Proof of concept: Nitrogen use efficiency of contrasting spring wheat varieties grown in greenhouse and field

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Abstract

Aims Major aims were to test and evaluate a new concept for assessment of nitrogen use efficiency (NUE) of crops by growing six spring wheat varieties in greenhouse and field environments. NUE was calculated with a plant based concept integrating the entire crop life history and separating plant characteristics from environmental factors affecting NUE. Specific hypotheses were tested related to the varieties' drought and nutrient fertilisation responses for NUE components, and coherence of those responses in field and greenhouse.

Methods The wheat (*Triticum aestivum* L.) cultivated varieties 'Diskett', 'Granary', 'Quarna', 'Stilett', 'Vinjett', and a Swedish landrace ('Dala') were grown in field and greenhouse environments in Central Sweden. Two fertilisation treatments were included in a field and greenhouse experiment, and in the greenhouse also drought. The NUE components N uptake efficiency (U_N), grain-specific N efficiency ($E_{N,g}$) and grain N concentration ($C_{N,g}$) were assessed.

Results Drought reduced yield and NUE through $E_{N,g}$, and more so when drought occurred prior to anthesis than after anthesis. Effect of fertilisation treatment on NUE components was similar in the two set-ups, but there were fewer variety x fertilisation interactions in the field. U_N was higher in the field and $E_{N,g}$ was higher in the greenhouse, while $C_{N,g}$ and overall NUE were similar in the two environments. Ranking of varieties regarding NUE and U_N was similar in the greenhouse and field, but different regarding $E_{N,g}$ and $C_{N,g}$.

Conclusions The NUE concept is a useful tool to describe and integrate important NUE components for crops grown in different treatments (nutrient fertilisation, drought) and experimental set-ups, i.e. greenhouse and field. Similar variety ranking in overall NUE across experimental set-ups indicates stable results in different environments.

Abbreviations

N Nitrogen

NUE Nitrogen use efficiency

Introduction

Agricultural crops are often fertilised with nutrients to increase yields. However, the use of fertilisers also has negative consequences, e.g. emissions of the potent greenhouse gas N_2O and increased nutrient leaching to the environment causing eutrophication (Canfield et al. 2010). At the same time, use of fertilisers, especially nitrogen, is driven by economic pressure on farmers to maintain high crop yield and quality, and a demand for secure food supplies for the world's population. The importance of in particular nitrogen (N) for production in conjunction with the possible negative environmental consequences of its use make N use efficiency (NUE) important in the development of sustainable food production.

Many methods have been used to asses NUE. In research on cereals the concept presented by Moll et al. (1982) is often used. It is defined as the grain yield per unit available N in the soil and is hereafter referred to as NUE_{Moll}. It can be divided into uptake efficiency (units of plant N per unit of soil N) and utilisation efficiency (units of grain yield produced per unit plant N). These two components have often been compared between varieties and fertilisation levels in order to determine which component is more important for overall NUE_{Moll}, but the results are inconsistent (Le Gouis et al. 2000; Moll et al. 1982). The approach by Moll et al. (1982) considers only the crop N and grain biomass at harvest, which is the *outcome* of growth and development processes occurring over a long period in which N not always is the most limiting factor for growth. However, N use efficiency is most relevant during the major growth period when N is limiting for growth. In this study we used an approach that considers aspects from grain sowing to harvested product, which is presented in detail by Weih et al.

(2011) and referred to as NUE_{Weih}. The NUE components in this approach address similar processes to the Moll et al. (1982) definition, but an additional component is added and two are redefined to include N retranslocation and N use during the major growth period. The components are (1) N uptake efficiency (U_N) based on initial plant N, (2) grain-specific N efficiency ($E_{N,g}$), which is the efficiency of converting plant N to grain biomass, and (3) grain N concentration ($C_{N,g}$) which is related to N retranslocation (Table 1). In this approach, the possible significance of seed N resources for early growth is recognized, and the plant's ability to multiply the N available in seeds is compared and evaluated in detail by means of the three NUE components. Environmental factors are assumed to affect the NUE and its components, but are not an intrinsic part of the equation. This means that increased external resource supply like added nutrient supply may increase NUE_{Weih}, while it would typically decrease NUE_{Moll}. The clear separation of plant characteristics and environmental factors affecting NUE facilitates identification of desirable crop traits for improved NUE by variety selection (e.g. variety ranking) and plant breeding.

In general, efficiency of nutrient use has been studied independently in different kinds of experiments (here referred to as experimental set-ups), like in the greenhouse or field. However, to the best of our knowledge there are only few reports of studies in which efficiency of nutrient use is investigated with the same plant material grown in greenhouse and field set-ups. For example, twenty-five winter wheat cultivars had different phosphorous use efficiency in the greenhouse compared to field (Gunes et al. 2006) while 40 bread and durum wheat cultivars responded similarly to Zn fertilisation in the greenhouse and field in another study (Kalayci et al. 1999). Greenhouse experiments offer several advantages compared to field experiments: The conditions are often easier to control and to repeat, resulting in reduced uncontrolled variation and thereby increased possibilities of detecting significant differences between treatments. Furthermore, experimental treatments are often

easier to apply in the greenhouse and costs are often lower. It is often more feasible to include extreme conditions in a greenhouse experiment, making it easier to find genotype environment interactions. There are however drawbacks regarding how the results can be interpreted in their proper context in the field. Some of these drawbacks are related to the pot environment. Pots are often saturated with water at least in the bottom, leading to hypoxia. Pot soil also often has a higher temperature than both the greenhouse air and normal field soil temperatures, due to the sun shining on the (often black) surface of the pot (Passioura 2006). Growth in (small) pots generally reduces plant biomass (Poorter et al. 2012). There could also be effects related to the aboveground conditions, which may differ between a plant located in a dense crop stand under full natural radiation in a field and a plant in a greenhouse with artificial lighting and often less shading from neighbouring plants. A comparison of nutrient use efficiencies especially regarding N (i.e. NUE) using contrasting genotypes grown under differing conditions, such as in the greenhouse and field, could improve our understanding of plant – soil – environment interactions and facilitate interpretation of results deriving from different experimental set-ups.

Table 1 close to here

The availability of water for agricultural production will decrease in many parts of the world according to future scenarios on the effects of climate change on agriculture. For example, large parts of Sweden are predicted to face more severe summer droughts in the future (Swedish Commission on Climate and Vulnerability 2007). The impact of drought on wheat production depends on the timing of the drought event. Early-season drought reduces the formation of flower structures and grain number, and differs from the Mediterranean-type terminal drought affecting grain filling and reducing grain size (Ferris et al. 1998; Ji et al. 2010). The effect of drought on grain number occurring around flowering is often considered the main contributor to yield losses under drought (Ji et al. 2010). In terms of NUE, those yield losses are expected to affect especially the efficiency of converting plant N to grain

biomass (i.e. the grain-specific N efficiency, $E_{N,g}$ in the terminology by Weih et al. 2011). Apart from timing of the drought event, the performance of wheat under drought compared with irrigation is affected by genotype and genotype × drought interactions (Fischer and Maurer 1978). Also the effect of nutrient fertilisation is dependent on the genotype (i.e., genotype × fertilisation interaction) (Górny and Garczynski 2008). In addition, crop water and N use are interrelated but few studies deal with NUE in different varieties exposed to various combinations of fertiliser and drought treatments (Cabrera-Bosquet et al. 2007; Giuliani et al. 2011).

Apart from concept (Weih et al. 2011) test and evaluation, the specific objectives of this study were to evaluate the effects of genotype and environment on different NUE components across a set of spring wheat varieties grown in different experimental set-ups. We tested the hypotheses that (i) early drought (before and at anthesis) reduces grain yield, grain-specific N efficiency and NUE more than late drought (after anthesis); (ii) the effects of drought and fertilisation treatments on NUE and its components vary between different varieties (i.e. G × E interaction); and (iii) ranking in NUE aspects of different varieties is similar in different experimental set-ups. We tested these hypotheses with six varieties of spring wheat grown in a field experiment with two fertilisation treatments and in a greenhouse pot experiment with two fertilisation and three drought treatments.

Materials and methods

Plant material

The spring wheat (*Triticum aestivum* L.) cultivated varieties 'Diskett', 'Granary', 'Quarna', 'Stilett', 'Vinjett', and a natural variety (landrace) from Dalecarlia, here called 'Dala' were used. The varieties represented the span of variation in grain yield, grain protein content, grain size, plant height and maturation time recorded in the 2008 Swedish variety trials (Larsson et al. 2008), or experience in the case of Dala. Our aim in selection was to ensure that the varieties included were dissimilar, but still well adapted to the growth conditions in Sweden. Granary is a high-yielding late maturing variety, Quarna has high grain protein concentration and early maturity and Stilett is a short variety with low grain weight. Vinjett is used for comparisons in Swedish spring wheat variety trials, and is a relatively tall variety. The traits of Diskett are intermediate. The Dala landrace is very tall and low yielding, with heavy grains and high protein concentration, and had been grown in the area of the field experiment for 10 generations. Diskett, Granary, Stilett and Vinjett seeds were treated with bitertanol and fuberidazole, while Quarna seeds were treated with guazatine. The seeds of the Dala landrace were untreated.

Experimental design

The field experiment was designed as a complete block split-plot with four replications. Main plot factor was fertilisation treatment, F_L and F_H (fertilisation low or high), and varieties were randomized subplots within each fertilisation treatment. The greenhouse experiment also had a complete split-plot design with four replications, and single pots as experimental units. Main plot factors were combinations of fertilisation (F) treatment, drought (D) treatment and harvest time (H), and the sub-plot factor was variety (V). The fertilisation treatments F_L and F_H; the drought treatments D0 (no drought), D1 (drought before anthesis) and D2 (drought

after anthesis); and three harvest times H1 (seedling stage), H2 (before anthesis and drought treatments) and H3 (ripening), in all relevant combinations (e.g. the combination D2 and H1 is not relevant), were randomised within each block. The six varieties of spring wheat were randomised within each treatment combination.

Experimental management

Field experiment

The field experiment was conducted in 2010 and was situated near Uppsala, Sweden (59°50'N, 17°47'E). The mean temperatures for May, June, July and August were 11.0 °C, 15.0 °C, 20.4 °C and 16.5 °C respectively, and the precipitation sums were 54, 38, 69 and 89 mm, respectively (climate data from the Ultuna meteorological station situated about 8 km from the experimental site). The previous crop was pea. The experimental plots were 2 × 16 m. Destructive sampling was limited to the three outermost meters in each end of the plots, while 10 m in the centre were kept intact for grain yield determination. Sowing took place on 29 April, with 550 viable seeds m^{-2}, which is the standard seed rate for spring wheat in variety trials in Sweden. The row spacing was 12-13 cm and sowing depth 3-4 cm. On 30 April 2010 the high fertilisation treatment, F_H, received 81 kg N ha^{-1} as ammonium nitrate mixed with calcium carbonate and sulfur (0.27 g g^{-1} N). The low fertilisation treatment, F_L, did not receive any fertiliser. There were sufficient amounts of P and K in the soil of the field experiment, and plant growth could be assumed to be N-limited in both F_L and F_H. Herbicides Ariane S plus Hormotex were applied once to control weeds. There was no need for any pest or disease control.

Soil samples were taken in each block to determine soil type (6-7 November 2009) and soil mineral N (14-15 April 2010). At each sampling occasion, twenty subsamples per block were taken at the level 0-30 cm, and 10 subsamples from the levels 30-60 and 60-90 cm; the

samples were pooled for each depth. After storage in the freezer, samples for ammonium and nitrate analysis were milled and extracted using 2 M KCl at a 125 g fresh soil: 250 mL KCl ratio and concentrations were determined using an auto analyser (TRAACS 800, Germany). The top 30 cm of the soil was silty clay (British Standards Institution) with 0.056 g g^{-1} organic matter content. The soil pH (H_2O) was 6.4, 6.9 and 7.1 (0-30, 30-60, 60-90 cm). The mean total amount of ammonium and nitrate N in 0–90 cm of the soil was 95 kg ha^{-1} before addition of fertiliser in spring.

Greenhouse experiment

The greenhouse experiment was carried out from 8 February to 21 May 2010 in a greenhouse in Uppsala, Sweden (59°49'N, 17°39'O). The light regime was ambient light supplemented with 16 h artificial light per day. Day temperature was set to 18 °C and night temperature to 12 °C, and the maximum and minimum hourly mean temperatures were 29.4 °C and 9.2 °C respectively. The overall mean temperature was 16.7 °C. Photosynthetically active radiation (PAR, 400-700 nm) was recorded during three days in March at the top of the pots and ranged between 400 and 130 µmol m^{-2} s^{-1} at daytime. White metal stands were placed around each pot to prevent lodging. The experimental units were 5.5-L pots placed on individual plates. A 50 cm x 50 cm square of woven plastic cloth was placed in the bottom of each pot. The pots were filled with 4.5 L fine Perlite and washed with 2 L deionised water. The seeds were placed on the moist surface and covered with 0.5 L Perlite, creating a sowing depth of 2-3 cm. Sowing was performed on 8 February 2010 and seven days later most seeds had germinated and the first leaves were 1-2 cm above the Perlite surface. Hence 15 February was used as the day of emergence, day 1 of the experiment. The 19 seeds sown per pot were thinned down to 15 plants on day 17. This corresponds to a plant density of 550 plants m^{-2}. The plants were

watered every 2-3 days and treatments were circulated within blocks in a systematic manner on the watering occasions. All pots were placed close to each other without paths. No pests or diseases were observed.

Fertiliser was applied 3 times a week as 50 mL solution. The following standard nutrient mix was used (g L^{-1}): N 51, Ca 3, P 10, Mg 4, K 43, S 4, Mn 0.2, Fe 0.17, Cu 0.015, Zn 0.03, B 0.1, Mo 0.004. The mix was diluted in deionised water and applied in increasing amounts as the plants grew larger, so that the N supply ranged between 2.5 and 400 mg N pot^{-1} $week^{-1}$ in the high fertilisation treatment (F_H) and 1/8 of those levels in the low fertilisation treatment (F_L). In the greenhouse experiment, nutrients other than N were added in their corresponding proportions (i.e. higher concentrations in the high than low fertilisation treatment) to avoid that other nutrients than N would limit plant growth. The F_H treatment received a total of 2256 mg N per pot and F_L received 287 mg N per pot (corresponding to 150 mg and 19 mg N per plant, respectively). The low fertilisation level was intended to represent a condition with nutrient supply far below optimum, and the high level a condition with nutrient supply close to or above optimum.

Three different drought treatments were applied. In the D0 treatment plants were watered throughout the whole experiment. In the D1 treatment drought started on day 45 when plants in the most developed pot had reached beginning of anthesis (BBCH 61 according to Lancashire et al. 1991), and the flag leaf of the least developed plants was just visible (BBCH 37) (Table 2). In the D2 treatment drought started on day 64 after plants in all pots in all treatments had reached anthesis. The drought treatments consisted of withdrawn watering for 9 (early drought, D1) or 11 days (late drought, D2). The drought was ended and full watering resumed when there were visible differences between the pots in terms of plant condition and many had started wilting. Fertiliser was given throughout the drought periods.

Table 2 close to here

Measurements

Field experiment

Samples of five plants per plot were taken before the major growth period (H1, 24-31 May, around BBCH 13) and after the major growth period (H2, 5-8 July, BBCH 55-69). Each block was sampled within one day. At H1 five plants were chosen randomly from an area of 3 × 2 m at the ends of the plots, while at H2 five plants were chosen randomly only from the second outermost rows of the plots. The plants were uprooted to try and make sure all shoots were included and afterwards cut with scissors at ground level. The plants were stored in plastic bags in a fridge for maximum 2 days, and dried in 60 °C for minimum 3 days. The dried plant biomass was ground using a knife mill, thereafter with a ball mill. The ball mill grinding and the nitrogen analysis were carried out by Waikato Stable Isotope Unit (The University of Waikato, Hamilton, New Zealand) using a Dumas elemental analyser (Europa Scientific ANCA-SL) interfaced to an isotope mass spectrometer (Europa Scientific 20-20 Stable Isotope Analyser) (Europa Scientific Ltd, Crewe, U.K.).

The number of plants m^{-2} was assessed on 28 May and 1 June 2010 by counting plants on four running metres in each plot. They were counted on 2 adjacent 1-m sections on the 3rd and 4th row from the side, on two locations in the plot situated diagonal to each other at each end of the plot. Grain yield was determined from the inner 20 m^2 in each plot on 28 August 2010. Subsamples of grains were analysed for water and N concentrations (based on a conversion factor of 5.7 from protein concentration) using the near infrared transmittance (NIT) method (Infratec™ 1241 Grain Analyzer, Foss, Denmark).

A final harvest to determine aboveground biomass (B) was carried out on 20 August. A total area of 0.5 m^2 was sampled from each plot, i.e., one square of 0.5 × 0.5 m in each end of the plot. The samples were dried in 60 °C for 3 days.

Greenhouse

Harvest 1 (H1) was performed on days 10-12 (BBCH 11), harvest 2 (H2) on days 39-40 (BBCH 41-49) and harvest 3 (H3) on days 93-96, around BBCH 91. Separate pots were allocated to each harvest. At H1 and H2, a representative sample of five plants per pot was taken at surface level. At H3 all plants in the pots were harvested and threshing was performed with a sample threshing machine (Saatmeister, Bad Godesberg, Germany). Seedlings, straw and ears were all dried at 60 °C for at least 2 days and weighed.

Nitrogen concentration was analysed in aboveground biomass from all harvests, at H3 separately in straw and grain, but not including the chaff. Chaff was assumed to have the same N concentration as the straw. The dried plant biomass was ground using a knife mill and then a ball mill. The ball mill grinding and N analysis were carried out by the Waikato Stable Isotope Unit (University of Waikato, Hamilton, New Zealand). The N analysis was performed with a Dumas elemental analyser (Europa Scientific ANCA-SL) interfaced to an isotope mass spectrometer (Europa Scientific 20-20 Stable Isotope Analyser, Europa Scientific Ltd, Crewe, U.K.) or a LECO (Truspec CN determinator, LECO Corporation, US).

Nitrogen use efficiency

Nitrogen use efficiency and NUE components were calculated according to the method of Weih et al. (2011a) (Table 1). The major growth period was the period between the harvests H1 and H2, and H2 in the greenhouse was performed before the initiation of any drought treatment. This means that U_N was calculated based on N uptake prior to any drought treatment. We determined harvest dates and initiation of drought treatments based on fixed points in time rather than the developmental stage that was used by Weih et al. (2011). This difference was considered necessary to ensure that all plants experienced similar environmental conditions between the harvests, thus avoiding different varieties being

exposed to different environments when grown in the same experimental treatment. For the field experiment, NUE and its components were calculated per m^2, while in the greenhouse NUE was calculated per plant. The measures are still comparable since extrapolating the pot values to m^{-2} would in fact not change the values of NUE and its components. The plant density was instead included as a covariate in the statistical analysis since we expect plant density to affect NUE. For grain and total aboveground biomass (B_g and B, respectively) the values are dependent on the choice of denominator, and we have presented results per plant both from the greenhouse and the field. The variety patterns were unchanged when greenhouse values were extrapolated to an area based measure.

Statistical analysis

The statistics were performed separately for the two experiments. In both cases the NUE components were analyzed with the software SAS® procedure mixed, using the REML estimation method and the Kenward-Roger method (Kenward and Roger 1997) for calculating the fixed effects standard errors and degrees of freedom. Homogeneity of variances and normality were examined graphically. Fertilisation treatment and variety were treated as fixed effects and block as random effect. For the greenhouse experiment, drought was also considered a fixed effect while block x fertilisation x drought (for U_N only block × fertilisation) were treated as a random effects. Plant density was used as a covariate for all components in the analysis of field data. In the analysis of greenhouse data plant density was used as a covariate for NUE components related to the last harvest, since although the pots were thinned to 15 plants some re-emerged. For the field analysis, N uptake efficiency (U_N) and NUE were log-10 transformed. For the greenhouse analysis, NUE and U_N were log-10 transformed and grain-specific N efficiency ($E_{N,g}$) was square-root transformed.

In the greenhouse the variables grain N concentration ($C_{N,g}$), NUE and grain biomass (B_g) showed greater variability in the F_H-D1 treatment combination than in the other combinations.

For these variables, a model with residual error variance depending on treatment combination was fitted. This model included two residual error variances, as the F_H-D1 combination had a different residual error variance than the other combinations.

All statistics were computed with the software SAS version 9.3 (SAS Institute Inc., 2002-2008). Plots were made with the statistical programming language R version 2.14.2 (R Development Core Team, 2009).

Results

Effect of experimental set-up

Fertilisation treatment affected NUE components both in the field (Figure 1) and in the greenhouse (Figure 2). The comparison of the greenhouse and the field experiment showed similar ranking of the varieties regarding NUE and N uptake efficiency (U_N), in both low and high fertilisation condition (F_L and F_H) in the field compared to the low fertilised and fully irrigated (F_L-D0) treatment in the greenhouse (Figure 3). The variety ranking regarding grain-specific N efficiency ($E_{N,g}$) and grain N concentration ($C_{N,g}$) was different in the two experimental set-ups. When the values from the F_L-D0 treatment in the greenhouse were compared to the F_L and F_H treatments in the field, the U_N values were 3.0 and 5.6 times higher in the field than in the greenhouse, respectively. The corresponding $E_{N,g}$ values were 3.4 and 4.4 times higher in the greenhouse compared to the field. The $C_{N,g}$ in the F_L-D0 treatment in the greenhouse compared with the field with the factors 1.1 and 1.0 for F_L and F_H, respectively. The $C_{N,g}$ in the greenhouse (F_H-D0 treatment) was 2.3 times higher than $C_{N,g}$ at F_H in the field. Overall NUE was between 1.3 times higher in the greenhouse compared to the field at low fertilisation (F_L), and 0.8 times lower in the greenhouse compared to the field at high fertilisation (F_H).

Effect of experimental treatments

Fertilisation had similar effects on N uptake efficiency (U_N) in all varieties in both field and the greenhouse. There was however a significant fertilisation × variety interaction effect in the greenhouse, possibly due to a smaller increase in Granary than the other varieties at high fertilisation (F_H). Grain-specific N efficiency ($E_{N,g}$) decreased with increased fertilisation, and in the greenhouse Quarna had a smaller reduction than other varieties. Overall NUE increased at F_H both in the field and the greenhouse and the varieties ranked similar. The fertilisation × variety interaction for NUE was significant in the greenhouse, with the weakest fertilisation response seen in Dala. Of the NUE components, only grain N concentration ($C_{N,g}$) showed significant variety × fertilisation interaction effects in the field. Quarna had the highest $C_{N,g}$ at F_L in both experiments, but at F_H Quarna and Dala were similarly high in the field while all varieties were similar in the greenhouse.

Drought condition was applied only in the greenhouse and decreased grain biomass (B_g), $E_{N,g}$ and also overall NUE along with increased $C_{N,g}$ (Fig. 2, Table 4). The early drought (D1) treatment reduced grain biomass more than late drought (D2), resulting in greater effect of the early drought treatment on $E_{N,g}$ and NUE (Fig. 2). Drought response was different between varieties for some characteristics (drought × variety interaction, Table 4). For example, Dala had the lowest $C_{N,g}$ in D1 but the highest $C_{N,g}$ in D2, while Vinjett was among the highest in D1 but had the lowest $C_{N,g}$ in D2. In the field, where no drought condition was applied, the high fertilisation (F_H) treatment increased grain biomass (B_g). In the greenhouse, with all droughts pooled, F_H decreased B_g due to a negative effect of fertilisation in the drought treatments.

Discussion

There were large differences in the magnitude of the values of the NUE components between the two experimental set-ups (mainly in N uptake efficiency, U_N, and grain-specific N efficiency, $E_{N,g}$), but similar ranking of the varieties relative to each other in U_N and NUE in the two set-ups. Significant genotype environment interactions were found both in the greenhouse and in the field, but were more frequently observed in the greenhouse.

Nitrogen use and N productivity

Biomass production per unit nitrogen during the major growth period, or N productivity, is a central process for all plants grown in N-limited conditions (Ågren 1985), and our grain-specific N efficiency ($E_{N,g}$) corresponds to that N productivity. In contrast to $E_{N,g}$, the N utilisation efficiency defined by Moll et al. (1982) cannot be interpreted in the same functional way as N productivity. From a mechanistic perspective, N utilisation efficiency (of Moll et al. 1982) assumes that the final N pool is the functional N pool over the whole growing season, and therefore functionally greatly underestimates the N productivity. For example, for the low fertilisation – no drought (F_L-D0) treatment of our study, the mean N utilisation efficiency according to Moll et al. (1982) would be 38 g g^{-1}, whereas mean $E_{N,g}$ was 277 g g^{-1}. There are clear advantages of a functionally sound interpretation of $E_{N,g}$. Nevertheless, the start and end of the major growth period varied between the varieties, and those varietal differences in development are difficult to match in terms of correct sampling at many different points in time within the same experiment. To solve that problem, extrapolating mean N content during the major growth period based on measured values at similar points in time combined with a model accounting for differences in timing of the critical developmental stages assessed non-destructively, would be more appropriate than the

simple mean value proposed by Weih et al. (2011). That solution would also allow calculation of mean N uptake efficiency (U_N) in situations where destructive harvests at all critical plant stages are not feasible, as was the case in the drought treatments of our greenhouse study.

Yields, grain N and limiting factors in greenhouse vs. field

In contrast to field, yields in the greenhouse were relatively low, which was probably caused by the high temperature in combination with low light irradiance in the greenhouse (Van Oijen and Ewert 1999). Furthermore, a high biomass to substrate volume ratio in our greenhouse pot experiment could have been another factor limiting biomass production (Poorter et al. 2012). The low fertilisation (F_L) treatment was intended to simulate conditions in which nutrients, particularly N, strongly limit plant growth. Nitrogen-limited plant growth in this study is supported by harvested grain N concentrations being similar to sown grain N concentration and within the range of commonly observed field values. Drought increased harvested grain N concentration slightly, and the high fertilisation treatment (F_H) more than doubled grain N concentration compared with the sown grain, up to values that we consider extreme. The combination of high grain N concentration and low grain yield, here observed especially in the F_H treatment, could indicate low starch content. This has previously been reported under high temperature and nutrient supply along with low light intensities during grain filling (Grashoff and D' Antuono 1997; Triboi and Triboi-Blondel 2002), i.e. conditions characteristic of our F_H treatment in the greenhouse. The results indicate that in the greenhouse the plants grown in the F_L treatment were mostly N-limited, whereas the plants grown in the F_H treatment were mostly carbon (light)-limited. In the field experiment plants at both fertilisation treatments seemed to be N-limited, and this difference in the experimental set-up should be considered in the comparison between them.

405

406 Effect of drought treatments assessed in greenhouse

407 Drought condition significantly reduced yield and NUE, and more so when the drought
408 condition occurred prior to anthesis (D1 treatment) than after anthesis (D2 treatment). Those
409 results support other findings (e.g. Ferris et al. 1998; Ji et al. 2010) and are in line with our
410 first hypothesis that early drought reduces grain yield, grain-specific N efficiency and NUE
411 more than late drought. However, varietal differences in development made it difficult to
412 assess especially the effects of drought on NUE aspects, and we need to improve assessment
413 of N accumulation across varieties with differences in developmental timing in the way
414 previously discussed. We found strong interaction between drought and nutrient supply,
415 because increased nutrient supply decreased yield when the plants were subjected to drought.
416 A relevant finding in line with our observation is that higher nutrient availability can reduce
417 yields as a result of terminal drought, i.e. water deficit during grain filling (Van Herwaarden
418 et al. 1998). In our experiment water became available again during grain filling, but the
419 additional water apparently could not compensate for the greater drought-induced reduction in
420 yield at the higher fertilisation level. The results indicate that even the relatively short drought
421 periods applied here reduced yield and NUE through grain-specific N efficiency especially at
422 high nutrient supply. According to our results, a critical issue at least under the conditions in
423 Northern Europe is whether drought will become more frequent also early in the growing
424 season, an issue also pointed out by Mäkelä et al. (2008). Genotype by drought interaction for
425 some of the traits (e.g. Table 4) indicates a potential for breeding towards improved drought
426 adaptation (Fischer and Maurer 1978), but the limited amount of genotypes used here does not
427 allow any more detailed conclusions regarding desirable traits for wheat improvement under
428 drought.

429

430 Proof of NUE concept for crop and variety evaluation

431 The components N uptake efficiency (U_N) and grain-specific N efficiency ($E_{N,g}$) greatly
432 differed in magnitude between the experiments while NUE and grain N concentration ($C_{N,g}$)
433 did not. Great variation in U_N and $E_{N,g}$ between the experiments indicates differences in the
434 environmental factors affecting N uptake (e.g. nutrient availability) and grain production per
435 unit plant N. Despite great variation in U_N and $E_{N,g}$ between the two experiments, the overall
436 NUE was similar, partly because the variations in U_N and $E_{N,g}$ cancelled out each other. This
437 means that N accumulation in harvested grain per unit N in seed grain was relatively constant
438 between the two experiments, in spite of much greater variation in two out of the three major
439 NUE components. The results illustrate that NUE assessment, e.g. for identification of
440 desirable crop traits for improved NUE, should not be restricted to single NUE components,
441 but simultaneously analyze the various components contributing to NUE. Such integrated
442 NUE assessment greatly facilitates the interpretation of experiments carried out under
443 different environmental conditions, e.g. the greenhouse and field experiment studied here.

444 Assessment of NUE and its components can be used to evaluate crops and varieties in terms
445 of integrated crop characteristics important for yield and sustainability issues. In future, the
446 integrated crop characteristics investigated here need to be linked to key crop traits that can be
447 directly used as targets in variety selection and breeding. Identification of desirable crop traits
448 for improved nutrient use efficiency currently receives much attention. We conclude that the
449 NUE concept by Weih et al. (2011) can be a useful tool to describe and integrate important
450 NUE components for crops grown in different treatments (fertilisation, drought) and
451 experimental set-ups, i.e. greenhouse and field. We found similar variety ranking in N
452 accumulation (U_N) and overall NUE across experimental set-ups, but different variety ranking

in grain-specific N efficiency ($E_{N,g}$) and grain N concentration ($C_{N,g}$), which appear to depend more on interactions between specific variety characteristics and the environment. The absolute values of NUE components are often greatly influenced by experimental set-up and also sampling procedure.

A conceptual dilemma in using greenhouse and/or field experiments for crop variety testing and selection is an often untested assumption of similar variety ranking in greenhouse and field conditions on one hand, and the explicit aim to identify different variety responses to particular environmental conditions (genotype environment interaction) on the other hand. Caused by this conceptual dilemma, there are few reports in which the characteristics of identical varieties are investigated under both greenhouse and field conditions, as was done in this study. Similar to numerous other reports, we found partly strong influence of environmental conditions on variety ranking, both in terms of experimental set-ups and particular environmental factors manipulated within an experimental set-up. Major differences between greenhouse and field conditions include substrate and temperature (mean and diurnal course) issues. Interestingly, those differences between greenhouse and field conditions apparently had little influence on variety ranking for characteristics related to N accumulation (i.e. U_N), which is a major component of overall NUE, resulting in stable variety ranking for N accumulation and overall NUE despite of rather different values in absolute terms. Genotypic variation in N accumulation assessed in greenhouse may therefore be relevant also in many field conditions, but that conclusion requires further verification. Contrary, variety ranking differed between experimental set-ups regarding grain-specific N efficiency ($E_{N,g}$) and grain N concentration ($C_{N,g}$), which appear to more depend on interaction between specific variety characteristics and environment, and frequently showed corresponding pattern (i.e. higher $E_{N,g}$ along with lower $C_{N,g}$, and vice versa).

An interesting question is whether the observed similarities and differences between varieties and environments mostly reflect peculiarities of the applied method (here for NUE assessment by means of Weih et al. 2011), or true differences between varieties grown in particular environments. Especially if problems caused by varietal differences in development timing are eliminated, e.g. by incorporating a modelling approach adjusting N accumulation period to specific developmental timing of each variety, we believe that the method used here does reflect true differences between varieties, i.e., generated results are relevant for variety testing and selection.

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564

565 **Figure captions**

566 Figure 1. Effects of fertilisation and variety on NUE components and yield in the field
567 experiment. The symbols represent adjusted means and error bars (back transformed) 95th
568 percentile confidence intervals from the ANOVA (Table 3). Crosses represent low
569 fertilisation (F_L) and open circles high fertilisation (F_H). Abbreviations of variables according
570 to Table 1.

571

572 Figure 2. Effects of variety, drought and fertilisation on NUE components and grain biomass
573 in the greenhouse experiment. The symbols represent adjusted means and error bars (back
574 transformed) 95th percentile confidence intervals from the ANOVA (Table 4). Crosses
575 represent low fertilisation (F_L) and open circles high fertilisation (F_H). Filled squares represent
576 no drought treatment (D0), filled circles early drought (D1) and filled triangles late drought
577 (D2). Abbreviations of variables according to Table 1.

578

579 Figure 3. Comparison of greenhouse and field values of NUE components and other measured
580 variables. The values are the adjusted means from the statistical analysis. The x-axis shows
581 the greenhouse values at low fertilisation and no drought treatment (F_L-D0), and the y-axis
582 shows the field values at low fertilisation, F_L (small symbols) and high fertilisation, F_H (large
583 symbols). Abbreviations of variables according to Table 1.

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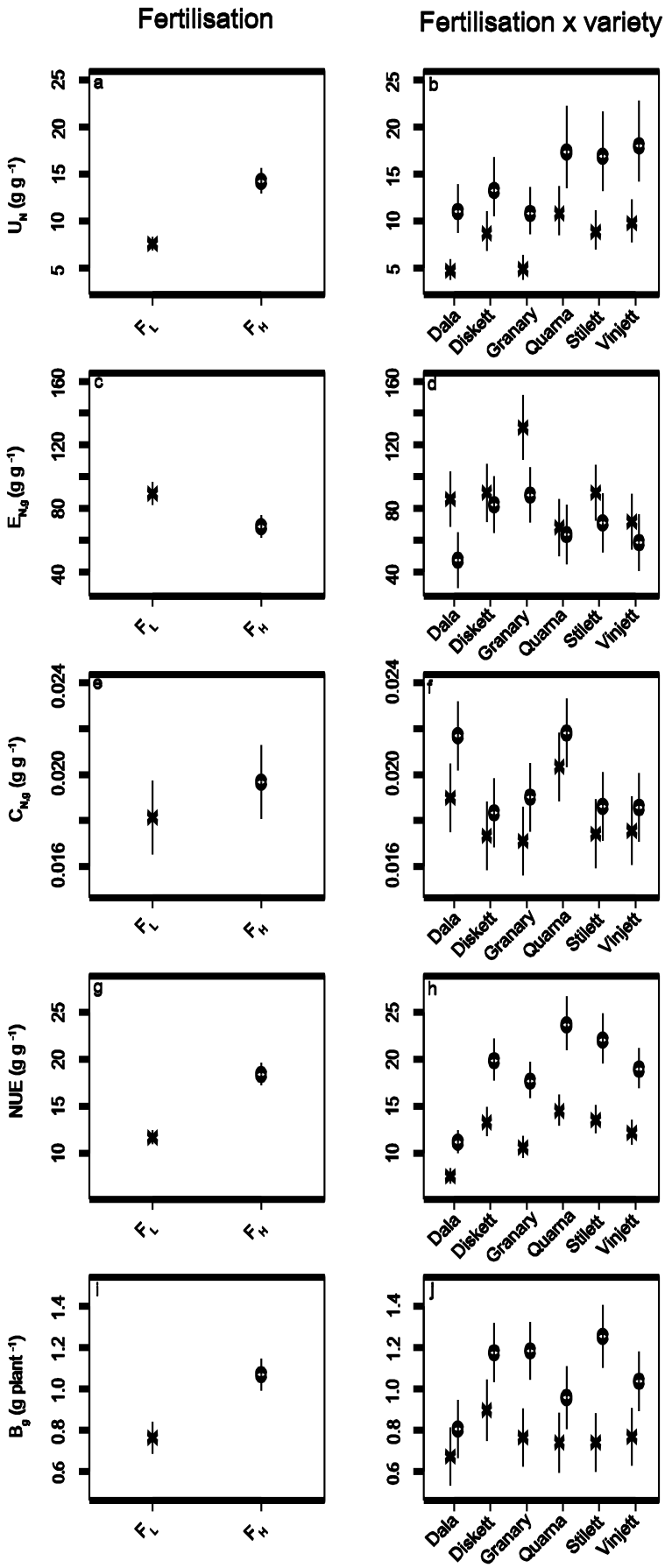

587

588 Asplund et al. Figure 1.

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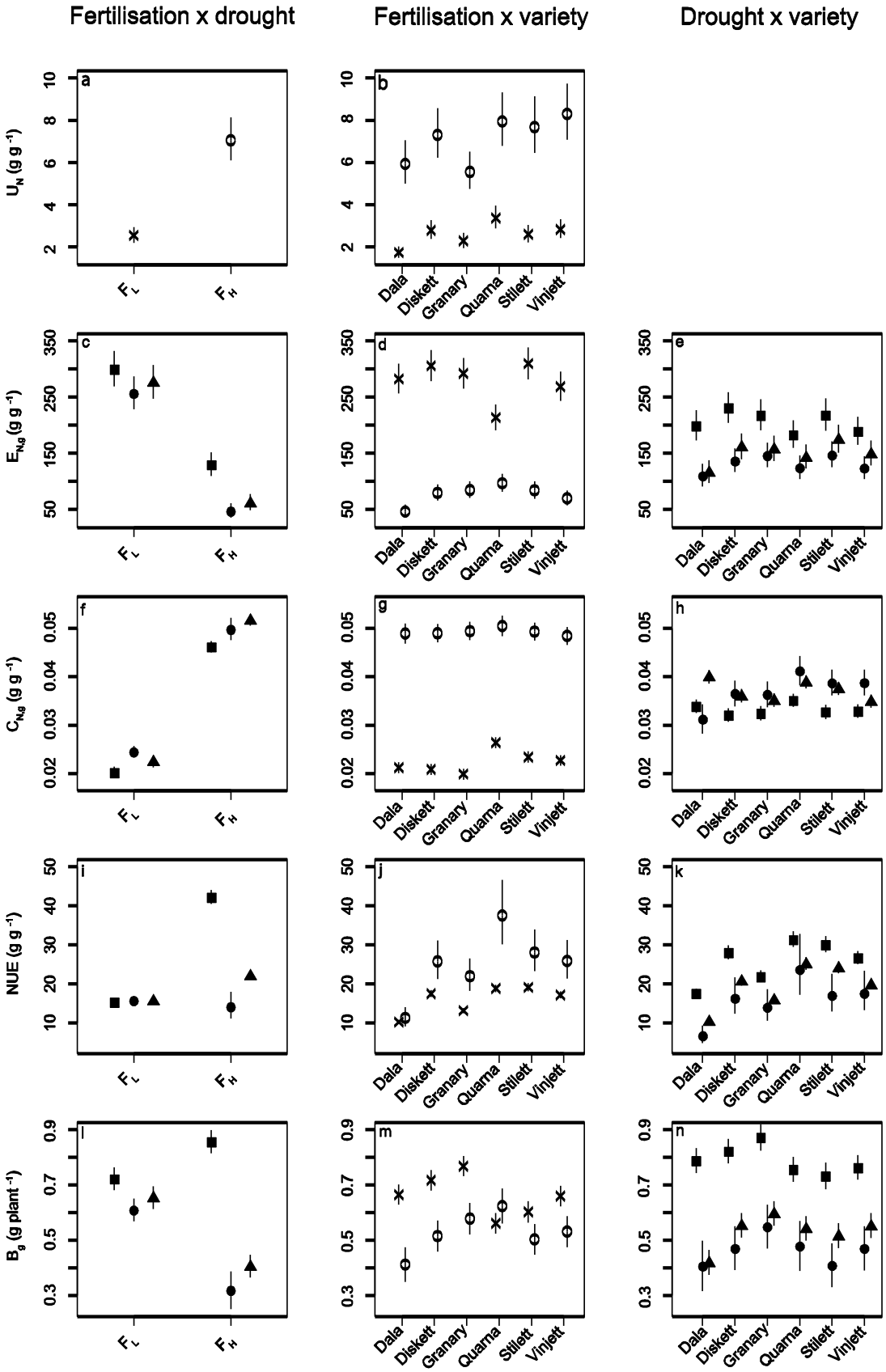
Fertilisation x drought
Fertilisation x variety
Drought x variety
a
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k
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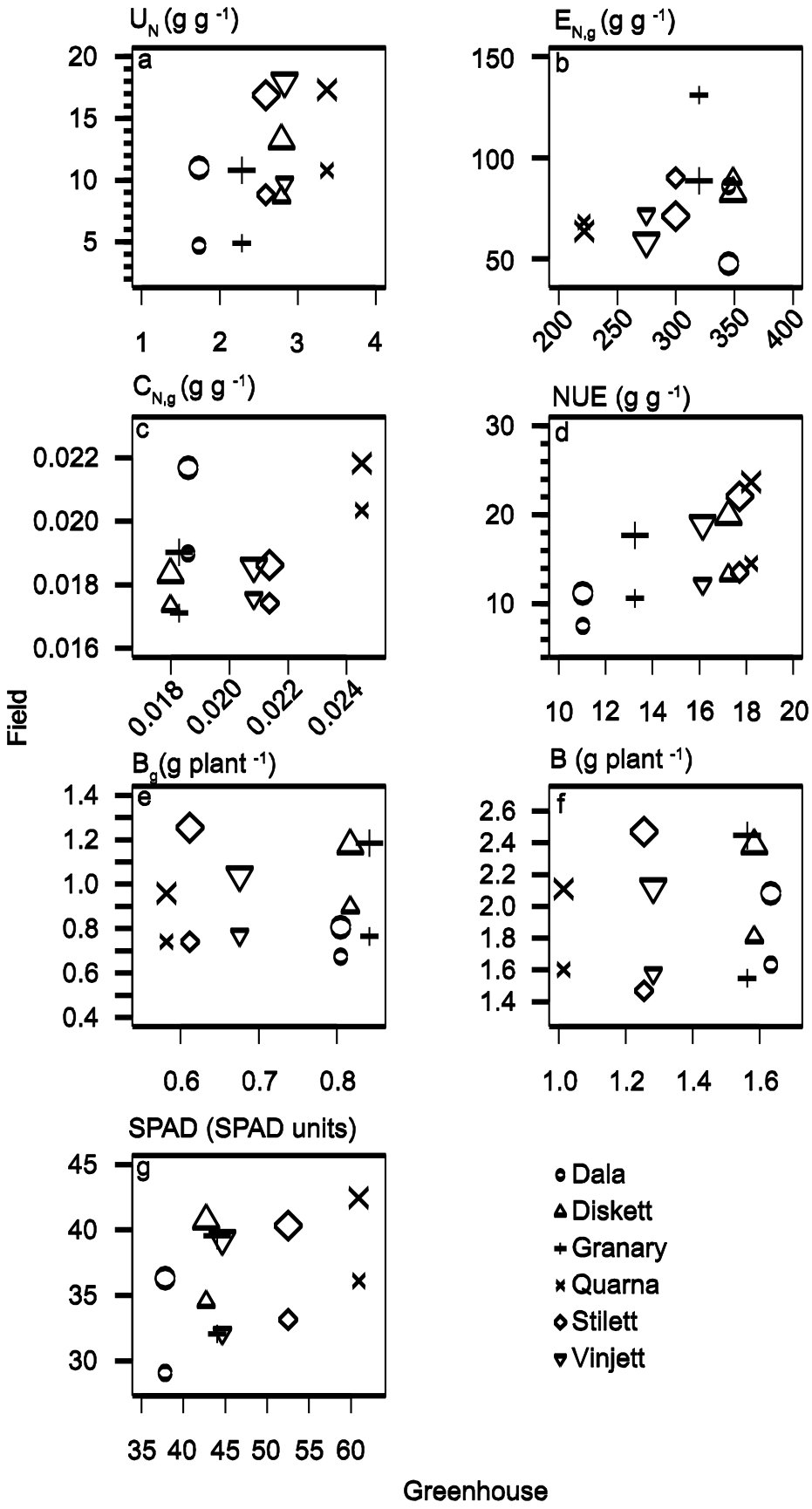
n
U_N (g g^{-1})
E_{Ng} (g g^{-1})
C_{Ng} (g g^{-1})
NUE (g g^{-1})
B_g (g plant^{-1})
F_L
F_H
Dala
Diskett
Granary
Quarna
Stilett
Vinjett

590

591 Asplund et al. Figure 2.



592

593 Asplund et al. Figure 3.

594 **Tables**

595 Table 1. Definitions of NUE components according to Weih et al. (2011).

Symbol	Component	Calculation	Unit
NUE	Nitrogen use efficiency	$U_N \times E_{N,g} \times C_{N,g} = N_g/N_s$	g g $^{-1}$
U_N	Mean N uptake efficiency during major growth period per N content in seed grain	N'/N_s	g g $^{-1}$
$E_{N,g}$	Grain-specific N efficiency	B_g/N'	g g $^{-1}$
$C_{N,g}$	Grain N concentration at final harvest	N_g/B_g	g g $^{-1}$
N_s	N content of seed (sown) grain		g
N_g	N content of produced grain at final harvest		g
N'	Mean plant N content during major growth period	Mean of plant N content at two time points: the beginning and the end of the major growth period.	g
B_g	Biomass of produced grain at final harvest		g
B	Plant biomass at final harvest		g

596

597

598 Table 2. Mean day degrees to anthesis averaged over all factors (SE 7.5 day degrees) and
599 median growth stage (Lancashire et al. 1991) one day after start of the early drought treatment
600 for six spring wheat varieties. The late drought treatment started after growth stage 61 for all
601 varieties.

Variety	Day degrees to anthesis	Growth stage at start of early drought
Dala	988	41
Diskett	935	42
Granary	893	45
Quarna	776	59
Stilett	747	60
Vinjett	821	59

602 Table 3. ANOVA table with *F* and *P* values for NUE components and biomass in the field experiment. Abbreviations of variables according to
603 Table 1. NUE and U_N were log-10 transformed prior to analysis

604

Source of variation	U_N		$E_{N,g}$		$C_{N,g}$		NUE		B_g		B (plant)		SPAD	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Fertiliser (F)	88.4	<.001	16.9	<.001	99.4	<.001	159.5	0.001	45.3	0.001	34.2	0.001	394.2	<.001
Variety (V)	12.4	<.001	7.2	<.001	44.3	<.001	46.3	<.001	4.5	0.004	0.8	0.568	26.8	<.001
F x V	1.1	0.403	1.6	0.180	3.1	0.023	0.4	0.828	1.9	0.121	1.1	0.365	0.4	0.855

605

606

607 Table 4. ANOVA table with *F* and *P* values for NUE components and biomass in the greenhouse experiment. Abbreviations of variables
608 according to Table 1. NUE and U_N were log-10 transformed prior to analysis, and $E_{N,g}$ was square-root-transformed prior to analysis

609

Source of variation	U_N		$E_{N,g}$		$C_{N,g}$		NUE		B_g		B (plant)		SPAD	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Fertiliser (F)	842.5	<.001	615.1	<.001	2333.4	<.001	104.4	<.001	60.0	<.001	37.4	<.001	0.41	0.525
Drought (D)			29.1	<.001	28.2	<.001	141.0	<.001	139.8	<.001	54.3	<.001		
D x F			10.1	0.002	5.7	0.010	159.0	<.001	68.5	<.001	20.1	<.001		
Variety (V)	18.6	<.001	10.5	<.001	6.6	<.001	37.3	<.001	9.3	<.001	33.1	<.001	26.4	<.001
F x V	2.6	0.045	20.1	<.001	3.4	0.012	3.4	0.022	11.6	<.001	2.4	0.045	1.6	0.182
D x V			1.3	0.224	4.6	<.001	3.7	0.001	2.5	0.021	1.4	0.178		
D x F x V			1.5	0.151	3.0	0.005	1.4	0.202	1.7	0.116	0.9	0.566		

610