



# Positive effects of free air CO<sub>2</sub> enrichment on N remobilization and post-anthesis N uptake in winter wheat

Markus Dier<sup>a,c</sup>, Jan Sickora<sup>a</sup>, Martin Erbs<sup>b</sup>, Hans-Joachim Weigel<sup>a</sup>, Christian Zörb<sup>c</sup>, Remy Manderscheid<sup>a,\*</sup>

<sup>a</sup> Thünen Institute of Biodiversity, Bundesallee 65, D-38116, Braunschweig, Germany

<sup>b</sup> Deutsche Agrarforschungsallianz (DAFA), German Agricultural Research Alliance, c/o Thünen Institute, Braunschweig, Germany

<sup>c</sup> Institute of Crop Science, Quality of Plant Products, University of Hohenheim, Emil-Wolff-Str. 25, D-70599, Stuttgart, Germany

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## ABSTRACT

Elevated atmospheric CO<sub>2</sub> concentration (e[CO<sub>2</sub>]) often increases cereal yield, but can also decrease vegetative and grain tissue nitrogen (N) concentration that might affect future food and feed quality. However, data about CO<sub>2</sub> × N interactions on key processes determining grain N yield and concentration, which are remobilization of vegetative N taken up before anthesis (Nrem) and post-anthesis N uptake (Nabs), are scarce. Therefore, a two-year Free Air CO<sub>2</sub> Enrichment (FACE) experiment was conducted with winter wheat grown under two CO<sub>2</sub> (~393 and 600 ppm) and three N levels (severe deficiency with N nutrition index (NNI) of 0.4, adequate with NNI of 0.8 and excess with NNI of 1.1).

e[CO<sub>2</sub>] did not influence the allometric relation between aboveground N concentration and biomass up to anthesis. At anthesis, e[CO<sub>2</sub>] increased N acquisition of stem and ear, but not of leaf. Correspondingly, e[CO<sub>2</sub>] increased Nrem of stem and chaff. Moreover, e[CO<sub>2</sub>] enhanced the efficiency of Nrem of stem and aboveground plant in the first year, indicating increased N mobilization from vegetative tissue. Nabs tended to be increased by e[CO<sub>2</sub>], especially in the second year. Finally, e[CO<sub>2</sub>] increased grain N yield (8 to 12%), N use efficiency (13 to 18%) and N uptake efficiency (10 to 12%). Grain N concentration was slightly decreased by e[CO<sub>2</sub>] in both years (-1 to -6%), while grain N concentration was considerably larger (9 to 19%) in the second year compared to the first year. There was a strong linear relation between grain N yield and grain number ( $r^2 = 0.98$ ) that was not influenced by e[CO<sub>2</sub>], suggesting grain number as important factor determining the grain N yield increase under e[CO<sub>2</sub>]. Grain N concentration was more strongly affected by e[CO<sub>2</sub>] than mean N content per grain.

## 1. Introduction

Wheat (*Triticum aestivum* L.) is an important basic food of a large part of the world population, with annual production has increased during the last half-century (Simoni, 2009) and is expected to further rise in future (Shewry and Hey, 2015). The atmospheric CO<sub>2</sub> concentration is predicted to continue to rise from current 408 to 730–1020 ppm by the end of this century (IPCC, 2013). Elevated atmospheric CO<sub>2</sub> concentration (e[CO<sub>2</sub>]) stimulates photosynthesis in C<sub>3</sub> crops, which is often associated with biomass and yield increases (Ainsworth and Long, 2005). However, these positive e[CO<sub>2</sub>] effects are often accompanied by a decrease of tissue nitrogen (N) concentration. For wheat, meta-analyses found reductions of -9 to -16% of vegetative tissue (Cotrufo et al., 1998; Wang et al., 2013) and -6 to -16% of grain (Taub et al., 2008; Myers et al., 2014). Moreover, a modelling study, taking climate change adaptations

concerning grain yield into account, suggested global mean decrease of grain protein concentration of -9% under e[CO<sub>2</sub>] in 2050 (Asseng et al., 2019). There is concern that those reductions will result in poor food (Myers et al., 2014), feed (Sinclair et al., 2000) and baking quality (Wieser et al., 2008; Panozzo et al., 2014).

In wheat, the highest proportion of grain N originates from remobilization of N acquired by the vegetative organs before anthesis to the grains during grain filling (Barbottin et al., 2005). Leaf and stem are the most important N sources of N remobilization (Nrem), contributing about 75% of the N originating from Nrem, whereas the residual share of Nrem is provided by chaff and roots (Gaju et al., 2014). Another important N source of the grains is post-anthesis N uptake (Nabs). Depending on environmental conditions, 40 to 90% of the grain N originates from Nrem and thus 10 to 60% from Nabs (Kong et al., 2016). Nabs depends on soil mineral N availability at early grain filling

\* Corresponding author.

E-mail address: [remy.manderscheid@thuenen.de](mailto:remy.manderscheid@thuenen.de) (R. Manderscheid).

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(Bancal, 2009). Many studies (e.g. Ma et al., 1995; Jamieson and Semenov, 2000) indicated that grain N acquisition is determined by the N source, i.e. N acquisition before anthesis and soil N availability at early grain filling. However, a strong inverse relationship between Nrem and Nabs exists (Barbottin et al., 2005; Kong et al., 2016), indicating a certain degree of sink regulation of grain N acquisition. According to Martre et al. (2003), accumulation of structural and metabolic proteins, making up about 30% of the grain protein in wheat, is sink regulated, whereas accumulation of storage protein (gluten) is source regulated. In addition to grain N acquisition, individual grain growth, i.e. starch acquisition, can be important in determining grain N concentration. The processes controlling grain N and starch acquisition act partly independently (Jenner et al., 1991).

The mechanism by which  $e[CO_2]$  decreases tissue N concentration is still elusive. Several mechanisms have been hypothesized, which could occur simultaneously or interact with each other. A hypothesis is growth dilution, implying that plant N acquisition is not directly influenced by  $e[CO_2]$  but cannot fully keep pace with the enhanced growth under  $e[CO_2]$  (Loladze, 2002). This hypothesis implies increased N acquisition to some extent in plants grown under  $e[CO_2]$ , which was observed in wheat in several Free Air  $CO_2$  Enrichment (FACE) experiments (e.g. Han et al., 2015; Cai et al., 2016; Tausz et al., 2017). However, Pleijel and Uddling (2012) and Feng et al. (2015) found that  $e[CO_2]$  can also decrease tissue N concentration under conditions of no growth stimulation by  $e[CO_2]$ . A potential mechanism for the decrease of N concentration is inhibition of  $NO_3^-$  assimilation by  $e[CO_2]$  (Bloom et al., 2010), which could explain a decrease of N concentration without enhanced growth. Another hypothesis is that  $e[CO_2]$  decreases leaf N concentration because of downregulation of ribulose-1,5-bisphosphat carboxylase/oxygenase (RuBisCO) gene expression, especially under N deficiency (Stitt and Krapp, 1999).

There are also other  $e[CO_2]$  induced processes that could enhance N acquisition and thus tissue N concentration. These include stimulation of root growth (Pacholski et al., 2015) and increase of whole-crop N sink strength (Feng et al., 2015).

Plant N concentration declines as biomass increases during crop development because of shading effects and change of the leaf: stem ratio (Justes et al., 1994; Sadras and Lemaire, 2014). Therefore, the decrease of N concentration under  $e[CO_2]$  can be, at least partly, explained by increased biomass according to the negative allometric relation between N concentration and biomass. Some crop models assume a reduction of critical N concentration, i.e. the concentration that is required to ensure potential production, when wheat is grown under  $e[CO_2]$  (Vanuytrecht and Thorburn, 2017). However, few experimental studies (e.g. Coleman et al., 1993) have considered the allometry between N concentration and biomass in their comparison of the effect of  $CO_2$  on N concentration.

It was proposed that a decrease of tissue N concentration by  $e[CO_2]$  at anthesis results in a decrease of Nrem (Rubio-Asensio and Bloom, 2016). In line with this, the extent of decrease of leaf and grain N concentration by  $e[CO_2]$  was strongly correlated in a FACE study (Kimball et al., 2001, 2002). It is also possible that while Nrem is unaffected the efficiency of Nrem, which is the proportion of the N taken up before anthesis that is remobilized, is decreased under  $e[CO_2]$ . This might occur when  $e[CO_2]$  enhances tillering (Cai et al., 2016), but decreases Nrem per tiller. However, regarding Nrem, processes possibly exist that could compensate for reduced vegetative tissue N concentration under  $e[CO_2]$ . For instance, studies showed that  $e[CO_2]$  enhances the grain N sink strength (Lam et al., 2012) and under ambient  $[CO_2]$  a large amount of N that is potentially available for Nrem, i.e. that is not structural N, remains in vegetative tissue at maturity (Pask et al., 2012).

Studies found that canopy senescence was accelerated under  $e[CO_2]$  at grain filling (Osborne et al., 1998; Brooks et al., 2000; Fangmeier et al., 2000) and thus, the time interval of Nabs might be reduced under  $e[CO_2]$ . Moreover, Nabs could be reduced by  $e[CO_2]$  because of inhibition of  $NO_3^-$  assimilation (Bloom et al., 2010), even if indication of such inhibition was not found in the present experiment (Dier et al., 2018a).

The decrease in tissue N concentration by  $e[CO_2]$  is stronger under low compared to high N supply in wheat (Kimball et al., 2002; Taub et al., 2008). The meta-analysis of Taub et al. (2008), which included chamber and field studies, showed for grain N concentration a decrease of -10% under high and of -16% under low N supply. The FACE study of Kimball et al. (2002) revealed a strong decrease of leaf and grain N concentration under severe N deficiency, but no decrease under ample N supply. However, other FACE studies showed no difference in the  $e[CO_2]$  effect on N concentration between low and high N supply (Ma et al., 2007; Erbs et al., 2010; Lam et al., 2012; Han et al., 2015) and little difference between adequate and excess N supply (Tausz et al., 2017). Therefore, the  $CO_2 \times N$  interaction on grain N concentration under field conditions is still unclear.

In the present study, a two-year FACE experiment was conducted with winter wheat under well-watered conditions, comprising two  $CO_2$  (393 and 600 ppm) and three N fertilizer levels (severe deficiency, adequate and excess). The main objective was to investigate  $CO_2 \times N$  interactions on Nrem, considering different vegetative organs, and Nabs. The following specific questions were addressed: (i) can the decrease of N concentration by  $e[CO_2]$  before anthesis be explained by the negative allometric relation between biomass and N concentration (ii) does decrease of N concentration by  $e[CO_2]$  at anthesis result in decreased Nrem and (iii) does  $e[CO_2]$  decrease Nabs because of accelerated canopy senescence and/or inhibited  $NO_3^-$  assimilation.

## 2. Material and methods

### 2.1. Study site and experimental design

The experiment was conducted on a field site (52°18'N, 10°26'E, 79 m a.s.l.) at the Thünen-Institut in Braunschweig, Germany in 2014 and 2015. Winter wheat (*Triticum aestivum* L. Variety “Batis”) was grown under ambient  $[CO_2]$  and  $e[CO_2]$  of 600 ppm and three levels of nitrogen (N) fertilizer. The  $CO_2$  treatments were conducted on circular plots with a diameter of 20 m and the N fertilizer treatments on rectangular sub-plots (3 m  $\times$  5 m), which were randomly established within the  $CO_2$  plots. Altogether, the experiment consisted of six different  $CO_2 \times N$  treatments that were replicated three times. The position of the  $CO_2$  and N treatments on the field site did not differ between the two years.

The soil in the 0–40 cm plough horizon is a luvisol of loamy sand texture consisting of 69% sand, 24% silt and 7% clay. The lower (-1.5 MPa soil water tension) and upper limit (-0.01 MPa soil water tension) of plant available soil water is a volumetric water content of 5% and 23%, respectively. Important soil parameters were measured of the 0–40 cm soil profile in each N subplot in March 2015. Soil was dried at 105 °C and passed through a 2 mm sieve before carbon (C) and N determination with an element analyzer (Leco TruSpec CNS, USA). Soil pH was measured in a soil suspension with water. The results were (mean  $\pm$  SD;  $n = 18$ ): pH,  $6.83 \pm 0.26$ ; C content,  $0.98 \pm 0.05\%$ ; and N content,  $0.09 \pm 0.00\%$ . Mineral N in the 0–40 cm soil profile measured in middle of March was  $14.2 \pm 2.4 \text{ kg N ha}^{-1}$  in 2014 and  $22.4 \pm 5.8 \text{ kg N ha}^{-1}$  in 2015. With respect to soil mineral N content, statistical analysis did not result in significant  $CO_2$ , N and  $CO_2 \times N$  effects.

### 2.2. Crop management and $CO_2$ enrichment

Wheat was sown with a density of 380 kernels per  $m^2$  on October 29 in 2014 and November 4 in 2015. Crop management was conducted according to local farm practice with sufficient pesticide application and nutrient supply. N fertilization was conducted with calcium ammonium nitrate (CAN; 27% N), which was scattered by hand. A severe deficient N level with 40 (2014) / 35  $\text{kg N ha}^{-1}$  (2015) (Nd), an adequate with 180 (2014) / 200  $\text{kg N ha}^{-1}$  (2015) (Nad) and an excess with 320  $\text{kg N ha}^{-1}$  (2014 and 2015) (Nex) were used. Table 1 presents an overview of the application dates with the corresponding N fertilizer doses. At anthesis in 2015, an aqueous solution of CAN labelled with

**Table 1**

N fertilizer treatments with application dates and quantities. *Nd* refers to the severe deficiency, *Nad* to the adequate and *Nex* to the excess N level. At mid-anthesis on June 11 in 2015  $^{15}\text{N}$  labeled N fertilizer with 5%  $^{15}\text{N}$  excess was applied.

2014						2015					
Quantity (kg N ha <sup>-1</sup> )						Quantity (kg N ha <sup>-1</sup> )					
N level	Mar 19 <sup>th</sup>	Apr 14 <sup>th</sup>	May 4 <sup>th</sup>	June 2 <sup>nd</sup>	Total	Mar 18 <sup>th</sup>	Apr 28 <sup>th</sup>	May 11 <sup>th</sup>	June 11 <sup>th</sup>	Total	
<i>Nd</i>	20	20			40	15	15		5 ( $^{15}\text{N}$ )	35	
<i>Nad</i>	70	35	35	40	180	70	35	35	60 ( $^{15}\text{N}$ )	200	
<i>Nex</i>	120	60	60	80	320	120	60	60	80 ( $^{15}\text{N}$ )	320	

$^{15}\text{N}$  (5% excess) was applied, whose amounts are shown in Table 1. The solution was prepared by mixing of a solution of CAN with a solution of  $^{15}\text{N}$  labelled ammonium nitrate ( $^{15}\text{NH}_4^+ \text{ }^{15}\text{NO}_3^-$ ; 98% excess). This solution was then carefully applied with a watering can. Manual irrigation was implemented to keep volumetric soil water content in the range of 14–21% (50–90% of field capacity) and irrigation was adapted to the differing water demand of the different  $\text{CO}_2 \times \text{N}$  treatments (for details see: Manderscheid et al., 2018).

$\text{CO}_2$  enrichment was conducted with a FACE system constructed according to the Brookhaven National Laboratory design (Lewin et al., 1992).  $\text{CO}_2$  enrichment started at the four and three leaf stage on March 31 in 2014 and March 12 in 2015, respectively.  $\text{CO}_2$  enrichment took place during the daytime hours and was interrupted when air temperature fell below 5 °C or wind speed exceeded 6 m s<sup>-1</sup>.

### 2.3. Determination of N concentration, atom% $^{15}\text{N}$ excess and N yield

Plant samples were taken from an area of 0.5 m<sup>2</sup> at three stages (first node stage, flag leaf stage, anthesis) and from an area of 1.8 m<sup>2</sup> at maturity. At the samplings up to anthesis, a small subsample consisting of 30 plants was then separated into stems (including leaf sheaths), leaf blades and ears followed by drying at 105 °C and biomass determination. The remaining subsample was dried at 105 °C followed by biomass determination. Both subsamples were used for biomass determination. At maturity, ears were sampled from the whole (1.8 m<sup>2</sup>) sampling area. After threshing, the grain and chaff fraction were determined by weight and dry weight was determined from a grain and chaff subsample after drying at 105 °C. Biomass data have already been published (Dier et al., 2018b).

Tissue N concentration was determined using an element analyzer (Leco TruSpec CNS, USA). Before analysis, the stem, leaf blade and chaff fraction were dried at 105 °C and ground to a fine powder in a rotor mill (Brabender, Germany). The grain samples were ground to pass a 0.75 mm sieve in an ultracentrifugal mill (Retsch type ZM1, Germany) after drying at 105 °C. To determine atom%  $^{15}\text{N}$  excess, a subsample of the ground plant material was further ground in a ball mill (MM 400, Retsch, Germany) followed by atom%  $^{15}\text{N}$  determination with an isotope ratio mass spectrometer (IRMS) (Delta Plus, Thermo Fisher Scientific, USA). Atom %  $^{15}\text{N}$  excess was calculated as atom%  $^{15}\text{N}$  in the sample minus  $^{15}\text{N}$  natural abundance. To test whether there is a  $\text{CO}_2$  or N effect on atom%  $^{15}\text{N}$  of unlabelled plants at maturity, a plant sample was taken from the area fertilized with unlabelled CAN. However, neither a significant  $\text{CO}_2$  and N effect nor  $\text{CO}_2 \times \text{N}$  interaction were detected.

### 2.4. Determination of soil mineral N and $^{15}\text{N}$ excess

Soil samples of the 0–40 cm soil profile were taken at the beginning and the end of the main growing season in both years, and at anthesis in 2015 (before application of the  $^{15}\text{N}$  labelled fertilizer). Subsamples of approx. 40 g soil were taken from six random positions, pooled and extracted with KCl (2 M) followed by photometrical determination of soil mineral N (N<sub>min</sub>) with a Continuous-Flow Analyzer (Model SA3000/5000, Scalar, Netherlands).

$^{15}\text{N}$  excess was determined as described in 2.3. Before determination, all visible plant material was removed from the soil samples before

drying of the soil at 105 °C and grinding to fine powder in a ball mill (MM 400, Retsch, Germany).

### 2.5. Determination of N nutrition index

N nutrition index (NNI) was determined as the ratio of the actual aboveground N concentration and critical N concentration belonging to the actual aboveground biomass. The critical N concentration was determined according to the critical dilution curve described for wheat (Justes et al., 1994). NNI was measured at flag leaf stage (DC39) and anthesis (DC65) because of the strong connection between NNI during this period and grain yield (Ravier et al., 2017).

### 2.6. Determination of N remobilization and post-anthesis N uptake

N remobilization (Nrem) and post-anthesis N uptake (Nabs) were estimated with the apparent method (Kichey et al., 2007) in both years. Nrem was calculated by subtracting N yield of the vegetative plant fractions (stem + leaf sheath, leaf blade and chaff) at maturity from the ones at anthesis. Efficiency of Nrem was calculated as the ratio of Nrem to N yield at anthesis. Nabs was calculated from the difference between N yield of all plant fractions at maturity and at anthesis.

Nrem and Nabs were additionally estimated by  $^{15}\text{N}$  isotope labelling in 2015. This was conducted according to Kichey et al. (2007) on the basis of the following equations:

$$N_{\text{rem}} = \frac{[N_{\text{ant}}(E_{\text{abs}} - E_{\text{ant}})] - [N_{\text{mat}}(E_{\text{abs}} - E_{\text{mat}})]}{E_{\text{rem}} - E_{\text{abs}}} \quad (1)$$

$$N_{\text{abs}} = \frac{[N_{\text{mat}}(E_{\text{mat}} - E_{\text{ant}})] - [N_{\text{ant}}(E_{\text{ant}} - E_{\text{rem}})]}{E_{\text{abs}} - E_{\text{rem}}} \quad (2)$$

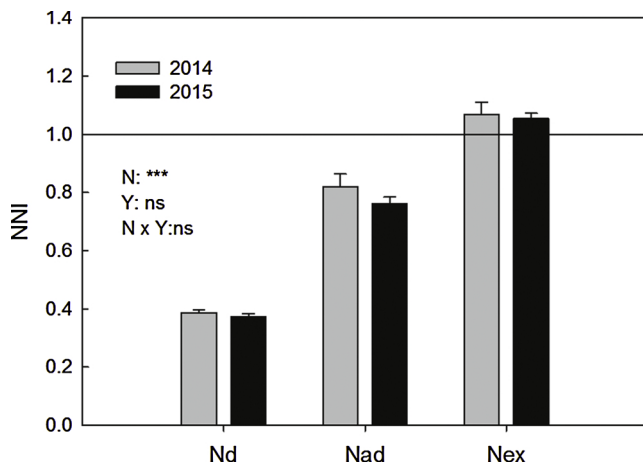
where  $N_{\text{ant}}$  is N yield at anthesis;  $N_{\text{mat}}$ , N yield at maturity;  $E_{\text{ant}}$ ,  $^{15}\text{N}$  excess at anthesis;  $E_{\text{mat}}$ ,  $^{15}\text{N}$  excess at maturity;  $E_{\text{rem}}$ ,  $^{15}\text{N}$  excess derived from Nrem;  $E_{\text{abs}}$ ,  $^{15}\text{N}$  excess derived from Nabs. Because there was no  $^{15}\text{N}$  excess in the plant fractions at anthesis,  $E_{\text{rem}}$  was regarded as zero.  $E_{\text{abs}}$  was calculated with the following equation:

$$E_{\text{abs}} = \frac{(N_{\text{fer}} * E_{\text{fer}}) + (N_{\text{soil}} * E_{\text{soil}}) + (N_{\text{minr}} * E_{\text{minr}})}{N_{\text{fer}} + N_{\text{soil}} + N_{\text{minr}}} \quad (3)$$

where  $N_{\text{fer}}$  is the amount of labelled fertilizer applied at anthesis;  $E_{\text{fer}}$ ,  $^{15}\text{N}$  excess of the fertilizer (5%);  $N_{\text{soil}}$ , soil N<sub>min</sub> at anthesis;  $E_{\text{soil}}$ ,  $^{15}\text{N}$  excess in the soil at anthesis;  $N_{\text{minr}}$ , N derived from mineralization from anthesis up to maturity, which was calculated as total N (aboveground plant + N<sub>min</sub>) under *Nd* at maturity minus total N at anthesis under *Nd*; and  $E_{\text{minr}}$ ,  $^{15}\text{N}$  excess derived from mineralization from anthesis up to maturity.  $E_{\text{minr}}$  was regarded as zero because of the absence of  $^{15}\text{N}$  excess in the soil at anthesis.

### 2.7. Determination of relative greenness

Relative greenness (SPAD) of the flag leaf was measured with a portable chlorophyll meter (Model SPAD 502, Minolta, Japan) starting at medium milk development (DC75). In each N subplot, 10 randomly chosen plants were used and SPAD measurements were conducted at three leaf positions (distal, medial, and proximal).



**Fig. 1.** Nitrogen nutrition index (NNI) of the three N treatments (severe deficiency (Nd), adequate (Nad) and excess (Nex)) in both years of the experiment. NNI was determined at the flag leaf stage and anthesis and shown are the mean values over the two growth stages and CO<sub>2</sub> levels ( $\pm$  S.E.M; n = 12) and the F-test result of the N and year effect. The horizontal line indicates the NNI of one, from which onwards the crop N status can be considered as non-limiting for growth. \*\*\*P < 0.001.

## 2.8. Determination of N use efficiency, its components and N harvest index

N use efficiency (NUE) was calculated by dividing grain biomass by Nmin at the beginning of the main growing season plus fertilizer N (Moll et al., 1982). N uptake efficiency (NUpE) was calculated by dividing total aboveground N yield by the same denominator used to calculate NUE. N utilization efficiency (NUtE) was calculated by dividing grain biomass by total aboveground N yield. N harvest index (NHI) was calculated by dividing grain N yield by total aboveground N yield. Grain specific N uptake efficiency (gNUpE) was calculated by multiplying NUpE with NHI.

## 2.9. Statistical analysis

The experiment was analyzed with SAS (version 9.4) proc mixed as split plot design with the CO<sub>2</sub> treatment as main plot factor and the N treatment as sub plot factor. If the year was added as third factor to the mixed model, then statistical analysis was conducted as repeated measurements. The year was treated as fixed effect and the variance/covariance between the two years of the random main plot and residual error was modelled with the UN (1) covariance model. This model applies no covariance between both years, but different error variances between the two years. Least square difference tests were implemented with SAS proc glimmix and mean values were regarded as significantly different if P < 0.05.

The CO<sub>2</sub> effect on the allometric relation between aboveground N concentration (N%) and aboveground biomass (W), which follows the equation:

$$N \% = aW^{-b} \quad (4)$$

(Sadras and Lemaire, 2014), was examined by transforming Eq. (4) to:

$$\log(N\%) = \log(a) - b \log(W) \quad (5)$$

and subjecting this linear relation to analysis of covariance.

Analysis of covariance of the effect of CO<sub>2</sub> and aboveground biomass on aboveground N concentration as well as the effect of CO<sub>2</sub> and grain number on variables describing N acquisition at maturity (e.g. Nrem and Nabs) was implemented by sequential F-tests with SAS proc mixed. The interaction effect was analyzed by testing the full model against the model without the interaction effect. The main effects were analyzed by testing the model without the interaction against the model further reduced by the main effect to be tested.

## 3. Results

### 3.1. CO<sub>2</sub> enrichment and climatic conditions

CO<sub>2</sub> enrichment took place during 99.0% of the target time in 2014 and 97.4% in 2015. The one minute average [CO<sub>2</sub>] was within  $\pm$  10% of the target concentration of 600 ppm for 95.6% of the operating time in 2014 and for 95.7% in 2015. Monthly mean temperature and global radiation as well as rainfall were in the range of normal variation in both years. In 2014, temperature in March and April was warmer compared to 2015 and the long term mean, respectively. A detailed description of the environmental conditions is shown elsewhere (Dier et al., 2018a,b). In 2015 rainfall was low in May and June and thus intensive irrigation was necessary (Manderscheid et al., 2018).

### 3.2. Characterization of the N treatments

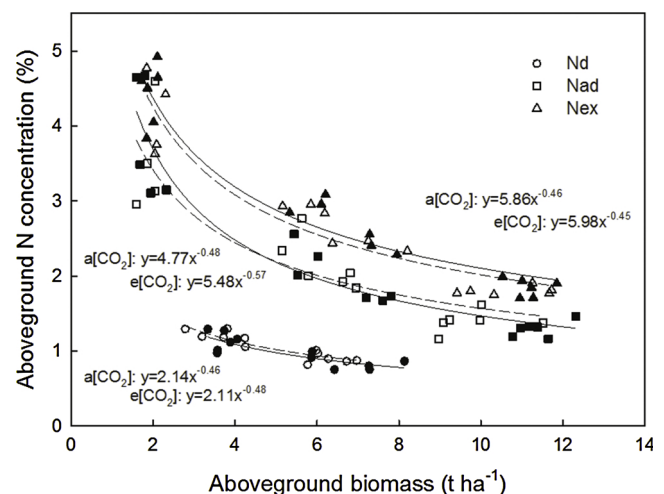
Fig. 1 shows the N nutrition index (NNI) measured at the flag leaf stage and anthesis of the three N levels (Table 1). Rising N supply significantly increased NNI and over both growth stages, CO<sub>2</sub> levels and years NNI was 0.4 under the N deficiency (Nd), 0.8 under the adequate (Nad) and 1.1 under the excess N level (Nex). Neither a significant CO<sub>2</sub> and year effect nor a CO<sub>2</sub> x N, N x year and CO<sub>2</sub> x N x year interaction on NNI were detected.

### 3.3. Allometric relation between plant N concentration and biomass

Fig. 2 shows the allometric relation between aboveground biomass and aboveground N concentration from 1 st node stage up to anthesis for each CO<sub>2</sub> x N combination comprising the data of both years. Rising N supply shifted the allometric curves to generally larger N concentration, but e[CO<sub>2</sub>] did not significantly influence the allometric relation under any N level (Table S1).

### 3.4. Tissue N concentration at anthesis and maturity and mean N content per grain

N concentration of all wheat fractions were strongly increased by increasing N level at anthesis and maturity (Tables 2, 3 and S2). Most of these N concentrations were influenced by the year, especially grain N concentration was considerably larger in 2015 compared to 2014 (16%



**Fig. 2.** Allometric relation between plant N concentration and biomass for each CO<sub>2</sub> x N combination comprising the data of both years. Open symbols and dashed regression line = ambient [CO<sub>2</sub>]; closed symbols and solid regression line = e[CO<sub>2</sub>]. Result of analysis of covariance of the linearized allometric relation is shown in Table S1. Only data of the biomass range of 1.55–12 t ha<sup>-1</sup> were considered for analysis in accordance with Justes et al. (1994).



**Table 2**

F-test result of the effect of the two CO<sub>2</sub> and three N levels on tissue N concentration (Ncon); N yield; mean N content per grain; percentage of N in stem, leaf and ear as a proportional of total aboveground N yield (%N); N use efficiency (NUE); N uptake efficiency (NUpE); N utilization efficiency (NuTE); N harvest index (NHI) and N uptake efficiency of grain (NUpEg). A refers to the sampling at anthesis and M to the one at maturity.

Variable	Growth stage	CO <sub>2</sub>	N	Year	CO <sub>2</sub> x N	CO <sub>2</sub> x Y	N x Y	CO <sub>2</sub> x N x Y
Nconc stem (mg N g <sup>-1</sup> )	A	ns	***	*	ns	ns	(*)	ns
	M	*	***	***	ns	(*)	***	ns
Nconc leaf (mg N g <sup>-1</sup> )	A	ns	***	(*)	ns	ns	ns	ns
	M	*	***	**	(*)	ns	**	(*)
Nconc ear/chaff (mg N g <sup>-1</sup> )	A	ns	***	ns	*	ns	ns	ns
	M	ns	***	ns	ns	ns	ns	ns
Nconc grain (mg N g <sup>-1</sup> )	M	(*)	***	***	*	ns	***	ns
N content per grain (mg)	M	ns	***	*	ns	ns	***	ns
N yield stem (g N m <sup>-2</sup> )	A	*	***	*	ns	ns	ns	ns
	M	(*) <sup>1</sup>	*** <sup>1</sup>	* <sup>1</sup>	ns <sup>1</sup>	ns <sup>1</sup>	ns <sup>1</sup>	ns <sup>1</sup>
N yield leaves (g N m <sup>-2</sup> )	A	ns	***	ns	ns	ns	ns	ns
	M	ns	***	***	ns	ns	**	ns
N yield chaff (g N m <sup>-2</sup> )	A	*	***	ns	ns	ns	ns	ns
	M	ns	***	(*)	ns	ns	**	ns
N yield total (g N m <sup>-2</sup> )	A	ns	***	ns	ns	ns	ns	ns
	M	(*)	***	***	ns	ns	**	ns
N yield grain (g N m <sup>-2</sup> )	M	*	***	***	(*)	ns	***	ns
%N of stem	A	*** <sup>2</sup>	*** <sup>2</sup>	*** <sup>2</sup>	ns <sup>2</sup>	(*) <sup>2</sup>	ns <sup>2</sup>	ns <sup>2</sup>
%N of leaf	A	(*) <sup>2</sup>	*** <sup>2</sup>	(*) <sup>2</sup>	ns <sup>2</sup>	ns <sup>2</sup>	ns <sup>2</sup>	ns <sup>2</sup>
%N of ear	A	ns <sup>2</sup>	*** <sup>2</sup>	ns <sup>2</sup>	ns <sup>2</sup>	ns <sup>2</sup>	ns <sup>2</sup>	ns <sup>2</sup>
NUE (g g <sup>-1</sup> N)	M	** <sup>1</sup>	*** <sup>1</sup>	* <sup>1</sup>	ns <sup>1</sup>	ns <sup>1</sup>	ns <sup>1</sup>	ns <sup>1</sup>
NUpE (g N g <sup>-1</sup> N)	M	*	***	ns	ns	ns	ns	ns
NuTE (g g <sup>-1</sup> N)	M	ns	***	**	ns	ns	**	ns
NHI	M	ns	***	**	ns	(*)	ns	ns
NUpEg (g N g <sup>-1</sup> N)	M	* <sup>1</sup>	*** <sup>1</sup>	ns <sup>1</sup>	ns <sup>1</sup>	ns <sup>1</sup>	ns <sup>1</sup>	ns <sup>1</sup>

(\*)P < 0.1 \*P < 0.05 \*\*P < 0.01 \*\*\*P < 0.001.

<sup>1</sup> Prior to analysis, data were log-transformed to ensure variance homogeneity and normal distribution of the residual error.

<sup>2</sup> Data were square root-transformed to ensure variance homogeneity and normal distribution of the residual error.

at Nd, 19% at Nad, 9% at Nex). Additionally, mean N content per grain N was larger under Nd (14%) and Nad (13%) in 2015 (Tables 2 and 3).

e[CO<sub>2</sub>] did not affect stem N concentration at anthesis, but significantly (P < 0.1) reduced it under all N levels by -9 to -17% at maturity in 2014 (Tables 2 and S2). Leaf N concentration was not significantly affected by e[CO<sub>2</sub>] at anthesis, but was significantly (P < 0.05) reduced at maturity in both years (-7% (Nd), -16 (Nad), -10% (Nex)). Ear N concentration was significantly (P < 0.05) reduced under Nad (-6%) at anthesis in both years.

Grain N concentration was slightly reduced by e[CO<sub>2</sub>] in both years (-1% (Nd), -6% (Nad) and -4% (Nex)), which was significant (P < 0.05) only under Nad (Tables 2 and 3). e[CO<sub>2</sub>] did not significantly affect mean N content per grain.

### 3.5. N yield at anthesis and maturity and N allocation at anthesis

Total aboveground N yield ranged from 5.3 to 21.6 at anthesis and from 7.9 to 28.3 g N m<sup>-2</sup> at maturity (Table 4). Grain N yield ranged

from 6.5 to 21.5 g N m<sup>-2</sup>. Rising N level strongly increased N yield of all wheat fractions at anthesis and maturity and most of these variables were significantly different in both years (Tables 2 and 4 and S3).

At anthesis, e[CO<sub>2</sub>] did not significantly influence total aboveground N yield (Tables 2 and 4), but increased N yield of stem (5–12%) and ear (12%) under Nad and Nex in both years and these variables under Nd in 2014 (Tables 2 and S3). At maturity, e[CO<sub>2</sub>] increased aboveground N yield on average by 8% (Nd), 9% (Nad) and 9% (Nex) and grain N yield by 8% (Nd), 10% (Nad) and 12% (Nex) (Table 4) where the former effect was significant for all N levels (P < 0.1) and the latter only under Nad and Nex (P < 0.1) (Table 2). Stem N yield was increased by e[CO<sub>2</sub>] primarily in 2015, although no significant CO<sub>2</sub> x year interaction was detected (Table 2 and S3).

Rising N supply increased the relative contribution of N in leaf as a proportional of the total N contained in the aboveground compartment whereas the relative contribution of N contained in the ear was decreased (Table 2 and Fig. S1). The relative contribution of N contained in the stem was largest under Nd and smallest under Nad. e[CO<sub>2</sub>]

**Table 3**

Effect of the two CO<sub>2</sub> and three N levels on grain N concentration and mean N content per grain. Shown are mean values (n = 3) and the percentage effect of e[CO<sub>2</sub>] (Δ (%)). Different small letters indicate significant differences among the marginal means of the N treatments. If the F-test resulted in a significant CO<sub>2</sub> x N interaction (Table 2), then different capital letters indicate significant differences for CO<sub>2</sub> means separate for each N level and different small letters significant differences for N means separate for each CO<sub>2</sub> treatment (letters are in bold for e[CO<sub>2</sub>]). All letters refer to the mean over both years.

	2014			2015		
	Nd	Nad	Nex	Nd	Nad	Nex
Nconc grain (mg N g <sup>-1</sup> )						
a[CO <sub>2</sub> ]	13.9 A c	18.6 A b	21.7 A a	16.0 A c	22.1 A b	23.7 A a
e[CO <sub>2</sub> ]	13.6 A c	17.5 B b	20.9 A a	15.9 A c	20.9 B b	22.7 A a
Δ (%)	-2	-6	-4	-1	-6	-4
N content per grain (mg)						
a[CO <sub>2</sub> ]	0.642 b	0.852 a	0.934 a	0.716 b	0.955 a	0.899 a
e[CO <sub>2</sub> ]	0.625 b	0.828 a	0.947 a	0.731 b	0.939 a	0.887 a
Δ (%)	-3	-3	1	2	-2	-1

**Table 4**

Effect of the two CO<sub>2</sub> and three N levels on total aboveground (AGN) and grain N yield. Shown are mean values (n = 3) and the percentage effect of e[CO<sub>2</sub>] (Δ (%)). Different small letters indicate significant differences among the marginal means of the N treatments. If the F-test resulted in a significant CO<sub>2</sub> × N interaction (Table 2), then different capital letters indicate significant differences for CO<sub>2</sub> means separate for each N treatment and different small letters significant differences for N means separate for each CO<sub>2</sub> treatment (letters are in bold for e[CO<sub>2</sub>]). All letters refer to the mean over both years.

		2014			2015		
		<i>Nd</i>	<i>Nad</i>	<i>Nex</i>	<i>Nd</i>	<i>Nad</i>	<i>Nex</i>
AGN yield (g N m <sup>-2</sup> )							
Anthesis	a[CO <sub>2</sub> ]	5.50 c	13.2 b	18.7 a	5.83 c	14.2 b	19.8 a
	e[CO <sub>2</sub> ]	6.11 c	14.7 b	19.6 a	5.34 c	14.8 b	21.6 a
	Δ (%)	11	11	4	-8	4	9
Maturity	a[CO <sub>2</sub> ]	7.92 c	19.1 b	24.8 a	7.85 c	22.1 b	26.1 a
	e[CO <sub>2</sub> ]	8.60 c	20.3 b	27.2 a	8.48 c	24.5 b	28.3 a
	Δ (%)	9	7	10	8	11	9
Grain N yield (g N m <sup>-2</sup> )							
	a[CO <sub>2</sub> ]	6.55 A c	15.2 B b	17.7 B a	6.68 A c	18.4 B b	19.7 B a
	e[CO <sub>2</sub> ]	7.18 A c	16.6 A b	20.3 A a	7.08 A c	20.3 A b	21.5 A a
	Δ (%)	10	9	14	6	10	9

significantly ( $P < 0.1$ ) decreased the relative contribution of N in the leaf in both years on average by -4, -6 and -7% but significantly ( $P < 0.001$ ) increased the one of stem by 4, 4 and 5% under *Nd*, *Nad* and *Nex*, respectively. There was a significant ( $P < 0.1$ ) CO<sub>2</sub> × Y interaction on the relative contribution of N in the stem because of a slightly stronger increase by e[CO<sub>2</sub>] in 2015.

### 3.6. N use efficiency, its components and N harvest index

N use efficiency (NUE) ranged from 24.1 to 100 g g<sup>-1</sup> N, N uptake efficiency (NUpE) from 0.74 to 1.64 g N g<sup>-1</sup> N, N utilization efficiency (NUEt) from 31.8 to 61.5 g g<sup>-1</sup> N, N harvest index (NHI) from 0.714 to 0.851 and grain specific N uptake efficiency (NUpEg) from 0.53 to 1.37 g N g<sup>-1</sup> N (Table 5). These variables were significantly decreased by rising N fertilization (Tables 2 and 5). Regarding year, NUE under all N levels and NUEt under *Nd* and *Nad* were larger in 2014, whereas NHI was larger under all N levels in 2015.

e[CO<sub>2</sub>] significantly increased NUE ( $P < 0.01$ ), NUpE ( $P < 0.05$ ) and gNUpE ( $P < 0.05$ ) in both years on average by 13, 18 and 17% (NUE); 12, 11 and 10% (NUpE); and 11, 12, 13% (gNUpE) under *Nd*, *Nad* and *Nex*, respectively (Tables 2 and 5). NHI was slightly but significantly ( $P < 0.1$ ) increased by e[CO<sub>2</sub>] in 2014 (1–4%). NUEt was not significantly influenced by e[CO<sub>2</sub>].

### 3.7. N remobilization and N remobilization efficiency

Fig. 3 shows the effect of CO<sub>2</sub>, N and year on N remobilization (Nrem) and N remobilization efficiency (NRE) for stem, leaf, chaff and aboveground plant based on the apparent method. Over both years and all CO<sub>2</sub> and N levels, Nrem was 3.47, 4.10 and 2.07 g N m<sup>-2</sup> and NRE 61, 76 and 73% for stem, leaf and chaff, respectively. Nrem was strongly correlated with N yield at anthesis with  $r^2$  values of 0.92 (stem), 0.98 (leaf) and 0.96 (chaff).

Rising N fertilization strongly increased Nrem of all wheat fractions. NRE of stem and total aboveground plant were decreased by rising N supply and NRE of leaf and chaff were similar under *Nd* and *Nad*, but were larger compared to *Nex*. Nrem and NRE were significantly influenced by year; in particular leaf NRE was considerably larger in 2015.

e[CO<sub>2</sub>] increased Nrem of stem and chaff on average by 15–23% and 13–16%, respectively under *Nad* and *Nex* in both years and by 11% and 10% under *Nd* in 2014. However, e[CO<sub>2</sub>] did not significantly influence Nrem of leaf and aboveground plant. Determination of Nrem by <sup>15</sup>N labelling, which was only conducted in 2015, showed no influence of e[CO<sub>2</sub>] on total plant Nrem (Fig. S2). Linear regression comparing the apparent with the <sup>15</sup>N labelling method yielded a slope of 1.03 ( $r^2 = 0.97$ ), indicating similar values for total plant Nrem (Fig. S3).

**Table 5**

Effect of the two CO<sub>2</sub> and three N levels on N use efficiency (NUE), N uptake efficiency (NUpE), N utilization efficiency (NUEt), N harvest index (NHI) and N uptake efficiency of grain (NUpEg). Shown are mean values (n = 3) and the percentage effect of e[CO<sub>2</sub>] (Δ (%)). Different small letters indicate significant differences among the marginal means of the N treatments. All letters refer to the means over both years.

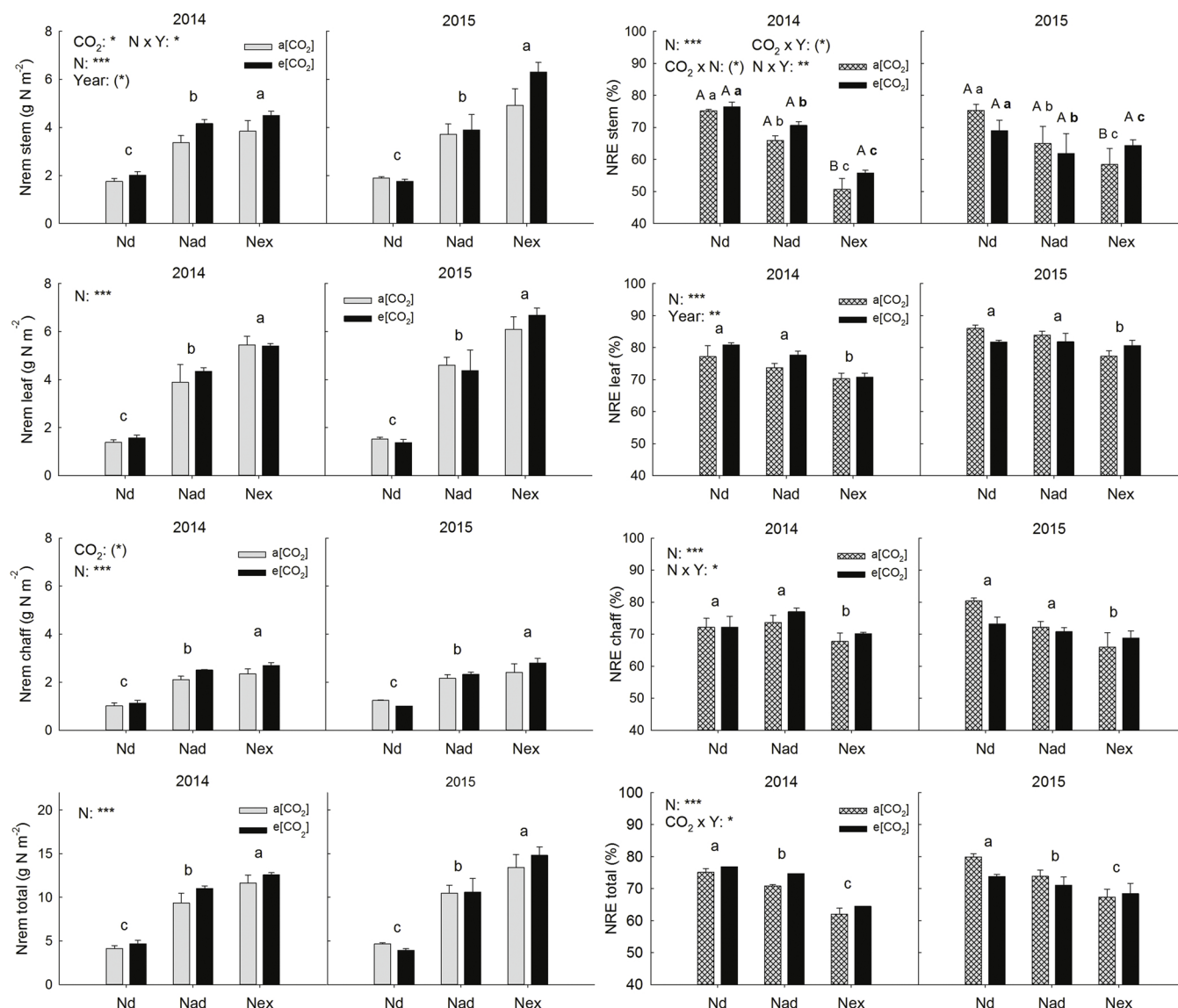
	2014			2015		
	<i>Nd</i>	<i>Na</i>	<i>Ne</i>	<i>Nd</i>	<i>Na</i>	<i>Ne</i>
NUE (g g <sup>-1</sup> N)						
a[CO <sub>2</sub> ]	84.7 a	41.7 b	24.3 c	75.8 a	37.2 b	24.1 c
e[CO <sub>2</sub> ]	100 a	49.3 b	29.2 c	81.5 a	44.2 b	27.6 c
Δ (%)	18	18	20	7	19	15
NUpE (g N g <sup>-1</sup> N)						
a[CO <sub>2</sub> ]	1.42 a	0.97 b	0.74 c	1.42 a	0.99 b	0.76 c
e[CO <sub>2</sub> ]	1.64 a	1.06 b	0.82 c	1.54 a	1.11 b	0.82 c
Δ (%)	15	8	11	9	13	9
NUEt (g g <sup>-1</sup> N)						
a[CO <sub>2</sub> ]	59.5 a	43.1 b	33.0 c	53.2 a	37.7 b	31.8 c
e[CO <sub>2</sub> ]	61.5 a	46.8 b	35.7 c	52.7 a	39.7 b	33.5 c
Δ (%)	3	8	8	-1	5	5
NHI						
a[CO <sub>2</sub> ]	0.827 a	0.798 b	0.714 c	0.851 a	0.833 b	0.755 c
e[CO <sub>2</sub> ]	0.835 a	0.816 b	0.744 c	0.835 a	0.829 b	0.761 c
Δ (%)	1	2	4	-2	-1	1
NUpEg (g N g <sup>-1</sup> N)						
a[CO <sub>2</sub> ]	1.17	0.77	0.53	1.21	0.82	0.57
e[CO <sub>2</sub> ]	1.37	0.86	0.61	1.29	0.92	0.63
Δ (%)	16	11	16	6	12	10

In 2014, e[CO<sub>2</sub>] significantly increased NRE of stem ( $P < 0.1$ ) by 2, 7 and 10% and of aboveground plant ( $P < 0.05$ ) by 2, 6 and 4% under *Nd*, *Nad* and *Nex*, respectively (Fig. 3). However, under *Nex*, stem NRE was significantly ( $P < 0.1$ ) increased in both years on average by 10%.

### 3.8. Flag leaf senescence, post-anthesis N uptake and soil mineral N at maturity

In both years, relative greenness of the flag leaf increased with rising N supply (Fig. 4). Starting from milk-ripe stage, flag leaf greenness declined with progressing growth stage, whereby this decline was delayed by rising N supply. Flag leaf greenness was not affected by e[CO<sub>2</sub>].

The apparent (Fig. 5) and the <sup>15</sup>N labelling method (Fig. S2) indicate that post-anthesis N uptake (Nabs) was increased by rising N fertilization from *Nd* to *Nad*, but further increases in *Nex* did not result in increased Nabs. Nabs considerably exceeded the amount of N fertilizer applied at anthesis under *Nd* and *Nad* (Figs. 5 and S2). Regarding year, Nabs was significantly ( $P < 0.05$ ) larger under *Nad* in 2015 than



**Fig. 3.** Effect of the two CO<sub>2</sub> and three N levels on N remobilization (Nrem) and N remobilization efficiency (NRE) of stem, leaf, chaff and aboveground plant based on the apparent method. Shown are the means values ( $\pm$  S.E.M;  $n = 3$ ) and the F-test result of the significant effects. Different small letters indicate significant differences among the marginal N means (mean over both CO<sub>2</sub> treatments and years). With a significant CO<sub>2</sub>  $\times$  N interaction: different capital letters indicate significant differences between the CO<sub>2</sub> means separate for each N level and different small letters significant differences among the N means separate for each CO<sub>2</sub> treatment (letters are in bold for e[CO<sub>2</sub>]). (\*) $P < 0.1$  \*\* $P < 0.05$  \*\*\* $P < 0.01$  \*\*\*\* $P < 0.001$ .

in 2014. Both the apparent method and <sup>15</sup>N labelling, which showed a strong correlation ( $r^2 = 0.94$ ) with respect to Nabs (Fig. S3), indicate a trend of increased Nabs under e[CO<sub>2</sub>] at Nad in 2015, even if a significant interaction with CO<sub>2</sub> was not detected.

Soil mineral N at maturity was significantly ( $P < 0.01$ ) decreased under e[CO<sub>2</sub>] under all N levels in both years (Fig. 5) on average by -22, -22 and -26% under Nd, Nad and Nex, respectively.

### 3.9. Correlation between grain number and variables describing grain N acquisition

Fig. 6 shows linear regression of grain number on aboveground and grain N yield, grain N concentration, mean N content per grain, Nrem and Nabs. In both years, e[CO<sub>2</sub>] increased grain number on average by 8% under Nd and 12% under Nad and Nex, respectively (Dier et al., 2018b). All variables were strongly positive correlated with grain number, whereby  $r^2$  values were highest for grain N yield (0.98, ambient [CO<sub>2</sub>]; 0.97, e[CO<sub>2</sub>]) and lowest for Nabs (0.86, ambient [CO<sub>2</sub>];

0.71, e[CO<sub>2</sub>]). Analysis of covariance (Fig. 6) resulted in significant grain number effects on all variables ( $P < 0.001$ ) and significant CO<sub>2</sub> effects on aboveground N yield ( $P < 0.1$ ), grain N concentration ( $P < 0.001$ ) and mean N content per grain ( $P < 0.1$ ). Significant CO<sub>2</sub> effects imply that the regression line with respect to e[CO<sub>2</sub>] runs below the one regarding ambient e[CO<sub>2</sub>]. This was also the case for Nrem, although no significant CO<sub>2</sub> effect was detected.

### 4. Discussion

The main objective of the present FACE study was to investigate in winter wheat grown under well-watered conditions the CO<sub>2</sub>  $\times$  N interactions on key processes determining grain N acquisition and thus grain N concentration, which are N remobilization (Nrem) and post-anthesis grain N acquisition (Nabs). The data show that under widely differing N levels, comprising a N nutrition index (NNI) between 0.4 and 1.1, e[CO<sub>2</sub>] improved Nrem and tended to increase Nabs that resulted in increased grain N yield and only slightly reduced grain N concentration.

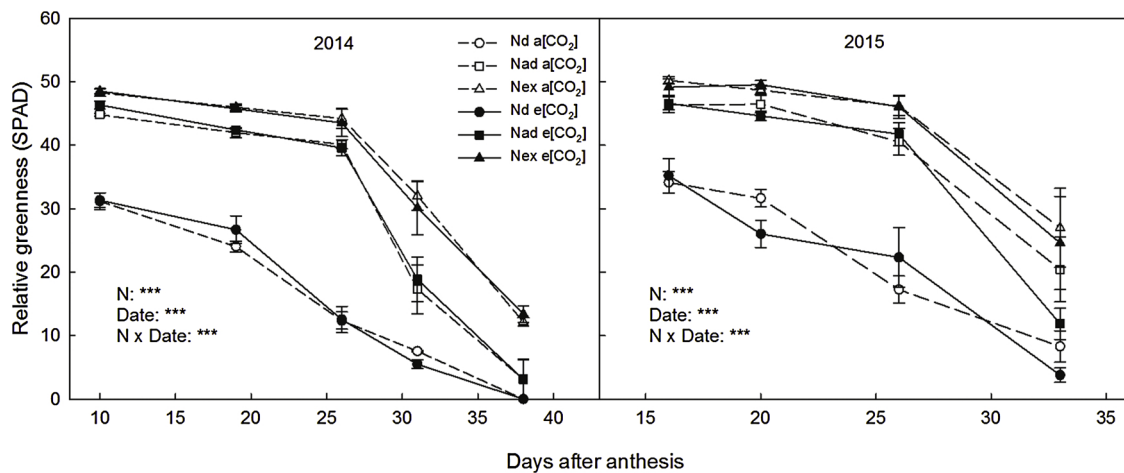


Fig. 4. Effect of the two  $\text{CO}_2$  and three N levels on relative greenness (SPAD) of the flag leaf during grain filling. Shown are mean values ( $\pm$  S.E.M;  $n = 3$ ) and the F-test results of the significant effects.

#### 4.1. Allometry between plant N concentration and biomass

In the present study, tissue N concentration at anthesis was slightly reduced by  $\text{e}[\text{CO}_2]$  which corresponds to previous FACE studies (Han et al., 2015; Tausz et al., 2017). If the negative allometric relation between N concentration and biomass (Justes et al., 1994; Sadras and Lemaire, 2014) is considered, where plants are compared at common biomass, then N concentration was unaffected by  $\text{e}[\text{CO}_2]$ . Consistent results were also found in a growth chamber study with annual  $\text{C}_3$  weed (*Abutilon theophrasti*) where decrease of N concentration by  $\text{e}[\text{CO}_2]$  disappeared when N concentration was compared at common biomass with regard to  $\text{CO}_2$  level (Coleman et al., 1993). Similarly, in the present experiment green leaf N concentration was not decreased by  $\text{e}[\text{CO}_2]$  when this variable was compared at common leaf area index (Dier et al., 2018b). Thus, there is indication that the decrease of N concentration by  $\text{e}[\text{CO}_2]$  before anthesis results only from increased growth under  $\text{e}[\text{CO}_2]$ , but not from direct physiological effects on N acquisition such as downregulation of RuBisCO gene expression in leaf

(Stitt and Krapp, 1999). Consequently, it might not be necessary to adjust the critical N concentration to  $\text{e}[\text{CO}_2]$  in wheat growth models as it was previously discussed by Vanuytrecht and Thorburn (2017).

#### 4.2. N acquisition

Aboveground plant N acquisition up to anthesis was not significantly increased by  $\text{e}[\text{CO}_2]$ . This result contrasts with other FACE studies where significant increases of 13–17% were found (Han et al., 2015; Tausz et al., 2017). In the present study, N allocation to the different organs was altered by  $\text{e}[\text{CO}_2]$  because  $\text{e}[\text{CO}_2]$  increased the proportion of stem in aboveground N but decreased that of leaf. This effect is consistent with Han et al. (2015) and could be explained by the larger stimulation of stem compared to leaf growth by  $\text{e}[\text{CO}_2]$  (Dier et al., 2018b).

At maturity, a FACE study conducted under subtropical climate and well-watered conditions found increased grain N yield of 17% under  $\text{e}[\text{CO}_2]$  at ample N supply (about  $460 \text{ kg N ha}^{-1}$ ), but grain N yield was

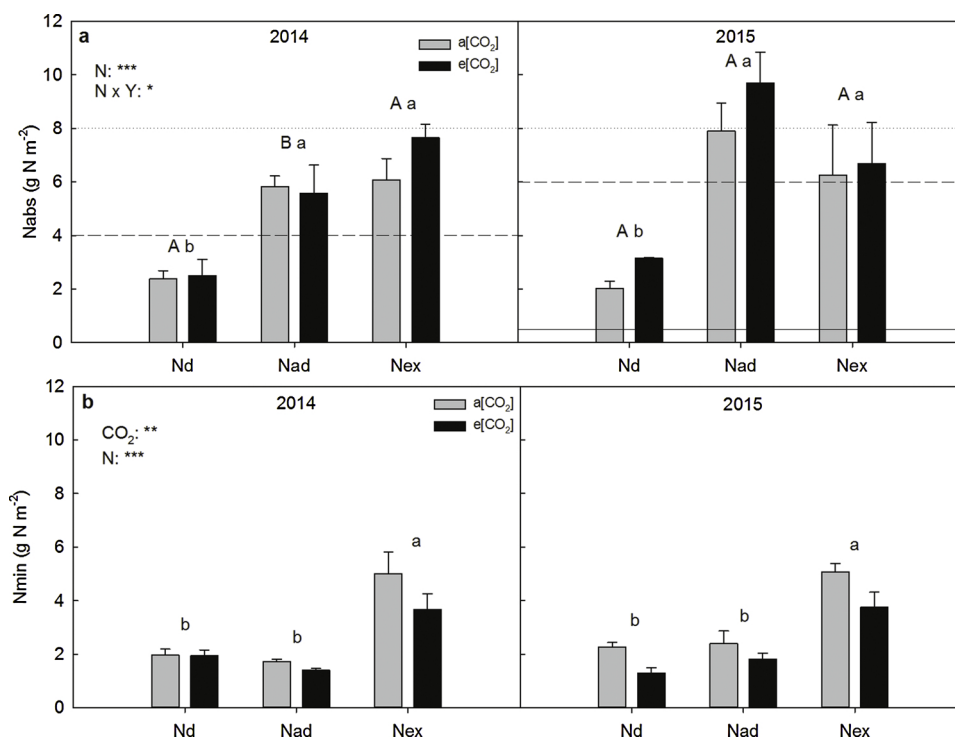


Fig. 5. Effect of the two  $\text{CO}_2$  and three N levels on (a) post-anthesis N uptake (Nabs) based on the apparent method and (b) mineral N in the 0–40 cm soil layer (Nmin) at maturity. Shown are mean values ( $\pm$  S.E.M;  $n = 3$ ) and the F-test result of the significant effects. The lines indicate the amount of N applied at anthesis (solid = Nd; dashed = Nad; dotted = Nex). Different small letters indicate significant differences between the marginal N means (mean over both  $\text{CO}_2$  treatments). With a significant Y x N interaction different capital letters indicate significant differences between both years of the marginal N means within each N level and different small letters significant differences between the marginal N means within each year. \* $P < 0.05$  \*\*\* $P < 0.001$ .



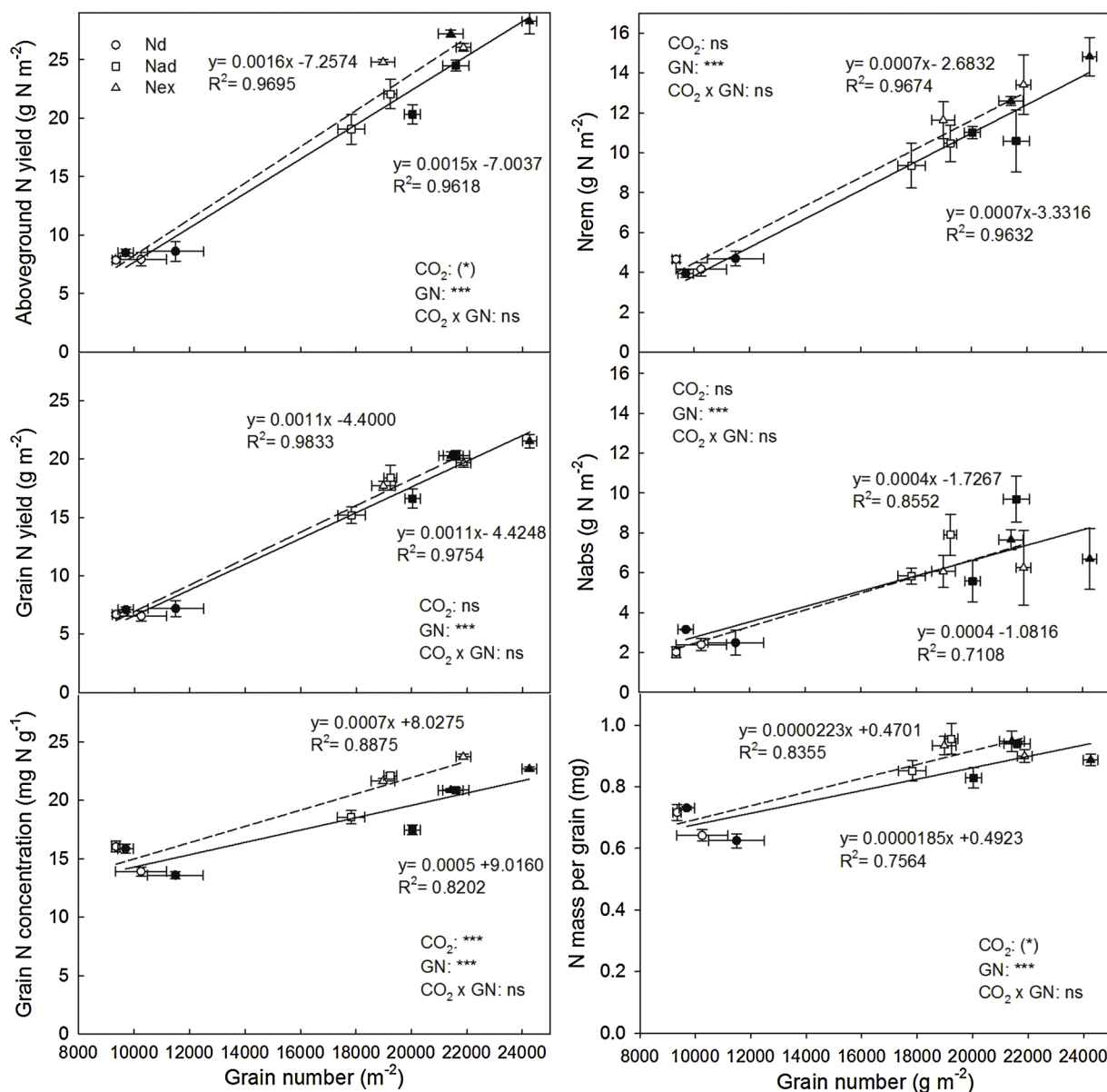


Fig. 6. Linear regression of grain number on variables describing N acquisition at maturity (aboveground and grain N yield; grain N concentration; Nrem; Nabs and; mean N content per grain). Shown are the mean values ( $\pm$  S.E.M;  $n = 3$ ) for each CO<sub>2</sub> x N x year combination. Open symbols and dashed regression line = ambient [CO<sub>2</sub>]; closed symbols and solid regression line = e[CO<sub>2</sub>]. Each diagram includes the result of the analysis of covariance. (\*)  $P < 0.1$  \*\*\*  $P < 0.001$ .

uninfluenced under N deficiency (about 100 kg N ha<sup>-1</sup>) (Sinclair et al., 2000; Kimball et al., 2001). However, other FACE studies conducted under similar conditions (Ma et al., 2007; Han et al., 2015) and in a semi-arid environment where water availability is particularly low at grain filling (Tausz et al., 2017) showed increased aboveground N yield of 9–21% under a low (100–180 kg N ha<sup>-1</sup>) and 9–22% under a high N treatment (170–240 kg N ha<sup>-1</sup>). In the present study under temperate climate and well-watered conditions, e[CO<sub>2</sub>] increased grain and total aboveground N yield under all N levels, but the e[CO<sub>2</sub>] effect on grain N yield was slightly less pronounced under Nd (8%) compared to Nad (10%) and Nex (12%). The very strong linear relation ( $r^2 = 0.98$ ) between grain number and grain N yield (Fig. 6) indicates that increase of grain number by e[CO<sub>2</sub>], which was 8% under Nd and 12% under Nad and Nex, respectively (Dier et al., 2018b), was the reason for the enhanced grain N yield.

The finding that e[CO<sub>2</sub>] increased N use efficiency primarily through the increase of N uptake efficiency, being consistent with the FACE study of Tausz et al. (2017), can be attributed to the enhanced N acquisition of stem and grain.

#### 4.3. Nitrogen sources contributing to the increased grain N acquisition under e[CO<sub>2</sub>]

##### 4.3.1. Nitrogen remobilization

In the present study, rising N supply increased Nrem but decreased the efficiency of Nrem (NRE). While the former effect is consistent with other studies (Barbottin et al., 2005; Gaju et al., 2014), various effects of rising N supply on NRE were found (Barbottin et al., 2005; Gaju et al., 2011, 2014). It is established that a high post-anthesis plant N status delays senescence, being a major N source for Nrem (Kong et al., 2016), which might explain the decrease of NRE by rising N supply. Moreover, grain sink strength might be a reason because N yield at anthesis per individual grain decreased with decreasing N supply and linear regression resulted in a stronger negative correlation between this variable and NRE ( $r^2 = -0.76$ ) compared to N yield at anthesis and NRE ( $r^2 = -0.67$ ) (data not shown). Consistent with other studies (Pask et al., 2012; Gaju et al., 2014) leaves had the highest NRE (76%) followed by chaff (73%) and stems (61%).

It was suggested that the decrease of vegetative tissue N concentration by e[CO<sub>2</sub>] at anthesis result in a decrease of Nrem (Kimball et al.,

2001; Rubio-Asensio and Bloom, 2016). However, a FACE study showed no  $e[CO_2]$  effect on total plant Nrem (Tausz et al., 2017). In the present study,  $e[CO_2]$  did not affect leaf Nrem and enhanced Nrem of stem and chaff as well as NRE of stem and aboveground plant. While the increase of Nrem is consistent with the increased stem and ear N yield at anthesis, the  $e[CO_2]$  effect on NRE of stem and total aboveground plant indicate that  $e[CO_2]$  enhanced mobilization of vegetative N during grain filling. Indication that  $e[CO_2]$  increased the rate of mobilization of N from vegetative tissue was found in other FACE studies, where  $e[CO_2]$  accelerated the decline of leaf proteins (Nie et al., 1995; Osborne et al., 1998) and of N in stem tissue during grain filling (Lam et al., 2012) and stimulated the gene expression of glutamine synthetase (Buchner et al., 2015), whose activity correlates with Nrem (Kichey et al., 2007).

Under *Nd* and *Nad*, stem and aboveground plant NRE as well as NHI were increased by  $e[CO_2]$  only in 2014, while NHI and mean N content per grain were generally larger in 2015. These effects could be associated with larger post-anthesis soil N availability in 2015, whereby in 2014 it was possibly insufficient to serve an increased grain N sink strength due to the grain number increase by  $e[CO_2]$ . Reasons for increased post-anthesis soil N availability per grain in 2015 are the increased N fertilizer supply at anthesis under *Nad* (Table 1) and the lower grain number in 2015 (Dier et al., 2018b) but similar Nabs between both years under *Nd*.

#### 4.3.2. Post-anthesis N uptake

Judging from relative greenness of the flag leaf during grain filling, senescence was not accelerated by  $e[CO_2]$ , which contrasts with previous studies (Osborne et al., 1998; Brooks et al., 2000; Fangmeier et al., 2000) possibly due to differences in climatic conditions and the effect on leaf temperature. Correspondingly, Nabs was not significantly affected, but tended to be increased by  $e[CO_2]$ , which is consistent with the significantly lower soil mineral N content at maturity under  $e[CO_2]$  compared to ambient  $[CO_2]$ . Moreover,  $e[CO_2]$  enhanced N acquisition more at maturity compared to anthesis, indicating that Nabs was important to cover an increased grain N sink demand under  $e[CO_2]$ . The fact that Nabs considerably exceeded the amount of N applied at anthesis under *Nd* and *Nad* suggests that there was a source limit of post-anthesis soil N availability under these N levels. It is difficult to conclude, therefore, whether higher N application at anthesis would have led to a significant increase of Nabs by  $e[CO_2]$  under *Nd* and *Nad*. However, there is indication from a glasshouse study that  $e[CO_2]$  can strongly increase Nabs, leading to no decrease of grain N concentration under  $e[CO_2]$ , when wheat is grown under relatively low pre-anthesis, but high post-anthesis N availability (Fernando et al., 2017). Because water availability is critical for N uptake, Nabs might be unimportant to serve an increased grain N demand under  $e[CO_2]$  in a semi-arid environment despite sufficient N availability in the soil at grain filling.

Under *Nex*, the relative low Nabs despite non-limiting N availability might be explained by decreased post-anthesis root N uptake. It is established that root  $NO_3^-$  uptake is decreased under a high plant N status (Glass et al., 2002; Barneix, 2007). That the plant N status was high under *Nex* is indicated by the NNI of 1.1. Moreover, individual grain weight was smaller under *Nex* compared to *Nd* and *Nad* (Dier et al., 2018b), suggesting limited C assimilation per grain and thus limited energy supply for this process. Energy costs are higher for N than C assimilation (Munier-Jolain and Salon, 2005) and thus increased competition between N and C assimilation for energy might also be a reason for the relatively low Nabs.

The high Nabs under *Nd* and *Nad* suggest that  $NO_3^-$  assimilation was not affected by  $e[CO_2]$ , which is consistent with the finding that  $e[CO_2]$  did not reduce gene expression and activity of  $NO_3^-$  reductase before and at grain filling (Dier et al., 2018a).

#### 4.4. Grain N concentration

In previous FACE studies under subtropical and semi-arid conditions,  $e[CO_2]$  decreased grain protein concentration by -9 and -10% under N

deficiency (Kimball et al., 2001; Walker et al., 2017). Those results contrast with the present study, in which hardly any reduction of grain N concentration (-1%) was found under *Nd* (NNI: 0.4). The decrease under *Nad* (NNI: 0.8) (-6%) is consistent with other FACE studies using adequate N levels in which reductions were found to be -5 to -9% (Kimball et al., 2002; Erbs et al., 2010; Lam et al., 2012; Tausz et al., 2017). The decrease under *Nex* (NNI: 1.1) (-4%) contrasts with the FACE study of Kimball et al. (2001) where no reduction was found under ample N supply.

Grain N concentration was considerably larger in 2015 than 2014, which strongly exceeded the  $e[CO_2]$  effect. This could be partly explained under *Nd* and *Nad* by the increased post-anthesis N availability per grain due to the smaller grain number under *Nd* (Dier et al., 2018b) and the higher N fertilizer supply at anthesis under *Nad* in 2015. Moreover, under *Nad* and *Nex*, grain number was increased but individual grain weight was decreased in 2015 (Dier et al., 2018b). Therefore, dilution of N content per grain by grain growth was decreased in 2015 compared to 2014.

The smaller relative reduction of grain N concentration by  $e[CO_2]$  under *Nd* compared to high N supply could be attributed to the smaller grain yield stimulation under *Nd* (Dier et al., 2018b) and that grain N accumulation is partly sink regulated, especially under low grain N concentration (Martre et al., 2003). The contrasting results of grain N concentration under N deficiency between the present (-1%) and the Kimball et al. (2001) study (-9%) could be explained by differences in post-anthesis soil N availability. While a large proportion of grain N originated from Nabs in the present study, post-anthesis soil N availability was possibly deficient in the study of Kimball et al. (2001) as, unlike the present study, flag leaf senescence was accelerated under  $e[CO_2]$  (Brooks et al., 2000).

The decrease of grain N concentration by  $e[CO_2]$  could be attributed to several factors. As indicated by the regression of grain number on aboveground and grain N yield (Fig. 6), the N source per grain (i.e. vegetative N) was reduced under  $e[CO_2]$ . Secondly, the finding that grain N concentration was more strongly affected by  $e[CO_2]$  than mean N content per grain while individual grain weight was increased (Dier et al., 2018b) indicate that growth dilution was another reason. Moreover, the  $e[CO_2]$  induced increase of the proportion of stem in aboveground N at the expense of leaf, having much larger NRE than stem, possibly led to a decreased whole-plant Nrem potential.

#### 4.5. Recommendation of N fertilization in future

The results herein show that grain N yield increased with rising grain number and this relation was not affected by  $e[CO_2]$  (Fig. 6). Hence, increased N fertilization in the phase between flag leaf emergence and anthesis, when NNI (Ravier et al., 2017) and  $e[CO_2]$  (Fisher and Aguilar, 1976) have their greatest influence on grain number, might enhance the grain number and thus N yield increase by  $e[CO_2]$ . Moreover, as indicated by the positive  $e[CO_2]$  effect on Nabs, supply of additional N at anthesis could enable the plant to increase grain N concentration under  $e[CO_2]$ , provided that the plant N status before anthesis is not excessive.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.fcr.2019.02.013>.

## References

- Ainsworth, E.A., Long, S.P., 2005. What have we learned from 15 years of free-air CO<sub>2</sub> enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO<sub>2</sub>. *New Phytol.* 165, 351–371.
- Asseng, S., Martre, P., Maïorano, A., Rötter, R.P., O'Leary, G.J., Fitzgerald, G.J., Girousse, C., Motzo, R., Giunta, F., Babar, M.A., Reynolds, M.P., Kheir, A.M.S., Thorburn, P.J., Waha, K., Ruane, A.C., Aggarwal, P.K., Ahmed, M., Balković, J., Basso, B., Biernath, C., Bindu, M., Cammarano, D., Challinor, A.J., De Sanctis, G., Dumont, B., Rezaei, E.E., Fereres, E., Ferrise, R., Garcia-Vila, M., Gayler, S., Gao, Y., Horan, H., Hoogenboom, G., Izaurralde, R.C., Jabloun, M., Jones, C.D., Kassie, B.T., Kersebaum, K.-C., Klein, C., Koehler, A.-K., Liu, B., Minoli, S., San Martin, M.M., Müller, C., Kumar, S.N., Nendel, C., Olesen, J.E., Palosuo, T., Porter, J.R., Priesack, E., Ripoche, D., Semenov, M.A., Stöckle, C., Stratonovitch, P., Streck, T., Supit, I., Tao, F., Van der Velde, M., Wallach, D., Wang, E., Webber, H., Wolf, J., Xiao, L., Zhang, Z., Zhao, Z., Zhu, Y., Ewert, F., 2019. Climate change impact and adaptation for wheat protein. *Glob. Chang. Biol.* 25 (1), 155–173.
- Bancal, P., 2009. Decorrelating source and sink determinism of nitrogen remobilization during grain filling in wheat. *Ann. Bot.* 103 (8), 1315–1324.
- Barbottin, A., Lecomte, C., Bouchard, C., Jeuffroy, M.H., 2005. Nitrogen remobilization during grain filling in wheat. *Crop Sci.* 45 (3), 1141–1150.
- Barneix, A.J., 2007. Physiology and biochemistry of source-regulated protein accumulation in the wheat grain. *J. Plant Physiol.* 164 (5), 581–590.
- Bloom, A.J., Burger, M., Rubio-Asensio, J.S., Cousins, A.B., 2010. Carbon dioxide enrichment inhibits nitrate assimilation in wheat and Arabidopsis. *Science* 328, 899–903.
- Brooks, T.J., Wall, G.W., Pinter, P.J., Kimball, B.A., LaMorte, R.L., Leavitt, S.W., Matthias, A.D., Adamsen, F.J., Hunsaker, D.J., Webber, A.N., 2000. Acclimation response of spring wheat in a free-air CO<sub>2</sub> enrichment (FACE) atmosphere with variable soil nitrogen regimes. 3. Canopy architecture and gas exchange. *Photosyn. Res.* 66, 97–108.
- Buchner, P., Tausz, M., Ford, R., Leo, A., Fitzgerald, G.J., Hawkesford, M.J., Tausz-Pösch, S., 2015. Expression patterns of C- and N-metabolism related genes in wheat are changed during senescence under elevated CO<sub>2</sub> in dry-land agriculture. *Plant Sci.* 236, 239–249.
- Cai, C., Yin, X., He, S., Jiang, W., Si, C., Struik, P.C., Luo, W., Li, G., Xie, Y., Xiong, Y., Pan, G., 2016. Responses of wheat and rice to factorial combinations of ambient and elevated CO<sub>2</sub> and temperature in FACE experiments. *Glob. Chang. Biol.* 22, 856–874.
- Coleman, J.S., McConnaughey, K.D.M., Bazzaz, F.A., 1993. Elevated CO<sub>2</sub> and plant nitrogen-use: is reduced tissue nitrogen concentration size-dependent? *Oecologia* 93, 195–200.
- Cotrufo, M.F., Ineson, P., Scott, A., 1998. Elevated CO<sub>2</sub> reduces the nitrogen concentration of plant tissues. *Glob. Chang. Biol.* 4, 43–54.
- Dier, M., Meinen, R., Erbs, M., Kollhorst, L., Baillie, C.K., Kaufholdt, D., Weigel, H.J., Zörb, C., Hänsch, R., Manderscheid, R., 2018a. Effects of Free Air Carbon Dioxide Enrichment (FACE) on nitrogen assimilation and growth of winter wheat under nitrate and ammonium fertilization. *Glob. Chang. Biol.* 24, e40–e54.
- Dier, M., Sickora, J., Erbs, M., Weigel, H.J., Zörb, C., Manderscheid, R., 2018b. Decreased wheat grain yield stimulation by Free air CO<sub>2</sub> Enrichment under N deficiency is strongly related to decreased radiation use efficiency enhancement. *Eur. J. Agron.* 101, 38–48.
- Erbs, M., Manderscheid, R., Jansen, G., Seddig, S., Pacholski, A., Weigel, H.J., 2010. Effects of free-air CO<sub>2</sub> enrichment and nitrogen supply on grain quality parameters and elemental composition of wheat and barley grown in a crop rotation. *Agric. Ecosyst. Environ.* 136 (1), 59–68.
- Fangmeier, A., Chrost, B., Högy, P., Krupinska, K., 2000. CO<sub>2</sub> enrichment enhances flag leaf senescence in barley due to greater grain nitrogen sink capacity. *Environ. Exp. Bot.* 44 (2), 151–164.
- Feng, Z., Rütting, T., Pleijel, H., Wallin, G., Reich, P.B., Kammann, C.I., Newton, P.C.D., Kobayashi, K., Luo, Y., Uddling, J., 2015. Constraints to nitrogen acquisition of terrestrial plants under elevated CO<sub>2</sub>. *Glob. Change Biol.* 21 (8), 3152–3168.
- Fernando, N., Hirotsu, N., Panozzo, J., Tausz, M., Norton, R.M., Seneweera, S., 2017. Lower grain nitrogen content of wheat at elevated CO<sub>2</sub> can be improved through post-anthesis NH<sub>4</sub><sup>+</sup> supplement. *J. Cereal Sci.* 74, 79–85.
- Fisher, R.A., Aguilar, I., 1976. Yield potential in a dwarf spring wheat and effect of carbon-dioxide fertilization. *Agron. J.* 68, 749–752.
- Gaju, O., Allard, V., Martre, P., Snape, J.W., Heumez, E., LeGouis, J., Moreau, D., Bogard, M., Griffiths, S., Orford, S., Hubbart, S., Foulkes, M.J., 2011. Identification of traits to improve the nitrogen-use efficiency of wheat genotypes. *Field Crops Res.* 123 (2), 139–152.
- Gaju, O., Allard, V., Martre, P., Le Gouis, J., Moreau, D., Bogard, M., Hubbart, S., Foulkes, M.J., 2014. Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. *Field Crops Res.* 155, 213–223.
- Glass, A.D., Britto, D.T., Kaiser, B.N., Kinghorn, J.R., Kronzucker, H.J., Kumar, A., Okamoto, M., Rawat, S., Siddiqui, M.Y., Unkles, S.E., Vidmar, J.J., 2002. The regulation of nitrate and ammonium transport systems in plants. *J. Exp. Bot.* 53 (370), 855–864.
- Han, X., Hao, X., Lam, S.K., Wang, H., Li, Y., Wheeler, T., Ju, H., Lin, E., 2015. Yield and nitrogen accumulation and partitioning in winter wheat under elevated CO<sub>2</sub>: a 3-year free-air CO<sub>2</sub> enrichment experiment. *Agric. Ecosyst. Environ.* 209, 132–137.
- IPCC, 2013. In: Stocker, T.F.D., Qin, G.K., Plattner, M., Tignor, S.K., Allen, J., Boschung, A., Nauels, Y., Xia, V., Bex, P.M., Midgley (Eds.), *Climate Change (2013): The Physical Science Basis Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA 1535 pp.
- Jamieson, P.D., Semenov, M.A., 2000. Modelling nitrogen uptake and redistribution in wheat. *Field Crops Res.* 68 (1), 21–29.
- Jenner, C.F., Ugalde, T.D., Aspinall, D., 1991. The physiology of starch and protein deposition in the endosperm of wheat. *Funct. Plant Biol.* 18 (3), 211–226.
- Justes, E., Mary, B., Meynard, J.M., Machet, J.M., Thelier-Huché, L., 1994. Determination of a critical nitrogen dilution curve for winter wheat crops. *Ann. Bot.* 74 (4), 397–407.
- Kichey, T., Hirel, B., Heumez, E., Dubois, F., Le Gouis, J., 2007. In winter wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers. *Field Crops Res.* 102, 22–32.
- Kimball, B.A., Morris, C.F., Pinter, P.J., Wall, G.W., Hunsaker, D.J., Adamsen, F.J., LaMorte, R.L., Leavitt, S.W., Thompson, T.L., Matthias, A.D., Brooks, T.J., 2001. Elevated CO<sub>2</sub>, drought and soil nitrogen effects on wheat grain quality. *New Phytol.* 150 (2), 295–303.
- Kimball, B.A., Kobayashi, K., Bindi, M., 2002. Responses of agricultural crops to free-air CO<sub>2</sub> enrichment. *Adv. Agron.* 77, 293–368.
- Kong, L., Xie, Y., Hu, L., Feng, B., Li, S., 2016. Remobilization of vegetative nitrogen to developing grain in wheat (*Triticum aestivum* L.). *Field Crops Res.* 196, 134–144.
- Lam, S.K., Han, X., Lin, E., Norton, R., Chen, D., 2012. Does elevated atmospheric carbon dioxide concentration increase wheat nitrogen demand and recovery of nitrogen applied at stem elongation? *Agric. Ecosyst. Environ.* 155, 142–146.
- Lewin, K.F., Hendrey, G.R., Kolber, Z., 1992. Brookhaven National Laboratory free-air carbon-dioxide enrichment facility. *Crit. Rev. Plant Sci.* 11, 135–141.
- Loladze, I., 2002. Rising atmospheric CO<sub>2</sub> and human nutrition: toward globally imbalanced plant stoichiometry? *Trends Ecol. Evol.* (Amst.) 17 (10), 457–461.
- Ma, Y.Z., MacKown, C.T., Van Sanford, D.A., 1995. Kernel mass and assimilate accumulation of wheat: cultivar responses to 50% spikelet removal at anthesis. *Field Crops Res.* 42, 93–99.
- Ma, H.L., Zhu, H.G., Liu, G., Xie, Z.B., Wang, Y.L., Yang, L.X., Zeng, Q., 2007. Availability of soil nitrogen and phosphorus in a typical rice-wheat rotation system under elevated atmospheric CO<sub>2</sub>. *Field Crops Res.* 100, 44–51.
- Manderscheid, R., Dier, M., Erbs, M., Sickora, J., Weigel, H.J., 2018. Nitrogen supply - A determinant in water use efficiency of winter wheat under free air CO<sub>2</sub> enrichment. *Agric. Water Manag.* 210, 70–77.
- Martre, P., Porter, J.R., Jamieson, P.D., Tribou, E., 2003. Modeling grain nitrogen accumulation and protein composition to understand the sink/source regulations of nitrogen remobilization for wheat. *Plant Physiol.* 133 (4), 1959–1967.
- Moll, R.H., Kamprath, E.J., Jackson, W.A., 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization 1. *Agron. J.* 74 (3), 562–564.
- Munier-Jolain, N.G., Salon, C., 2005. Are the carbon costs of seed production related to the quantitative and qualitative performance? An appraisal for legumes and other crops. *Plant Cell Environ.* 28 (11), 1388–1395.
- Myers, S.S., Zanolletti, A., Kloog, I., et al., 2014. Increasing CO<sub>2</sub> threatens human nutrition. *Nature* 510, 139–142.
- Nie, G.Y., Long, S.P., Garcia, R.L., Kimball, B.A., Lamorte, R.L., Pinter, P.J., Wall, G.W., Webber, A.N., 1995. Effects of Free-Air CO<sub>2</sub> enrichment on the development of the photosynthetic apparatus in wheat, as indicated by changes in leaf proteins. *Plant Cell Environ.* 18, 855–864.
- Osborne, C.P., La Roche, J., Garcia, R.L., Kimball, B.A., Wall, G.W., Pinter, P.J., LaMorte, R.L., Hendrey, G.R., Long, S.P., 1998. Does leaf position within a canopy affect acclimation of photosynthesis to elevated CO<sub>2</sub>? Analysis of a wheat crop under free-air CO<sub>2</sub> enrichment. *Plant Physiol.* 117 (3), 1037–1045.
- Pacholski, A., Manderscheid, R., Weigel, H.J., 2015. Effects of free air CO<sub>2</sub> enrichment on root growth of barley, sugar beet and wheat grown in a rotation under different nitrogen supply. *Eur. J. Agron.* 63, 36–46.
- Panozzo, J.F., Walker, C.K., Partington, D.L., Neumann, N.C., Tausz, M., Seneweera, S., Fitzgerald, G.J., 2014. Elevated carbon dioxide changes grain protein concentration and composition and compromises baking quality. A FACE study. *J. Cereal Sci.* 60 (3), 461–470.
- Pask, A.J.D., Sylvester-Bradley, R., Jamieson, P.D., Foulkes, M.J., 2012. Quantifying how winter wheat crops accumulate and use nitrogen reserves during growth. *Field Crops Res.* 126, 104–118.
- Pleijel, H., Uddling, J., 2012. Yield vs. Quality trade-offs for wheat in response to carbon dioxide and ozone. *Glob. Change Biol.* 18, 596–605.
- Ravier, C., Meynard, J.M., Cohan, J.P., Gate, P., Jeuffroy, M.H., 2017. Early nitrogen deficiencies favor high yield, grain protein content and N use efficiency in wheat. *Eur. J. Agron.* 89, 16–24.
- Rubio-Asensio, J.S., Bloom, A.J., 2016. Inorganic nitrogen form: a major player in wheat and Arabidopsis responses to elevated CO<sub>2</sub>. *J. Exp. Bot.* 68, 2611–2625.
- Sadras, V.O., Lemaire, G., 2014. Quantifying crop nitrogen status for comparisons of agronomic practices and genotypes. *Field Crops Res.* 164, 54–64.
- Shewry, P.R., Hey, S.J., 2015. The contribution of wheat to human diet and health. *Food Energy Secur.* 4, 178–202.
- Simoni, S., 2009. World cereal production brief aspects privind productia mondiala de cereale. *Lucrari Stiintifice, Universitatea de Stiinte Agricole Si Medicina Veterinara a Banatului, Timisoara, Seria I, Management Agricol.* 11 (4), 183–188.
- Sinclair, T.R., Pinter, P.J., Kimball, B.A., Adamsen, F.J., LaMorte, R.L., Wall, G.W., Hunsaker, D.J., Adam, N., Brooks, T.J., Garcia, R.L., Thompson, T., Leavitt, S., Matthias, A., 2000. Leaf nitrogen concentration of wheat subjected to elevated [CO<sub>2</sub>]

- and either water or N deficits. *Agric. Ecosyst. Environ.* 79, 53–60.
- Stitt, M., Krapp, A., 1999. The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant Cell Environ.* 22, 583–622.
- Taub, D.R., Miller, B., Allen, H., 2008. Effects of elevated CO<sub>2</sub> on the protein concentration of food crops: a meta-analysis. *Glob. Change Biol.* 14, 565–575.
- Tausz, M., Norton, R.M., Tausz-Posch, S., Löw, M., Seneweera, S., O'Leary, G., Armstrong, R., Fitzgerald, G.J., 2017. Can additional N fertiliser ameliorate the elevated CO<sub>2</sub>-induced depression in grain and tissue N concentrations of wheat on a high soil N background? *J. Agron. Crop. Sci.* 203, 574–583.
- Vanuytrecht, E., Thorburn, P.J., 2017. Responses to atmospheric CO<sub>2</sub> concentrations in crop simulation models: a review of current simple and semicomplex representations and options for model development. *Glob. Change Biol.* 23, 1806–1820.
- Walker, C., Armstrong, R., Panozzo, J., Partington, D., Fitzgerald, G., 2017. Can nitrogen fertiliser maintain wheat (*Triticum aestivum*) grain protein concentration in an elevated CO<sub>2</sub> environment? *Soil Res.* 55 (6), 518–523.
- Wang, L., Feng, Z.Z., Schjoerring, J.K., 2013. Effects of elevated atmospheric CO<sub>2</sub> on physiology and yield of wheat (*Triticum aestivum* L.): a meta-analytic test of current hypotheses. *Agric. Ecosyst. Environ.* 178, 57–63.
- Wieser, H., Manderscheid, R., Erbs, M., Weigel, H.J., 2008. Effects of elevated atmospheric CO<sub>2</sub> concentrations on the quantitative protein composition of wheat grain. *J. Agric. Food Chem.* 56, 6531–6535.