675

Europ. J. Agronomy 29 (2008) 184–190

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Estimating the nitrogen nutrition index using spectral canopy reflectance measurements

Bodo Mistele, Urs Schmidhalter*

Department of Plant Science, Life Science Centre Weihenstephan, Technical University of Munich, Am Hochanger 2, 85354 Freising, Germany

ARTICLE INFO

Article history:
Received 11 January 2008
Received in revised form 2 May 2008
Accepted 21 May 2008

Keywords:
Biomass
Hyperspectral
N content
N status
N uptake
Precision farming
Precision phenotyping
Spectroscopy
Wheat

ABSTRACT

Spectral measurements are useful for estimating the nitrogen status of crops, thereby enabling site-specific fertilizing in precision farming systems. To date, however, spectral information was associated primarily to nitrogen (N) uptake or to chlorophyll content per unit area, but using it to determine the effective nitrogen (N) need of a crop requires additional information given that N uptake increases with canopy development.

The nitrogen nutrition index (NNI) describes the nitrogen status of the entire crop directly, analogous to what the SPAD meter does on the leaf level. The index is the ratio between the actual nitrogen content of the crop and the critical content indicating the minimum N content required for the maximum biomass production of a canopy. As such, the index indicates whether the N content is higher or lower than the optimum level for a specific crop biomass and so matches the exact needs of the farmer. We examine the relationship between the NNI and the canopy reflectance intensity (CRI) as a prelude to their use in a site-specific farming framework. The NNI/CRI correlation was validated in a three-year field experiment with winter wheat with an overall average R^2 of 0.95. The NNI was more closely correlated with the CRI than with any of total aerial N, N content or biomass dry weight. As such, it appears that the NNI can indeed be determined in a rapid, cost-effective fashion using spectra-based measurements.

1. Introduction

Many scientists describe the biomass production of crops as a function of nitrogen (N) content in plants (Lemaire et al., 2005; Farruggia et al., 2004; Gastal and Lemaire, 2002; Devienne-Barret et al., 2000). However, accurately summarizing N content as a prelude to optimal N fertilizer application is complicated by the complex distribution of this variable. The N content of plants is highest at early growth stages and decreases continually up to the stage of senescence, with this reduction in N content typically being interpreted as a dilution effect of growth through differential tissue N contents. For example, whereas metabolic tissues such as leaf lamina are known to be rich in N (6.5%), structural tissues such as veins are N poor (0.8%) (Lemaire and Gastal, 1997). During leaf growth, the proportion of structural tissue increases relative to the metabolic tissue such that the N content is highest in young leaves and decreases in the leaves of isolated plants without shadowing effects when the leaf area increases. This same phenomenon affects not only the leaves but also the entire plant, demonstrating that N content depends on the plant biomass. Thus, if two different plants have the same N content but differ in biomass, it is possible that the plant with the higher biomass is well supplied with N, whereas the one with the lower biomass may suffer from N shortage (Lemaire et al., 1995; Lemaire et al., 1992).

For plants in crop stands, N distribution follows these same rules, but with a further N-distribution gradient vertically within the canopy. This gradient is directly correlated to light intensity in the canopy (Eichelmann et al., 2005; Gastal and Lemaire, 2002; Grindlay et al., 1995). In the upper leaf layers, plants show the highest N contents, and ones that correspond to the relative proportions of metabolic and structural tissues. N content for the underlying leaf layers decreases linearly with decreasing light intensity up to the light compensation point, where the leaves become senescent. Beyond this point, the plant material dies and consists solely of structural tissue and N content is as low as 0.8% (Lemaire and Gastal, 1997).

Thus, optimal nitrogen fertilizer application regimes in crop production have two requirements: (1) knowledge of the adequate N content for a given amount of biomass and (2) the development of fast, accurate methods to determine the actual N content and biomass (or N demand) of the crop plant to relate it to (1) as well as the soil N-supply (Schmidhalter, 2005). For winter wheat, much

---

* Corresponding author. Tel.: +49 8161 713390; fax: +49 8161 714500.
E-mail address: Schmidhalter@wzw.tum.de (U. Schmidhalter).

1161-0301/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.eja.2008.05.007

work has already been done for (1), with several studies having examined the minimum ("critical") N content required for maximum biomass production from tillering up to flowering (Lemaire et al., 2005; Devienne-Barret et al., 2000; Lemaire et al., 1992; Greenwood et al., 1991). This information is incorporated in the nitrogen nutrition index (NNI) (Lemaire et al., 1992; Greenwood et al., 1991) to indicate the relative N status of a plant. The index, which is calculated as the ratio between the measured N content and the critical N content is greater than one in canopies in an ample N state and less then one in ones lacking nitrogen.

The NNI can be easily transformed to the critical amount of N, plants should have absorbed at a certain amount of biomass. Subtracting the critical amount of N from the N uptake by the crop, one can calculate the canopy N deficit in kg ha^{-1} (Houlès et al., 2007).

For the second requirement, spectral measurements appear to offer several advantages over more traditional methods. Destructive methods for estimating the N content and calculating the NNI are indeed very accurate, but also time consuming and expensive. Optical transmission measurements with a handheld chlorophyll meter (SPAD) are equally time consuming with the values also being influenced by the leaf water content and in that way distorted by daytime and weather conditions influencing the specific leaf weight (Schlemmer et al., 2005). More crucially, the measurements are accurate only for the measured spot (Cartelat et al., 2005; Blackmer et al., 1994), which is not sufficient on the canopy level because of differential measurement positions in the canopy. Such devices measure only one point of the last fully developed leaf, but N content is neither homogeneous within the flag leaf (Cartelat et al., 2005) nor within the canopy (Eichelmann et al., 2005; Lemaire and Gastal, 1997; Pons et al., 1993).

Values derived from spectral measurements admittedly depend predominantly on the amount of chlorophyll. The best relationships between chlorophyll and reflectance measurements have been obtained on the leaf level (Cartelat et al., 2005; Gitelson et al., 2003). Unfortunately, the relationship between chlorophyll content and N content, upon which spectral based measurements of NNI would depend, is disputed: Read et al. (2002) found only a weak correlation between these two variables ($R^2 = 0.32$) in contrast to the very strong correlation found by Cartelat et al. (2005) (R^2 of 0.97). On the canopy level, however, spectral measurements appear to correlate well with total aerial N (Lukina et al., 2001; Schmidhalter et al., 2003; Mistele and Schmidhalter, 2008).

However, the apparent relationship between reflectance and light distribution within the canopy means that spectral measurements may indeed be well suited to record the N distribution within the canopy (Vouillot et al., 1998). In the upper leaf layer, both the N content and illumination are high (Gastal and Lemaire, 2002). Lower leaf layers are increasingly less illuminated and a lower proportion of the reflection signal is to be expected here. As such, the mixed canopy reflectance promises to give a weighted signal from all leaf layers that might possibly also represent the N content from the different canopy layers.

Specific knowledge about the relationship between NNI and spectral canopy reflectance is limited. Link and Jasper (2003) used the optimum N rate for maximum yield and related this to spectral measurements. However, this method worked only in the reverse direction such that it was not useful for accurate estimates of the crop canopy N status. In addition, Schepers et al. (1998) defined an analogous nitrogen sufficiency index (NSI) based on either canopy reflectance or transmittance information. The index excludes years and sites effects, but the system requires reference plots in that the optical canopy information must be referenced against that from plants where N is not limited. For example, nitrogen fertilizer is not applied in the stress-based N treatment until reflectance of corn reached less than 96% of the reflectance of well-fertilized corn. A report fund that estimates of NNI and values of the leaf reflectance measured with a NIR/red ratio were related to each other with $R^2 = 0.78$ (Vouillot et al., 1998). Additionally, N uptake calculated as foliar N content (leaf N content per m^2 × LAI) was found to be related to spectral reflectance measurements with $R^2 = 0.93$. However, these investigations were limited in the present context insofar as they were based on leaf level measurements.

The objective of this study was to further assess the utility of spectral methods with respect to optimal N fertilization regimes by investigating the relationship between spectral reflectance values and NNI in winter wheat. A particular point of focus was whether or not this index is more closely related to spectral measurement values than are values of total aerial N (N uptake).

2. Material and methods

2.1. Experimental fields

All experiments were conducted on fields at the Dürnast Research Station of the Technical University of Munich in Freising to the north of Munich in southeast Germany. Winter wheat (*Triticum aestivum* cv. Ludwig, cropped with 50% Tommi in 2004) was examined in a three-year field experiment (years 2002–2004). The average annual temperature was 7.5 °C and the average annual precipitation was 800 mm per year with the highest values occurring in the summer. The experiments were conducted on different fields each about 3 ha in size. Heterogeneous fields were chosen to obtain differences in both the N status and biomass. Soil characteristics differed within and among the fields, with the soil heterogeneity being cogently indicated by the apparent electrical conductivities varying between 20 and 50 mS m^{-1} (Mistele and Schmidhalter, 2008). The experimental design consisted of five fertilization rates (0, 90, 130, 170 and 210 kg N ha^{-1}) each with five replicates for a total of 25 plots. Each plot was 15 m in width and 50–60 m in length. Fertilizer was applied at the four growth stages BBCH 22, 30, 40 and 50 (Bleiholder et al., 2001). BBCH stands for Biologische Bundesanstalt, Bundessortenamt und Chemical industry. The BBCH-scale is based on the well-known cereal code developed by Zadoks et al. (1974). 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.

2.2. Spectral measurements

A tractor mounted field spectrometer similar to the Yara sensor (Yara GmbH & Co. KG, Dülmen, Germany) was used to get spectral information of the crop canopy reflectance. In contrast to the Yara sensor configuration the sensor was not placed on the tractor roof, but in front of the tractor that allowed positioning the sensor setup at comparable heights above the crop stand during the season. This sensor contained two spectrometers with bandwidths of 3.3 nm and measured canopy reflectance and incident radiation simultaneously. The sensor can measure 256 bands with a spectral detection range from 400 to 1000 nm. Whereas in the years 2002 and 2003 a five-wavelength scan mode was used, another sensor with a modified electronic (tec5, Oberursel, Germany) allowed to take hyperspectral readings for the measurements in 2004. This sensor was identical in construction, but equipped with different electronics, software and calibration algorithms.

The unit measuring the global radiation was linked to a cosine-corrected diffuser and the second unit was linked to a four-in-one light fibre that took measurements at all four edges of the tractor with an oblique oligo view optic. The advantage of this optical

Table 1
Spectral measurement conditions in the years 2002, 2003 and 2004

Sampling	Year							
	2002		2003			2004		
	First	Second	First	Second	Third	First	Second	Third
Growth Stage (BBCH)	32	55	30	39	65	27	32	71
Date	May 15	June 3	May 5	May 19	June 5	April 26	May 12	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	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	62 ± 0
Solar radiation ($W m^{-2}$)	844	790	750	100	840	580	710	410
Weather conditions	Sunny	Sunny	Sunny	Sunny hazy	Sunny	Sunny-cloudy	Sunny hazy	Sunny-cloudy

setup was that one optic always measured the sunlight exposed side of the plants whereas another always measured the shady side, so as to minimize any effects of the zenith angle (Reusch, 2003; Mistele and Schmidhalter, 2008). A further advantage was that the measured area (four ellipses at the four edges of the tractor) fell outside of the shadow of the tractor. Each ellipse was 1.8 m^2 in area, altogether 7.6 m^2 in size. When the tractor moved along the track, 1.5 m wide strips on each sides of the tractor were measured. Spectral measurement conditions in the years 2002, 2003 and 2004 are indicated in Table 1.

The canopy reflectance intensity was measured at 670, 700, 740 and 780 nm. To depict the spectral canopy information, we calculated the red edge inflection point (REIP) (Guyot et al., 1988), which has been shown to describe N uptake the best (Mistele et al., 2004; Plenet and Cruz, 1997):

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

2.3. Biomass sampling

Information about the biomass and the N status of the crops within the plots was obtained from samples taken from exactly the area measured by the sensor. The plants were cut with a green forage chopper equipped with a weighing unit (Schmidhalter et al., 2003) to record the biomass immediately after cutting. The sampled areas on each side of the tramline were each 1.5 m in width and around 4 m in length and were exactly within the field of view of the sensor. A representative subsample was oven dried to estimate the dry matter. The subsamples were subsequently 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). Total aerial N was calculated as dry matter yield × N content.

2.4. 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):

$$N_c = 5.35 \times W^{-0.442},$$

where N_c is the critical N content as a percentage of dry matter and W is the dry weight of above-ground biomass in Mg ha^{-1}. This equation can be applied most robustly when shoot dry weight is in the range 1.55–12 Mg ha^{-1} and for growth stages ranging from BBCH 30 to 65. Although the critical N content may be constant at a mean value of 4.4% for shoot dry weight values between 0.2 and 1.55 Mg ha^{-1} (Justes et al., 1997), we did not pay attention to this restriction because the relationship between spectral measurements and plant parameters is a continual function that does not fit to a discontinual function like this. To indicate the N status for each plot, independent of the growth stage and differing biomasses, the nitrogen nutrition index (NNI) was calculated as follows:

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

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

For the statistical analysis, we used SPSS 11 (SPSS Inc., Chicago, USA). Simple regressions were mainly calculated. Curvilinear models were also examined and coefficients of determination averaged over all plots in one field were calculated. As such, differences in soil conditions, fertilizer application rates, slopes and water supply were not considered. Mean values were not included in the analyses because we expected large interplot differences due to heterogeneous soil and field conditions being independent of the fertilizer rate.

3. Results

The results of the destructive analysis of the biomass samples are indicated in Fig. 1. The N status during crop growth differs between the three experimental years. In 2002, 10% and 20% of the plots possessed adequate N statuses at the first and second samplings, respectively. In 2003, all plots displayed N deficiency in the first two samplings, although 20% were in an optimum N state by the third sampling; a clear improvement in the N status from the first to the third samplings was clearly evident. The results in 2004 were different compared to the other two years. Here, 4%, 22%, and 11% of the plots indicated a sufficient N status of the crops in the first (very early growth stage) to third samplings, respectively. For all samplings, the N status was considerably improved compared to the previous years.

An increase in the N status could be observed for all three experimental years from tillering to flowering as indicated in Fig. 1(B, D and F).

The relationship between NNI and canopy reflectance intensity (CRI) was curvilinear (Fig. 2), except for some of the early measurements in 2003 where all plots had a NNI lower than 1. For these early measurements, Fig. 2 shows that the relationship between NNI and CRI is increasingly linear the lower the average NNI. Similar observations also apply to the relationship between N uptake and canopy reflectance. The R^2 between NNI and CRI was higher than that between total aerial N and CRI for all measurements, with the average R^2 across all measurements being greater than 0.92 and even higher than 0.96, except for one early measurement in 2004.

The fitted curves between NNI and CRI for the different samplings within each of 2002 and 2003 paralleled one other (Fig. 2). In 2002, the curves for the different samplings were also closer together than those between N uptake and CRI. The pattern of the relationships between CRI and each of N uptake and NNI were

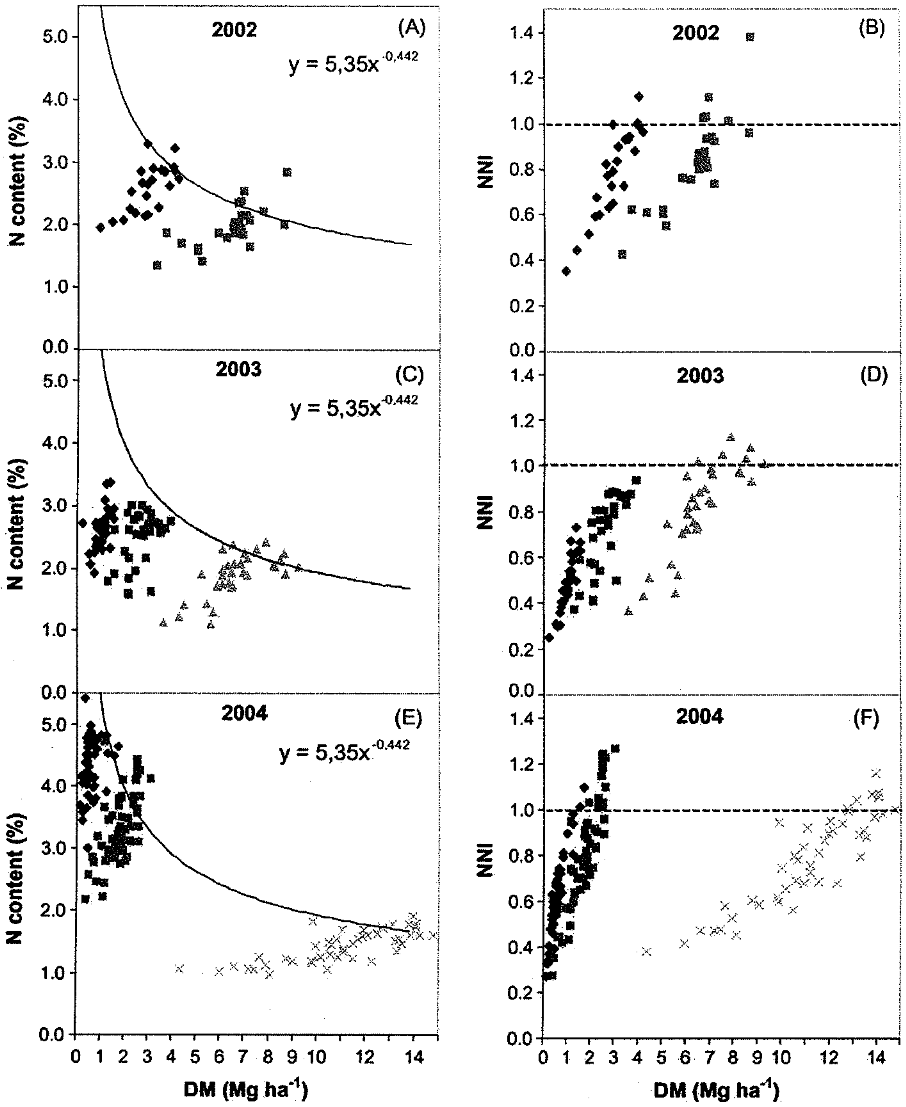

Fig. 1. Destructive analysis of the biomass samples. (A, C and E) Relationships between biomass dry weight and N content with critical N curves (Justes et al., 1997). (B, D and F) Relationships between biomass dry weight and nitrogen nutrition index (NNI). All results are split into the first, second, third, and third samplings (◆, ■, ▲, and ×, respectively) for each of the years from 2002 to 2004.

generally similar in 2002, the only difference being that the lines for the two samplings reversed their relative positions. For the relationships between NNI and CRI the slope of the regression curves tended to increase 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 tended to decrease and the values decreased. By contrast, a slightly different pattern was observed in 2004 in that the different sampling curves for N uptake and CRI paralleled one other strongly, but not those for NNI and CRI.

Finally, the total range of REIP values observed differed from year to year. In each year, the minimum REIP value was 719 nm, but the maximum value increased from 728 to 738 nm from 2002 to 2004.

4. Discussion

Our results demonstrate that spectral measurements are apparently useful for describing the N status of wheat canopies, being closely related to both NNI and total aerial N (and then slightly more so to the former). The use of NNI to describe the N status of crop canopies is particularly desirable because it reflects whether or not the plants have an optimal N concentration for maximum biomass production in relation to their actual biomass. By contrast, N uptake or N content values, which do not consider the amount of biomass in the field, cannot contain any direct information about the specific N need for the actual amount of biomass. For example, in a sandy area within the field where plants are suffering because of insufficient water, N uptake and the biomass may both be low, but the N content may still be similar to other parts of the field with good growth conditions that enable higher biomass and N uptake (Mirschel et al., 2005; Eck, 1988). The NNI, however, would present a lower value for the drought affected crop stand because it considers biomass directly and not simply the growth stage. Therefore, the NNI can deliver valuable information for fertilizer application decisions.

Moreover, this information can be achieved fast, easily and non-destructively through spectral-based measurements. Canopy reflectance measurements based on the REIP showed no important

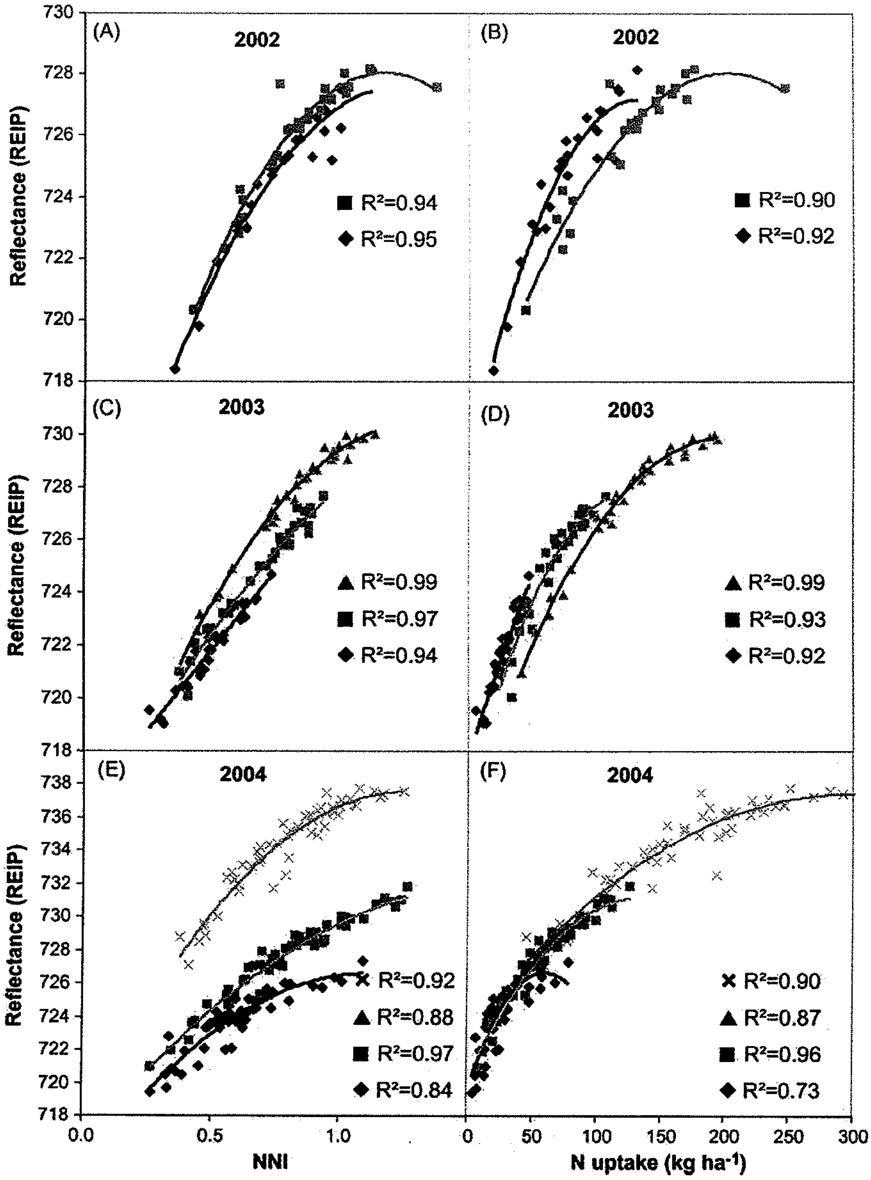

Fig. 2. Spectral detection of the N status with. (A, C and E) Relationships between the nitrogen nutrition index (NNI) and reflectance intensity of the crop canopy calculated as REIP. (B, D and F) Relationships between total aerial N (kg ha^{-1}) and crop canopy reflectance calculated as REIP. All results are split into the first, second, third, and third samplings (◆, ■, ▲, and ×, respectively) for each of the years from 2002 to 2004.

differences in their relationship to either the NNI or the total aerial N. The relationships in both cases were very strong and slightly more so for NNI. This result differs from those reported in other papers that found closer relationships between spectral measurements and total aerial N than for other crop canopy parameters (Link et al., 2005; Mistele et al., 2004). In particular, we found a closer relationships with NNI ($R^2 = 0.94$) than that reported by Vouillot et al. (1998) ($R^2 = 0.78$).

If spectral measurements could also detect the biomass of a canopy, the NNI would be useful to calculate the amount of N which should be applied to the crop (Houlès et al., 2007). But spectral measurements describe N status ($R^2 = 0.94$) better than biomass ($R^2 = 0.75$) (Mistele et al., 2004).

The critical N content for a given biomass is the basis upon which the NNI depends. This relationship is based on many experiments in France with increasing rates of N application, and is reported to be largely independent of growth rate, density, cultivar and pedoclimatic conditions (Justes et al., 1997). To our knowledge, however, the model has not yet been validated in Germany. For our investigations we also tested other parameters within the model for critical N content, but none improved the relationship between NNI and REIP.

The curves relating NNI and the REIP measurements for the different biomass samples showed mainly a shift in the values, and most strongly so in 2004. However, the first measurement was arguably performed too early in 2004 because the average biomass was only 580 kg ha^{-1}, whereas the model described by Justes et al. (1997) is really only validated for values in excess of 1500 kg ha^{-1} biomass, the lower value examined. As such, it might be difficult to differentiate crop canopies in earlier growth stages because less structural tissue is found and the same relationship between structural tissue and metabolic tissue applies. The last measurements might have been late because nitrogen becomes accumulated in the kernels after flowering, with this stored nitrogen not deriving from either structural or metabolic tissue and so distorting the NNI (Lemaire and Gastal, 1997), but the values were still closely related to each other at $R^2 = 0.92$ being only slightly lower than for earlier measurements. Additionally, a number of factors might explain the shift of the curves in 2004. Two different cultivars that resembled one another strongly (Sticksel et al., 2004) were used accidentally in 2004, a fact that we did not discover until afterwards. Finally, the use of a modified sensor with different electronics, software and calibration methods could also well be responsible for the different behaviour observed in 2004. However, shift in the measurements were also observed in 2002 and 2003, albeit of a far smaller magnitude. Houlès et al. (2007) found that the relationship between NNI and the leaf chlorophyll content could differ as well where points were either well grouped in one year or more distinct from one date to another in a different year. Such behaviour has been explained to be eventually linked to the dilution phenomenon, the nitrogen content in the plant falling more rapidly than the chlorophyll content (Houlès et al., 2007). It is noteworthy, however, that the curves obtained between N uptake and CRI were much more grouped together than between NNI and CRI in 2004.

The efficiency offered by spectral measurements is offset slightly by the field calibrations required to relate CRI values to NNI or total aerial N absolutely (to account for the shifts along the x-axes observed). This ordinarily needs destructive methods to estimate the N content and biomass. For farmers, however, this solution is not practical because it is time-consuming and costly. Use of other aids such as the SPAD meter may include new sources of error as explained in Section 1. As such, a reasonable compromise would be to define absolute curves for different amounts of biomass. For this calibration, further investigations have to be performed to determine the main factors influencing the relationship between NNI and REIP (and therefore underlying the differences observed in 2004). A number of potential factors were already outlined above: the use of another sensor, different cultivars, dilution phenomena. All these factors need to be quantified to create a generalized calibration curve and the accuracy of this curve would then need to be determined empirically to determine whether or not it is acceptable under field conditions.

5. Conclusions

Spectral measurements were useful to describe the NNI in winter wheat, whether or not the canopy was deprived or adequately supplied with N in particular. The results showed high R^2-values between canopy reflectance (REIP) and NNI, ones that were slightly better than those between canopy reflectance and total aerial N. This information about the N status of crop stands by using spectral reflectance measurements is useful to support nitrogen fertilizer application within a Precision Farming or Precision Phenotyping framework, especially because the tractor-mounted measurements allowed N status to be estimated quickly and non-destructively. However, because there was still a small shift in the REIP values between the different biomass samplings, the field spectrometer has to be calibrated according to the given field. The factors, that potentially are responsible for this shift, and therefore need to be accounted for in any generalized calibration methods, have to be investigated further. It remains a particular challenge to test whether remotely acquired information as compared to proximal detection of the nitrogen status can be done as precisely and effectively.

Acknowledgment

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

References

Bleiholder, H., Buhr, L., Eicke, H., Feller, C., Hack, H., Hess, M., Klose, R., Lancashire, P.D., Meier, U., Stauss, R., Weber, E., von den Boom, T., 2001. In: Meier, U. (Ed.), Growth Stages of Mono- and Dicotyledonous Plants. BBCH Monograph. Wissenschafts-Verlag, Blackwell.

Blackmer, T.M., Schepers, J.S., Varvel, G.E., 1994. Light reflectance compared with other nitrogen stress measurements in corn leaves. Agronomy Journal 86, 934–938.

Cartelat, A., Cerovic, Z.G., Goulas, Y., Meyer, S., Lelarge, C., Prioul, J.L., Barbottin, A., Jeuffroy, M.H., Gate, P., Agati, G., Moya, I., 2005. Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (*Triticum aestivum* L.). Field Crops Research 91, 35–49.

Devienne-Barret, F., Justes, E., Machet, J.M., Mary, B., 2000. Integrated control of nitrate uptake by crop growth rate and soil nitrate availability under field conditions. Annals of Botany 86, 995–1005.

Eck, H.V., 1988. Winter wheat response to nitrogen and irrigation. Agronomy Journal 80, 902–908.

Eichelmann, H., Oja, V., Rasulov, B., Padu, E., Bichele, I., Pettai, P., Mänd, P., Kull, O., Laisk, A., 2005. Adjustment of leaf photosynthesis to shade in a natural canopy: reallocation of nitrogen. Plant, Cell and Environment 28, 389–401.

Farruggia, A., Gastal, F., Scholefield, D., 2004. Assessment of the nitrogen status of grassland. Grass and Forage Science 59, 113–120.

Gastal, F., Lemaire, G., 2002. N uptake and distribution in crops: an agronomical and ecophysiological perspective. Journal of Experimental Botany 53, 789–799.

Gitelson, A.A., Gritz, Y., Merzlyak, M.N., 2003. Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. Journal of Plant Physiology 160, 271–282.

Greenwood, D.J., Gastal, F., Lemaire, G., Draycott, A., Millard, P., Jeeteson, J.J., 1991. Growth rate and %N of field grown crops: theory and experiments. Annals of Botany 67, 181–190.

Grindlay, D.J.C., Sylvester-Bradley, R., Scott, R.K., 1995. The relationship between canopy green area and nitrogen in the shoot. In: Lemaire, G., Burns, I.G. (Eds.), Diagnostic Procedures for Crop N Management. INRA Editions, pp. 53–60.

Guyot, G., Baret, F., Major, D.J., 1988. High spectral resolution: Determination of spectral shifts between the red and the near infrared. International Archives of Photogrammetry and Remote Sensing 11, 750–760.

Houlès, V., Guérif, M., Mary, B., 2007. Elaboration of a nitrogen nutrition indicator for winter wheat based on leaf area index and chlorophyll content for making nitrogen recommendations. European Journal of Agronomy 27, 1–11.

Justes, E., Jeuffroy, M.H., Mary, B., 1997. Wheat, barley, and durum wheat. In: Lemaire, G. (Ed.), Diagnosis of the Nitrogen Status in Crops. Springer-Verlag, pp. 73–92.

Lemaire, G., Avice, J.-C., Kim, T.-H., Ourry, A., 2005. Developmental changes in shoot N dynamics of lucerne (*Medicago sativa* L.) in relation to leaf growth dynamics as a function of plant density and hierarchical position within the canopy. Journal of Experimental Botany 56, 935–943.

Lemaire, G., Gastal, F., 1997. N uptake and distribution in plant canopies. In: Lemaire, G. (Ed.), Diagnosis of the Nitrogen Status in Crops. Springer-Verlag, pp. 3–41.

Lemaire, G., Gastal, F., Plenet, D., 1995. Dynamics of N uptake and N distribution in plant canopies. Use of crop N status index in crop modelling. In: Lemaire, G., Bruns, I.G. (Eds.), Diagnostic Procedures for Crop N Management. INRA Editions, pp. 15–29.

Lemaire, G., Khaity, M., Onillon, B., Allirand, J.M., Chartier, M., Gosse, G., 1992. Dynamics of accumulation and partitioning of N leaves, stems and roots of lucerne in a dense canopy. Annals of Botany 70, 429–435.

Link, A., Jasper, J., 2003. Site-specific N fertilization based on remote sensing - is it necessary to take yield variability into account? In: Stafford, J., Werner, A. (Eds.), Precision Agriculture Proceedings of the 4th European Conference on Precision Agriculture. Academic Publishers, Wageningen, pp. 353–359.

Link, A., Jasper, J., Reusch, S., 2005. Suitability of different crop parameters for the determination of site-specific nitrogen fertilizer demand. In: Stafford, J. (Ed.), Precision Agriculture '05 Proceedings of the 5th European Conference on Precision Agriculture. Academic Publishers, Wageningen, pp. 297–303.

Lukina, E.V., Freeman, K.W., Wynn, K.J., Thomason, W.E., Mullen, R.W., Stone, M.L., Solie, J.B., Klatt, A.R., Johnson, G.V., Elliott, R.L., Raun, W.R., 2001. Nitrogen fertilization optimization algorithm based on in-season estimates of yield and plant nitrogen uptake. Journal of Plant Nutrition 24, 885–898.

Mirschel, W., Wenkel, K.O., Schultz, A., Pommerening, J., Verch, G., 2005. Dynamic phenological model for winter rye and winter barley. European Journal of Agronomy 23, 123–135.

Mistele, B., Gutser, R., Schmidhalter, U., 2004. Validation of field-scaled spectral measurements of the nitrogen status in winter wheat. In: Mulla, D.J. (Ed.), Proceedings of the 7th International Conference on Precision Agriculture and other Precision Resources Management. Minneapolis, MN, USA, pp. 1187–1195 (CD ROM).

Mistele, B., Schmidhalter, U., 2008. Spectral measurements of the total aerial N and biomass dry weight in maize using a quadrilateral-view optic. Field Crops Res. 106, 94–103.

Plenet, D., Cruz, P., 1997. Maize and sorghum. In: Lemaire, G. (Ed.), Diagnosis of the Nitrogen Status in Crops. Springer-Verlag, pp. 93–105.

Pons, T.H., Rijnberk, V.H., Scheurwater, I., Wert, A., 1993. Importance of the gradient in photosynthetically active radiation in a vegetation stand for leaf nitrogen allocation in two monocotyledons. Oecologia 95, 416–424.

Read, J.J., Tarpley, L., McKinion, J.M., Reddy, K.R., 2002. Narrow-waveband reflectance ratios for remote estimation of nitrogen status in cotton. Journal of Environmental Quality 31, 1442–1452.

Reusch, S., 2003. Optimisation of oblique-view remote measurement of crop N-uptake under changing irradiance conditions. In: Stafford, J., Werner, A. (Eds.), Precision Agriculture Proceedings of the 4th European Conference on Precision Agriculture. Academic Publishers, Wageningen, pp. 573–578.

Schepers, J.S., Blackmer, T.M., Francis, D.D., 1998. Chlorophyll meter method for estimating nitrogen content in plant tissue. In: Kalra, Y.P. (Ed.), Handbook on Reference Methods for Plant Analysis. CRC Press, pp. 129–135.

Schlemmer, M.R., Francis, D.D., Shanahan, J.F., Schepers, J.S., 2005. Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. Agronomy Journal 97, 106–112.

Schmidhalter, U., 2005. Development of a quick on-farm test to determine nitrate levels in soil. Journal of Plant Nutrition and Soil Science 168, 432–438.

Schmidhalter, U., Jungert, S., Bredemeier, C., Gutser, R., Manhart, R., Mistele, B., Gerl, G., 2003. Field-scale validation of a tractor based multispectral crop scanner to determine biomass and nitrogen uptake of winter wheat. In: Stafford, J., Werner, A. (Eds.), Precision Agriculture: Papers from the 4th European Conference on Precision Agriculture. Wageningen Academic Publishers, pp. 615–619.

Sticksel, E., Huber, G., Liebler, J., Schächtel, J., Maidl, F.X., 2004. The effect of diurnal variations of canopy reflectance on the assessment of biomass formation in wheat. In: Mulla, D.J. (Ed.), Proceedings of the 7th International Conference on Precision Agriculture. Minneapolis, MN, USA, pp. 1177–1186 (CD ROM).

Vouillot, M.O., Huet, P., Boissard, P., 1998. Early detection of N deficiency in a wheat crop using physiological and radiometric methods. Agronomie 18, 117–130.

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Research 14, 415–421.