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**Influence of nitrogen and water supply on evapotranspiration,
yield and agronomic water use efficiency of winter wheat**

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Zusammenfassung

Die Prognose der klimatischen Veränderungen in Nordwesteuropa, welche im Vergleich zur derzeitigen Situation erhöhte Temperaturen und geringere Niederschläge in den Sommermonaten vorhersagt, wird die zukünftige Nahrungsmittelproduktion vor neue Herausforderungen stellen. Vor diesem Hintergrund stellt sich die Frage, welchen Einfluss die Stickstoffdüngung auf die Evapotranspiration (ET_a) und die agronomische Effizienz der Wassernutzung (WUE_y) hat. Da aus der Literatur wenig über Trockenstressreaktionen von Winterweizen (*Triticum aestivum*, L.) in temperat humiden Klimaten in Nord-West Europa bekannt ist, wurden in den Jahren 2013-2015 Feldversuche mit unterschiedlichem Stickstoff- und Wasserangebot am Institut für Pflanzenernährung und Umweltforschung in Dülmen durchgeführt. Zur Einstellung der unterschiedlichen Wasserversorgung (voll gewässerte Kontrolle, früher Trockenstress, früher und später Trockenstress) wurden Regenabdeckungen und Tropfbewässerungssysteme eingesetzt. Eine unterschiedliche N-Versorgung (ungedüngte Kontrolle, 120 und 230 kg N ha⁻¹) wurde mittels Düngung mit Kalkammonsalpeter (27% N) eingestellt. Eine möglichst realistische Berechnung der ET_a ist für die Quantifizierung des Einflusses der Stickstoffversorgung auf die WUE_y von zentraler Bedeutung. Zunächst wurden deshalb zwei Berechnungsmethoden für die Abschätzung der ET_a nach Methode FAO 56 verglichen. Die ET_a wurde anhand von handspektrometrisch gemessenen Pflanzenkoeffizienten (Kc-Werten) (NDVI-Ansatz) und auf Grundlage publizierter Kc-Werte und Kc-Phasenlängen (tabellierter-Ansatz) für nicht Wasser- und Stickstoff-limitierte Weizenbestände berechnet. Insgesamt zeigte sich, dass die ET_a -Berechnungsmethode nach FAO 56 die Wasserverbräuche der Pflanzenbestände sehr realistisch abbildete. Die Daten zeigen weiterhin, dass der NDVI-Ansatz im Vergleich mit dem tabellierten-Ansatz eine realistische Berechnung der ET_a ermöglicht. Tabellierte Werte können lediglich retrospektiv für eine Berechnung der ET_a herangezogen werden, währenddessen der NDVI-Ansatz auch während der Vegetationsperiode zur Abschätzung der ET_a genutzt werden kann und wachstumsbeeinflussende Faktoren (Wetter, Nährstoffmangel, Krankheiten) berücksichtigt. Deshalb erfolgte die Berechnung der ET_a für Stickstoff- und Wasser-limitierte Prüfglieder mit dem NDVI-Ansatz. Bei ausreichendem Wasserangebot erhöhte die Stickstoffdüngung den Korn-ertrag vergleichsweise stärker als die ET_a . Der positive Effekt der Stickstoffdüngung auf die WUE_y beruhte hauptsächlich auf einer relativen Verminderung der Bodenevaporation. Dieser Effekt war unter Bedingungen ausreichender Wasserversorgung ausgeprägter als unter Wasser-limitierten Bedingungen. Die Ergebnisse zeigen, dass mittels des NDVI-Ansatzes nicht nur die ET_a , sondern auch das Ausmaß der Trockenstress bedingten Ertragsreduktion (K_y -Wert) quantifiziert werden konnten. Weiterhin stellte sich heraus, dass auch ein Trockenstress während des Schossens den Ertrag negativ beeinflusste. Die Ergebnisse dieser Arbeit zeigten, dass eine hohe N-Versorgung im Vergleich zu einer moderaten N-Düngung unter frühem und andauernden Trockenstress zu höheren Korn-erträgen führte und gleichzeitig eine höhere ET_a verursachte, was in einer höheren WUE_y resultierte. Wir schreiben den positiven N-Effekt den nahezu wassergesättigten Böden zu Vegetationsbeginn nach Winter zu.

Summary

The predicted climate change for North West Europe, which will be characterized by higher temperatures and lower rainfall during the summer months, will challenge future food production. Against this background, the question is how nitrogen supply influences the evapotranspiration (ET_a) and the agronomic water-use-efficiency (WUE_y). Literature regarding drought stress reactions of winter wheat (*Triticum aestivum*, L.) grown in the temperate humid climate of North West Europe is scarce. Because of this a field trial with variable nitrogen and water supply was conducted during 2013-2015 at the Research Center for Crop Nutrition Hanninghof in Dülmen. To induce different water regimes (fully watered control, early drought, early and late drought) rain-out-shelter and drip irrigation systems were installed. Different N-supply treatments (unfertilized control, 120 and 230 kg N ha⁻¹) were induced using calcium ammonium nitrate (27% N). A realistic calculation of ET_a is the key for the quantification of the impact of nitrogen on WUE_y . Therefore two different calculation approaches for estimating ET_a according to the FAO 56 method were compared. ET_a was calculated based on handspectrometer measurements converted to crop coefficients (Kc-values) (NDVI-approach) and on the other hand based on published Kc-values and Kc duration periods (tabulated-approach) for plots that were not water and nitrogen limited. In general, it could be concluded that the method for estimating ET_a according to FAO 56 showed realistic results. Furthermore, the data showed that the NDVI-approach, in contrast to the tabulated-approach, allowed a realistic calculation of ET_a . Tabulated values could only be used retrospectively for estimating ET_a , whereas the NDVI-approach can take growth influencing parameters (weather, pests, lack of nutrients) into account and can therefore be used to quantify ET_a during the vegetation season. For this reason we used the NDVI-approach to calculate the ET_a for plots that were limited in their water and nitrogen supply. If water was not limited a higher nitrogen rate increased grain yields of wheat more than ET_a . The positive effect of nitrogen fertilization on WUE_y was mainly caused by a reduction of soil evaporation. This effect was more pronounced under wet than under drought conditions. The results of this study also showed that the NDVI-approach can be used not only to quantify ET_a but also to measure yield reductions caused by drought stress (K_y -value) during the vegetation period. Drought stress during booting also caused grain yield reductions. This study showed that a high N-supply compared to moderate N-supply under early drought and continuous drought conditions increased both grain yields and ET_a in a more water use efficient way. We refer this positive N-effect to the fully water saturated soils at the start of the vegetation period in spring.

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Abbreviations

DOY	Day of year
E	Evaporation
ET	Evapotranspiration
ET _a	Actual evapotranspiration
FA	Fischer Allen approach
FWB	Field water balance
GDD	Growing Degree Day
HI	Harvest index
K _c	Crop Coefficients
NDVI	Normalized Difference Vegetation Index
n.a.	not applicable
PASW	Plant-available soil water
Tr	Transpiration
VI	Vegetation index
WS+	Well-watered plots
WS31	Early drought
WS31 1/3	Early drought with approximate irrigation amounts of 1/3 of crop water use after the drought period
WS31 2/3	Early drought with approximate irrigation amount of 2/3 of crop water use after the drought period
WUE	Water use efficiency
WUE _B	Biomass WUE
WUE _Y	Agronomic WUE

General Introduction

Irrigated agriculture is the dominant user of global freshwater resources and improvements in water management are considered key to simultaneously enhance food production and to secure regional water resources (Molden, 2007; Siebert et al., 2010). Irrigation management requires reliable estimates of crop water demand, which have been extensively developed during the last five decades (e.g. Doorenbos and Pruitt, 1971; Allen et al., 2006). About 70% of global freshwater use is allocated to irrigation (Siebert et al., 2010) and increasing food demand puts further pressure on land and water resources (FAO, 2011; UNEP, 2014). An analysis of present and future crop water use in consideration of environmental water demand illustrated the importance of improved water management to enhance food production and secure both food security and regional water resources (Molden et al., 2007). Freshwater resources are threatened by nutrient and pesticide loads from agriculture as well as increasing domestic and industrial freshwater demand in some world regions. Freshwater availability per capita and year has decreased in e.g. South-East Asia and North Africa (Reuveny, 2007; McDonald et al., 2011). In future, water will become increasingly scarce particularly in semi-arid regions. Improvements in agricultural water management are necessary to enhance agricultural productivity in order to meet food demands of the growing world population. The International Water Management Institute (IWMI, 2007) stated that there will be enough land, water and human capacity to produce enough food for a growing population, if water use in agriculture is improved. The water use efficiency (WUE) has therefore become one of the most important indices for benchmarking optimal water management practices.

To quantify the water use efficiency by plants, it is important to have a realistic estimation of the amount of water used by the plant. Estimates of evapotranspiration (ET) presented in the FAO 56 guidelines (Allen et al., 2006) rely on input data for the calculation of crop water use. Input data are meteorological information, crop-specific coefficients (K_c values), which are multipliers of crop water use relative to the reference ET, lengths of crop growth stages, and plant-available soil water (PASW) (Pereira et al., 2015). K_c values and crop-specific information about duration of growth stages are available in several sources (e.g., Allen et al., 2006; Stetson and Mecham, 2011), but these data are strongly focused on climate zones with regularly applied irrigation. The duration of crop growth stages for winter wheat in a temperate humid climate is reported by Fischer et al. (2000). However, estimates of stage length are relative to the final harvest date, allowing stage length prediction only in the

retrospective mode and, therefore, not straightforward applicable for irrigation purposes. Furthermore, these data are not variety specific and adjustments are necessary particularly for temperate climates, where crop development is highly variable due to interannual variability of temperatures during the pre- and post-winter growth period.

Local adjustments for stage duration and basal crop coefficients are expected to be more suitable for estimating ET and crop water demand than the use of published data for stage duration and K_c values (Bausch, 1995; Allen et al., 2011; Peireira et al., 2015). Such local adjustments are usually based on site-specific measurements or observations of crop growth and, consequently, vegetation index (VI) based approaches are increasingly recommended for irrigation management. The Normalized Difference Vegetation Index (NDVI) is one of the most commonly used VI (Pinter et al., 2003) and several studies showed good correlations between the NDVI and plant growth parameters in wheat, e.g. biomass (Pinter et al., 2003), fraction of soil covered by plants (Er-Raki et al., 2007) and leaf area (Duchemin et al., 2006; Chattaraj et al., 2013). The documented advantages of using VI-based crop coefficients are the ability to account for variations in plant growth due to local weather conditions, site-specific differences in sowing dates and seed densities, cultivars, pests and nutrient supply (Tasumi and Allen, 2007; Pôças et al., 2015; Hunsaker et al., 2007). Estimates of crop coefficients derived from NDVI are possible due to the generally close relationship between vegetation cover and crop canopy size. Nutrient deficiency has a pronounced effect on crop canopy development and growth rate. In this case, optically derived information about canopy cover is a suitable option to calculate crop water use as tabulated data of stage length and crop coefficients implicitly assume that crops grow without nutrient limitations (Allen et al., 2011). Hunsaker et al. (2005) demonstrated that remotely-sensed NDVI values enable the determination of real-time K_{cb} and crop evapotranspiration of wheat. Furthermore these authors illustrated that length of growth stages as well as the crop coefficient during the mid-stage were affected by different N management of wheat and that NDVI-derived crop coefficients were more suitable than tabulated values for estimating ET (Hunsaker et al., 2007).

Climate change scenarios indicate increasing occurrence and intensity of droughts (Sheffield and Wood, 2008). Lehner et al. (2006) analyzed drought scenarios for South Eastern Europe from the last century and projected in a model study that in the year 2070 severe drought events, occurring actually once per century, will happen every forty years. Water scarcity is a

regional to local event which is highly affected by soil type, soil profile depth and cropping pattern (Ehlers and Goss, 2003; Rickmann and Sourell, 2014). This underlines the necessity to predict water demand and water deficit at a site- and crop specific level. Water stress is expected to decrease yield and farm management of field sites regularly responds by adjusting fertilizer amount accordingly to the expected yield decrease. A phenomenon known as ‘Haying-off’ has been illustrated in semiarid environments showing that grain yield is negatively influenced by excessive N supply. High N rates in that case caused an increase in shoot biomass during the vegetative growth phase and resulted in a depletion of soil water reserves during the grain filling period and related yield decreases (van Herwaarden et al., 1998). As WUE is related to total crop water use, which is sum of evaporation and transpiration, several management options, such as the reduction of non-productive loss through soil evaporation, avoidance of runoff and drainage exist (Gregory, 2000). Application of fertilizer, provided that it allows a more rapid growth of the canopy that shades the soil surface, thereby reduces the proportion of the total water that is evaporated (Cooper et al., 1983). Options to increase yield and WUE by integrated water and nitrogen management are indicated by several studies (Eck, 1988; Musick et al., 1994; Hussain and Jaloud, 1995; Schjoerring et al., 1995; Oweis et al., 1998; Lenka et al., 2009).

Chapter 1 aims to assess the suitability of using NDVI-based estimates of ET compared to tabulated values of K_c and stage length. To compare both approaches, post-winter growth of well-watered and fertilized winter wheat (*Triticum aestivum*, L.) was closely monitored and predicted ET was used to calculate a daily field water balance (FWB). The calculated FWB of both approaches was compared with the measured FWB during 2014 and 2015. In order to quantify the effect of variation in stage length and K_c values on ET estimates, a sensitivity analysis was finally performed.

To quantify the effects of N fertilizer supply on evapotranspiration (ET) of winter wheat, three nitrogen rates (N0, N120 and N230) were applied during a 2 year field experiment. Plots were watered when required to avoid water limitations. The Normalized Difference Vegetation Index (NDVI) was used to derive crop coefficients which were used to calculate ET with the dual-coefficient approach. The extent to which N supply modified bare soil evaporation (E), transpiration (Tr), ET, grain yield, aboveground biomass and harvest index (HI) was tested and summarized in chapter 2.

The aim of of chapter 3 was i) to quantify the extent of different N-rates on grain yield under conditions of drought in a temperate humid climate in NW Europe ii) to quantify differences in water-use-efficiency under varying nitrogen supply of winter wheat grown under climates with fully saturated soils at vegetation start after winter, compared to plants grown under arid environment. Therefore we induced drought spells with rain-out-shelters on different fertilized plots (N120, N230) and compared them with treatments (N120, N230) that were not limited in water supply.

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Chapter 1

Evapotranspiration of winter wheat estimated with the FAO 56 approach and NDVI measurements in a temperate humid climate of NW Europe

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Abstract

According to a higher food demand under a projected climate change in the future, a more efficient use of freshwater in agriculture is required. This could be reached by maximizing the water use efficiency. Therefore a precise estimate of crop water (ET_a) use is necessary, not at least to ensure a sufficient water requirement. Information about ET_a are needed during the growth period to support agronomic water use efficiency (WUE_y) relevant crop management options (e.g. fertilization, irrigation). During the 2013/14 and 2014/15 growing seasons at Duellen, Germany, field trials with winter wheat (*Triticum aestivum*) were established under locally recommended nitrogen supply (230 kg N ha^{-1}), while a sufficient water supply was ensured by drip irrigation. Crop growth dynamics were measured by handspectrometer and canopy analyzer, derived phenological stages were documented. ET_a was calculated in two different ways: Firstly based on published Crop Coefficients (K_c) and stage durations (FA-approach) and secondly based on remote sensed K_c -values (NDVI-approach). A comparison of measured (FDR-techniques) and estimated field water balance (FWB), based on the two calculated ET_a 's, showed a good correlation (RMSE 0.7, 0.6 (FA-approach), 0.6, 0.8 (NDVI-approach)). Based on calculated ET_a 's (403 mm, 430 mm (FA) and 377 mm, 463 mm (NDVI) in 2014 and 2015, respectively) and derived grain yields (10.4 and 10.5 t ha^{-1} in 2014 and 2015, respectively) the corresponding WUE_y ranged between 1.93–2.76 g/l. The results indicate that an approach to estimate ET_a (NDVI) which can be used during the growing season, compared to estimates of ET_a based on published values (FA) which can only be used retrospectively, predicts ET_a in a sufficient way. A remote sensing approach also provides the

potential to calculate ET_a of plants grown under suboptimal conditions (e.g. lack of nutrients), while a calculation with published values is not recommended.

1. Introduction

Irrigated agriculture is the dominant user of global freshwater resources and improvements in water management are considered key to simultaneously enhance food production and to secure regional water resources (Molden, 2007; Siebert et al. 2010). Irrigation management requires reliable estimates of crop water demand, which have been extensively developed during the last five decades (e.g., Doorenbos and Pruitt, 1977; Allen et al., 2006). Estimates of evapotranspiration (ET) presented in the FAO 56 guidelines (Allen et al., 2006) rely on input data for the calculation of crop water use. Input data are meteorological information, crop-specific coefficients (K_c values), which are multipliers of crop water use relative to the reference ET, lengths of crop growth stages, and plant-available soil water (PASW) (Pereira et al., 2015). K_c values and crop-specific information about duration of growth stages are available in several sources (e.g., Allen et al., 2006; Stetson and Mecham, 2011), but these data are strongly focused on climate zones with regularly applied irrigation. The duration of crop growth stages for winter wheat in a temperate humid climate is reported by Fischer et al. (2000). However, estimates of stage length are relative to the final harvest date, allowing stage length prediction only in the retrospective mode and, therefore, not straightforward applicable for irrigation purposes. Furthermore, these data are not variety specific and adjustments are necessary particularly for temperate climates, where crop development is highly variable due to interannual variability of temperatures during the pre- and post-winter growth period.

Local adjustments for stage duration and basal crop coefficients are expected to be more suitable for estimating ET and crop water demand than the use of published data for stage duration and K_c values (Bausch, 1995; Allen et al., 2011; Peireira et al., 2015). Such local adjustments are usually based on site-specific measurements or observations of crop growth and, consequently, vegetation index (VI) based approaches are increasingly recommended for irrigation management. The documented advantages of using VI-based crop coefficients are the ability to account for variations in plant growth due to local weather conditions, site-specific differences in sowing dates and seed densities, cultivars, pests and nutrient supply (Tasumi and Allen, 2007; Pôças et al., 2015; Hunsaker et al., 2007). The Normalized

Difference Vegetation Index (NDVI) is one of the most commonly used VI (Pinter et al., 2003) and several studies showed good correlations between the NDVI and plant growth parameters in wheat, e.g. biomass (Pinter et al., 2003), fraction of soil covered by plants (Er-Raki et al., 2007) and leaf area (Duchemin et al., 2006; Chattaraj et al., 2013).

This study aims to assess the suitability of using NDVI-based estimates of ET compared to tabulated values of K_c and stage length. To compare both approaches, post-winter growth of well-watered and fertilized winter wheat (*Triticum aestivum*, L.) was closely monitored and predicted ET was used to calculate a daily field water balance (FWB). The calculated FWB of both approaches was compared with the measured FWB in a 2-years field study. It was speculated that the NDVI-based estimate of ET_a was more realistic than estimates based on tabulated data. In order to quantify the effect of variation in stage length and K_c values on ET estimates, a sensitivity analysis was finally performed.

2. Materials and methods

The study was carried out on the experimental farm Hanninghof, Research Center for Crop Nutrition Hanninghof (IPU), Duermen, North-Rhine Westphalia, Germany (51° 50' 22" N latitude, 7° 15' 18.5" E longitude). Local average annual rainfall and temperature (1969-2012) are 888 mm and 9.9 °C, respectively. The post-winter growth period of winter wheat usually starts in March and wheat is harvested at the end of July to mid-August. During this period, rainfall is quite evenly distributed with April being the driest and July being the wettest months (Table 1). Temperatures and ET_o regularly increase from March to July.

The two experimental sites were 300 m apart from each other and had a similar soil texture (0-100 cm soil depth, n=4) with 86% ($\pm 2\%$) sand, 7% ($\pm 3\%$) silt and 7% ($\pm 1\%$) clay. Table 2 different site coefficients. The soil type is a Stagnosol brown earth with a usable field capacity (0-80 cm soil depth) of 128 l m⁻³ (Mueller, 2015).

The bread wheat variety *Inspiration* (Breun KG, Herzogenaurach, Germany) was drilled at 4 cm depth on Oct. 7 in 2013 and Oct 22 in 2014 with a row spacing of 11.5 cm and 330 seeds m⁻². All plots received the same doses of Patentkali (25% K, 6% Mg, 17% S), Triple-super-phosphate (20% P) and YaraVita Gramitel (150 g l⁻¹ Mg, 50 g l⁻¹ Cu, 150 g l⁻¹ Mn and 80 g l⁻¹ Zn) to ensure a sufficient supply of plants with P (51.5 kg ha⁻¹), K (112.5 kg ha⁻¹), Mg

(21.8 kg ha⁻¹), S (71 kg ha⁻¹), B (0.38 kg ha⁻¹), Fe (0.9 kg ha⁻¹), Mn and Zn (0.05 kg ha⁻¹). Nitrogen fertilizer (CAN, 27% N) was supplied at rates which established plant-available N amounts of 230 kg N ha⁻¹ in three split applications (100/90/40) at post-winter vegetation start (March 7 in 2014 and March 5 on 2015), at BBCH 31 (April 14 in 2014 and April 27 in 2015) and at BBCH 39 (May 8 in 2014 and May 26 in 2015). Directly plant-available mineral soil nitrogen (NO₃-N and NH₄-N extracted with 0.0125 M CaCl₂) at the post-winter vegetation start (33 and 30 kg N ha⁻¹ in 2014 and 2015, respectively, in 0-90 cm soil depth) was considered at the first N dressing. Weed and pest control were done according to best practice.

Table 1

Growing season rainfall and supplementary irrigation, average temperature and average daily reference evapotranspiration (ET_O) in 2014 and 2015, and long-term average (LTA).

	Rainfall (Irrigation) (mm)			Average temperature (°C)			Average daily ET _O (mm)		
	2014	2015	LTA ¹	2014	2015	LTA ¹	2014	2015	LTA ²
Mar.	20 (-)	78 (-)	68 ± 50	8.8	5.9	5.5 ± 2.0	2.6	2.2	2.1 ± 0.9
Apr.	33 (78)	40 (30)	57 ± 51	12.2	8.8	9.2 ± 1.8	3.1	2.9	3.6 ± 1.1
May	109 (72)	50 (72)	70 ± 48	12.6	12.2	13.8 ± 2.0	3.5	3.5	4.6 ± 1.9
Jun.	91 (90)	46 (96)	80 ± 46	16.3	15.5	16.6 ± 1.6	5.1	5.0	5.3 ± 1.8
Jul.	134 (-)	95 (12)	86 ± 50	20.2	19.1	18.5 ± 2.2	5.3	5.1	5.3 ± 1.9
Aug.	106 (-)	173 (-)	80 ± 52	16.5	19.4	18.2 ± 1.8	3.8	3.8	4.5 ± 1.4
Total	493 (240)	482 (210)	441 ± 50	14.4	13.4	13.6 ± 1.9	3.9	3.8	4.2 ± 1.5

¹ Weather station data (1969 – 2012) at Duermen, IPU

² Weather station data (2008 – 2015) at Duermen, IPU

The plots were located in a two-factorial split-plot design with four replicates with water as the main treatment factor and nitrogen as sub-factor. In this paper we present results of irrigated plots supplied with sufficient N. Only these plots (but not the water-or N-limited treatments) were suitable for a comparison of the dual-coefficient approach of ET estimation with either tabulated or measured basal crop coefficients (K_{cb} values). The size of the plots was 3 x 3 m with additionally 0.5 m border lines. Drip irrigation tubes (Netafim Ltd., Hatzerim, Israel) were installed in every second sowing row. Drippers were placed at a distance of 40 cm apart and supplied with 6 l m⁻² water per hour with a minimum operational water pressure of 0.08 MPa. The water deficit was calculated from the estimated daily crop water use according to FAO 56 (Allen et al., 2006). The water deficit was compensated by

irrigation (see Table 1 for amount of irrigation water applied). Notably, these high irrigation volumes are not representative for farm conditions, but were selected in order to avoid any water stress throughout the post-winter growth period.

The field water balance (FWB) was calculated according to FAO 56 (Allen et al., 2006) with the parameters listed in Table 2. Reference ET (ET_o) was calculated from climate data collected at an on-field weather station (Pessl Instruments GmbH, Weiz, Austria):

$$ET_o = \frac{0.408 * \Delta * (R_n - G) + \gamma * \frac{900}{(T + 273)} * u_2 * vpd}{\Delta * \gamma * (1 + 0.34 * u_2)} \quad (1),$$

where R_n is the net radiation at the crop surface ($MJ m^{-2} d^{-1}$), G is soil heat flux ($MJm^{-2} d^{-1}$); T and u_2 are air temperature ($^{\circ}C$) and wind speed ($m s^{-1}$) at 2 m height; vpd is the vapour pressure deficit (kPa); γ is the psychrometric constant; λ the latent vapor heat; and Δ the slope of vapour pressure deficit.

Table 2
Parameters used to calculate ET_a of winter wheat in 2014 and 2015 according to the FAO 56 approach (Allen et al., 2006). Data were either measured or taken from literature.

Parameter	Unit	Value	Source
Base temperature	$^{\circ}C$	0	Ewert 1996
$K_{cb \max}$	dim.less	1.15	Allen et al., 2006
Plant height to vegetation start	m	0.05	measured
Plant height during initial stage	m	0.1	measured
Final plant height	m	0.9	measured
Rooting depth at vegetation start after winter	m	0.3	measured
Maximum root depth in 2014, 2015	m	0.75, 0.9	measured
Field capacity in 2014 and 2015	Vol. %	23	Mueller et al., 2015
Water at wilting point in 2014, 2015	Vol. %	13,10	Mueller et al., 2015
Soil evaporations depth	m	0.15	Allen et al., 2006
Uncovered soil fraction at vegetation start	% / 100	0.95	Allen et al., 2006
Uncovered soil fraction at end of stem elongation	% / 100	0.05	Allen et al., 2006
Evaporations reduction factor	dim.less	0.55	Allen et al., 2006

Crop evapotranspiration (ET_a) was calculated from ET_o multiplied by a basal crop coefficient (K_{cb}) and a soil evaporation coefficient (K_e):

$$ET_a = ET_o * (K_{cb} + K_e) \quad (2)$$

We compared two different approaches of estimating basal crop coefficients (K_{cb}) and the lengths of crop developmental stages. On the one hand, we used a combined Fischer-Allen approach (FA approach) with stage duration of winter wheat in humid temperate climates taken from Fischer et al. (2000) who recommended calculating post-winter stage lengths retrospectively as percentages of the whole post-winter growth period. The initial, developmental, mid- and late-growth stages were, according to Fischer et al. (2000), assumed to take 10%, 30%, 35% and 25% of the entire post-winter growth period, respectively. K_{cb} values for the initial, mid- and end-phase of winter wheat were taken from Allen et al. (2006) and increase and decrease for K_{cb} values during the developmental and late stage were linearly approximated. On the other hand, K_{cb} values were derived from NDVI measurements according to a modified approach of Er-Raki et al. (2007):

$$K_{cb} = 1.15 \left[1 - \left(\frac{NDVI_{max} - NDVI}{NDVI_{max} - NDVI_{min}} \right) \right] \quad (3).$$

In contradiction to Er-Raki et al. (2007) who used 1.07 as $K_{cb,max}$ for durum wheat in a semiarid climate of Morocco we used a $K_{cb,max}$ of 1.15 for winter wheat (Allen et al., 2006). Er-Raki et al. (2007) used an exponent, which was derived from experimental analyses of the relationships between NDVI and LAI and ET_a/ET_o and LAI (see Duchemin et al., 2006 for further details). This exponent was not used in this study to quantify the relationship between LAI and ET_a/ET_o as lysimeter studies are rarely available (Kang et al., 2003). $NDVI_{max}$ and the $NDVI_{min}$ were the maximal and minimal measured NDVI-values during the growing period. We took an initial K_{cb} -value of 0.15 (Allen et al., 2006) at the vegetation start (DOY 70 in 2014 and DOY 65 in 2015) and integrated it linearly until the first K_{cb} -values were derived from the NDVI-measurements (DOY 83 in 2014 and DOY 69 in 2015). The NDVI was measured at least weekly with a MMS1-handheld spectrometer (tec5 AG, Oberursel, Germany) in four replicates per plot with a perpendicular view angle. Measurements were conducted at a distance of approximately 1.8 m from canopy height with a minimum solar altitude of 35°. To consider possible shading effects measurements were conducted from each of the four cardinal directions of the plot corners and the four data were pooled. The spectrometer measured wavelengths (R) from 400 nm to 1000 nm in 10 nm increments. The NDVI was calculated according to Rouse et al. (1974):

$$\text{NDVI} = (\text{R } 800 - \text{R } 670) / (\text{R } 800 + \text{R } 670) \quad (4)$$

The leaf area was non-destructively measured almost weekly during the post-winter growth period with a Sun Scan SS1 (Delta-T Devices Ltd., Cambridge, Great Britain). In order to check the validity of Sun Scan SS1 based LAI estimates, 31 wheat samples from 0.5 m² ground surface were harvested during the growth period from BBCH 23 to BBCH 39 (DOY 65-140 in 2015, DOY 78-112 in 2014) and aboveground samples were separated into leaves and stems. The leaf area meter LI3000 A (LI-COR®, Lincoln, Nebraska USA) was used to measure the leaf area of the plant samples.

K_{cb} values of both approaches (FA or NDVI), were daily adjusted to wind speed at 2 m height (u_2), RH and plant height according to Eq. (5).

$$K_{cbadj.} = K_{cb} + [0.04 (u_2 - 2) - 0.004 (RH_{min} - 45)] (h/3)^{0.3} \quad (5)$$

RH_{min} is the daily minimum relative humidity (%) and h is the plant height (m).

K_e in the FA-approach was calculated according to FAO 56 (Allen et al., 2006):

$$K_e = K_r (K_{cmax} - K_{cb}) \leq f_{ew} K_{cmax} \quad (6),$$

K_{cmax} is the maximum value of K_c following rainfall or irrigation. K_r is an evaporation reduction coefficient depending on the cumulative depth of water evaporated from the topsoil. K_r was calculated according to Eq. 74 of the FAO 56 guidelines (Allen et al., 2006) for stage 1, as plots were frequently wetted by rainfall or irrigation (≈ 2.5 days) in order to avoid any water limitations. f_{ew} is the fraction of soil that is exposed to evaporation.

K_e in the NDVI-approach was calculated according to Er-Raki et al. (2007):

$$K_e = 0.9 * (1 - f_c) \quad (7)$$

The value 0.9 was determined according to Fig. 29 of FAO 56 (Allen et al., 2006) based on the observed frequency of irrigation and rainfall. From the post-winter vegetation start until the $NDVI_{max}$ (DOY 70-113 in 2014 and DOY 66-128 in 2015) 20 and 27 irrigation/rainfall events of $>0.2 * ET_0$ occurred (for relevant rainfall events, see Allen et al. 2006, p. 153). This was equivalent to rainfall events every 2.5 days. With an average ET_0 of 3.3 mm/day during

the growing season this corresponds to a coefficient of 0.9. Fraction of soil covered by plants is determined as f_c .

Vegetation cover (f_c) was calculated according to Er-Raki et al. (2007):

$$f_c = 1.18 * (NDVI - NDVI_{min}) \quad (8)$$

Volumetric soil moisture content was measured with FDR soil moisture probes (Sentek®, Stepney, Australia). In the center of two of the four experimental plots, tubes were installed and the soil water content hourly logged at five soil depths (10, 30, 50, 70, 90 cm) from DOY 69 in 2014 and 2015 (BBCH 21) to harvest. The measured FWB was compared with estimates of the FWB derived from the FA and NDVI approach. Relevant parameters of these FAO 56 calculations are summarized in Table 2. The initial values of the calculated FWB at DOY 69 in 2014 and DOY 69 in 2015 were matched with the measured value of the FWB on those days (183 l m^{-3} and 186 l m^{-3}).

A core area of 4 m^2 was harvested from each plot on July 2 and August 2 in 2014 and 2015, respectively and grain yield determined after drying samples at 60°C to constant weight. The grain yield was reported with 14% residual water content. The agronomic water-use efficiency was calculated from the grain yield (with 0% residual water content) and cumulative ET from post-winter vegetation start to maturity. Biomass water-use-efficiency was calculated from harvested biomass (0% residual water content) and cumulative ET from post-winter vegetation start to maturity.

The relationship between non-destructive and destructive LAI measurements was analyzed with linear regression and the relationship between the LAI and the NDVI with a quadratic-linear plateau function. Regression analyses were carried out with R (R, Core Team, 2015). The suitability of predicted compared to measured dynamics of FWB was tested with root mean square error (RMSE) and efficiency (Eff.) (Chai and Draxler, 2014; Nash and Sutcliffe, 2010). RSME quantifies the variance of error, while Eff. is a normalized statistic determining the relative magnitude of the residual variance compared to the measured data variance. The target value for Eff. is 1.0, while zero or negative values indicate that even the arithmetic mean of all observations is as good predictor as the model.

3. Results

According to the modified concept of Er-Raki et al. (2007), the start of the mid-stage with an K_{cb} value of 1.15 is reached at canopy closure (see Eq. 3). NDVI values at canopy closure were 0.95 in 2014 and 2015. Pooled over both years, the corresponding LAI, derived from a quadratic-linear plateau regression (Fig. 1a), was 2.61. The LAI derived from Sun Scan SS1 and leaf area meter estimates from destructive sampling were highly positively correlated (Fig. 1b) but exhibited an off-set with lower LAI estimates of the non-destructive method (SunScan SS1) compared to the destructive method.

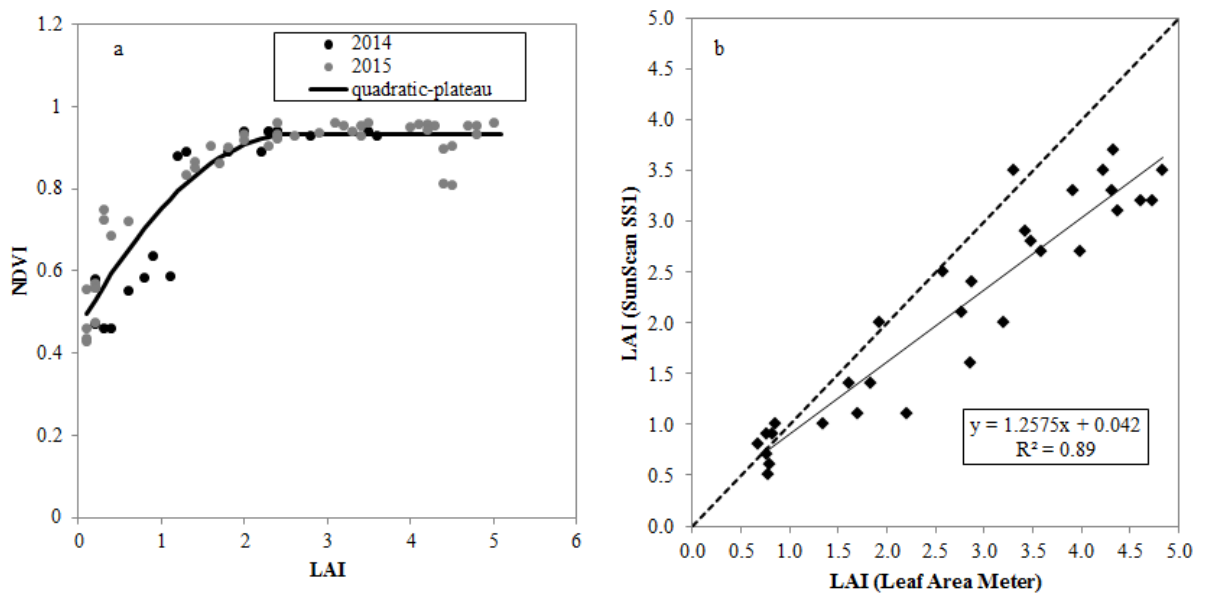


Fig. 1. (a) Relationship between measured leaf area index (LAI) and NDVI in 2014 (black circles) and 2015 (grey circles) derived from plots with no water limitation and supplied with 230 kg N ha⁻¹. (b) Regression between LAI derived from destructive sampling (Leaf Area Meter) and non-destructive field measurements (Sun Scan SS1). 1:1 line broken.

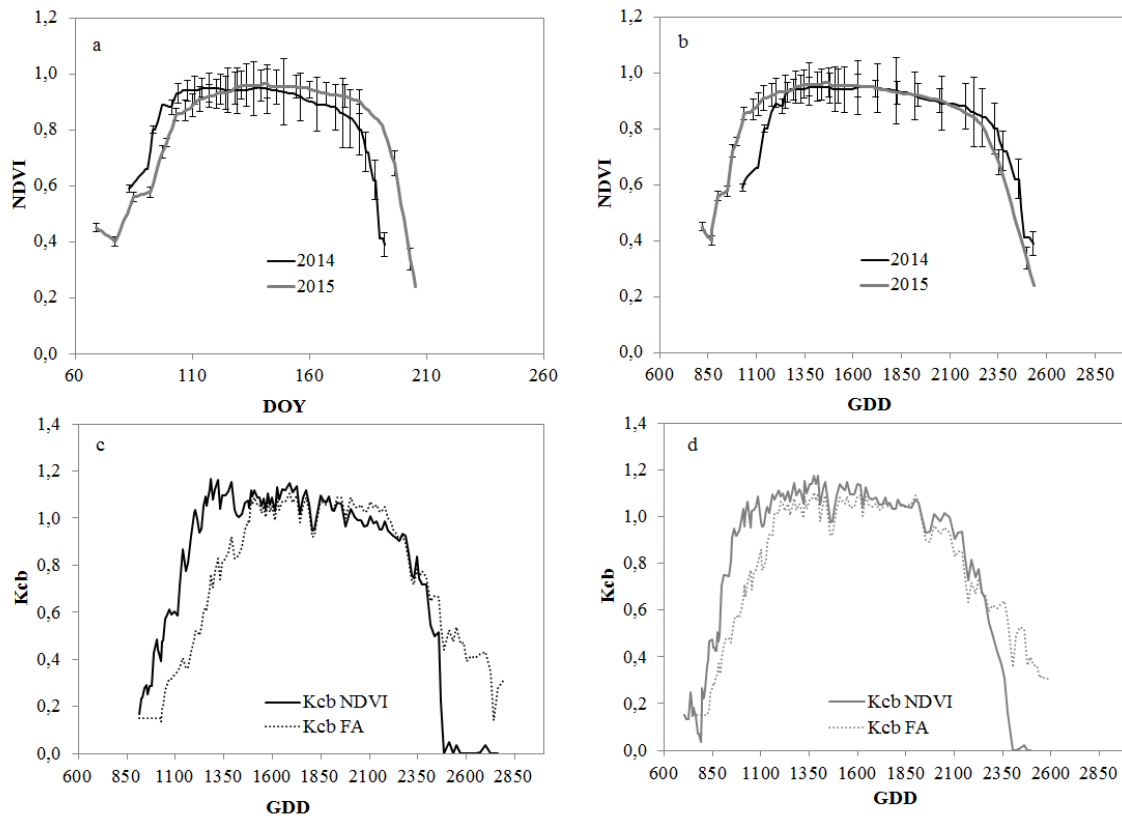


Fig. 2. Measured Normalized Difference Vegetation Index (NDVI) in 2014 and 2015 (a) based on Day of Year (DOY) and (b) based on Growing Degree Days (GDD; °C). Post-winter seasonal dynamics of K_{cb} values according to the FA approach (K_{cb} FA) and with K_{cb} values derived from measured NDVI (K_{cb} NDVI) in (c) 2014 and (d) 2015.

Seasonal dynamics of NDVI, expressed on a DOY basis, indicated an earlier crop development and beginning of senescence in 2014 compare to 2015 (Fig. 2a), while the dynamics were more synchronal when expressed on a GDD basis (Fig. 2b). For example canopy closure was reached at DOY 102 in 2014 and 10 days later (DOY 112) in 2015, while a GDD of 1185°C at canopy closure in 2015 was very similar to that in 2014 (1244 GDD).

Compared with the FA approach, which uses fixed values of stage lengths and tabulated K_{cb} values with linear extrapolations during developmental and senescence stage, K_{cb} values derived from NDVI measurements reached the mid-stage substantially earlier and exhibited a steeper declining slope towards maturity (Fig. 2c,d). These differences in K_{cb} estimate between the FA and NDVI approach, however, did not result in substantial differences in ET_a (Fig. 3). Comparing cumulative ET_a during relevant phenological growth stages of winter wheat, NDVI-based estimates of ET_a were slightly higher than FA-based estimates during the early post-winter growth stage (vegetation start until begin booting) in 2014 and 2015. FA-based estimates during the late growth stages (ripening) were higher in both years compared

to NDVI-based estimates, while for other growth stages no (2014) or small differences (2015) occurred.

Estimated cumulative transpiration of the FA-approach was in both years only 12 mm and 31 mm lower compared to the NDVI-approach (Table 3) and estimated ET_a of the FA approach was 26 mm higher in 2014 and 33 mm lower in 2015 than with the NDVI approach.

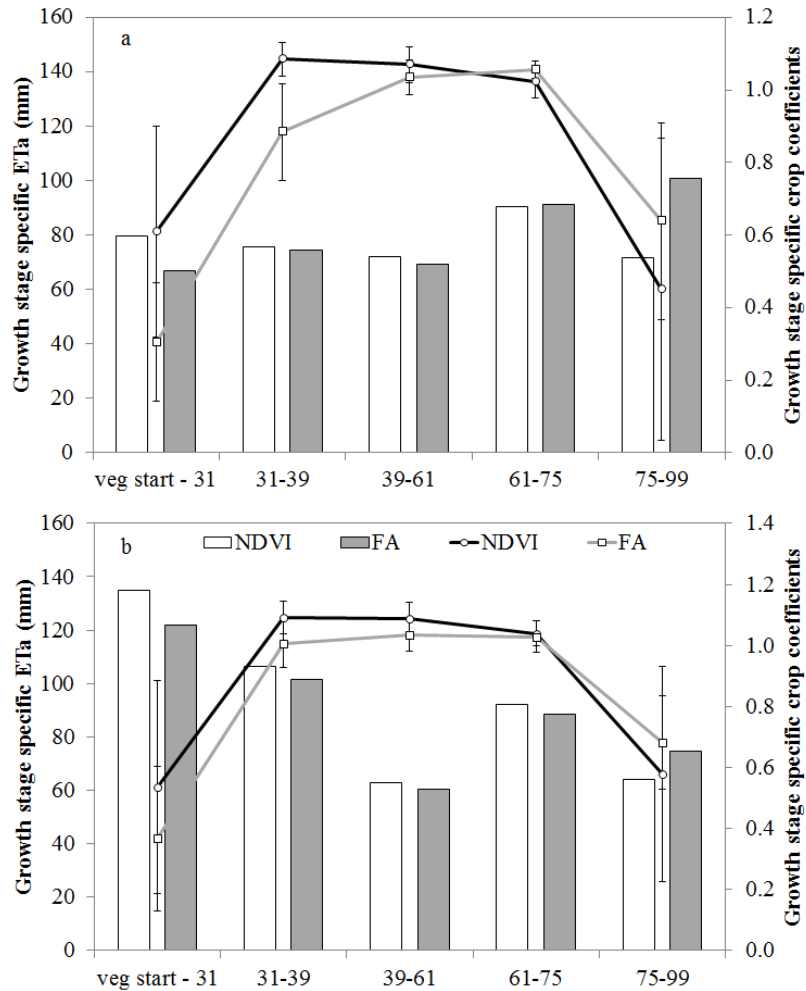


Fig. 3. Growth-stage specific estimates of ET_a and K_{cb} values derived from measured NDVI and according to the FA approach in 2014 (a) and 2015 (b). The x-axis indicates phenological stages: veg start: post winter vegetation start, begin of booting (31), end of booting (39), begin flowering (61), milk stage (75) and maturity (99).

Estimated evaporation of the NDVI approach was higher in 2015 compared to 2014. Both approaches estimated substantially higher drainage in 2014 compared to 2015. Almost half of the drainage could be explained by heavy rainfall events (RF) in 2014: 59 mm RF (DOY 128-135), 30 mm RF (DOY 143) and 35 mm RF (DOY 146-148) resulted in drainage estimates of 38 mm, 12 mm and 20 mm, respectively. Agronomic (WUE_Y) and biomass water use efficiencies (WUE_B) were similar between both years for the FA and NDVI approaches.

Grain yields were nearly almost the same in both years, while in 2015 the straw yield was 0.6 t ha⁻¹ higher than in 2014.

The FWB of irrigated plots exhibited a similar dynamic with increases in water availability from post-winter vegetation start until a GDD of 1.500°C and minor fluctuations (between 190 and 230 mm) of the FWB during the rest of the season (Fig. 4) in both years. Suitability of predicting FWB with the FA and NDVI approaches depended on the growth stages considered (Table 4). Both approaches were able to predict the dynamics of FWB during the early post-winter growth period, while, as indicated by goodness-of-fit parameter Eff, the dynamics of FWB were not well predicted during booting (growth stage 31-39). The water demand of wheat from end of booting to flowering (growth stage 39-61) was well predicted by both approaches in 2014, but not in 2015. Taking Eff as an indicator, the NDVI approach tended to be a better predictor of ET than the FA approach in seven of the ten growth periods of 2014 and 2015 (Table 4).

Table 3

NDVI and FA-approach based estimates of cumulative transpiration (T), evaporation (E), evapotranspiration (ET_a), and drainage (D) during the growth periods 2014 (DOY 70-205) and 2015 (DOY 65-214) and grain yield (Yield; 14% residual water content), shoot biomass (Biomass; 0% residual water content) and agronomic (WUE_Y) and biomass water-use efficiencies (WUE_B). WUE was calculated with yield and total aboveground biomass with 0% residual water content. WUE_{Y,w} and WUE_{B,w} are weighted by average VPD during the post-winter growth period.

	2014		2015	
	NDVI ±	FA	NDVI ±	FA
T [mm]	339 ± 6.8	327	381 ± 5.4	350
E [mm]	38 ± 0.8	76	82 ± 2.4	80
ET _a [mm]	377 ± 6.1	403	463 ± 3.1	430
D [mm]	197 ± 6.3	175	62 ± 2.9	60
WUE _Y [g l ⁻¹]	2.76 ± 0.1	2.22	1.93 ± 0.2	2.08
WUE _B [g l ⁻¹]	4.83 ± 0.2	4.52	3.94 ± 0.3	4.33
WUE _{Y,w} [g kPa l ⁻¹]	1.42 ± 0.1	1.46	1.03 ± 0.1	1.11
WUE _{B,w} [g kPa l ⁻¹]	2.90 ± 0.1	2.98	2.09 ± 0.1	2.30
Yield [t ha ⁻¹]	10.4 ± 0.4		10.5 ± 0.5	
Biomass [t ha ⁻¹]	18.2 ± 0.7		18.8 ± 1.4	

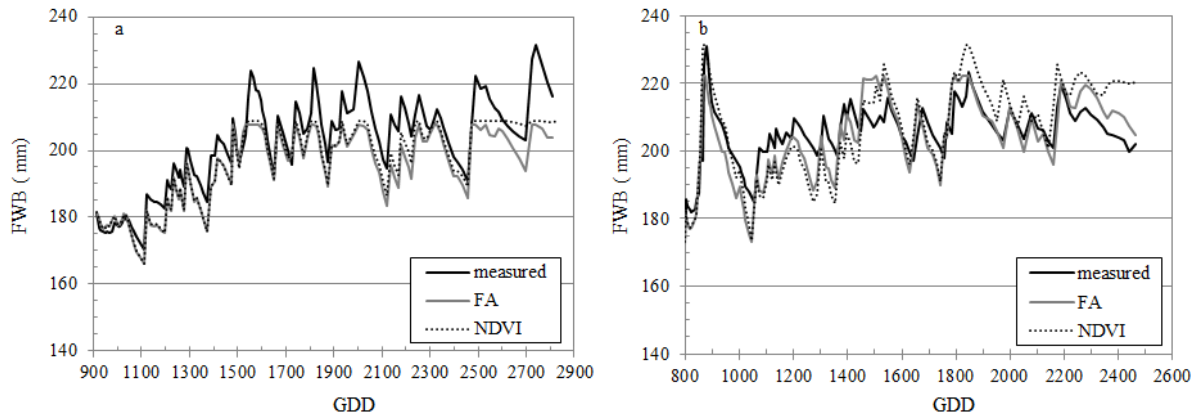


Fig. 4. Measured and simulated (FA and NDVI approach) field water balance (FWB) during the post-winter growth periods of 2014 (a) and 2015 (b). Seasonal dynamics are expressed on the growing-degree days (GDD, °C) basis.

Table 4

Root mean square error (RMSE; mm day⁻¹) and efficiency (Eff.; dim.less) of simulated (NDVI-Approach and FA-Approach) soil water content in 2014 and 2015 during several growth stages (BBCH code) of winter wheat. For BBCH codes, see Fig. 3.

Growth period	Statistical parameter	2014		2015	
		NDVI	FA	NDVI	FA
V.S. ¹ – 31	RMSE	0.7	0.7	1.1	1.1
	Eff.	0.96	0.95	0.87	0.79
31 – 39	RMSE	1.3	1.3	1.8	1.5
	Eff.	0.29	0.28	-0.21	-2.10
39 – 61	RMSE	1.6	1.8	2.4	2.3
	Eff.	0.68	0.60	-2.63	-1.15
61 – 75	RMSE	2.2	2.5	1.7	1.4
	Eff.	0.40	0.19	-0.60	0.49
75 – 99	RMSE	1.4	1.8	1.8	1.2
	Eff.	0.58	0.33	-0.57	0.22
V.S. ¹ – 99	RMSE	0.6	0.7	0.8	0.6
	Eff.	0.76	0.67	0.36	0.56

¹ V.S.: Post-winter vegetation start

A sensitivity analysis with relative changes of $\pm 30\%$ for the developmental stage length and basal K_c values indicated that cumulative ET estimates were affected to a different extent (Fig. 5).

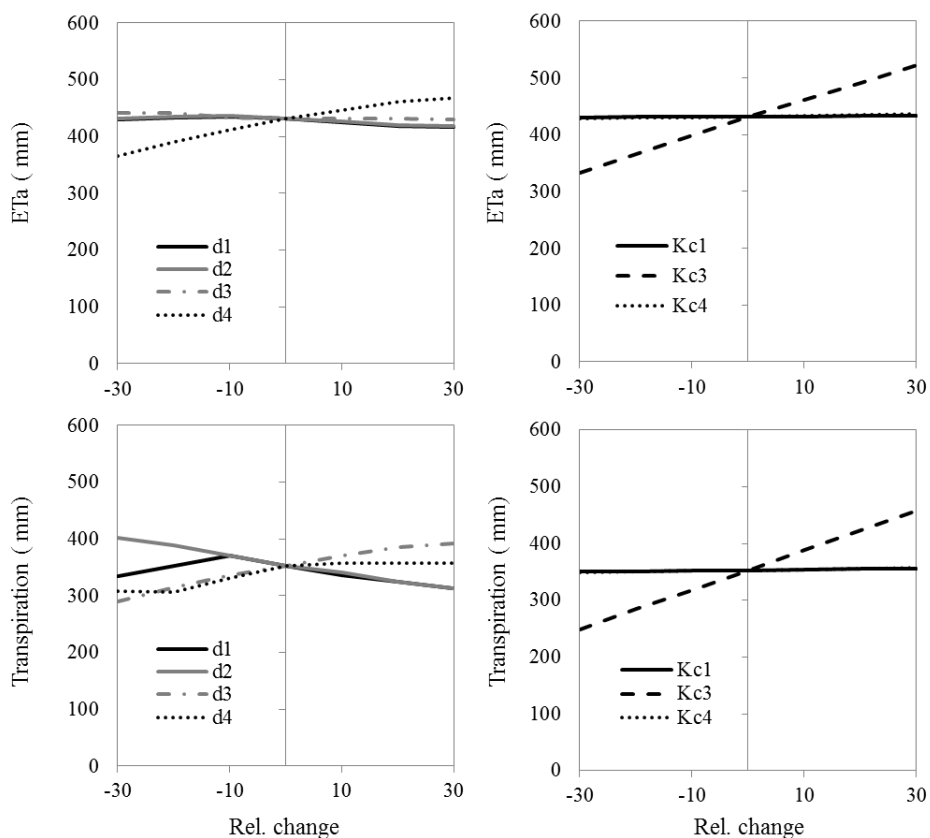


Fig. 5. Effect of relative changes (Rel. change) of stage lengths (d1 to d4, days) and basal crop coefficients (Kc1 to Kc3, dim. less) on estimates of cumulative crop evapotranspiration (ET_a, mm) and cumulative transpiration illustrated for the FA approach and the climate of 2014.

Changes of stage length d4 (senescence phase) and Kc3 (mid-stage) affected ET_a, while ET_a was almost unaffected by changes of duration of other stage lengths and basal crop coefficients Kc1 and Kc4. Transpiration reacted more sensitively to changes in stage duration than ET_a. The FA approach inherently assumes a fixed length of total post-winter growth period, e.g., a shorter duration of d2 (booting stage) implies a longer duration of d3 (full canopy cover). Therefore, shorter duration of phases d1 and d2 increased transpiration estimates. Similarly, longer duration of d3 increased transpiration as d4 was proportionally shorter. The effects of variation of basal crop coefficients on transpiration were similar to those for ET_a with relative changes of Kc3 having a relevant impact on transpiration estimates.

4. Discussion

The FAO56 dual coefficient approach is widely used for irrigation management and estimates of crop water use (Allen et al., 2011; Peireira et al., 2015) and local adjustments of stage

duration and basal crop coefficients are recommended (Ko et al., 2009; Allen et al., 2011). Consequently, site-specific measurements or observations of crop growth are expected to be more suitable for estimating ET and crop water demand than the application of published data of stage duration and K_c values. In the present study the suitability of estimating ET_a using the NDVI compared to the use of tabulated K_c values and stage length information (FA approach) was tested in well-watered and fertilized winter wheat.

The basal crop coefficients of the FA and NDVI approach exhibited differences during the post-winter growth period (Fig. 2). The NDVI approach resulted in higher K_{cb} estimates during the early growth stage and an extended mid-stage (Fig. 3). These differences in NDVI, however, translated into only moderately higher estimates of transpiration compared with the FA approach (Table 3). This conclusion of higher transpiration inherently assumes i) that NDVI, or potentially other VIs, are reliably monitoring *in-situ* crop growth and ii) that crop growth and transpiration are closely and linearly correlated.

Information about *in-situ* crop growth is required for estimating temporal dynamics of ground cover (see Eq. 6) and optical methods are increasingly used for this purpose (López-Urrea et al., 2009; Nielsen et al., 2012). A ground cover of 80% is assumed to indicate the transition point between the developmental and mid-stage in the FAO56 approach (Allen et al., 2006). The most suitable proxy of ground cover for agricultural crops is LAI, which was measured destructively and non-destructively (Fig. 1b) and could be predicted by the NDVI as indicated by the quadratic-plateau relationship between both parameters (Fig. 1a). We found a plateau value of the NDVI 0.93 with a corresponding LAI of 2.61 at the beginning of the plateau-stage. Non-destructively measured LAI (Fig. 1b) was underestimated compared to the destructively measured leaf area by approximately 0.5 as the upward view of the sensor-bar did not allow the LAI measurement of wheat plants until a plant height of 0.1 m was reached. Due to this, the NDVI-plateau was likely reached at a LAI of around 3.1. This estimate is similar to the findings of Duchemin et al. (2006) and Chattaraj et al. (2013), who found the NDVI in wheat to saturate when LAI was >3.5 .

In the FAO56 approach, temporal dynamics of ground cover are predicted by linear extrapolation between vegetation start ($>10\%$ ground cover) to the mid stage (see Table 2), while NDVI allows for site-specific consideration of crop growth. This is relevant as the growth of winter wheat in temperate-humid climate exhibits a large year-to-year variability

due to potential frost damage during winter and variable onset of growth after winter. Tabulated data of stage lengths are, therefore, difficult to apply under these conditions as time of cardinal events (canopy closure, onset of senescence, maturity) was variable (Fig. 2a) and the approach of Fischer et al. (2000) in which fixed portions of the total post-winter growth period are allocated to developmental stages cannot be used for irrigation as it relies on information about final harvest date. In summary, the NDVI appears as a promising monitoring parameter for crop development during the growth period. Furthermore, it gives the opportunity to calculate online and not retrospectively like the FA-approach crop water use and water demand under non-optimal growth conditions such as water or nutrient stress.

The assumption of linearity between NDVI and transpiration is based on evidence of linear correlations between NDVI and cumulative ET estimates in cross-site analyses as summarized in e.g. Glenn et al. (2007). The largest differences between K_{cb} values of the NDVI and FA approach were observed during the post-winter vegetation start and during ripening (Fig. 3). However, our sensitivity analysis indicated that changes in $Kc1$ and $Kc4$ had only a small impact on estimated transpiration (Fig. 5). Changes in crop coefficients, e.g. through pests or diseases of leaves or generally reduced growth by a lack of nutrients, is not taken into account by the FA-approach in contrast to the NDVI-approach. Er-Raki et al. (2010) showed the importance of an accurate estimate of the $Kc3$ value when analyzing effects of sowing data and the development of the vegetation on ET. Satti et al. (2004) showed a 15% change in irrigation requirements when changing K_c -values by 10%. Both, ET and T responded sensitively to changes in K_{cb} values during the mid-stage. We found that the $Kc3$ -value had a relevant impact on ET and T estimates (Fig. 5). For example, a decrease of 10% in the $NDVI_{max}$ (from 0.95 to 0.85) resulted in a decrease of transpiration by 35 mm. From the sensitivity analysis it can be concluded that i) reliable information about stage duration (ideally derived directly from the NDVI), and ii) estimation of $Kc3$ (mid stage) are crucial for accurate estimation of actual crop water use.

ET and T reacted sensitively to changes in stage duration (Fig. 5). The lengths of stage durations of winter wheat are defined by photoperiod and temperature and can be successfully predicted for NW European climates (e.g., Ewert 1996). The use of tabulated data of stage lengths (FA approach) is recommended if no site-specific information is available or required (such as in national water footprint accounting, e.g. Chapagain et al., 2004), while the NDVI-based estimates of K_{cb} do not require stage lengths and are, therefore, closer to year- and site-

specific crop growth. The post-winter growth of winter wheat is difficult to predict in terms of the onset of growth and canopy cover both of which are crucial information for ET estimated during the initial growth stage (K_c1). These particular environmental conditions, with mild to very cold winter seasons and potential frost damage during spring, renders the application of tabulated stage lengths and K_c values and clearly indicated that optical information such as NDVI should be preferred.

Winter wheat is not regularly irrigated in NW-European climate, although transient droughts occur and irrigation would be profitable on light-textured soils (El Chami et al., 2015). NDVI-based estimates of seasonal crop water use indicate rather small differences with 26 mm less in 2014 and an additional water demand of 33 mm in 2015 compared with the FA approach (Table 3). This can be expected as, according to Eq. 6, increases in K_{cb} inevitably result in lower values of the soil coefficient K_e , if, under irrigated conditions, $K_r=1$. Similarly, Caviglia and Sadras (2001) reported that expansion of LAI increased transpiration and reduced evaporation while ET remained almost constant. The small differences in ET estimates between the NDVI and FA approach in this study can therefore be explained by the fact that soil evaporation was high in this humid climate and well-watered plots. Differences between both approaches are expected to be more pronounced in semiarid and arid environments.

Both approaches of seasonal ET dynamics agreed with the measured FWB data (Fig. 4, Table 4) and our results are in line with Sanchez et al. (2012), who compared NDVI-derived K_{cb} -values with measured soil moisture. Pooled over the post-winter growth period, RMSE and Eff. varied from 0.6-0.8 and 0.36-0.76, respectively. However, the observed FWB compared to modelled FWB deviated substantially during the growth periods BBCH 39-61 and BBCH 61-75 in 2015. Discrepancies between measured and modelled data can be partially explained by systematic offsets between calculated (FA- and NDVI-approach) and measured FWB. For short time periods (after bigger rainfall events, e.g. in 2014), the measured soil water content was higher than the total available water (TAW) and soils drained back to 'realistic' TAWs. Differences in 2015, however could not be explained by high rainfall, but indicate systematic off-sets with an overestimation of ET during early growth stage (900-1000 GDD) and an underestimation of ET at the later growth stages (1400-1500 GDD and 1800-2000 GDD). This either indicates that the NDVI or tabulated data are not reliable when considering specific growth stages or that site-specific hydrology of the

experimental site (e.g. capillary rise) could not be properly considered in the simple FWB calculation approach of FAO56.

Estimated ET of the two years varied between 377 and 463 mm and, related to grain yield, gave agronomic water-use efficiencies (WUE_y) between 1.93 and 2.76 g litre⁻¹ (Table 3). These estimates are high, but not unrealistic compared with estimates of WUE_y of winter wheat from a global survey (Zwart and Bastiaanssen, 2004). These results indicate that winter wheat, not limited by water and nutrients (and well protected from diseases) was very efficient in water use. We speculated that year differences in WUE_y could be explained by climate differences as the same variety and sowing density was used in both years. However, normalization by post-winter growing season average VPD did not result in similar values of WUE_y in both years. Furthermore, consideration of vegetative biomass (leaves and stems), which was higher in 2015, did not explain year differences (as indicated by differences in $WUE_{B,w}$). From this data, it can be concluded that season-averaged VPD is not a suitable normalization factor.

5. Conclusions

To compare the suitability of tabulated K_c -values and K_c -values which derived from remote sensing measurements in terms of accuracy estimating crop water use a two year field study was established. These two approaches worked in almost the same quality regarding the estimated ET_a . Tabulated values can only be used retrospectively. An additional benefit of remote sensed K_c -values and stage duration is that these measurements take all crop growth influenced parameters (weather, pests, lack of nutrients) into account. With the remote sensed approach there are more application possibilities to quantify crop water use than with tabulated K_c -values and stage duration.

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Chapter 2 NDVI-based estimates of evapotranspiration of winter wheat indicate positive effects of N fertilizer application on agronomic water-use efficiency

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Abstract

Biomass production is positively correlated with transpiration and yield increase by fertilizer application or other yield-increasing measures necessarily impose higher crop water use. However, yields of wheat, rice and maize, in a global survey, were shown to be positively correlated with agronomic water-use efficiency (WUE_Y) (Zwart and Bastiaansen, 2004). To quantify these effects of N fertilizer supply on evapotranspiration (ET) of winter wheat, a field experiment with three nitrogen rates (N0, N120 and N230) was performed during 2014 and 2015. Plots were watered when required to avoid water limitations. Normalized Difference Vegetation Index (NDVI) was used to derive crop coefficients which were used to calculate ET with the dual-coefficient approach. The extent to which N supply modified bare soil evaporation (E), transpiration (Tr), ET, grain yield, aboveground biomass and harvest index (HI) was tested. Bare soil evaporation during the early post-winter growth period was measured with micro-lysimeters and compared with two model estimates of E. It was speculated that E increases under conditions of low N supply, as canopy cover is less complete and that WUE_Y increases partially due to increases in HI. N supply resulted in lower cumulative E and model predictions of E agreed reasonably with measured rates and cumulative E. N application increased grain yield more than ET resulting in a higher WUE_Y . HI of N120 was higher than that of N230 indicating that HI was not the main reason of higher WUE_Y . It is concluded that estimates of ET under variable N supply requires consideration of N-induced effects on canopy development which were successfully monitored by NDVI

measurements. It is concluded that N supply related increases of both transpiration and WUE_Y potentially impose a trade-off between water conservation and efficiency of water use for crop production.

Introduction

About 70% of global freshwater use is allocated to irrigation (Siebert et al. 2010) and increasing food demand puts further pressure on land and water resources (FAO 2011, UNEP 2014). An analysis of present and future crop water use in consideration of environmental water demand illustrated the importance of improved water management to enhance food production and secure both food security and regional water resources (Molden et al. 2007). Such water-wise management of crops requires reliable estimates of crop water demand which have been developed during the last four decades (Doorenbos and Pruitt 1971, Allen et al. 2006).

Optically derived estimates of crop growth parameters, such as NDVI, are increasingly used for estimating crop water use and water stress related yield depression (Jongschaap and Schouten 2005) and site-specific irrigation (Glenn et al. 2007, Er-Raki et al. 2007, Jayanthi et al. 2007, López-Urrea et al. 2009, Kamble et al. 2013). A previous study indicated that, under sufficient water and nutrient supply, NDVI-based estimates of evapotranspiration (ET) were comparable to estimates using tabulated crop coefficients and stage lengths for winter wheat in NW Europe (Chapter 1). Estimates of crop coefficients derived from NDVI are possible due to the generally close relationship between vegetation cover and crop canopy size. Nutrient deficiency has a pronounced effect on crop canopy development and growth rate. In this case, optically derived information about canopy cover is a suitable option to calculate crop water use as tabulated data of stage length and crop coefficients implicitly assume that crops grow without nutrient limitations (Allen et al. 2011). Hunsaker et al. (2005) demonstrated that remotely-sensed NDVI values enable the determination of real-time K_{cb} and crop evapotranspiration of wheat. Furthermore these authors illustrated that length of growth stages as well as the crop coefficient during the mid-stage were affected by different N management of wheat and that NDVI-derived crop coefficients were more suitable than tabulated values for estimating ET (Hunsaker et al. 2007).

Biomass production is positively correlated with transpiration (Steduto et al. 2007) and yield increases by fertilizer application or other yield-increasing measures necessarily impose higher crop water use (Hunsaker et al. 2007, Liu et al. 2015). However, in a global survey it was demonstrated that yield of wheat, rice and maize are positively correlated with agronomic water-use efficiency (Zwart and Bastiaansen, 2004), indicating that low-productive sites are less water-use efficient when expressed as grain yield per unit of evapotranspiration (WUE_y). Several studies showed that N supply increased WUE_y (Garabet et al. 1988, Anderson 1992, Zhang et al. 1998, Caviglia and Sadras 2001, Wang et al. 2013). N supply induced increases of both transpiration and WUE_y indicating that N fertilizer application imposes a potential trade-off between water conservation and efficiency of water use for crop production.

Positive N effects on WUE_y of wheat can be explained by four hypotheses: i) reduced bare soil evaporation due to more rapid canopy closure (Ritchie 1972), ii) increase in harvest index (Barraclough et al. 2010), iii) increases in carbon gain per unit transpiration (transpirational WUE) (Brueck 2008), and iv) a smaller fraction of carbon allocation to the root system (Poorter and Nagel 2000). We focused on hypothesis i) and ii) as hypothesis iii) and iv) are difficult to quantify over extended time periods under field conditions.

Assessments of N application effects on WUE_y of field-grown wheat rely on estimates of ET which have been performed in arid and semiarid environments (see references above) but rarely in humid temperate climate (Klapp 1962). In the present study, we investigated N supply effects on WUE_y and aimed at separating ET into transpiration and evaporation. We used NDVI as a proxy to quantify temporal dynamics of canopy development and basal crop coefficients and used the FAO 56 dual coefficient approach to estimate daily rates and cumulative water consumption, separated into transpiration and evaporation. Cumulative ET was used to calculate WUE_y and biomass WUE (WUE_B) as ET was expected to be more closely correlated with aboveground biomass than with grain yield. Finally, temporal dynamics of biomass increase until end of booting stage were derived from measurements of the water index (Penuelas et al. 1997). These readings, in conjunction with leaf area index measurements, allowed us to compare the FAO 56 approach with a method which derives at transpiration and evaporation rate estimates independently from the crop coefficient approach of FAO 56.

Table 1: Growing season rainfall and supplementary irrigation, average temperature and average daily reference evapotranspiration (ET_O) in 2014 and 2015, and long-term average (LTA).

	Rainfall (Irrigation) (mm)			Temperature (°C)			Daily ET _O (mm)		
	2014	2015	LTA ¹	2014	2015	LTA ¹	2014	2015	LTA ²
Mar.	20 (-)	78 (-)	68 ± 50	8.8	5.9	5.5 ± 2.0	2.6	2.2	2.1 ± 0.9
Apr.	33 (78)	40 (30)	57 ± 51	12.2	8.8	9.2 ± 1.8	3.1	2.9	3.6 ± 1.1
May	109 (72)	50 (72)	70 ± 48	12.6	12.2	13.8 ± 2.0	3.5	3.5	4.6 ± 1.9
Jun.	91 (90)	46 (96)	80 ± 46	16.3	15.5	16.6 ± 1.6	5.1	5.0	5.3 ± 1.8
Jul.	134 (-)	95 (12)	86 ± 50	20.2	19.1	18.5 ± 2.2	5.3	5.1	5.3 ± 1.9
Aug.	106 (-)	173 (-)	80 ± 52	16.5	19.4	18.2 ± 1.8	3.8	3.8	4.5 ± 1.4
Total	493 (240)	482 (210)	441 ± 50	14.4	13.4	13.6 ± 1.9	3.9	3.8	4.2 ± 1.5

¹ Weather station data (1969 – 2012) at Duermen, IPU

² Weather station data (2008 – 2015) at Duermen, IPU

Table 2: Parameters used to calculate ET_a of winter wheat in 2014 and 2015 according to the FAO 56 approach (Allen et al. 2006). Data were either measured (meas.) or from literature.

Parameter	Unit	Value	Source
Base temperature	°C	0	Ewert 1996
K_{cb max}	dim.less	1.15	Allen et al., 2006
Plant height to vegetation start	m	0.05	measured
Plant height during initial stage	m	0.1	measured
Final plant height	m	0.9	measured
Rooting depth at vegetation start after winter	m	0.3	measured
Maximum root depth in 2014, 2015	m	0.75, 0.9	measured
Field capacity in 2014 and 2015	Vol. %	23	Mueller et al., 2015
Water at wilting point in 2014, 2015	Vol. %	13,10	Mueller et al., 2015
Soil evaporations depth	m	0.15	Allen et al., 2006
Uncovered soil fraction at vegetation start	% / 100	0.95	Allen et al., 2006
Uncovered soil fraction at end of stem elongation	% / 100	0.05	Allen et al., 2006
Evaporations reduction factor	dim.less	0.55	Allen et al., 2006

Materials and methods

The study was carried out on the experimental farm Hanninghof, Research Center for Crop Nutrition Hanninghof (IPU), Duellmen, North-Rhine Westphalia, Germany (51° 50` 22`` N latitude, 7° 15` 18.5`` E longitude). Average local annual rainfall and temperature (1969-2012) are 888 mm and 9.9 °C, respectively. The post-winter growth period of winter wheat usually starts in March and wheat is harvested at the end of July to mid-August. During this period, rainfall is quite evenly distributed with April being the driest and July being the wettest months (Table 1). Temperatures and ET_o increase from March to July.

Average soil texture of the Stagnosol brown earth (0-100 cm soil depth, 4 samples per site) of the two experimental sites is 86% ($\pm 2\%$) sand, 7% ($\pm 3\%$) silt and 7 ($\pm 1\%$) clay, with a usable field capacity (0-80 cm soil depth) of 128 l m⁻³ (Mueller 2015).

The bread wheat variety *Inspiration* (Breun KG, Herzogenaurach, Germany) was drilled at 4cm depth on Oct. 7 in 2013 and Oct. 22 in 2014 with a row spacing of 11.5 cm and 330 seeds m⁻². All plots received the same doses of Patentkali (25% K, 6% Mg, 17% S), Triple-super-phosphate (20% P) and YaraVita Gramitel (150 g l⁻¹ Mg, 50 g l⁻¹ Cu, 150 g l⁻¹ Mn und 80 g l⁻¹ Zn) to ensure a sufficient supply of plants with P (51.5 kg ha⁻¹), K (112.5 kg ha⁻¹), Mg (21.8 kg ha⁻¹), S (71 kg ha⁻¹), B (0.38 kg ha⁻¹), Fe (0.9 kg ha⁻¹), Mn and Zn (0.05 kg ha⁻¹).

Three different N-levels were imposed by applying different doses of CAN (27% N): unfertilized control (N0), N120 with 120 kg N ha⁻¹ applied in three splits (50/30/40) and N230 with 230 kg N ha⁻¹ (100/90/40). The three splits took place at post-winter vegetation start (March 7 in 2014 and March 5 in 2015), at phenological stage BBCH 31 (beginning of booting stage) (April 14 in 2014 and April 27 in 2015) and at BBCH 39 (flag leaf appearance) (May 8 in 2014 and May 26 in 2015). Directly plant-available mineral soil nitrogen (N_{min} = NO₃⁻ and NH₄-N extracted with 0.0125 M CaCl₂) at post-winter vegetation start (33 and 30 kg N ha⁻¹ in 2014 and 2015, respectively, in 0-90 cm soil depth) were considered at the first N dressing. Weed and pest control were conducted according to best practice.

Plots (four replicates) with a size of 3 by 3 m with 0.5 m border lines were located in a two-factorial split-plot design with the main treatment factor water and the sub-factor nitrogen. In this paper we present results of irrigated plots supplied with three different N levels. Irrigation tubes (Netafim Ltd., Hatzetim, Israel) were installed in every second sowing row to keep plots

well watered. Drippers had a distance of 40 cm and supplied $6 \text{ l m}^{-2} \text{ h}^{-1}$ with a minimum operational water pressure of 0.08 MPa. Water deficit was calculated from estimated daily crop water use according to FAO 56 (Allen et al. 2006). Water deficit was compensated by irrigation (see Table 1 for amount of irrigation water applied). Notably, these high irrigation volumes are not representative for farm conditions but were selected in order to avoid any water stress throughout the post-winter growth period.

The field water balance (FWB) was calculated with parameters summarized in Table 2. Reference ET (ET_o) was calculated from climate data collected by an on-field weather station (Pessl Instruments GmbH, Weiz, Austria):

$$ET_o = \frac{0.408 * \Delta * (R_n - G) + \gamma * \frac{900}{(T + 273)} * u_2 * vpd}{\Delta * \gamma * (1 + 0.34 * u_2)} \quad (1),$$

where R_n is the net radiation at the crop surface ($\text{MJ m}^{-2} \text{ d}^{-1}$), G is soil heat flux ($\text{MJ m}^{-2} \text{ d}^{-1}$); T and u_2 are air temperature ($^{\circ}\text{C}$) and wind speed (m s^{-1}) at 2 m height; vpd is the vapour pressure deficit (kPa); γ is the psychrometric constant; λ the latent vapor heat; and Δ the slope of vapour pressure deficit. To compare FAO 56-based estimates of the FWB with measured FWB, the initial value of the calculated FWB at DOY 70 in 2014 and DOY 66 in 2015 were set equal to the measured values of the FWB at these days. Measured FWB was derived from Frequent Domain Reflectometry (FDR) soil moisture tube readings (Sentek, Stepney, Australia). Tubes were installed in all three N treatments ($n=2$) and soil water content was hourly monitored at 5 soil depths (10, 30, 50, 70, 90 cm).

Crop evapotranspiration (ET_a) was calculated from ET_o multiplied by a basal crop coefficient (K_{cb}) and a soil evaporation coefficient (K_e):

$$ET_a = ET_o * (K_{cb} + K_e) \quad (2)$$

K_{cb} values were derived from NDVI measurements according to a modified approach of Er-Raki et al. (2007):

$$K_{cb} = 1.15 \left[1 - \left(\frac{NDVI_{max} - NDVI}{NDVI_{max} - NDVI_{min}} \right) \right] \quad (3).$$

In contradiction to Er-Raki et al. (2007) who used 1.07 as $K_{cb,max}$ for durum wheat in a semiarid climate of Morocco we used a $K_{cb,max}$ of 1.15 for winter wheat (Allen et al. 2006).

Er-Raki et al. (2007) used an exponent, which was derived from experimental analyses of the relationships between NDVI and LAI and ET_a/ET_o and LAI (see Duchemin et al. 2006 for further details). This exponent was not used as lysimeter studies are rarely available to quantify the *in-situ* relationship between LAI and ET_a/ET_o . $NDVI_{max}$ and $NDVI_{min}$ were the maximal and minimal measured NDVI-values during the growing period. We took an initial K_{cb} -value of 0.15 (Allen et al. 2006) at vegetation start (DOY 70 in 2014 and DOY 65 in 2015) and integrated linearly until the first K_{cb} -values were derived from NDVI-measurements (DOY 83 in 2014 and DOY 69 in 2015).

K_{cb} values were daily adjusted to u_2 , RH and plant height according to Eq. (4).

$$K_{cb\ adj.} = K_{cb} + [0.04 (u_2-2)-0.004 (RH_{min}-45)] (h/3)^{0.3} \quad (4)$$

where RH_{min} is daily minimum relative humidity (%) and h is the daily plant height (m).

K_e was calculated according to Er Raki et al. (2007):

$$K_e = 0.9 * (1-f_c) \quad (5)$$

The value 0.9 was determined according to Fig. 29 of FAO 56 (Allen et al. 2006) based on observed frequency of irrigation and rainfall: from post-winter vegetation start until $NDVI_{max}$ (DOY 70-113 in 2014 and DOY 66-128 in 2015) 20 and 27 irrigation / rainfall events of $>0.2*ET_o$ occurred (for relevant rainfall events, see Allen et al. 2006, p. 153). This was equivalent to rainfall events every 2.5 days. With an average ET_o of 3.3mm / day during the growing season this corresponds to a coefficient of 0.9.

Vegetation cover (f_c) was calculated according to Er-Raki et al. (2007):

$$f_c = 1.18 * (NDVI-NDVI_{min}) \quad (6)$$

The NDVI was measured at least weekly with a MMS1-handheld spectrometer (tec5 AG, Oberursel, Germany) in four replicates per plot with a perpendicular view angle. Measurements were conducted at a distance of approximately 1.8 m to the plant surface with a minimum solar altitude of 35°. To consider possible shading effects measurements were conducted from each of the four cardinal directions of the plot corners and the four data were pooled. MMS1 measures wavelengths (R) from 400 nm to 1000 nm in 10 nm increments. The NDVI was calculated according to Rouse et al. (1974):

$$\text{NDVI} = (\text{R } 800 - \text{R } 670) / (\text{R } 800 + \text{R } 670) \quad (7)$$

The relationship between biomass and spectrometer readings was investigated by sampling 96 destructive wheat samples from 1 m² plots (with four replicates) during several growth stages of winter wheat in 2014 and 2015 (DOY 73-136 in 2014 and DOY 64-148 in 2015, respectively) on the same fields where trials were conducted. After taking spectrometer measurements, aboveground plant samples were harvested and dried until a constant weight. For calculation of biomass based on spectrometer measurements, the water index according to Penuelas et al. (1997) was used:

$$\text{Water Index} = (\text{R}900/970) \quad (8)$$

The relationship between measured biomass and water index was fitted with a Gompertz function in R (R Core Team 2015) (Fig. 1):

$$f(x) = y_0 + a e^{-e - ((x - x_0)/b)} \quad (9),$$

where $y_0 = 0.003078$; $a = 18.786706$; $b = 12.737944$; $x_0 = 16.988818$; $x = \text{water index}$.

The correlation between water index and biomass was only applicable until growth stage BBCH 49 (end of booting). Further biomass increases could not be detected by the water index (data not shown). Spectrometer-based estimates of biomass development from post-winter vegetation start to BBCH 49 were used for daily estimates of biomass increase by linear interpolating between measurement dates.

Daily estimates of biomass increase (ΔB ; g m⁻²) were used to calculate transpiration (T) (Eq. 10). To derive at T, we used the approach of Steduto et al. (2009), who used estimates of T to derive at B, reversely. We took a ET_0 normalized water productivity (WP) of 17 g biomass per m² transpired water (Raes et al. 2011).

$$T = \frac{\Delta B \cdot ET_0}{WP} \quad (10)$$

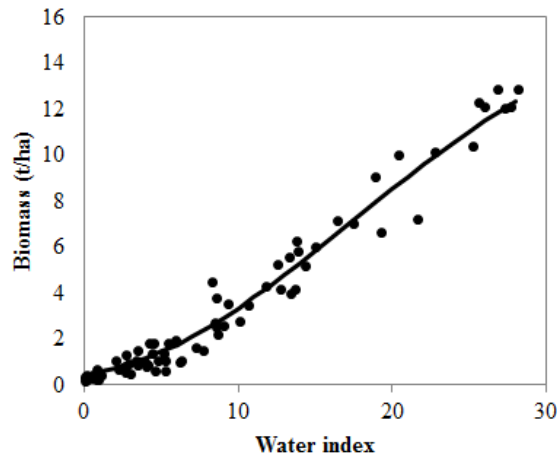


Figure 1: Non-linear regression between water index and measured biomass. The water index was derived from spectrometer measurements and dry mass sampled during the post-winter growth periods of 2014 and 2015 until the end of booting stage. For function parameters, see Eq. 9, $n=96$.

Estimates of bare soil evaporation (E) following the dual-coefficient approach of FAO 56 (Allen et al. 2006) were compared with an approach of Raes et al. (2009) in which information of leaf area index (LAI) measurements were used for estimating E:

$$E = (1-CC) * K_{c, \text{Stage1}} * ET_o \quad (11),$$

where $K_{c, \text{Stage1}}$ is the evaporation coefficient according to Allen et al. (2006) with a value of 1.1. CC is the fraction of soil covered by plants according to Hsaio et al. (2009):

$$CC = 94 [1 - \exp(-0.43 \text{ LAI})]^{0.52} \quad (12)$$

Leaf area measurements were taken almost weekly during the post-winter growth period with a Sun Scan SS1 (Delta-T Devices, Cambridge, Great Britain). Sun Scan SS1 based LAI estimates were compared with 31 destructive samples taken from 0.5 m² ground surface area, harvested during the growth period from BBCH 23 – BBCH 39 (DOY 78-112 in 2014, DOY 65-140 in 2015). Aboveground plant samples were separated into leaves and stems and LAI was measured with an Leaf Area Meter LI3000 A (Li-Cor, Lincoln, Nebraska USA). LAI derived from Sun Scan SS1 and in-situ leaf area index were linearly highly correlated (R^2 : 0.89) (Chapter 1).

Calculated daily bare soil evaporation rates of 2015 were compared with measured evaporation rates. Bare soil evaporation was measured with the micro-lysimeter method (Daamen et al. 1993) on experimental plots, additionally included in the experimental layout.

Casing tubes (radius 5 cm, height 15 cm) were installed in the soil of different fertilized plots (N0, N80 and N230) in four replicates. Smaller PVC-tubes (radius 4.55 cm, height 14.7) were placed into these casing tubes, and weight losses recorded once a day by weighing. These tubes were filled with dried, sieved (2 mm) soil from the experimental field site (1250 g, bulk density 1.35 Mg m^{-3}). Micro-lysimeters were regularly watered to 75% water holding capacity to mimic frequent rainfall and irrigation events.

A core-area of 4 m^2 was harvested from each plot at July 24 and August 2 in 2014 and 2015, respectively and grain yield determined after drying samples to constant weight with 14% residual water content. Agronomic water-use efficiency was calculated from grain yield (with 0% residual water content) and cumulative ET from post-winter vegetation start to maturity. Biomass water-use-efficiency was calculated from harvested biomass (0% residual water content) and cumulative ET from post-winter vegetation start to maturity.

Regression analysis (relationship between water index and biomass) and analysis of variance (ANOVA) (test of significance of N supply and year effects on parameters) were analyzed with R (R, Core Team 2015). Model performance of FWB calculations was evaluated with the root mean square error (RMSE) and the efficiency index (E) (Chai and Draxler 2014, Nash and Sutcliffe 1970). RSME quantifies variance of error while E is a normalized statistic determining the relative magnitude of the residual variance compared to the measured data variance. The target value for E is 1.0, while null or negative values indicate that even the arithmetic mean across observations is as good predictor as the model.

Results

Higher temperatures in March and April 2014 (Table 1) led to earlier plant development than in 2015 (Fig. 2). In 2014 the high fertilized plots reached the maximal NDVI of 0.95 approximately 13 days earlier than in 2015. Maximum NDVI of treatments N0 and N120 was reached 10 and 28 days earlier than in 2015. Onset of senescence and maturity in 2014 were reached earlier (DOY 192) in all treatments than in 2015 (DOY 203). In both years, fertilized plots (N120, N230) reached substantially higher NDVI values than N0 plots. Differences in NDVI between treatments N120 and N230 were small in 2014 and, in 2015, only evident from DOY 100 to DOY 130. Treatment N120 reached its maximum NDVI 11 and 13 days

later than N230 in 2014 and 2015, respectively. Senescence of treatment N120 started 6 and 5 days earlier in 2014 and 2015, respectively, than in treatment N230.

N effects on LAI of N230 compared to N120 were more obvious than on NDVI (Fig. 2). Due to the measurement protocol of LAI determination in which the sensor bar was positioned on the soil surface with a 90° upward view, early plant growth could not be reliably detected.

For example, while NDVI increased from DOY69 to 98 in 2015, LAI measurements indicated hardly any leaf growth. Year effects on LAI were similar to those on NDVI with higher LAI of all N treatments in 2014 than in 2015. Final shoot biomass increased linearly with LAID until LAID exceeded values of >450 (Fig. 3a). N effects on NDVI in terms of temporal dynamics and values resulted in clearly differentiating values of cumulative NDVI which were linearly correlated with final biomass (Fig. 3b). Differences between years were small but final shoot biomass tended to be higher in 2015 than in 2014 for the same cumulative NDVI. Treatment averages of N supply in 2013/14 and 2014/15 are summarized in Table 3 and statistical effects in Table 4.

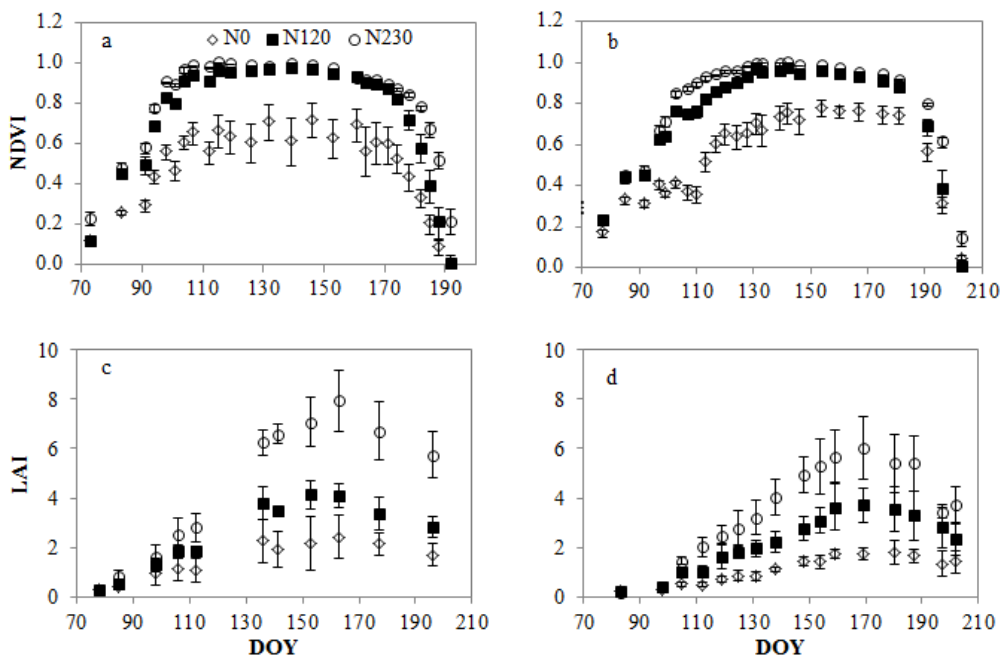


Figure 2: (a, b) Measured Normalized Differenced Vegetation Index (NDVI), (c, d) measured leaf area index (LAI) for 2014 (a, c) and 2015 (b, d). Different N-supply: unfertilized control (open diamond), 120 kg N/ha⁻¹ (closed square) and 230 kg N/ha⁻¹ (open circle), all treatments were not limited by water supply.

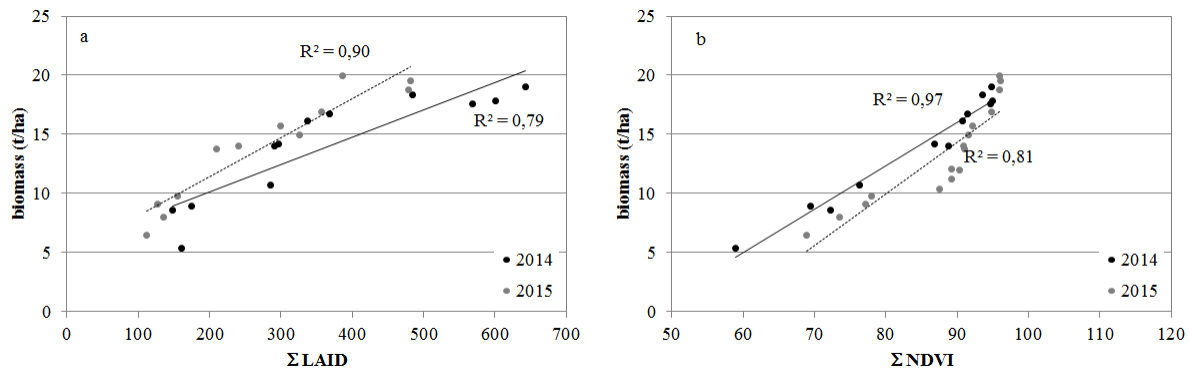


Figure 3: Relationship between biomass (0% residual water content) plotted against Leaf Area Duration (Σ LAID) (a) and cumulative measured Normalized Differenced Vegetation Index (Σ NDVI) (b) in 2014 (black) and 2015 (dotted grey). NDVI measurements were cumulated for DOY 83-192 in both years. Σ LAID was cumulated from DOY 78-196 in 2014 and DOY 65-202 in 2015, respectively. Regression was based on plots with 0,120 and 230 kg N ha⁻¹ supply in 2014 and 0,120 and 230 kg N ha⁻¹ supply in 2015.

Grain yields and shoot biomass were significantly different between N treatments but not between years. N treatment effects between the unfertilized treatment and N120 and N230 were pronounced while differences in grain yield between N120 and N230 were small though significant (Table 4). Shoot biomass was similar in both years and increased significantly with increasing N supply. HI was not significantly different between N0 and N230 and significantly higher in N120 compared to other N treatments in 2014. ET was significantly higher in 2015 than in 2014 and, in both years, ET increased with N supply. Both, agronomic and biomass water use efficiency increased with increasing N supply. N0 had a significantly lower WUE than treatments N120 and N230. WUE_Y of treatment N120 tended to be lower than that of treatment N230. WUE_B of treatment N120 was significantly lower than that of treatment N230. WUE was significantly higher in 2014 than in 2015. These differences in WUE between years were also evident if WUE was corrected by average VPD during the post-winter growth period (data not shown).

In order to test the suitability of NDVI-based estimation of ET under variable N supply, the approach was compared with an alternative approach in which T was estimated from crop growth until BBCH 49 (see Fig. 1 and Eq. 10) and E from LAI (see Eq. 11). N effects on crop growth were less pronounced in 2014 compared to 2015 (Fig. 4). Taking calculated biomass of N230 at BBCH 49 as 100%, treatments N120 and N0 realized 96% and 44% in 2014 and 71% and 33%, respectively, in 2015.

Evaporation estimates of the NDVI- and Raes approach were similar for N0, but estimates were lower with the NDVI approach for treatments N120 and N230 (Table 5). On the contrary, transpiration estimates of both approaches were similar for N230, but higher with the NDVI approach for treatment N0. ET estimates tended to be higher with the Raes and Steduto approach for treatments N120 and N230 but were lower than with the NDVI approach for treatment N0. Estimates of ET compared to measured ET fit better for the NDVI approach (treatments N0 and N230) compared to estimates of ET based on the Raes and Steduto approach, while ET of N120 was better estimated by the Raes and Steduto approach compared to the NDVI approach (Table 6). The evaporative portion of ET increased in both approaches with lower N supply and was higher with the Raes and Steduto approach.

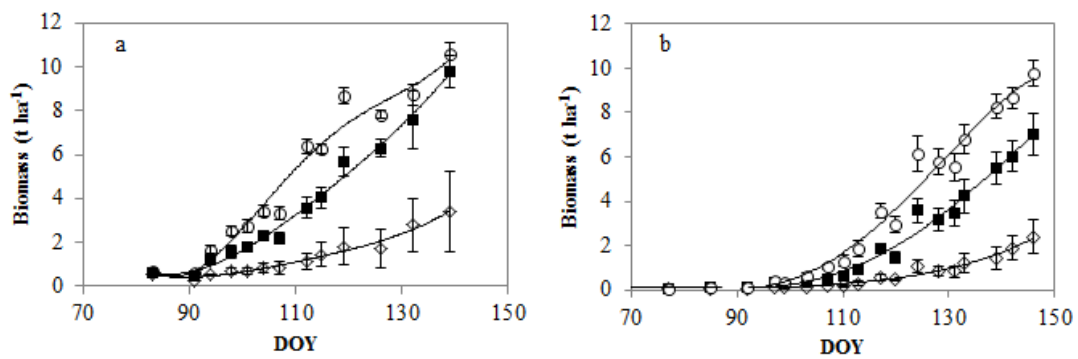


Figure 4: Biomass estimates from spectral readings for three nitrogen levels: 0 kg N ha⁻¹, 120 kg N ha⁻¹ and 230 kg N ha⁻¹ in the years 2014 (a) and 2015 (b). Different N-supply: unfertilized control (open diamond), 120 kg N ha⁻¹ (closed square) and 230 kg N ha⁻¹ (open circle), all treatments were not limited by water supply

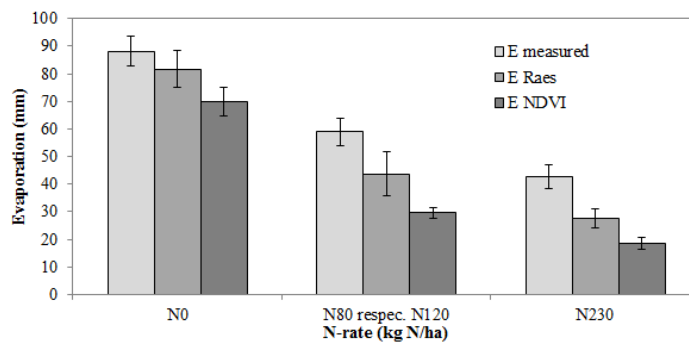


Figure 5: Measured and calculated cumulative evaporation during DOY 98-144 in 2015. N-rates were an unfertilized control N0 (0 kg N ha⁻¹), 80 kg N ha⁻¹ for measured and 120 kg N ha⁻¹ for calculated E, and 230 kg N ha⁻¹.

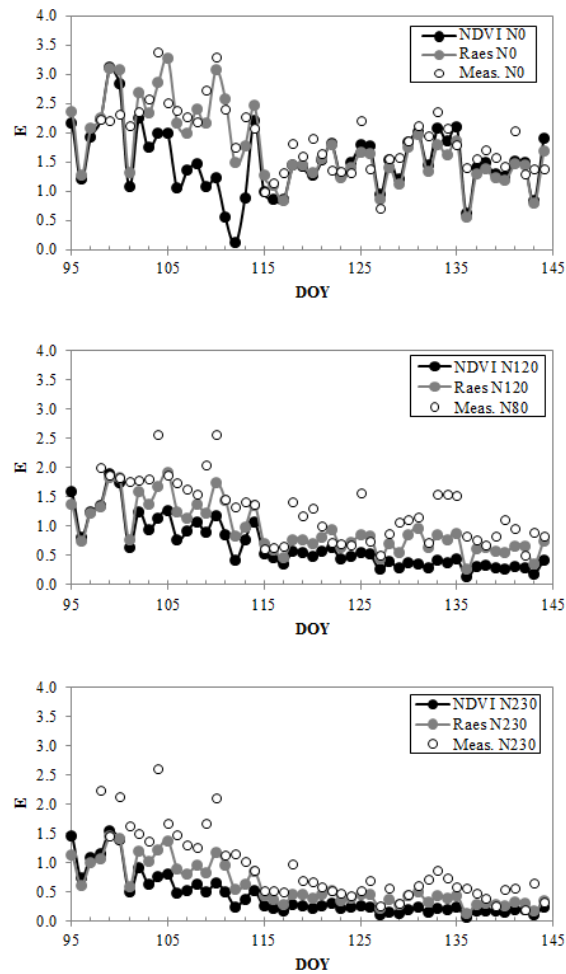


Figure 6: Daily evaporation rates (E , mm day^{-1}) of the three N treatments (N0, N120, N230) during DOY 95-144 in 2015. E was estimated from NDVI measurements (NDVI), LAI measurements (Raes), or was measured (Meas.).

Calculated E was compared with experimentally measured E for DOY 98-144 in 2015. Similar to measured cumulative E , both approaches predicted decreases of E with increasing N supply but both approaches underestimated cumulative E compared to measured cumulative E (Fig. 5). The LAI-based approach (E_{Raes}) predicted higher cumulative E than the NDVI approach. A comparison of measured and modelled daily E rates indicates that E_{Raes} agreed well with measured E of treatment N0 while the NDVI approach during the early growth phase (DOY 95-115) was less suitable. Except of underestimations of E during the early growth phase, both approaches were able to predict the observed rapid decrease of E in treatment N230. Both approaches were not able to predict some high E rates in treatment N120, however, NDVI readings and LAI measurements were from plots with a N supply of 120 kg N ha^{-1} while evaporation was measured in plots supplied with 80 kg N ha^{-1} (see Material and methods).

Table 3: Grain yield ($t\ ha^{-1}$; 14% residual water content), shoot biomass ($t\ ha^{-1}$; 0% residual water content), harvest index (HI), evapotranspiration (ET_{Total} ; mm) during the post-winter growth period (2014: DOY 70-205; 2015: DOY 65-214), agronomic (WUE_Y ; g grain per l ET) and biomass water-use efficiencies (WUE_B ; g shoot biomass per l ET) of the 3 N treatments in 2014 and 2015. WUE and HI were calculated with yield and total aboveground biomass of 0% residual water content.

N treatment	N0		N120		N230	
Year	2014	2015	2014	2015	2014	2015
Grain yield	4.9	4.8	9.8	8.9	10.4	10.5
Shoot biomass	8.4	8.3	15.3	14.6	18.2	18.8
HI	0.51	0.50	0.55	0.52	0.50	0.48
ET_{Total}	315	410	370	444	377	462
WUE_Y	1.56	1.17	2.65	2.00	2.76	2.27
WUE_B	2.67	2.02	4.13	3.29	4.83	4.07

Table 4: Differences between N treatment means ($\Delta\mu$) and confidence interval (lower limit, lwr and upper limit, upr; $\alpha:0.05$). Treatment means are pooled over both years.

Parameter	N-comparison	95% Confidence interval		
		$\Delta\mu$	lwr	upr
Grain yield	120-0	4.46	3.40	5.53
	230-0	5.58	4.50	6.64
	230-120	1.11	0.05	2.18
Biomass	0-120	6.59	4.90	8.28
	0-230	10.10	8.41	11.79
	120-230	3.51	1.82	5.20
HI	120-0	0.04	0.02	0.05
	230-0	-0.01	-0.03	0.003
	230-120	-0.05	-0.06	-0.31
ET_{Total}	120-0	45	-11	100
	230-0	64	8	120
	230-120	20	-36	75
WUE_Y	120-0	0.55	0.24	0.86
	230-0	0.63	0.31	0.94
	230-120	0.08	-0.24	0.39
WUE_B	120-0	0.78	0.31	1.26
	230-0	1.15	0.67	1.63
	230-120	0.37	-0.11	0.84

Parameter	Pr (< F)		
	year	N	year * N
Grain yield	0.353	<0.0001	0.462
Biomass	0.966	<0.0001	0.694
HI	<0.0017	<0.0001	0.151
ET _{Total}	<0.0001	<0.0001	0.099
WUE _y	<0.0001	<0.0001	0.190
WUE _B	<0.0001	<0.0001	0.480

Table 5: Effect of N rate (kg N ha⁻¹) on soil evaporation estimates (mm) based on NDVI (NDVI) or LAI (Raes) and transpiration estimates (mm) based on NDVI (NDVI) or aboveground biomass (Steduto) and the portion which E contributed to ET (E%). Calculations for DOY 70-139 and 70-144 in 2014 and 2015, respectively.

	N-rate	N0		N120		N230	
	Year	2014	2015	2014	2015	2014	2015
Evaporation	NDVI	74	111	33	65	21	53
	Raes	75	122	67	83	63	63
Transpiration	NDVI	84	90	130	155	156	171
	Steduto	48	40	128	124	147	172
Evapotranspiration	NDVI	158	201	163	220	177	224
	Raes and Steduto	123	162	195	207	199	235
E%	NDVI	47	55	20	30	12	24
	Raes and Steduto	61	75	34	40	32	27

Table 6: Statistical analysis of model performance of predicting soil water content with the NDVI K_{cb} approach (NDVI) or based on Raes and Steduto (RS) of winter wheat grown under three N levels (N0, N120, N230) during DOY 70-144 in 2015. RMSE: root mean square error (mm day⁻¹) and Eff.: efficiency (dimensionless).

Approach	Statistical parameter	N0	N120	N230
NDVI	RMSE	1.0	1.5	1.0
	Eff.	0.57	0.30	0.58
RS	RMSE	1.7	1.0	1.5
	Eff.	-0.14	0.67	0.06

Discussion

Compared with irrigation based on tabulated values of both K_c and growth stage lengths, site-specific modifications are recommended (Allen 2006) and optically-derived estimates of crops, such as NDVI are increasingly used for estimation of crop water use and related (site-

specific) irrigation (Glenn et al. 2007, Er-Raki et al. 2007, Allen et al. 2011). Estimates of crop coefficients derived from NDVI are possible due to the generally close correlation between canopy size and transpiration. Advantages of using vegetation index-based crop coefficients are the ability to account for variation in plant growth due to weather conditions, differences in sowing dates (Tasumi and Allen 2007, Er-Raki et al. 2010) or post-winter vegetation start, plant density, cultivars, pests and nutrient supply (Hunsaker et al. 2007, Pocas et al. 2015). NDVI is one of the most frequently used vegetation index and several studies showed good correlations of NDVI with plant growth parameters, e.g. in wheat (Pinter et al. 2003), measured fraction of soil cover with plants (Er-Raki et al. 2007) and leaf area (Duchemin et al. 2006, Chattaraj et al. 2013).

In this study the NDVI approach is the only option for estimating K_{cb} values and to calculate crop water use under non-optimal growth conditions (here limited N supply) under field conditions. Confirming results of Hunsaker et al. (2007) that wheat growth stage length and the maximum K_{cb} value were affected by different N management in wheat our results similarly showed that K_{cb} values derived from NDVI measurements were affected by N supply in both years (Fig. 2). Low N supply decreased maximal K_{cb} values, delayed canopy closure and induced earlier onset of senescence. These differences in NDVI were consistently reflected in final shoot biomass (Fig. 3), indicating that cumulative NDVI was a suitable index of carbon gain over the post-winter growth period. High winter wheat yields (>10 tons of grain/ha in this region) required cumulative NDVI values of >95.

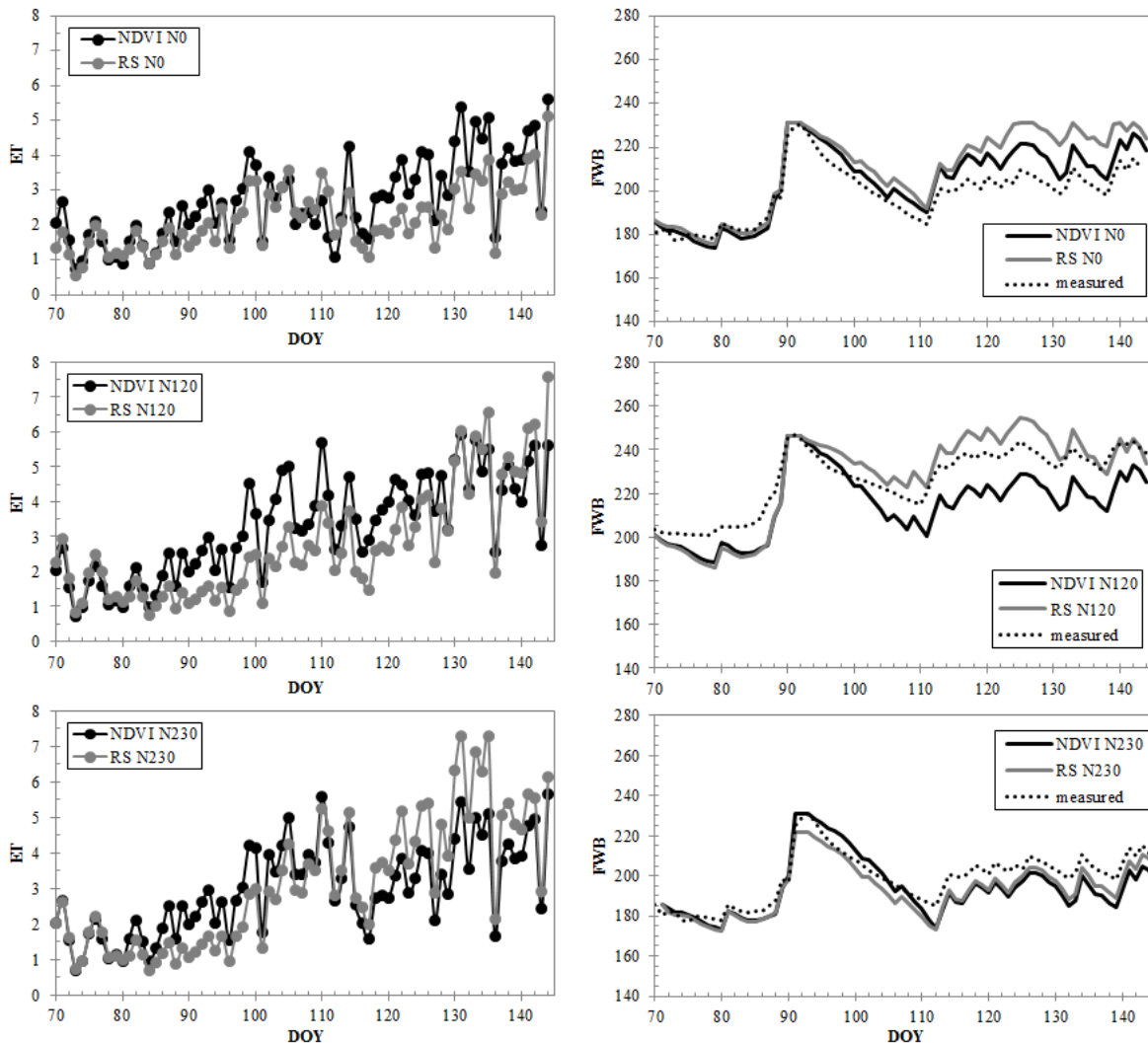


Figure 7: Evapotranspiration (ET, mm) and field water balance (FWB, mm) estimated as the sum of ERAes and TSteduto (RS) or ET derived from FAO 56 based on NDVI-measurements (NDVI) for 3 different N-level with an unfertilized control (N0), 120 kg N ha⁻¹ (N120) and 230 kg N ha⁻¹ (N230) for winter wheat in the year 2015 from DOY 70-144.

In 2014, plants of treatments N0 and N120 tended to realize higher final shoot biomass than in 2015 with the same cumulative NDVI. This possibly indicates that the conversion efficiency of light absorption was higher in 2014 than in 2015, and was likely related to seasonal differences in temperatures which are not considered by this simple cumulative NDVI approach. LAID also appeared to be a suitable indicator of final biomass, the critical LAID for high yields was roughly >450. However, continuous or at least frequent measurements of LAI would require the permanent installation of rather expensive equipment in the field, whereas NDVI can be derived rather simply from remote sensing devices.

Efficient water management in agriculture is a key for sustainable water use and nutrient management of cropping systems is closely related to water resources for two reasons, crop

water use and potential pollution of freshwater by overdosing of nutrients. Intensive agriculture, including fertilizer application, has been identified as a threat to regional long-term water availability of cropping systems (Liu et al. 2015). However, yield of wheat, rice and maize, in a global survey, were shown to positively correlate with agronomic water-use efficiency (Zwart and Bastiaanssen, 2004), indicating that low-input agriculture is less water-use efficient when expressed as product per unit of ET, indicating a trade-off between water conservation and efficient water use for food production.

Yield (and biomass) increases with fertilizer application necessarily imply higher ET due to the conservative nature of biomass WUE (Steduto et al. 2007). Field studies indicated a rather variable picture with N-related increases of ET varying from >20% (Garabet et al. 1998, Caviglia and Sadras 2001, Huang et al. 2003) to <15% (Zhang et al. 1998, Hunsaker et al. 2007, Karam et al. 2009). Our results indicate that cumulative post-winter season ET of N230 was 24% and 12% higher than that of treatment N0 (Table 3) in 2014 and 2015, respectively. Variability in reported N effects on increases of ET can be explained by differences in yield response to variable N supply (the smaller the yield response, the smaller the differences in ET) and site- and crop-specific effects on the partitioning of ET between E and T. N-induced increases in T can be small if E dominates ET such as in irrigated row crops with poor ground cover before canopy closure. Results of our study indicate that increases in ET due to N applications were small for winter wheat in temperate humid climate, as the decrease of T under low N was strongly off-set by corresponding increases of evaporation. This finding cannot be extrapolated to drier climates with less rainfall events as high evaporation in the present study was realized by frequent wetting, keeping the soil more or less continuously in the high evaporation stage 1 (Ritchie 1972).

Bare soil evaporation is controlled by available energy, RH, wind speed, topsoil water content and canopy cover. It can be expected that estimates of E are improved by measurements of the fraction of soil wetted and exposed to direct radiation (Peirera et al. 2015), thereby adding more accuracy to the estimation of E and total ET. Johnson and Troust (2012) found a good linear correlation between NDVI and canopy cover of vegetable crops by comparing NDVI measurements and estimates of canopy cover derived from images taken by a digital ground camera. We carried out a more detailed estimate of N effects on ET for the post-winter growth period until BBCH 49. Our results show that the NDVI and Raes and Steduto approaches consistently indicated increases of E under low N supply, particularly when comparing a

sparse crop canopy (N0) with plots receiving fertilizer (N120 and N230) (Table 5). However, estimates of E differed substantially between both approaches (NDVI versus Raes). As calculated E in both cases relied on the same climate data, differences between both approaches were related to differences in canopy cover estimates. In the NDVI approach the soil evaporation coefficient was derived from NDVI measurements (see Eq. 5 and 6) while E_{Raes} was estimated from LAI measurements (see Eq. 11 and 12). Cumulative estimates of E were similar for treatment N0 (Table 5) although a comparison with measured E suggests that daily rates were better predicted by the Raes approach (Fig. 6). For treatments N120 and N230 the Raes approach estimated higher rates of E than the NDVI approach and, again, agreed better with measured E (Fig. 6). Both approaches were not able to predict the high rates of E observed during some days. However, the microclimate of the micro-lysimeter set-up was not identical to those plots on which NDVI and LAI were measured, as installation of the unplanted micro-lysimeters disturbed growth of the surrounding plants and likely resulted in overestimation of E compared to undisturbed N120 and N230 plots. Furthermore, in one of the N treatments, E was measured in plots which received only 80 kg N ha⁻¹. This resulted in less vigorous growth compared to treatment N120. These two factors likely explain the higher measured E compared to predicted E consistently observed in the two fertilized N treatments (Fig. 5). Summarizing, NDVI or LAI are suitable to estimate the fraction of canopy cover particularly under conditions of nutrient limitation and should be preferred over tabulated data of crop coefficients. Compared with winter wheat, the relevance of monitoring in-situ canopy development will be even higher in row crops with extended periods of incomplete ground cover.

Both approaches (NDVI and Raes and Steduto) derived at different transpiration estimates. Transpiration derived from NDVI was higher than that of T_{Steduto} particularly for treatments N120 and N0 (Table 5). Both approaches followed different methods to estimate transpiration: the NDVI approach derived at transpiration by subtracting E from ET (with ET derived from ET_0 and soil and crop coefficients, see Eq. 2), whereas transpiration in the Steduto approach was derived from estimates of daily biomass increments (see Eq. 10). Differences in E and transpiration estimates of both approaches led to contrasting estimates of N induced differences in ET. When ET was estimated from NDVI, ET of N0 was, in both years, only ~10% lower than that of N230, whereas the Raes and Steduto approach indicated that ET of unfertilized plots was substantially lower (38% and 31% in 2014 and 2015,

respectively) than that of treatment N230. As ET estimates based on Raes and Steduto could not be extended beyond BBCH 49, it remained unclear, to what extent N differences of total post-winter ET based on the NDVI approach (Table 3) were underestimated as compared with the Raes and Steduto approach, but we assume that NDVI-based estimates represent the lower range of N effects on ET. However, both approaches should be considered as estimates of ET as they could not be validated in this field study. Comparison with measured evaporation rates and the field water balance (Fig. 6, 7) indicate that both methods were equally suitable to predict ET but the comparison with field water balance data is not straightforward as the extent of drainage and capillary rise remains unknown under the experimental conditions (high rainfall and supplemental irrigation, see Table 1).

The Raes and Steduto approach indicated that the proportion of evaporation relative to ET decreased with N supply from >60% to <30% until BBCH 49 in this study (Table 5) while the same transpirational WUE was assumed for the three N treatments. If N fertilizer application reduced E while T increased in proportion to dry mass gain, N application effects on WUE would be large in 'high E' environments such as humid climates with frequent soil surface wetting and small (or even absent) in 'low E' environments such as in arid climates with subsurface irrigation. Rainfall and irrigation in our experiment generated a humid environment so that increases in ET from N0 to N230 were small compared to N-induced yield increases, resulting in a substantially lower WUE_Y of N0 compared to N120 and N230 (Table 3). Differences in ET and WUE_Y between moderate (N120) to high (N230) N supply were small in both years. Consequently, positive N effects on WUE_Y could be explained by a shift from evaporation to transpiration and are in line with other field studies which were conducted in arid and semiarid environments (e.g. Eck 1988, Zhang et al. 1998, Asseng et al. 2001).

Results of the FAO 56 approach and the Raes and Steduto approach consistently indicate that increases in yields were higher than increases in ET supporting hypothesis i) (see introduction) that fertilizer application contributes to more efficient water use by reducing evaporation, particularly in humid climates.

Contrary to our expectation, hypothesis ii) which assumed an increase of WUE_Y via increases in HI could not be confirmed. Wheat in treatment N230 produced more biomass than in treatment N120 but failed to increase correspondingly yield components, such as number of

ears, spikelets per ear or thousand grain weight (data not shown). WUE_Y of N230 was not significantly higher than that of N120, while differences in WUE_B between both treatments were more obvious (Table 3). This finding suggests that high N supplied plants tended to produce ‘luxury’ leaf and stem reserves and that high N rates do not automatically guarantee the highest WUE_Y .

Conclusions

Estimates of ET under variable N supply require consideration of N-induced effects on canopy development which were successfully monitored with NDVI measurements. N application increased grain yield more than ET resulting in a higher agronomic WUE. This was mostly due to a proportional shift between E and T, indicating that N effects on WUE are more pronounced under wet than dry environmental conditions. As canopy development and crop growth in temperate-humid climates is highly variable during the post-winter growth period and strongly affected by N supply, NDVI should be monitored in a high-time resolution until canopy closure and during the senescence phase. Use of tabulated data of stage lengths and crop coefficients cannot be used reliably to quantify ET of winter wheat under N limitations.

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Appendix Chapter 1

Measured FWB was derived from Frequent Domain Reflectometry (FDR) soil moisture tube readings (Sentek, Stepney, Australia). Tubes were installed in all three N treatments (n=2) and soil water content was hourly monitored at 5 soil depths (10, 30, 50, 70, 90 cm). The measured FWB presented in Fig. 7 was the average of 2 tubes per treatments which were installed in two experimental plots. As depicted in Fig. A1, both tubes in treatments N0 and N120

exhibited a very similar temporal dynamic during DOY 70 and 190. The measured FWB presented in Fig. 7 does not include standard deviations. Due to technical failure, in treatment N230, only one tube was available until DOY 160. Notably, in other experimental plots and in 2014, differences in FWB estimates between replicates were larger due to spatial differences in soil texture. However, seasonal changes were similar.

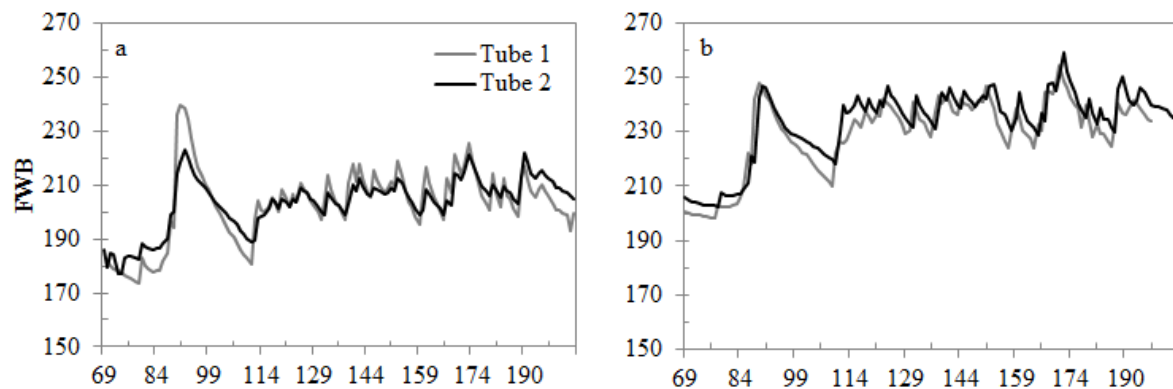


Figure A1: Field water balance (FWB, mm) of treatment N0 (a) and N120 (b) measured in two experimental plots per treatment (Tube 1 and 2) during DOY 70-205 in 2015.

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Chapter 3

NDVI-based estimates of evapotranspiration of winter wheat indicate positive effects of N fertilizer application on agronomic water-use efficiency under drought conditions

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Abstract

Broad acre crops grown in NW Europe, like winter wheat, are the main constituent of diets for humans and to feed livestock. Winter wheat is more and more confronted with drought periods during the post-winter vegetation growth period. Findings from the literature, mostly based on experiments in arid climates, indicate that high nitrogen fertilization is not the most water efficient way to generate grain yields in combination with maximal water use efficiency.

To quantify the effects of N fertilizer supply on grain yield and evapotranspiration (ET) of winter wheat, a field experiment with two nitrogen rates (N120 and N230) and three (WS+, WS31 and WS31 1/3) respective four (WS+, WS31, WS31 2/3 and WS31 1/3) different water supply levels was performed during 2014 and 2015. Well-watered plots (WS+) were irrigated when required to avoid water limitations, while drought was induced on plots (WS31, WS31 2/3 and WS31 1/3) by the installation of rain-out-shelters. All plots were equipped with drip irrigation to supply water when intended. The Normalized Difference Vegetation Index (NDVI) was measured to derive crop coefficients which were used to calculate ET with the FAO 56 dual-coefficient approach. The extent to which N supply modified bare soil evaporation (E), transpiration (Tr), ET, grain yield, water use efficiency (WUE) and above-ground biomass was tested.

We found that treatment N230 compared with treatment N120 in a temperate humid climate in NW Europe led to higher crop water use, but in the most water efficient way, irrespective of water stress treatments. N-supply increased the share of Tr on ET overall water treatments. Furthermore, N application increased grain yield more than ET resulting in a higher WUE. These findings from plants grown under climates with full saturated soils at vegetation start after winter are contrary compared to findings from wheat grown under arid environments.

1. Introduction

Freshwater resources are threatened by nutrient and pesticide loads from agriculture as well as increasing domestic and industrial freshwater demand in some world regions. Freshwater availability per capita and year has decreased in e.g. South-East Asia and North Africa (Reuveny, 2007; Mc Donald et al., 2011). Water demand has increased over the last decades, primary due to agricultural use of fresh water for irrigation (Siebert et al., 2010). In future, water will become increasingly scarce particularly in semi-arid regions. Improvements in agricultural water management are necessary to enhance agricultural productivity in order to meet food demands of the growing world population. The International Water Management Institute (IWMI, 2007) stated that there will be enough land, water and human capacity to produce enough food for a growing population, if water use in agriculture is improved. The water use efficiency (WUE) has therefore become one of the most important indices for benchmarking optimal water management practices.

Climate change scenarios indicate increasing occurrence and intensity of droughts (Sheffield and Wood, 2008). Lehner et al. (2006) analyzed drought scenarios for South Eastern Europe from the last century and projected in a model study that in the year 2070 severe drought events, occurring actually once per century, will happen every forty years. Water scarcity is a regional to local event which is highly affected by soil type, soil profile depth and cropping pattern (Ehlers and Goss, 2003; Rickmann and Sourell, 2014). This underlines the necessity to predict water demand and water deficit at a site- and crop specific level.

As WUE is related to total crop water use, which is the sum of evaporation and transpiration, several management options, such as the reduction of non-productive loss through soil evaporation, avoidance of runoff and drainage exist (Gregory, 2000). Application of fertilizer, provided that it allows a more rapid growth of the canopy that shades the soil surface, thereby

reduces the proportion of the total water that is evaporated (Cooper et al., 1983). Options to increase yield and WUE by integrated water and nitrogen management are indicated by several studies (Eck, 1988; Musick et al., 1994; Hussain and Jaloud, 1995; Schjoerring et al., 1995; Oweis et al., 1998; Lenka et al., 2009).

Water stress is expected to decrease yield and farm management of field sites regularly responds by adjusting fertilizer amount accordingly to the expected yield decrease. A phenomenon known as ‘Haying-off’ has been illustrated in semiarid environments showing that grain yield is negatively influenced by excessive N supply. High N rates in that case caused an increase in shoot biomass during the vegetative growth phase and resulted in a depletion of soil water reserves during the grain filling period and related yield decreases (van Herwaarden et al., 1998).

Steduto et al. (2007) assessed transpirational water loss of different crops in several environments which contrasted each other in rainfall and vapor pressure deficit and showed constant agronomic water-use efficiency (WUE_y) of $20 \text{ kg kPa grain ha}^{-1} \text{ mm}^{-1}$. This implies that plants react biologically in the same way when their biomass is increased (e.g. due to higher nitrogen supply) by using more water. We therefore expected that effects of nitrogen supply and drought on transpirational WUE are small. As evaporation is a relevant pathway of latent heat flux in humid climates, we further speculate that N supply improves agronomic WUE mainly by reducing evaporation. Finally, as evaporation of dry soil surfaces is low, we speculate that N effects on agronomic WUE are small in treatments which are subject to drought.

The aim of this study was i) to quantify the extent of different N-rates on grain yield under conditions of drought in a temperate humid climate in NW Europe. ii) to quantify the extent of water use efficiency with varying nitrogen supply of winter wheat grown under climates with full saturated soils at vegetation start after winter, compared to plants grown under arid environments.

2. Materials and methods

2.1. Study area, climate and soils

The study was carried out at the Experimental Farm Hanninghof, Institute of Plant Nutrition and Environmental Research, Duermen North-Rhine Westphalia, Germany (51° 50' 22" N latitude, 7° 15' 18.5" E longitude). The climate is humid-temperate and classified according to Köppen and Geiger (1936) as Cfb (July being the hottest and January the coldest month) with mean annual rainfall of 888mm (April is the driest month with mean annual rainfall of at least 57 mm, July is the wettest month with a mean annual rainfall of 86mm). The average soil texture (0-100 cm soil depth, 4 soil samples per site) of the two experimental sites used in 2014 and 2015 was 86% ($\pm 2\%$) sand, 7% ($\pm 3\%$) silt and 7% ($\pm 1\%$) clay. The soil type was a Stagnosol brown earth with a usable field capacity (0-80 cm soil depth) of 128 l m⁻³ (Mueller, 2015).

2.2. Field experiment and layout

The bread wheat variety *Inspiration* (Breun KG, Herzogenaurach, Germany) was drilled at 4 cm depth on Oct. 7 in 2013 and Oct. 22 in 2014 with a row spacing of 11.5 cm and 330 seeds m⁻². Two N-levels were imposed by applying different doses of CAN (27% N): N120 with 120 kg N ha⁻¹ applied in three splits (50/30/40) and N230 with 230 kg N ha⁻¹ (100/90/40). The three splits took place at post-winter vegetation start (March 7 in 2014 and March 5 on 2015), at BBCH 31 (April 14 in 2014 and April 27 in 2015) and at BBCH 39 (May 8 in 2014 and May 26 in 2015). Directly plant-available mineral soil nitrogen (N_{min}) at post-winter vegetation start (33 and 30 kg N ha⁻¹ in 0-90 cm soil depth in 2014 and 2015, respectively) were considered at the first N dressing. All plots received the same doses of Patenkali (25% K, 6% Mg, 17% S), Triple-super-phosphate (20% P) and YaraVita Gramitel (150 g l⁻¹ Mg, 50 g l⁻¹ Cu, 150 g l⁻¹ Mn and 80 g l⁻¹ Zn) to ensure a sufficient supply of plants with K (112.5 kg ha⁻¹), Mg (21.8 kg ha⁻¹), S (71 kg ha⁻¹), P (51.5 kg ha⁻¹), B (0.38 kg ha⁻¹), Fe (0.9 kg ha⁻¹), Mn and Zn (0.05 kg ha⁻¹). Weed and pest control was according to best practice. Four different water regimes were induced to the plots: i) no limitation in water supply (WS+), ii) early drought (WS31) from DOY 76-128 in 2014 and DOY 77-146 in 2015 and sufficient water supply thereafter, iii) early drought with approximate irrigation amounts of 1/3 of crop water use after the drought period (WS31 1/3), iiiii) early drought with approximate irrigation amount of 2/3 of crop water use after the drought period (WS31 2/3). Weed and pest control

was conducted according to best practice. Plots were arranged in a two-factorial split-plot design with main treatment factor water and sub-factor nitrogen with four replicates (Table 1). Monitoring of growth stages (BBCH) were done according to Hack et al. (1992). Several growth stages were documented according to the development of the plants if minimum 50% of the plants in the plots reached a BBCH-stage.

2.3. Rain-out-shelter

To ensure that the crop grew under the intended water availability, rain-out-shelters were used to exclude rainfall. These shelters were based on a steel frame with the size of 3 by 3 meters. Plots were covered with a UV-permeable 200 µm thick foil (Polydress® SPR5, RKW SE, Michelstadt, Germany). Own measurements showed 84% permeability (manufacturer specification 86%) of light (wavelength 400-700 nm). The steel frame with foil was attached to 2 vertical steel bars of 2 m aboveground height which were installed in the soil down to depth of 0.8 m. The steel frame covers were movable so that they could be positioned at a variable height above the crop canopy. To prevent wind-driven rain to enter the plots, steel frame covers were positioned 0.5 m above the crop canopy and steel frame cover height was regularly adjusted during the growing season. The incoming rainfall intercepted by the rain-out-shelter roof was guided with tubes from the roofs into soak aways (1m depth) at a distance of 2-3 m of the sheltered plots. For measurements, plant protection and fertilizer application shelters were moved up with a hand winch and afterwards repositioned again.

In 2014 (DOY 76-128) 75 mm and in 2015 (DOY 77-146) 139 mm of rainfall were excluded from drought plots during the growth stage booting. Drought plots received no additional irrigation during that growth stage, while the well-watered plots received 90 mm irrigation in 2014 and 84 mm in 2015. Only 6 mm irrigation were supplied to drought plots at DOY 104 in 2014 and DOY 120 in 2015 after the second fertilizer application in order to ensure solution of surface-applied fertilizer. After the termination of early drought (DOY 128 in 2014 and DOY 146 in 2015) drought plots received irrigation according to the listed volumes for well-watered plants (Table 1).

Table 1: Water regimes in 2014 (DOY 70-205) and 2015 (DOY 66-214) for 2 different N levels (N120 and N230). 4 different water regimes were induced to the plots: i) no limitation in water supply (WS+), ii) early drought (WS31) from DOY 76-128 in 2014 and DOY 77-146 in 2015, iii) early drought with an approximate irrigation amount of 1/3 of crop water demand after drought period (WS31 1/3), iiiii) early drought with an approximate irrigation amount of 2/3 of crop water demand after drought period (WS31 2/3). Water regimes during the vegetative growing period (Veg. per.) and generative growing period (Gen. per.) were: no limitation in water (opt.), 6 mm watered (scares), approximately 1/3 of calculated crop water use watered (little) and approximately 2/3 of calculated crop water use watered (moderate).

Year	N-supply (kg N/ha ⁻¹)	Water regime	Water supply during		Total available water (mm)	Rainfall (mm)	Irrigation (mm)
			Veg. per	Gen. per.			
2014 ^{*1}	120,230	WS+	opt.	opt.	599	359	240
	120,230	WS31	scares	opt.	482	290	192
	120,230	WS31 1/3	scares	little	62	-	62
2015 ^{*2}	120,230	WS+	opt.	opt.	517	307	210
	120,230	WS31	scares	opt.	339	156	183
	120,230	WS31 1/3	scares	little	48	-	48
	120,230	WS31 2/3	scares	moderate	98	-	98

*1 from DOY 70-205

*2 from DOY 66-214

Table 2: Parameters used to calculate ET_a of winter wheat in 2014 and 2015 according to the FAO 56 approach (Allen et al., 2006). Data were either measured (meas.) or from literature.

Parameter	Unit	Value	Source
Base temperature	°C	0	Ewert 1996
K _{cb max}	dim.less	1.15	Allen et al., 2006
Plant height to vegetation start	m	0.05	measured
Plant height during initial stage	m	0.1	measured
Final plant height	m	0.9	measured
Rooting depth at vegetation start after winter	m	0.3	measured
Maximum root depth in 2014, 2015	m	0.75, 0.9	measured
Field capacity in 2014 and 2015	Vol. %	23	Mueller et al., 2015
Water at wilting point in 2014, 2015	Vol. %	13,10	Mueller et al., 2015
Soil evaporations depth	m	0.15	Allen et al., 2006
Uncovered soil fraction at vegetation start	% / 100	0.95	Allen et al., 2006
Uncovered soil fraction at end of stem elongation	% / 100	0.05	Allen et al., 2006
Evaporations reduction factor	dim.less	0.55	Allen et al., 2006

We measured air temperature and relative air humidity with TinyTags (BMC, Pochheim, Germany), which were placed approximately 10 cm below canopy height. The Data logger were carried out in the sheltered and unsheltered plots by binding them at a plastic stick which was arranged approximately 10 cm below canopy height. Air temperature of sheltered plots was maximal increased by 1°C, air humidity was approximately 10% increased when comparing sheltered and unsheltered plots. Own results of phenology, biomass and grain yield showed a negligible effect on micro climate of the covered plots compared to uncovered plots (see appendix).

Plots were 3x3 m in size with 0.5 m border lines. To keep plots well-watered irrigation tubes (Netafim, Hatzetim, Israel) were installed in every second sowing row. Drippers had a distance of 40 cm and supplied 6 l m² per hour with a minimum operational water pressure of 0.8 bar water deficit between estimated crop water use according to FAO 56 (Allen et al., 2006) and rainfall were compensated by irrigation (see Table 1). Additional irrigation of 240 and 210 mm in 2014 and 2015 respectively, was applied to experimental plots. Notably these high irrigation volumes are not applicable to farm conditions, but were designed to avoid water stress throughout the post-winter growth period.

2.4. Field water balance and soil moisture measurements

The field water balance (FWB) of treatments was calculated with the dual-coefficient approach according to FAO 56 (Allen et al., 2006) with parameters summarized in Table 2. To compare FAO 56 estimates of the FWB with measured FWB, the initial value of the calculated FWB at DOY 70 in 2014 and DOY 66 in 2015 were matched with the measured value of the FWB at that day. Measured FWB was derived from Frequent Domain Reflectometry (FDR) soil moisture tube readings (Sentek, Stepney, Australia). Tubes were installed in both N in WS+ and WS31 treatments (n = 2) and soil water content was hourly monitored at 5 soil depths (10, 30, 50, 70, 90 cm).

2.5. Spectrometer measurements

Spectrometer measurements were done at least weekly with a MMS1-Handspectrometer (tec5 AG, Oberursel, Germany) in four replicates per plot with a perpendicular view angle during the whole growing season. Measurements were done in a distance of approximately 1.8 m to the plant surface with a minimum solar altitude of 35°. Measurements were taken from each

of the four directions (N, E, S, W) of the plot corners to consider possible shading effects from sunlight.

MMS1 measures wavelengths (R) from 400 nm to 1000 nm in 10 nm increments. The Normalized Difference Vegetation Index (NDVI) was calculated according to Rouse et al. (1974):

$$\text{NDVI} = (\text{R } 800 - \text{R } 670) / (\text{R } 800 + \text{R } 670) \quad (1)$$

and was used for calculation of Kcb-values (Eq. 2) modified to Er-Raki et al. (2007). NDVI values were linearly interpolated between measurement days and summed over the measuring period to calculate the cumulative NDVI (NDVI_{cum})

$$\text{Kcb} = 1.15 \left[1 - \left(\frac{\text{NDVI}_{\text{max}} - \text{NDVI}}{\text{NDVI}_{\text{max}} - \text{NDVI}_{\text{min}}} \right) \right] \quad (2)$$

For our calculations we set Kcb-value at vegetation start (DOY 70 in 2014 and DOY 65 in 2015) according to the Kcb initial value given from Allen et al., 2006 at 0.3 and integrated linearly until first Kcb-values derived from NDVI-measurements (DOY in 2014 83 and DOY 69 in 2015).

2.6. Leaf area index and canopy coverage

Leaf area measurements were taken nearly weekly during the post-winter growth period with a Sun Scan SS1 (Delta-T Devices, Cambridge, Great Britain). Sun Scan SS1 based LAI estimates were compared with 31 destructive samples taken from 0.5 m² ground surface area which were harvested during the growth period from BBCH 23-BBCH 39 (DOY 65-140 in 2015, DOY 78-112 in 2014). Aboveground plant samples were separated into leaves and stems and LAI was measured with a Leaf Area Meter LI3000 A (Li-Cor, Lincoln, Nebraska USA). LAI derived from Sun Scan SS1 and *in-situ* leaf area index were linearly highly correlated (R²: 0.89) (Chapter 1).

2.7. Grain yield and final biomass

A core center area of 4 m² of each plot was hand-harvested in July 24 and August 2 in 2014 and 2015, respectively. The whole samples were dried in a forced oven at 70°C to constant

weight and weighed. After drying samples were threshed with a combine harvester Zürn 150 (Waldenburg, Germany).

2.8. Yield reduction factor

The yield reduction factor K_y describes the relationship between grain yield and crop water use which is a proxy for water use efficiency (Smith and Steduto, 2012). Intensity of drought is expressed as the ratio of actual ET_a and ET_x of a reference plot with no limitation in water supply. Under optimal growth conditions actual grain yield (Y_a) is equal to maximal attainable yield (Y_x). If the water requirements of the plants are not fulfilled, plants cannot achieve the maximal grain yield ($Y_a < Y_x$) (Doorenbos and Kassam, 1981). In order to quantify the extent of water stress the linear regression between relative yield reduction and relative ET-decrease is plotted and K_y is the slope of this relationship. $K_y < 1$ means a proportional lower decrease of yield due to water stress. $K_y > 1$ means a proportional higher decrease of grain yield. The yield reduction factor K_y is calculated according to Doorenbos and Kassam (1981):

$$\left(1 - \frac{Y_a}{Y_x}\right) = K_y \left(1 - \frac{ET_a}{ET_x}\right) \quad (3)$$

ET and yields of N230 plots with no limitation in water (WS+) were set as reference Y_x and ET_x .

2.9. Statistical analysis

Regression analysis (relationship between water index and biomass) and analysis of variance (ANOVA) (test of significance of N supply and year effects on parameters) were analyzed with R (R Core Team, 2015). Due to limited number of rain-out-shelters, there was no unfertilized control (N0) in all water requirements. Statistical analysis was only completed for plots that received N120 and N230 in 3 water requirements (WS+, WS31, WS31 1/3) in 2014 and 4 water requirements (WS+, WS31, WS31 1/3, WS31 2/3) in 2015.

The model was specified as: $\text{fit} \rightarrow \text{lme}(\text{grain} \sim \text{n} * \text{water}, \text{tmp}, \text{random} = \sim 1/\text{year}/\text{water}/\text{n})$

Model performance of FWB calculations was evaluated with the root mean square error (RMSE) and the efficiency index (Eff.) (Chai and Draxler, 2014; Nash and Sutcliffe, 2010).

3. Results

Induced early drought (GDD 968°C-1544°C in 2014 (Fig. 1a) and 866°C-1522°C in 2015) resulted in a decrease of soil water content. The lowest measured FWB was 150 l m⁻³ (GDD 1550°C) of plots where drought was induced compared to well-watered plots (Fig. 1b) where measured FWB at that point was approximately 200 l m⁻³.

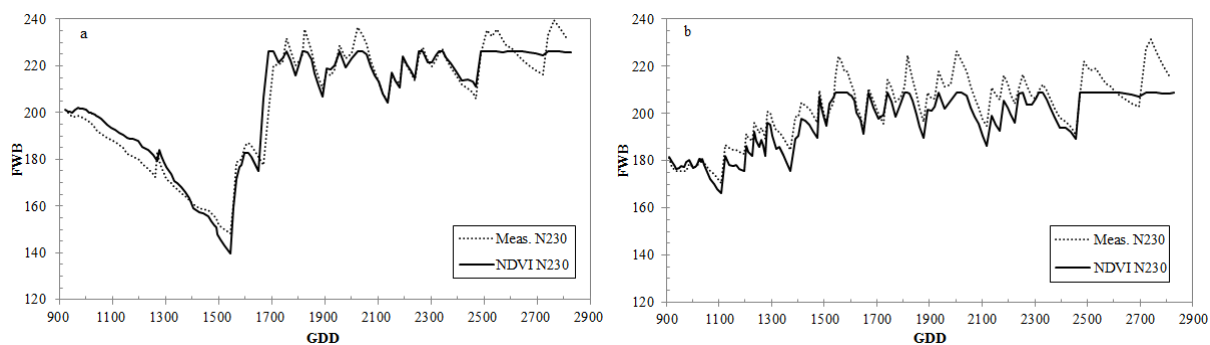


Figure 1: Calculated (grey line) and measured (black line) field water balance (FWB) (l m⁻²) for plots with induced early drought (a), where drought was induced during GDD 968°C-1544°C, and well-watered plots (b) both with high nutrient supply (230 kg N ha⁻¹) in 2014. Root mean square (RMSE) and Efficiency (EF) from measured FWB compared to calculated FWB was: (a) 1.0 and 0.90, (b) 0.7 and 0.88 (see Table 3)

The calculated FWB with Kcb-values derived from NDVI-measurements showed almost the same temporal trend of soil water content in early-drought and well-watered plots indicating the suitability of predicting the FWB with NDVI measurements in combination with the FAO 56 approach (Table 1). For both, water and N treatments, RMSE varied from 0.8 (N120 in 2015) to 1.5 (N230 in 2015) and the efficiency was > 0.5 except of treatment N120 WS+ in 2015.

Table 3: Statistics analysis of calculated soil water content and measured soil water content by FDR-techniques of winter wheat growing under 2 different nitrogen levels (N120, N230) in 2 years field experiment (2014 and 2015) grown under well-watered (WS+) and early drought (WS31) conditions. RMSE: root mean square error (mm day⁻¹) and efficiency (Eff.) (no units).

Year	Water requirement	WS+		WS31	
	Statistical parameter	N120	N230	N120	N230
2014	RMSE	1.5	0.7	1.4	1.0
	Eff.	0.85	0.88	0.91	0.90
2015	RMSE	1.5	1.0	0.8	1.5
	Eff.	0.30	0.58	0.91	0.52

The drought stress index (KS-value) of early drought plots, calculated according to FAO 56, dropped from 1 (no stress) to 0 (extreme stress) (Fig. 2) during the developmental stages BBCH 29 to BBCH 39. First onset of drought (decrease of KS-values) was calculated for 1304°C in 2014 and 1286°C in 2015, respectively. Until re-watering of drought plots at BBCH 39 (1556°C in 2014 and 1628°C in 2015) drought was more pronounced in 2015 than in 2014. While the shelters were built up and removed in both years at the same phenological stage (post winter vegetation start until BBCH 39), the drought period in 2014 lasted for 252°C, while the drought period in 2015 was longer (342°C). KS-value of well-watered plots in 2014 and 2015 were almost always 1.0 during the post-winter vegetation growth period. Regular visual inspection of plots during both years indicated that leaf rolling was observed at KS-values of roughly 0.6.

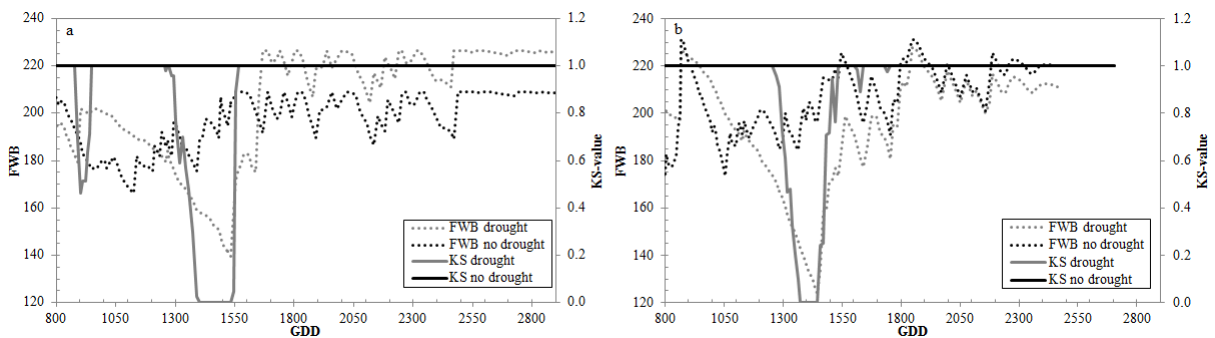


Figure 2: Measured field water balance (FWB) in 2014 (a) and 2015 (b) for well-watered plots (black dotted line (no drought)) and plots with induced early water stress (grey dotted line (drought)). Related drought stress index (KS-value) ranging from 0-1 for well-watered plots (black line (no drought)) and water stressed plots (grey line (drought)). All plots received 230 kg N ha⁻¹.

Stress-induced reduction of biomass, either caused by the lack of nitrogen or water, was reflected by NDVI and LAI measurements during the post winter growth period (Fig. 3). Cumulative NDVI and LAI were positively correlated with final shoot biomass (R^2 : 0.94 and 0.89). A single regression function between shoot biomass and cumulative NDVI (Σ NDVI) did apply across all N supply levels and to plants grown under drought conditions or when well-watered (Fig. 3). Leaf Area Duration (Σ LAID) of well-watered plots responded in the same way to changes of shoot biomass like plots grown under conditions of early drought. Due to reduced growth under drought stress, maximum values for Σ NDVI (> 85) and Σ LAID (> 330) could only be reached by plots with ample water supply.

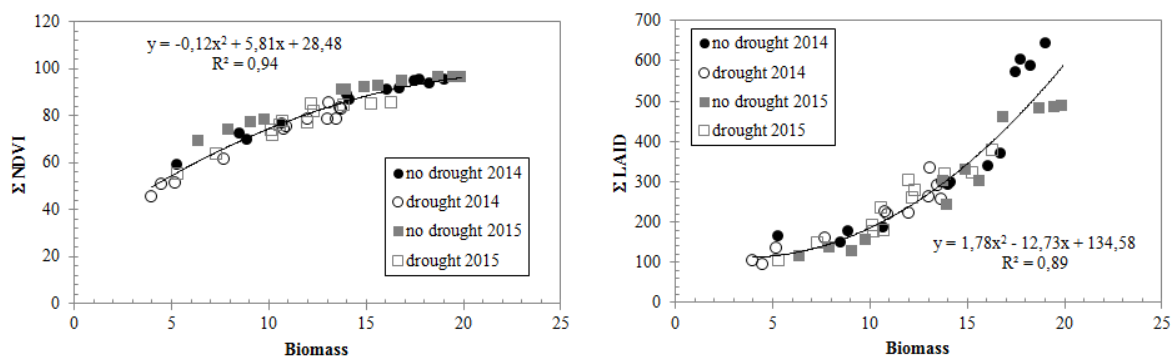


Figure 3: Regression for cumulative NDVI (Σ NDVI) and final shoot biomass ($t\ ha^{-1}$) of well-watered (closed symbols) and drought stressed (open symbols) plots in 2014 (circles) and 2015 (square). NDVI and LAI were measured during DOY 83-192 for three different N-levels (N0, N120 and N230). Regression between cumulative Leaf Area Duration (Σ LAID) and shoot biomass ($t\ ha^{-1}$) for the same duration and treatments.

Table 4: Calculated cumulative soil evaporation (E) and transpiration (T) according to FAO 56 and based on NDVI measurements. Calculated cumulative evapotranspiration (ET) is the sum of E and T. E% is the calculated share of E relative to ET. Calculations for days of year (DOY) 66-205 and 70-205 in the years 2014 and 2015, respectively. WS31 2/3 was tested in 2015, in 2014 it was not applicable (n.a.).

	Water supply	N120		N230	
		2014	2015	2014	2015
Evaporation	WS+	51	116	42	82
	WS31	29	42	27	42
	WS31 2/3	n.a.	24	n.a.	24
	WS31 1/3	2	24	2	25
Transpiration	WS+	319	310	350	381
	WS31	245	278	263	307
	WS31 2/3	n.a.	196	225	253
	WS31 1/3	172	192	n.a.	237
Evapotranspiration	WS+	370	445	392	462
	WS31	274	320	290	350
	WS31 2/3	n.a.	220	n.a.	277
	WS31 1/3	174	216	227	261
E%	WS+	14	26	11	18
	WS31	11	13	9	12
	WS31 2/3	n.a.	11	n.a.	9
	WS31 1/3	1	11	1	10

Evapotranspiration of all treatments was higher in 2015 than in 2014 (15%, 17% and 13% for N230 and 17%, 14% and 13% for N120). Highest transpiration and evapotranspiration was calculated for plots with high nitrogen supply (N230) and no limitation in water supply

(WS+) in both years. High fertilized plots used less water via evaporation compared to low N fertilized plots (Table 4). Differences between N230 and N120 in E were higher (9 mm and 34 mm in 2014 and 2015, respectively) for treatment WS+, while estimated E under drought conditions (WS31 1/3) were mostly similar for treatments N230 and N120 (≤ 1 mm). The share of E on ET was highest under unlimited water supply without fertilizer use and decreased with the (higher) use of fertilizer and less water supply (WS31 1/3).

In both years, the highest grain yields and shoot biomass, regardless of water supply levels, were reached by plots with highest N supply. WUE_y and WUE_B were highest with high N supply (Table 5).

Table 5: Grain yield (14% residual water content), shoot biomass (0% residual water content), derived from field trials in 2014 (day of year 66-205) and 2015 (day of year 70-205), agronomic water use efficiency (WUE_y) and biomass water use efficiency (WUE_B) for 3 nitrogen rates N0 (0 kg N ha⁻¹) N120 (120 kg N ha⁻¹) and N230 (230 kg N ha⁻¹) under conditions of early drought (WS31) with no rainfall and irrigation during DOY 76-128 in 2014 and DOY 77-146 in 2015, respectively, early drought with approximately 1/3 (WS31 1/3) or 2/3 (WS31 2/3) of calculated crop water use after early drought and well-watered (WS+) plots.

Year	Water supply	N-supply (kg N ha ⁻¹)	Grain yield* ¹ (t ha ⁻¹)	Shoot biomass* ² (t ha ⁻¹)	WUE_y	WUE_B
2014	WS+	120	9.8 ± 0.8	15.3 ± 1.4	2.6	4.1
		230	10.4 ± 0.5	18.2 ± 0.7	2.7	4.6
	WS31	120	7.2 ± 0.8	11.8 ± 1.3	2.6	4.3
		230	8.8 ± 1.2	13.4 ± 0.3	3.0	4.6
	WS31 1/3	120	5.7 ± 1.1	11.0 ± 1.6	3.3	6.3
		230	7.1 ± 0.7	12.2 ± 1.0	3.1	4.7
2015	WS+	120	8.9 ± 0.5	14.6 ± 0.9	2.0	3.3
		230	10.5 ± 0.8	18.8 ± 1.3	2.3	4.1
	WS31	120	6.3 ± 0.8	11.3 ± 1.2	2.0	3.5
		230	8.6 ± 1.6	14.4 ± 1.9	2.5	4.1
	WS31 1/3	120	5.5 ± 1.2	8.8 ± 1.6	2.5	4.1
		230	7.4 ± 0.9	11.4 ± 1.3	2.8	4.4
	WS31 2/3	120	5.3 ± 0.9	9.3 ± 2.1	2.4	4.2
		230	8.2 ± 0.9	12.3 ± 1.2	3.0	4.4

*¹ 14% residual water content

*² 0% residual water content

Table 6: Differences between N treatment means ($\Delta\mu$) and confidence-interval (lower limit, lwr and upper limit, upr; $\alpha:0.05$). Treatment means are pooled over both years.

Parameter	N230-N120	95% Confidence interval		
	Water requirements	$\Delta\mu$	lwr	upr
Grain yield	230-120	1.69	1.03	2.34
	WS31-WS+	-2.27	-3.14	-1.40
	WS31 1/3-WS+	-3.49	-4.36	-2.62
	WS31 2/3-WS+	-3.12	-4.18	-2.05
	WS31 1/3-WS31	-1.22	-2.09	-0.36
	WS31 2/3-WS31	-0.85	-1.92	0.22
	WS31 2/3-WS31 1/3	0.38	-0.69	1.44
ET	230-120	33.78	13.40	54.16
	WS31-WS+	-107.13	-139.58	-74.68
	WS31 1/3-WS+	-196.93	-229.38	-164.48
	WS31 2/3-WS+	-194.22	-236.04	-152.40
	WS31 1/3-WS31	-89.80	-122.25	-57.35
	WS31 2/3-WS31	-87.09	-128.91	-45.26
	WS31 2/3-WS31 1/3	2.71	-39.11	44.54
Biomass	230-120	2.63	1.64	3.62
	WS31-WS+	-3.99	-5.57	-2.41
	WS31 1/3-WS+	-5.79	-7.37	-4.21
	WS31 2/3-WS+	-5.83	-7.77	-3.90
	WS31 1/3-WS31	-1.80	-3.38	-0.22
	WS31 2/3-WS31	-1.84	-3.77	0.10
	WS31 2/3-WS31 1/3	-0.04	-1.97	1.90
WUE _y	230-120	0.23	0.05	0.41

Parameter	Pr (< F)		
	N	water	N * water
Grain yield	0.006	0.021	0.325
ET	0.022	0.010	0.390
Biomass	0.006	0.026	0.489
WUE _y	0.045	0.050	0.295
WUE _B	0.340	0.273	0.423

Water and N deficiency decreased yield and evapotranspiration. Yield reduction caused by water stress was more pronounced (higher K_y -value) under reduced N-supply compared to high N-supply for all drought treatments (Table 7). Due to the strength of drought, differences in K_y were calculated (Fig. 4). A short early drought stress (WS31) showed a stronger drought-induced yield reduction compared to an early drought with suboptimal water supply

after the early drought period (WS 31 1/3) (Table 7). This effect was more pronounced for treatment N120 compared to N230. Focusing on the same duration of drought stress (WS31 1/3 and WS 31 2/3 in 2015) from vegetation start after winter until harvest, yield reduction of higher stressed plots (WS31 1/3) reduced ET nearly in the same amount (relative changes) like the relative change of yield, so that K_y was similar.

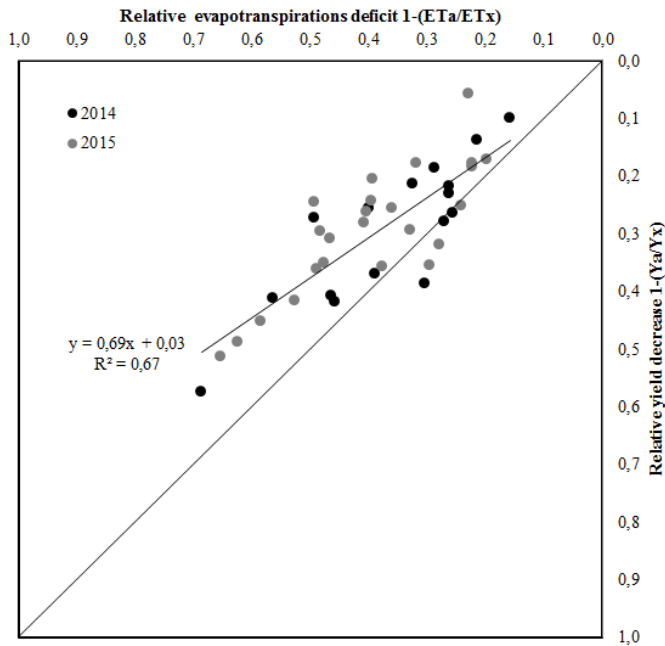


Figure 4: Influence of nitrogen and water supply on the relative evapotranspiration deficit and the relative yield decrease of winter wheat in 2014 (black circles) and 2015 (grey circles). Data are pooled over three N-rates (N0, N120, and N230).

Table 7: Influence of water supply and nitrogen supply on evapotranspiration (ET_a) compared to evapotranspiration of well-watered plots (ET_x) and on grain yield (Y_a) compared to yield of well-watered plots (Y_x) in 2014 and 2015. Derived yield reduction factors (K_y) calculated as $1-(Y_a/Y_x)/1-(ET_a/ET_x)$.

Year	Water supply	N-supply	$1-(ET_a/ET_x)$	$1-(Y_a/Y_x)$	K_y
2014	WS31	120	0.25 ± 0.06	0.26 ± 0.12	1.0 ± 0.26
		230	0.26 ± 0.03	0.19 ± 0.04	0.75 ± 0.12
	WS31 1/3	120	0.53 ± 0.13	0.42 ± 0.13	0.78 ± 0.12
		230	0.42 ± 0.08	0.32 ± 0.09	0.76 ± 0.18
2015	WS31	120	0.28 ± 0.08	0.28 ± 0.09	1.02 ± 0.14
		230	0.24 ± 0.03	0.19 ± 0.11	0.76 ± 0.37
	WS31 2/3	120	0.51 ± 0.10	0.38 ± 0.11	0.74 ± 0.07
		230	0.43 ± 0.06	0.29 ± 0.05	0.68 ± 0.06
	WS31 1/3	120	0.50 ± 0.13	0.40 ± 0.09	0.80 ± 0.07
		230	0.40 ± 0.07	0.22 ± 0.03	0.55 ± 0.05

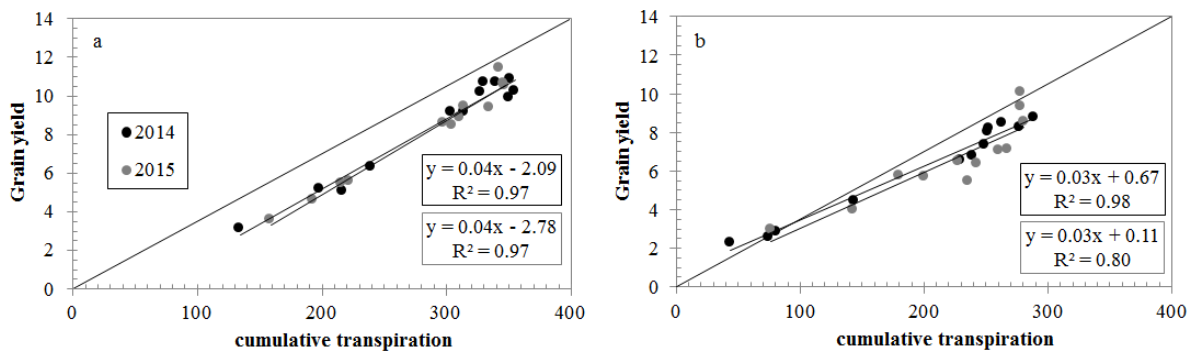


Figure 5: Influence of nitrogen supply (N0, N120 and N230) on grain yield and cumulative transpiration in 2014 and 2015 under well-watered conditions (a) and early drought (b).

Estimates of transpired water were linearly correlated to grain yield of plots which received three different N rates (Fig. 5). Under well-watered conditions (Fig. 5a) unfertilized control plots (cumulative transpiration ranged from 134 mm-240 mm) had a less efficient use of the transpired water converted into grain yield compared to N120 and N230 between which almost no differences were found. Surprisingly, the unfertilized control (Fig. 5b) tended to be more efficient under drought compared to medium (N120) and high (N230) fertilized plots. By comparing N120 to N230 a relative increase in grain yield caused by nitrogen, is followed by a similar relative increase of transpiration, so that the slope of the regression between grain yield and cumulative transpiration was almost equal.

4. Discussion

4.1 Impact of rain-out-shelter on radiation, relative air humidity and temperature

To investigate drought-related yield reduction in a humid temperate climate in North West Europe is challenging due to the frequency of rainfall. To make sure in our experiments that the intended water supply levels could be implemented in the field plots, we installed rain-out-shelters to prevent plots from rainfall during defined growth periods.

The main-driver for yield reduction of sheltered plots was shortage of water. However, it must be kept in mind that yield reduction may also have been caused by microclimatic effects of the rain-out-shelter. Reduced photosynthetically active radiation (PAR) (22%) caused by the usage of a rain-out-shelter reduced grain yield of winter wheat between 6.4 and 9.9% (Mu et al., 2010). A systematic comparison of rain-out-shelter treatments in 2013 indicated no yield differences between rain-out-shelter plots and adjacent plots which received ambient

radiation throughout the growing season (see appendix) and differences in relative air humidity and temperature were considered marginal. Based on the preliminary study in 2013, (see appendix) the shelters are considered as a suitable option to induce growth stage specific drought spells in a humid temperate climate with almost saturated soil moisture conditions after winter in North West Europe and to investigate drought-related reactions in plant growth, water use and yield.

4.2 Use of NDVI under varying nitrogen supply and water shortage

The prediction of ET_a based on crop coefficients (K_c) and drought stress coefficients (K_s) as a function of growth period is very important for determining crop water use and the scheduling of irrigation at a local scale (Allen et al., 2006). However, information about K_c , K_s and ET_a of winter wheat grown under drought conditions in a humid climate in North West Europe are scarce (Schittenhelm et al., 2014). Estimates of crop coefficients derived from NDVI are possible due to the generally close correlation between canopy size and transpiration. Advantages of using vegetation index-based crop coefficients are the ability to account for variation in plant growth due to weather conditions, differences in sowing dates (Tasumi and Allen, 2007; Er-Raki et al., 2010) or post-winter vegetation start, plant density, cultivars, pests and nutrient supply (Hunsaker et al., 2007; Pocas et al., 2015). NDVI is one of the most frequently used vegetation index and several studies showed good correlations of NDVI with plant growth parameters, e.g. in wheat (Pinter et al., 2003), measured fraction of soil cover with plants (Er-Raki et al., 2007), and leaf area (Duchemin et al., 2006; Chattaraj et al., 2013). We used NDVI for estimating K_{cb} values as it was the only option to calculate crop water use under different growth conditions (here varying N and water supply) under field conditions. Confirming our results that Σ NDVI values derived from spectral measurements were affected by N and water supply in both years (Fig. 3) Hunsaker et al. (2007) and Chapter 1 have shown a successful use of NDVI based K_{cb} values for calculating crop water use of different with N supplied plots.). Chattaraj et al. (2013) illustrated that both, wheat growth stage length and the maximum K_{cb} value, were affected by different water management and Low N supply and drought decreased green canopy cover and induced earlier onset of senescence. These differences in NDVI and LAID were consistently reflected in final shoot biomass (Table 5). NDVI-based estimates of FWB showed good correlations with the measured FWB (Table 3). With this approach crop water use could be estimated for crop stands influenced by drought or the lack of nitrogen. We conclude that such estimates of ET could be potentially used for an

integrated irrigation recommendation or to calculate water use efficiency of field sites which differ in N availability. It would be interesting to further investigate crop water use of plants that are limited in other nutrients (such as P, K, Mg, or S) and if a remote sensed based estimate of crop water use could lead to comparably promising results as indicated for N supply in this study. In summary, higher N supply resulted in increasing transpirational crop water use, but in a more efficient way, which resulted in higher grain yields under drought conditions compared to low fertilized plots.

Drought induces a cascade of physiological and morphological reactions of plants. Short-term reactions are the reductions of cell division, cell expansion, and stomatal conductance. Long-term reactions are shifts in phenology (e.g. drought escape via shortening of growth phases) and alterations of carbon allocation between leaves, stems and roots (e.g. preference of root growth). Under extended drought periods, plants skip their ambitions for maximizing growth and shift to a growth strategy aiming at grain formation and survival (Doorenbos and Kassam, 1981; Larcher, 2001). The extent of drought-induced yield reduction of winter wheat in temperate climate is influenced by soil type (water holding capacity) and soil water content at post-winter vegetation start. High initial soil moisture is considered essential for high grain yields of winter wheat which faces transient drought stress (Bruns and Croy, 1983; Eck, 1988). Schittenhelm et al. (2014) investigated the impact of extended drought (from tillering to maturity) on grain yield in a comparable climate zone and experimental set-up (North Germany, light-textured soil, rain-out-shelter) and found a 63% decrease of grain yield compared to a well-watered control treatment. We found a 32% (2014) and 30% (2015) drought-related decrease of grain yield compared to the well-watered control. Our results are in line with the findings of Schittenhelm et al. (2014) but yield depressions were not as great as treatment WS31 1/3 which received some 'life-saving' irrigation after flowering stage. That scenario of extended drought from tillering to maturity represented a worst-case scenario.

Treatment WS31 allowed us to quantify drought effects on grain yield during the distinct phenological stage of booting. Compared to well-watered plots grain yields were reduced by 1.6 (N230) t ha⁻¹ and 2.6 (N120) t ha⁻¹ in 2014 and 1.9 (N230) t ha⁻¹ and 2.6 (N120) t ha⁻¹ in 2015. Although several field studies illustrated drought-related yield reductions in winter wheat (Aparicio et al., 2000; Dencic et al., 2000), the effect of a distinct drought stress during booting stage in combination with N supply has not been reported for winter wheat in NW

Europe. Geesing et al. (2014) highlighted the relevance of interactions between rainfall, water holding capacity and N supply and indicated that a higher N-supply under drought conditions can potentially support grain yield formation due to better root growth and exploitation of water at greater soil depths. We could show that yield reduction was less with high compared to low N supply (N230 versus N120) irrespective of water stress treatments (Table 7). These results are in line with Brown (1971) and Nielsen and Halvorson (1991), however the reasons of positive N effects likely are not solely related to better access to water at deeper soil layers but due as well to compensatory stress responses of wheat when water was supplied after the transient drought stress. The comparison between treatment WS31 with optimal water supply after transient drought and treatment WS31 1/3 with low water supply afterwards allowed us to illustrate such compensatory effects of wheat yield formation. Plants that were stressed after early drought (WS31 1/3) lost compared to WS31 additional 1.7 (N230) t ha⁻¹ and 1.5 (N120) t ha⁻¹ grain yield in 2014 and 1.2 (N230) t ha⁻¹ and 0.8 (N120) t ha⁻¹.

Firstly, in our study, plants benefited from a fully water-saturated soil at post-winter vegetation start. This water, in combination with nitrogen, was used to build-up biomass until water became scarce and growth was reduced. According to Blum (1998) grain yield could partially be built up by stem reserve mobilization. Secondly, plants that were exposed to an early drought and got re-watered afterwards reacted with a reduced extent of tiller reduction after flowering resulting in an increased number of grain-bearing stems at harvest and showed a visible longer stay-green of the plants, so that these plants were able to increase the number and the weight of kernels and partially compensated that the number of ears per square meter were decreased compared to well-watered plants. This is in line with Gholamreza et al. (2013). Highest nitrogen supply could not avoid drought induced yield reduction, but high fertilized plots realized the highest yield under drought (compared to low and unfertilized plots).

Estimates of crop water use of wheat vary between 200 and 500 mm of ET depending on the climatic growth conditions and grain yield potential (Asseng, 2012). Doorenbos and Kassam (1981) showed that drought-induced yield reduction intensity depends on the growth stage when drought occurs. The most sensitive growth stage of wheat, in terms of drought stress, is around flowering with a yield reduction factor (K_y) of 0.6. Less sensitive are the yield formation period (K_y 0.5) and the period of vegetative growth (K_y 0.2). A longer drought period resulted in a more severe yield reduction when the evapotranspiration deficit was

increased. If drought intensity was increased, while drought duration was equal, K_y did not change. Current studies dealing with sensitivity of growth stages to drought have been contrary to the findings of Doorenbos and Kassam (1981) (Asseng, 2012). Turner (1997) found, that the growth period of booting and flowering until grain filling is the most sensitive one to drought. During these periods grain number is defined and with that final grain yield. Fujun et al. (1996) found lower K_y values compared to this study, with K_y values of 0.8-1.0 (heading until flowering) and a K_y for 0.6-0.7 from vegetative growth until heading. Our results showed that an early drought (WS31) increased K_y compared to an extended drought scenario (WS31 1/3). However, the concept of a linear response between relative yield decrease and relative evapotranspiration deficit according to Doorenbos and Kassam (1981) was supported by the findings of this study (Fig. 4). In the literature K_y -values are only available for plants without limited nutrient supply. So only results of treatment N230 could be compared with that of Doorenbos and Kassam (1981). Comparing results of N230 and N120 showed that K_y -values of N120 plots were higher compared to those of N230 implying that lower N supply lead to higher yield reduction under drought. The beneficial effects of N supply on grain yield under transient drought, as indicated by these results, underline the requirements to identify the optimal N rate under drought conditions. In this context it appears that the linear regression between K_s and K_y is potentially useful for a drought-induced yield reduction estimate during the booting growth stage and related adaptation of the third N fertilizer application.

5. Conclusion

Results of this study indicate that a higher N supply, compared to low N supply, increased grain yields even under early and extended drought conditions in a humid temperate climate in NW Europe. These findings are in contrast to findings in arid environments where a higher N supply negatively affected grain yield compared to low N supply. We attribute this positive N effects to conditions of almost fully saturated soils at post-winter vegetation start and the ability of wheat to compensate yield reduction during booting stage by increased number of ear-bearing stems, higher grain kernel weight and delayed senescence, if wheat received water during flowering and grain filling.

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General Discussion (Synopsis)

Evapotranspiration of winter wheat estimated with the FAO 56 approach and NDVI measurements in a temperate humid climate of NW Europe

Winter wheat is not regularly irrigated in the NW-European climate, although transient droughts occur and irrigation would be profitable on light-textured soils (El Chami et al., 2015). The FAO56 dual coefficient approach is widely used for irrigation management and estimates of crop water use (Allen et al., 2011; Peireira et al., 2015) and local adjustments of stage duration and basal crop coefficients are recommended (Ko et al., 2009; Allen et al., 2011). Information about *in-situ* crop growth is required for estimating temporal dynamics of ground cover and optical methods are increasingly used for this purpose (López-Urrea et al., 2009; Nielsen et al., 2012). A ground cover of 80% is assumed to indicate the transition point between the developmental and mid-stage in the FAO56 approach (Allen et al., 2006). The most suitable proxy of ground cover for agricultural crops is LAI, which was measured destructively and non-destructively and could be predicted by the NDVI as indicated by the quadratic-plateau relationship between both parameters. In the FAO56 approach, temporal dynamics of ground cover are predicted by linear extrapolation between vegetation start (>10% ground cover) to the mid stage while NDVI allows for site-specific consideration of crop growth. This is relevant as the growth of winter wheat in temperate-humid climate exhibits a large year-to-year variability due to potential frost damage during winter and variable onset of growth after winter. Tabulated data of stage lengths are therefore difficult to apply under these conditions as time of cardinal events (canopy closure, onset of senescence, maturity) was variable and the approach of Fischer et al. (2000) in which fixed portions of the total post-winter growth period are allocated to developmental stages cannot be used for irrigation as it relies on information about final harvest date. NDVI-based estimates of seasonal crop water use of well-watered winter wheat with 230 kg of plant available N per hectare were compared with the FA approach. Differences in evapotranspiration (ET) estimates between both approaches were rather small with 26 mm less water in 2014 and an additional water demand of 33 mm in 2015 compared with the FA approach. Both approaches of seasonal ET dynamics agreed with the measured FWB data and our results are in line with Sanchez et al. (2012), who compared NDVI-derived K_{cb} -values with measured soil moisture. Discrepancies between measured and modelled data can be partially explained by systematic

offsets between calculated (FA- and NDVI-approach) and measured FWB. For short time periods (after bigger rainfall events, e.g. in 2014), the measured soil water content was higher than the total available water (TAW) and soils drained back to 'realistic' TAWs. In summary, the NDVI appears as a promising monitoring parameter for crop development during the growth period. Furthermore, it gives the opportunity to calculate online and not retrospectively like the FA-approach crop water use and water demand under non-optimal growth conditions such as water or nutrient stress.

ET and transpiration reacted sensitively to changes in stage duration. The lengths of stage durations of winter wheat are defined by photoperiod and temperature and can be successfully predicted for NW European climates (Ewert, 1996). The use of tabulated data of stage lengths (FA approach) is recommended if no site-specific information is available or required (such as in national water footprint accounting, e.g. Chapagain et al., 2004), while the NDVI-based estimates of K_{cb} do not require stage lengths and are, therefore, closer to year- and site-specific crop growth. The post-winter growth of winter wheat is difficult to predict in terms of the onset of growth and canopy cover both of which are crucial information for ET estimated during the initial growth stage. These particular environmental conditions, with mild to very cold winter seasons and potential frost damage during spring, renders the application of tabulated stage lengths and K_c values and clearly indicated that optical information such as NDVI should be preferred. Estimated ET of the two years varied between 377 and 463 mm and, related to grain yield, gave agronomic water-use efficiencies (WUE_y) between 1.93 and 2.76 g l⁻¹. These estimates are high, but not unrealistic compared with estimates of WUE_y of winter wheat from a global survey (Zwart and Bastiaanssen, 2004). These results indicate that winter wheat, not limited by water and nutrients (and well protected from diseases) was very water use efficient. We speculated that year differences in WUE_Y could be explained by climate differences as the same variety and sowing density was used in both years.

Calculated field water balance (FWB) based on the NDVI-approach showed a good correlation with the measured FWB, but it must be kept in mind that other parameters like soil texture (and with that water holding capacity of the soil) are influencing the calculated FWB. For further improvements of irrigation decision tools, information about soil texture distribution over depth and effective rooting depth would be helpful to increase the accuracy of FWB estimates. Further research is also needed for a better prediction of post-winter

vegetation start as reactions of the NDVI signal were regularly seen when plants already had started to grow. Alternative indices might be better suited to predict early growth stages.

NDVI-based estimates of evapotranspiration of winter wheat indicate positive effects of N fertilizer application on agronomic water-use efficiency

The NDVI approach is the only option for estimating basal crop coefficients (K_{cb} values) and to calculate crop water use under non-optimal growth conditions (here limited N supply) under field conditions. Confirming results of Hunsaker et al. (2007) that wheat growth stage length and the maximum K_{cb} value were affected by different N management in wheat our results similarly showed that K_{cb} values derived from NDVI measurements were affected by N supply in both years. Low N supply decreased maximal K_{cb} values, delayed canopy closure and induced earlier onset of senescence. These differences in NDVI were consistently reflected in final shoot biomass (Fig. 3), indicating that cumulative NDVI was a suitable index of carbon gain over the post-winter growth period. Efficient water management in agriculture is key for sustainable water use and nutrient management of cropping systems is closely related to water resources for two reasons, crop water use and potential pollution of freshwater by overdosing of nutrients. Intensive agriculture, including fertilizer application, has been identified as a threat to regional long-term water availability of cropping systems (Liu et al., 2015). However, yield of wheat, rice and maize in a global survey, were shown to positively correlate with agronomic water-use efficiency (Zwart and Bastiaanssen, 2004), indicating that low-input agriculture is less water-use efficient when expressed as product per unit of ET, indicating a trade-off between water conservation and efficient water use for food production. Results of our study indicate that increases in ET due to N applications were small for winter wheat in temperate humid climate, as the decrease of T under low N was strongly off-set by corresponding increases of evaporation. This finding cannot be extrapolated to drier climates with less rainfall events as high evaporation in the present study was realized by frequent wetting, keeping the soil more or less continuously in the high evaporation stage 1 (Ritchie, 1972). Bare soil evaporation is controlled by available energy, RH and wind speed, topsoil water content and canopy cover. It can be expected that estimates of E are improved by measurements of the fraction of soil wetted and exposed to direct radiation (Peirera et al., 2015), thereby adding more accuracy to the estimation of E and total ET. Johnson and Troust (2012) found a good linear correlation between NDVI and canopy

cover of vegetable crops by comparing NDVI measurements and estimates of canopy cover derived from images taken by a digital ground camera. NDVI or LAI are suitable to estimate the fraction of canopy cover particularly under conditions of nutrient limitation and should be preferred over tabulated data of crop coefficients. Compared with winter wheat, the relevance of monitoring in-situ canopy development will be even higher in row crops with extended periods of incomplete ground cover. Comparison with measured evaporation rates and the field water balance indicate that both methods were equally suitable to predict ET but the comparison with field water balance data is not straightforward as the extent of drainage and capillary rise remains unknown under the experimental conditions (high rainfall and supplemental irrigation). Our results indicate that the proportion of evaporation relative to ET decreased with N supply from >60% to <30% until BBCH 49 while the same transpirational WUE was assumed for the three N treatments. If N fertilizer application reduces E while T increases in proportion to dry mass gain, N application effects on WUE would be large in 'high E' environments such as humid climates with frequent soil surface wetting and small (or even absent) in 'low E' environments such as in arid climates with subsurface irrigation. Rainfall and irrigation in our experiment generated a humid environment so that increases in ET from N0 to N230 were small compared to N-induced yield increases, resulting in a substantially lower WUE_Y of N0 compared to N120 and N230. Differences in ET and WUE_Y between moderate (N120) to high (N230) N supply were small in both years. Consequently, positive N effects on WUE_Y could be explained by a shift from evaporation to transpiration and are in line with other field studies which were conducted in arid and semiarid environments (e.g. Eck, 1988; Zhang et al., 1998; Asseng et al., 2001). An increase of WUE_Y via increases in HI could not be confirmed. Wheat in treatment N230 produced more biomass than in treatment N120 but failed to increase correspondingly yield components, such as number of ears, spikelets per ear or thousand grain weight (data not shown). WUE_Y of N230 was not significantly higher than that of N120, while differences in WUE_B between both treatments were more obvious. This finding suggests that high N supplied plants tended to produce 'luxury' leaf and stem reserves and that high N rates do not automatically guarantee the highest WUE_Y .

As nutrient limitation can strongly affect plant growth, it would be interesting to investigate crop water use of plants that are limited by other nutrients (such as P, K, Mg, or S) and if a

remote sensing based estimate of crop water use could lead to comparably promising results as indicated for N supply in this study.

NDVI-based estimates of evapotranspiration of winter wheat indicate positive effects of N fertilizer application on agronomic water-use efficiency under drought conditions

With the NDVI-approach crop water use could be estimated for crop stands influenced by drought or the lack of nitrogen. We conclude that such estimates of ET could be potentially used for an integrated irrigation recommendation or to calculate water use efficiency of field sites which differ in N availability. Our results showed, that higher N supply resulted in increasing transpirational crop water use, but in a more efficient way, which resulted in higher grain yields under drought conditions compared to low fertilized plots. We could show that yield reduction was less with high compared to low N supply (N230 versus N120) irrespective of water stress treatments (Table 3). These results are in line with Brown (1971) and Nielsen and Halvorson (1991), however the reasons of positive N effects likely are not solely related to better access to water at deeper soil layers but due as well to compensatory stress responses of wheat when water was supplied after the transient drought stress. But why could high fertilized plots cope better with drought compared to low fertilized plots? Firstly, in our study, plants benefited from a fully water-saturated soil at post-winter vegetation start. This water, in combination with nitrogen, was used to build-up biomass until water became scarce and growth was reduced. According to Blum (1998) grain yield could partially be build up by stem reserve mobilization. Secondly, plants that were exposed to an early drought and got re-watered afterwards reacted with a lower extent of tiller reduction after flowering resulting in an increased number of grain-bearing stems at harvest and showed a visible longer stay-green of the plants, so that these plants were able to increase the number and the weight of kernels and partially compensated that the number of ears per square meter were decreased compared to well-watered plants. Highest nitrogen supply could not avoid drought induced yield reduction, but high fertilized plots realized the highest yield under drought (compared to low and unfertilized plots).

Furthermore the concept of a linear response between relative yield decrease and relative evapotranspiration deficit according to Doorenbos and Kassam (1981) was supported by the findings of this study. In the literature K_y -values are only available for plants without limited nutrient supply. So only results of treatment N230 could be compared with that of Doorenbos

and Kassam (1981). A comparison of treatments N230 and N120 showed that K_y -values of N120 plots were higher compared to those of N230 implying that lower N supply lead to higher yield reduction under drought. The beneficial effects of N supply on grain yield under transient drought, as indicated by these results, underline the requirements to identify the optimal N rate under drought conditions. In this context it appears that the linear regression between K_s and K_y is potentially useful for a drought-induced yield reduction estimate during the booting growth stage and related adaptation of the third N fertilizer application.

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Appendix Chapter 3

Table A1: Grain yield (t ha^{-1}) and dry matter (with 14% water in the plants) of plots with rain-out-shelter (WS+) and without rain-out-shelter (WSO+) at different N-rates (kg N ha^{-1}) in 2013.

N-rate	Grain yield		Dry matter	
	WS+	WSO+	WS+	WSO+
120	8.9 ± 0.4	8.9 ± 0.3	12.9 ± 0.1	13.2 ± 0.6
150	9.9 ± 0.5	10.4 ± 0.2	13.8 ± 0.5	14.5 ± 0.5
170	10.6 ± 0.7	10.4 ± 0.2	16.1 ± 0.6	16.5 ± 0.6
180	10.7 ± 0.2	10.8 ± 0.1	15.2 ± 0.4	15.5 ± 0.4
200	10.7 ± 0.4	11.1 ± 0.1	16.6 ± 1.1	17.0 ± 0.9
230	10.8 ± 0.1	11.1 ± 0.5	17.7 ± 0.9	18.0 ± 0.5

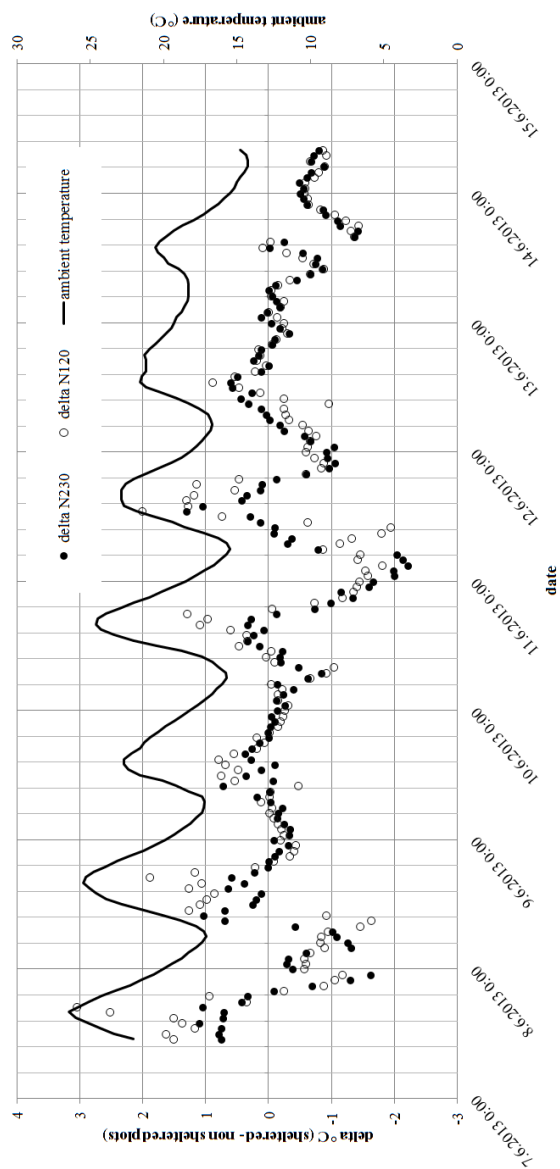


Figure A1: Measured temperature difference (Δ °C) of plots with rain-out-shelter (sheltered) and plots without rain-out-shelter (none sheltered) are shown on the left y-axis for hourly data starting at 11:00 a.m. on June 6 in 2013 until 8:00 a.m. at June 15 in 2013. Plots received two different nitrogen rates 120 kg N ha^{-1} (N120, open circle) and 230 kg N ha^{-1} (N230, closed circle), all treatments were not limited in water supply. Ambient temperature (°C) (black line) is shown at the right y-axis.

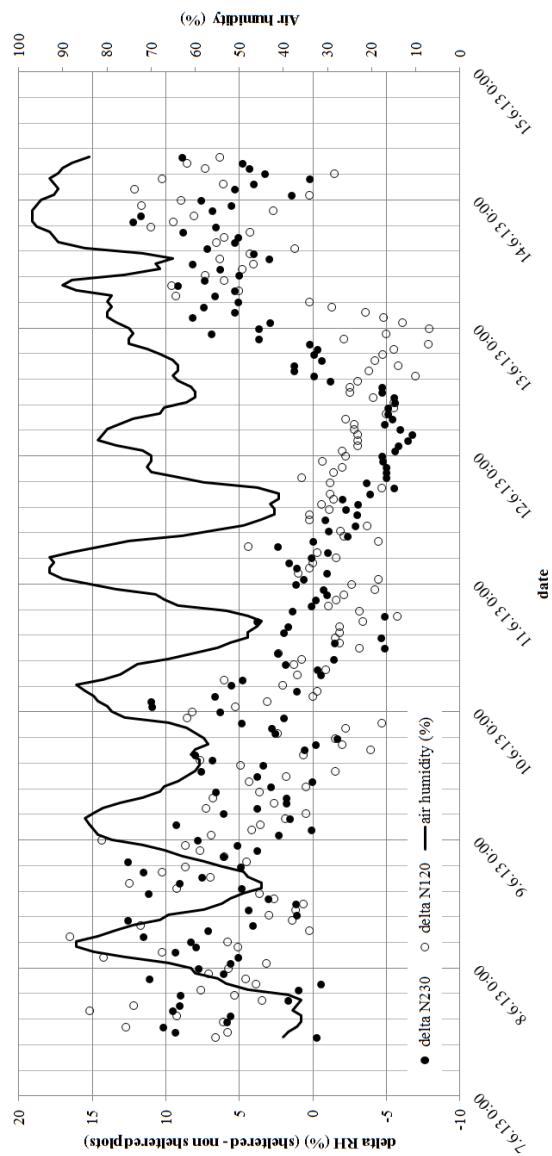


Figure A2: Measured air humidity difference (delta RH) of plots with rain-out-shelter (sheltered) and plots without rain-out-shelter (none sheltered) are shown on the left y-axis for hourly data starting at 11:00 a.m. on June 6 in 2013 until 8:00 a.m. at June 15 in 2013. Plots received two different nitrogen rates 120 kg N ha^{-1} (N120, open circle) and 230 kg N ha^{-1} (N230, closed circle), all treatments were not limited in water supply. Ambient air humidity (%) (black line) is shown at the right y-axis.

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