



# Spectral high-throughput assessments of phenotypic differences in biomass and nitrogen partitioning during grain filling of wheat under high yielding Western European conditions

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## ABSTRACT

Grain filling, the last phase of final yield development, is characterised by the transfer of assimilates and nitrogen compounds from vegetative plant tissues (source) to grains in the spike (sink). In this study, we spectrally assessed source and sink components to characterise the grain filling of wheat cultivars. In 2009, six high-yielding wheat (*Triticum aestivum* L.) cultivars were grown and fertilised with 160 kg nitrogen ha<sup>-1</sup>. At four development stages during grain filling, the crops were sampled to determine the phenotypic variation in dry weight translocation as well as in the nitrogen uptake of the spikes and of the leaves combined with the stems. The final grain yield and grain N content was assessed, and the grain dry matter content was calculated as the ratio of the dry weight to the fresh weight. The harvest index was further calculated. At each sampling date, tractor-based high-throughput canopy reflectance measurements were carried out, and multiple vegetation indices were calculated. Spikes characteristics were best related to the final grain yield. While the water index,  $R_{970}/R_{900}$ , explained the biomass partitioning between spikes and vegetative plant parts, the NIR/NIR-based index,  $R_{760}/R_{730}$  described nitrogen partitioning and the spikes dry matter with an  $r^2$  of up to 0.89. The final grain dry matter was best assessed by spectral indices offering information about physiological maturity. The cultivars strongly differed in yield relevant crop traits, as illustrated by their relative ranking of the crop traits. Observing yield relevant crop traits by spectral reflectance may allow to rapidly and non-destructively classify cultivars with regard to source–sink relationships.

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## 1. Introduction

Increasing the grain yield of wheat is a continual effort of breeders, farmers and plant physiologists. It is well known that single crop traits play an important role in establishing the final grain yield. These crop traits are in turn influenced by the phasic development of wheat (Stapper and Fischer, 1990; Slafer et al., 2009; Fischer, 2011). Spectral remote sensing is well known in agriculture and crop management as a non-destructive method to estimate traits of crops such as wheat but is mainly limited to the vegetative phase (Raun et al., 2001; Aparicio et al., 2002; Prasad et al., 2007). The amount of vegetative biomass as the source of assimilates and proteins for grain filling is a prerequisite for a high potential yield (Richards, 2000; Shearman et al., 2005; Reynolds et al., 2009) and can be estimated by sensor based crop measurements. However, although the photosynthetic activity decreases rapidly during grain filling (Mi et al., 2000; Acreche and Slafer, 2009), to a certain degree,

the leaves and stems are still active in N uptake and photosynthesis after anthesis (Masoni et al., 2007) and thus, maintaining assimilate production (Sinclair and Jamieson, 2006). Cultivar differences in yield are strongly related to their efficiency of transferring resources to the spike (Fischer, 2011) which is reflected in biomass and N compound partitioning. Furthermore, in Europe, small grain cereals harvested during grain filling play a significant role in the production of methane through anaerobic digestion. The knowledge about the state of kernel filling and dry matter content is crucial for successful silage and effective biomass digestion. Thus, post-anthesis spectral observations of these crop traits might be useful to assess the optimal plant status to harvest for methane production among other production goals.

Aiming at the final grain yield of wheat, the focus is primarily laid on the yield components spikes per area, grains per spike and the grain weight. With the exception of the grain weight, the formation of the single components already start pre-anthesis (Brooking and Kirby, 1981; Miralles et al., 2007; Reynolds et al., 2009; Foulkes et al., 2010; Fischer, 2011). The development of grain weight, however, depends on post-anthesis conditions in the phase of grain filling. Considering a constant chaff dry weight after approximately

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20 days after anthesis (DAA) (Abbate et al., 1997), the spike's dry weight during grain filling is thought to mirror the phase of grain development and thereby the yield (Ehdaie et al., 2008). Additionally, the nitrogen transferred from the leaves and stems to the grains is an indirect measurement for its protein content which is an important quality parameter of bread wheat. A method to indirectly estimate crop traits controlling yield and grain quality by tracking the spike development would be helpful to understand the yield components.

Several parameters linked to the source–sink relationship of wheat have already been assessed by breeders and physiologists to evaluate new crossbreds for grain yield. However, the traditional screening methods in breeders' gardens are highly laborious, time consuming and costly. Spectral remote sensing offers a rapid tool to estimate crop productivity before harvesting (Ferrio et al., 2005; Babar et al., 2006a,b). Babar et al. (2006a,b) and Prasad et al. (2007) already assessed genotypic yield potential with spectral measurements performed during heading and grain filling. However, the majority of these investigations were based on strongly varying environmental conditions. In Western Europe, crops are grown under temperate climate conditions in environments characterised by fertile soils and sufficient plant available water representing potential environments in which genetic differences are hard to find (Ferrio et al., 2005). Furthermore, modern wheat cultivars lack of broad genetic variation and are thought to be co-limited by source and sink (Shearman et al., 2005; Acreche and Slafer, 2009). Nevertheless, breeders depend on the evaluation of new crossbreds also in those high yielding environments.

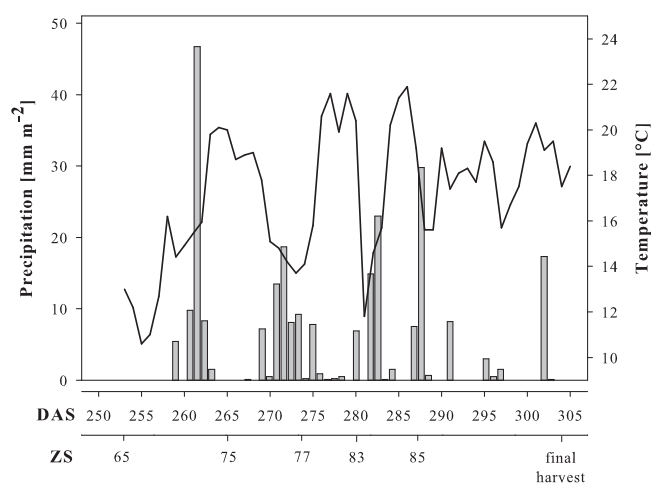
Both, the source as well as the sink, are combinations of several crop traits. While the potential yield is specified as the product of intercepted light, radiation use efficiency and biomass partitioning (Reynolds et al., 2005), the latter factor can be split into source and sink. Thus, the source can be defined as the leaves + stems and its photosynthetic capacity, and the sink is specified as the spike minus the photosynthetic capacity of the chaff (Wardlaw and Moncur, 1976), respectively. Due to the strong temporal influence on the source–sink relationships during grain filling, the observation of crop development after anthesis must be traced back to the single source and sink components and defined time frames. Although the final grain yield is known to be a consequence of the interplay between single crop traits, as far as we know, there has yet been no investigation observing single source–sink components via canopy reflectance measurements during grain filling. This experiment thus is a yet unrivalled approach to find a viable tool for breeders in Europe to evaluate the yield development of wheat crossbreds during the crucial phase of grain filling. Therefore, a single year approach was conducted to examine the sensors ability in non-destructively assessing source and sink components.

The objectives of this study were thus, (i) to evaluate the use of spectral vegetation indices within the grain filling phase, (ii) to estimate the effect of source–sink components on canopy reflectance data and (iii) to select vegetation indices to distinguish cultivars or cultivar groups by their grain-filling characteristics.

## 2. Materials and methods

### 2.1. Study site and biomass sampling

The field experiment was conducted at the Dürnast research station of the Technische Universität München in southwestern Germany (11°41'60"E, 48°23'60"N). The soil was characterised as a mostly homogeneous Cambisol of silty clay loam with a water holding capacity of about 160 mm within a soil depth of 1 m. The field was located in a hilly region sloping northwards at approximately 0.09 m m<sup>-1</sup>. In this geographic area, the average



**Fig. 1.** Daily sums of precipitation and course of average temperatures during grain filling from 253 to 303 days after anthesis (DAS). Arrows indicate 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.

yearly precipitation is approximately 800 mm, and the average temperature is 7.5 °C.

During grain filling in 2009, the precipitation totalled 262 mm m<sup>-2</sup> at a mean temperature of 17.3 °C (Fig. 1). Grain yields in this area vary between 6 and 10 t ha<sup>-1</sup>, depending on the climate and soil conditions. In the experimental field, the soil was sampled before planting, and a representative subsample was analysed for P (43 mg P per 100 g soil) and K (28 mg K per 100 g soil) to ensure an adequate supply of these macronutrients. Residual soil nitrate was determined before regreening by a simplified soil nitrate quick-test method (Schmidhalter, 2005) that indicated soil NO<sub>3</sub>-N levels of 45 kg ha<sup>-1</sup>. The field was managed conventionally, adopting local standards.

The experiment was designed as a randomised block design with four replicates. Six high-yielding winter wheat (*Triticum aestivum* L.) varieties were sown at 320 kernels per square metre: Tommi, Solitär, Impression, Pegassos, Cubus and Ellvis. The 160 kg ha<sup>-1</sup> of nitrogen fertiliser application was split following the practice of local farmers and applied at the stem-elongation (60 kg ha<sup>-1</sup>), booting (60 kg ha<sup>-1</sup>) and late-flowering (40 kg ha<sup>-1</sup>) stages. No fertiliser was applied as pre-planting dressing. Fertiliser application at the late-flowering stage is frequently used to increase the protein quality of small grain cereals in Germany. The experimental plots were 2 m wide and 15 m long.

Biomass sampling was performed at four times during grain filling, at Zadoks growth stages (Zadoks et al., 1974) (ZS) of 75 – medium milk, 77 – late milk, 83 – early dough and 85 – soft dough, referring to 265, 273, 280 and 287 days after sowing (DAS), respectively (Fig. 1). For biomass sampling, two parallel sowing strips (0.12 m<sup>2</sup>), twice 0.5 m, at the plot centre were cut at ground level, resulting in about 70 spiked wheat culms plus leaves. The plant samples were put into plastic bags and immediately weighed as a whole. Next, the spikes were separated, and the remaining biomass (leaves + stems) was weighed again and then dried. The fresh weight (FW in t ha<sup>-1</sup>) and the dry weight (DW in t ha<sup>-1</sup>) were recorded, and the dry matter content (DM in %) was calculated as the percentage of the DW to the FW. The aboveground N uptake (N<sub>up</sub> in kg ha<sup>-1</sup>) of the spikes and leaves + stems was calculated, respectively, as the dry weight multiplied by the total N content in percent detected by mass spectrometry using an Isotope Radio Mass Spectrometer with an ANCA SL 20-20 preparation unit (Europe Scientific, Crewe, UK). The relationships of the DW

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