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High-throughput phenotyping of biomass, N status and yield development in wheat (*Triticum aestivum* L.)

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Zusammenfassung

Eine einfache und kostengünstige Erfassung von Pflanzenparametern und Sorteneigenschaften ist sowohl für die Pflanzenproduktion wie auch für die Pflanzenzüchtung erwünscht. Spektrale Reflektionsmessungen bieten hierbei eine Möglichkeit Pflanzeneigenschaften schnell zu bestimmen. Sensorsysteme wurden nur selten auf Basis destruktiv erfasster Bestandesparameter verglichen. Die spektrale Beobachtung von Beständen war bisher meist auf das vegetative Wachstum von Getreide beschränkt, obwohl die Kornfüllungsphase großen Einfluss auf den Kornertrag besitzt. Ziel dieser Arbeit war es, passive und aktive Sensoren in ihrer Messqualität und in der Erfassung von Bestandesparametern bei Weizen zu vergleichen. Spektrale Parameter wurden auch getestet, inwiefern durch diese die Verlagerung von Trockenmasse während der Kornfüllung verschiedener Weizensorten unterschieden werden kann.

In den Jahren 2009 und 2010 wurden auf der Versuchsstation Dürnast der Technischen Universität München Feldversuche angelegt, in denen bis zu sieben Hochertragssorten bei einer Stickstoffversorgung von 0 kg N ha^{-1} bis 420 kg N ha^{-1} angebaut wurden. Zwischen Vegetationsbeginn und Ernte wurden regelmäßig Biomasseproben entnommen, deren Frisch- und Trockenmassen bestimmt und Parameter des Ernährungszustandes ermittelt. Vor den jeweiligen Zwischenernten fanden Reflektionsmessungen mit der Messplattform Phenotrac IV statt, wobei die Messwerte mit hochgenauen GPS-Daten georeferenziert wurden. Zusätzlich wurden neunzig Weizensorten sowohl mittels visueller Bonitur als auch mit Spektralmessungen bezüglich ihrer Bestandesdichte klassifiziert.

Auf Grund ihrer technischen Voraussetzungen unterschieden sich die getesteten Sensorsysteme bezüglich der Variation ihrer Einzelmessungen. Auf Plot-Ebene zeigten sie jedoch große Ähnlichkeiten in der Differenzierung der Bestandesparameter, aber nur wenn ähnliche Spektralparameter verglichen wurden. Die Spektralparameter selbst wurden stark vom Entwicklungsstadium und dem Ernährungszustand der Pflanzen beeinflusst. Bei hohen Bestandesdichten zeigten Reflektionswerte, welche auf sichtbarem Licht basieren, starke Sättigungseffekte, während auf Nahinfrarotlicht basierende Parameter vergleichsweise unempfindlich reagierten. Die Biomasseverlagerung während der Kornfüllung konnte mit Spektralmessungen gut erfasst werden. Ährengewicht und -trockensubstanz zeigten enge Zusammenhänge mit dem Kornertrag als auch mit Spektralparametern. Mit den Spektralmessungen konnte die Korn-Trockensubstanz und damit die physiologischen Reife am besten widergegeben werden. Durch die Kombination von Spektral- und Ultraschallmessungen wurde die Sichtbonitur der Bestandesdichte verbessert. Reflektionsmessungen stellen somit eine effektive Methode dar, traditionelle Boniturmethoden zu optimieren und Getreidesorten bezüglich ertragsrelevanter Pflanzenparameter zu bewerten.

Summary

The easy and cheap assessment of plant development is relevant in crop production and for a rapid evaluation of crossbreds in plant breeding. Spectral reflectance measurements offer a possibility to rapidly determine plant traits. However, sensor systems and spectral parameters have not extensively been compared in their ability to assess crop attributes based on destructively obtained plant traits. Furthermore, most spectral crop observations were concentrated on the vegetative phase of development, although the grain filling phase is crucial for yield formation. In this study, we compared four passive and active sensor systems in their quality of signal recording and in differentiating crop biomass and nitrogen status. Consequently, post-anthesis measurements were run to distinguish wheat cultivars in grain filling characteristics.

In 2009 and 2010, field trials were set up at the research station Dürnast of the Technische Universität München, Germany. Up to seven high yielding wheat cultivars were grown at nitrogen supply levels of 0 kg N ha^{-1} to up to 420 kg N ha^{-1}. Biomass samples were taken regularly between tillering and final harvest. Weight and dry matter content of different plant tissues were recorded and indicators of N status were assessed. Before biomass sampling, spectral reflectance was measured by a tractor-based measuring platform, Phenotrac IV, and were co-recorded with GPS data. Similar to that, 90 wheat cultivars were rated visually and spectrally for crop density.

As a consequence of their technical design, the spectral devices tested in this study strongly varied in the within-plot variation of their recordings. However, on plot level they offered comparable results in differentiating crop parameters but only when similar spectral parameters were chosen. Spectral parameters were strongly affected by the development stage and the nutritive status of the crop. Whereas visible light-based reflectance showed saturation effects with dense crop stands, near-infrared-based reflectance was more resistant to saturation at high N levels. Compared to relationships between spectral parameters and final grain yield, better relationships were found in the spectral assessment of source-sink related plant traits. Spike parameters such as spike dry weight and dry matter content were well related to spectral reflectance and to final grain yield. The successful assessment of final grain dry matter content offers a method to easily determine the physiological maturity of wheat. Visual ratings of canopy density was optimised by the combination of spectral and ultrasound reflectance measurements. Spectral reflectance measurements may offer an effective method to rapidly and objectively evaluate crossbreds due to yield related plant traits and allow for optimisation of traditional rating systems.

1 Introduction

The world is facing increasing population going along with the loss of agronomic productive area. The natural loss of areas under cultivation due to changing climatic trends and its implications is even increased by progressive urbanization and rural depopulation. Attested by rising food prices around the world, despite the decrease of crop land, the demand for food and plant-based energy is strongly increasing. As a consequence, plant and food production urges for high yielding and adaptive crops to countervail this imbalance.

Plant breeding is one keystone for solutions meeting the challenges mentioned above. Over the past decades methods of locating and characterizing genetic traits for plant breeding have become highly sophisticated. Using these opportunities, great efforts were done to develop genotypes being resistant to abiotic and biotic stresses, showing high resource efficiencies and thus, own the potential to produce acceptable yields under limited conditions. To test the genetic potential of modern crossbreds, phenotypic specification must be evaluated in field experiments. However, methods in breeders' field-based experiments to obtain phenotypic traits of crossbreds seem to advance quite slowly compared to the lab methods. Rating of plot-grown crops is still done either visually or manually and thus highly labour intensive, time consuming and limited to easily detectable traits. A non-destructive and rapid tool to estimate crop development and physiological or morphological traits would be a powerful improvement in plant breeding (Ma et al., 2001; Ferrio et al., 2005; Babar et al., 2006a).

1.1 Reflectance measurements

One tool to non-destructively assess plant traits is the recording of plants spectral reflectance. Light is measured in the visible (VIS, app. 400-700 nm) and near-infrared (NIR, app. 700-2500 nm) ranges. Referred to plants, VIS-light is mainly used for photosynthesis and its reflectance is therefore dependent on the chlorophyll content of the leaves. Several investigations proved that measuring chlorophyll of leaves gives an indirect estimation of leaves N content (Hinzman et al., 1986; Daughtry et al., 2000; Gitelson, 2003; Gitelson et al., 2003; Fitzgerald et al., 2010; Fitzgerald, 2010). However, the chlorophyll content is also affected by other abiotic stress factors, such as water availability (Peñuelas et al., 1993; Babar et al., 2006a; Babar et al., 2006b) or different nutrient deficiencies (Pinter et al., 2003; Hatfield et al., 2008). Most early spectral measurements for relationships to plant traits were done on single leaf levels where good estimations of chlorophyll concentration via reflectance measurements were found (Guyot et al., 1992; Blackburn, 1998; Daughtry et al., 2000; Gitelson, 2003). On canopy level, which is a step towards the utilization in agriculture and breeding, relationships between optical reflectance and

chlorophyll concentration had to be optimised because of interferences due to the soil background and leaf shading (Guyot et al., 1992; Blackburn, 1998; Daughtry et al., 2000).
To assess plant dry weight, the combination of VIS and NIR waveband reflectance proved to be a meaningful method. Reflectance patterns of the NIR are based on the diffusion of light due to water saturated cell walls and air-cell interfaces in the leaves (Campbell, 2002). Most NIR light is reflected by the spongy mesophyll because of the random orientation of the cell walls (Guyot et al., 1992) and therefore is well related to the amount of biomass (Shaver et al., 2010).
Additional to spectral reflectance measurements, sound reflectance can be used to characterise canopy structures in crop stands. Ultrasound sensors were frequently used to measure crop structure (Shibayama et al., 1985; Miller, 2000; Katoka et al., 2002) . The theory of these measurements is based on pulses of ultrasound which are sent by a transmitter from above onto the canopy. The first object coming in touch with the sound wave bounces back the signal which is then detected by a receiver. While the speed of the signal is known, the distance between the sensor and the object can be calculated out of the time between sending and detecting the signal. However, as the sound is neither sent nor reflected punctually, the detected signal is a superposition of the reflectance of any horizontal plant tissues reached by the sound signal (Shibayama et al., 1985; Mc Kerrow and Haper, 2001). Rather than assessing the actual height of the canopy, ultrasound measurements reproduce the canopy structure implying height, leave position and crop density. Ultrasound sensors were already used in combination with spectral reflectance measurements to add canopy structure characteristics to exclusively spectral assessments of crops (Scotford and Miller, 2003b; Scotford and Miller, 2003a; Scotford and Miller, 2004).

1.2 Spectral sensor systems

Spectral measurements work similarly, independent of the system used in a specific case. The light reflected by the crop canopy can be measured by sensors that convert the light signal into electrical output. For remote sensing, early sensor systems worked primarily passive, dependent on sunlight as light source. With advancing technical developments, measuring units became smaller and spectral resolution increased from broad wavebands of several wavelengths to even single wavelengths of modern systems (Jackson, 1986; Moran et al., 1997). To increase the spatial resolution of reflectance measurements, observation levels changed from satellite and airborne to ground-based remote sensing with hand held or tractor-based sensor units. Satellite and airborne remote sensing is mainly used for the assessment of land use on a regional scale or on large field experiments. Although results of these remote measurements attested to be of good quality, time slots for observations are constricted, highly dependent on weather conditions and measurements itself are costly due to the complexity of the carrying systems (Moran et al., 1997). With tractor-based systems, there is a positive trade-off between spatial resolution and the flexible and quick assessment of spectral information (Jackson, 1986). The widely used passive sensor systems depend on adequate solar radiation, must be calibrated precisely and regularly or need a second measuring unit for reference measurements. But spectral resolution is high and light can be measured in any spectral region needed for vegetation observations. In contrast, active sensor systems use artificial light sources like light emitting diodes (LED) or xenon lights and thus are independent of solar radiation. As a consequence, active spectral remote sensing is more flexible in terms of timeliness and illumination conditions. Since the radiation output of artificial light sources are known, calibration is only done once and no reference measurements are necessary. Active sensor measurements are mostly restricted to only few single wavelengths because the LEDs emit light only in specific wavebands (Solie et al., 2002; Holland et al., 2004; Jasper et al., 2009). Therefore, the scope of application of active sensors is limited compared to the passive sensor systems. Nevertheless, both sensor systems are used in science and plant production to assess crop reflectance patterns for the understanding of plant physiology and supporting agronomic decisions.

1.3 Spectral reflection and plant traits

The differences between the reflected VIS and the NIR wavebands are characteristic for plant canopies. Since the contrast of reflectance between soil and vegetation is highest with combinations of red and NIR wavebands, ratios and indices of these bands are frequently used to describe plant parameters on canopy level (Guyot et al., 1992). In early observations of vegetation canopies, the simple ratio (SR) (Pearson and Miller, 1972) between the wavebands around NIR (780 nm) and red (640 nm) was used to describe green vegetation cover. The normalized difference vegetation index (NDVI) (Rouse et al., 1974; Tucker, 1979), deduced from the SR, is one of the most frequently used reflectance indices to describe green biomass, leaf are index (LAI), plant health or yield of crops (Aparicio et al., 2000; Serrano et al., 2000; Raun et al., 2001; Pinter et al., 2003; Mistele et al., 2004; Prasad et al., 2007; Heege et al., 2008; Trotter et al., 2008). To better describe the vigour of winter wheat throughout a growing season, the NDVI was already combined with ultrasound reflectance data rendering a combination of green biomass and structural information of the canopy (Scotford and Miller, 2004). With the opportunity of increasing spectral resolution, advanced reflectance parameters could be calculated to improve the airborne estimations of canopy characteristics. The transition between the red and the NIR spectral course promised to be a powerful predictor for canopy specifications. The red edge inflection point (REIP) was modelled linearly to determine vegetation conditions and showed high resistance to atmospheric effects with airborne data recordings (Guyot et al., 1992). Later, the REIP was also used in ground-based remote sensing as an indicator for crop canopy characteristics (Mistele et al., 2004; Reusch, 2005; Heege et al., 2008; Strenner and Maidl, 2010). Guyot´s formula of the REIP is based on either two wavelengths of the red, 640 and 700 nm, and the NIR, 740 and 780 nm, respectively. Because of the low arithmetic values of the red reflectance, the wavelengths 740 and 780 nm mainly determine the modulus of the REIP. Due to this supposition, Mistele et al. (2004) developed a new spectral parameter, exclusively based on the reflectance of the two NIR wavelengths 740 and 780 nm. This and similarly calculated indices (R_{760}/R_{730}) proved to be good estimators for nitrogen uptake and crop biomass even at dense crop stands (Jasper et al., 2009; Mistele and Schmidhalter, 2010b; Reusch et al., 2010; Erdle et al., 2011).

In the NIR spectral bands, the water absorption band at around 970 nm is also found when measuring canopy reflectance. Peñuelas et al. (1993) developed the water index (WI) using the ratio of the water absorption band, 970 nm, in reference to 900 nm. Thus, the WI expresses structural changes in the plant due to varying cell water content. The close relationships between the WI and the water status of crops were adapted to irrigation purposes and the observation of crop productivity in arid environments (Peñuelas and Filella, 1998; Sims and Gamon, 2003; Babar et al., 2006a).

In opposition to exclusively NIR-based spectral parameters, like mentioned before, VIS-based spectral parameters are known to best describe chlorophyll status in plants. The photochemical reflectance index (PRI) is calculated out of reflectance data between the blue and green spectral bands (Gamon et al., 1992). The PRI correlates with the photosynthetic efficiency and the nitrogen status of the plant canopies (Gamon et al., 1992; Peñuelas et al., 1995; Gamon et al., 1997; Aparicio et al., 2002; Bannari et al., 2007). The visible atmospherically resistant vegetation index (VARI) has been described as a good indicator of the fraction of green vegetation over the soil surface (Gitelson et al., 2002) and is based on green, red and blue light bands. In several studies, the VARI proved to well describe chlorophyll status and green leaf area of crops (Gitelson et al., 2003; Steddom et al., 2003; Vina et al., 2004; Stow et al., 2005).

The reflectance spectrum of vegetation therefore offers broad information about its health and nutritional status.

1.4 Sensor application on plot level

Compared to practical crop management, the adaption of spectral sensing to breeders´ gardens is based on strongly differing areal scales and differentiation levels. For crop management, the required resolution of the canopy status is dependent on the technical equipment which is controlled online by the sensor. The combination of working width, driving speed and controlling accuracy of the agricultural equipment results in subareas of up to several hundred square meters. In breeders´ gardens, however, plot sizes decrease along with the generation of the crossbreds from several square meters to a few plants in a row. With decreasing plot size, the variation within plots, based on plants or sensor related deviations, is crucial to interpret averaged sensor recordings. Solari (2006) showed that point-to-point variations of two active sensor devices were based on the detected area of each sensor and the speed of moving forward. However, it is unknown if this within-plot variation is rather based on sensor signal deviation or on natural canopy structures and if it has a considerable effect on the plot averages. The knowledge of these factors, though, directly influences the confidence in the sensors output and the implementation of sensor systems into small scaled field trials.

Furthermore, while in crop management the sensors have to differentiate heterogeneities within a field of a single cultivar, in a breeders´ garden, mostly equally treated plots of multiple crossbreds must be evaluated. In such environments, only genetic differences are to be distinguished and classified aiming at a specific character. Already in earlier investigations visual ratings were compared with destructively measured parameters (Wanous et al., 1991; Xu et al., 2000; Riday, 2009). However, the comparison between two systems of indirect estimations, the visual and the sensor-based, is still missing. Both systems are, due to their indirect way of assessment, afflicted with errors when expressing the actual crop status. A direct comparison with a measured dimensions is not always feasible because some estimated attributes such as crops´ vigour or canopy density are not measurable in definite units.

Sensor technologies and reflectance patterns must be evaluated due to their potential to support the understanding of crop development, yield formation and finally the application in practical breeding.

1.5 Objectives

In most studies using remotely sensed spectral reflectance data of crop canopies, the multiple data points detected by the sensors are averaged over differently extended areas. Hardly any study yet investigated the assessment of the likely variation within subareas or plots. Moreover, if any certain within-plot variation is found, it is questionable if this variation is based on crop or measurement heterogeneities. The aims of *Section I* of this study therefore were i) to observe within-plot variations of multiple sensor measurements and ii) to evaluate the quality of signal reproduction of within-plot variations and testing the sensors detection regime.

While spectral sensors are sophisticated and plant reflectance measurements are already used successfully in agronomy, a comparison of lately available sensor systems based on destructively assessed plant traits, is still missing. *Section II* of this study therefore investigated the comparison of passive and active spectral sensor systems with each of several reflectance indices i) to identify the crop nitrogen status, ii) to evaluate the quality of their relationship to crop attributes in the field, and iii) to find a possible ranking in the prediction of several crop characteristics.

Considering the introduction of remote sensing to agronomic purposes, its application is mainly limited to the vegetative period of plant development. Only few investigations of spectral reflectance measurements considered the grain filling period of cereal crops, although this phase is crucial for final yield development. The aims of *Section III* of this study were therefore i) to observe the temporal effect of the grain filling phase on spectral parameters, ii) to estimate the influence of either leaves and stems or spikes on canopy reflectance data and iii) to select reflectance parameters to distinguish cultivars or cultivar groups by their grain filling characteristics.

While yield related plant traits could be well estimated under uniform conditions, a strong influence is expected with varying nutrient supply. Therefore the objectives of *Section IV* were i) to observe genetic differences in yield related plant traits by spectral remote sensing throughout grain filling and ii) to estimate the influence of varying N supply on the relationship between plant traits and reflectance indices of high yielding wheat cultivars.

Most attributes assessed by spectral measurements could be related to definitely measurable units such as weights or concentrations. Phenotypic evaluation of cultivars is still done manually by breeders when visually classifying cultivar characteristics. Parameters like canopy density represent a combination of several crop traits which until now could only be rated visually. In *Section V* we investigated i) the estimation of canopy density by spectral reflectance measurements, ii) the comparison of visual ratings with sensor classifications and ii) the combination of different sensors (sensor fusion) to improve the relationships to crop density.

2 Materials and Methods

2.1 Study site, biomass sampling and visual rating

The field experiments for *Sections I* to *IV* were conducted at the Dürnast research station of the Technische Universität München in southwestern Germany (11°41´60” E, 48°23´60” N). The soils were mostly of homogeneous Cambisols of silty clay loam. The fields are located in a hilly region sloping northwards with approximately 0.09 m m^{-1}. In this geographic area, the average yearly precipitation is approximately 800 mm at an average temperature of 7.5 °C. Yields vary between 6 and 10 tons per hectare, depending on the climate and soil conditions. The soil was sampled and a representative subsample was analysed for P (8.7 mg P per 100 g soil) and K (19.0 mg K per 100 g soil) to ensure an adequate supply of these macronutrients. Field management was done conventionally, adopting local standards. Residual soil nitrate was determined before tillering by a simplified soil nitrate quick-test method (Schmidhalter, 2005) indicating soil NO_3-N levels of 45 kg ha^{-1} and 52 kg ha^{-1} in 2009 and 2010, respectively.

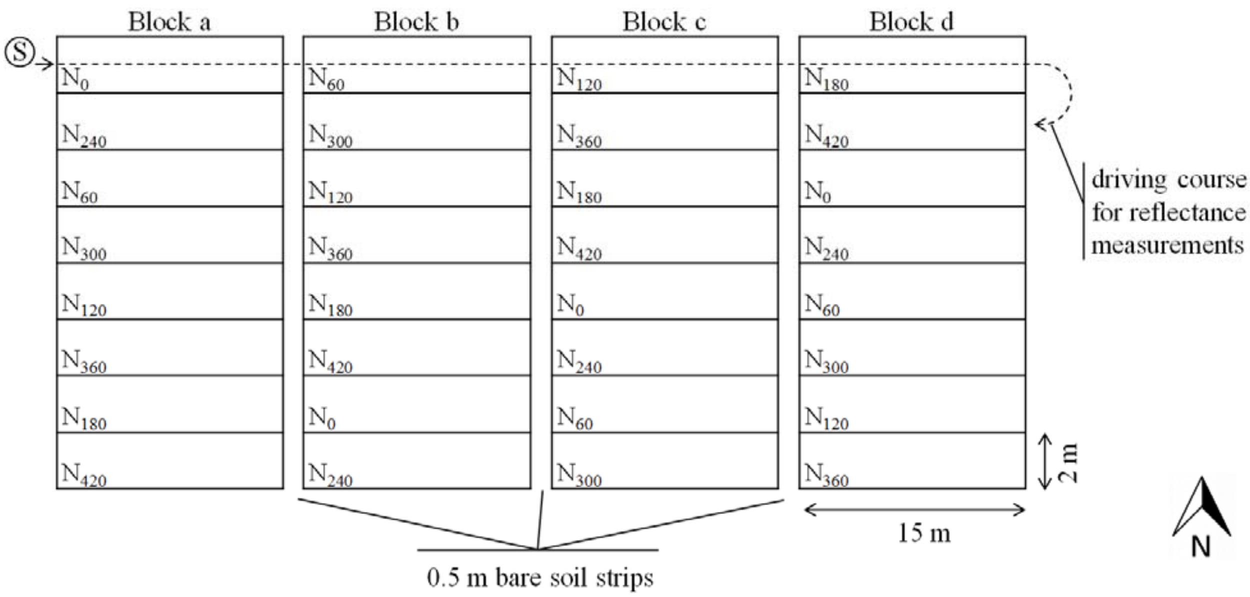


Figure 2.1a: Map of the single cultivar (*Tommi*) trial layout for *Section I* with plot dimensions, bare soil strips and the driving direction for reflectance measurements. N treatment levels increased from 0 kg N ha^{-1} to 420 kg N ha^{-1} in steps of 60 kg N ha^{-1} (N_0, N_{60}, N_{120}, N_{240}, N_{300}, N_{360} and N_{420}). All spectral measurements were started at starting point (S).

For the observation of within-plot variations in *Section I*, in 2010 exclusively one wheat (*Triticum aestivum* L.) cultivar *Tommi* was grown in a randomised block design with four replicates and a plot area of 2 m wide and 15 m long (Figure 2.1a). The plots were separated by strips of 0.5 m of bare soil. Eight N levels were adopted increasing from 0 kg N ha^{-1} to 420 kg N ha^{-1} in steps of 60 kg N ha^{-1} (N_0, N_{60}, N_{120}, N_{240}, N_{300}, N_{360} and N_{420}) each. The extremely high amount of 420 kg N ha^{-1} was chosen to evaluate the differentiation of biomass and spectral parameters also at high N supply. No fertiliser was applied pre-planting. Fertiliser application was done at the Zadoks stages (Zadoks et al., 1974) tillering (ZS 2), stem elongation (ZS 3) and booting (ZS 4) receiving 50 %, 30 % and 20 % of the total N amount applied, respectively. At Zadoks stage ZS 3 biomass samples were taken manually covering an area of 1 m^2 per plot. The fresh biomass was put into plastic bags, immediately weighed, then dried and the dry weight (DW in t ha^{-1}) was recorded. The plant samples were milled and the N content (%) was detected with mass spectrometry using an isotope radio mass spectrometer with an ANCA SL 20-20 preparation unit (Europe Scientific, Crewe, UK). Additionally, the aboveground N uptake (N_{up} in kg ha^{-1}) was calculated as the shoot dry weight multiplied by the total N content.

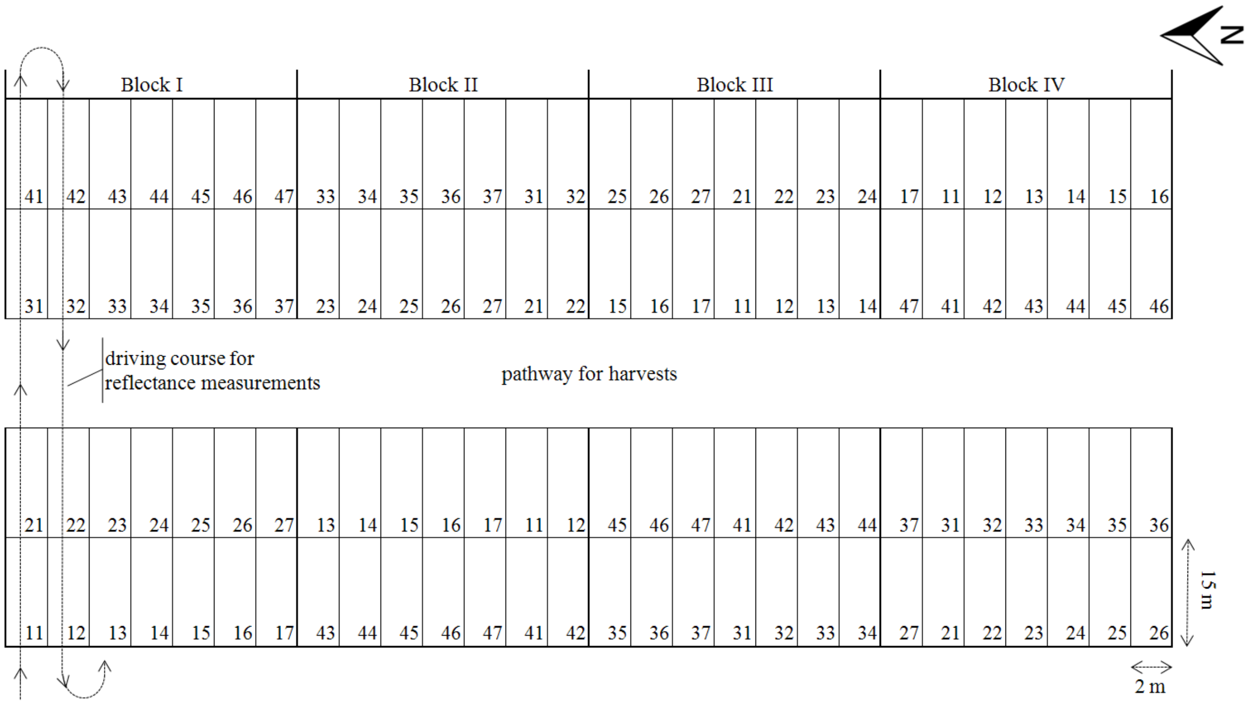


Figure 2.1b: Map of the trial layout with plot dimensions and the driving direction for reflectance measurements. Numbers indicate N treatment levels (first digit) with 0 kg N ha^{-1} = 1, 100 kg N ha^{-1} = 2, 160 kg N ha^{-1} = 3, 220 kg N ha^{-1} = 4 and cultivars (second digit) including *Tommi*, 1, *Solitär*, 2, *Impression*, 3, *Pegassos*, 4, *Ludwig*, 5, *Cubus*, 6 and *Ellvis*, 7.

For *Sections II, III and IV*, seven high yielding winter wheat cultivars were grown, arranged in a randomised block design with four replicates (Figure 2.1b). The seven cultivars *Tommi*, *Solitär*, *Impression*, *Pegassos*, *Ludwig*, *Cubus* and *Ellvis* were cultivated on plots with an area of 2 m wide and 15 m long. Four N treatments were applied including rates of 0 (N_0), 100 (N_{100}), 160 (N_{160}) and 220 (N_{220}) kg N ha^{-1}. No fertiliser was applied pre-planting. Fertiliser application was done at the stem-elongation (ZS 3), booting (ZS 4) and heading stages (ZS 5). At each of the first and second application date 30 kg N ha^{-1}, 60 kg N ha^{-1}, and 90 kg N ha^{-1} were given for the N treatments N_{100}, N_{160}, and N_{220} kg N ha^{-1}, respectively. A nitrogen supply of 40 kg ha^{-1} was given at the heading stage which is frequently used in Germany to increase the quality of small grain cereals.

Pre-anthesis biomass sampling was performed three times in both years, at the Zadoks growth stages ZS 3, stem elongation, ZS 4, booting, and ZS 6, anthesis, (Zadoks et al., 1974) and was checked via growing degree days (GDD) for similar development in the two years of the study. For biomass sampling, a green forage chopper was used to cut an area 2 m wide by 3 m long of each plot (Thoren and Schmidhalter, 2009). The fresh biomass was put into plastic bags, immediately weighed and then dried. The fresh weight (FW in t ha^{-1}) and the dry weight (DW in t ha^{-1}) were recorded and dry matter content (DM) calculated as shoot dry weight divided by shoot fresh weight and expressed as a percentage value. The N content (%) was detected and the aboveground N uptake (N_{up} in kg ha^{-1}) was calculated as described earlier. The Nitrogen Nutrition Index (NNI) was determined by:

$$\mathrm{NNI} = \frac{\mathrm{N_{act}}}{\mathrm{N_c}}$$ (Lemaire and Gastal, 1997)

where N_c is the critical N content of the plant dry weight described by the equation

$$\mathrm{N_c} = 5.35\ \left(\text{dry weight}\ \left[t\ \mathrm{ha}^{-1}\right]^{-0.442}\right)$$ (Justes et al., 1997)

based on investigations in winter wheat. The critical N content is assumed to be nearly constant at a mean value of 4.4 % for shoot dry weight values between 0.2 and 1.55 t ha^{-1} (Justes et al., 1997). Despite this assumption, we calculated the critical N content for each plot to reflect the individual N status of replicates and N treatments. This calculation takes into account that the relationship between spectral measurements and plant N status is represented by a continual function and therefore cannot be fitted to an absolute term (Mistele and Schmidhalter, 2008b).

Post-anthesis biomass sampling was performed only in 2009 and was limited to six cultivars excluding cultivar *Ludwig*. Known as a high growing and early maturing variety, *Ludwig* was strongly affected by the wet conditions during grain filling (Figure 2.1c) followed by an incomparable low grain yield in 2009. Because of that, this cultivar was excluded from analysis to avoid inappropriate variations of the crop attributes. Biomass sampling of the six cultivars took place at ZS 75, medium milk, ZS 77, late milk, ZS 83, early dough, and ZS 85, soft dough, referring to 265, 273, 280, and 287 days after sowing (DAS), respectively (Figure 2.1c). Two times 0.5 meters of two parallel sowing strips (0.12 m^2) of the plots centre were cut at ground level, resulting in about 70 spiked wheat culms with leaves. Like before, the fresh biomass was put into plastic bags and immediately weighed as a whole. Afterwards, the samples were partitioned into spikes and remaining biomass (leaves+stems) and FW and DW in t ha^{-1} of each subsample was recorded. The total N content (%) was measured and the dry matter content (DM in %) and the aboveground N uptake (N_{up} in kg ha^{-1}) of spikes and leaves+stems was calculated, respectively, following the procedure above. The relations of each, DW development and N uptake behaviour, between spikes and leaves+stems were calculated as

$$\text{DW index} = \frac{\text{spikes DW}}{\text{leaves+stems DW}}$$

$$N_{up} \text{ index} = \frac{\text{spikes } N_{up}}{\text{leaves+stems } N_{up}}$$

, respectively.

To assess final grain yield (t ha^{-1} at 86 % dry matter content) of both years, a combine was used to cut the whole plot area of an equally treated plot next to the sampled one. Grain dry matter content (Grain DM in %) was assessed and grain N content (Grain N in %) was measured with a Vario Max CNS Elementanalyser (Elementar, Hanau, DE). The N yield was calculated by multiplying grain N content with final grain yield to determine the N use efficiency (NUE), the quotient of N yield and the N supply (soil, 45 kg N ha^{-1}, and application, 160 kg N ha^{-1} in N_{160}). The harvest index (HI) as the ratio between grain yield and whole shoot biomass was calculated. For the detailed observation of nutrition effects on biomass and N partitioning during grain filling, data assessed in *Section IV* were downscaled to a square meter level.

The field experiment for *Section V* was conducted on a breeders´ garden near Munich, Germany (11°86'87"39 E, 48°44'05" N) with humic Cambisols of silty clay loam. Representative soil samples were analysed for P (14 mg P per 100 g soil) and K (35 mg K per 100 g soil) to ensure sufficient supply of these nutrients. Field management was done conventionally, adopting local standards. Residual soil nitrate was determined before tillering indicating a soil NO_3-N level of 92 kg ha^{-1}. The high NO_3 level was due to a leguminous preceding crop (field pea) in the year 2009. In this trial 90 wheat (*Triticum aestivum* L.) cultivars were grown in a randomised design with two replicates of plots of 1.75 m widths and 7 m length. Due to a broad range of years of origin (approximately 1930 to 2005), the cultivars represented a broad genetic variation and breeding history. The field was equally supplied with 190 kg N ha^{-1} split in amounts of 50 kg ha^{-1}, 60 kg ha^{-1} and 80 kg ha^{-1} at Zadoks stages tillering, ZS 2, booting, ZS 4, and heading, ZS 5, respectively.

In this trial no biomass sampling took place. The cultivars were rated visually for canopy density at ZS 4 in relative classification ranges between 1 (very sparse) and 9 (highly dense). During visual rating, the evaluator walked through the trial passing every plot and attributing a classification number to every replication independent of the cultivar evaluated.

2.2 Spectral reflectance measurements

Passive and active optical sensor systems were mounted on a frame in front of a tractor-based measuring platform. The sensors were driven from west to east to avoid any shading of the measuring unit (Figure 2.1b). Independent of the sensor system, all devices were used at a nadir position of approximately 1.40 m above the ground across the season. This height was chosen to constantly sense the plots' central area and avoid the unintended detection of the plot borders. While collecting information in the field, the sensor outputs were co-recorded along with the GPS coordinates from a RTK-GPS (real-time kinematic global positioning system) (Trimble, Sunnyvale, CA, USA). The frequency of recordings was primarily dependent on the output frequency of the RTK-GPS with approximately five readings per second. For each position, the actual sensor output was co-referenced and recorded. Afterwards, readings within one plot were averaged to single values per plot with the exception of *Section I*, investigating in within-plot variation.

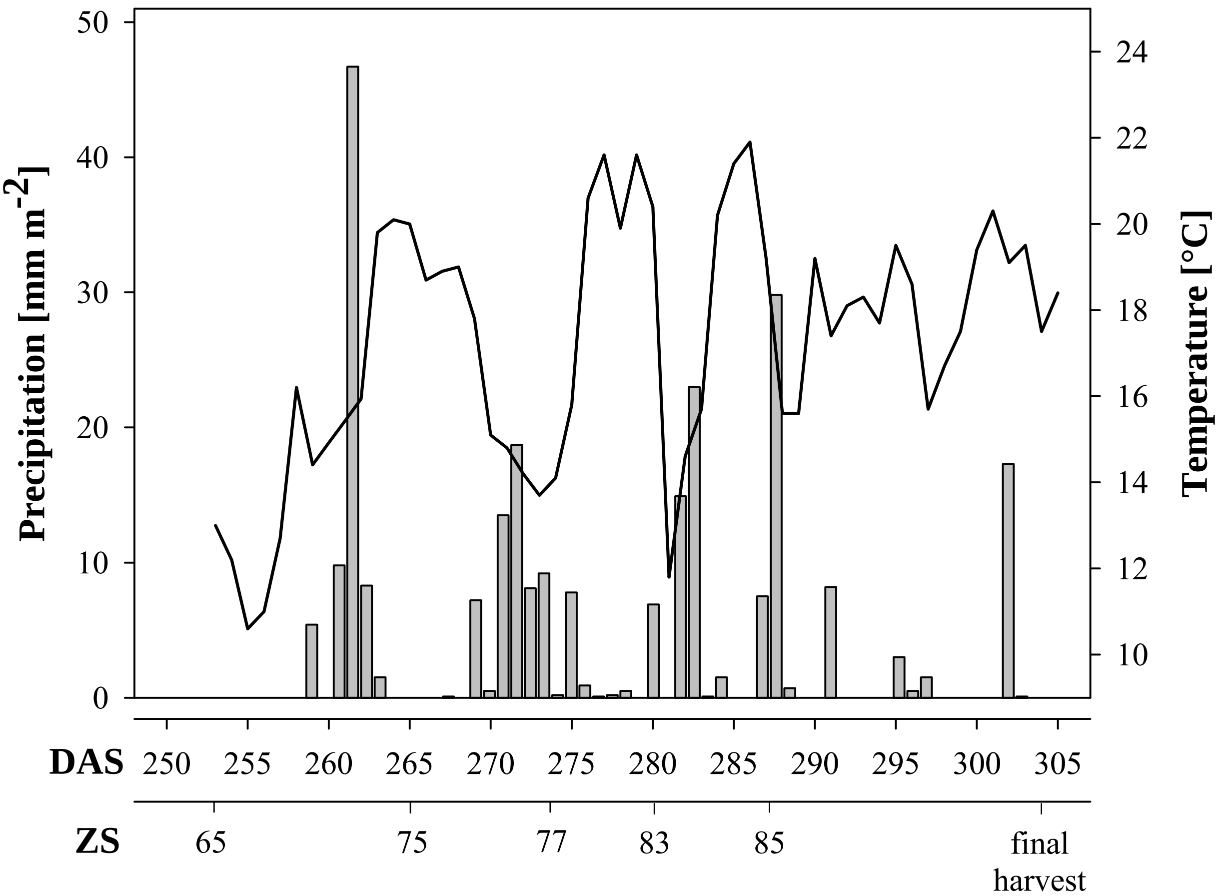


Figure 2.1c: Daily sums of precipitation and course of average temperatures during grain filling from 253 to 303 days after anthesis (DAS) in 2009. The second abscissa indicates the dates of anthesis at Zadoks stage (ZS) 65, the four sampling dates ZS 75, 77, 83 and 85, and the final harvest at 303 DAS.

To estimate the quality of signal reproduction in *Section I*, a single strip of four plots (N_0, N_{60}, N_{120}, N_{180}) within the single cultivar trial was measured three times in succession from west to east resulting in measurements M_A, M_B and M_C. Time brakes of maximum five minutes appeared between each measurement to check the sensor performance and the equal driving direction from east to west. Only one strip was measured to avoid unintended changes of measurement and light conditions during extended field measurements especially for the passive bi-directional spectrometer. With this limitation, each measurement took one minute passing through the four plots of 15 m length. Considering the constant RTK-GPS output frequency, the vehicle speed and the plot length, a number of 40 to 60 single data points were recorded within each plot. Differences in the number of data points, thus, the resolution, due to difficulties in holding a constant driving speed in the field, were revised manually. The data points of the four plots could easily be separated by strongly negative peaks of the spectral parameters course, representing the bare soil strips the plots were separated with. To further equalise recording differences due to the varying driving speed, a manual adjustment was carried out considering the bare soil strips and individual spectral course patterns within the plots. Time laps between the data points were extended if necessary to bring in line the selected patterns within M_A, M_B and M_C.

Table 2.2a: Spectral reflectance parameters selected for the comparison of active and passive sensors.

Abbreviation	Formula	Reference
NDVI	$(R_{780} - R_{670})/(R_{780} + R_{670})$	(Rouse et al., 1974)
SR	R_{780}/R_{670}	(Pearson and Miller, 1972)
NIR/NIR	R_{780}/R_{740}	(Mistele and Schmidhalter, 2010b)
WI	R_{900}/R_{970}	(Peñuelas et al., 1993)
REIP	$700 + 40 ((R_{670} + R_{780})$ $/2 - R_{700})/(R_{740} - R_{700}))$	(Guyot et al., 1988)
PRI	$(R_{531} - R_{570})/(R_{531} + R_{570})$	(Gamon et al., 1992)
VARI	$(R_{550} - R_{650})/(R_{550} + R_{650} - R_{470})$	(Gitelson et al., 2002)

For spectral measurements in *Section I* and *Section II*, a passive bi-directional spectrometer (tec5, Oberursel, Germany) and three active sensor devices were used. This bi-directional spectrometer (BDS) with modified electronics to enable hyperspectral readings contained two Zeiss MMS1 silicon diode array spectrometers with a spectral detection range of 340 to 1000 nm and a bandwidth of 3.3 nm (Mistele and Schmidhalter, 2010b). One unit was linked to a diffuser detecting solar radiation as a reference signal. Simultaneously, the second unit measured the canopy reflectance with a 12° field of view (FOV), resulting in about 0.28 m^2 at the fixed height of 1.40 m above the ground. The measurements were taken under clear sky conditions before or at noon to provide the best possible conditions for passive recordings. One of the three active devices used was an active flash sensor (AFS) similar to the N-Sensor ALS® (YARA International, ASA) but limited to a single sensor and USB interface (Mistele and Schmidhalter, 2010b). The light source for this system was a flashing xenon light producing a spectral range of 650 nm to 1100 nm with 10 flashes per second and a circular FOV of about 0.15 m^2. The four detectable wavelengths could be selected independently, but in this experiment filters similar to those of the YARA ALS® system were chosen: 730 nm, 760 nm, 900 nm and 970 nm (Jasper et al., 2009).

Furthermore, the commercially available sensor systems GreenSeeker RT100® (NTech Industries, Inc., Ukiah, CA) and Crop Circle ACS-470® (Holland Scientific, Inc, Lincoln, NE) were used. The GreenSeeker emits light by two LEDs and detects the reflection of each in the VIS (656 nm, ~25 nm band width) and NIR (774 nm, ~25 nm band width) spectral regions. The FOV of this device is a narrow strip of about 61 cm by 1.5 cm (0.009 m^2) at a height of 66 cm to 112 cm above the plant canopy (NTech Industries, 2007).

The Crop Circle operates similarly like the GreenSeeker but allows for more flexibility in the wavelengths detected because it emits white light and offers a choice of selectable interference filters. Filters for the wavelengths 670 nm, 730 nm, and 760 nm were chosen to record reflectance data. The FOV of the Crop Circle is an oval of ~32° by ~6° range (Holland-Scientific, 2008), resulting in approximately 0.09 m^2 at 1.40 m above the ground. In the year 2009, the active sensors were not available all along the season. Therefore, at ZS 3, spectral parameters cannot be provided for all active devices. At ZS 4 and ZS 6, AFS data were not available but Crop Circle and GreenSeeker data were collected. In 2010, all the sensor devices were available throughout the season. Although some sensors offer calculated spectral parameters as output, we used single wavelength reflection values to calculate the reflectance parameters aimed for in this project. Seven spectral parameters were selected to represent the different reflectance intensities in the VIS and NIR ranges of all the sensor systems used (Table 2.2a). Because the active devices did not always detect exactly the wavelengths typical for these parameters, similar wavelengths and combinations were used to calculate parameters (Table 2.2b) based on the seven initial spectral parameters.

The normalised difference vegetation index (NDVI) and the simple ratio (SR) were calculated reflecting spectral parameters of both the VIS and NIR spectral regions. The red edge inflection point (REIP) contains information about the chlorophyll absorption and cell wall reflection

(Guyot et al., 1988; Guyot et al., 1992). The REIP lies between the VIS and NIR spectral information but is sensitive to changes on either side. An index of two similar NIR reflectances (NIR/NIR), which has been shown to be closely related to the N status of crops, was calculated (Mistele and Schmidhalter, 2010b). The water index (WI) was developed to estimate the water status of plants (Peñuelas et al., 1993) and is based on the water absorbtion band at 970 nm. Two VIS-based reflectance indices, the photochemical reflectance index (PRI) (Gamon et al., 1992) and the visible atmospherically resistant index (VARI) (Gitelson et al., 2002), were chosen to represent parameters of the VIS spectral bands.

Table 2.2b: Wavelengths and spectral reflectance parameters of the four sensor systems and their abbreviations. A bi-directional passive (BDS) and three active sensors (Crop Circle, CC, GreenSeeker, GS, active flash sensor, AFS) were compared.

Device	Wavelengths used	Parameters abbreviation
Bi-directional spectrometer	R_{670}, R_{780}	BDS_NDVI
	R_{670}, R_{730}	BDS_R_{730}/R_{670}
	R_{670}, R_{760}	BDS_R_{760}/R_{670}
	R_{780}, R_{650}	BDS_R_{780}/R_{650}
	R_{730}, R_{760}	BDS_R_{760}/R_{730}
	R_{900}, R_{970}	BDS_WI
	R_{670}, R_{700}, R_{740}, R_{780}	BDS_REIP
	R_{531}, R_{570}	BDS_PRI
	R_{470}, R_{550}, R_{650},	BDS_VARI
Crop Circle	R_{670}, R_{760}	CC_NDVI
	R_{670}, R_{730}	CC_R_{730}/R_{670}
	R_{670}, R_{760}	CC_R_{760}/R_{670}
	R_{730}, R_{760}	CC_R_{760}/R_{730}
GreenSeeker	R_{656}, R_{774} *)	GS_NDVI
	R_{656}, R_{774} *)	GS_R_{774}/R_{656}
Active flash sensor	R_{730}, R_{760}	AFS_R_{760}/R_{730}
	R_{900}, R_{970}	AFS_WI

*) ~25 nm spectral width at 50 % of peak (NTech Industries, 2007)

Measurements for *Section III*, *Section IV* and *Section V* were run exclusively using the passive bi-directional spectrometer, BDS. Measurements for the canopy density classification in *Section V* took place one day before visual canopy density rating at or around noon under clear sky conditions for unbiased passive measurements. The spectral reflectance data between 340 nm and 1000 nm per plot were used as raw spectra with no parameters calculated afore. An ultrasound sensor (UM38, Sick AG, Germany), was used to detect the canopy structure via ultra sound reflection measurements. The ultrasound sensor, US, was fixed in the height of 1.40 m next to the spectral sensor and scanned the distance to the canopy surface. Subtracting the sensor-canopy distance from the maximum height of 1.40 m, a weighted canopy height could be calculated. The US reflectance data were as well co-referenced with RTK-GPS data.

2.3 Data processing and statistical analysis

SPSS 11 (SPSS Inc., Chicago, USA) was used for statistical analysis. Depending on the objectives investigated, different methods of analysis were preferred.

Section I:

A one-way analysis of variance (ANOVA) with Duncan's multiple comparison procedure based on the studentised range test with a p-value of 0.05 was applied to differentiate the means of the N levels N_0, N_{60}, N_{120}, N_{240}, N_{300}, N_{360} and N_{420}. To evaluate the within-plot variation compared to the whole trial variation, a CV-ratio for each plot was calculated following the formula

$$\text{CV-ratio} = \frac{CV_{plot}}{CV_{trial}}$$

with the single plots´ coefficients of variation (CV_{plot}) and the trials´ coefficient of variation including all treatments of the field trial (CV_{trial}).

Section II:

Linear and curvilinear models were used to establish relationships between spectral parameters and dry matter content, dry weight, fresh weight, N content, N uptake, and the NNI for pre-anthesis biomass samplings. The Akaike Information Criterion (AIC) (Webster and McBratney, 1989) was used to assess the goodness-of-fit, and hence the best choice of linear or curvilinear models. The AIC criterion is based on a threepart equation of which one term is a constant value which therefore could be ignored. We used the shortform equation recommended by Webster and McBratney based on the number of observations, the residual sum of squares and the number of coefficients of a model. For further explanations of AIC we refer to Webster and McBratney (1989). A one-way analysis of variance (ANOVA) with Duncan's multiple comparison procedure based on the studentised range test with a p-value of 0.05 was applied to differentiate the means of the N treatments.

Section III:

A one-way analysis of variance (ANOVA) with Duncan’s multiple comparison procedure based on the studentised range test with a p-value of 0.05 was applied to differentiate the means of the cultivars. The main effects and interactions between the cultivars, replications and development stages were tested via GLM. Linear models were used to establish relationships between a) the yield and the plant traits destructively sampled during grain filling and b) the spectral parameters and the yield related plant traits. To allow for a qualitative ranking of the cultivars, the cultivar means of destructively assessed plant traits and the spectral parameters were normalised using the equation:

$$x' = \frac{x - x_{min}}{x_{max} - x_{min}}$$

where x is the average value of one cultivar´s replications, x_{min} is the minimum value of all the cultivar means and x_{max} is the maximum value of all the cultivar means. Conclusively, the resulting values lie in the range of ≥ 0 and ≤ 1. Thus, all of the plant traits and spectral parameters could be compared regardless of their prior dimensions and timely differences in absolute values while maintaining the quality of the relationships.

Section IV:

A one-way analysis of variance (ANOVA) with Duncan’s multiple comparison procedure based on the studentised range test with a p-value of 0.05 was applied to differentiate the means of the cultivars. Furthermore, main effects and interactions between cultivars, replications and development stages were tested via GLM. Linear models were used to establish relationships for each N level between a) yield and plant traits destructively sampled during grain filling and b) spectral parameters and the yield related plant traits.

Section V

To exclude influences of technical drifts in the spectral curve due to varying canopy height or light conditions, the first derivative following the formula

$$\Delta \text{ Reflection} = \frac{R(x_2) - R(x_1)}{x_2 - x_1} = \frac{\delta \text{ Reflection}}{\delta \text{ Wavelength}}$$

of each plots spectral curve (90 cultivars x 2 replications) was used to extract information strictly based on plants´ spectral reflectance. Linear models were used to establish relationships between the spectral reflectance values and the respective visually detected canopy density classes (DC_{vis}). The reflectively detected ranges (spectral and ultrasound) were divided in three and four regular classes, DC_3 and DC_4, respectively, to develop explicitly sensor-based classification. Selected spectral values were combined with ultrasound data by simple multiplication.

To directly evaluate the quality of reproducing the visual or sensor-based classification, the two cultivar replications were checked for being classified equally. The number of cultivars with equally classified replications was set in relation to the number of 90 cultivars resulting in % of positive matches.

3 Results

3.1 Section I: Sensor-based observation of within-plot variation and quality of signal reproduction during successive measurements

3.1.1 Within-plot variation of spectral parameters and N level differentiation

Despite of the high variation in N supply between 0 kg ha^{-1} and 420 kg ha^{-1} within the single cultivar trial, the biomass and spectral parameters variation were difficult to compare: While the REIPs´ output values increased by about 2 % when rendering the 420 kg N increase, the NDVI values increased by about 500 % (data not shown). Due to the differences in the parameters magnitudes, the plots´ coefficients of variation (CV_{plot}) were set in relation to the whole-trial coefficient of variation (CV_{trial}) of the biomass and spectral values resulting in a CV-ratio for each parameter and each plot (Tab 3.1a).

In the N level N_0, the replicate *d* shows high CV-ratios of up to 178 % throughout the spectral parameters assessed. The NDVIs´ CV-ratio of the BDS, the CC and the GS decreased with increasing N level, whereas the WI of the BDS and the AFS were strongly different. While the BDS_WI assessment constantly resulted in plot CV-ratios of up to 79 %, the AFS_WI never increased its CV-ratios higher than 31 %. However, the exact opposite was found for the R_{760}/R_{730} index of the two sensors. The AFS_R_{760}/R_{730} index showed considerably higher CV-ratios throughout the trial compared to the BDS_R_{760}/R_{730} index. The CC_R_{760}/R_{730} index resulted in even higher CV-ratios of up to 91 % in the 32 observed plots. Measurements of the BDS_REIP resulted in CV-ratios very similar to those of the BDS_R_{760}/R_{730} index.

The mean values of each treatment were compared to describe the significant differences of treatments reflected by the biomass or spectral parameters (Table 3.1b). With increasing N supply, the crop dry weight, DW, could not be significantly distinguished between the treatments. In strong contrast to that, the N content showed significant differences of all treatments to up to a N supply of 360 kg ha^{-1}. Only the two highest N levels N_{360} and N_{420} could not be significantly differed by N content measurements. Differences in N uptake, the product of DW and the respective N content, were less significant compared to the N content but more specific than for DW.

Treatment differences could well be expressed by spectral measurements. However, with increasing N level, differences were hardly detected by the spectral parameters used in the study. Only the highest N level, N_{420}, was differentiated precisely by any spectral parameter. The CC_NDVI showed higher differentiation ability compared to the BDS_NDVI and the GS_NDVI which were quite similar in distinguishing the trials N treatments. The R_{760}/R_{730} indices of both, the

Crop Circle and the BDS were similar in their differentiation pattern. The BDS_WI was able to differentiate higher N levels compared to the AFS_WI

3.1.2 Sensors quality of signal reproduction

Observing the quality of reproduction of within-plot variation in differently fertilised plots, each data point recorded by the sensors was depicted graphically to visualize any canopy heterogeneity within a plot (Figure 3.1). For each sensors´ spectral course, the four plots can easily be separated by strongly negative peaks of the index´ values, representing bare soil strips between the plots. Independent of the order of measurement, M_A, M_B or M_C , within the plots of N_0, N_{60}, and N_{120}, an increase of the spectral parameter in the direction east to west was observed. In contrast, within the plot of N_{180}, no such trend was apparent. The sensor recordings differed strongly in their within-plot variation (Table 3.1c). While the BDS and the AFS showed maximum values of CV 6 % and 3 %, respectively, the Crop Circle and the GreenSeeker reached CV values of 17 % and 15 %, respectively.
The individual recording patterns formed by positive and negative peaks within spectral courses were used to evaluate the sensors´ signal reproduction. The AFS and its R_{760}/R_{730} index best reproduced the patterns within the three successive measurements M_A, M_B and M_C. Similar to that, the BDS also proved to reproduce small scale differences expressed by special output patterns of the BDS_R_{760}/R_{730} index. However, the BDS offered a less distinct reproduction compared to the AFS.
The high within-plot variation of the index values of the Crop Circle and GreenSeeker made it difficult to find specific patterns of the spectral parameters used. Although less specific peaks were found for these sensors, trends of lower or higher parameter values within the courses were well reproduced in the single measurement repetitions. Sensors plot averages of M_A, M_B and M_C were comparable varying by maximum 4 %. Thus, the reproductive quality of average plot parameters was high for every sensor system used in this study.

Table 3.1a: Mean values of the plant traits dry weight, DW, N content and N uptake for the N levels of 0 kg N ha^{-1} to 420 kg N ha^{-1} increasing in steps of 60 kg N ha^{-1} (N_0, N_{60}, N_{120}, N_{240}, N_{300}, N_{360} and N_{420}). Standard deviation is added in brackets. Coefficient of variation ratios of each replication (R) are shown for the spectral parameters obtained from one bi-directional passive spectrometer (BDS) and three active sensors (Crop Circle, CC, GreenSeeker, GS, active flash sensor, AFS).

N level	DW	N content	N uptake	R	CV-ratio								
					BDS				CC		GS	AFS	
	t ha^{-1}	%	kg ha^{-1}		NDVI	WI	REIP	R_{760}/R_{730}	NDVI	R_{760}/R_{730}	NDVI	WI	R_{760}/R_{730}
N_0	5.81 (8.2)	1.4 (0.1)	81.6 (15.0)	a	1.03	0.79	0.39	0.37	1.13	0.64	0.68	0.29	0.37
				b	0.37	0.52	0.11	0.12	0.89	0.83	0.68	0.18	0.39
				c	0.43	0.54	0.23	0.23	0.68	0.52	0.63	0.24	0.38
				d	1.78	0.79	0.71	0.85	1.55	0.82	0.71	0.75	0.65
N_{60}	7.94 (8.1)	2.3 (0.2)	178.3 (16.6)	a	0.56	0.69	0.21	0.25	0.50	0.67	0.48	0.23	0.45
				b	0.32	0.49	0.14	0.16	0.82	0.82	0.58	0.22	0.35
				c	0.19	0.44	0.17	0.19	0.30	0.50	0.40	0.31	0.58
				d	0.25	0.23	0.09	0.13	0.44	0.62	0.50	0.19	0.26
N_{120}	7.79 (9.2)	2.6 (0.2)	204.8 (31.5)	a	0.27	0.48	0.23	0.28	0.30	0.54	0.36	0.26	0.55
				b	0.18	0.47	0.14	0.16	0.41	0.77	0.50	0.26	0.49
				c	0.26	0.59	0.15	0.15	0.55	0.81	0.12	0.31	0.64
				d	0.28	0.30	0.17	0.22	0.46	0.82	0.56	0.26	0.38
N_{180}	8.91 (14.6)	3.1 (0.2)	273.5 (52.4)	a	0.17	0.53	0.26	0.27	0.22	0.59	0.41	0.25	0.50
				b	0.12	0.50	0.14	0.15	0.32	0.75	0.55	0.24	0.61
				c	0.11	0.47	0.15	0.16	0.24	0.54	0.45	0.24	0.65
				d	0.24	0.17	0.17	0.19	0.51	0.91	0.45	0.21	0.39
N_{240}	9.38 (2.9)	3.4 (0.2)	317.7 (6.1)	a	0.07	0.44	0.16	0.17	0.37	0.66	0.45	0.19	0.48
				b	0.11	0.44	0.18	0.18	0.23	0.75	0.38	0.24	0.45
				c	0.16	0.35	0.18	0.17	0.16	0.57	0.40	0.18	0.52
				d	0.12	0.21	0.15	0.18	0.24	0.63	0.36	0.23	0.52
N_{300}	9.40 (12.7)	3.7 (0.1)	344.5 (48.8)	a	0.07	0.62	0.20	0.20	0.17	0.51	0.26	0.17	0.58
				b	0.12	0.47	0.17	0.19	0.26	0.60	0.29	0.23	0.56
				c	0.23	0.54	0.21	0.22	0.22	0.51	0.38	0.22	0.66
				d	0.14	0.31	0.22	0.24	0.16	0.54	0.26	0.23	0.65
N_{360}	9.70 (10.5)	3.9 (0.0)	383.0 (41.8)	a	0.07	0.55	0.17	0.17	0.18	0.55	0.24	0.21	0.71
				b	0.19	0.56	0.25	0.25	0.18	0.51	0.33	0.22	0.56
				c	0.18	0.22	0.25	0.26	0.24	0.60	0.40	0.23	0.65
				d	0.12	0.20	0.19	0.20	0.18	0.54	0.32	0.23	0.56
N_{420}	9.81 (7.5)	4.0 (0.2)	391.3 (45.1)	a	0.04	0.48	0.13	0.12	0.13	0.51	0.27	0.21	0.62
				b	0.04	0.41	0.14	0.13	0.17	0.62	0.42	0.19	0.55
				c	0.09	0.29	0.23	0.24	0.17	0.48	0.25	0.20	0.61
				d	0.07	0.18	0.14	0.15	0.18	0.45	0.58	0.28	0.58

Table 3.1b: Mean values comparisons of the N levels of 0 kg N ha^{-1} to 420 kg N ha^{-1} increasing in steps of 60 kg N ha^{-1} (N_0, N_{60}, N_{120}, N_{240}, N_{300}, N_{360} and N_{420}). Significant differentiation is indicated by different letters are depicted for the plant traits dry weight, DW, N content and N uptake as well as for the spectral parameters obtained from one bi-directional passive spectrometer (BDS) and three active sensors (Crop Circle, CC, GreenSeeker, GS, active flash sensor, AFS). Different letters show significant differences at $p \leq 0.05$ and n = 4.

N level	DW	N content	N uptake	BDS				CC		GS	AFS	
				NDVI	WI	REIP	R_{760}/R_{730}	NDVI	R_{760}/R_{730}	NDVI	WI	R_{760}/R_{730}
N_0	a	a	a	d	a	a	a	a	a	a	a	a
N_{60}	b	b	b	b	b	b	b	b	b	b	b	b
N_{120}	bc	c	b	bc	bc	bc	bc	bc	bc	b	bc	b
N_{180}	bcd	d	c	c	cd	cd	cd	cd	cd	c	cd	c
N_{240}	cd	e	cd	c	de	de	de	de	d	cd	cd	cd
N_{300}	cd	f	cde	c	de	de	de	ef	de	d	d	cd
N_{360}	d	g	e	c	de	de	de	ef	de	d	d	cd
N_{420}	d	g	e	c	e	e	e	e	e	d	d	d

Table 3.1c: Plot averages (Aver.) and coefficients of variation (CV) of the four N levels 0 kg ha^{-1} (N_0), 60 kg ha^{-1} (N_{60}), 120 kg ha^{-1} (N_{120}) and 180 kg ha^{-1} (N_{180}). Three successive measurements, M_A, M_B and M_C, of the bi-directional passive spectrometer (BDS) and three active sensors (Crop Circle, CC, GreenSeeker, GS, active flash sensor, AFS) and their spectral parameters are shown.

	N level	BDS_R_{760}/R_{730}		CC_R_{760}/R_{730}		GS_NDVI		AFS_R_{760}/R_{730}	
		Aver.	CV [%]	Aver.	CV [%]	Aver.	CV [%]	Aver.	CV [%]
M_A	N_0	1.43	5	1.57	16	0.68	9	1.46	3
	N_{60}	1.82	2	2.16	17	0.63	8	1.74	3
	N_{120}	1.99	4	2.47	13	0.58	12	1.91	3
	N_{180}	2.13	4	2.75	15	0.45	15	2.08	3
M_B	N_0	1.39	5	1.52	15	0.67	8	1.47	4
	N_{60}	1.77	6	2.23	13	0.63	10	1.73	3
	N_{120}	1.97	6	2.48	11	0.58	11	1.90	3
	N_{180}	2.17	3	2.80	12	0.47	14	2.05	2
M_C	N_0	1.38	4	1.55	14	0.66	9	1.44	4
	N_{60}	1.76	3	2.19	14	0.63	9	1.72	3
	N_{120}	1.97	3	2.49	12	0.57	11	1.90	3
	N_{180}	2.14	2	2.80	13	0.45	12	2.04	3

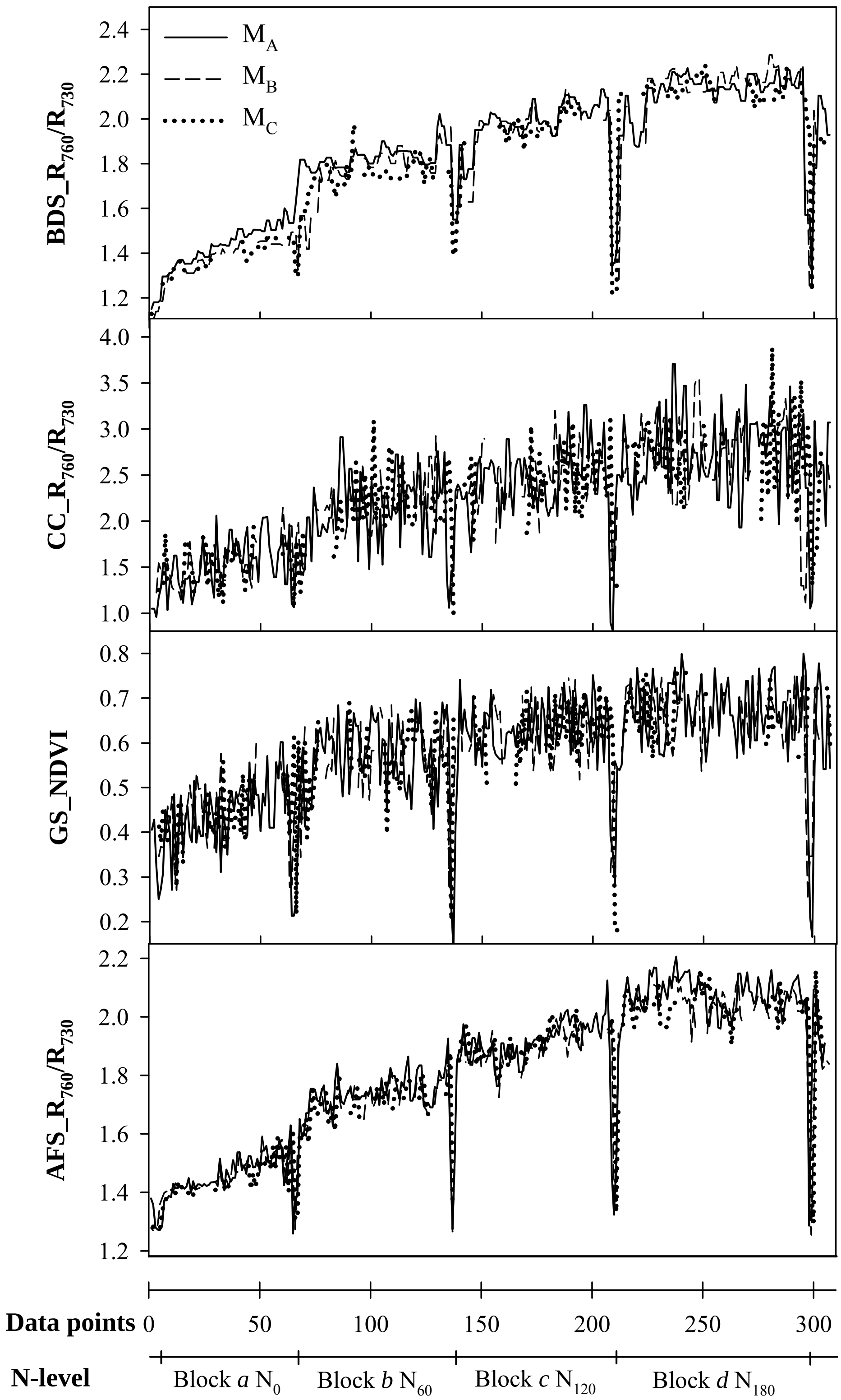


Figure 3.1: Course of the recorded spectral parameters of the bi-directional passive spectrometer (BDS) and three active sensors (Crop Circle, CC, GreenSeeker, GS, active flash sensor, AFS). The successive measurements M_A, M_B and M_C are shown for each plot of the respective N levels 0 kg ha^{-1} (N_0), 60 kg ha^{-1} (N_{60}), 120 kg ha^{-1} (N_{120}) and 180 kg ha^{-1} (N_{180}) located in blocks *a*, *b*, *c* and *d*.

3.2 Section II: Comparison of active and passive spectral sensors in discriminating biomass parameters and nitrogen status in wheat

3.2.1 Differentiation of agronomic plant traits

For all sampling dates across the seasons 2009 and 2010, the mean values of the destructively assessed plant traits DM, DW, N content, aboveground N uptake, FW, and the NNI as influenced by the four N treatments were compared. In the growing season 2009, all the agronomic traits except DW could be distinguished significantly (Figure 3.2a) and showed the same differentiation patterns. For the DW at ZS 3, the two highest N application treatments, 160 kg ha^{-1} and 220 kg ha^{-1}, could not be differentiated significantly. Overall, the differentiation patterns of the plant traits in 2010 (Figure 3.2b) were less distinct than those in 2009. The effects of high N levels on the dry matter in 2010 could not be separated across the whole season as they were in the shoot fresh weight for ZS 3 and ZS 6.

a) Zadoks stage 3

kg N ha^{-1}	DM [%]				DW [t ha^{-1}]				N content [%]				N uptake [kg ha^{-1}]				FW [t ha^{-1}]				NNI			
0	*31.7 1.44*				1.07 0.32				2.07 0.29				21.9 5.85				3.38 1.07				0.39 0.05			
100		28.2 1.42				1.36 0.24				2.64 0.23				35.8 6.11				4.86 0.95				0.56 0.05		
160			*26.5 1.38*				*1.62 0.40*				*3.17 0.29*				*51.3 12.9*				6.17 1.65				0.73 0.09	
220				24.6 1.60			1.76 0.30					3.55 0.38				62.0 10.8				7.17 1.33				0.85 0.10
kg N ha^{-1}	**BDS_NDVI**				**BDS_R_{730}/R_{670}**				**BDS_R_{760}/R_{670}**				**BDS_R_{780}/R_{650}**				**BDS_R_{760}/R_{730}**				**BDS_WI**			
0	0.79 0.06				6.32 1.76				9.43 3.05				7.80 2.21				1.47 0.07				1.11 0.01			
100		0.89 0.02				9.42 1.71				16.8 3.43				13.8 2.49				1.78 0.07				1.13 0.01		
160			0.91 0.03				11.4 2.43				22.9 5.55				19.2 4.29				5.00 0.11				1.15 0.02	
220			0.93 0.02					12.6 2.51				27.4 6.68				23.2 5.34				2.16 0.13				1.16 0.02
kg N ha^{-1}	**BDS_REIP**				**BDS_PRI**				**BDS_VARI**				**CC_NDVI**				**CC_R_{730}/R_{670}**				**CC_R_{760}/R_{670}**			
0	721.4 0.75				-0.09 0.01				0.30 0.09															
100		724.4 0.68				-0.07 0.01				0.43 0.06			**no data**				**no data**				**no data**			
160			726.4 0.87				-0.06 0.01				0.50 0.08													
220				727.8 0.97				-0.05 0.01			0.53 0.07													
kg N ha^{-1}	**CC_R_{760}/R_{730}**				**GS_NDVI**				**GS_R_{774}/R_{656}**				**AFS_R_{760}/R_{730}**				**AFS_WI**							
0																								
100	**no data**				**no data**				**no data**				**no data**				**no data**							
160																								
220																								
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d

b) Zadoks stage 4

kg N ha^{-1}	DM [%]				DW [t ha^{-1}]				N content [%]				N uptake [kg ha^{-1}]				FW [t ha^{-1}]				NNI			
0				25.4 *0.93*	3.3 *5.22*				1.26 *0.16*				41.1 *6.70*				12.9 *3.85*				0.39 *0.05*			
100			21.6 *0.70*			5.1 *7.34*				1.95 *0.37*				99.2 *23.1*				23.6 *6.12*				0.75 *0.15*		
160		19.8 *1.39*					5.7 *7.45*				2.52 *0.28*				141.8 *19.4*				28.7 *31.1*				1.01 *0.10*	
220	18.8 *1.22*							6.4 *6.67*				2.84 *0.33*				181.8 *18.9*				34.5 *31.1*				1.22 *0.13*

kg N ha^{-1}	BDS_NDVI				BDS_R_{730}/R_{670}				BDS_R_{760}/R_{670}				BDS_R_{780}/R_{650}				BDS_R_{760}/R_{730}				BDS_WI			
0	0.65 *0.05*				3.43 *0.55*				4.73 *0.91*				4.69 *0.85*				1.37 *0.05*				1.19 *0.02*			
100		0.88 *0.02*				7.70 *1.06*				14.8 *2.24*				14.2 *0.38*				1.92 *0.07*				1.31 *0.02*		
160			0.92 *0.01*				10.2 *1.32*				22.7 *3.54*				21.7 *3.27*				2.22 *0.10*				1.34 *0.03*	
220			0.93 *0.01*					11.2 *1.90*				26.2 *5.87*				25.1 *5.56*				2.33 *0.14*			1.34 *0.04*	

kg N ha^{-1}	BDS_REIP				BDS_PRI				BDS_VARI				CC_NDVI				CC_R_{730}/R_{670}				CC_R_{760}/R_{670}			
0	720.8 *0.6*				-0.07 *0.01*				0.09 *0.05*				0.34 *0.06*				1.45 *0.13*				2.08 *0.30*			
100		726.2 *0.64*				-0.01 *0.01*				0.35 *0.06*				0.64 *0.05*				2.26 *0.28*				4.68 *0.75*		
160			728.7 *0.81*				0.02 *0.01*				0.48 *0.04*				0.74 *0.03*				2.83 *0.36*				6.81 *0.96*	
220				729.7 *1.03*				0.03 *0.001*				0.53 *0.02*			0.78 *0.04*					3.20 *0.40*				8.26 *1.21*

kg N ha^{-1}	CC_R_{760}/R_{730}				GS_NDVI				GS_R_{774}/R_{656}				AFS_R_{760}/R_{730}				AFS_WI							
0	1.43 *0.08*				0.36 *0.04*				2.14 *0.04*															
100		2.06 *0.11*				0.54 *0.04*				3.40 *0.34*														
160			2.41 *0.09*				0.63 *0.04*				4.45 *0.63*		no data				no data							
220				2.58 *0.15*				0.68 *0.05*				5.30 *0.90*												
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d

c) Zadoks stage 6

kg N ha^{-1}	DM [%]				DW [t ha^{-1}]				N content [%]				N uptake [kg ha^{-1}]				FW [t ha^{-1}]				NNI			
0				26.6 *1.67*	4.5 *0.55*				1.15 *0.17*				51.7 *104*				16.9 *2.2*				0.42 *0.07*			
100			24.9 *1.79*			7.4 *0.75*				1.58 *0.24*				115.9 *21.7*				29.5 *2.46*				0.71 *0.11*		
160		22.1 *1.74*					7.8 *0.77*				2.02 *0.28*				156.5 *21.3*				35.3 *3.43*				0.93 *0.12*	
220	21.1 *1.57*							8.7 *0.88*				2.32 *0.27*				201.8 *27.5*				41.3 *3.46*				1.12 *0.02*

kg N ha^{-1}	BDS_NDVI				BDS_R_{730}/R_{670}				BDS_R_{760}/R_{670}				BDS_R_{780}/R_{650}				BDS_R_{760}/R_{730}				BDS_WI			
0	0.70 *0.05*				4.09 *0.82*				5.77 *1.38*				5.54 *1.19*				1.40 *0.06*				1.22 *0.02*			
100		0.88 *0.01*				7.94 *0.82*				14.8 *1.73*				13.8 *1.45*				1.87 *0.06*				1.32 *0.02*		
160			0.91 *0.01*				9.44 *0.71*				20.2 *1.61*				18.6 *1.30*				2.14 *0.04*				1.36 *0.02*	
220			0.92 *0.01*					9.97 *0.95*				22.4 *2.30*				20.6 *1.87*				2.25 *0.06*				1.37 *0.02*

kg N ha^{-1}	BDS_REIP				BDS_PRI				BDS_VARI				CC_NDVI				CC_R_{730}/R_{670}				CC_R_{760}/R_{670}			
0	720.8 *0.66*				-0.06 *0.01*				0.14 *0.07*				0.37 *0.07*				1.54 *0.14*				2.21 *0.37*			
100		725.6 *0.58*				-0.02 *0.01*				0.36 *0.05*				0.63 *0.04*				2.25 *0.20*				4.45 *0.62*		
160			728.1 *0.41*				0.001 *.0001*				0.43 *0.04*				0.71 *0.03*				2.64 *0.22*				5.96 *0.59*	
220				729.3 *0.51*				0.001 *.0001*			0.45 *0.03*					0.74 *0.03*				2.85 *0.24*				6.87 *0.81*

kg N ha^{-1}	CC_R_{760}/R_{730}				GS_NDVI				GS_R_{774}/R_{656}				AFS_R_{760}/R_{730}				AFS_WI							
0	1.43 *012*				0.42 *0.04*				2.47 *0.28*															
100		1.97 *0.12*				0.63 *0.04*				4.40 *0.62*														
160			2.26 *0.09*				0.70 *0.03*				5.84 *0.66*		no data				no data							
220				2.41 *0.13*				0.74 *0.02*				6.77 *0.64*												
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d

Figure 3.2a: Mean value comparisons indicated separately for each trait of biomass and nitrogen status as well as spectral parameters for the three sampling dates Zadoks stage 3, a), 4, b), and 6, c), in 2009. Differentiation patterns are depicted for spectral parameters obtained from one bi-directional passive spectrometer (BDS) and three active sensors (Crop Circle, CC, GreenSeeker, GS, active flash sensor, AFS) as well as for the agronomic plant traits dry matter content (DM), dry weight (DW), N content, aboveground N uptake, fresh weight (FW), and nitrogen nutrition index (NNI). Different letters show significant differences at $p \leq 0.05$. For all parameters (each $n = 28$) mean values and standard deviations (*italic*) are added.

3.2.2 Differentiation of spectral parameters

In 2009, AFS data were not available, whereas for the Crop Circle and GreenSeeker, only data for the first sampling date (ZS 3) were missing. Means of the SR indices (R_{730}/R_{670}, R_{760}/R_{670}, R_{780}/R_{650}), the R_{760}/R_{730} for all sensors, the BDS_REIP and the GS_NDVI could clearly be separated over all N application levels at all dates. Only the NDVI values obtained for the N rates 160 kg ha^{-1} and 220 kg ha^{-1} could not be distinguished in 2009 (Figure 3.2a). As for the destructive differentiation of the plant traits, spectral differentiations were less distinct in the growing season 2010 compared to 2009 (Figure 3.2b).

In 2010, all sensors were available throughout the whole season. Through that time, only the indices BDS_R_{760}/R_{730} and ALS_R_{760}/R_{730} could successfully distinguish all N levels. In contrast, other spectral parameters could also separate the different N levels but only for a limited number of spectral assessments over time. This finding was the case for the majority of the Crop Circle and GreenSeeker outputs, as well as for BDS_REIP.

Inspecting only the higher N application rates of 160 kg ha^{-1} and 220 kg ha^{-1}, differentiation was not possible by the bi-directional spectrometer simple ratios BDS_R_{760}/R_{670}, BDS_R_{780}/R_{650} and WI, as well as by the CC_NDVI and the ALS_WI for all three recording dates in 2010. This differentiation pattern was also obtained with other parameters from the BDS, Crop Circle, and GreenSeeker; However, it was restricted to specific spectral assessment dates. Especially at ZS 6, the averages of the passively assessed indices BDS_NDVI, BDS_R_{730}/R_{670}, BDS_PRI, and BDS_VARI could not be distinguished between the N levels of 100 kg ha^{-1} to 220 kg ha^{-1} (Figure 3.2b). There was only one spectral parameter at a time, the GS_R_{774}/R_{656} at ZS 6, that did not show any differentiation between the four N levels.

In comparing the differentiation patterns of both plant traits and spectral parameters, a number of similarities were observed, especially in the growing season 2009. Biomass parameters and spectral recordings behaved quite similarly in this growing season and could mostly differentiate individual N levels. While not separating the N levels 160 kg ha^{-1}and 220 kg ha^{-1}, the DW at the first sampling date was well reflected by the BDS_NDVI and BDS_VARI. Through the course of the two subsequent sampling dates, only a few spectral parameters from 2009 did not match the differentiation pattern of the dedicated plant traits. Differentiation patterns were less distinct in 2010. Decreased biomass production in 2009 as compared to 2010 was possibly caused by lower temperatures and wet conditions in spring. The majority of the spectral parameters showed patterns similar to the biomass attributes in this growing season. Mainly at the third sampling date in 2010, certain differentiation patterns of the spectral parameters did not correspond to those of the plant traits and could hardly distinguish higher N rates.

a) Zadoks stage 3

kg N ha^{-1}	DM [%]				DW [t ha^{-1}]				N content [%]				N uptake [kg ha^{-1}]				FW [t ha^{-1}]				NNI			
0				26.6 *2.86*	1.43 *0.58*				2.14 *0.25*				31.2 *15.1*				5.61 *2.85*				0.46 *0.12*			
100			23.7 *1.25*			2.0 *0.42*				3.18 *0.22*				63.6 *13.7*				8.46 *1.96*				0.80 *0.09*		
160		21.5 *1.56*					2.45 *0.37*				3.92 *0.25*				95.6 *12.1*				11.5 *1.87*				1.08 *0.06*	
220	20.4 *1.66*						2.49 *0.57*					4.37 *0.28*				108.4 *22.4*			12.4 *3.38*					1.21 *0.11*

kg N ha^{-1}	BDS_NDVI				BDS_R_{730}/R_{670}				BDS_R_{760}/R_{670}				BDS_R_{780}/R_{650}				BDS_R_{760}/R_{730}				BDS_WI			
0	0.78 *0.10*				7.27 *3.09*				10.5 *5.56*				8.72 *4.41*				1.38 *0.16*				1.10 *0.03*			
100		0.90 *0.03*				11.6 *2.64*				20.1 *5.29*				17.1 *4.21*				1.72 *0.10*				1.14 *0.02*		
160			0.93 *0.01*				13.7 *2.17*				26.3 *4.62*				22.8 *3.72*				1.91 *0.08*				1.16 *0.02*	
220			0.93 *0.01*				14.1 *2.50*				28.3 *6.67*				24.8 *5.58*					1.99 *0.13*			1.17 *0.02*	

kg N ha^{-1}	BDS_REIP				BDS_PRI				BDS_VARI				CC_NDVI				CC_R_{730}/R_{670}				CC_R_{760}/R_{670}			
0	718.6 *2.34*				-0.03 *0.03*				0.35 *0.14*				0.32 *0.04*				1.45 *0.09*				*1.96* *0.18*			
100		723.0 *0.89*				0.01 *0.02*				0.52 *0.09*				0.58 *0.06*				2.05 *0.25*				3.91 *0.80*		
160			724.8 *0.70*				0.03 *.001*				0.58 *0.06*				0.68 *0.05*				2.42 *0.35*				5.50 *1.20*	
220			725.5 *1.12*				0.03 *.001*				0.60 *0.07*				0.70 *0.05*				2.51 *0.37*				5.77 *1.39*	

kg N ha^{-1}	CC_R_{760}/R_{730}				GS_NDVI				GS_R_{774}/R_{656}				AFS_R_{760}/R_{730}				AFS_WI							
0	1.35 *0.04*				0.47 *0.09*				2.89 *0.72*				1.44 *0.10*				0.99 *0.01*							
100		*1.89* *0.14*				0.60 *0.06*				4.05 *0.72*				1.66 *0.07*				1.00 *0.01*						
160			2.26 *0.16*				0.66 *0.04*				5.02 *0.72*				1.82 *0.08*				1.01 *0.01*					
220			2.28 *0.19*				0.68 *0.06*				5.42 *1.09*					1.89 *0.10*			1.02 *0.01*					
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d

b) Zadoks stage 4

kg N ha^{-1}	DM [%]				DW [t ha^{-1}]				N content [%]				N uptake [kg ha^{-1}]				FW [t ha^{-1}]				NNI			
0				26.5 *2.46*	4.15 *1.20*				1.21 *0.19*				50.9 *19.2*				16.1 *5.75*				0.42 *0.10*			
100			22.9 *1.56*			5.75 *0.88*				1.63 *0.24*				93.5 *19.3*				25.6 *3.47*				0.66 *0.10*		
160		20.8 *1.10*					6.84 *0.92*				2.02 *0.33*				137.4 *24.7*				32.7 *2.86*				0.88 *0.13*	
220		20.0 *3.24*					7.23 *1.29*					2.32 *0.28*				167.3 *33.5*				36.4 *4.76*				1.03 *0.14*

kg N ha^{-1}	BDS_NDVI				BDS_R730/R670				BDS_R760/R670				BDS_R780/R650				BDS_R760/R730				BDS_WI			
0	0.71 *0.10*				4.48 *1.66*				6.82 *3.02*				6.23 *2.62*				1.37 *0.14*				1.19 *0.04*			
100		0.88 *0.01*				9.35 *1.17*				16.0 *2.20*				14.21 *1.56*				1.71 *0.06*				1.27 *0.02*		
160			0.91 *0.01*				10.9 *1.72*				21.2 *3.78*				19.0 *3.10*				1.93 *0.07*				1.30 *0.02*	
220			0.91 *0.01*				11.1 *1.85*				22.2 *4.23*				20.0 *3.48*					1.99 *0.07*			1.30 *0.02*	

kg N ha^{-1}	BDS_REIP				BDS_PRI				BDS_VARI				CC_NDVI				CC_R730/R670				CC_R760/R670			
0	719.5 *2.06*				-0.03 *0.02*				0.23 *0.11*				0.34 *0.14*				1.51 *0.32*				2.20 *0.81*			
100		723.7 *0.79*				0.02 *0.01*				0.48 *0.05*				0.64 *0.04*				2.39 *0.32*				4.72 *0.80*		
160			725.9 *0.65*				0.04 *.0001*				0.56 *0.04*				0.75 *0.03*				3.05 *0.33*				7.13 *0.93*	
220				726.6 *0.72*			0.04 *0.01*				0.57 *0.04*				0.78 *0.03*					3.31 *0.37*				8.13 *1.12*

kg N ha^{-1}	CC_R760/R730				GS_NDVI				GS_R774/R656				AFS_R760/R730				AFS_WI							
0	1.41 *0.20*				0.43 *0.10*				2.71 *0.74*				1.40 *0.08*				0.96 *0.01*							
100		1.97 *0.09*				0.64 *0.04*				4.81 *0.71*				1.63 *0.04*				0.99 *0.01*						
160			2.34 *0.09*				0.72 *0.06*				6.69 *0.84*				1.79 *0.04*				0.99 *0.01*					
220				2.45 *0.10*				0.76 *0.04*				7.62 *0.96*				1.84 *0.04*			1.00 *0.01*					
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d

c) Zadoks stage 6

kg N ha^{-1}	DM [%]				DW [t ha^{-1}]				N content [%]				N uptake [kg ha^{-1}]				FW [t ha^{-1}]				NNI			
0				38.7 *2.76*	7.31 *1.57*				1.01 *0.14*				74.2 *21.1*				19.0 *4.66*				0.45 *0.09*			
100			36.2 *2.15*			10.6 *1.29*				1.37 *0.21*				145.8 *32.8*				29.3 *4.11*				0.73 *0.13*		
160		32.7 *1.94*					11.3 *1.22*				1.58 *0.24*				179.6 *33.6*				34.7 *4.07*				0.86 *0.14*	
220	31.0 *1.97*					11.0 *1.19*	11.0 *1.19*					1.85 *0.25*				204.1 *36.4*			35.6 *4.10*					1.00 *0.15*

kg N ha^{-1}	BDS_NDVI				BDS_R730/R670				BDS_R760/R670				BDS_R780/R650				BDS_R760/R730				BDS_WI			
0	0.78 *0.07*				6.28 *1.90*				9.10 *3.86*				7.75 *3.19*				1.41 *0.16*				1.16 *0.04*			
100		0.89 *0.02*				9.24 *1.19*				16.3 *2.69*				14.0 *2.24*				1.76 *0.09*				1.22 *0.02*		
160		0.90 *0.02*				9.64 *1.31*					18.9 *3.15*				16.6 *2.59*				1.96 *0.08*				*1.26* 0.02	
220		0.91 *0.01*				9.66 *1.18*					19.5 *2.81*				17.2 *2.29*					2.01 *0.07*			1.27 *0.02*	

kg N ha^{-1}	BDS_REIP				BDS_PRI				BDS_VARI				CC_NDVI				CC_R730/R670				CC_R760/R670			
0	719.4 *2.29*				-0.03 *0.02*				0.35 *0.10*				0.40 *0.11*				1.65 *0.27*				2.43 *0.69*			
100		723.9 *0.91*				.001 *.0001*				0.49 *0.04*				0.64 *0.03*				2.35 *0.19*				4.60 *0.52*		
160			726.1 *0.70*			0.01 *0.001*				0.50 *0.03*					0.71 *0.03*				2.72 *0.02*				6.08 *0.71*	
220			726.7 *0.66*			0.01 *0.005*				0.50 *0.03*					0.73 *0.03*				2.86 *0.23*					6.61 *0.74*

kg N ha^{-1}	CC_R760/R730				GS_NDVI				GS_R774/R656				AFS_R760/R730				AFS_WI							
0	1.45 *0.16*				0.48 *0.09*				2.82 *0.57*				1.39 *0.07*				0.99 *0.01*							
100		1.95 *0.09*				0.66 *0.04*			4.98 *0.63*					1.62 *0.04*				1.01 *0.01*						
160			2.23 *0.12*				0.72 *0.03*		6.42 *0.73*						1.75 *0.05*				1.01 *0.01*					
220				2.30 *0.11*			0.74 *0.03*		7.16 *0.79*							1.79 *0.06*			1.02 *0.01*					
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d

Figure 3.2b: Mean value comparisons indicated separately for each trait of biomass and nitrogen status as well as spectral parameters for the three sampling dates Zadoks stage 3, a), 4, b), and 6, c), in 2010. Differentiation patterns are depicted for spectral parameters obtained from one bi-directional passive spectrometer (BDS) and three active sensors (Crop Circle, CC, Green-Seeker, GS, active flash sensor, AFS) as well as for the agronomic plant traits dry matter content (DM), dry weight (DW), N content, aboveground N uptake, fresh weight (FW), and nitrogen nutrition index (NNI). Different letters show significant differences at $p \leq 0.05$. For all parameters (each $n = 28$) mean values and standard deviations (*italic*) are added.

3.2.3 Relationship between plant traits and spectral parameters

Linear and curvilinear relationships were chosen depending on the AIC criterion (Webster and McBratney, 1989) to assess the relationship between plant traits and spectral parameters. In general, the majority of relationships was best depicted by quadratic relationships.

Over the three sampling dates, all 17 spectral parameters of the four sensor systems were correlated with the plant traits and r^2 values are shown for each growing season in Table 3.2a. At ZS 3, the r^2 values obtained for the relationships between sensor outputs and plant traits were higher in 2010 than in 2009, with the coefficients being as high as 0.96. However, it must be considered that only the bi-directional spectrometer was available at the first sampling date 2009, and the differences among the r^2 values in 2009 and 2010 were between 0.02 and 0.39. Highest coefficients were found for the relationship with N content. The largest differences in r^2 values in the two growing seasons were found for the indices BDS_WI followed by BDS_VARI and BDS_R_{730}/R_{670} over all plant traits. In contrast, at the second and third sampling dates, the values for the coefficients of determination of most spectral parameters increased in 2009 relative to 2010. At ZS 4, the plant trait dry matter content had r^2 values ranging from 0.70 to 0.84 in 2009 compared to values ranging from 0.48 to 0.62 in 2010. However, at ZS 6, the r^2 values of this plant trait were lower in both growing seasons than for the other plant traits.

Over all three sampling dates, the best relationships between spectral and biomass data were found for the plant traits aboveground N uptake, fresh weight, and NNI, independent of the chosen spectral parameter. On the other hand, independent of the plant traits or recording dates, four spectral parameters appeared to be highly and consistently related to the sampling data: BDS_R_{760}/R_{730}, BDS_REIP, CC_NDVI, and CC_R_{760}/R_{730}.

By augmenting these findings with information on the differentiation patterns, the relationship between biomass attributes and spectral parameters can be even better illustrated. The coefficients of determination for which the patterns of the spectral parameters and the plant traits agree are highlighted in Table 3.2a. For the first sampling date, a quite controversial picture between the two growing seasons was obtained: on the one hand, most of the passively assessed parameters in 2009 (except BDS_NDVI and BDS_VARI) matched the patterns of the plant traits DM, N content, aboveground N uptake, FW and NNI. On the other hand, in the growing season 2010, only one index, BDS_R_{760}/R_{730}, behaved analogously, except for FW. But the r^2 values in 2010 were almost constantly higher than those in 2009. For FW, each spectral parameter agreed with the plant trait´s N level in both years at ZS 3 except for three spectral indices BDS NDVI, R_{760}/R_{730} and VARI.

At the second sampling date, the sensor parameters of the Crop Circle and the GreenSeeker, except for CC_NDVI, did match the pattern of differentiation for FW or for all three nitrogen indicators. In 2010 this was only the case for BDS_R_{760}/R_{730} and BDS_REIP. In the case of the bi-directional spectrometer in 2009, the patterns' analogies of each plant trait behaved comparably: All spectral parameters, except for BDS_NDVI and BDS_WI, fit the dedicated pattern and showed even higher r^2 values than in 2010. Similarly, at ZS 6 in 2009, the spectral outputs from the Crop Circle and the GreenSeeker as well as most of the BDS parameters were in line with the patterns for each plant trait. Additionally, at the same date, relationships between the spectral and biomass data showed higher r^2 values in 2009 than in 2010.

Regarding both the differentiation patterns and the r^2 values, the passively assessed BDS_ R_{760}/R_{730} was most closely related to all crop attributes over both seasons.

Table 3.2a: Coefficients of determination for the relationships between spectral parameters obtained from one bi-directional passive spectrometer (BDS) and three active sensors (Crop Circle, CC, GreenSeeker, GS, active flash sensor, AFS) and the plant traits dry matter content (DM), dry weight (DW), N content, N uptake, fresh weight (FW) and nitrogen nutrition index (NNI) for the three sampling dates at Zadoks stages 3, 4, and 6 in 2009 and 2010. Models follow linear (indicated by an asterisk) or quadratic courses, appropriateness was assessed by the variable part of the AIC. Highlighted values indicate an analogous pattern of differentiation between a spectral parameter and a plant trait. All results were significant at $p < 0.001$ for $n = 112$.

Zadoks stage 3	DM		DW		N content		N uptake		FW		NNI	
	´09	´10	´09	´10	´09	´10	´09	´10	´09	´10	´09	´10
BDS_NDVI	0.53	0.82	0.67	0.84	0.50	0.78	0.72	0.86	0.71	0.89	0.68	0.85
BDS_R_{730}/R_{670}	0.49$^{*)}$	0.68$^{*)}$	0.60	0.80	0.39	0.67	0.61	0.79	0.65	0.83	0.55	0.75
BDS_R_{760}/R_{670}	0.60	0.71$^{*)}$	0.62	0.83	0.49	0.73	0.71	0.86	0.71	0.87	0.69	0.82
BDS_R_{780}/R_{650}	0.64	0.72$^{*)}$	0.63	0.85	0.53	0.76	0.75	0.88	0.73	0.89	0.71	0.85
BDS_R_{760}/R_{730}	0.76$^{*)}$	0.78$^{*)}$	0.63	0.88	0.75	0.90	0.86	0.97	0.75	0.92	0.87	0.96
BDS_WI	0.59	0.80$^{*)}$	0.66	0.85	0.45	0.84	0.70	0.91	0.73	0.90	0.63	0.90
BDS_REIP	0.76$^{*)}$	0.78$^{*)}$	0.60	0.91	0.79	0.91	0.86	0.97	0.73	0.94	0.89	0.96
BDS_PRI	0.72$^{*)}$	0.78$^{*)}$	0.66	0.89	0.70	0.81	0.85	0.91	0.77	0.92	0.84	0.88
BDS_VARI	0.49$^{*)}$	0.73	0.65	0.80	0.40	0.68	0.64	0.80	0.69	0.84	0.57	0.76
CC_NDVI		0.77$^{*)}$		0.81$^{*)}$		0.83		0.90		0.89		0.89
CC_R_{730}/R_{670}		0.59$^{*)}$		0.71$^{*)}$		0.62		0.74$^{*)}$		0.74$^{*)}$		0.70$^{*)}$
CC_R_{760}/R_{670}		0.59$^{*)}$		0.73		0.64		0.77$^{*)}$		0.76$^{*)}$		0.74$^{*)}$
CC_R_{760}/R_{730}		0.73$^{*)}$		0.82		0.81		0.89		0.87		0.88
GS_NDVI		0.70		0.73		0.73		0.79		0.78		0.78
GS_R_{774}/R_{656}		0.59$^{*)}$		0.65$^{*)}$		0.63		0.70		0.69		0.69$^{*)}$
AFS_R_{760}/R_{730}		0.75$^{*)}$		0.83		0.84		0.92		0.88		0.91
AFS_WI		0.73$^{*)}$		0.72$^{*)}$		0.76$^{*)}$		0.83		0.81		0.82

Zadoks stage 4	DM		DW		N content		N uptake		FW		NNI	
	´09	´10	´09	´10	´09	´10	´09	´10	´09	´10	´09	´10
BDS_NDVI	0.83	0.61	0.87	0.78	0.80	0.59	0.92	0.81	0.94	0.90	0.90	0.77
BDS_R_{730}/R_{670}	0.75	0.52	0.75	0.62	0.72	0.60	0.82	0.73	0.84	0.74	0.80	0.71
BDS_R_{760}/R_{670}	0.73$^{*)}$	0.53	0.72$^{*)}$	0.63	0.70	0.65	0.80	0.78	0.82$^{*)}$	0.78	0.77	0.75
BDS_R_{780}/R_{650}	0.73$^{*)}$	0.54	0.72$^{*)}$	0.64	0.71	0.68	0.80	0.80	0.82$^{*)}$	0.80	0.77	0.78
BDS_R_{760}/R_{730}	0.82	0.60	0.79	0.73	0.82	0.73	0.90	0.88	0.89	0.91	0.89	0.85
BDS_WI	0.71	0.58	0.81	0.71	0.73	0.61	0.80	0.78	0.83	0.83	0.79	0.75
BDS_REIP	0.84	0.61	0.80	0.76	0.85	0.73	0.91	0.89	0.90	0.92	0.90	0.86
BDS_PRI	0.84	0.62	0.84	0.77	0.83	0.66	0.93	0.86	0.93	0.91	0.91	0.82
BDS_VARI	0.82	0.58	0.82	0.72	0.81	0.64	0.92	0.82	0.91	0.85	0.90	0.78
CC_NDVI	0.82	0.61	0.83	0.76	0.80	0.70	0.91	0.87	0.92	0.90	0.89	0.84
CC_R_{730}/R_{670}	0.70$^{*)}$	0.50	0.70	0.61	0.68	0.69	0.79	0.79	0.80	0.76	0.76	0.78
CC_R_{760}/R_{670}	0.75$^{*)}$	0.50	0.70	0.61	0.74	0.71	0.83	0.81	0.82$^{*)}$	0.78$^{*)}$	0.81	0.80
CC_R_{760}/R_{730}	0.83	0.58	0.77	0.71	0.83	0.75	0.91	0.89	0.88	0.88	0.90	0.86
GS_NDVI	0.83	0.58	0.74	0.72	0.81	0.70	0.87	0.85	0.85	0.86	0.86	0.83
GS_R_{774}/R_{656}	0.75	0.48	0.55	0.61	0.78$^{*)}$	0.70	0.75	0.80	0.68	0.77	0.77	0.79
AFS_R_{760}/R_{730}		0.57		0.69		0.75		0.88		0.86		0.86
AFS_WI		0.58		0.70		0.61		0.77		0.82		0.74

Zadoks stage 6	DM		DW		N content		N uptake		FW		NNI	
	´09	´10	´09	´10	´09	´10	´09	´10	´09	´10	´09	´10
BDS_NDVI	0.47	0.53	0.90	0.65	0.71	0.52	0.88	0.68	0.93	0.72	0.84	0.64
BDS_R_{730}/R_{670}	0.57*)	0.43	0.80	0.49	0.73	0.44	0.86	0.53	0.89	0.55	0.82	0.52
BDS_R_{760}/R_{670}	0.61*)	0.52	0.79	0.53	0.78	0.55	0.89	0.63	0.91	0.62	0.86	0.62
BDS_R_{780}/R_{650}	0.62*)	0.55	0.80	0.54	0.79	0.58	0.90	0.66	0.91	0.64	0.87	0.65
BDS_R_{760}/R_{730}	0.58*)	0.64	0.84	0.61	0.82	0.69	0.94	0.77	0.93	0.74	0.91	0.76
BDS_WI	0.51	0.63	0.84	0.62	0.72	0.60	0.86	0.72	0.90	0.74	0.82	0.70
BDS_REIP	0.59*)	0.64	0.84	0.66	0.83	0.68	0.94	0.79	0.93	0.78	0.91	0.77
BDS_PRI	0.52	0.52	0.84	0.65	0.70	0.52	0.84	0.69	0.90	0.73	0.81	0.65
BDS_VARI	0.53*)	0.42	0.81	0.56	0.69	0.41	0.83	0.57	0.89	0.61	0.79	0.53
CC_NDVI	0.56*)	0.61	0.85	0.69	0.76	0.68	0.89	0.81	0.92	0.79	0.86	0.78
CC_R_{730}/R_{670}	0.63*)	0.58	0.71	0.58	0.75	0.63	0.83	0.72	0.85	0.70	0.82	0.71
CC_R_{760}/R_{670}	0.67*)	0.63*)	0.69	0.57	0.79	0.67	0.85	0.74	0.86	0.72	0.84	0.73
CC_R_{760}/R_{730}	0.63*)	0.67	0.76	0.63	0.79	0.70	0.87	0.79	0.88	0.77	0.86	0.78
GS_NDVI	0.57	0.61	0.84	0.65	0.78	0.68	0.91	0.79	0.93	0.76	0.88	0.77
GS_R_{774}/R_{656}	0.63*)	0.62*)	0.80*)	0.54	0.78	0.66	0.86	0.72	0.87*)	0.70*)	0.85	0.72
AFS_R_{760}/R_{730}		0.67*)		0.60		0.71		0.78		0.76		0.78
AFS_WI		0.47		0.63		0.52		0.67		0.69		0.63

3.3 *Section III: Tracking phenotypic differences in biomass and nitrogen partitioning by spectral high-throughput assessments during grain filling in wheat*

3.3.1 Wheat cultivar differences in destructively obtained plant traits

The GLM results (Table 3.3a) revealed that the main effects of both the cultivar and the development stage were highly significant over all sampling dates and spectral parameters. Therefore, we expect to find cultivar differences in biomass and N deposition with a variation between the development stages. The plant traits of four intermediate biomass samplings collected during grain filling are listed in Table 3.3b. While no differences were found in the onset of the single vegetation stages, the six observed winter wheat cultivars did significantly differ in their biomass and nitrogen accumulation.

Solitär showed the highest values for leaves+stems DW which were significantly different from the remaining cultivars except at ZS 83 for both. In contrast, the cultivars *Tommi* and *Cubus* exhibited only low leaves+stems DW values. All the cultivars except *Cubus* and *Ellvis* showed slight increases in leaves+stems DW between ZS 75 and ZS 77 followed by a strong decrease in these attributes at ZS 83.

The spikes DW increased consistently throughout the grain-filling period for most of the cultivars. The increase in spikes DW between ZS 75 and ZS 77 was highest for *Pegassos* with a DW gain of 3.9 t ha^{-1}, resulting in the highest spikes DW yield of 11.1 t ha^{-1} at ZS 85.

Table 3.3a: F-values, degrees of freedom (df) and significance levels of the general linear model (GLM) for the four spectral parameters assessed by the passive bi-directional spectrometer (BDS) across six wheat cultivars, four replications and four development stages (Dev. stage). Significant main effects and interactions are indicated as $p \le 0.05$ (*), $p \le 0.01$ (**) and $p \le 0.001$ (***).

Source of variation	df	BDS_NDVI	BDS_WI	BDS_REIP	BDS_R_{760}/R_{730}
Model	41	238.5^{***}	97.9^{***}	95.0^{***}	157.4^{***}
Cultivar	5	174.3^{***}	54.2^{***}	49.7^{***}	94.6^{***}
Replication	3	9.9^{***}	0.2	8.0^{***}	6.6^{***}
Dev. stage	3	2866.8^{***}	1218.8^{***}	1166.8^{***}	1950.5^{***}
Cultivar x Replication	15	2.3^{*}	1.3	2.3^{*}	2.3^{*}
Cultivar x Dev. stage	15	16.2^{***}	4.5^{***}	5.9^{***}	4.9^{***}
Residual	54				
Total	96				

Expressing the transfer dynamics between the leaves+stems and the spikes, the two plant traits DW index and N_{up} index revealed obvious differences between the six cultivars. The persistently high leaves+stems DW values in contrast to the low spikes DW of the cultivar *Solitär* resulted in a low DW index between 0.39 and 1.18 throughout the grain filling period. In contrast, *Cubus* exhibited a constantly high DW index of 0.68 to 2.06. Similar to the DW index, *Solitär* reflected the lowest N_{up} index among the six cultivars.

The final combine harvest resulted in grain yields of more than 9.0 t ha^{-1} for the cultivars *Impression*, *Pegassos* and *Cubus*, whereas the cultivar *Solitär* yielded only 8.2 t ha^{-1}, showing the lowest grain yield in this experiment. In terms of the final grain DM content, *Solitär* proved to have the significantly lowest grain DM content of 83.5 %, in contrast to *Cubus* with 85.2 %. The cultivar differences in grain DM content tended to follow the differences in the DW indices and N_{up} indices. The final grain N content was highest for *Tommi* (2.2 %) and lowest for *Cubus* (2.0 %) but was not correlated to any N uptake data throughout grain filling. The NUE was highest for the cultivar *Impression* with a value of 0.97 and lowest for *Solitär* with 0.84. However, the NUE was hardly correlated to any biomass sampling or harvest data of this experiment (data not shown).

The harvest index, HI, differed significantly for the cultivars observed. Whereas *Solitär* showed the lowest HI with 0.45, the group of *Tommi*, *Impression* and *Cubus* exhibited the significantly highest HI of up to 0.55.

3.3.2 Relationships between grain yield and plant traits

Linear relationships were chosen to assess the influence of single plant traits on the final yield. Figure 3.3a shows the predictive accuracy in terms of the coefficient of determination (r^2). The final grain yield was best correlated to the spikes DW, the spikes DM content and the DW index with significant r^2 values of up to 0.85 for the spikes DM content. These three plant traits were significantly correlated to final grain yield throughout the sampling dates from ZS 75 to ZS 83. Only the spikes DW also resulted in a good relationship to the final grain yield at ZS 85. Leaves+stems DW was also highly related to the final grain yield but was restricted to the sampling date at ZS 83. Similarly, the interrelation of the grain yield and the N_{up} index was only significant at ZS 77. The leaves+stems N_{up} and the spikes N_{up} did not show significant relationships to the final grain yield at the observed growth stages.

The harvest index (HI) was correlated to final grain yield with an r^2 of 0.39 which is similar to the DW index at ZS 85 with a r^2 of 0.36.

Table 3.3b: Mean value comparisons between six wheat cultivars indicated separately for each plant trait and final harvest data. For the four sampling dates at Zadoks stage (ZS) 75, 77, 83 and 85, the values of dry weight (DW), dry matter content (DM) and N uptake (N_{up}) for the leaves+stems and the spikes, as well as the dry weight index (DW index), N uptake index (N_{up} index), final grain yield, grain dry matter content (grain DM), grain N content (Grain N) and the harvest index (HI) are shown. Different letters within each row show significant differences between the cultivars at $p \leq 0.05$. For all parameters (each n = 4), the mean values and standard deviations (±) are indicated.

Plant traits	ZS	Tommi		Solitär		Impression		Pegassos		Cubus		Ellvis	
leaves+stems DW	75	7.2^{a}	(1.04)	9.3^{b}	(1.45)	7.9^{ab}	(0.46)	8.7^{ab}	(1.29)	8.5^{ab}	(1.14)	8.8^{ab}	(0.73)
[t ha^{-1}]	77	7.3^{ab}	(0.69)	9.6^{c}	(2.12)	8.8^{bc}	(0.38)	9.0^{bc}	(1.27)	6.1^{a}	(1.05)	8.0^{bc}	(1.21)
	83	6.4^{a}	(1.01)	6.8^{a}	(1.12)	5.9^{a}	(0.69)	6.1^{a}	(0.64)	5.6^{a}	(1.35)	6.7^{a}	(1.01)
	85	5.5^{a}	(0.92)	7.8^{a}	(1.79)	6.1^{a}	(0.39)	7.2^{a}	(0.71)	5.1^{a}	(0.63)	5.7^{a}	(1.01)
spikes DW	75	3.9^{a}	(0.39)	3.6^{a}	(0.28)	5.1^{b}	(0.10)	4.8^{b}	(1.11)	5.8^{b}	(0.60)	4.9^{b}	(0.55)
[t ha^{-1}]	77	6.8^{a}	(1.45)	6.2^{a}	(0.71)	8.7^{bc}	(0.68)	8.7^{bc}	(1.86)	7.4^{abc}	(0.38)	7.2^{ab}	(1.03)
	83	8.6^{a}	(0.29)	7.7^{a}	(0.84)	9.4^{c}	(1.19)	8.6^{a}	(0.95)	9.1^{a}	(1.39)	8.6^{a}	(0.32)
	85	9.3^{a}	(1.76)	9.0^{a}	(1.19)	10.2^{a}	(1.13)	11.1^{a}	(1.35)	10.5^{a}	(1.75)	9.9^{a}	(1.99)
DW index	75	0.55^{b}	(0.03)	0.39^{a}	(0.04)	0.65^{c}	(0.04)	0.55^{b}	(0.05)	0.68^{c}	(0.03)	0.56^{b}	(0.03)
	77	0.93^{b}	(0.13)	0.66^{a}	(0.09)	0.99^{b}	(0.06)	0.97^{b}	(0.16)	1.25^{c}	(0.30)	0.90^{b}	(0.06)
	83	1.32^{ab}	(0.20)	1.14^{a}	(0.11)	1.59^{bc}	(0.26)	1.41^{abc}	(0.12)	1.64^{c}	(0.17)	1.31^{ab}	(0.23)
	85	1.70^{bc}	(0.17)	1.18^{a}	(0.10	1.68^{bc}	(0.23)	1.55^{ab}	(0.19)	2.06^{c}	(0.21)	1.78^{bc}	(0.48)
leaves+stems N_{up}	75	105.9^{ab}	(19.4)	125.1^{b}	(20.2)	94.4^{ab}	(9.50)	107.1^{ab}	(28.2)	110.1^{ab}	(22.2)	106.4^{ab}	(13.3)
[kg ha^{-1}]	77	93.5^{c}	(17.7)	102.9^{c}	(17.5)	90.7^{bc}	(15.1)	89.5^{bc}	(10.8)	63.9^{a}	(16.0)	82.4^{abc}	(5.6)
	83	73.3^{c}	(21.0)	70.3^{bc}	(16.0)	56.2^{abc}	(4.6)	51.6^{c}	(8.6)	51.1^{ab}	(13.3)	54.0^{ab}	(7.5)
	85	53.1^{b}	(18.7)	53.9^{b}	(11.6)	34.5^{a}	(10.2)	70.9^{c}	(6.0)	40.9^{ab}	(7.6)	43.9^{ab}	(7.8)
spikes N_{up}	75	74.4^{ab}	(6.8)	63.9^{a}	(4.2)	90.7^{c}	(1.5)	81.9^{bc}	(19.2)	97.7^{c}	(8.8)	93.1^{c}	(9.9)
[kg ha^{-1}]	77	130.1^{abc}	(24.3)	105.4^{a}	(12.9)	140.0^{bc}	(14.0)	151.7^{bc}	(32.8)	124.6^{ab}	(4.4)	128.9^{abc}	(8.7)
	83	165.1^{a}	(5.5)	133.8^{a}	(18.0)	162.4^{a}	(20.1)	152.7^{a}	(14.6)	160.1^{a}	(22.1)	162.0^{a}	(7.5)
	85	184.6^{a}	(40.1)	161.8^{a}	(15.9)	187.6^{a}	(18.1)	210.7^{a}	(30.8)	202.7^{a}	(36.6)	194.3^{a}	(7.8)
N_{up} index	75	0.72^{b}	(0.15)	0.52^{a}	(0.07)	0.97^{c}	(0.12)	0.77^{bc}	(0.10)	0.91^{cd}	(0.14)	0.88^{cd}	(0.11)
	77	1.41^{ab}	(0.29)	1.03^{a}	(0.08)	1.56^{abc}	(0.15)	1.69^{bc}	(0.29)	2.06^{cd}	(0.62)	1.56^{abc}	(0.18)
	83	2.23^{a}	(0.12)	1.95^{a}	(0.30)	2.91^{b}	(0.48)	3.00^{b}	(0.40)	3.21^{b}	(0.45)	3.05^{b}	(0.52)
	85	2.23^{ab}	(0.48)	1.95^{a}	(0.64)	2.91^{c}	(0.23)	3.00^{a}	(0.19)	3.21^{cd}	(0.37)	3.05^{bc}	(1.42)
spikes DM	75	26.8^{a}	(0.01)	26.2^{a}	(0.00)	29.5^{c}	(0.01)	30.3^{c}	(0.01)	29.9^{c}	(0.01)	28.7^{b}	(0.00)
[%]	77	36.1^{ab}	(0.05)	32.1^{a}	(0.01)	36.9^{ab}	(0.01)	39.0^{bc}	(0.03)	37.3^{b}	(0.01)	35.9^{ab}	(0.01)
	83	38.9^{b}	(0.01)	37.2^{a}	(0.01)	41.0^{c}	(0.00)	41.4^{cd}	(0.00)	42.2^{d}	(0.00)	41.1^{c}	(0.01)
	85	43.9^{a}	(0.01)	43.3^{a}	(0.01)	46.1^{b}	(0.01)	47.8^{c}	(0.01)	49.4^{c}	(0.01)	48.3^{c}	(0.00)
Grain yield [t ha^{-1}]		8.7^{b}	(0.17)	8.2^{a}	(0.24)	9.5^{c}	(0.06)	9.4^{c}	(0.19)	9.4^{c}	(0.22)	8.6^{b}	(0.31)
Grain DM [%]		83.9^{ab}	(0.71)	83.5^{a}	(0.83)	84.6^{bc}	(0.73)	84.4^{abc}	(0.49)	85.2^{c}	(0.15)	84.9^{c}	(0.65)
Grain N [%]		2.2^{b}	(0.11)	2.1^{ab}	(0.06)	2.1^{ab}	(0.09)	2.1^{ab}	(0.04)	2.0^{a}	(0.09)	2.1^{ab}	(0.03)
HI		0.54^{c}	(0.00)	0.45^{a}	(0.03)	0.52^{c}	(0.02)	0.49^{b}	(0.01)	0.55^{c}	(0.02)	0.48^{b}	(0.03)

3.3.3 Relationships between spectral parameters and plant traits

To assess the ability of the reflectance measurements to reveal differences of grain filling characteristics between cultivars, linear relationships between the spectral parameters and the plant traits were established. In Table 3.3c, the predictive accuracy in terms of the coefficient of determination (r^2) between the spectral parameters and the plant traits are shown. Only relationships between spectral parameters and plant traits which were additionally well related to final grain yield were depicted graphically (Figures 3.3b, 3.3c, 3.3d).

Although expressing rather low but temporarily stable coefficients of determination, the final grain yield was related to three spectral parameters, BDS_NDVI, BDS_REIP and BDS_R_{760}/R_{730}. But depending on the spectral parameters used and the plant trait chosen, far better relationships were obtained to the plant traits chosen in this work being closely related to grain yield.

The BDS_NDVI was well related to the spikes DM content, the DW index, the grain DM content and the leaves+stems N_{up} throughout grain filling. However, the predictive quality strongly varied between the development stages showing r^2 values between 0.05 and 0.84 (for leaves+stems N_{up}) for a single plant trait.

The water index, BDS_WI, was the only spectral parameter significantly related to the leaves+stems DW and the HI. The predictive qualities of the BDS_WI in terms of the leaves+stems DW and the DW index were variable among the different dates of observation and were lowest at ZS 83.

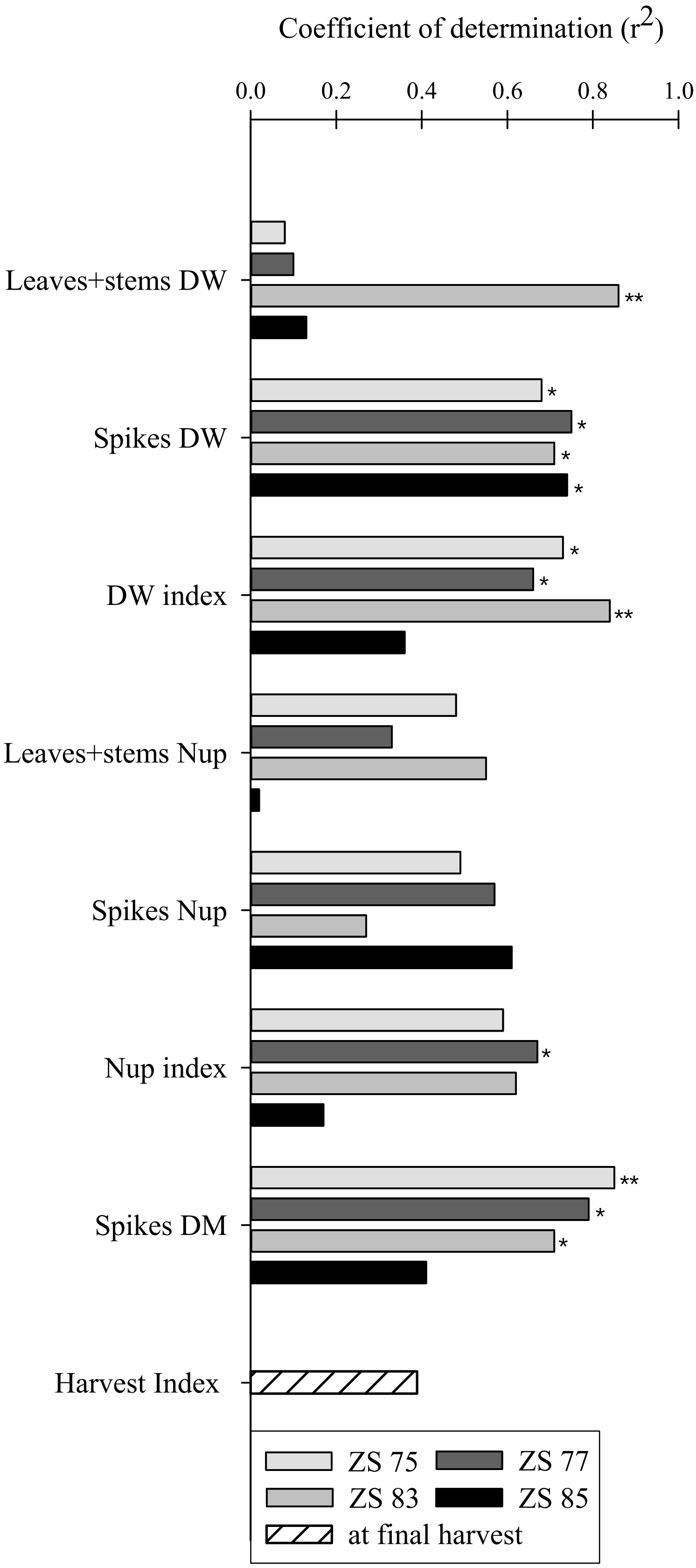
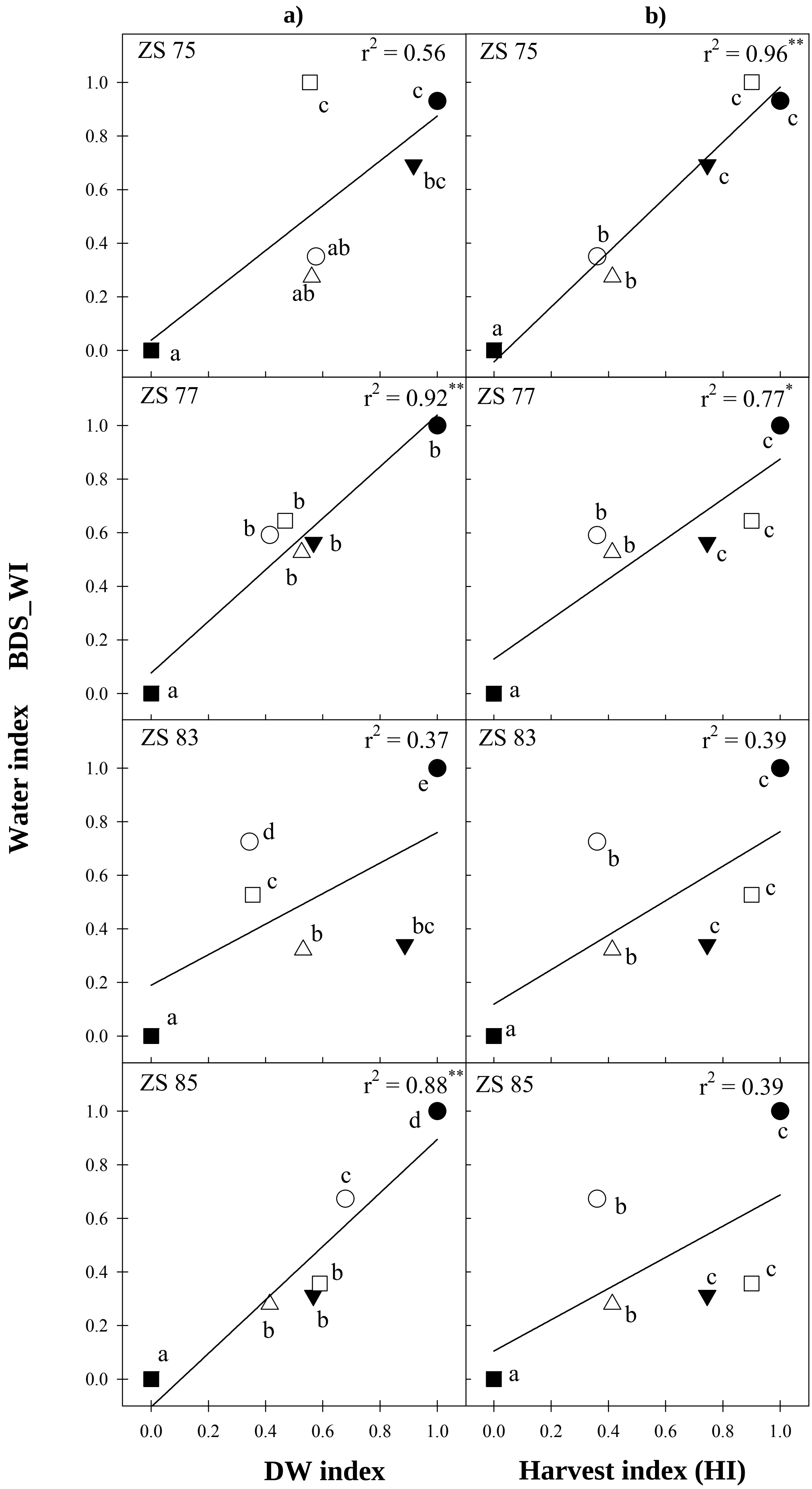


Figure 3.3a: Coefficients of determination for the relationships between the final grain yield and the plant traits of six wheat cultivars. Dry weight (DW), dry matter content (DM) and N uptake (N_{up}) for the leaves+stems and the spikes, as well as the dry weight index (DW index) and the N uptake index (N_{up} index), were obtained at four sampling dates at Zadoks stages (ZS) 75, 77, 83 and 85. At final harvest, the harvest index (HI) was assessed. Linear relationships are shown with the significance indicated as $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***) for n = 6.

The relationships quality of the HI to the four spectral parameters decreased with progressing of grain filling. Best correlations to the HI were found for the WI with an r^2 value of up to 0.96 at ZS 75 (Figure 3.3b).

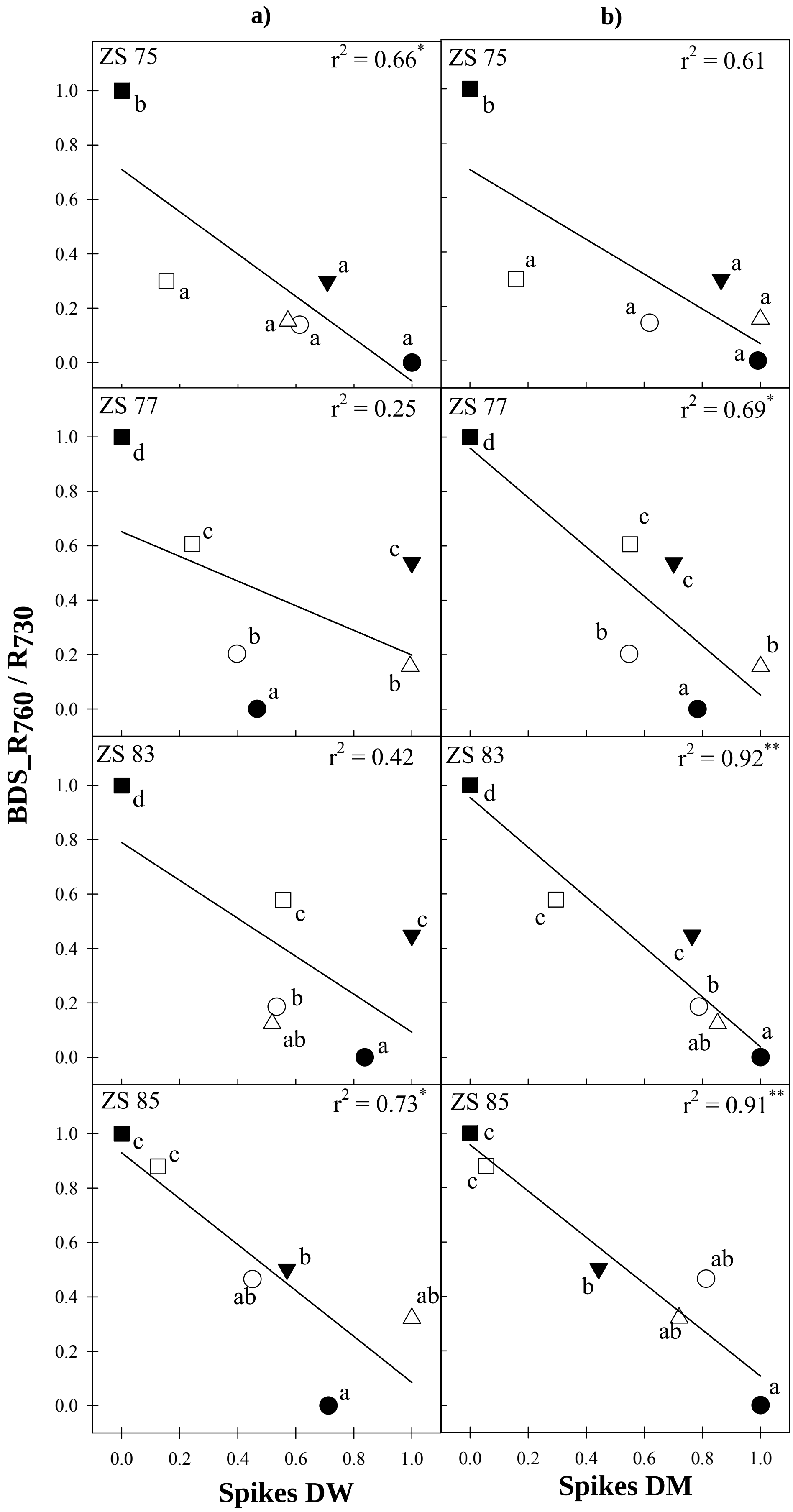
The BDS_REIP was significantly related to several plant traits at the three sampling dates of ZS 75, 77 and 83. This was observed for the spikes DM content, the N_{up} index and the final grain DM content with r^2 values ranging between 0.67 and 0.92. A similar trend with lower coefficients of determination was found for the relationships to the DW index and the spikes N_{up}. The BDS_REIP was the only spectral parameter being significantly related to the final grain yield, but restricted to the sampling dates at ZS 75 and ZS 83.

Figures 3.3c and 3.3d show the relationships of the index BDS_R_{760}/R_{730} to the spikes DW, spikes DM content, spikes N_{up}, N_{up} index and grain DM content. The spikes DW and spikes N_{up} both resulted in good relations to BDS_R_{760}/R_{730} but only at the sampling dates at ZS 75 and ZS 85. Compared to the BDS_REIP, the BDS_R_{760}/R_{730} also successfully predicted the spikes DM content throughout the grain filling period, with r^2 values of up to 0.92. The predictive qualities for the relationships between BDS_R_{760}/R_{730} and the spikes DM content increased during grain filling resulting in a distinct differentiation of four cultivar groups at ZS 85. This trend was also found for the index´ relationship to the grain N content. Within the same period as the BDS_REIP, ZS 75 to ZS 83, the index BDS_R_{760}/R_{730} was strongly related to the N_{up} index. The N_{up} course of the spikes was significantly assessed by the index BDS_R_{760}/R_{730} at ZS 75 and ZS 85. Like the BDS_WI, the BDS_R_{760}/R_{730} index was well related to the final grain DM content throughout grain filling.



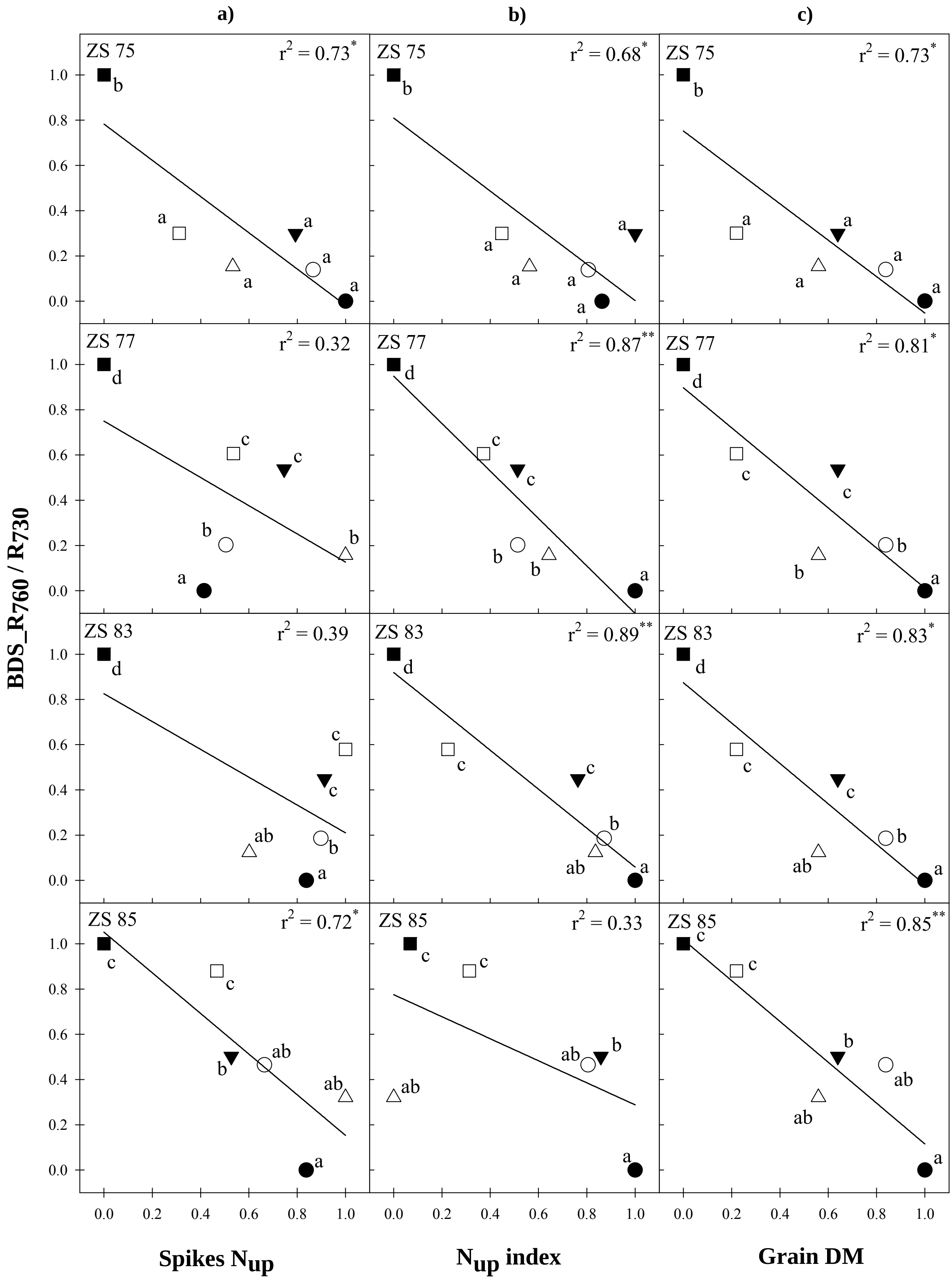
a)
b)
ZS 75
r2 = 0.56
r2 = 0.96**
ZS 77
r2 = 0.92**
r2 = 0.77*
ZS 83
r2 = 0.37
r2 = 0.39
ZS 85
r2 = 0.88**
r2 = 0.39
Water index BDS_WI
DW index
Harvest index (HI)

Figure 3.3b: Linear relationships between the normalised mean values (n = 4) of the water index (BDS_WI) and the plant traits **a)** the dry weight index (DW index) and **b)** the harvest index (HI) of the four sampling dates at Zadoks stage (ZS) 75, 77, 83 and 85. Significant coefficients of determination (r^2) are indicated by the significance levels of $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***) for n = 6. Different letters indicate significant differences of the WI among the six wheat cultivars *Cubus* (●), *Ellvis* (○), *Impression* (▼), *Pegassos* (Δ), *Solitär* (■) and *Tommi* (□).



a)
b)
ZS 75
$r^2 = 0.66^*$
ZS 75
$r^2 = 0.61$
ZS 77
$r^2 = 0.25$
ZS 77
$r^2 = 0.69^*$
ZS 83
$r^2 = 0.42$
ZS 83
$r^2 = 0.92^{**}$
ZS 85
$r^2 = 0.73^*$
ZS 85
$r^2 = 0.91^{**}$
BDS_R760 / R730
Spikes DW
Spikes DM

Figure 3.3c: Linear relationships between the normalised mean values (n = 4) of the spectral index BDS_R_{760}/R_{730} and the plant traits **a)**, spikes dry weight (DW), **b)** spikes dry matter content (DM) of the four sampling dates at Zadoks stage (ZS) 75, 77, 83 and 85. Significant coefficients of determination (r^2) are indicated by the significance levels of $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***) for n = 6. Different letters indicate significant differences of the index R_{760}/R_{730} among the six wheat cultivars *Cubus* (●), *Ellvis* (○), *Impression* (▼), *Pegassos* (∆), *Solitär* (■) and *Tommi* (□).



a)
b)
c)
ZS 75
ZS 77
ZS 83
ZS 85
r² = 0.73*
r² = 0.68*
r² = 0.32
r² = 0.87**
r² = 0.81*
r² = 0.39
r² = 0.89**
r² = 0.83*
r² = 0.72*
r² = 0.33
r² = 0.85**
BDS_R760 / R730
Spikes Nup
Nup index
Grain DM

Figure 3.3d: Linear relationships between the normalised mean values ($n = 4$) of the spectral index BDS_R_{760}/R_{730} and the plant traits **a)** spikes N uptake (N_{up}), **b)** N uptake index and (N_{up} index) and **c)** grain dry matter content (DM) of the four sampling dates at Zadoks stage (ZS) 75, 77, 83 and 85. Significant coefficients of determination (r^2) are indicated by the significance levels of $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***) for $n = 6$. Different letters indicate significant differences of the index R_{760}/R_{730} among the six wheat cultivars *Cubus* (●), *Ellvis* (○), *Impression* (▼), *Pegassos* (Δ), *Solitär* (■) and *Tommi* (□).

3.4 Section IV: Spectral assessments of phenotypic differences in spike development during grain filling affected by varying N supply in wheat

3.4.1 Cultivar differences in yield and destructively obtained plant traits

The plant traits of the six high yielding wheat cultivars used in this study were compared within each N application level and development stage (Table 3.4a). For the six cultivars up to four differentiation levels were found. Spikes DW was best differentiated at ZS 75. Differentiation of spikes DW decreased with progressing grain filling. In contrast to that, the spikes dry matter contents (spikes DM) of the cultivars could well be separated in later growth stages, too. Whereas *Tommi* and *Solitär* mostly showed lower spikes DM across all N application rates, the four remaining cultivars showed higher spikes DM values especially with increasing N application.

The dry weight index (DW index) represents the biomass partitioning between leaves+stems and the spikes of wheat plants. With increasing DW index, the spikes portion on the whole plant biomass increases compared to that of leaves+stems. In contrast to the later development stages the cultivars differences in the DW index were more inconsistent at ZS 75 and ZS 77. Throughout the different development stages and N application levels, the cultivar *Solitär* showed the lowest DW index within the group of the six wheat cultivars. As a notable fact, the cultivar *Cubus* reached three times the significantly highest DW index, namely at ZS 75, 77 and 85, but limited to the application level N_{100}.

Similar to the DW index, the N_{up} index describes the relationship between the N uptake of the spike and that of the leaves+stems. A N_{up} index of higher than 1.0 indicates a spike N uptake exceeding the N uptake of leaves+stems. Comparable to the DW index, the cultivar *Solitär* showed the lowest values of the N_{up} index throughout the period of grain filling within all N application levels. The cultivar *Cubus* expressed the highest N_{up} index at ZS 75, 77 and 85 within N_{100}.

Throughout the four N treatments, the final grain yield was recorded for each wheat cultivar. No significant differences of grain yield were found within N_0. The three remaining N levels revealed similar yield patterns. In accordance with all other destructively obtained plant traits, the cultivar *Solitär* showed the lowest yield compared to the remaining cultivars. The group of *Impression*, *Pegassos* and *Cubus* reached the highest yield within each of the three N application levels N_{100}, N_{160} and N_{220}.

Table 3.4a: Mean value comparisons between six wheat cultivars depicted for each plant trait and final grain yield. Data are separated for the four sampling dates at Zadoks stage (ZS) 75, 77, 83 and 85 and the nitrogen application levels 0 kg ha^{-1} (N_0), 100 kg ha^{-1} (N_{100}), 160 kg ha^{-1} (N_{160}) and 220 kg ha^{-1} (N_{220}). Significant differences between the cultivars are indicated by different letters for the values of spikes dry weight (spikes DW), spikes dry matter content (spikes DM), dry weight index (DW index) and N uptake index (N_{up} index) and final grain yield at $p \leq 0.05$.

	Cultivars	Spikes DW				Spikes DM				DW index				N_{up} index				Grain yield			
		N_0	N_{100}	N_{160}	N_{220}	N_0	N_{100}	N_{160}	N_{220}	N_0	N_{100}	N_{160}	N_{220}	N_0	N_{100}	N_{160}	N_{220}	N_0	N_{100}	N_{160}	N_{220}
ZS 75	Tommi	ab	ab	a	ab	a	ab	a	a	ab	ab	b	ab	a	ab	b	ab	a	ab	b	b
	Solitär	ab	a	a	ab	a	a	a	a	ab	a	a	ab	a	a	a	ab	a	a	a	a
	Impression	bcd	cd	b	bc	a	c	c	b	d	bc	c	b	b	c	c	bc	a	d	c	d
	Pegassos	d	cd	b	c	a	c	c	c	cd	b	b	b	b	bc	b	c	a	cd	c	c
	Cubus	cd	d	b	c	a	d	c	b	cd	c	c	b	b	d	bc	c	a	d	c	c
	Ellvis	abc	bc	b	bc	a	bc	b	b	bc	b	b	ab	b	bc	bc	bc	a	bc	b	ab
ZS 77	Tommi	a	a	a	ab	ab	a	ab	b	ab	b	b	b	a	ab	ab	ab	a	ab	b	b
	Solitär	ab	a	a	a	a	a	a	a	a	a	a	ab	a	a	a	ab	a	a	a	a
	Impression	ab	ab	b	ab	bc	b	b	cd	bc	c	b	bc	a	bc	bc	b	a	d	c	d
	Pegassos	ab	b	b	b	c	b	b	d	cd	c	b	bc	b	c	bc	bc	a	cd	c	c
	Cubus	b	ab	ab	ab	c	b	b	bc	cd	d	c	c	b	d	c	c	a	d	c	c
	Ellvis	ab	ab	ab	ab	bc	b	ab	bc	d	c	b	b	b	c	bc	bc	a	bc	b	ab
ZS 83	Tommi	a	a	a	ab	b	b	b	b	b	b	ab	a	ab	a	a	a	a	ab	b	b
	Solitär	a	ab	a	a	a	a	a	a	a	a	a	ab	a	a	a	a	a	a	a	a
	Impression	a	ab	a	bc	c	c	c	cd	c	c	bc	ab	ab	b	b	ab	a	d	c	d
	Pegassos	a	b	a	abc	c	c	cd	d	c	c	abc	ab	c	c	b	ab	a	cd	c	c
	Cubus	a	ab	a	c	c	c	d	d	c	c	c	b	c	c	b	ab	a	d	c	c
	Ellvis	a	ab	a	ab	c	c	c	c	c	c	ab	ab	bc	c	b	b	a	bc	b	ab
ZS 85	Tommi	a	ab	a	a	a	a	a	a	b	bc	bc	ab	a	ab	ab	a	a	ab	b	b
	Solitär	a	a	a	ab	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	Impression	a	ab	a	b	b	b	b	ab	b	c	bc	ab	a	b	c	b	a	d	c	d
	Pegassos	a	b	a	b	c	b	c	bc	b	b	ab	b	a	c	a	b	a	cd	c	c
	Cubus	a	ab	a	ab	c	c	d	c	b	d	c	b	a	d	c	b	a	d	c	c
	Ellvis	a	ab	a	ab	c	b	cd	c	b	bc	bc	b	a	cd	bc	b	a	bc	b	ab

3.4.2 Relationships between yield and plant traits

As grain yield is known to be dependent on the development of spikes and grains and the translocation of assimilates from leaves and stems, the four plant traits spikes DW, spikes DM in %, DW index and N_{up} index were well related to the final grain yield. Because the varying plant trait values are inseparably connected to the single cultivars, the following results also consider cultivar, thus, phenotypic effects. The relationships between plant traits and final grain yield throughout the four development stages ZS 75, 77, 83 and 85 and for the four N application rates are shown in Table 3.4b.

Good relationships were found between the final grain yield and all destructively obtained plant traits, however, strongly varying dependent on the different development stages and the N treatments. Compared to the N levels N_0, N_{100} and N_{160}, relationships within the N level N_{220} were least. With regard to the development stages, the majority of the relationships between grain yield and the attributes spikes DW, spikes DM, the DW index and the N_{up} index were less close at the latest sampling date ZS 85. Regarding the relationships of grain yield to spikes DW, coefficients of determination were highest within N_0 at ZS 75 with an r^2 value of 0.95 decreasing with increasing N level. At the remaining sampling dates, r^2 values of the same relationship increased with the N supply except for the N_{220} at ZS 83. Good predictive qualities were obtained at the N_{100} level throughout grain filling. Spikes DM was best correlated to final grain yield at the two sampling dates ZS 77 and 83 within all N treatments with r^2 values ranging from 0.56 to 0.88. Particularly at N_{100} and N_{160}, good predictive qualities were also found at ZS 75. The DW index was similarly as the spikes DW correlated to the final grain yield. While good relationships were found at the N level N_{100} with r^2 values up to 0.85, low predictive accuracies were found at ZS 85 in N_{160} and N_{220}. The most inconsistent relationships were found between the final grain yield and the N_{up} index. While the predictive qualities were comparable to relationships between grain yield and spikes DW or DW index at medium N levels, low and high N rates, N_0 and N_{220}, were expressed less precisely.

3.4.3 Relationships between plant traits and spectral parameters

To increase the understanding of the influence of the crops nutritional status and yield formation on spectral reflectance, two well established and one lately developed spectral parameter were tested. The spectral parameters were related to the plant traits during the period of grain filling combined with varying N supply.

The BDS_NDVI and the index BDS_R_{760}/R_{730} were mainly influenced by the effect of the development stage closely followed by the effect of nitrogen supply (Table 3.4c). In contrast, the BDS_REIP was firstly influenced by the N treatment similarly to the influence of the development stage. The cultivars effect on spectral parameters was on third place for the BDS_NDVI and the BDS_REIP. The BDS_R_{760}/R_{730} index was additionally strongly influenced by the interactive effect of the development stage and the N treatment.

For all spectral parameters used in this study, the outputs magnitude of the reflectance parameters decreased chronologically but increased with the amount of nitrogen applied. While the BDS_NDVI and the BDS_REIP increased numerically by only 40 % and 70 % from N_0 to N_{220}, the index BDS_R_{760}/R_{730} increased its index values up to 250 %, enabling a potentially better differentiation within each N treatment. In contrast to the BDS_NDVI, the variation of BDS_R_{760}/R_{730} was highest at ZS 75 and decreased with progressing grain filling.

Table 3.4b: Coefficients of determination for the relationships between four plant traits and the final grain yield. Spikes dry weight (spikes DW), spikes dry matter content (spikes DM), dry weight index (DW index) and N uptake index (N_{up} index) were assessed at the four nitrogen application levels 0 kg ha^{-1} (N_0), 100 kg ha^{-1} (N_{100}), 160 kg ha^{-1} (N_{160}) and 220 kg ha^{-1} (N_{220}) at Zadoks stages (ZS) ZS 75, 77, ZS 83 and ZS 85. The models follow linear courses. The significant coefficients of determination are highlighted as p ≤ 0.05 (*), p ≤ 0.01 (**) and p ≤ 0.001 (***) for n = 6.

Plant trait	ZS	Coefficient of determination for grain yield			
		N_0	N_{100}	N_{160}	N_{220}
Spikes DW	75	0.95***	0.92**	0.68*	0.47
	77	0.61	0.75*	0.75*	0.39
	83	0.08	0.64	0.71*	0.79*
	85	0.29	0.20	0.74*	0.36
Spikes DM	75	0.26	0.82*	0.85**	0.34
	77	0.74*	0.88**	0.79*	0.64
	83	0.70*	0.80*	0.71*	0.56
	85	0.56	0.59	0.41	0.06
DW index	75	0.68*	0.81*	0.73*	0.58
	77	0.28	0.85**	0.66*	0.67*
	83	0.70*	0.84**	0.84**	0.74*
	85	0.76*	0.59	0.36	0.37
N_{up} index	75	0.47	0.80*	0.59	0.43
	77	0.32	0.63	0.67*	0.26
	83	0.77*	0.58	0.62	0.23
	85	0.70*	0.50	0.17	0.21

In detail, spikes DW increased thoroughly with progressing grain filling and N supply for all cultivars observed with values between 200 and 1100 g m^{-2} of spikes biomass (Figure 3.4a). Relationships between the spikes DW and the BDS_NDVI were poor compared to those of the BDS_REIP and the BDS_R_{760}/R_{730} index with a maximum of r^2 0.68 at ZS 75 for N_{160}. At N_{100}, N_{160} and N_{220}, the BDS_REIP was well correlated to spikes DW at ZS 75 to 83. In treatment N_0, the BDS_REIP described the spikes DW well at ZS 85 with r^2 values up to 0.72, however, in contrasting regressions´ orientation. Though, with increasing N supply the relationships' slope decreased and finally became negative at latest within N_{220}.

Table 3.4c: F-values and degrees of freedom (df) of the general linear model (GLM) for three spectral parameters assessed by the passive bi-directional spectrometer (BDS) across six wheat cultivars, four development stages (Dev. stage) and four N treatments. All main effects and interactions were significant at $p \leq 0.001$.

Source of variation	df	BDS_NDVI	BDS_REIP	BDS_R_{760}/R_{730}
Model	50	387.7	207.1	468.0
Cultivar	5	229.2	60.2	211.2
Dev. stage	3	4419.4	1466.3	4437.3
N treatment	3	1444.2	1676.0	2091.0
Cultivar x Dev. stage	15	21.2	12.6	9.6
Cultivar x N treatment	15	5.7	5.6	15.7
Dev. Stage x N treatment	9	27.2	39.3	264.2
Residual	335			
Total	384			

The index BDS_R_{760}/R_{730} well expressed spikes DW at the development stage ZS 75 with r^2 values ranging between 0.66 and 0.74. Despite the increased numerical range of variation of the index BDS_R_{760}/R_{730} at higher N rates, the relationships between the index and the cultivars´ spikes DW were highly varying with the development stages and the N levels.

The BDS_NDVI better predicted spikes DM with progressing grain filling within each N treatment (Figure 3.4b). Best relationships were found at ZS 83 and 85 with r^2-values ranging between 0.65 and 0.93. The BDS_REIP at N_{100}, N_{160} and N_{220} increased its predictive accuracy of spikes DM to up to r^2 0.94 with progressing grain filling. Though, there was a strong decrease of the relationships quality at ZS 85. As mentioned earlier this was due to the change of the regressions´ orientation with increasing development stages. Therefore, spikes DM did hardly correlate with the BDS_REIP at ZS 85. The index BDS_R_{760}/R_{730} was the only spectral parameter that reached highly predictive qualities of spikes DM throughout all development stages with r^2 values of up to 0.92 but restricted to the N rates N_{100} to N_{220}. Within N_0, the spectral variation between cultivars was low resulting in poor differentiations of the cultivars.

The DW index of all cultivars increased with advancing grain filling, however, did not change strongly with increased N supply, reaching values from 0.38 to 2.06 (Figure 3.4c). Especially within the N treatment N_{220}, the differences between the DW indices at ZS 83 and 85 were smaller. Compared to these development stages, the cultivars variation in terms of the BDS_NDVI was lowest at ZS 75. With progressing grain filling, the variation of this index increased parallel to the N supply. Good relationships were found between the BDS_NDVI and the DW index within the N application level N_{100} throughout the grain filling phase with r^2 values of up to 0.77. With any N supply in N_{100} and N_{220} at ZS 75 to ZS 85, the BDS_REIP – DW index relationship resulted in high r^2 values of up to 0.87. Results at ZS 85 were repeatedly affected by the inverted direction of the regressions. Similar to the BDS_REIP, the index BDS_R_{760}/R_{730} was well correlated with the DW index until ZS 83. Best relationships between the DW index and the BDS_R_{760}/R_{730} were found within N_{100} with r^2 values of up to 0.95.

Compared to the previous plant attributes, best relationships were found between the N_{up} index and the three calculated spectral parameters (Figure 3.4d). The majority of the relationships to the BDS_NDVI were highly significant with r^2 values of up to 0.96. Only at single dates and for the N treatments N_0 at ZS 75 and N_{160} at ZS 85, coefficients of determination were lower than 0.30.

For the BDS_REIP, best relationships to the N_{up} index were found at ZS 75 to ZS 83 with r^2 values up to 0.98, similar to the relationship to the other observed parameters. Due to the strongly changing slope of the correlations of the BDS_REIP at ZS 85, relationships within this development stage were strongly inconsistent.

The quotient BDS_R_{760}/R_{730} was the most stable spectral index describing the N_{up} index. Throughout all N application rates and development stages the relationships were close with r^2 values ranging between 0.62 and 0.97, except for N_0 and N_{160} at ZS 85.

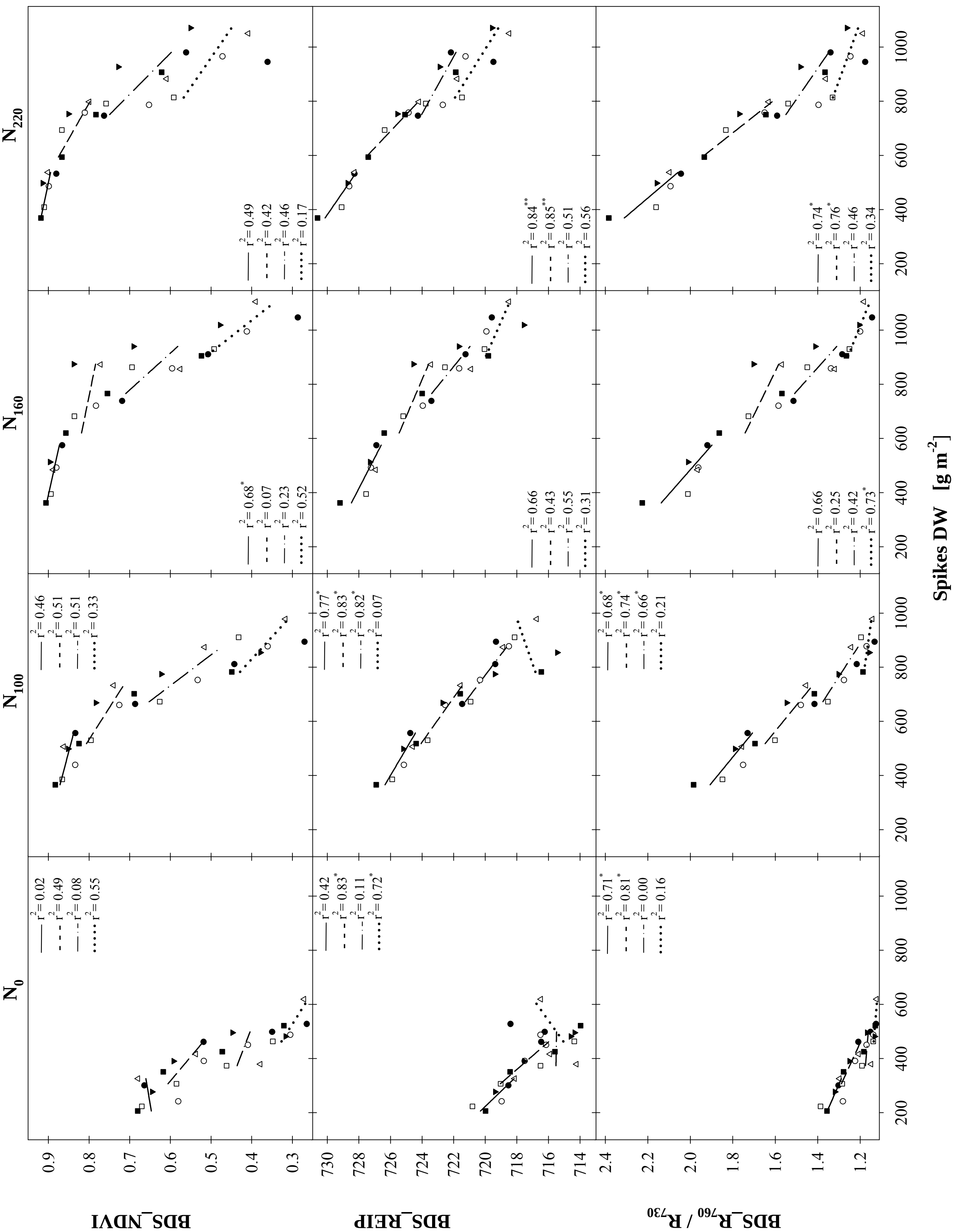
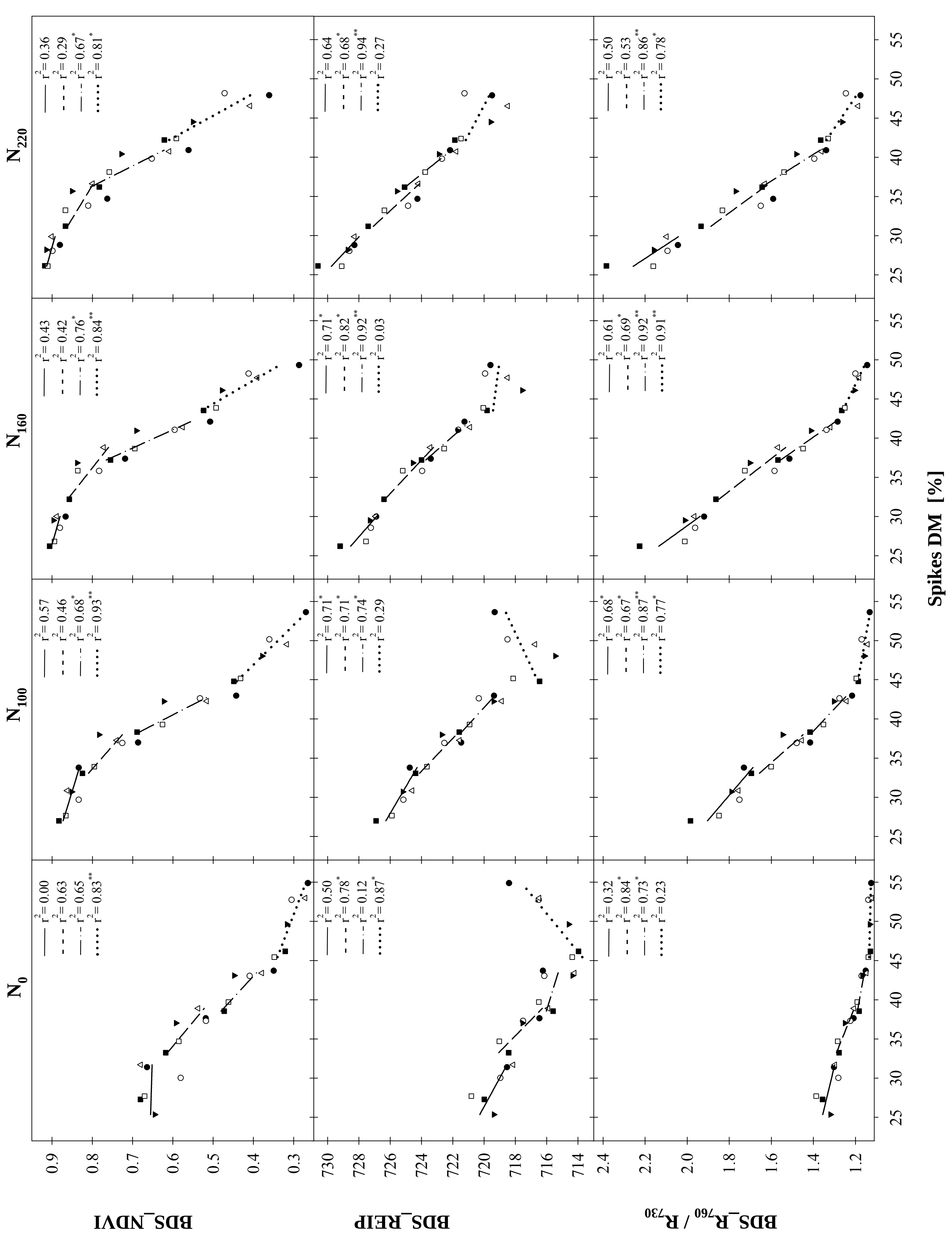
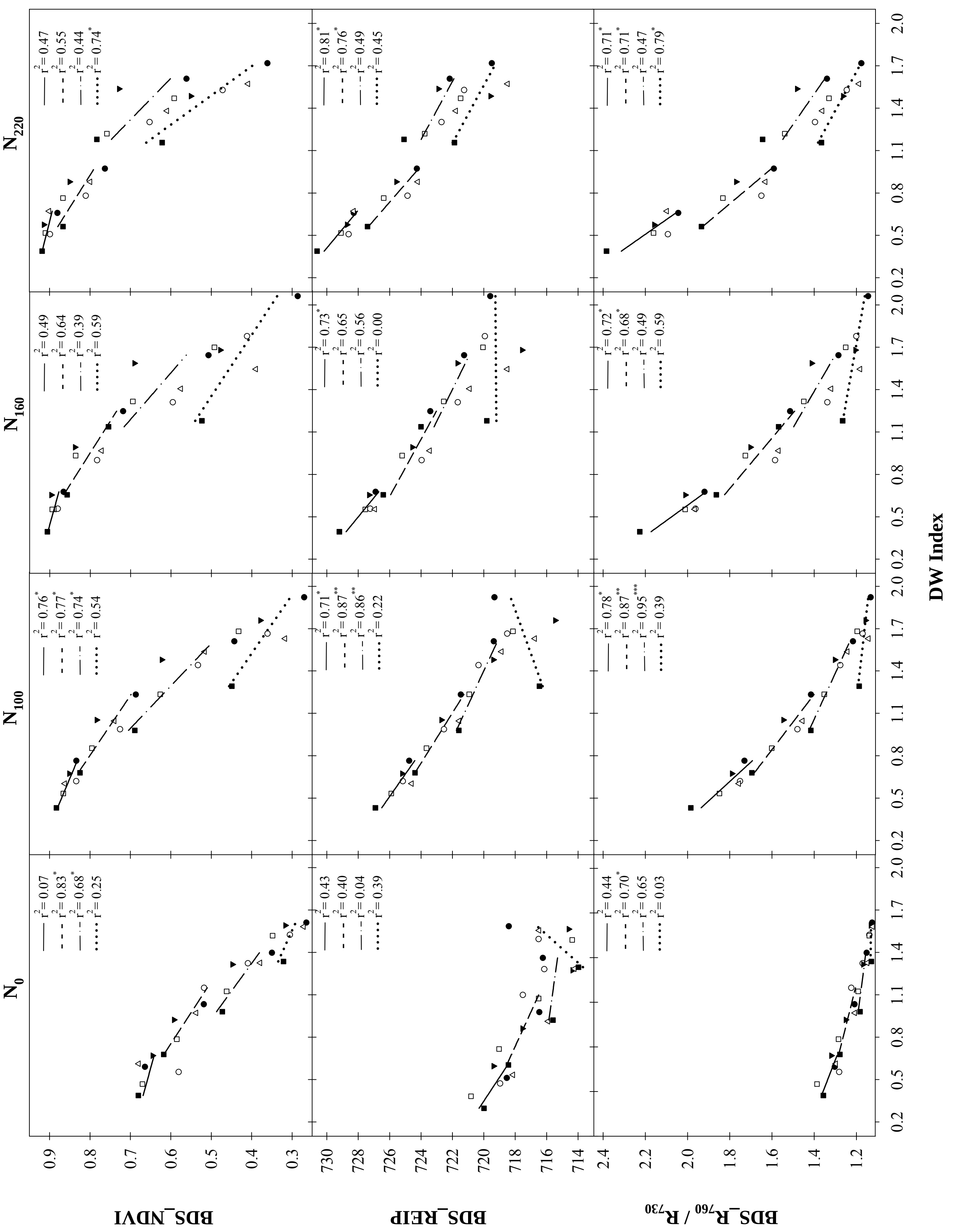

N0
N100
N160
N220
BDS_NDVI
BDS_REIP
BDS_R760 / R730
Spikes DW [g m-2]
r2 = 0.02
r2 = 0.49
r2 = 0.08
r2 = 0.55
r2 = 0.42
r2 = 0.83*
r2 = 0.11
r2 = 0.72*
r2 = 0.71*
r2 = 0.81*
r2 = 0.00
r2 = 0.16
r2 = 0.46
r2 = 0.51
r2 = 0.51
r2 = 0.33
r2 = 0.77*
r2 = 0.83*
r2 = 0.82*
r2 = 0.07
r2 = 0.68*
r2 = 0.74*
r2 = 0.66*
r2 = 0.21
r2 = 0.68*
r2 = 0.07
r2 = 0.23
r2 = 0.52
r2 = 0.66
r2 = 0.43
r2 = 0.55
r2 = 0.31
r2 = 0.66
r2 = 0.25
r2 = 0.42
r2 = 0.73*
r2 = 0.49
r2 = 0.42
r2 = 0.46
r2 = 0.17
r2 = 0.84**
r2 = 0.85**
r2 = 0.51
r2 = 0.56
r2 = 0.74*
r2 = 0.76*
r2 = 0.46
r2 = 0.34

Figure 3.4a: Linear relationships between the mean values ($n = 4$) of the BDS_NDVI, the BDS_REIP and the BDS_R_{760}/R_{730} index assessed by the passive bi-directional spectrometer (BDS) and the plant trait of spikes dry weight (spikes DW) are shown for the nitrogen application levels 0 kg ha^{-1} (N_0), 100 kg ha^{-1} (N_{100}), 160 kg ha^{-1} (N_{160}) and 220 kg ha^{-1} (N_{220}). Cultivars are indicated as *Cubus* (●), *Ellvis* (○), *Impression* (▼), *Pegassos* (∆), *Solitär* (■) and *Tommi* (□). The regression lines for the single sampling dates are indicated as Zadoks stages ZS 75, ——, ZS 77, - - -, ZS 83, —·—·—, and ZS 85, · · · ·, and their respective coefficients of determination (r^2). Significance levels were chosen at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***) for $n = 6$.



N0
N100
N160
N220
BDS_NDVI
BDS_REIP
BDS_R760 / R730
Spikes DM [%]
r² = 0.00
r² = 0.63
r² = 0.65
r² = 0.83**
r² = 0.57
r² = 0.46
r² = 0.68*
r² = 0.93**
r² = 0.43
r² = 0.42
r² = 0.76*
r² = 0.84**
r² = 0.36
r² = 0.29
r² = 0.67*
r² = 0.81*
r² = 0.50
r² = 0.78*
r² = 0.12
r² = 0.87*
r² = 0.71*
r² = 0.71*
r² = 0.74*
r² = 0.29
r² = 0.71*
r² = 0.82*
r² = 0.92**
r² = 0.03
r² = 0.64
r² = 0.68*
r² = 0.94**
r² = 0.27
r² = 0.32
r² = 0.84*
r² = 0.73*
r² = 0.23
r² = 0.68*
r² = 0.67*
r² = 0.87**
r² = 0.77*
r² = 0.61
r² = 0.69*
r² = 0.92**
r² = 0.91**
r² = 0.50
r² = 0.53
r² = 0.86**
r² = 0.78*

Figure 3.4b: Linear relationships between the mean values ($n = 4$) of the BDS_NDVI, the BDS_REIP and the BDS_R_{760}/R_{730} index assessed by the passive bi-directional spectrometer (BDS) and the plant trait of spikes dry matter content (spikes DM) are shown for the nitrogen application levels 0 kg ha^{-1} (N_0), 100 kg ha^{-1} (N_{100}), 160 kg ha^{-1} (N_{160}) and 220 kg ha^{-1} (N_{220}). Cultivars are indicated as *Cubus* (●), *Ellvis* (○), *Impression* (▼), *Pegassos* (∆), *Solitär* (■) and *Tommi* (□). The regression lines for the single sampling dates are indicated as Zadoks stages ZS 75, ——, ZS 77, - - -, ZS 83, —·—·—, and ZS 85, · · · ·, and their respective coefficients of determination (r^2). Significance levels were chosen at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***) for $n = 6$.



N0
N100
N160
N220
BDS_NDVI
BDS_REIP
BDS_R760 / R730
DW Index

Figure 3.4c: Linear relationships between the mean values ($n = 4$) of the BDS_NDVI, the BDS_REIP and the BDS_R_{760}/R_{730} index assessed by the passive bi-directional spectrometer (BDS) and the plant trait of dry weight index (DW index) are shown for the nitrogen application levels 0 kg ha^{-1} (N_0), 100 kg ha^{-1} (N_{100}), 160 kg ha^{-1} (N_{160}) and 220 kg ha^{-1} (N_{220}). Cultivars are indicated as *Cubus* (●), *Ellvis* (○), *Impression* (▼), *Pegassos* (Δ), *Solitär* (■) and *Tommi* (□). The regression lines for the single sampling dates are indicated as Zadoks stages ZS 75, ——, ZS 77, - - -, ZS 83, —·—·—, and ZS 85, · · · ·, and their respective coefficients of determination (r^2). Significance levels were chosen at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***) for $n = 6$.

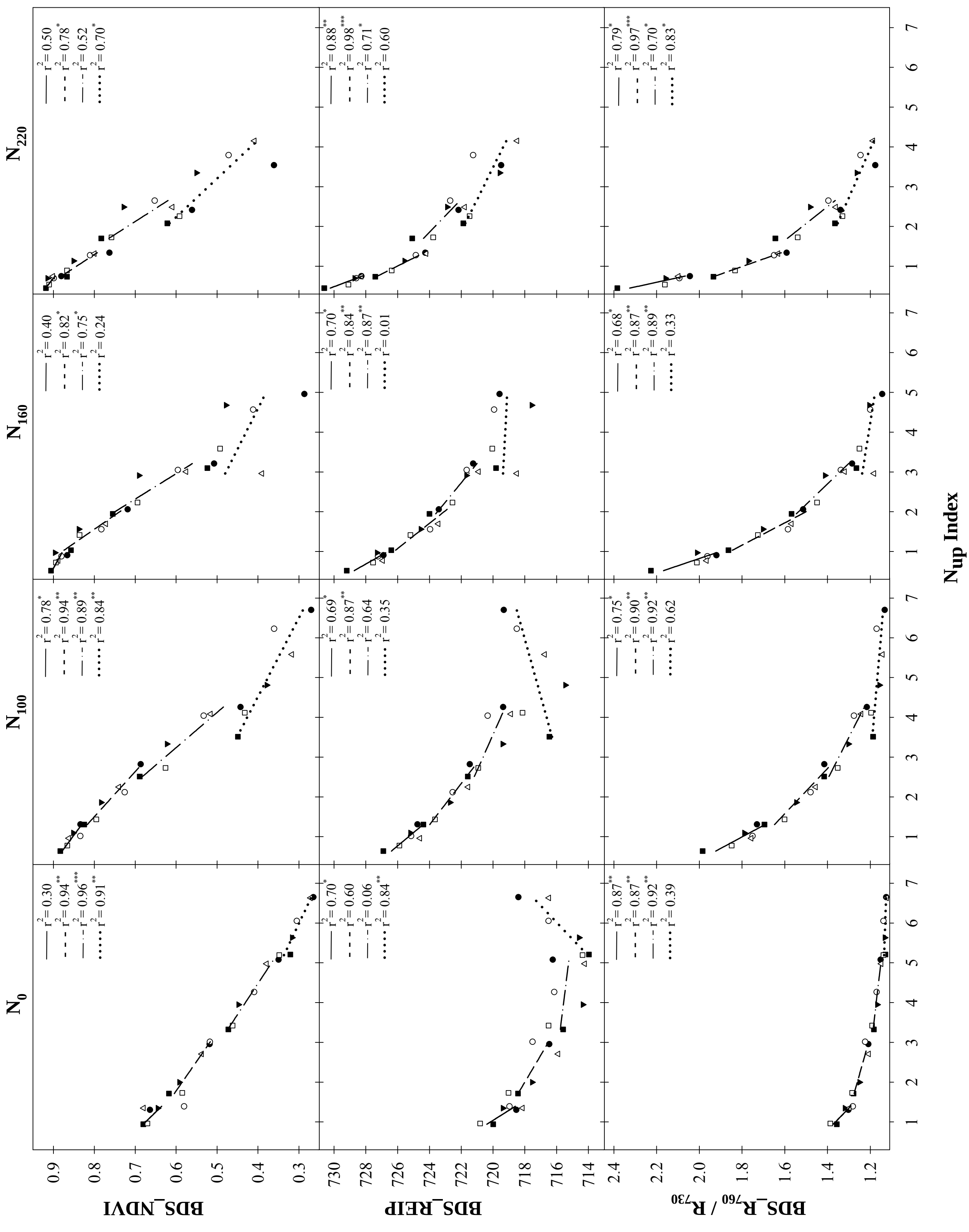

N0
N100
N160
N220
BDS_NDVI
BDS_REIP
BDS_R760 / R730
Nup Index

Figure 3.4d: Linear relationships between the mean values ($n = 4$) of the BDS_NDVI, the BDS_REIP and the BDS_R_{760}/R_{730} index assessed by the passive bi-directional spectrometer (BDS) and the plant trait of N uptake index (N_{up} index) are shown for the nitrogen application levels 0 kg ha^{-1} (N_0), 100 kg ha^{-1} (N_{100}), 160 kg ha^{-1} (N_{160}) and 220 kg ha^{-1} (N_{220}). Cultivars are indicated as *Cubus* (●), *Ellvis* (○), *Impression* (▼), *Pegassos* (Δ), *Solitär* (■) and *Tommi* (□). The regression lines for the single sampling dates are indicated as Zadoks stages ZS 75, ——, ZS 77, - - -, ZS 83, —·—·—, and ZS 85, · · · ·, and their respective coefficients of determination (r^2). Significance levels were chosen at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***) for $n = 6$.

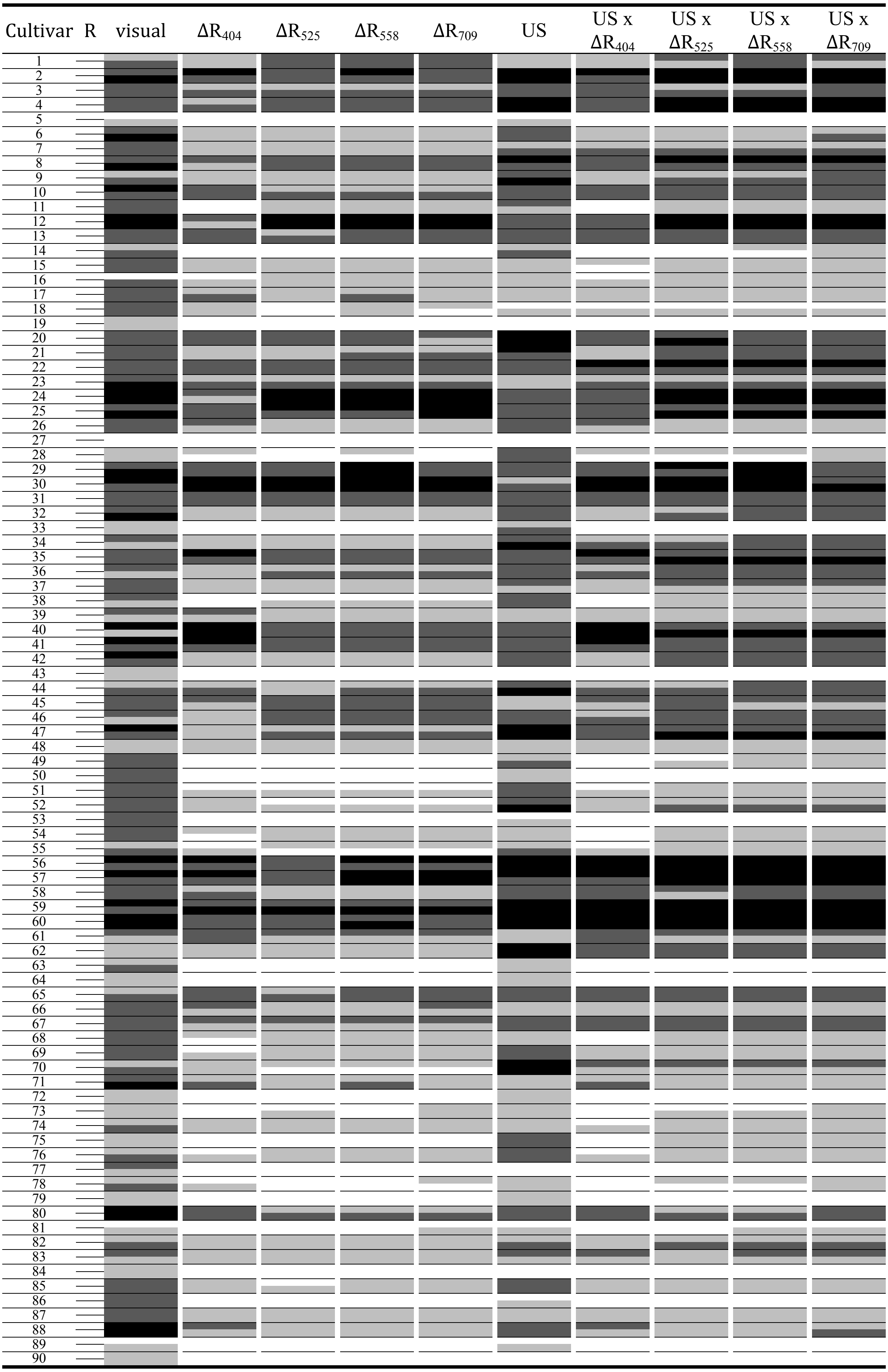
3.5 Section V: Testing spectral sensing and sensor fusion for the detection of canopy density based on visual ratings and spectral assessments

3.5.1 Relationships between reflectance data and visual ratings

The visual ratings run on the ninety wheat cultivars and their respective replications (n = 180) revealed only four density classes (DC_{vis}), 6 to 9. Therewith, the cultivars´ canopies appeared to be in the range between average to highly dense. Only six plots were rated as comparably sparse (DC 6), while 50, 97 and 27 plots were rated as less sparse (DC 7), dense (DC 8) and highly dense (DC 9), respectively (Table 3.5a).

Using the first derivative of the spectral curves of each plot, already a rough differentiation between the breeders visually assessed canopy density classes, DC_{vis}, could be seen (Figure 3.5). Especially in the spectral ranges between 500 nm and 600 nm and around 700 nm, the DC_{vis} classes could be distinguished while varying in the peaks´ magnitude. In contrast to wavelengths of up to 800 nm, the spectral curve between 800 nm and 1000 nm showed irregularly and noisy reflectance patterns. The coefficients of determination, r^2, between the DC_{vis} and the single wavelengths revealed maximum r^2 values of 0.39 especially in the blue (± 400 nm), green (± 500 nm) and red (± 700 nm) spectral ranges. Highest r^2 values were found for wavelengths of 404 nm, 525 nm, 558 nm and 709 nm with r^2 values of 0.31, 0.36, 0.39 and 0.38, respectively. The ultrasonic (US) values were less well related to the DC_{vis} data explaining only 19 % of its variance (Table 3.5b). Combining spectral and ultrasound information, the relationship to the DC_{vis} data hardly improved resulting in r^2 values between 0.33 and 0.37.

Table 3.5a: Results of the visual rating of the ninety wheat cultivars and their two replications (R) depicted as two lines per cultivar. The sensor-based density classification with four classes, DC_4, is shown for the single reflection information (ΔR and US) and their combinations. Four density classes are indicated as sparse, less sparse, dense and highly dense.


Cultivar
R
visual
ΔR_{404}
ΔR_{525}
ΔR_{558}
ΔR_{709}
US
US x ΔR_{404}
US x ΔR_{525}
US x ΔR_{558}
US x ΔR_{709}
1
2
3
4
5
6
7
8
9
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11
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14
15
16
17
18
19
20
21
22
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65
66
67
68
69
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90

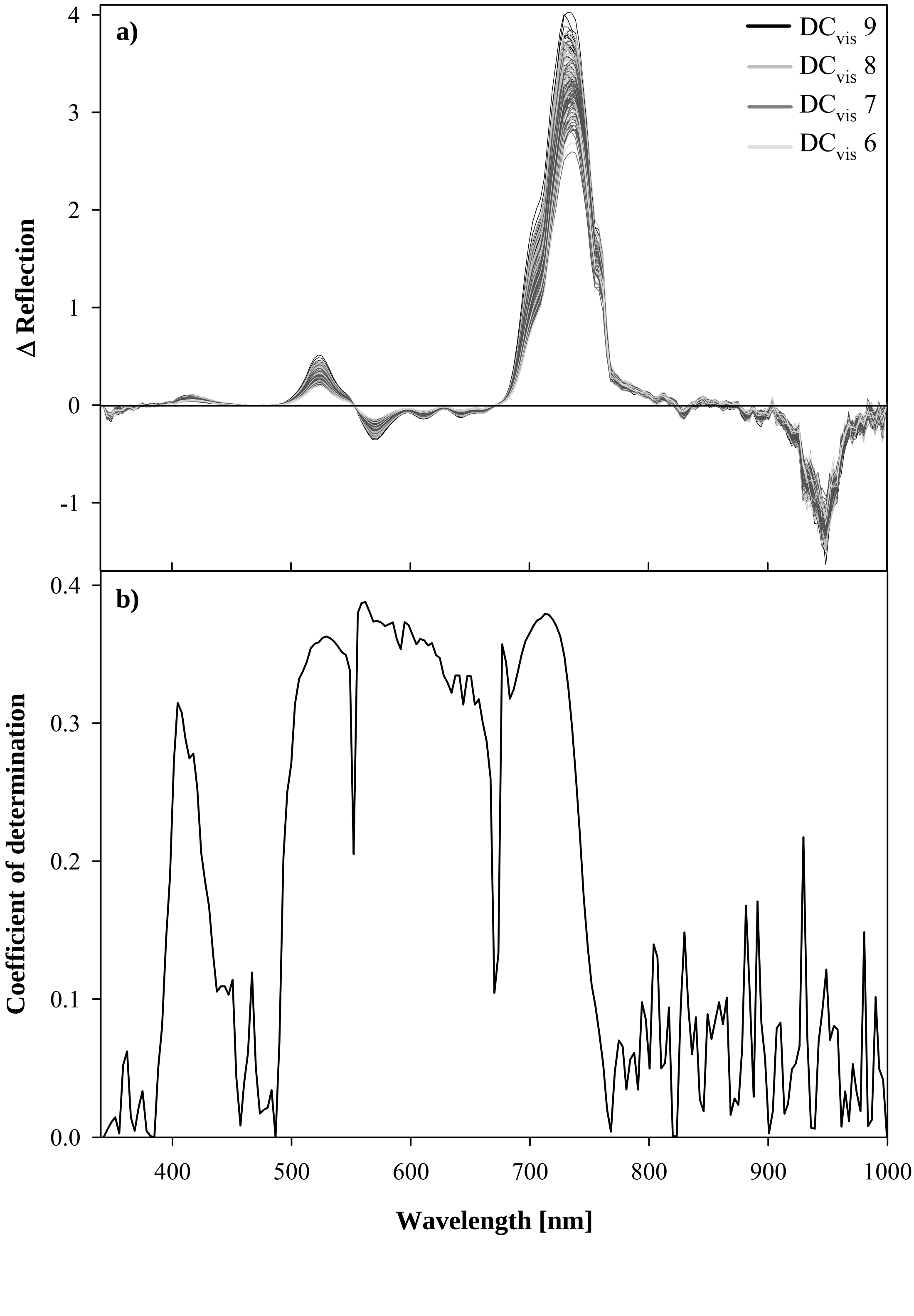


Figure 3.5: **a)** First derivative of the spectral reflectance curves of the 90 cultivars with each two replications obtained by the passive bi-directional spectrometer (BDS). Curves are coloured differently considering the visually assessed density classes DC_{vis} 6, sparse, DC_{vis} 7, less sparse, DC_{vis} 8, dense and DC_{vis} 9, highly dense. **b)** Coefficients of determination of the relationships between the density classes (DC_{vis}) and the single wavelengths are shown.

Table 3.5b: Coefficients of determination (r^2) between the density class systems, DC_{vis}, visually assessed, DC_3, sensor-based with three classes, and DC_4, sensor-based with four classes and the single reflection information (ΔR and US) and their combinations. Positive classification matches of cultivar replications are depicted in % of all 90 cultivars.

		DC_{vis}	ΔR_{404}	ΔR_{525}	ΔR_{558}	ΔR_{709}	US	US x ΔR_{404}	US x ΔR_{525}	US x ΔR_{558}	US x ΔR_{709}
r^2	DC_{vis}		0.31	0.36	0.39	0.38	0.19	0.33	0.36	0.37	0.36
	DC_3		0.83	0.81	0.82	0.83	0.80	0.86	0.86	0.86	0.85
	DC_4		0.90	0.90	0.90	0.90	0.90	0.92	0.92	0.91	0.91
Match [%]	DC_{vis}	54									
	DC_3		82	83	82	82	76	87	87	81	82
	DC_4		66	79	74	76	74	72	69	73	77

3.5.2 Comparison of visual ratings and sensor-based classification scales

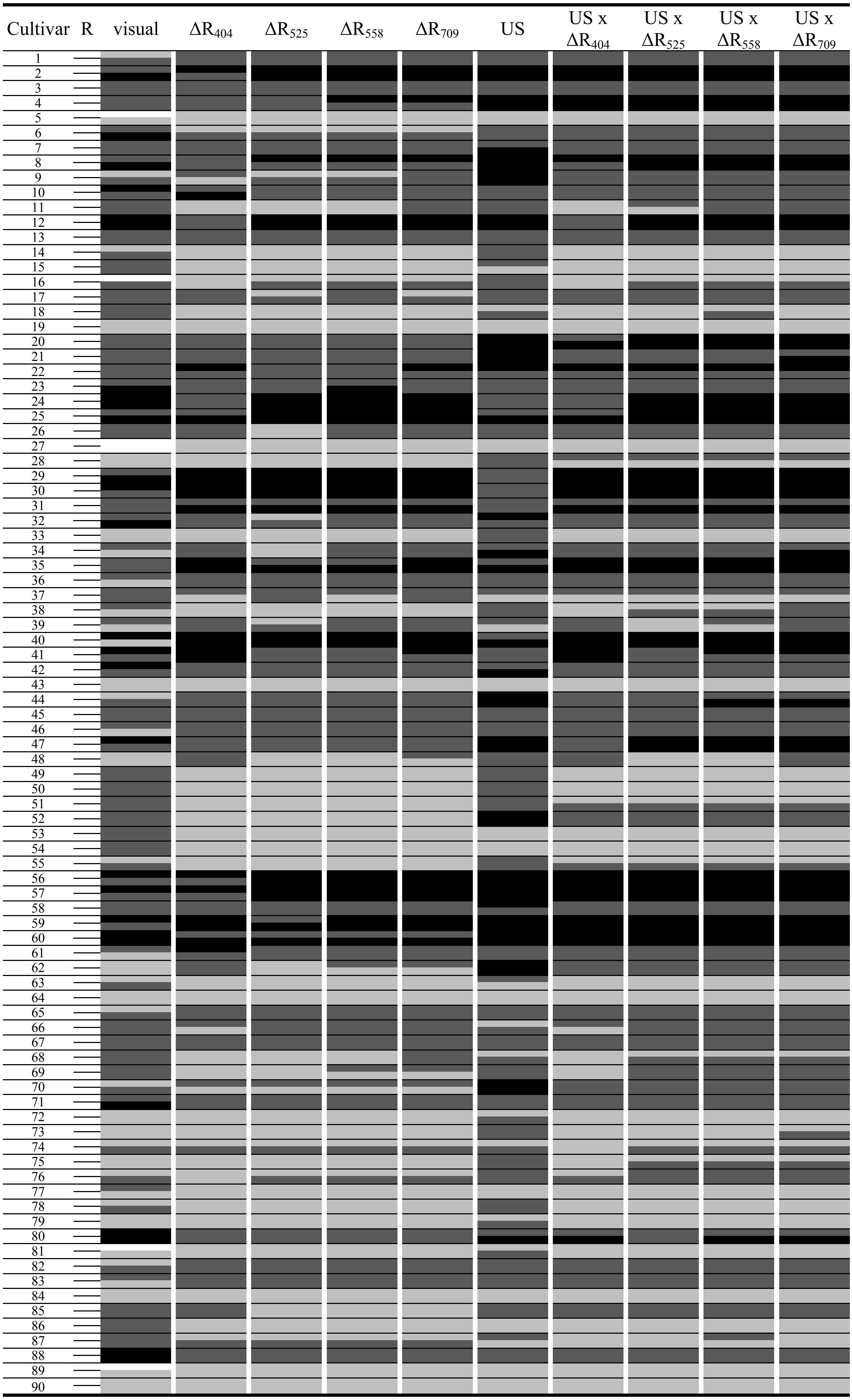
Producing new classification scales by dividing the single reflectance variations into three or four equal classes, the sensor-based classification systems DC_3 and DC_4 were applied to classify canopy density. Table 3.5a shows the system DC_4 which has the same class number as the visual ratings. It can be seen that compared to the visual ratings, the sensor-based ratings show many more plots classified as sparse (n = 48 - 55) and less plots classified as highly dense (n = 8 - 15). The combination with the US data partly levelled out this diversity classifying a number of up to 26 plots as highly dense and up to 60 plots as sparse.

Using the DC_3 system, the number of plots classified as dense with spectral data was similar to that of the visual rating with 27 and up to 28, respectively (Table 3.5c). The missing of a fourth class resulted in plot numbers of 71 to 86 plots rated as sparse or average. The combination with US reflectance data did not change this partitioning between the three classes.

Certainly, the sensor-based classification scales, DC_3 and DC_4, were well related to the reflectance data, the two DC systems are based on, with r^2 values between 0.81 and 0.92 (Table 3.5b). Due to its additional class, the scale DC_4 resulted in higher coefficients of determination of minimum 0.90 compared to DC_3. However, presuming a phenotypic stability, meaning that both replications of each cultivar show the same canopy density, the real quality of the three classification systems can be assessed testing the equal DC of both cultivar replications. In Table 3.5b the positive matches of both cultivar replicates based on the DC systems DC_{vis}, DC_3 and DC_4 are shown. While the DC_{vis} system rated only 54 % of the cultivars replicates equally, the DC_3 and DC_4 systems, both based on the reflectance data, showed positive matches between 66 % and 87 %. Compared to the DC_3 system, the DC_4 system resulted in lower positive matches of less than

80 %. Within the DC_3 system, the combination of the reflectance data ΔR_{404} and ΔR_{525} with the US information, US x ΔR_{404} and US x ΔR_{525}, resulted in an increase of positive matches from 82 % and 83 %, respectively, to both 87 %.

Table 3.5c: Results of the visual rating of the ninety wheat cultivars and their two replications (R) depicted as two lines per cultivar. The sensor-based density classification with three classes, DC_3, is shown for the single reflection information (ΔR and US) and their combinations. Four density classes are indicated as sparse, average and dense.


Cultivar R
visual
ΔR404
ΔR525
ΔR558
ΔR709
US
US x ΔR404
US x ΔR525
US x ΔR558
US x ΔR709
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90

4 Discussion

4.1 Section I: Sensor-based observation of within-plot variation and quality of signal reproduction during successive measurements

Observing the within-plot variation in a single wheat cultivar experiment, the CV-ratio revealed that especially plots of block *a* in the very west showed high variations of the otherwise less varying spectral information. Due to the fact that this situation diminished with increasing N supply compared to the remaining blocks, a higher residual soil N content could have been the reason for this disparity of block *a*. This is also approved by the detailed successive measurements M_A, M_B and M_C running across the blocks. In the direction west to east a positive spectral trend was observed most probably being related to this soil heterogeneity. With increasing N levels, this effect, however, was not apparent anymore.

The NDVI of the devices BDS, Crop Circle and GreenSeeker decreased its CV-ratio with increasing N supply due to the saturation of spectral bands. The NDVI is well known to be strongly affected by high crop densities going in line with sufficient N supply in wheat (Aparicio et al., 2002; Mistele et al., 2004; Heege et al., 2008; Erdle et al., 2011). With increasing green biomass red light-based reflectance reaches a minimum and the NDVI, which depends on the reflection information of the red spectral bands, becomes saturated. While this saturation led to a low differentiation quality between higher N levels it also resulted in a low signal variation within the well-supplied plots.

Similar to the NDVI, the water index, WI, lost within-plot variation with increasing N supply, however, to a much lower extent. The WI is known to be sensitive to changes of the crops water status (Peñuelas et al., 1993; Pinter et al., 2003; Sims and Gamon, 2003; Babar et al., 2006a; Babar et al., 2006b). The high soil qualities and regular and high precipitation amounts offered sufficient supply of plant available water in the year 2010 and the water status did not show high variations. Due to this fact, the WI was less suitable to detect variation in differently nourished crop stands. Crop water related spectral information can also be an indicator for the amount of water captured by the above ground biomass (Winterhalter et al., 2011). This amount of water increases with increasing N supply which accelerates biomass growth. As the additional DW accumulation slows down at high N levels, also the biomass related amount of water reaches a maximum, thus, the variation of WI within the plots decreases.

In contrast to the stagnating gain of DW with increasing N supply, the N contents of the single N levels were significantly differentiated up to 360 kg N ha^{-1}. The N contents of the plots´ subsamples, thus, were sufficient to represent the whole plot N status. It was already found that, depending on the character estimated, already small samples can adequately reflect a whole plots´ status (De Souza et al., 1993). The REIP and the R_{760}/R_{730} index have been proven to be good indicators for the N status of wheat (Mistele et al., 2004; Mistele and Schmidhalter, 2008a; Strenner and Maidl, 2010). The representativeness of the N content, therefore, explains the low CV-ratio of the two N-sensitive spectral parameters. But the CV-ratios of the R_{760}/R_{730} index of the Crop Circle and the AFS showed far higher within-plot variations for the same plots. This can be ascribed to the negative relationship between the size of the sensors field of view (FOV) and the within-plot variation. As described earlier, the BDS offers the largest FOV with about 0.28 m^2, while the AFS, the Crop Circle and the GreenSeeker measure areas of 0.15 m^2, 0.09 m^2 and 0.009 m^2, respectively. The consequences of these differences are strongly varying detection patterns of the single sensor systems. At a similar measurement speed, compared to a larger FOV, a small FOV results in the measurement of more different canopy spots per distance, thus, resulting in a higher variation. Additionally, the shape of the FOV contributed also to this variation. With respect to a circular shape, a narrow strip is much more sensitive to small-scale variations in a crop canopy such as small gaps of bare soil or bushels of narrowly grown plants. Compared to the BDS and the AFS, the combination of both, small and narrow FOVs most probably were the reasons for the higher within-plot CV of the Crop Circle and the GreenSeeker. The high point-to-point variation of the Crop Circle and the GreenSeeker was already found in literature and was as well related to the relationship between FOV and the speed of measurement (Solari, 2006). The BDS and the AFS, both working with a circular FOV with 0.28 m^2 and 0.15 m^2, thus, end up with a lower within-plot variation.

In terms of the quality of signal reproduction, the sensor systems could be divided into two groups. Due to the strong output variations of the Crop Circle and the GreenSeeker based on the FOV-effect described above, their reproducibility could hardly be evaluated. Only rough trends within the plots were confirmed by the successive measurements. The two devices BDS and AFS, however, showed their ability to reproduce within-plot variations with only small deviations. Within-plot variation of these sensors therefore can be mainly ascribed to crop heterogeneities within the plot rather than to sensors´ detection variation. This ability may offer the opportunity to detect small scaled canopy structures and their temporal variation during crop development.

However, despite high within-plot variations and block differences, calculating the plots averages out of the multiple single measurements, the plot values were highly stable for any sensor system used in this study. Conclusively, the sensors offer a promising tool to assess crop canopy characteristics based on plot level and replications.

4.2 Section II: Comparison of active and passive spectral sensors in discriminating biomass parameters and nitrogen status in wheat

Four reflectance sensors, including one passive bi-directional hyperspectral and three active sensors were tested over two seasons to detect differences in the nitrogen nutritional status and in crop attributes. Spectral parameters were related to six destructively assessed plant traits at ZS 3, ZS 4 and ZS 6. The observed plant traits were well described by selected spectral parameters throughout the two growing seasons, if only considering the coefficients of determination. However, the spectral outputs differed markedly in their ability to reflect the influence of the single N levels on destructively sampled plant traits as reflected in the ANOVA results (Figures 3.2a and 3.2b). This lack of predictive quality can be ascribed to the lack of distinction between higher N supply levels.

Saturation effects with increasing N rates and dense canopies are more likely when using red light-dependent spectral parameters. With high biomass and LAI values, the reflectance of red spectral ranges tends to be saturated sooner than when using NIR-based reflectance parameters (Hatfield et al., 2008; Mistele and Schmidhalter, 2008b). Therefore, reflectance patterns are strongly influenced by growing seasons and developmental stages (Prasad et al., 2007). This influence was evident from the first sampling date, when the shoot fresh weight could well be distinguished by the majority of the spectral parameters. Such a differentiation was less distinct for the N-status representatives, N content, aboveground N uptake and NNI. At this stage, the N concentration was highest in all treatments with only small differences being observed. Detection of the N status of canopies with incomplete ground cover is quite complex due to the antagonism of high N concentration going along with low biomass amounts (Fitzgerald et al., 2010). With further biomass increases, the effect of dilution of N progresses because N uptake cannot keep pace with the biomass increase (Justes et al., 1997). At later stages, the saturation effect of red reflection increases with biomass, but a better segregation of N parameters is possible due to a favourable balance between biomass and N concentration.

Generally, for all sensors, the closest relationships were observed between spectral parameters and the indicators for plant nitrogen status, N content, aboveground N uptake and NNI. These relationships are therefore primarily discussed. Spectral proximal sensing allowed us to differentiate the influence of the four N levels ranging from 0 to 220 kg N ha^{-1} on the N status indicators across the seasons. Closer relationships were observed with N uptake and NNI than with N content. In agreement with this observation, similar findings have previously been reported for aboveground N uptake as compared to N content, independent of the illumination source or the viewing angle (Schmidhalter et al., 2003; Mistele and Schmidhalter, 2008a; Li et al., 2009; Fitzgerald et al., 2010). The BDS_REIP was comparably well related to NNI and aboveground N uptake, thus, contrasting with a previous report that indicated a significant closer relationship between REIP and NNI for wheat plants (Mistele and Schmidhalter, 2008a). Whereas our measurements were conducted in the nadir direction, information was obtained from an oblique view in this previous study.

For all sensors, throughout the two years of observation, the R_{760}/R_{730} index best predicted the investigated crop attributes. Among the passive spectral parameters, the REIP was comparable to the BDS_R_{760}/R_{730} index in its accuracy of prediction. Similar results were repeatedly found in wheat experiments (Mistele et al., 2004; Mistele and Schmidhalter, 2008b; Strenner and Maidl, 2010) and also for growth stages between ZS 3 and ZS 6, when these spectral parameters were found to best predict aboveground N uptake. In accordance with Strenner and Maidl (2010), saturation of the REIP was observed with high N supply. This behaviour of the REIP can be explained by its dependency on red light being prone to saturation. Hence, although displaying high r^2 values, the REIP cannot entirely reflect the N status of the crop. The R_{760}/R_{730} index of the passive spectrometer has been shown to be more resistant to saturation effects (Heege et al., 2008; Strenner and Maidl, 2010) and is therefore preferred for reflecting the N status in wheat. A high predictive quality was also found for the CC_R_{760}/R_{730}, which was the best index provided by the Crop Circle. But the high predictive quality of this index was limited to the later growth stages, ZS 4 and ZS 6.

As has been shown previously, the NDVI is strongly prone to saturation with increasing biomass or LAI exceeding 2.5 – 3.0 (Aparicio et al., 2000; Serrano et al., 2000; Li et al., 2008). Saturation is frequent for dense crop stands in Europe with LAI values up to 8.0 (Heege et al., 2008). Considering that mostly quadratic relationships best described the spectral parameters, reflectance differences between higher N levels decrease, causing saturation. This response was observed earlier for the NDVI than for other spectral parameters. The saturation response was apparent for any NDVI-based parameter throughout the experiment, except for GS_NDVI at ZS 4. This finding contrasts with other investigations done with the GreenSeeker in which the NDVI showed strong saturation effects in maize (Hong et al., 2007) and in wheat (Li et al., 2010) at similar growth stages. On the other hand, due to its characteristic asymptotic course with high crop densities, the NDVI is particularly sensitive at small biomass and low ground coverage. Therefore, this spectral parameter may be more suitable for differentiating plant canopies at early growth stages or in more sparsely grown crop stands.

The CC_NDVI reflected a similar behaviour related to the N parameters. This behaviour was also demonstrated in several comparisons of the Crop Circle and GreenSeeker sensors, primarily in maize canopies (Hong et al., 2007; Devadas, 2009; Sudduth et al., 2010). However, due to the flexibility in choosing specific wavelengths filters for the Crop Circle, we were able to calculate a R_{760}/R_{730} index as well for that device. This capability turned out to be strongly advantageous because, as for the bi-directional passive spectrometer, the CC_R_{760}/R_{730} did not display any tendency to saturation with high coefficients of determination. In previous investigations, different spectral parameters have been calculated for the Crop Circle. Trotter et al. (2008) and Fitzgerald et al. (2010) used simple ratios, the soil adjusted vegetation index (SAVI) and the NDVI for Crop Circle measurements, but mainly limited their study to biomass and LAI characteristics of small grain cereals. As far as we know, no attempt has yet been made to choose interference filters to record wavelengths depicting NIR/NIR spectral indices with the Crop Circle. The results of this study show that by choosing a high-performing spectral parameter for the active Crop Circle sensor, we were able to successfully identify the wheat nitrogen status.

Disregarding the fact that the AFS was only available in 2010, its R_{760}/R_{730} index turned out to recognise the N status about as well as the other devices. Similar results have been found for comparable active flash devices by Jasper et al. (2009) and Mistele et al. (2010a), however, with oblique viewing angles. As in our work, high r^2-values were observed and the ability to discriminate different N levels was demonstrated. Moreover, the coefficients of determination were as high as those obtained for the passive reflectance sensor.

The predictive quality for DM content, and also water content and DW differed strongly between growing seasons. The best relationships were found at the second sampling date, ZS 4, when leaves were fully developed but the spikes had not yet appeared. As structural components such as spikes strongly influence spectral reflectance, they can impact the sensitivity of the spectral information, which may decrease the predictive accuracy at later stages (Aparicio et al., 2002). This influence of spikes on spectral reflection, however, may offer a chance to characterise crop development after anthesis, thus, during grain filling and yield development.

4.3 *Section III: Tracking phenotypic differences in biomass and nitrogen partitioning by spectral high-throughput assessments during grain filling in wheat*

For cereals, grain filling is the last period of its life cycle and the last phase of yield development (Slafer et al., 2009). Using spectral reflectance measurements, Babar et al. (2006a, 2006b) and Prasad et al. (2007) described good correlations between water band indices and the final grain yield during grain filling with r^2 values of up to 0.70. However in these studies drought-stressed conditions and a wide range of genetic material were used. In contrast to that, in our study with high yielding and non-stressed crops only the spectral information of the BDS_REIP was well correlated to final grain yield; However, the predictive quality of the used spectral parameters was increased when the spectral information was related to plant traits influencing yield. Especially the spike attributes, spikes DW, spikes DM content and the DW index explaining the biomass transfer to the spikes, were strongly related to final grain yield. The BDS_REIP was well correlated to the plant traits at the beginning of grain filling. But this spectral parameter was strongly affected by decreasing green biomass due to its dependence on the red light bands (Guyot et al., 1992), thus, loosing predictive quality at late grain filling.

The BDS_R_{760}/R_{730} index best explained the spikes DW and spikes DM content of the cultivars, though, with differing qualities. Both traits are closely related to spikes kernel number which is the major determinant of the final grain yield (Stapper and Fischer, 1990; Reynolds et al., 2009). Spikes dry weight around anthesis is known to be strongly correlated to final grain yield (Fischer, 2011). In agreement with findings of Ehdaie et al. (2008) who directly related spikes DW to final grain yield, our results support these relationships. Representing the reflectance patterns of structure-influenced NIR wavebands, the BDS_R_{760}/R_{730} index best reflected the fraction of the structural tissues in the spike. Spikes can have considerable influence on spectral assessments in wheat (Chance and LeMaster, 1978; Shibayama et al., 1986). During spike development, the structural components increase due to the rising number of spikelets and fertile florets and therewith the kernel number (Brooking and Kirby, 1981; Fischer, 2011) and potential yield (Reynolds et al., 2009). The dependence between the structural components, the spikes DM content and the kernel number decreases with progressive grain filling. At advanced grain filling, the spikes´ structure becomes increasingly influenced by the stored assimilates mirrored by the spikes DW development. Furthermore, as the dry matter content and the dry weight development are important traits for the supply of qualitative biomass for biogas production (Rincón et al., 2010), the assessment of these traits can be worthwhile for renewable energy resources.

The differentiation of the leaves+stems DW by the BDS_WI resembled that of the DW index, which expresses the relationship of the biomass accumulation between spikes and leaves+stems. The DW index is similarly assessed like the HI in terms of expressing plant traits controlling grain yield such as spikes and grains, in relation to the shoot biomass. This affinity can also be seen in the very similar correlation to final grain yield of the DW index at ZS 85 and the HI at final harvest (Figure 3.3a). However, this correlation is comparably weak with r^2 values between 0.36 and 0.39, respectively, which makes the DW partitioning at early grain filling (r^2 up to 0.84) being a more meaningful trait for final grain yield. The DW index at early grain filling mirrors the actual state of important source-sink traits during grain filling which is known to be of high importance for yield development (Evans, 1993; Fischer, 2008). As the BDS_WI is able to render this relationship, spectral reflectance measurements can be a rapid tool to observe dry weight partitioning during grain filling of new crossbreds.

A differentiation pattern quite similar to the spikes DW was found for the spikes N_{up} at the same sampling dates. N uptake is presumed to be dependent on the sink capacity and, therefore, the dry weight of the spikes (Mi et al., 2000; Reynolds et al., 2005; Masoni et al., 2007; Acreche and Slafer, 2009). Comparing the spikes DW and the spikes N_{up} dynamics, the relationship between sink capacity and N uptake could clearly be seen. N uptake indicators were less well related to final grain yield but can be an important indication for nitrogen use efficiency (NUE) and protein content, thus, the quality of grains (Ercoli et al., 2008). Although high NUEs were apparent for the cultivars in our study, no direct relationship to the N uptake during grain filling was found. Nevertheless, the N_{up} values of leaves+stems and spikes show that even at late developmental stages, there was considerable plant N uptake by the wheat cultivars. This most likely resulted from a continued uptake of mineral nitrogen from the soil pool. A long uptake activity of wheat can be supported by a sufficient water supply during the grain filling period (Masoni et al., 2007; Ercoli et al., 2008). While the transfer of N compounds like amino acids from the leaves to the spikes decreases photosynthesis, the continuing N uptake from the soil avoids the untimely degradation of chlorophyll and therewith maintains assimilate production (Sinclair and Jamieson, 2006).

There is a strong similarity between the BDS_R_{760}/R_{730} index´ relationship to the N_{up} index and to the grain DM content (Figure 3.3d). This parallelism might be related to the assimilate-storing characteristics of the plants. While transferring assimilates and N containing proteins to the grains, grain dry weight and dry matter content increase until the maximum kernel weight is achieved, indicating physiological maturity (Calderini et al., 2000). This increase of dry matter depends on the kernel filling rate which is strongly cultivar dependent (Fischer, 2011). Accordingly, such a variation may well be identified by the BDS_R_{760}/R_{730} index. However, the predictive quality for the N_{up} index decreases at comparable water contents of the wheat cultivars achieved at progressive physiological maturity (Calderini et al., 2000; Borrás et al., 2004).

The grain DM was the plant trait best described by any spectral parameter assessed. The grain DM content was shown by Calderini et al. (2000) and Slafer et al. (2009) to be a promising trait to assess the physiological maturity of grains. With physiological maturity, the deposition of assimilates into the grain has been completed and only the water content has to be decreased by natural or artificial ripening (Slafer et al., 2009). Early maturing cultivars were found to be best adapted to high-yielding conditions (Stapper and Fischer, 1990). With spectral detection of the grain DM, which allows the observation of the physiological maturity of wheat, favoured crossbreds can be found and advanced harvests are possible by applying desiccants without losing yield during natural ripening.

Observing the partitioning characteristics of well-nourished wheat cultivars enabled a better understanding of the influence of physiological processes during grain filling on spectral reflectance. However, wheat cultivars may differ strongly in transfer efficiencies or yield stability when they become stressed or nutrient deficient.

4.4 Section IV: Spectral assessments of phenotypic differences in spike development during grain filling affected by varying N supply in wheat

Grain filling is one of the most crucial periods in yield development of cereal crops. The cultivars performance varies in terms of spike development and is affected by a strongly reduced nitrogen supply. In this study, these phenotypic variations were observed by spectral assessments of spike development under varying N supply throughout the grain filling phase.

The BDS_NDVI, was mainly affected by saturation effects shortly after anthesis which is well known for this index at high crop densities (Aparicio et al., 2000; Serrano et al., 2000; Li et al., 2008; Erdle et al., 2011). The difficulties in differentiating cultivars decreased with senescence but recurred at very low chlorophyll densities especially with low N supplies. Insufficient N supply is known to accelerate senescence of wheat (Spiertz and Ellen, 1978; Spiertz and De Vos, 1983; Dreccer et al., 2000; Ercoli et al., 2008), thus, missing chlorophyll light absorbance. The BDS_REIP showed a similar behaviour going along with low amounts of green biomass. With progressing grain filling, the slope of any BDS_REIP regression was changing from strongly negative to a positive orientation at low N levels. As the leaf area index (LAI) and the chlorophyll content decrease with grain filling, the BDS_REIP moves towards shorter wavelengths until a minimum is reached and is increasing again although the LAI is further decreasing (Guyot et al., 1992; Railyan and Korobov, 1993). The rank change of interrelations at late development stages due to senescence is therefore based on this dissymmetric relationship, however, becoming less intense with increasing N supply. Comparisons between the REIP and NIR-based spectral parameters showed that NIR-based spectral parmeters were less sensitive to saturation effects during the time of increasing green biomass (Heege et al., 2008; Mistele and Schmidhalter, 2010a; Erdle et al., 2011). After anthesis and with decreasing chlorophyll contents and crop density, a similar effect was apparent: While the BDS_REIP was more sensitive to the decreases of green biomass and tended to lose efficacy with strong senescence, the BDS_R_{760}/R_{730} index was more insensitive to low chlorophyll contents. However, similar to the BDS_REIP, the NIR-based index BDS_R_{760}/R_{730} was increasing the phenotypic variation with N supply but decreasing with development stages. While advanced senescence leads to a less abrupt ascent between VIS and NIR reflected wavebands (Hinzman et al., 1986), this effect trims down the ratio of BDS_R_{760}/R_{730}. Therewith, the correlation quality decreases with phenotypic variation.

The close relationships observed between spikes DW and final grain yield can be nicely related to the assumption that changes in spikes DW mirror grain filling, thus, yield development (Ehdaie et al., 2008). The decrease of the relationships quality between spikes DW and grain yield especially at low N levels accounts for an influence of nutrient supply on this conclusion. Earlier results show that when assimilates were limited during spike growth, an imbalance between the development of chaff and grains appeared (Fischer and Stockman, 1980; Stockman et al., 1983; Abbate et al., 1997). With variable N supply spike size and chaff portion is influenced parallel to the dry weight partitioning between chaff and grains (Dreccer et al., 2000; Ercoli et al., 2008). While running out of nutrient supply at low N levels and progressing grain filling, these variable effects might have initiated the loss of relation between spike DW and final grain yield. In combination with the low chlorophyll contents of the spikes per se (Zhou and Wang, 2003), and even accelerated by senescence, spectral reflection was hardly able to detect cultivar differences satisfactorily during late grain filling. However, the BDS_REIP and the BDS_R_{760}/R_{730} index were less affected by saturation after anthesis and thus were able to predict spikes DW adequately until senescence.

Final grain yield was more closely related to spikes DM content which can be ascribed to the relationship of spikes structural components and kernel number (Brooking and Kirby, 1981; Fischer, 2011), being the principle base for final grain yield (Reynolds et al., 2009). Structure´s spectral information must be found with strong orientation to NIR wavebands (Campbell, 2002), especially when senescence decreases the spectral signal of chlorophyll. As the BDS_REIP is developing a dissymmetric course when senescence gets severe, its validity for describing spikes DM at late growth stages diminishes. The BDS_NDVI and the BDS_R_{760}/R_{730} index, both rather linked to NIR wavebands, described the spikes DM with good quality. While the BDS_NDVI was strongly affected by saturation effects at early grain filling, the BDS_R_{760}/R_{730} index showed fair resistance to either high chlorophyll contents or to senescent biomass, and thus, was able to predict spikes DM throughout grain filling for any N supply.

The DW index indirectly describes the ability to transfer biomass from the leaves+stems to the spikes and kernels. Fischer (2011) described the rate of transfer to be strongly cultivar dependent. The increasing range of cultivars DW index with progressing grain filling supports this finding. Due to the hardly changing spikes DW and the DW index of the cultivars at high N supply at ZS 85 a maximum grain filling must be reached. In combination with the still changing DW indices at lower N levels, a sink limitation might be postulated at high N levels. Low N supply resulting in advanced senescence hindered spectral parameters to adequately describe cultivar differences in the DW index. Due to its extensive resistance to saturation effects, the

R_{760}/R_{730} index was best explaining the dry weight transfer from vegetative to generative plant tissues throughout grain filling.

The N_{up} index, reflecting the imbalance of plants between taking up nitrogen from the soil or to transfer N to the spikes, was unsteadily related to the final grain yield. In contrast to that, the N_{up} index was strongly related to any spectral parameter assessed in this study. As both, VIS and NIR reflectance is correlated to the N status of the plant (Lamb et al., 2002; Shaver et al., 2010) this relationship was already shown frequently in literature (Schmidhalter et al., 2003; Moges et al., 2004; Reusch, 2005; Mistele and Schmidhalter, 2008a). However, most of these experiments were run during the vegetative growth period of crops, hardly observing the senescent effect on the N status during grain filling. Zhou and Wang (2003) did not find any influence of N supply on the NIR/red reflection of rice spikes, but a significant influence on vegetative plant parts. The N_{up} index might therefore rather be an expression of phenotypic differences in providing N by leaves+stems than of the variation in N uptake by the spike itself. The N_{up} index predictions were influenced by the effect of senescence within the observed spectral parameters. While the BDS_NDVI is prone to saturation in dense crop stands, it was able to rather predict the N_{up} index at lower N levels affected by advanced senescence. This behaviour of VIS-based spectral parameter during grain filling was already found for durum wheat (Bort et al., 2005). Similar to that, the BDS_REIP and the BDS_R_{760}/R_{730} index were also influenced by the development stages, however, in an opposite direction. Like mentioned earlier, the BDS_REIP could hardly handle senescent crops showing good correlations within less senescent crop stands. The BDS_R_{760}/R_{730} index was similarly affected by the decay of chlorophyll but was less prone to the effect of missing differentiation with advanced senescence.

Despite the high potential of phenotypic attributes such as spike development or partitioning efficiency which were assessed spectrally in this study, breeders still rely on visual ratings. A comparison of visual and spectral methods therefore can be another way to introduce spectral remote sensing in breeders gardens.

4.5 Section V: Testing spectral sensing and sensor fusion for the detection of canopy density based on visual ratings and spectral assessments

Visual ratings are still a standard method to evaluate phenotypic characters of crop canopies in breeders´ gardens. The majority of breed selection in the field is based on this method. With experienced evaluators, the visual rating of crop traits can explain up to 95 % of its variation (Wanous et al., 1991; Riday, 2009).

When visual ratings were set in relation to the spectral reflectance data, a clear relationship to spectral ranges was found representing the visible spectral bands, thus, photosynthetic active biomass. The blue, green and red spectral ranges express the visual information a human eye can evaluate. While the green light can directly be seen, the reflection of the blue and red spectral ranges decreases with increasing biomass or crop density. As a consequence, less blue and red (seen as yellow-brownish colour) but more green reflected light is correlated with increasing crop density and the subsequent visual ratings.

The relationships between spectral and visual information in our study were not as good as already found in literature where visual ratings were very well related to the spectrally assessed chlorophyll contents with r^2 values of up to 0.80 (Xu et al., 2000). The repeated visual ratings of the same cultivar in terms of its replication in our study resulted in positive matches of 54 %. In observations of forage amounts in clover, up to 80 % of equally repeated classifications were reached by experienced evaluators (Riday, 2009). The divergence between measured and visually estimated values combined with a lower reproducibility allowed relationships between spectral and visual estimates of r^2 with 0.39 in our study.

Spectral systems like the SPAD meter were already recommended to support visual ratings in plant breeding (Xu et al., 2000; Reynolds et al., 2001). Using the visual ratings as a draft for the development of the sensor-based classification systems DC_3 and DC_4, a reproducibility of 79 % to up to 87 %, respectively, were reached. Here, the high objectivity of sensor devices proves its advantage. Thus, spectral remote sensing can actually support cultivar classifications already with traditional rating parameters.

The differences between the positive matches of the DC_3 and DC_4 classification systems are due to the increased likelihood of wrong hits when the number of classes increases.

The character of crop density represents an undefined combination of green biomass and the structure of the canopy. As described above, the spectral information directly related to classes of crop density, are mainly based on the reflected or absorbed ranges of light. Due to the dispersed reflectance of the ultrasound signal, the measurements of canopy height by US reflection rather describe the horizontal structure within the canopy including depth, leaves layering and density (Shibayama et al., 1985; Mc Kerrow and Haper, 2001) than the definite crop height. A specific height in terms of the highest point of a plant or canopy cannot be assessed by dispersed signal reflection. The combination of both, the spectral reflectance data expressing biomass quantity and the ultrasound information expressing the canopy structure, resulted in an improvement of the relationships to the crop density classes. However, r^2 improvements were low with a maximum of 6 % of explained variation. The low level of improvement by including US information may be due to the low differences in US reflective canopy surface at this development stage. Scotford et al. (2003a; 2003b; 2004) observed a decrease of variation of US reflection between wheat plots of different seeding rates. In their study, at development stage ZS

4 variation was too low to be detected by the ultrasonic sensor. This was dedicated to the fact, that independent of the crop density, after ZS 4, the canopies offer a very similar reflection surface and thus low US reflection variation.

While especially the spectral ranges of blue and green improved their relationship to crop density when being combined with US reflectance data, spectral ranges of the far green or red did not improve r^2 significantly. This may be based on the fact that the closer the spectral ranges are related to the NIR spectral bands, whose reflectance is already dependent on canopy structure (Campbell, 2002), the less extra information about structure can be added by US data. This is also supported by the higher coefficients of determination of the relationships between the wavelengths lying closer to the NIR ranges, ΔR_{558} and ΔR_{709}, and any DC classes.

Conclusively, spectral assessments strongly improved the repeatability of crop density classifications in the field. Sensor fusion of spectral and ultrasound devices have shown to be advantageous, however, specific development stages have to be chosen when using this signal combination.

5 General discussion

Plot-based field trials were the basis for all experiments of this study. Therefore, the within-plot variations of the sensors outputs were assessed to evaluate the output regimes of the different systems. The measurements revealed that the four sensors systems strongly differed in the variation of their output values. The coefficients of variation (CV) of each single plot were set in relation to the whole trial CV. Therewith, it could be assessed if the variation within the plot is higher than the variation of wheat grown at N levels between 0 kg ha^{-1} and 420 kg ha^{-1}. The magnitude of the CV-ratio was strongly dependent on the spectral parameter and the sensor system used. Due to the saturation of red spectral reflection with high crop densities (Aparicio et al., 2002; Mistele et al., 2004; Heege et al., 2008; Erdle et al., 2011), the NDVI of all sensor devices decreased its variation with increasing N supply. However, also the variations´ quality of the NIR-based R_{760}/R_{730} index varied strongly between the sensor systems. This effect was shown to be due to the differences in the field of view (FOV) of the sensor systems (Solari, 2006). Whereas the passive bi-directional spectrometer (BDS) offered the largest FOV, the detected canopy area decreases with the active sensors of AFS, Crop Circle and GreenSeeker. Especially in this order, the within-plot variation increased. With decreasing and narrowing FOV, the sensors record a smaller canopy area per data point resulting in a higher variance within the plot. Repeated measurements of the same canopy area could successfully reproduce trends and reflectance patterns within the plots. The lower the within-plot variation of the sensor device, the better within-plot heterogeneities could be identified. Calculating the plot averages of the spectral outputs, however, the spectral parameters of any sensor system were highly significant in expressing crop attributes.

Wheat N status of the crops was best reflected by spectral assessments until anthesis. Although analysing small subsamples of one square meter per 30 m^2 plots, the N content was significantly distinguished at N levels up to 360 kg ha^{-1}. The differentiation of plant traits such as dry weight, fresh weight or dry matter content was strongly dependent on the development stage and the year of observation. Independent of the sensor system, the R_{760}/R_{730} index was the spectral parameter best and temporarily stable explaining the N status of wheat. Due to its dependence on NIR wavelengths, the R_{760}/R_{730} index was less affected by effects of saturation, thus, this index can be preferred to assess crop attributes also at development stages of high biomass (Mistele et al., 2004; Heege et al., 2008; Reusch et al., 2010). The red light-based spectral parameters SR, NDVI and REIP were affected by saturation already at earlier development stages. Sensors like GreenSeeker, only offering the NDVI, thus, are less suited for measurements in dense wheat crop stands. The Crop Circle, with selectable interference filters, the BDS and the AFS were able to calculate the R_{760}/R_{730} index and therewith, were well adapted to the high crop stands typical of Western Europe. The BDS offered the highest flexibility in the development of spectral parameters. Although the active sensors showed similar results when using the same spectral parameters, due to its broad spectral information, the BDS was used for further investigations where multiple spectral parameters were compared.

While spectral reflectance measurements are well known for pre-anthesis crop development, the observations of post-anthesis development were limited to only few studies. The direct relationship between spectral assessments during grain filling and the final grain yield were only successful when stressed plants or a broad genetic variation was considered (Babar et al., 2006a; Babar et al., 2006b; Prasad et al., 2007). Due to the missing variation of genetically similar and high yielding wheat cultivars, in our investigation the development of single plant traits which were well related to final grain yield were observed by spectral sensing. Although being the main source of transferable assimilates, plant traits of leaves together with the stems were hardly related to final grain yield. Good relationships to grain yield, though, were found for the spike attributes spikes dry weight and spikes dry matter content. The dry matter content of spikes increases with the structural components within the spike. Being the framework for the kernel formation, the number of spikelets and fertile flowers directly influences the structural components and the dry matter content of the spike (Brooking and Kirby, 1981; Fischer, 2011). Whereas it is well known, that the kernel number is the mayor influent of final grain yield (Reynolds et al., 2009), an increase in the spikes dry matter then is indirectly related to the kernel yield. Accordingly, with the finalised growth of the chaff at about 20 days after anthesis, any gain of weight of the spike can be ascribed to the filling up of the kernels, thus, the development of kernel yield (Ehdaie et al., 2008). However, with varying N supply, the relationships quality between spikes DW and grain yield decreased especially at low N levels. It was shown, that in deficient wheat an imbalance between the development of chaff and grains appeared (Fischer and Stockman, 1980; Stockman et al., 1983; Abbate et al., 1997). This imbalance led to an unstable relationship between spike DW and final grain yield. The biomass partitioning between the leaves+stems and the spikes was strongly affected by N supply. Well-nourished cultivars showed a stagnation of DW transfer to the spikes indicating a sink-limitation, whereas N-deficient cultivars still increased the DW index.

Spectral parameters recorded during grain filling revealed similar effects like at pre-anthesis. As mentioned before, the BDS_NDVI was strongly affected by saturation around anthesis and relationships qualities varied between the parameters and the development stages. This effect was even increased by varying N supply. The BDS_REIP being dependent on red spectral ranges, as well, was affected by both high and very low chlorophyll contents. Besides the already shown saturation at high crop densities, with low N supply and decreasing chlorophyll contents at late grain filling, the BDS_REIP showed strongly dissymmetric relationships to crop attributes (Guyot et al., 1992; Railyan and Korobov, 1993). At anthesis or late grain filling, the chlorophyll contents were too high or too low, respectively, to result in detectable differences between the cultivars.

The BDS_R_{760}/R_{730} index and the water index, BDS_WI, both depending on exclusively NIR wavebands were less affected by signal saturation and well described yield related plant traits throughout grain filling. The BDS_R_{760}/R_{730} index, representing the reflectance patterns of structure-influenced NIR wavebands, best described the fraction of the structural tissues in the spike. Therewith, good relationships were found between the BDS_R_{760}/R_{730} index and the spikes´ dry matter content fairly independent of the N status. Also the DW transfer from leaves+stems to the spike, expressed by the DW index, as well as the spikes dry weight were well correlated to the BDS_R_{760}/R_{730} index. This is because with the continuing storage of assimilates in the kernels, the assimilates as structural material itself increased the spikes structure. Known to be a good indicator for crop N status (Mistele et al., 2004; Mistele and Schmidhalter, 2008b; Strenner and Maidl, 2010), the BDS_R_{760}/R_{730} index was well related to the N partitioning between the leaves+stems and the spike. Although, N status indicators were neither related to final grain yield, nor to grain protein or grain N content, the spectral assessments expressed well significant cultivar differences in soil nitrogen uptake and the N transfer to the spike.

The BDS_WI, reflecting the spectral water bands and the water status of crops, successfully estimated the cultivar differences of grain dry matter content. Although the grain DM was not related to final grain yield, it can be an important indicator for small cereals physiological maturity (Calderini et al., 2000; Slafer et al., 2009). With physiological maturity, the deposition of assimilates into the grain has been completed followed by the loss of water by ripening (Slafer et al., 2009). Early physiological maturity of wheat cultivars was found to favour high yields (Stapper and Fischer, 1990). The remotely detected state of maturity and therewith the grain DM content, could accelerate the development of favoured crossbreds in plant breeding.

While the crop attributes mentioned above until now are rarely used in practical crossbred classification, characteristics which can be assessed visually are widely accepted in plant breeding. The attribute of canopy density was tested for 90 cultivars of a broad genetic range for repeatability of the classification result. Whereas positive repetitions were run for stay green differentiation or biomass estimations with a reproducibility of up to 80 % (Xu et al., 2000; Riday, 2009) visual classification in our study resulted in a reproducibility of 54 %. Spectral devices can be a supporting tool for visual ratings (Xu et al., 2000; Reynolds et al., 2009) and resulted in a classification reproducibility of up to 87 % in our study when using sensor-based rating classes. The combination of spectral and ultrasound reflectance data was already used to describe growth and vigour of wheat (Jones et al., 2004) but in our investigation only slightly improved the relationship to crop density. Scotford et al. (2003a; 2003b; 2004) found that at development stages around ZS 4 the differentiation between high and low seed densities in wheat is difficult because canopy surfaces of both, dense and sparse canopies, show similar ultrasound reflectance. However, although the effect of sensor fusion was affected by the development stage, the attempt to optimise visual ratings with reflectance information resulted in better predictions of the canopy density. Spectrally assessing phenotypic differences of plant traits proved to be a promising tool for future plant breeding.

6 Conclusions

Overall, our results show, that technical conditions of sensor devices strongly affect within-plot variations of sensor outputs and the signal reproduction of within-plot heterogeneities. However, all sensors used in this study proved to offer stable and significant estimates of crop attributes when plot averages were calculated. When wheat of varying N supply was observed, red light-based parameters of all sensor systems were affected by saturation effects in dense crop stands. Independent of the sensor system, spectral parameters relying on NIR wavelengths were more resistant to saturation effects. The R_{760}/R_{730} index proved to be the most powerful and temporarily stable spectral parameter to estimate crop status.

Post-anthesis measurements were influenced by strongly varying chlorophyll contents and the transfer of dry weight and N compounds from the vegetative to the regenerative part of the plant. VIS-based spectral parameters were prone to the initially high but rapidly decreasing chlorophyll contents and therewith were less suitable to describe spike attributes during grain filling. Spike dry weight, spike dry matter content and the N transfer to the spike were best reflected by the R_{760}/R_{730} index independent of the nutritional status of the crop. Although the final grain dry matter content was not related to final grain yield, this plant trait was well related to the water band index, WI. The grain dry matter content is a good indicator for the physiological maturity of cereal crops and can help to find optimal harvest dates for energy crops or superior wheat crossbreds.

Consequently, visual ratings were set in relation to reflectance measurements whereas the direct comparison resulted in poor relationships. Developing explicitly sensor-based classification scales strongly improved the repeatability of classification results for 90 wheat cultivars and proved that spectral sensors can be a promising tool to optimise breeders visual ratings.

While the demand for stable yields and highly adaptable crops is increasing with increasing global population and changing climate trends, traditional methods in crossbred phenotyping will soon struggle to meet these challenges. With the identification of spectral parameters to rapidly and objectively estimate conventional plant attributes or plant traits of high potential for yield predictions, our study showed, that spectral remote sensing can be a promising tool to support the phenotypic classification in the field and thus, selective breeding.

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