Effects on nutrients and on grain quality in spring wheat crops grown under elevated CO_2 concentrations and stress conditions in the European, multiple-site experiment 'ESPACE-wheat'

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Abstract

Nutrient element concentrations and grain quality were assessed in spring wheat grown under elevated CO_2 concentrations and contrasting levels of tropospheric ozone at different nitrogen supply rates at several European sites. Carbon dioxide enrichment proved to affect nutrient concentrations in a complex manner. In green leaves, all elements (with exception of phosphorus and iron) decreased. In contrast, effects on the element composition of grains were restricted to reductions in nitrogen, calcium, sulphur and iron. Ozone exposure resulted in no significant effects on nutrient element concentrations in different tissues in the overall analysis. The nitrogen demand of green tissues was reduced due to CO_2 enrichment as shown by reductions in the critical leaf nitrogen concentration and also enhanced nitrogen use efficiency. Reductions in the content of ribulose-bisphosphate carboxylase/oxygenase and repression of the photorespiratory pathway and reduced nitrogen allocation to enzymes driving the photosynthetic carbon oxidation cycle were chiefly responsible for this effect. Thus, nitrogen acquisition by the crop did not match carbon acquisition under CO_2 enrichment. Since crop nitrogen uptake from the soil was already completed at anthesis, nitrogen allocated to the grain after anthesis originated from vegetative pools—causing grain nitrogen concentrations to decrease under CO_2 enrichment (on average by 15% when CO_2 concentrations increased from 360 to 680 µmol mol^{-1}). Correspondingly, grain quality was reduced by CO_2 enrichment. The Zeleny value, Hagberg value and dry/wet gluten content decreased significantly with increasing $[CO_2]$. Despite the beneficial impact of CO_2 enrichment on growth and yield of C_3 cereal crops, declines in flour quality due to reduced nitrogen content are likely in a future, $[CO_2]$-rich world. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: CO_2 enrichment; Grain quality; Nutrients; *Triticum aestivum*; Tropospheric ozone

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1. Introduction

CO_2 enrichment not only affects growth and yield of C_3 crops, but may also affect crop quality. Since CO_2 is the sole carbon source for higher plants in terrestrial ecosystems, it must be regarded as the nutrient that presently limits growth of all terrestrial C_3 plants (Sinclair, 1992). Thus, CO_2 enrichment is expected to interact with the uptake and utilization of nutrients since nutrient metabolism is balanced in a complex manner (Nátr, 1992; Rastetter and Shaver, 1992; Bergmann, 1993; Roy and Garnier, 1994). Most studies carried out so far have shown that C/N ratios in tissues of C_3 plants increase under CO_2 enrichment (Conroy, 1992; Cotrufo et al., 1998), indicating that nutrient balance is affected.

Interactions between CO_2 enrichment and nitrogen nutrition have been studied rather intensively (see Conroy, 1992; Petterson and McDonald, 1994). Nitrogen concentrations in green tissues of C_3 species have been found to be reduced under CO_2 enrichment and several explanations proposed (Conroy, 1992). The first target of CO_2 is ribulose-bisphosphate carboxylase/oxygenase (RuBisCO). Since oxygen and CO_2 compete for the same reaction sites on this enzyme, the oxygenase reaction of RuBisCO is suppressed under CO_2 enrichment (Sharkey, 1985; Webber et al., 1994); model calculations (Sharkey, 1988), indicating that the rate of photorespiration will be depressed to approximately half of the current values with a doubling of the atmospheric CO_2 concentration. Thus, the demand for enzymes of the glycolate pathway will decrease, which may in turn lower the nitrogen demand of green tissues (Webber et al., 1994). Reductions in leaf nitrogen demand may also result from the optimization of resources within the photosynthetic apparatus (Webber et al., 1994). Under CO_2 enrichment, carboxylation by RuBisCO will no longer limit the rate of CO_2 assimilation; rather, ribulose-bisphosphate- (RuBP) and phosphate- (P_i) regeneration will be limiting (Harley and Sharkey, 1991). There may, therefore, be a reallocation of nitrogen away from RuBisCO to light harvesting and sucrose synthesis (Sage et al., 1989; Webber et al., 1994; Woodrow, 1994), increasing the nitrogen use efficiency (the amount of carbon acquired per unit nitrogen taken up) because of the large amounts of leaf nitrogen bound in RuBisCO (up to 58% of soluble leaf protein, Jacob et al., 1995). Critical foliar nitrogen concentrations (the N concentration at which 90% of maximum biomass or yield is achieved) are, thus, significantly reduced by CO_2 enrichment, suggesting that fertilizer management regimes may have to be reassessed in the future (Conroy, 1992).

Reduced nitrogen concentrations in crops grown under CO_2 enrichment suggest that future changes in atmospheric composition may affect crop quality, e.g. wheat quality (Hocking and Meyer, 1991; Manderscheid et al., 1995; Blumenthal et al., 1996; Fangmeier et al., 1997). In particular, such relationships exist between grain protein content and protein-linked quality parameters such as the Zeleny value and gluten content. Bread-making qualities are, in part, based on the retention of CO_2 in the dough—a quality parameter mainly dependent upon the insoluble protein content of the grain (i.e. gluten contents; constituted mainly by gliadin and glutenin). Four indices are used to assess bread-making quality:

(I) the dry gluten concentration—a measure of the total gluten content (quantity of gluten);

(II) the wet gluten concentration—a measure of the water-retaining capacity of the gluten (quality of gluten);

(III) the Zeleny index—an indication of the quantity of proteins and quality of the gluten; a high Zeleny index corresponds with a high quantity and/or quality of the gluten (Biston et al., 1986; Czuchajowska and Pomeranz, 1990); and

(IV) the Hagberg value—an indication of the α-amylase activity of the flour. The lower the Hagberg value (high α-amylase activity), the more starch is transformed into fermentable sugars upon which yeast may act to produce the CO_2, which enables the dough to rise during bread-making.

Rather less is known about interactions between CO_2 enrichment and phosphorus nutrition. Leaf phosphorus demand is thought to increase with increasing CO_2 because the photosynthetic carbon reduction (PCR) cycle is favoured over the photosynthetic carbon oxidation (photorespiratory or PCO) cycle and, thus, more P_i is

required (Sharkey, 1985; Conroy, 1992; Morin et al., 1992). Conroy (1992) and Rogers et al. (1993) found that critical phosphorus concentrations in leaf tissues increased under CO_2 enrichment and that leaf phosphorus concentrations showed no, or little, reduction compared with nitrogen under CO_2 enrichment (Overdieck, 1993; Fangmeier et al., 1997).

Information on the effects of CO_2 enrichment on nutrients other than N or P in herbaceous species is scarce. Reduced concentrations of macro- and micronutrients have been reported after exposure to CO_2 enrichment by some authors (Overdieck, 1993: *Trifolium repens* L., *T. pratense* L., *Lolium perenne* L. and *Festuca pratensis* Huds.; Heagle et al., 1993: *Trifolium repens*; Huluka et al., 1994: *Gossypium hirsutum* L.; Manderscheid et al., 1995: *Triticum aestivum* L. and *Hordeum vulgare* L.; Fangmeier et al., 1997: *Triticum aestivum*). In contrast, much attention has been paid to the effects of tropospheric ozone on tissue nutrient concentrations—particularly in forest trees because of concern over the role of O_3 in the decline of forests in both Europe and the USA (McLaughlin, 1985; Krause et al., 1986; Kickert and Krupa, 1990). Research suggests that the impacts of O_3 on nutrient relations are dependent on the species and environmental conditions. Increased concentrations of Ca, Mg, and N have been reported in *Picea abies* (L.) Karst. (Keller and Matyssek, 1990) and in *Pinus taeda* L. (Baker et al., 1994) after ozone fumigation, an effect attributed to ozone-induced premature abscission of older needles. However, Bytnerowicz et al. (1990) did not detect ozone effects on nutrients in *Pinus ponderosa* Dougl. needles, and Ogner (1993) found ozone effects on nutrients in only two of six clones of *Picea abies*. Rather less attention has been paid to the effects of O_3 on nutrient concentrations in herbaceous species. However, decreased foliar concentrations of Ca, Mg, Fe and Mn, and increased pod concentrations of K, P and Mo, have been reported in snapbean (*Phaseolus vulgaris* L.) (Tingey et al., 1986), whereas the nutrient composition (Ca, Mg, K, P) of grain of spring wheat has been found to be increased by ozone exposure (Fuhrer et al., 1990).

Little is known about the interactive effects of elevated CO_2 and O_3 on element composition in plant tissues. Nutrient concentrations in leaves of white clover exposed to CO_2 and/or O_3 were analysed by Heagle et al. (1993). However, the authors did not state whether treatment interactions on nutrient concentrations occurred. The same holds for a four-factor study by Lippert et al. (1996) when Norway spruce was exposed to different levels of CO_2, O_3, nitrogen nutrition and drought. Pfirrmann et al. (1996) also used Norway spruce saplings to investigate the effects of CO_2 enrichment, O_3 and K deficiency on element concentrations in fine roots, current year needles and previous-year needles. With the exception of Cu in needle samples, they did not detect any significant interactive effects (at $P < 0.05$) of CO_2 and O_3 on any of the elements analysed.

Within the ESPACE-wheat study, several of the participating groups analysed the concentrations of nitrogen in various tissues from different treatments. Two of the groups also analysed additional nutrients, and grain quality (1000 kernel weight, crude protein content, starch content) was assessed by several groups, with one of the groups carrying out a comprehensive analysis of grain quality. In this contribution, a summary of findings from ESPACE-wheat is presented to show that grain quality and nutrient relations may be important parameters that deserve more attention in future efforts to model the impacts of rising CO_2 concentrations on crop yield.

2. Materials and methods

The same cultivar of spring wheat (*Triticum aestivum* L. cv. Minaret) was used by all participating groups. The growth and exposure conditions followed standard protocols developed for the ESPACE-wheat study as described elsewhere (Jäger et al., 1999).

Data on foliar nutrient concentrations and grain quality parameters (treatment means, respectively) were requested from all participating groups in the ESPACE-wheat study. Nitrogen data were available from seven groups and data on additional nutrients from two groups. However, individual participants in the project analysed the nutrient

composition of different organs. Analyses have therefore been restricted to situations where data were available from at least four different sites. In Table 1, a summary of the data-set used for the overall analysis of treatment effects on nitrogen concentrations is presented. Nitrogen data for green leaf blades, senescent leaf blades, stems, ears and grain were subjected to analysis of variance (ANOVA). Data from ambient field plots were excluded from the analysis—most of the groups

reported significant chamber effects when the growth and yield from ambient field plots was compared with that of open-top chambers ventilated with ambient air. Also, data from treatments other than ozone and nitrogen deficiency (e.g. soil moisture deficit or variation of temperature) were excluded from such analysis, since data were too few to permit a rigorous analysis of effects. Thus, CO_2 concentration (ambient, ambient plus 160 μl l^{-1} CO_2, or ambient plus 320 μl l^{-1}

Table 1
Summary of the data set used for overall analysis of treatment effects on nitrogen concentrations

Site[a]	CO_2 levels	O_3 levels	Nitrogen nutrition levels	Organs sampled	At growth stages[b]
ROTH	2	1 (growth chamber experiment)	3	Green leaves	31/65
				Senescent leaves	65/93
				Stems	31/65
				Ears	65
				Grains	93
GIES	3	2	Up to 5	Green leaves	31/65
				Senescent leaves	65/93
				Stems	31/65/93
				Ears	65
				Grains	93
TERV	3	2	1	Green leaves	65
				Senescent leaves	93
				Stems	65/93
				Stems	65
				Grains	93
ROSK	3	1	2	Green leaves	31/65
				Senescent leaves	65/93
				Stems	31/65/93
				Stems	65
				Grains	93
BRAU	4/3[c]	2	1	Green leaves	65
				Stems	65
CARL	2/3[d]	2	1	Green leaves	65
				Stems	65
				Grains	93
WAGE	2	1	1	Green leaves	65
				Stems	65/93

[a] Site abbreviations:
ROTH: Biochemistry and Physiology Department, IACR Rothamsted, Harpenden, UK
GIES: Institute for Plant Ecology, Justus-Liebig-University, Giessen, Germany
TERV: Veterinary and Agrochemical Research, Tervuren, Belgium
ROSK: National Environmental Research Institute, Roskilde, Denmark
BRAU: Institute for Production- and Ecotoxicology, FAL, Braunschweig, Germany
CARL: Agricultural and Food Development Authority, Oak Park Research Center, Carlow, Ireland
WAGE: Department of Theoretical and Production Ecology, Agricultural University, Wageningen, The Netherlands.
[b] Growth stages according to Tottman and Broad (1987).
[c] Four different CO2 levels in 1994 and 1995, three different CO2 levels in 1996.
[d] Two different CO2 levels in 1994, three different CO2 levels in 1995 and 1996.

CO_2), O_3 level (non-filtered or non filtered plus ozone addition), nitrogen supply, growth stage at sampling date and site served as independent variables for ANOVA. The variation in nitrogen supply was restricted to three of the participating groups. The nitrogen supply was regarded either as sufficient (high) when the amount of fertilization was 150 kg N ha^{-1} or more, or as insufficient (low) when it was below 150 kg ha^{-1}. Some groups who did not vary the nitrogen supply did not report the amount of fertilizer actually applied. In these cases, nitrogen supply was regarded as sufficient, since the standard protocol required an optimum nutrient supply. When data for particular organs such as stems, green or senescent leaf blades were available at different growth stages, the growth stage was added as an independent variable. Most groups provided data from three growth stages: beginning of stem elongation, anthesis and ripening. Data from additional growth stages, although provided by some groups, were excluded from the analysis.

Since different methods were used by the particular groups for nitrogen determination, and since no method comparison was carried out to check for systematic errors in the determinations, nitrogen data were calculated as a percentage of the respective ambient air treatment data for each site (i.e. 100% relates to samples obtained at ambient CO_2 level, non-filtered O_3 level, and sufficient nitrogen supply). These calculations were performed separately for different growth stages. Regression analyses were carried out to test for CO_2 effects. In these cases, data were calculated as a percentage of ambient CO_2 exposure concentrations separately for different growth stages, N supply, and ozone exposure conditions, respectively.

Data for nutrients other than nitrogen were limited to two groups (GIES and ROSK, see Table 1 for site abbreviations). Data were first normalized as described for nitrogen, then subjected to ANOVA [CO_2 by N supply, by growth stage (if applicable), by site]. Since exposures at ROSK were restricted to ambient ozone concentrations, only GIES data from NF chambers were included in the analyses.

Data relating to shoot nitrogen use efficiency (NUE) at different growth stages were available from four different sites. At two of these sites (GIES and TERV), the crop was grown at different ozone levels, and these data were subjected to ANOVA to check for ozone effects on NUE. Since there were no significant effects of ozone (P between 0.42 and 0.89 according to site and growth stage), data from different ozone treatments were pooled for subsequent ANOVA using CO_2, N supply, and site as independent variables (separately at different growth stages).

Concerning grain quality parameters:

(I) starch content was determined by polarimetry based on Ewers method (Method 2.3.4.; 72/199/EEC; PB L123, 29/5/1972);

(II) the Zeleny index (sedimentation index according to Zeleny) using a near-infra-red method (ISO 5529-1978);

(III) the Hagberg value (Method Hagberg-Perten; I.C.C. Standard 107) was determined as the time (expressed in seconds) an agitator-viscosity metre needs to penetrate an aqueous flour suspension;

(IV) dry and wet gluten content of flour was determined using the glutomatic system (Falling number AB), by separating gluten and soluble starch and protein fraction (I.C.C. Standard 137).

Data relating to starch content and 1000 kernel weight (TKW) were obtained from five sites. At three sites, data for different ozone levels and for two sites, data on different N supply were available. The data were normalized (as for nitrogen) and then subjected to ANOVA to check for CO_2, ozone and N effects on starch content and 1000 kernel weight. A regression analysis was carried out to test for CO_2 effects. For these analyses, data were split into three different N supply ranges: low (N supply < 100 kg ha^{-1}), normal (100–160 kg ha^{-1}) and high (> 160 kg ha^{-1}).

Bread-making quality data were only available from one site (TERV). Four quality parameters were determined over three successive years. The data were subjected to ANOVA to test for the effects of CO_2 enrichment on quality. The effects of ozone were not studied since the data were restricted to filtered and non-filtered air. Chambers removing NO_x through air filtration may have

negative effects on wheat quality as shown in previous experiments (Vandermeiren et al., 1992; De Temmerman et al., 1993).

3. Results and discussion

3.1. Nitrogen concentrations in different tissues

In general, CO_2 enrichment caused decreases in the nitrogen content of green leaves, senescent leaves, ears and grains. There was, however, no statistically sigificant effect in stems (see Table 2 for the results of ANOVA). A compilation of data is shown in Fig. 1, which illustrates the impacts of CO_2 enrichment on the nitrogen concentration of green leaves and grains in the different treatments. There was a site-to-site variation in the response of tissue nitrogen concentrations to CO_2 enrichment. For example, the greatest reduction in grain nitrogen concentration at high nitrogen supply (680 vs. 360 $\mu mol\ mol^{-1}$ CO_2) was observed at GIES (−31%, see Table 1 for site abbreviations), followed by ROSK (−23%), ROTH (−14%), TERV (−8%) and CARL (−8%), respectively. The corresponding reductions in the nitrogen content of green leaves from plants well supplied with nitrogen and harvested at anthesis were: −31% (GIES), −13% (ROSK), −12% (BRAU), −12% (WAGE), −12% (CARL), −6% (ROTH), and −5% (TERV). It is not clear from the data-set why there are such discrepancies between the data at the different sites, but several environmental and exposure conditions differed between sites.

Reductions in nitrogen content of plant tissues under CO_2 enrichment might have several ecological impacts. The increase in C/N ratio in green leaf tissues means a reduction in the quality for herbivorous insects that respond to enriched CO_2-grown foliage by increasing their consumption rate by 20–80% (Lincoln et al., 1993). There is some debate as to whether or not decomposition rate of litter will be influenced by CO_2 enrichment. If the C/N ratio in litter is increased after exposure under CO_2 enrichment, the rate of litter decomposition is expected to slow down (Couteaûx et al., 1995). However, a reduction in litter C/N has not always been observed (O'Neill and Norby, 1996; Hirschel et al., 1997). Since CO_2 enrichment caused significant reductions in the nitrogen content of senescent leaves in this study (on average by 21% over all sites and treatments), there may be some effects on litter decomposition.

The nitrogen supply affected the tissue nitrogen content of all sampled tissues at all three sites where the canopies were grown under different fertilizer regimes, as expected. However, interactions between CO_2 enrichment and nitrogen treatments were detected in very few cases. ROSK and GIES data that were subjected to two-way ANOVA revealed significant interactive effects on the nitrogen content of senescent leaves, and CO_2 and N supply interacted on grain nitrogen content at GIES, with a high nitrogen supply increasing the reduction in tissue nitrogen content enforced by CO_2 enrichment.

Table 2
Results of ANOVA on treatment effects on nitrogen concentrations in different tissues of spring wheat. Interactions were excluded from the analyses since a complete factorial design was restricted to certain sites (compare Table 1)

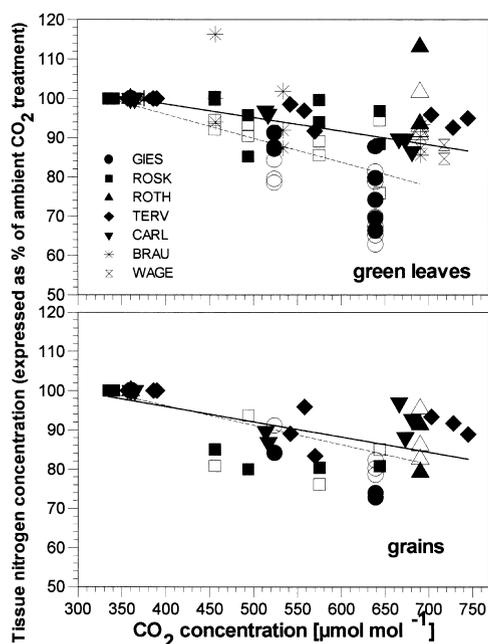| Tissue	Independent variable				
	CO_2	O_3	N supply	Growth stage	Site
Green leaves	$P < 0.001$	ns	$P < 0.001$	$P < 0.001$	$P < 0.001$
Senescent leaves	$P < 0.001$	ns	$P < 0.001$	ns	$P < 0.001$
Stems	ns	ns	$P < 0.05$	ns	$P < 0.05$
Ears	$P < 0.01$	ns	$P < 0.01$	Harvested only at growth stage 65	$P < 0.001$
Grains	$P < 0.001$	ns	$P < 0.001$	Harvested only at growth stage 93	$P < 0.05$

ns: not significant ($P > 0.05$).

Fig. 1. Response of nitrogen concentrations in green leaves (at anthesis) and in grains (at ripening) of spring wheat to CO2 enrichment. Data are given as a percentage of the concentrations at ambient CO2, respectively. Closed symbols, stars, and hourglass represent data from plots with high nitrogen supply, and open symbols represent data from plots with a low nitrogen supply. For an explanation of the site abbreviations, see Table 1. Regression lines were calculated separately for high (solid) and low (broken) nitrogen supply. The regressions followed the equations:

green leaves, high N: $y=112.5-0.03475x$ $(P<0.001)$,
green leaves, low N: $y=120.5-0.06241x$ $(P<0.001)$,
grains, high N: $y=111.4-0.03871x$ $(P<0.001)$,
grains, low N: $y=116.1-0.04974x$ $(P<0.001)$,

where $y=$ concentration [%] in relation to concentrations found at ambient CO2, and $x=$ CO2 concentration [mol mol−1].

Ozone at concentrations higher than ambient did not affect tissue nitrogen concentrations. Effects of ozone on foliar nutrient composition have been reported in some studies with wheat. Mortensen and Jorgensen (1996) detected increased tissue nitrogen concentrations in one spring wheat wheat cultivar (cv. Minaret) exposed to ambient air or ambient air enriched with ozone in comparison with filtered air, but no effects were found in another cultivar (cv. Eridano). Scotti et al. (1994) did not observe any significant effects of non-filtered air compared with filtered air on

grain nitrogen concentrations in winter wheat, whereas Fuhrer et al. (1990) reported a tendency toward increased grain nitrogen concentrations under elevated ozone exposure. Individual groups participating in ESPACE-wheat reported significant effects of ozone on tissue nitrogen concentrations in some cases. However, over the whole data-set, ozone effects were not significant.

3.2. Nutrient concentrations other than nitrogen in different tissues

Two sites (GIES and ROSK) provided extensive data on element concentrations other than nitrogen (P, K, S, Ca, Mg, Fe, Mn, Zn). The statistical design at both sites comprised a full factorial variation of CO_2 by N supply, and thus the data were subjected to three-way ANOVA ($CO_2 \times$ N $\times$ site) or four-way ANOVA ($CO_2 \times$ N $\times$ growth stage $\times$ site), where applicable. Only data from NF chambers were included for GIES.

Table 3 provides a compilation of the results of ANOVA. Element concentration data in green leaves at anthesis and in grains at ripening are shown in Fig. 2. In green leaves, CO_2 enrichment significantly decreased the concentrations of all elements except iron and phosphorous. ANOVA detected a slightly significant effect on phosphorous concentrations in green leaves, but that was not reproduced in the regression analysis. The lack of a pronounced reduction of P concentrations in green leaves supports earlier suggestions that the P demand of leaves increases under CO_2 enrichment (Conroy et al., 1992), since the photosynthetic carbon reduction cycle is favoured over the photosynthetic carbon oxidation cycle at higher CO_2 partial pressures (Bowes, 1993; Sage, 1994) and, thus, more P_i is consumed (Sharkey, 1985). Iron concentrations in green leaves were closely related to P concentrations and showed no significant changes due to CO_2 enrichment. The concentrations of Ca, S, Mg, Zn, K in green leaves, however, responded similarly to nitrogen.

In senescent leaves, effects of CO_2 enrichment were usually less pronounced than in green leaves. The alteration of element concentrations in senescing leaves is affected by several means: immobile elements such as Ca or Fe increase in concentration during senescence because of further uptake and

Table 3
Results of ANOVA on treatment effects on element concentrations in different tissues of spring wheat

Element	Tissue	Independent variable				Interactions	
		CO_2	N supply	Growth stage	Site	CO_2 by N supply	CO_2 by site
P	Green leaves	$P<0.05$	$P<0.05$	$P<0.01$	$P<0.05$	ns	$P<0.05$
	Senescent leaves	ns	ns	ns	ns	ns	ns
	Stems	ns	ns	$P<0.001$	$P<0.01$	ns	ns
	Ears	ns	ns	–	$P<0.001$	ns	ns
	Grains	ns	ns	–	ns	ns	ns
K	Green leaves	$P<0.001$	$P<0.01$	ns	$P<0.001$	ns	ns
	Senescent leaves	$P<0.001$	$P<0.01$	$P<0.05$	$P<0.05$	ns	ns
	Stems	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.01$	ns	ns
	Ears	$P<0.05$	$P<0.05$	–	$P<0.001$	ns	ns
	Grains	ns	ns	–	ns	ns	ns
Ca	Green leaves	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	ns	$P<0.001$
	Senescent leaves	$P<0.05$	$P<0.001$	ns	$P<0.001$	ns	$P<0.01$
	Stems	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	ns	$P<0.01$
	Ears	ns	$P<0.05$	–	$P<0.001$	ns	ns
	Grains	$P<0.001$	$P<0.001$	–	$P<0.01$	ns	ns
S	Green leaves	$P<0.001$	$P<0.001$	ns	$P<0.001$	ns	ns
	Senescent leaves	$P<0.001$	$P<0.05$	$P<0.01$	$P<0.001$	ns	$P<0.001$
	Stems	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	ns	$P<0.001$
	Ears	ns	$P<0.05$	–	$P<0.01$	ns	ns
	Grains	$P<0.001$	$P<0.001$	–	$P<0.01$	ns	ns
Zn	Green leaves	$P<0.001$	$P<0.001$	$P<0.01$	$P<0.001$	ns	$P<0.001$
	Senescent leaves	ns	$P<0.001$	ns	$P<0.01$	ns	ns
	Stems	ns	$P<0.05$	$P<0.01$	$P<0.001$	ns	$P<0.05$
	Ears	ns	ns	–	ns	ns	ns
	Grains	ns	ns	–	ns	ns	ns
Fe	Green leaves	ns	$P<0.01$	ns	$P<0.01$	ns	ns
	Senescent leaves	ns	ns	ns	ns	ns	ns
	Stems	ns	ns	ns	ns	ns	ns
	Ears	ns	ns	–	ns	ns	ns
	Grains	ns	ns	–	$P<0.05$	ns	ns
Mn	Green leaves	$P<0.001$	$P<0.001$	$P<0.05$	ns	ns	ns
	Senescent leaves	$P<0.01$	$P<0.001$	ns	$P<0.001$	ns	ns
	Stems	ns	$P<0.05$	ns	ns	ns	ns
	Ears	ns	ns	–	ns	ns	ns
	Grains	ns	ns	–	ns	ns	ns

n.s.: not significant ($P>0.05$).
–: not applicable (assessed at one growth stage only).

because of a loss in dry weight due to the remobilization of non-structural carbohydrates and proteins (Schnyder, 1993). On average, Ca and Fe concentrations were found to increase by 112 and 46%, respectively, from anthesis (when leaves were green) until ripening (when complete senescence was achieved). Common mobile elements, such as K, S or Mn, were not retracted from senescing leaves, with the concentrations of these elements remaining similar to those in green leaves. In contrast, elements needed during grain filling (in particular P and N, and to a lesser extent Zn) were almost entirely reallocated from the leaves during senescence (leaf concentrations of P, N and Zn dropping by 83, 77 and 53%, respectively, from anthesis to ripening). All these processes interacted with CO_2 enrichment, making it difficult to draw any further conclusions.
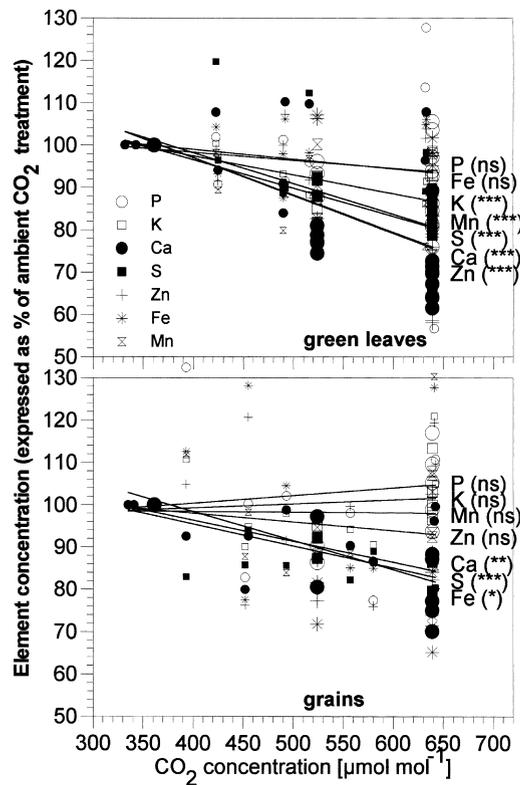
Fig. 2. Response of element concentrations other than nitrogen in green leaves (at anthesis) and grains (at ripening) of spring wheat to CO_2 enrichment. Data are given as a percentage of concentrations at ambient CO_2, respectively. Data were derived from two sites (GIES and ROSK; see Table 1 for site abbreviations). Larger symbols refer to GIES data and smaller symbols to ROSK data. Data sets for high and low N supply were pooled since ANOVA (see Table 3) did not detect CO_2 by N supply interactions for either element in any of the sampled tissues. Regression lines were calculated for the pooled data sets of the two sites for any of the elements, respectively. The significance of the regression analyses is indicated in the graph (ns: not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

In general, in stems, a 'dilution' of nutrients occurred following exposure to CO_2 enrichment. This was most pronounced at anthesis and at ripening and was most probably due to the accumulation of non-structural carbohydrates (Schnyder, 1993; Fangmeier et al., 1996; Hofstaetter et al., 1996). Only manganese did not follow this general pattern.

Element concentration in ears at anthesis were marginally affected by the treatments. In grains at final harvest, CO_2 enrichment resulted in no significant changes in the concentration of P, K, Zn and Mn, but decreased the concentration of Ca, S and Fe. These findings are very similar to results obtained by Manderscheid et al. (1995) who worked on two different cultivars of wheat. This suggests that the observed reaction pattern of change in the nutrient concentrations of wheat grains under CO_2 enrichment may be a ubiquitous response.

3.3. Nitrogen amounts acquired by the crop in different tissues

The amount of nitrogen taken up by the crop and stored in the shoot was not affected by CO_2 enrichment at either growth stage ($P = 0.49$ to 0.54 according to ANOVA). In Fig. 3, N amounts in shoots at different growth stages are shown for each site from different CO_2 and N supply treatments. There was no CO_2 effect on nitrogen acquisition at either site. It is also clear from the data shown in Fig. 3 that, in general, no further uptake of nitrogen took place after anthesis (growth stage 65), i.e. the nitrogen supplied to the grains after anthesis originated from vegetative pools—a finding consistent with previous observa-
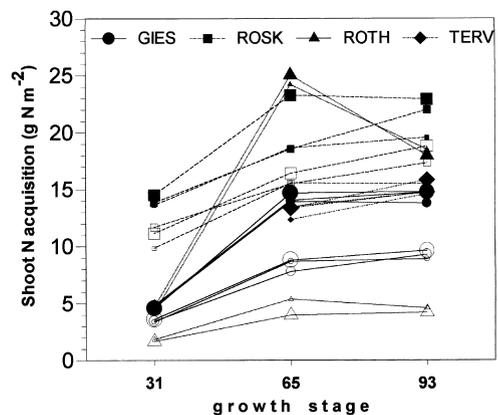

Fig. 3. Effects of nitrogen supply and CO_2 enrichment on nitrogen amounts acquired by spring wheat canopies at different sites. Open symbols refer to plots with low nitrogen supply and closed symbols to plots with high nitrogen supply. Symbol size corresponds to CO_2 level (small: 360 $\mu mol\ mol^{-1}$, medium: 520 $\mu mol\ mol^{-1}$, large: 680 $\mu mol\ mol^{-1}$).

tions (Van Kraalingen, 1990; Peeters and Van Laere, 1994; Palta and Fillery, 1995).

Nitrogen acquisition and supply of mineral nitrogen were linearly related at those sites where the N supply was varied (see Fig. 4). At ROTH and GIES, the crops took up roughly the amount of nitrogen supplied via the fertilizer, whereas N acquisition by the crop at ROSK far exceeded supply (data for ROSK not shown in Fig. 4). This is due to the fact that different soils were used at the experimental sites. For example, at GIES, a substrate very low in organic matter ($<1\%$) was used, preventing significant nitrogen mineralization, whereas mineralized N was available at ROSK.

To further elucidate the role of vegetative nitrogen pools in the supply of N to the grains, the effects of CO_2 enrichment and N supply on nitrogen pools in stems and green leaves at anthesis are plotted in Fig. 5 (data from GIES). CO_2 enrichment was found to decrease the nitrogen pool in green leaves by more than 20%—an effect that was, in part, compensated by an increase in the
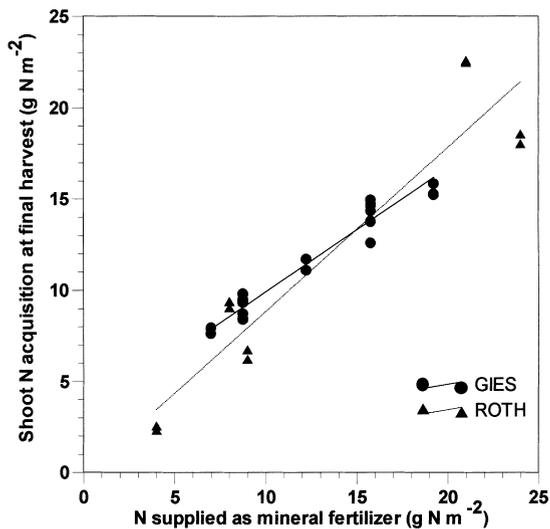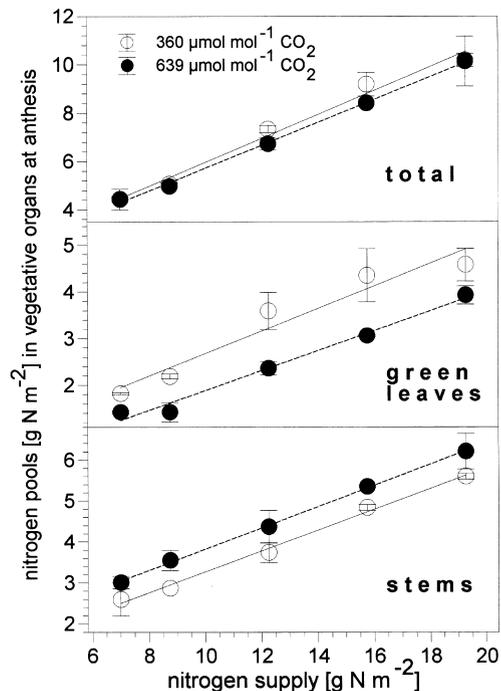

Fig. 5. Effect of CO_2 enrichment and nitrogen supply on nitrogen pools in vegetative tissues of spring wheat at anthesis (data from GIES).

stem N pool (which in turn may be attributed to the large increase in stem biomass at anthesis; Fangmeier et al., 1996). The total pool size was slightly, but not significantly, decreased by CO_2 enrichment and responded linearly to nitrogen supply as shown in Fig. 4.

Thus, the amount of nitrogen available during grain filling appears to be dependent on the pool size stored in vegetative tissues, which in turn is determined by the nitrogen supply. It might be argued that decreases in the nitrogen content of grain under CO_2 enrichment could be overcome by increasing the N supply to the crop. However, CO_2 enrichment decreased the nitrogen demand of green leaves—a conclusion supported by lower critical nitrogen concentrations (reduction by 10% in flag leaves and by 13% in lower leaves; Fangmeier et al., 1997) and by the increase in nitrogen use efficiency observed at all of the sites (data not shown). Thus, a further N supply might translate into a higher yield without improving the nitrogen status of the crop.

Fig. 4. Response of shoot nitrogen acquiration in spring wheat canopies at ripening to supply of mineral nitrogen fertilizer at ROTH and GIES. Data from ROSK were not included since data were restricted to two fertilizer regimes (7.5 and 15 g N m^{-2}), thus preventing regression analysis. The regression lines follow the equations:
ROTH: $y = -0.15 + 0.90x$ ($P < 0.001$)
GIES: $y = 3.13 + 0.68x$ ($P < 0.001$).

To clarify whether optimization of the photosynthetic apparatus and/or reduction of the photorespiratory carbon and nitrogen flux were responsible for the reduced nitrogen demand of leaves under CO_2 enrichment, the amount of RuBisCO protein was assessed at GIES and at ROTH, and photorespiratory enzymes were assessed at GIES. In addition, acclimation of the photosynthetic apparatus was studied by gas exchange measurements carried out at seven sites within the ESPACE-wheat study (Mitchell et al., 1999). The amount of RuBisCO in flag leaves (data from GIES) is shown in Fig. 6 together with the corresponding content of total soluble leaf protein. Both the amount of RuBisCO and that of total soluble protein were significantly ($P < 0.05$) reduced under CO_2 enrichment at growth stages 47 and 65 [growth stages according to Tottman and Broad (1987)], whereas the relationship between RuBisCO and total soluble protein remained unchanged. This is in agreement with earlier findings in *Scirpus olneyi* Grey. exposed to CO_2 enrichment in a long-term field study (Jacob

et al., 1995). However, these authors observed a downward acclimation of photosynthesis, which was not found in wheat exposed within ESPACE-wheat (at least not before anthesis). Gas exchange measurements indicated that there was no evidence of any reduction in photosynthetic capacity of flag leaves under elevated CO_2 before anthesis, whereas it was usually reduced after anthesis, suggesting that acclimation of photosynthesis to CO_2 enrichment occurred slowly (Grüters et al., 1998; Mitchell et al., 1999).

Reductions in the amount of RuBisCO can in part explain the reduced nitrogen demand of leaves under CO_2 enrichment. Since the amount of total soluble protein was affected by CO_2 enrichment similar to RuBisCO, other pathways must also have been influenced. Photorespiratory carbon and nitrogen flux and, thus, the amounts of enzymes driving the PCO cycle (Webber et al., 1994) appear to be another candidate to explain the reduced nitrogen demand of leaves under CO_2 enrichment. Model calculations show that C and N flux through the PCO cycle are approximately halved when CO_2 concentrations are raised from the present ambient levels to the highest levels used within ESPACE-wheat (Sharkey, 1988). Assuming an optimal use of nitrogen, the amount of the driving enzymes might be expected also to be halved. Taking into account that the N flux through the PCO cycle exceeds the flux during N assimilation by around one order of magnitude (Leegood, 1996) and that most of the enzymes involved in the PCO cycle are solely responsible for photorespiratory C and N flux and can contribute a substantial fraction of the protein N bound in leaves (Raman and Oliver, 1997), it might be argued that the suppression of photorespiration by CO_2 enrichment will cause leaf nitrogen demand to decrease. The activity of two photorespiratory enzymes was assessed at GIES from full emergence until the beginning of senescence in wheat flag leaves. The data obtained for the maximum in-vitro activity of glycolate oxidase and hydroxypyruvate reductase (which strongly relates to enzyme amounts) are shown in Fig. 7. Both enzymes were down-regulated to approximately half of the control values under CO_2 enrichment,
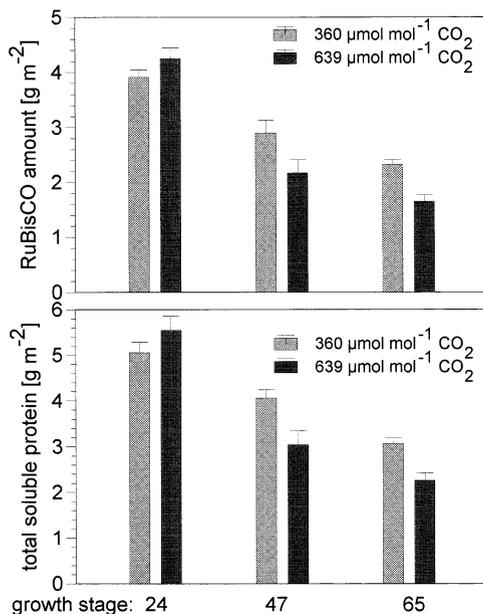

Fig. 6. Effect of CO_2 enrichment on amounts of RuBisCO and total soluble protein in flag leaves of spring wheat at different growth stages at GIES [growth stages according to Tottman and Broad (1987)].
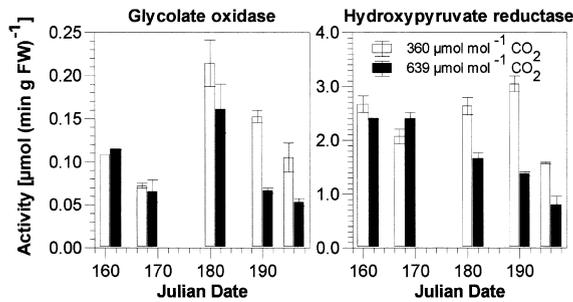
Fig. 7. Effect of CO_2 enrichment on photorespiratory enzymes in wheat flag leaves from full extension until the beginning of senescence (data from GIES).

which is in agreement with the conclusions drawn above.

3.4. Grain quality

The TKW was not significantly influenced by elevated CO_2 or by ozone exposure. Rudorff et al. (1996) also found no effect of ozone exposure, but for wheat exposed at elevated CO_2, they reported an increase (of borderline significance) in TKW, whereas Blumenthal et al. (1996) found that TKW decreased. These conflicting results suggest that elevated CO_2 is not the major cause of changes in TKW. Indeed, nitrogen fertilization had a significant effect ($P<0.05$) on TKW and may be one further contribution to the variable nature of the available data. As shown in Fig. 8, there was a slight increase in the TKW as a function of elevated CO_2 (not significant) at normal N supply and a decrease at a high N supply. As there was no significant effect of elevated CO_2 on the TKW, it can be concluded that the increase in yield was based on the development of the number of grains (more ears per m²) rather than heavier kernels.

As a result of the higher photosynthetic activity under CO_2 enrichment, a higher starch content caused by an increase in carbohydrate translocation from source (leaves and stems) to sink (grain) might be expected. However, the significant ($P<0.01$) increase in starch content was restricted to crops with a high N supply (Fig. 8). No effect of ozone exposure was found on the starch content, but, again, the N supply significantly ($P<0.05$) changed the starch content.
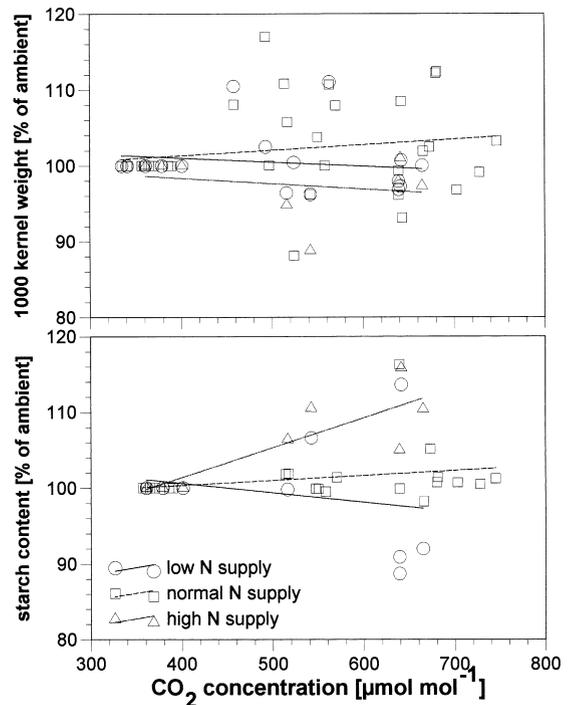

Fig. 8. Response of 1000 kernel weight and starch content in grains of spring wheat to CO_2 enrichment (data from five sites and three growing seasons). Data are given as a percentage of concentrations at ambient CO_2, respectively. The data are split into low nitrogen supply (<100 kg N ha^{-1}), normal nitrogen supply (100–160 kg N ha^{-1}) and high nitrogen supply (>160 kg N ha^{-1}). Neither of the regressions was significant with the exception of the response of starch content to CO_2 enrichment at a high N supply, which followed the equation: $y=85.6+0.0393x$ ($P<0.01$).

In concert with the decreased nitrogen, and consequently protein concentration of the grain, also the protein-linked quality parameters (the Zeleny value and dry and wet gluten content) were significantly ($P<0.001$) decreased under CO_2 enrichment, indicating a reduction in baking quality (Fig. 9). Flour of a good quality has a Zeleny value between 25 and 40 ml (Sinnaeve et al., 1997). In spite of the decline in the Zeleny value, the range of the values observed at elevated CO_2 remained acceptable, except in the 1994 experiment. Wheat grown in filtered air also showed a reduced Zeleny value at elevated CO_2. This may be attributed to a decrease in the protein content of the grain as a result of filtering NO$_x$
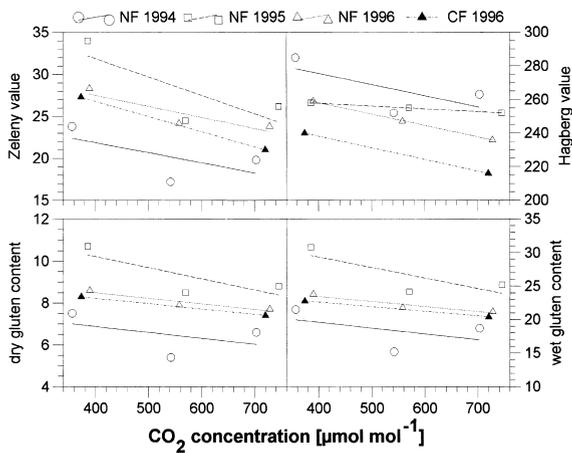
Fig. 9. Effect of CO_2 enrichment on the quality of wheat grain (Zeleny value in ml, dry and wet gluten content in % d.w., and Hagberg value in s). Data are from Tervuren; NF = data from chambers driven with non-filtered air, and CF = data from chambers driven with charcoal-filtered air.

(Vandermeiren et al., 1992; De Temmerman et al., 1993).

The Hagberg index depends mainly on the harvest date and storage conditions of the grain. The optimum Haberg value is reported to be between 220 and 350 s (Sinnaeve et al., 1997). Elevated CO_2 was found to decrease the Hagberg value ($P < 0.05$) and, thus, increased α-amylase activity. Air filtration also decreased the Hagberg value, which can be explained by a delayed senescence and retarded maturity (Vandermeiren et al., 1992).

4. Conclusions

CO_2 enrichment has been proven to be beneficial for the growth and yield of C_3 crops (e.g. Poorter, 1993). The results from the ESPACE-wheat experiments confirm previous findings and have improved our understanding of the underlying physiological processes that will help to improve the modeling of crop growth responses in a future, CO_2-rich world. It is not surprising, however, that an increased supply of the element most severely restricting C_3 crop growth at present, i.e. carbon, will improve biomass acquistion. It is clear, however, that nutrient imbalances may result from CO_2 enrichment not only because the supply of one nutrient is enhanced, but also because CO_2 alters the demand of green tissues for other elements (decrease in nitrogen and those elements most closely coupled to nitrogen such as calcium, sulphur or magnesium, increase in phosphorus). Thus, nutrient uptake, distribution and redistribution are influenced by CO_2 enrichment in a complex manner.

From an agricultural perspective, CO_2 enrichment will have several consequences. First, guidelines for fertilizer management will have to be reassessed since critical nitrogen concentrations in the tissues are decreased (Conroy, 1992) and since the total element uptake by the crop is influenced in a different manner for each particular nutrient. Second, grain quality experiences negative effects of CO_2 enrichment. Because of the underlying mechanisms, the reduction in grain quality may not easily be overcome by additional nitrogen fertilization since this may translate into greater carbon acquisition and not into enhanced nitrogen redistribution to the grains. Rather, it will be a challenge for plant breeders to create genotypes with altered nitrogen uptake and redistribution characteristics.

References

Baker, T.R., Allen, H.L., Schoeneberger, M.M., Kress, L.W., 1994. Nutritional response of loblolly pine exposed to ozone and simulated acid rain. Can. J. For. Res. 24, 453–461.

Bergmann, W., 1993. Ernährungsstörungen bei Kulturpflanzen. Entstehung, visuelle und analytische Diagnose. Gustav Fischer, Jena.

Biston, R., Castille, J.P., Crohain, A., Dardenne, P., Falise, A., Frankinet, M., Herman, J.L., Monfort, B., Noulard, L., Rixhon, L., 1986. Qualité et teneur en protéines des blés.

Actualisation 1985. Note technique du centre de recherches agronomiques de l'état. Gembloux 42, 4–22.

Blumenthal, C., Rawson, H.M., McKenzie, E., Gras, P.W., Barlow, E.W.R., Wrigley, C.W., 1996. Changes in wheat grain quality due to doubling the level of atmospheric CO_2. Cereal Chem. 73, 762–766.

Bowes, G., 1993. Facing the inevitable: plants and increasing atmospheric CO_2. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44, 309–332.

Bytnerowicz, A., Poth, M., Takemoto, B.K., 1990. Effects of photochemical smog and mineral nutrition on Ponderosa pine seedlings. Environ. Pollut. 67, 233–248.

Conroy, J.P., 1992. Influence of elevated atmospheric CO_2 concentrations on plant nutrition. Aust. J. Bot. 40, 445–456.

Conroy, J.P., Milham, P.J., Barlow, E.W.R., 1992. Effect of nitrogen and phosphorus availability on the growth response of *Eucalyptus grandis* to high CO_2. Plant Cell Environ. 15, 843–847.

Cotrufo, M.F., Ineson, P., Scott, A., 1998. Elevated CO_2 reduces the nitrogen concentration of plant tissues. Glob. Change Biol. 4, 43–54.

Couteaûx, M.M., Bottner, P., Berg, B., 1995. Litter decomposition, climate and litter quality. Trends Ecol. Evolut. 10, 63–66.

Czuchajowska, Z., Pomeranz, Y., 1990. Quest for a universal test of commercial gluten quality for breadmaking. Cereals Food World 35, 458–463.

De Temmerman, L.O., Trappeniers, M., Vandermeiren, K., 1993. Nitrogen cycling and quality aspects of wheat exposed to ozone and nitrogen dioxide. In: Jäger, H.-J., Unsworth, M., De Temmerman, L., Mathy, P. (Eds.), Proceedings of the CEC symposium 'Effects of Air Pollution on agricultural crops in Europe', Air Pollution Report 46, C.E.C. Brussels, 281–295..

Fangmeier, A., Grüters, U., Hertstein, U., Sandhage-Hofmann, A., Vermehren, B., Jäger, H.-J., 1996. Effects of elevated CO_2, nitrogen supply and tropospheric ozone on spring wheat, I. Growth and yield. Environ. Pollut. 91, 381–390.

Fangmeier, A., Grüters, U., Högy, P., Vermehren, B., Jäger, H.-J., 1997. Effects of elevated CO_2, nitrogen supply and tropospheric ozone on spring wheat—II. Nutrients (N, P, K, S, Ca, Mg, Fe, Mn, Zn). Environ. Pollut. 96, 43–59.

Fuhrer, J., Lehnherr, B., Moeri, P.B., Tschannen, W., Shariat-madari, H., 1990. Effects of ozone on the grain composition of spring wheat grown in open-top field chambers. Environ. Pollut 65, 181–192.

Grüters, U., Hoffstadt, J., Fangmeier, A., Jäger, H.-J., 1998. Ein Photosynthese-Modell zeigt: keine Akklimation von Weizen an erhöhtes CO_2. Verh. Ges. Ökol. 28, 363–372.

Harley, P.C., Sharkey, T.D., 1991. An improved model of C_3 photosynthesis at high CO_2—reversed O_2 sensitivity explained by a lack of glycerate reentry into the chloroplast. Photosynth. Res. 27, 169–178.

Heagle, A.S., Miller, J.E., Sherrill, D.E., Rawlings, J.O., 1993. Effects of ozone and carbon dioxide mixtures on two clones of white clover. New Phytol. 123, 751–762.

Hirschel, G., Körner, C., Arnone, J.A., 1997. Will rising atmo-spheric CO_2 affect leaf litter quality and in situ decomposition rates in native plant communities? Oecologia 110, 387–392.

Hocking, P.J., Meyer, C.P., 1991. Effects of CO_2 enrichment and nitrogen stress on growth, and partitioning of dry matter and nitrogen in wheat and maize. Aust. J. Plant Physiol. 18, 339–356.

Hofstaetter, B., Grüters, U., Fangmeier, A., Jäger, H.-J., 1996. Der Halm als Kohlenstoffsenke prägt die Reaktion von Weizen auf erhöhte CO_2-Konzentration. Verh. Ges, Ökol. 26, 613–617.

Huluka, G., Hileman, D.R., Biswas, P.K., Lewin, K.F., Nagy, J., Hendrey, G.R., 1994. Effects of elevated CO_2 and water stress on mineral concentration of cotton. Agr. For. Meteorol. 70, 141–152.

Jacob, J., Greitner, C., Drake, B.G., 1995. Acclimation of photosynthesis in relation to Rubisco and nonstructural carbohydrate contents and in situ carboxylase activity in *Scirpus olneyi* grown at elevated CO_2 in the field. Plant Cell Environ. 18, 875–884.

Jäger, H.J., Hertstein, U., Fangmeier, A., The European Stress Physiology and Climate Experiment—project 1: wheat (ESPACE-wheat): introduction, aims and methodology. Eur. J. Agron., 10, 155–162.

Keller, T., Matyssek, R., 1990. Limited compensation of ozone stress by potassium in Norway spruce. Environ. Pollut. 67, 1–14.

Kickert, R.N., Krupa, S.V., 1990. Forest responses to tropospheric ozone and global climate change—an analysis. Environ. Pollut. 68, 29–65.

Krause, G.H.M., Arndt, U., Brandt, C.J., Bucher, J., Kenk, G., Matzner, E., 1986. Forest decline in Europe: development and possible causes. Water Air Soil Pollut. 31, 647–668.

Leegood, R.C., 1996. Primary photosynthate production: Physiology and metabolism. In: Zamski, E., Schafer, A.A. (Eds.), Photoassimilate Distribution in Plants and Crops. Marcel Dekker, New York, pp. 21–41.

Lincoln, D.E., Fajer, E.D., Johnson, R.H., 1993. Plant insect herbivore interactions in elevated CO_2 environments. Trends Ecol. Evolut. 8, 64–68.

Lippert, M., Häberle, K.H., Steiner, K., Payer, H.D., Rehfuess, K.E., 1996. Interactive effects of elevated CO_2 and O_3 on photosynthesis and biomass production of clonal 5-year-old Norway spruce [*Picea abies* (L.) Karst.] under different nitrogen nutrition and irrigation treatments. Trees—Struct. Funct. 10, 382–392.

McLaughlin, S.B., 1985. Effects of air pollution on forests. A critical review. JAPCA 35, 512–534.

Manderscheid, R., Bender, J., Jäger, H.-J., Weigel, H.J., 1995. Effects of season long CO_2 enrichment on cereals. II. Nutrient concentrations and grain quality. Agr. Ecosyst. Environ. 54, 175–185.

Mitchell, R.A.C., Burke, J.I., de Temmerman, L., Fangmeier, A., Mulholland, B.J., Manscheid, R., van Oijen, M., Photosynthetic gas exchange responses in spring wheat crops grown under elevated CO_2 concentrations and stress condi-

tions in the European, multiple-site experiment 'ESPACE-wheat'. Eur. J. Agron., 10, 205–214.

Morin, F., André, M., Betsche, T., 1992. Growth kinetics carbohydrate and leaf phosphate content of clover (*Trifolium subterraneum* L.) after transfer to a high CO_2 atmosphere or to high light and ambient air. Plant Physiol. 99, 89–95.

Mortensen, L., Jorgensen, H.E., 1996. Responses of spring wheat (*Triticum aestivum* L.) to ozone produced by either electric discharge and dry air or by UV-lamps and ambient air. Environ. Pollut. 93, 121–127.

Nátr, L., 1992. Mineral nutrients—a ubiquitous stress factor for photosynthesis. Photosynthetica 27, 271–294.

O'Neill, E.G., Norby, R.J., 1996. Litter quality and decomposition rates of foliar litter produced under CO_2 enrichment. In: Koch, G.W., Mooney, H.A. (Eds.), Carbon Dioxide and Terrestrial Ecosystems. Academic Press, San Diego, CA, pp. 87–103.

Ogner, G., 1993. No general effect of ozone on foliar nutrient concentrations in mature scions of grafted *Picea abies* trees. Environ. Pollut. 82, 197–200.

Overdieck, D., 1993. Elevated CO_2 and the mineral content of herbaceous and woody plants. In: Rozema, J., Lambers, H., Van de Geijn, S.C., Cambridge, M.L. (Eds.), CO_2 and Biosphere. Kluwer Academic, Dordrecht, pp. 403–411.

Palta, J.A., Fillery, I.R.P., 1995. N application enhances remobilization and reduces losses of pre-anthesis N in wheat grown on a duplex soil. Aust. J. Agric. Res. 46, 519–531.

Peeters, K.M.U., Van Laere, A.J., 1994. Amino acid metabolism associated with N-mobilization from the flag leaf of wheat (*Triticum aestivum* L.) during grain development. Plant Cell Environ. 17, 131–141.

Pettersson, R., McDonald, A.J.S., 1994. Effects of nitrogen supply on the acclimation of photosynthesis to elevated CO_2. Photosynth. Res. 39, 389–400.

Pfirrmann, T., Barnes, J.D., Steiner, K., Schramel, P., Busch, U., Küchenhoff, H., Payer, H.D., 1996. Effects of elevated CO_2, O_3 and K deficiency on Norway spruce (*Picea abies*): nutrient supply, content and leaching. New Phytol. 134, 267–278.

Poorter, H., 1993. Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. In: Rozema, J., Lambers, H., Van de Geijn, S.C., Cambridge, M.L. (Eds.), CO_2 and Biosphere. Kluwer Academic, Dordrecht, pp. 77–97.

Raman, R., Oliver, D.J., 1997. Light-dependent control of photorespiratory gene expression. In: Pessarakli, M. (Ed.), Handbook of Photosynthesis. Marcel Dekker, New York, pp. 381–389.

Rastetter, E.B., Shaver, G.R., 1992. A model of multiple-element limitation for acclimating vegetation. Ecology 73, 1157–1174.

Rogers, G.S., Payne, L., Milham, P., Conroy, J., 1993. Nitrogen and phosphorus requirements of cotton and wheat under changing atmospheric CO_2 concentrations. Plant Soil 155 (156), 231–234.

Roy, J., Garnier, E., . A Whole Plant Perspective on Carbon--Nitrogen Interactions. SPB Academic Publishing, The Hague.

Rudorff, B.F.T., Mulchi, C.L., Lee, E.H., Rowland, R., 1996. Effects of enhanced O_3 and CO_2 enrichment on plant characteristics in wheat and corn. Environ. Pollut. 94, 53–60.

Sage, R.F., Sharkey, T.D., Seemann, J.R., 1989. Acclimation of photosynthesis to elevated CO_2 in five C_3 species. Plant Physiol. 89, 590–596.

Sage, R.F., 1994. Acclimation of photosynthesis to increasing atmospheric CO_2: the gas exchange perspective. Photosynth. Res. 39, 351–368.

Schnyder, H., 1993. The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling—a review. New Phytol. 123, 233–245.

Scotti, I.A., Silva, S., Botteschi, G., 1994. Effects of ozone on grain quality of wheat grown in open-top chambers: three years of experimentation. Environ. Pollut. 86, 31–35.

Sharkey, T.D., 1985. Photosynthesis in intact leaves of C_3 plants: physics, physiology and rate limitations. Bot. Rev. 51, 53–105.

Sharkey, T.D., 1988. Estimating the rate of photorespiration in leaves. Physiol. Plant. 73, 147–152.

Sinclair, T.R., 1992. Mineral nutrition and plant growth response to climate change. J. Exp. Bot. 43, 1141–1146.

Sinnaeve, G., Herman, J.L., Couvreur, L., Dardenne, P., Oger, R., Vrebos, D., 1997. Aperçu de la qualité des froments d'hiver en 1997. In: Falisse, A., Meeus, P. (Eds.), Livre blanc 'Céréales' Gembloux, 11 Septembre 1997, Centre de Recherches Agronomiques de l'état. Gembloux, Belgium.

Tingey, D.T., Rodecap, K.D., Lee, E.H., Moser, T.J., Hogsett, W.E., 1986. Ozone alters the concentrations of nutrients in bean tissue. Angew. Bot. 60, 481–493.

Tottman, D.R., Broad, H., 1987. The decimal code for the growth stages of cereals, with illustrations. Ann. Appl. Biol. 110, 441–454.

Van Kraalingen, D.W.G., 1990. Effects of CO_2 enrichment on nutrient-deficient plants. In: Goudriaan, J., Van Keulen, H., Van Laar, H.H. (Eds.), The Greenhouse Effect and Primary Productivity in European Agro-ecosystems. Pudoc, Wageningen, 42–45..

Vandermeiren, K., De Temmerman, L., Staquet, A., Baeten, H., 1992. Effects of air filtration on spring wheat grown in open-top field chambers at a rural site. II. Effects on mineral partitioning, sulphur and nitrogen metabolism and on grain quality. Environ. Pollut. 77, 7–14.

Webber, A.N., Nie, G.Y., Long, S.P., 1994. Acclimation of photosynthetic proteins to rising atmospheric CO_2. Photosynth. Res. 39, 413–425.

Woodrow, I.E., 1994. Optimal acclimation of the C_3-photosynthetic system under enhanced CO_2. Photosynth. Res. 39, 401–412.