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Does elevated atmospheric CO_2 allow for sufficient wheat grain quality in the future?

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Summary

To identify future impacts on biomass production and yield quality of important C_3 crops, spring wheat was grown in association with 13 weed species in a Mini-FACE (free-air carbon dioxide (CO_2) enrichment) system under ambient (375 µl l^{-1}) and elevated (526 µl l^{-1}) CO_2 concentrations. Wheat productivity was assessed at maturity and grain yield was subjected to various chemical analyses and baking quality tests.

CO_2 enrichment acted as carbon 'fertiliser' and increased the aboveground biomass production of wheat by 18.8% as there was a trend towards higher stem biomass. Although not statistically significant, wheat grain yield was increased by 13.4% due to a significant establishment of more grains per unit ground area. At the same time, thousand grain weight was non-significantly shifted towards smaller grain size classes, which may result in negative consequences for the crop market value. As a result of the CO_2-induced physiological and biochemical modifications, concentration of total grain protein was significantly decreased by 3.5%, reducing the wheat grain quality with potentially far-reaching impacts on the nutritional value and use for processing industry. Although often not significant, the concentrations of amino acids per unit of flour were decreased by 0.2 to 8.3% due to elevated CO_2 thereby affecting the composition of proteinogenic amino acids.

Furthermore, gluten proteins tended to decline. Within the significant decreased gliadins, α- and ω5-gliadins were significantly reduced under CO_2 enrichment; there was also a negative trend for ω1,2- and γ-gliadins. Changes in certain essential minerals were found as well, although not statistically significant. Concentrations of sodium, calcium, phosphorus and sulphur were slightly lowered and those of potassium and magnesium were slightly increased due to CO_2 enrichment. The micro-element molybdenum was increased, while concentrations of iron, zinc, copper, manganese and aluminium were decreased. With regard to rheological and baking parameters defining the cereal quality for industrial processing, the resistance of the dough was significantly reduced by about 30%, while the extensibility was non-significantly increased by 17.1% under CO_2 enrichment. Moreover, the bread volume was decreased non-significantly by about 9%. Elevated CO_2 is obviously affecting grain characteristics important for consumer nutrition and health, industrial processing and marketing. Experimental evidence for these changes is still poor but deserves further attention.

Introduction

Before the industrial revolution, the global atmospheric carbon dioxide (CO_2) concentration was around 280 µl l^{-1} and has increased since then considerably to approximately 380 µl l^{-1} today. A further increase to about 550 µl l^{-1} by the year 2050 has been predicted (IPCC, 2007). Beside indirect impacts via climate changes such as temperature increase and alterations in precipitation pattern, CO_2 enrichment is acting directly on C_3 crops such as wheat in agroecosystems (IPCC, 2007; ZISKA and RUNION, 2007). The CO_2-induced impacts may be determined by interactions with other components such as the presence of biotic (pests, diseases, weeds) or abiotic (temperature, light, soil water, nutrients) stresses (FANGMEIER and JÄGER, 2001; AINSWORTH and LONG, 2005).

Wheat (*Triticum aestivum* L.) is one of the most important C_3 crops worldwide. CO_2-induced consequences include increased photosynthesis as well as improved water- and nitrogen- (N) use efficiencies. Biomass and grain yield production of wheat in terms of tonnage is positively affected by CO_2 enrichment (e.g. AMTHOR 2001; ZISKA and BUNCE, 2007). In earlier chamber-based experiments, the gain in wheat grain yield was shown to occur by means of increases in thousand grain weight (TGW; MCKEE et al., 1997), grain number per ear (MCKEE et al., 1997; MULHOLLAND et al., 1997) or the number of flowering tillers per unit ground area (HAVELKA et al., 1984; FANGMEIER et al., 1996). A recently published data evaluation of free-air CO_2 enrichment (FACE) experiments by HÖGY and FANGMEIER (2008) showed that TGW was significantly increased by 3.8% under elevated CO_2, indicating a potential positive effect on wheat grain quality in terms of milling quality and hence economic value.

Since CO_2 enrichment alters the carbon- (C) and N-metabolism in vegetative plant parts (COTRUFO et al., 1998; LOLADZE, 2002), the availability of metabolites for the grain during filling may be changed, thus affecting the chemical composition of grains with regard to different market and utilisation requirements. Although grain quality is as important as yield, much less attention has been paid to possible effects of elevated CO_2 concentrations on grain quality traits. Potential CO_2 effects on grain quality aspects other than concentration of total grain protein are scarcely published (KIMBALL et al., 2002; LOLADZE, 2002; HÖGY and FANGMEIER, 2008).

The total concentration of grain protein is a major determinant of grain price and is strongly and positively correlated with breadmaking quality in particular due to its impact on dough strength (MACRITCHIE, 1978; LAWLOR and MITCHELL, 2001). It has been shown that 80% of the N present in the vegetative plant parts of wheat will be distributed to the grain after anthesis (OSAKI et al., 1991; CONROY and HOCKING, 1993; FANGMEIER et al., 1997). As a consequence of the CO_2-induced modifications in plant metabolism, grain protein concentration is generally decreased in FACE experiments (HÖGY and FANGMEIER, 2008; TAUB et al., 2008). Overall, the grain protein concentration is negatively related to grain yield (EVANS, 1993; PLEIJEL et al., 1999), but this is not simply caused by dilution due to increased concentrations of non-protein components (GIFFORD et al., 2000). Currently, the mechanisms by which CO_2 enrichment decreases proteins are not well understood.

Especially the unique chemical and physical properties of wheat grain protein fractions are the main factors explaining the widespread use of wheat (FINNEY, 1985; POMMERANZ, 1988). Accumulation of structural and metabolic proteins (albumins and globulins) appears to be sink-regulated and starts earlier (TRIBOÏ et al., 2003), whereas storage protein accumulation is constrained by N sources to the developing grain (MARTRE et al., 2003). While albumins and globulins have only a minor impact on dough strength and associated characteristics such as loaf volume, the storage proteins (monomeric gliadins and large disulfide linked polymeric glutenins) are primarily responsible for many aspects of grain quality (MacRitchie et al., 1990; Weegels et al., 1996). Especially the ratio between gliadins and glutenins affects plasticity, strength and elasticity of dough, which are traits fundamental to processing high-quality bread. Wieser et al. (2008) recently reported that the amount of albumins and globulins was unaffected by CO_2 enrichment, while the gluten proteins, especially the N-rich ω5-, ω1,2- and α-gliadins and high molecular weight glutenin subunits, were decreased in a FACE experiment using winter wheat. Moreover, metabolic proteins are rich in sulphur (S) containing amino acids such as cysteine (Cys) and methionine (Met) as well as essential amino acids, especially lysine (Lys), resulting in a high nutritive value, while gluten proteins are rich in glutamine (Gln) and proline (Pro). In previous open-top chamber (OTC) experiments, relatively more essential amino acids were found under CO_2 enrichment (Manderscheid et al., 1995; Högy et al., 1998), indicating positive effects on the nutritive value.

The composition of grain proteins and the balance of strength and elasticity are fundamental in determining the physical properties of dough formation and product quality (Weegels et al., 1996; Cornish et al., 2006). Blumenthal et al. (1996) reported that CO_2 enrichment did not cause significant or consistent impacts on dough properties except of the lowered peak resistance by 15.9%. Dough extensibility and farinograph dough development time were also affected by CO_2 enrichment in the same chamber-based experiment (Blumenthal et al., 1996), which was largely associated with the decline in total protein concentration. In a prior FACE experiment, Kimball et al. (2001) found a 6% increase in optimum mixing time for bread dough due to elevated levels of CO_2. Currently, data of dough rheological properties from FACE experiments are still missing. Moreover, significant decreases in volume of bread loaves were observed in chamber-based (Blumenthal et al., 1996; Fangmeier et al., 1999) and in FACE experiments (Kimball et al., 2001).

With regard to minerals, CO_2-induced impacts have been reported in OTCs by Manderscheid et al. (1995) for magnesium (Mg), calcium (Ca), S, iron (Fe) and zinc (Zn), by Fangmeier et al. (1999) for Ca, Fe and S, by De La Puente et al. (2000) for Mg, potassium (K) and Ca and by Wu et al. (2004) for P, K and Zn. Data from FACE field studies are not available.

The impacts of CO_2 enrichment on the quality traits of wheat are currently not well understood and the experimental outcomes are often contradictory, suggesting both negative and positive implications for the nutritional value and industrial processing of grains in the future. Therefore, in our field study we tested whether the CO_2-induced impacts on photosynthesis and nutrient allocation of wheat plants have consequences on grain yield and grain quality with regard to food safety. Wheat grains were subjected to analyses of general quality aspects (number of grains per unit area, grain yield, TGW, grain size pattern), several chemical analyses (concentrations of macro- and micro-elements, amino acids, total proteins and protein composition) as well as mixing, rheological and baking quality tests to comprise whether these parameters were affected by realistic future concentrations (ambient + 150 $\mu l\ l^{-1}$ CO_2) in a FACE system.

Materials and methods

Experimental CO_2 treatments, environmental monitoring and plant cultivation

The experiments were performed in 2005 at the Heidfeldhof farm managed by the Plant Breeding Station of the Universität Hohenheim (9°11´E, 48°42´N, 395 m above sea level) south of Stuttgart, Germany. Spring wheat (*Triticum aestivum* L. cv. Triso) as main crop and 13 associated weed species were grown on circular field plots (2 m in diameter). Experimental treatments were (i) ambient CO_2 concentrations without exposure system (CONTROL), (ii) ambient CO_2 concentrations with exposure system (AMBIENT) and (iii) elevated CO_2 concentrations with exposure system (FACE) with five replicates each. The principle of operation and the performance of the FACE system have been described by Erbs and Fangmeier (2006) and Erbs et al. (2008). The CO_2 exposure was started before emergence of the plants (04. April 2005). To achieve the target gas concentrations of 150 $\mu l\ l^{-1}$ above AMBIENT during daylight hours (average from 7.00 a.m. to 8.00 p.m.), pure CO_2 (Westfalen Gas, Germany) was added and concentrations achieved at canopy height were monitored at the centre of each field plot. Wind speed and direction (Siggelkow, Germany; reference height 1 m) as well as air temperature, relative humidity (Rotronic, Switzerland) and solar radiation (Kipp & Zonen, The Netherlands; reference height 2 m, respectively) were measured at the centre of the field site. Precipitation was recorded at the nearby weather station Stuttgart airport from the "Deutscher Wetterdienst", which is about 2 km from the field site. The amount of rain is similar at both sites (J. Franzaring, personal communication). Local soil (clayey to loamy) was used for field-grown plants in the 15 plots. Wheat (13 lines with 15 cm row spacing; 200 plants m^{-2}) and weed species were sown on 24. March 2005. Based on agronomic practice, plots were fertilised with 140 kg N ha^{-1}, 30 kg phosphorous (P) ha^{-1} and 60 kg K ha^{-1}, which was supplied in 50/25/25% portions at different development stages of the spring wheat (EC 13-29, EC 30-39 and EC 40-59, respectively; Tottman and Broad, 1987). Additionally, a trace element fertiliser (K&S Kali, Germany) was used at EC 30-39 containing 0.03 kg boron (B) ha^{-1}, 0.45 kg Mg ha^{-1}, 0.36 kg S ha^{-1} and 0.03 kg manganese (Mn) ha^{-1}. Time Domain Reflectometry (TDR) sensors for soil moisture (IMKO, Germany) were horizontally installed in a depth of 15 cm. Plants were manually irrigated when the volumetric soil moisture declined to 25% with the same amount of water applied to all treatments. Irrigation volumes were recorded and included into the calculation of total water supply.

Wheat plants of the central square metre of each plot were harvested at maturity (01. August 2005) and separated into leaves, stems and ears. The biomass of the different wheat fractions was determined after drying until constant weight at 60°C, except for the ears (30°C). Grains were removed from ears by threshing, ground to wholemeal flour using a laboratory mixer mill (MM 301, Retsch, Germany) and stored for quality analyses such as concentrations of macro- and micro-elements and amino acids. In a second step, the grains were milled to refined flour (Type 550) for analyses of protein types, mixing and rheological properties and baking tests using a Brabender Quadrumat Junior mill.

Determination of grain quality parameters

The TGW was determined (Condator "E", Pfeuffer, Germany) and grains were separated into different size classes (> 2.8 mm; 2.8-2.5 mm; 2.5-2.2 mm; < 2.2 mm) using a Sortimat (Type K, Pfeuffer, Germany).

The S and N concentrations of wholemeal flour were determined using an elemental analyser (Vario EL, Elementar Analysensysteme, Germany) according to ISO 10694 (1995), while the N concentration of refined flour was obtained by the DUMAS combustion method using a LECO FP-328 (LECO, USA). The total protein concentration was calculated by multiplying the N concentration by a conversion factor of 5.7 for wheat grain. The individual macro- and micro-elements were determined after acid digestion using (i) inductively-coupled plasma optical emission spectrometry (ICP-OES, Vista Pro radial, Varian, USA) for aluminium (Al), calcium (Ca), Fe, K, Mg, sodium (Na), P and (ii) inductively-coupled plasma mass-spectrometry (ICP-MS, Elan 6000, Perkin Elmer Sciex, USA) for B, copper

(Cu), Mn, molybdenum (Mo), Zn according to the method of VDLUFA VII 2.2.2.5. (2003) and VDLUFA VII 2.2.2.6. (2003). Prior to analysis the samples were digested with highly pure nitric acid (Baker Instra-Analysed, USA) at 220°C and approximately 10 MPa in quartz vessels using a high pressure digestion system (UltraClave III, MLS, Germany). The amino acid concentrations were analysed according to the European Commission Directive 98/64/EC (1998). The protein types in wheat flour were quantified by a modified Osborne fractionation followed by reversed-phase high-performance liquid chromatography (RP-HPLC) using the protocol of Wieser et al. (1998). The mixing and rheological properties and the baking quality tests were analysed according to micro-scale methods described previously (Kieffer et al., 1998).

Data, replication, statistics

The statistical significances of the CO_2 effects on wheat biomass fractions and grain quality aspects were tested by an analysis of variance (ANOVA) using SPSS PC (version 15 for Windows). Data from AMBIENT and FACE treatments were used for the analyses, while data from the CONTROL treatment was excluded from the evaluation since differences to AMBIENT treatments were not significant. Results were expressed as probability (P) levels (ns, $P > 0.1$; numbers in parenthesis indicate trends with $0.1 \geq P > 0.05$; numbers without parenthesis indicate $P \leq 0.05$). Data from FACE treatments (four replicates) were given as CO_2 response in relation to the AMBIENT treatments (five replicates) of the experiment (AMBIENT = 100%).

Results

Climatic conditions and experimental CO_2 concentrations

In 2005, the cultivation period (130 days) was rather moist with frequent thunderstorms and high precipitation in single rain events. From sowing to maturity, the plants received 425 mm of water. The averages ± standard deviations of daily minimum, mean and maximum temperatures averaged over the cropping season were 9.2 ± 4.8°C, 14.8 ± 5.3°C and 20.1 ± 6.4°C, respectively, and the growing degree days (baseline > 5°C) were 1120°C. The mean seasonal daylight CO_2 concentrations achieved were 375 ± 11 µl l^{-1} (AMBIENT) and 526 ± 5 µl l^{-1} (FACE), demonstrating that the exposure system worked satisfactorily.

Biomass production, grain yield and grain quality traits

CO_2 enrichment increased the wheat aboveground biomass at final harvest by 18.8% (P = ns) with a trend towards increased stem weight due to CO_2 enrichment (Tab. 1). Leaf and ear biomass were increased by 13.5 and 13.3%, respectively; however, these increases were not statistically significant. Grain yield increased non-significantly by 13.5%, resulting from a CO_2-induced increase in the number of ears (+22.4%, P = 0.031) and grains (+14.8%, P = ns) produced per unit of area. Concomitantly, the grain number per ear and the harvest index was not significantly affected under elevated CO_2. Also the TGW remained unaffected under CO_2 enrichment, although there were slight but non-significant shifts towards smaller grains according to the results obtained for different grain size classes.

The concentrations of macro- and micro-elements were not significantly affected by CO_2 enrichment (Tab. 2). However, the macro-elements K and Mg were increased, whereas Na, Ca, P and S were decreased. The concentrations of micro-elements such as Fe, Zn, Cu, Mn and Al were also decreased, while the only CO_2-induced increase was observed for Mo.

The total protein concentration in wheat grains was significantly decreased in the high-CO_2 treatment, whereas the protein yield (i.e. protein harvested per ground area) was not changed. The CO_2-induced impact on protein concentrations was slightly larger (-4.2%) when protein was measured in refined rather than whole-grain flour (data not shown).

Tab. 1: Aboveground biomass production, grain yield traits and grain size classes of wheat grown in the field at either ambient (AMBIENT) or elevated (FACE) CO_2 concentrations. Values represent means and standard deviations of the replicates per treatment, the calculated CO_2 effect (AMBIENT = 100%) and level of statistical significance of the one-way analysis of variance (ANOVA).

Wheat traits	AMBIENT	FACE	CO_2 effect	P-level
Wheat production [g DW m^{-2}]				
Aboveground	1041 ± 191	1236 ± 169	+18.8%	ns
Leaves	106.7 ± 26.9	121.1 ± 17.2	+13.5%	ns
Stems	450.4 ± 75.0	568.8 ± 78.1	+26.3%	(0.054)
Ears	479.4 ± 102.7	543.1 ± 139.2	+13.3%	ns
Yield parameters				
Ear number [No. m^{-2}]	435.2 ± 50.6	532.6 ± 58.4	+22.4%	0.031
Grain number [No. m^{-2}]	8747 ± 1892	10044 ± 2729	+14.8%	ns
Grain number [No. ear^{-1}]	19.99 ± 2.76	19.02 ± 5.46	-4.9%	ns
Grain yield [g DW m^{-2}]	350.9 ± 84.0	398.2 ± 129.8	+13.5%	ns
Harvest Index	0.34 ± 0.04	0.32 ± 0.08	-5.3%	ns
TGW [g 1000 $grains^{-1}$]	39.95 ± 1.00	39.19 ± 2.30	-1.9%	ns
Grain size classes [% grains]				
> 2.8 mm	55.6 ± 3.3	52.6 ± 7.7	-5.4%	ns
2.8 – 2.5 mm	32.9 ± 2.4	31.7 ± 2.7	-3.8%	ns
2.5 – 2.2 mm	7.6 ± 1.1	10.2 ± 3.1	+35.2%	ns
< 2.2 mm	3.9 ± 1.3	5.5 ± 2.1	+40.1%	ns

ns = no significance at $P \leq 0.05$; number in parenthesis indicates trend ($0.1 \geq P > 0.05$).

Tab. 2: Concentrations of macro- and micro-elements of wheat grains grown in the field at either ambient (AMBIENT) or elevated (FACE) CO_2 concentrations. Values represent means and standard deviations of the replicates per treatment, the calculated CO_2 effect (AMBIENT = 100%) and level of statistical significance of the one-way analysis of variance (ANOVA).

Nutrient elements	AMBIENT	FACE	CO_2 effect	*P*-level
Macro-elements [mg kg^{-1} DW]				
Na	10.2 ± 2.2	9.8 ± 1.6	-4.1%	ns
K	892 ± 202	4943 ± 144	+1.0%	ns
Ca	439 ± 13	434 ± 36	-1.1%	ns
Mg	1812 ± 54	1835 ± 44	+1.3%	ns
P	5070 ± 105	5060 ± 67	-0.2%	ns
S	1864 ± 47	1835 ± 47	-1.6%	ns
Micro-elements [mg kg^{-1} DW]				
Fe	63.4 ± 3.1	1.3 ± 2.2	-3.4%	ns
Zn	36.2 ± 1.6	36.0 ± 2.2	-0.6%	ns
Cu	6.85 ± 0.42	6.66 ± 0.24	-2.8%	ns
Mn	26.4 ± 3.0	25.5 ± 3.7	-3.4%	ns
Mo	0.75 ± 0.14	0.76 ± 0.15	+0.9%	ns
Al	260 ± 154	247 ± 118	-5.1%	ns

ns = no significance at $P \leq 0.05$.

In agreement with the responses in protein concentration, the concentrations of all amino acids per unit of flour weight were decreased by 0.2 to 8.3% in the FACE treatment (Fig. 1). Of the proteinogenic amino acids, some are called essential amino acids because they cannot be synthesised de novo by humans and must be obtained from food. The semi-essential amino acids, however, are not normally required in the diet, but must be supplied exogenously to specific populations because the metabolic pathways that synthesise these amino acids in adequate amounts are not fully developed. In the present study, the CO_2 effects were only significant for non-essential glycine (Gly) and essential valine (Val), while a trend was observed for non-essential Gln. There was no clear pattern whether different amino acid types were more or less reduced in the high-CO_2 treatment. Correspondingly, if amino acid concentrations were calculated on a per protein basis, there were decreases in the concentrations of Gly, valine (Val), methionine (Met), Gln, leucine (Leu), Pro, isoleucine (Ile) and aspartic acid (Asp) by 1.1 to 5.5% in the FACE treatment (Fig. 2). The only significant CO_2 effect was observed for Gly, while for Val only such a trend was found. Concomitantly, non-significant CO_2-induced increases of tryptophan (Trp), arginine (Arg), Lys, tyrosine (Tyr), Cys, phenylalanine (Phe), serine (Ser), alanine (Ala), histidine (His) and threonine (Thr) were observed by 0.5 to 3.3%.

The protein fractions of albumins/globulins were not affected by CO_2 enrichment (Fig. 3). Glutens tended to be decreased by CO_2 enrichment. The gluten-related gliadin fraction was significantly decreased under elevated CO_2, while the glutenins remained unaffected. As a consequence, the glutenin-gliadin ratio was increased, although this effect was not statistically significant. Within the gliadin proteins, ω5-gliadins and α-gliadins were significantly decreased in the high-

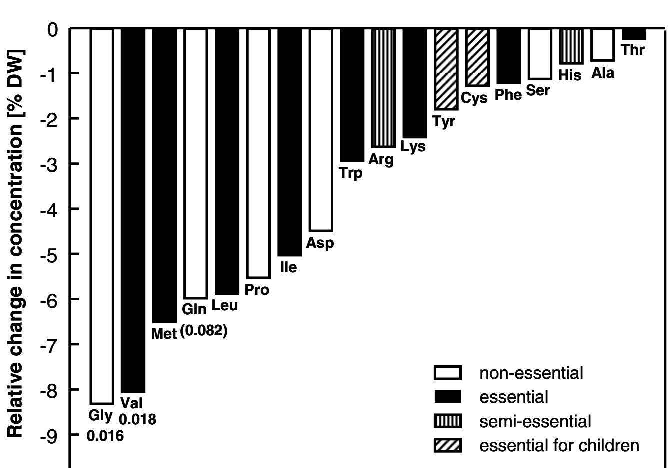

Fig. 1: Relative responses of amino acids [% DW] in wheat grains due to CO_2 enrichment. The results of the statistical analyses are denoted as levels of probability (*P*) of the one-way analysis of variance (ANOVA) at $P \leq 0.05$, numbers in parenthesis indicate a trend ($0.1 \geq P > 0.05$).

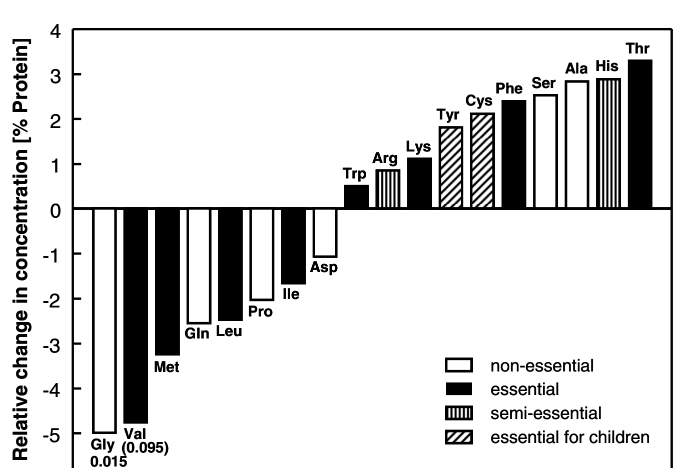

Fig. 2: Relative responses of amino acids [% protein] in wheat grains due to CO_2 enrichment. The results of the statistical analyses are denoted as levels of probability (*P*) of the one-way analysis of variance (ANOVA) at $P \leq 0.05$, numbers in parenthesis indicate a trend ($0.1 \geq P > 0.05$).

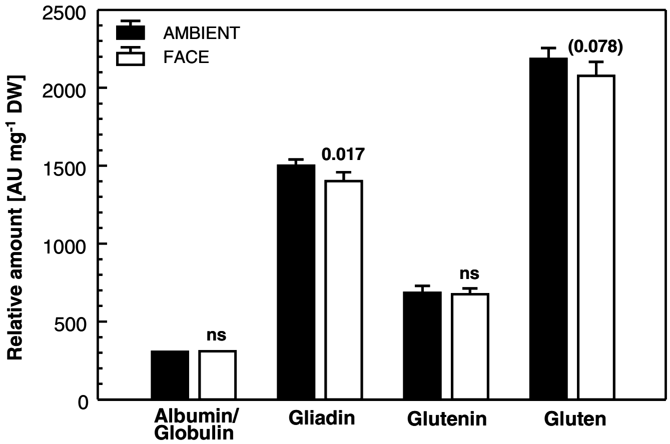

Fig. 3: Grain protein fractions of wheat grown in the field at either ambient (AMBIENT) or elevated (FACE) CO_2 concentrations. Values are means and standard deviations of all replicates per treatment. The results of the statistical analyses were expressed as levels of probability (P) of the one-way analysis of variance (ANOVA) at $P \leq 0.05$, ns = no significance at $P > 0.05$, numbers in parenthesis indicate a trend ($0.1 \geq P > 0.05$).

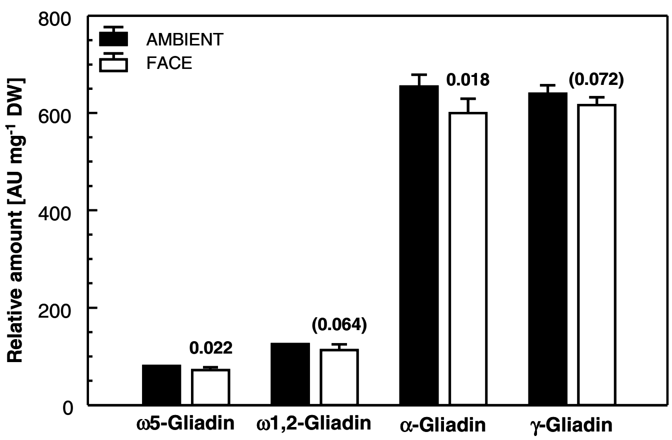

Fig. 4: Relative responses of gliadin fractions [% DW] in wheat grains due to CO_2 enrichment. The results of the statistical analyses are denoted as levels of probability (P) of the one-way analysis of variance (ANOVA) at $P \leq 0.05$, numbers in parenthesis indicate a trend ($0.1 \geq P > 0.05$).

CO_2 treatment (Fig. 4). Moreover, ω1,2-gliadins and γ-gliadins tended also to be decreased under elevated CO_2.

The mixing properties such as water absorption, dough development time and degree of dough softening showed no significant responses to elevated CO_2 concentrations (Tab. 3). With regard to the rheological properties, the dough resistance was significantly decreased in the high-CO_2 treatment. In contrast, the dough extensibility was increased by 17.1%, although this CO_2 effect was not statistically significant. The area of the extensogram was not affected. Furthermore, elevated CO_2 exposure non-significantly decreased the loaf volume by 8.9%.

Discussion

CO_2-induced impacts on biomass allocation, grain yield and yield components of wheat

In the present study, no significant CO_2 effect on aboveground biomass production of wheat was observed, although wheat production was increased by 18.8%. The biomass allocation towards stems tended to increase due to CO_2 enrichment, while that allocated to leaves and ears was not affected. Nevertheless, ear number per area was shown to significantly increase in response to CO_2 exposure, whereas no significant CO_2 effect was found for grain yield, although the latter was increased by 13.5%. The present grain yield gain is in accordance with increases by 10 to 16% found in previous FACE studies when CO_2 levels were raised from 380 to 550 µmol l^{-1} at ample supplies of N and water (KIMBALL, 2004, 2006; KIMBALL et al., 2001, 2002; LONG et al., 2006; SCHIMEL, 2006). However, the mean total grain yields were lower than in regional cultivation trials and reached a mean of 35 dt ha^{-1} (AMBIENT), which may have resulted from the lower plant density of wheat plants and the competitive pressure from weeds. In our study, other grain yield components such as grain number per unit area and grain number per ear as well as the harvest index remained unaffected in the high-CO_2 exposure. The TGW was also unaffected in the FACE treatment. This is in agreement with results from previous FACE studies (KIMBALL et al., 2001; MANDERSCHEID et al., 2004), while LI et al. (2001) observed an increase in TGW by 7% under high-CO_2 conditions. In summary, TGW may be affected due to elevated CO_2, but in an inconsistent fashion, depending on the experimental conditions. In the present study, a non-significant shift to smaller grain sizes was observed, resulting in a lower market value of the grains. No results on this topic are available from other FACE experiments, while significant shifts to smaller grain sizes were also found in OTCs (HÖGY, 2002).

Tab. 3: CO_2-induced impacts on wheat grains and consequences for mixing and rheological properties of flour and loaf volume. Values represent means and standard deviations of the replicates per treatment, the calculated CO_2 effect (AMBIENT = 100%) and level of statistical significance of the one-way analysis of variance (ANOVA). BE, Brabender units; N, Newton.

Grain quality traits	AMBIENT	FACE	CO_2 effect	P-level
Mixing properties				
Water absorption [ml]	4.66 ± 0.08	4.67 ± 0.03	+0.1%	ns
Dough development time [min]	12.6 ± 1.8	12.5 ± 0.6	-0.8%	ns
Degree of dough softening [BE]	45.0 ± 6.1	43.8 ± 11.1	-2.8%	ns
Rheological properties				
Dough resistance [mN]	298 ± 68	198 ± 48	-33.8%	0.041
Dough extensibility [mm]	78.9 ± 22.8	92.4 ± 9.9	+17.1%	ns
Area of extensogram [N x mm]	11.6 ± 4.0	11.2 ± 2.5	-2.9%	ns
Loaf volume [ml]	32.4 ± 9.6	29.5 ± 2.0	-8.9%	ns

ns = no significance at $P \leq 0.05$.

Alterations of mineral composition in wheat grains due to CO_2 enrichment

Since most of the minerals in wheat grains originate from the redistribution from vegetative pools during grain filling, CO_2 enrichment may cause serious alterations in the concentrations of macro- and micro-elements, probably diminishing the nutritional value. In our study, reductions due to elevated CO_2 were not statistically significant, although all concentrations except for K, Mg and Mo were decreased between 0.2 to 5.1%. Until today, knowledge with regard to CO_2-induced impacts on the elemental composition of wheat grains from FACE experiments is scarce.

Effects of CO_2 enrichment on concentration and composition of wheat grain proteins

In the present study, the total protein concentration per dry weight was 15.7% (AMBIENT) and 15.2% (FACE), which is quite high for summer wheat produced under N supply conventional for regional agronomic practice. The grain protein concentration was significantly decreased by 3.5% under CO_2 enrichment, which is in accordance with earlier findings from FACE experiments (KIMBALL et al., 2001, 2002; KIMBALL, 2004; HÖGY and FANGMEIER, 2008; TAUB et al., 2008). In contrast, WIESER et al. (2008) reported that the protein concentration of wheat grains was lowered by about 14% at comparable CO_2 enrichment (ambient + 150 µl l^{-1}). Since total protein concentration greater than 11.5% is required for adequate bread-making quality, crop fertilisation practices may need to be altered in a future high-CO_2 environment in order to achieve the quality requirements. Unfortunately, the CO_2-induced reductions in grain quality may not easily be overcome by increases in N fertilisation since this may translate into higher biomass and yield production rather than into enhanced redistribution of N to the grains (FANGMEIER et al. 1999; WEIGEL and MANDERSCHEID, 2005). Results from previous, European-wide studies in OTCs suggest that the loss in grain protein under CO_2 enrichment is similar irrespective of the amount of N supply to the wheat crop (FANGMEIER et al., 1999). Also TAUB et al. (2008) stated that even under high N treatments, wheat still experiences a reduction in protein concentration by 10%. Therefore, CO_2 enrichment is likely to make the wheat grain quality poorer in the future with regard to nutritional value and use for processing industry. It remains open whether more N fertilisation will compensate the CO_2-induced loss in grain protein. Moreover, in our FACE study the grain protein yield was unaffected, while PIIKKI et al. (2008) reported a significant increase of protein yield in chamber-based experiments, resulting from a higher grain yield caused by elevated CO_2 concentrations. TAUB et al. (2008) suggested that the CO_2 effect on human nutrition may differ between consumers of refined or whole-grain flour since CO_2 may affect the protein concentration in endosperm less than in other grain parts. Here we directly examined this possibility and found no clear evidence for this suggestion.

In the present study, the composition of grain protein fractions was affected by CO_2 enrichment, resulting in a serious impairment of the bread-making quality. The albumins and globulins were unaffected, whereas the gluten proteins tended to be decreased in the high-CO_2 treatment. Also WIESER et al. (2008) reported that both gluten proteins and their fractions were decreased in a comparable FACE experiment. Our results demonstrate that among the gluten proteins, the N- and Gln-rich gliadins showed a more pronounced CO_2-induced decrease, while the glutenins remained unaffected. Although not significant in the present study, comparable increases of the glutenin-gliadin ratio were reported in chamber-based (BLUMENTHAL et al., 1996) and FACE (WIESER et al., 2008) experiments. Within the gliadin fraction, the ω5- and ω1,2-gliadins with a high N requirement showed a more pronounced decrease due to CO_2 enrichment than did the α- and γ-gliadins, which is in accordance with WIESER et al. (2008). Currently, no further information on this topic is available in the literature.

CO_2-induced impacts on amino acid concentration and composition of wheat grains

Along with the reduced protein concentration and the observed changes in protein composition in wheat grains, the concentrations of amino acids were affected, too. All concentrations of amino acids were reduced between 0.2 and 8.3% in the present FACE study, although effects were only significant for Gly and Val, while a trend was observed for Gln. No clear CO_2-induced impacts on the pattern of different types of amino acids were observed in our FACE study. In contrast, MANDERSCHEID et al. (1995) and HÖGY et al. (1998) reported increases in the concentrations of essential amino acids in wheat grains under elevated CO_2 concentrations in chamber-based experiments. Moreover, in the present study changes in the composition of amino acids were found under CO_2 enrichment. No comparable results have been reported from other CO_2 experiments using FACE techniques. Based on OTC experiments, HÖGY et al. (1998) reported that the amino acid composition of wheat grains was affected by elevated CO_2, which likely indicated a decline in grain storage proteins, while metabolic proteins were unchanged or increased due to CO_2 enrichment. Currently, this view is supported by decreased gluten proteins found in high-CO_2 conditions (WIESER et al., 2008). Since amino acids such as Asn, and marginally Gln as well as Asp, are involved in the formation of acryl amide during industrial processes like baking, the possible concentration of acryl amide may decrease too in flour-derived products in a high-CO_2 world. However, there is no existing experimental database related to this topic to draw final conclusions.

Effects of CO_2 enrichment on mixing and rheological properties and loaf volume of wheat flour

In our study, the peak resistance was significantly decreased by 33.8%, which is in accordance with the results reported by BLUMENTHAL et al. (1996) from chamber-based experiments, although the present CO_2-induced impact was nearly twice as large. No significant CO_2 effects on dough extensibility and farinograph dough development time were found in the present FACE study, which is in contrast with the study by BLUMENTHAL et al. (1996). While in the present FACE experiment the volume of bread loaves was unaffected under CO_2 enrichment, significant decreases were reported in a previous experiment (KIMBALL et al., 2001). However, the change in grain protein is often more pronounced than CO_2-induced impacts on the functional properties for wheat processing (RUDORFF et al., 1996; LAWLOR and MITCHELL, 2001).

Conclusions

The present results demonstrate that especially the N-rich protein fractions, which are important for the visco-elastic properties of wheat grains, were negatively affected under CO_2 enrichment. Although it seems that CO_2-induced impacts on wheat grain quality may be relatively small, we should be concerned about it. Moreover, as the magnitude of CO_2-induced effects on grain quality aspects is often variable because of the sensitivity of the results to experimental conditions such as cultivar, location, climate, agronomic practice and more, it is important to realise multiple-year FACE field experiments at different sites. Despite the importance in terms of grain quality, the database concerning possible CO_2 effects is currently small. Further research is needed, especially with regard to potential technological adjustments by breeding, by agronomic management practices and by processing to maintain the quality requirements for all consumers of wheat grains in a future CO_2-enriched world.

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