this shift is probably caused by the incorrect cosine correction of the diffuser for the sun radiation. But measuring in the nadir, using a standard reflector like Spectralon entails the same problem, because the standard reflector is not a Lambertian reflector and changes its reflection with changing zenith angle (unpublished results).

5.1.3.1. Interference between soil and canopy reflectance

Another positive effect of the oblique view is that the LAI (leaf area index) increases theoretically with a factor F of 1.9, calculated as $F = (\sin 32°)^{-1}$, because the rays of light pass along a longer way across the canopy. Some crops and cultivars may have more planar leaves so that the factor is probably not as high but there is still an increased amount of biomass in the field of view to be expected.

Spectral reflectance of a canopy is always a mixed signal of soil reflectance and canopy reflectance and it is not possible to differentiate between both (Guyot, 1990; Major et al., 2003). But in spite of the increased amount of biomass in the field of view the signal intensity of the canopy reflectance was increased and the signal/noise ratio was reduced. In this way there is less interference from soil reflectance within the canopy reflectance signal, whereas other researchers reported clearly influenced canopy reflectance data from soil background (Broge and Mortensen, 2002).

5.1.3.2. Early vegetative stage measurements

The increased amount of biomass in the field of view caused by the optical setup includes a further positive effect. It increases the plant soil reflectance ratio and improves particularly early measurements with low soil coverage.

The results demonstrate that early measurements at BBCH 30 in wheat and at BBCH 14 in maize give a reliable estimation of the N status. In comparison with results from measurements in the nadir direction (Liebler et al., 2001) a positive effect of the oblique view could be observed. Other researchers showed, that measurements in the nadir were highly depending on the underlying soil at soil coverage up to 75 % (Heilman and Kress, 1987; Huete et al., 1985). Measurements with an oblique view decrease the interference between soil and canopy reflectance at this early growth stage, as indicated by high R^2-values for measurements at soil coverage less then 50 % of maize in heterogeneous fields with differing soil brightness and soil colour.

5.1.3.3. Optical saturation effects

A consequence of increased biomass in the field of view is a stronger saturation effect compared to nadir measurements (Broge and Mortensen, 2002), especially in the red area, because of the high light absorption. The differences between the reflectance values in this area are low in spite of the technical high contrast of the spectrometer because of the high sensitivity of the silicon array in this area. Also in nadir measurements saturation effects are known at LAI higher than 3, mainly by LAI but also by LAI*chlorophyll whereas the NDVI showed a perfect saturation and the NIR/R relationship was curvilinear (Aparicio et al., 2000; Serrano et al., 2000). These observations are in compliance with the results obtained in 2002 and 2004 in wheat; in 2003 however the saturation effect was less pronounced. This was probably directly related to the lower N status as described by the NNI. As an effect of this saturation the NIR/RR index was only useful to depict the N status up to BBCH 29. So far this behaviour had only been described by a model (Broge and Leblanc, 2001) whereas other scientists, measuring in the nadir, got best results up to senescence (Broge and Mortensen, 2002; Heege and Thiessen, 2002; Serrano et al., 2000). In maize this saturation effect was also observed but weaker. A possible reason for the saturation effect could be found in the competition for light among plants. It is reported to start at about LAI 2.5 and 1.6 t dry biomass per hectare (Justes et al., 1997).

5.1.4. Other non-contacting sensor systems

For the detection of the N status in crops on the field level, also active systems are available having their own light source. The GreenSeeker (Ntech Industries, Ukiah, California, USA) is equipped with a pulsed LED as light source and measures the canopy reflectance as a modulated signal (Beck and Vyse, 1994; Inman et al., 2005). Because of the limited light energy from the LED the maximum distance between the sensor and the canopy is 0.8 m and the field of view is 0.8 m. However the sensor is less sensitive to variable irradiance conditions (Lukina et al., 2001). Probably the sensor may also be used at dawn and in the night (Raun et al., 2002). The sensor measures only the NDVI and therefore it is probably less suited to detect the N status in western European agriculture, because the NDVI is rather suited to detect the biomass than the N status, the quality of which was least as indicated in Chapter 2.4. This observation was commonly made by others as well (Elwadie et al., 2005; Lukina et al., 2000).

Another active sensor concept is the laser induced chlorophyll fluorescence principle. This sensor uses a pulsed laser as light source and measures the chlorophyll fluorescence as a modulated signal. Two sensors are in the testing phase, the laser sensor from Planto (Planto

GmbH, Leipzig, Germany) (Bredemeier and Schmidhalter, 2003) and the MiniVeg N from Fritzmeier (Fritzmeier GmbH & Co. KG, Grosshelfendorf, Germany) (Schächtel et al., 2005). Both sensors are using point measurements, 5 mm in diameter. The Planto sensor uses a scanning mode that allows for a better areal representation. This principle allows to detect independently chlorophyll density and biomass (Bredemeier and Schmidhalter, 2005). These sensors are rather weakly sensitive to variable irradiance conditions and they perform also at dawn and likely in the night (Bredemeier, 2005).

5.2. Parameters influencing measurements on the canopy level

Canopies differ in their habitus between crops, sites and years. Different factors influence the yield and hence the structure of the plant canopy like soil conditions, inclination and exposition of the field, water status, disease (Auerswald et al., 1997) and variety (Schächtel, 2004). All this factors have the potential to influence spectral measurements directly or indirectly.

5.2.1. Soil conditions

Soil conditions influence plant growth and in this way indirectly influence spectral properties of the canopy (Thiessen, 2002). Sandy soils induce a high emergence rate and fast growing at the beginning but they are rather characterized by decreased nutrient resources and decreased water capacity. Loamy soils are cooler at the beginning of the season leading eventually to a lower emergence rate and a lower growth rate, but the nutrient availability and water capacity are higher and the plant growth is often better than on sandy soils in later growth stages. So the soil influence on canopy reflectance intensity is probably caused by differences in biomass and N content development within the crop. In our experiments soil conditions were “heterogeneous”. Some plots were on sandy soils and others on plots with loamy clay soil. Spectral measurements showed the ability to detect reliably the N status of crop canopies on different soils. In our experiments we did not find any influence of heterogeneous soil conditions.

5.2.2. Inclination and exposition

Regarding the position of the field, there are two points are of interest: The exposition and the inclination of the site.

If the inclination differs much within the field and if it contains partly strong slopes, two effects must be taken in consideration. One effect is the same observation as for different zenith angles because of the changing angle between diffuser optic of the sensor and the sun. The quality of the measurements depends on the quality of the cosine correction of the diffuser optic. The cosine correction of the used system is not exact (unpublished results), but the results in the fields were good enough to stabilize the measurements during 30 minutes to one hour and showed no decrease in quality as indicated by the high R^2-values. The second effect of different inclinations within a field are different angles between plants and soil. This creates different canopy architectures within the field of view. Theoretically the oblique oligo view optic eliminates the differences and the system detected no negative effect of the different slopes within the fields.

The exposition has no direct influence on spectral measurements, but an indirect influence through the row direction. In our experiments clear differences could be seen in wheat with regard to the row direction. It was oriented EES to WWN in 2002 and 2003 and N to S in 2004. In maize the row direction was N to S in 2003 and EES to WWN in 2002 and 2004. But the differences of the reflectance measurements in wheat could not be clearly assigned to the different row direction because in 2004 another sensor system and two different cultivars were used. In maize the decrease of the quality of spectral detection of the N status can also not be clearly assigned to the row direction because in the year 2003 drought or an additional factor influenced the plants. So there may be an effect of the row direction, which was not eliminated by the oblique oligo view optic, but further investigations are necessary to exclude the distortion and to describe this effect. These results are in line with others. Some indices were also strongly influenced by the row direction in their diurnal canopy reflectance (Jackson et al., 1979; Pinter et al., 1987).

5.2.3. Crop-specific differences

Crops differ in their spectral properties. They differ in their canopy architecture, height, seeding density, row spacing, leaf stem ratio, N content, leaf colour and so on.
In our experiments we compared wheat and maize canopies. These two canopies differ in many points. Both plants are monocotyledonous plants, but the height of maize is 3 times that of wheat and the leaf size of maize is also many times that of wheat. Row spacing is 0.75 m in maize and 0.13 m in wheat and the seeding density in wheat was 250 seeds per m² and in maize 6 to 14. Consequently the canopy architecture between both crops were totally different. The intercepted radiation per LAI of the special canopy architecture in maize is not as high as in other crops (Ehlert, 1996). Therefore measurements in maize

require at least 2.5 rows in the field of view, in order to overcome the variability caused by the interrow differences in the leaf area (Major et al., 2003).

Additionally the colour between these canopies was different and the N content of the maize plants was on average 0.5 % lower than for wheat, taken from canopies with equal biomass per area. This was observed by others too. This is because N content in C_4 plants is lower than in C_3 plants. Lemaire and Gastal (1997) indicated the N content of metabolic tissue of C_3 plants with 6.5 % N and C_4 plants with 4.8 % N. The differences in the values reflect the differences in the metabolic pathway for CO_2 assimilation and associated differences in leaf anatomy (Lawlor, 2002). This means, that C_4 plants require only 75 % of the N required by C_3 plants for the same biomass production. Further differences are that at similar plant mass, C_4 crops seem to contain a smaller proportion of metabolic tissues than C_3 crops, but have a 40 % higher radiation use efficiency (Lemaire and Gastal, 1997).

In wheat N uptake was the parameter that could be best predicted by spectral measurements. But in maize sometimes the biomass and sometimes the N uptake was best described by spectral measurements. This was probably because of the lower N content of the maize plants but it could also be an effect of the different canopy architecture. Further investigations are necessary to separate these effects.

5.2.4. Differences in reflectance among site-years, cultivars, abiotic and biotic stresses

5.2.4.1. Differences in reflectance between years

Especially in maize the crop growth between the experimental years differed very much and canopy parameters like biomass, N content and N uptake evolved in a different manner.

Spectral measurements also showed clear differences between years. In 2002 measurements in maize correlated best with N uptake and in 2004 best with biomass. But also the values of the spectral indices showed differences in wheat and maize between the years. The same observations were made by Schächtel (2004). He described the annual influence as the strongest “disturbance” in spectral measurements. However it was not clear, whether these annual effects resulted from different fields, or were influenced by an error of the spectrometers or were due to the special canopy development in each year.

5.2.4.2. Differences in reflectance between cultivars

Cultivars differ in their phenotype. Some cultivars are erectophil, others are planophil, or some are dark green and others more bright. In our experiments only once in 2004, two different cultivars of wheat were accidentally used. In this year results were less good than in the years before, but unfortunately in this year a new sensor was used and row directions differed, so the results can not clearly be assigned to different cultivars.
Other researchers detected differences between cultivars, but the influence on reflectance measurements was lower than the influence of the year and they did not take different biomass level and N status into account (Liebler et al., 2001; Schächtel, 2004; Sembiring et al., 2000). This might probably reduce differences.

5.2.4.3. Differences in reflectance between abiotic and biotic stresses

Even under drought, the sensor system used could determine N deficiency. But the results illustrate also, that spectral measurements were strongly influenced by water deficit. In 2003 in maize plants were partly wilting and the R^2 of the results decreased. This agrees with the results of others (Curran et al., 2001; Schlemmer et al., 2005; Serrano et al., 2000). Further activities have to be done to investigate the influence of water on reflectance measurements with this system.

A limitation of the system is the precondition that crop stands have to be healthy and free of weeds. If there is a fungal infestation of the plants, the sensor probably detects a lower N status (Carter, 1993) and increases the N rate, applied by a site specific fertilizer application. But the sensor has to reduce the N rate because the plants are perhaps well supplied or oversupplied and may not recover in the season. If weeds occur between the plants, the sensor may detect a higher N status, because there is a lot of biomass in the field of view and this may reduce the N rate applied (Gée et al., 2004). But the plants may perhaps suffer from insufficient N supply, because the weeds compete with nitrogen uptake. If plants suffer from stress situations that are not caused by nitrogen deficiency, but e.g. by other nutrients, water shortage, herbicide, ozone or salinity, plants probably decrease in chlorophyll content (Carter, 1993; Carter and Knapp, 2001; West et al., 2003). The sensor detects eventually a shortage in N but plants are well supplied with N. In our experiments the crops were free of weeds, diseases and sufficiently provided with nutrients.

5.2.6. Development of calibration factors

To develop calibration factors for a spectrometer system to fit the measurements to canopy parameters is very difficult and in some case impossible. For different cultivars calibration curves for each variety, or at least for groups of cultivars have to be created. For other influencing factors like drought, weeds, diseases and other nutrients it will be desirable to find a second specific index, which quantifies the strength of the factor and than a weighted factor can correct the influence on the reflectance index which indicates the N status. This could be for example a polyphenolics index as measured by the Dualex (Cartelat et al., 2005) device or a water index (Penuelas and Inoue, 1999).
The annual influence on spectral indices contains probably many different factors and can not be calibrated yet. First these factors have to be described and quantified in further investigations as depicted in Chapter 5.3.3, then a calibration might be possible.

5.3. Measuring principle

5.3.1. Nitrogen uptake

Spectral measurements in wheat correlated well with N uptake as the product between N content and above ground dry biomass. These results agree with others (Inman et al., 2005; Lukina et al., 2001; Reusch, 2003; Sembiring et al., 2000).
In maize spectral measurements showed clear differences between years. In the earliest readings, they best described the dry biomass for all three years and for all indices. Later in 2002, measurements described the N uptake best. At first we only found NIR/NIR and REIP and later also NIR/R and NIR/G describing N uptake best. In 2002 there was a N deficiency, indicated by the highest range of N content. In 2003 and 2004, spectral measurements described best the overall biomass, except the measurements for the third sampling in 2003. Probably because of drought, biomass detection was not possible.
One explanation for the good results between the biomass and the reflectance indices is that the N uptake was mainly determined by biomass as indicated in Table 3-4. It is reported, that if plants are well supplied with nitrogen, they differ mainly in biomass and less in N content and for nitrogen deficiency in an opposite manner (Ehdaie and Waines, 2001; Gastal and Lemaire, 2002). In wheat the NIR/RR index has been described as biomass index and that is probably the reason for the high R^2 of the N uptake relations up to 0.8 t per ha.

For all non-linear regressions the quadratic function was used. The expected situation for light reflectance in a high absorbance canopy is a saturation effect which is best described by a power function. But most of the regressions fitted best with quadratic functions which were also used by other researchers (Carter and Spiering, 2002; Read et al., 2002).

5.3.2. Nitrogen nutrition index

A rather new spectral feature to determine the N status of wheat canopies is the NNI. Spectral measurements correlated best with the NNI. The NNI seems to be particularly useful to describe the N status of crop canopies because it reflects whether they have an optimal N concentration for maximum biomass production in relation to their actual biomass or if they are nitrogen deficient. Information about the N uptake or the N content contains no direct information about the specific need for the actual amount of biomass, because the N uptake and N content do not consider the amount of biomass in the field. For example in a sandy area within the field, where plants are suffering because of insufficient water, the N uptake and the biomass may be low, but the N content may be similar as for other parts of the field with good growth conditions with higher biomass and N uptake (Eck, 1988; Mirschel et al., 2005). The NNI however indicates for the drought affected crop stand a lower value than for plants amply supplied with water and nitrogen because it takes the biomass in consideration and not the growth stage. Therefore the NNI can deliver valuable information for fertilizer application decisions.

With a tractor mounted spectrometer this information can be achieved fast, easily and non-destructively. Canopy reflectance measurements based on the REIP showed no big differences in the relationships to the NNI or the N uptake but with the NNI being ever closer related. This differs from results reported in other papers that found closer relationships between N uptake and spectral measurements than between spectral measurements and other crop canopy parameters (Link et al., 2005; Mistele et al., 2004). In this work closer relationships (R^2=0.94) were found as compared to Vouillot et al. (1998) in their attempt to estimate the NNI with spectral measurements (R^2=0.78). N uptake per area encompasses the total nitrogen of all parts of the plants, structural N and metabolic N. The concept of the NNI assumes that the structural N is constant at N deficiency or amply supplied crop stands, whereas the N content of metabolic tissue decreases with decreased N supply (Evans, 1972; Lemaire and Gastal, 1997). Not all N in metabolic tissue is contained in chlorophyll, but the ratio between chlorophyll and other components of the photosynthetic machinery increased linearly with increasing leaf N content (Lawlor, 2002). These observations support the hypothesis that the canopy reflectance signal is directly related to the gradient of N content in the metabolic tissue within the canopy. N content decreases in lower leaf layers and is directly correlated to the light intensity in these lower leaf layers (Gastal and Lemaire, 2002; Grindlay et al., 1995; Vouillot et al., 1998).

5.3.3. Generalization of the spectral measurement of N uptake and nitrogen nutrition index

Field calibration is necessary to match reflectance measurements (Flowers et al., 2003; Lammel et al., 2001) with the curve of NNI or N uptake. N uptake values increased during canopy growth and NNI values were plus minus one. This opposite behaviour is to be explained because the average NNI increased during canopy development.

For the relations between spectral measurements and N uptake or NNI in wheat and maize differences in absolute values between the years, daytime and probably variety sometimes existed. All these factors need to be quantified in further investigations or an in-field calibration is necessary. This calibration needs destructive methods to estimate the N content and biomass. For farmers this is not practical because it is time-consuming and expensive. To use aids like the SPAD meter may include new sources of error and is also not ideal. The SPAD meter measures also accurately the N status as the NNI (Ortuzar-Iragorri et al., 2005; Vouillot et al., 1998), but it measures only the last fully developed leaf and this can not represent the entire canopy. Additionally SPAD measurements are influenced by the leaf water content (Schlemmer et al., 2005), irradiance intensity and daytime (Martinez and Guiamet, 2004).

For an accurate calibration it is probably necessary to carry out several spectral measurement series to develop different calibration curves for different growth stages. For this calibration further investigations have to be done to better know main factors that were responsible for the strong shift of the curves between the NNI and the REIP in 2004. Different factors could account for this shift like the use of another sensor, different cultivars, pre crop, row direction or soil colour. All these factors have to be quantified to create calibration factors and finally a generalised calibration curve, or the sensor can only be used to detect relative differences (Flowers et al., 2003; Lammel et al., 2001) and no absolute relations between N uptake or NNI and spectral indices can be derived.

5.4. Fertilizer application systems

Soil conditions e.g. water and nutrient supply or exposition and inclination may vary on heterogeneous field sites leading to local differences in plant growth (Auerswald et al., 1997). Differences in soil organic matter content, soil water availability and soil temperature influence nitrogen supply (Cahn et al., 1994; Cambardella et al., 1994). Warm and humid soil conditions enhance nitrogen supply, whereas dry periods decrease it (Plenet and Cruz, 1997). Under rainfed conditions both situations may occur (Kolberg et al., 1999). In very dry periods plants can hardly use any nitrogen from the soil, so they will suffer from a deficiency for a certain period of time. This will lead the plant development to stagnate for some time and the nitrogen supply has to be adjusted (Bahrun et al., 2003). Because of this heterogeneity site specific fertilizer application is necessary. But the current fertilizer application management for maize around the world often disregards the site and annual specific nitrogen demand. Fertilizer application management for wheat in western Europe commonly splits the N in two, three or four rates along programs (Blankenau et al., 2002; Fischbeck et al., 1993) and in maize only one or two rates are common. Plant and soil (Schmidhalter, 2005) analysis is important to control the N status and detect the N demand during vegetation period; nevertheless destructive methods are not appropriate.

Such systems enable an innovative N fertilizer application concept especially for maize where the plants can be used as bioindicators for soil nutrient, growth condition and annual climatic conditions (Selige and Schmidhalter, 2001). Therefore they allow to further develop site-specific variable rate fertilizer application for maize crops. Fertilizer applications can be split either by applying the second rate at growth stage BBCH 14 with liquid fertilizer or with a high clearance tractor up to BBCH 36. In this way it is possible to detect the site-specific N status of the canopy and the applied N rate can be adjusted to the site-specific biomass development and nitrogen uptake for maize to increase yield and N use efficiency (Akbar et al., 1999).

For wheat the NNI offers a new opportunity to determine the N status, because the spectral measurements correlate best with the NNI. Up to now for site specific N application, SPAD meters were used to measure the N status in crops, but in the future the spectrometer will probably be used itself to describe the N status of canopies because it shows whether canopies are in optimal N concentration for maximum biomass production in relation to their actual biomass or if they are lacking nitrogen.

6. Summary

Fields with spatial differences in soil conditions require a variable, locally adjusted nitrogen fertiliser application during the growing season. Spectral measurements can be used to detect canopy N status. The present study investigated the potential of a tractor-based field spectrometer with an oligo-view optical setup to detect biomass, N content, N uptake and the nitrogen nutrition index in winter wheat and maize in a three-years´ field experiment. Validations were performed on large calibration areas of 25 m² for wheat and 10 m² for maize. Nitrogen supply and in maize additionally seeding density were varied to create different canopies on heterogeneous fields. The results obtained showed strong correlations between reflectance indices and N uptake in wheat with $R^2 = 0.92$ from the end of tillering to flowering. Coefficients of determination (R^2) between canopy reflectance and biomass or N content were on a lower level. In maize the results showed strong correlations between reflectance indices and N uptake from the four leaf stage up to flowering. But for the earliest measuring in all years and all measurings in 2004 the R^2 between biomass and canopy reflectance was higher than between N uptake and canopy reflectance.

In wheat additionally the nitrogen nutrition index was estimated and related to spectral measurements. This index is based on the relation between biomass and N content that shows for each biomass the optimal N content of the crop stand to produce maximum biomass during the vegetative stage. The index indicates whether the N content is higher or lower than the optimum for a specific biomass of the canopy. The results of the spectral detection of the NNI were compared to the results obtained for the spectral detection of N uptake. The nitrogen nutrition index was related with $R^2 = 0.95$ to canopy reflectance intensity measured with the REIP and showed better correlations than the N uptake for all measurements across all years in wheat. This parameter is probably desirable for farmers, needing information for fertilizer application decisions because they know directly if the canopy is insufficiently provided with nitrogen.

Thus, the tractor based passive sensor is a fast and suitable means to measure canopy N-status. The optical setup allows measurements being highly independent of day-time, azimuth angle and cloudiness. To support a fertilizer application, it is a useful method to detect nitrogen shortage and offers a possibility to adapt the N rate to the crop demand.

7. Zusammenfassung

Felder variieren kleinräumig in ihren Boden- und Standortseigenschaften. Die Bodentextur, Wassergehalte, Gehalte an organischer Substanz, Nährstoffgehalte und Hangneigung unterscheiden sich oft innerhalb eines Feldes. Eine kleinräumige Anpassung der Düngung, insbesondere der N-Düngung ist daher nötig, um dem unterschiedlichen N-Bedarf der Bestände innerhalb eines Feldes zu entsprechen.
Spektrale Reflexionsmessungen sind geeignet, um den N-Status von Pflanzenbeständen zu bestimmen. Es gibt viele Untersuchungen, die Biomasse, N-Konzentration und N-Aufnahme aus spektralen Messungen von Pflanzenbeständen ableiten. Für die Berechnung des N-Düngebedarfs eignet sich neben der N-Aufnahme auch der Stickstoffernährungs-Index (NNI). Dieser Index berechnet sich aus dem Verhältnis zwischen dem tatsächlichen N-Gehalt zum kritischen N-Gehalt der Pflanzen. Der kritische N-Gehalt beschreibt den minimalen N-Gehalt welcher eine maximale Biomasseproduktion ermöglicht.
Die meisten Untersuchungen wurden bisher mit handgehaltenen Sensoren auf kleinen Referenzflächen durchgeführt. Für unsere Untersuchungen wurde ein auf einem Traktor angebrachtes Feldspektrometer mit einer vierseitigen schrägen Messoptik eingesetzt. Das Gerät enthält zwei Spektrometer, die gleichzeitig die Bestandesreflexion und die Sonneneinstrahlung messen, damit die Messungen nicht von wechselnden Strahlungsbedingungen beeinflusst werden. Durch die schräge Messoptik sollten Messungen auch außerhalb des 10.00 – 14.00 Uhr Zeitfensters ermöglicht werden, im Gegensatz zu Nadirmessungen, die sich auf diese Zeiten beschränken.

In der vorliegenden Arbeit wurde die spektrale Erfassung des N-Status von Mais- und Weizenbeständen mit einer vierseitigen schrägen Messoptik validiert und die Erfassung des Stickstoffernährungs-Indexes mit spektralen Messungen überprüft.
Dazu wurde ein dreijähriger Feldversuch für Mais und Weizen auf heterogenen Böden angelegt, mit jeweils fünf N-Düngungsstufen und mindestens 5 Wiederholungen, um heterogene Pflanzenbestände zu erzeugen. Die Biomassebeprobung wurde auf 10 m² in Mais und 25 m² in Weizen je Parzelle jeweils kurz nach den spektralen Messungen durchgeführt. Die Proben wurden gewogen, getrocknet und im Labor auf N-Gehalt untersucht. Vor jeder Beprobung wurden zwei spektrale Messungen durchgeführt und verschiedene Indizes berechnet.

Die Messungen zeigten einen sehr guten Zusammenhang zwischen N-Aufnahme und den Indizes REIP und R_{780}/R_{740} mit einem konstanten R^2 über 0.90 in Weizen. Die anderen Indizes waren etwas schlechter, wobei die rot basierten Indizes wie der NDVI eher ungeeignet waren zur Bestimmung des N-Status. Dagegen wurde die Biomasse bei Weizen am besten durch die rot basierten Indizes wie den NDVI bestimmt, jedoch mit durchgängig etwas niedrigerem R^2 als die N-Aufnahme. Der N-Gehalt konnte wiederum am besten mit den Indizes REIP und R_{780}/R_{740} ermittelt werden, jedoch mit dem schwächsten R^2, das sich aber mit zunehmender Bestandesentwicklung verbesserte.
Durch die besondere Messoptik wurden die Ergebnisse weder durch die Heterogenität der Felder noch durch den Zenitwinkel der Sonne wesentlich beeinflusst. Lediglich bei stark wechselnden Strahlungsbedingungen und einem Zenitwinkel unter 25° verschlechterte sich das R^2. Durch die schräge Messoptik waren Messungen schon zu BBCH 27 möglich.

Durch die Berechnung des NNI konnte die Bestimmung des N-Status durch spektrale Messungen verbessert werden. Die Bestimmung des NNI durch spektrale Messungen der Bestandesreflexion ergaben für den REIP ein mittleres R^2 von 0.95. Damit korrelierten die Spektralmessungen besser mit dem NNI als mit der N-Aufnahme. Die Berechnungen des NNI zeigten in Weizen eine Verbesserung des Ernährungszustandes mit zunehmender Bestandesentwicklung. Der NNI gibt direkt Auskunft darüber, wie der Ernährungszustand des Pflanzenbestandes ist, wogegen eine Information über die N-Aufnahme zuerst mit einem Bedarfsalgorithmus überlagert werden muss, um den Ernährungszustand zu erkennen. Um die direkte Information des NNI über den Ernährungszustand nutzen zu können, müssen aber noch weitere Faktoren beschrieben werden, welche die absoluten Werte der spektralen Reflexionsmessungen beeinflussen, da die Werte nicht stabil zwischen den Jahren und verschiedenen Feldern waren.

In Mais dagegen waren zwischen den Versuchsjahren deutliche Unterschiede zu erkennen. Reflexionsmessungen korrelierten in einem Jahr mit der N-Aufnahme am besten, im anderen Jahr dagegen zeigte die Biomasse die höchsten R^2 zu den spektralen Messungen. Im dritten Jahr gab es starke Trockenheit und die Ergebnisse waren deutlich schlechter. Dennoch war es auch dann möglich die N-Aufnahme mit einem R^2 von 0.60 zu beschreiben. Da in gut ernährten Beständen die N-Aufnahme hauptsächlich durch den Biomassezuwachs bestimmt wird und der N-Gehalt in C_4 Pflanzen niedriger ist als in C_3 Pflanzen konnten bei Mais die spektralen Messungen möglicherweise nicht eindeutig der N-Aufnahme zugeschrieben werden wie bei Weizen. Bei Mais variiert die N-Nachlieferung des Bodens zwischen den Jahren und während der Vegetationsperiode stärker, da die Umsetzung von organischer Masse durch das Sommerklima stark beeinflusst wird.

Im Mais wurden damit Ergebnisse erzielt, die den Einsatz dieses Systems für eine teilflächenspezifische Düngung ermöglichen, obwohl Spektralmessungen bei Mais sehr deutlich von der Biomasse geprägt sind. Durch den Einsatz dieser Technik kann die N-Düngung mit Flüssigdünger der kleinräumigen Bestandesentwicklung in Mais bis BBCH 14 angepasst werden.

Feldspektroskopische Messungen mit einer vierseitigen Messoptik und simultaner Messung der Bestandesreflexion beziehungsweise der einfallenden Strahlung eignen sich gut zur Bestimmung des N-Status in Winterweizen und geben die N-Aufnahme in Winterweizen und Mais verlässlich wider. Daher eignet sich dieser Sensor für ein operatives System, um in Verbindung mit einem geeigneten Düngealgorithmus eine bedarfsgerechte teilflächenspezifische N-Düngung durchzuführen.

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Lebenslauf

Persönliche Angaben

Geboren	am 26.02.1966 in Heilbronn – Sontheim
Familienstand	verheiratet, 4 Kinder

Berufstätigkeit

1985 -1986	Obst- und Weingärtner bei Bernhard Mistele
1986 -1987	Zivildienst bei Ev. meth. Kirche in Esslingen
1987 -1988	Obst- und Weingärtner bei Bernhard Mistele
1988 -1989	Obst- und Weingärtner bei Rudolf Nanz in Stuttgart
1989 -1991	Obst- und Weingärtner bei Bernhard Mistele
1992 -1993	Gärtnermeister und Winzer bei Bernhard Mistele
1993 -1994	selbstständiger Betriebsleiter eines Obst- und Weinbaubetriebs
1995 -1996	Winzermeister beim Schlossgut Hohenbeilstein in Beilstein
2002 - heute	Wissenschaftlicher Mitarbeiter am Lehrstuhl für Pflanzenernährung der TUM

Ausbildung

1983 -1985	Ausbildung als Winzer
1991 -1992	Meisterschule zum Gärtnermeister an der LVWO Weinsberg
1997 -2002	Universität Hohenheim: Studium der allg. Agrarwissenschaften

Schulbildung

1972 -1981	Besuch der GHS-Ilsfeld
1981 -1983	Besuch der 2-jährigen landwirtschaftlichen Berufsfachschule
1996 -1997	Besuch des 1-jährigen Berufskolleg zur Fachhochschulreife
30.06.1997	Abiturprüfung beim Oberschulamt in Stuttgart (Begabtenprüfung)