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Elevated CO_2 alters grain quality of two bread wheat cultivars grown under different environmental conditions

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ABSTRACT

Bread wheat (*Triticum aestivum* L. cv. Yitpi and cv. Janz) was grown under field conditions in the Australian Grains Free-Air CO_2 Enrichment (AGFACE) facility. Ambient [CO_2] (a[CO_2], ~384 μmol mol^{-1}) and elevated [CO_2] (e[CO_2], ~550 μmol mol^{-1}) were combined with two soil water levels (rain-fed and irrigated) and two times of sowing (TOS) in three consecutive years to provide six environments (2007-TOS_1, 2007-TOS_2, 2008-TOS_1, 2008-TOS_2, 2009-TOS_1, 2009-TOS_2). Grain samples were assessed for a range of physical, nutritional and dough rheological properties. The effect of e[CO_2] on thousand grain weight (TGW) was significantly different in each growing environment: TGW was significantly increased under e[CO_2] only at 2007-TOS_2 (by 5%), 2009-TOS_1 (by 5%) and 2009-TOS_2 (by 15%) but not significantly changed under other conditions. The magnitude of reduction of grain protein concentration at e[CO_2] differed among the growing environments but was highly correlated with the percentage yield stimulation under e[CO_2] ($r^2 = 0.91$) suggesting that grain protein concentration under e[CO_2] was diluted by increased yield. Across all treatments, grain nutrient concentration was significantly reduced by e[CO_2] for Fe (3.9%, 6.2%), Cu (2.2%, 3.4%), Zn (5.9%, 5.7%), Ca (5.6%, 7.3%), Mg (5.6%, 5.8%), Na (21.2%, 30.4%), S (4.4%, 4.4%), P (4.1%, 3.2%) in cv. Yitpi and Janz, respectively. Effects of e[CO_2] on grain Zn, Mg and Na concentrations were dependent on the growing environment. Relative reduction of grain S, Fe, Mg, Zn, P at e[CO_2] were significantly correlated with grain yield stimulation at e[CO_2]. Reductions of these nutrients under e[CO_2] were not fully explained by biomass dilution as the relationships differed for each nutrient. Under e[CO_2], flour yield of cv. Janz was increased but that of cv. Yitpi was not changed. Even though grain protein concentrations of both cultivars were similar at e[CO_2], bread volume as inferred indirectly by dough rheology parameters was 12% greater for cv. Janz (185 ± 5 cm^3) than cv. Yitpi (162 ± 4 cm^3) at e[CO_2]. This disparity may be related to the compositional changes in wheat flour protein at e[CO_2], suggesting that future breeding and adaptation strategies to improve the grain quality under e[CO_2] should consider the prevailing hydro-thermal conditions.

1. Introduction

Increasing carbon dioxide concentration ([CO_2]) in the atmosphere together with rising temperature and changes in rainfall amount and patterns are current concerns for agricultural crop production and crop quality in the near future (Miraglia et al., 2009). Under most emission scenarios atmospheric [CO_2] (a[CO_2]) is expected increase to ~550 μmol mol^{-1} by 2050, causing global temperatures to increase by an average of 1.5–4.5 °C with more frequent occurrences of extreme climatic events such as heat waves and/or drought (Carter et al., 2007). Several studies have shown that wheat grain protein and mineral concentrations decrease under elevated [CO_2] (e[CO_2]) (Kimball et al., 2001; Taub et al., 2008; Högy et al., 2009; Fernando et al., 2012b). As wheat is a staple food crop for almost half the world's human population, and one of the main sources of minerals and protein in most developing countries (Cakmak, 2004), this is of concern for food security and human health. Grain protein concentration is also an important

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determinant of end product quality, because it influences dough properties and baking quality (Shewry and Halford, 2002).

It has been suggested that reduction of grain protein at $e[CO_2]$ is associated with accumulation of excess carbohydrates compared to N acquisition (Loladze, 2002). This phenomenon is referred to as growth dilution or biomass dilution (Taub and Wang, 2008). In a review, Taub and Wang (2008), argued that reduction of grain protein at $e[CO_2]$ could not be fully explained by growth dilution. This hypothesis was further supported by the variability in changes in grain mineral nutrient concentration in response to $e[CO_2]$. Several other mechanisms have been suggested to explain the reduction of grain protein concentration under $e[CO_2]$ (Taub and Wang, 2008; Bloom et al., 2010). However, the underlying physiological mechanism is still fully understood.

Better understanding how wheat grain quality changes under $e[CO_2]$ and its interaction with different growing conditions, is needed to underpin adaptation strategies. The current study was conducted in the AGFACE (Australian grains free air CO_2 enrichment) facility within the major wheat production area of Australia (Mollah et al., 2009). The research site receives an average of 250–300 mm rainfall during the growing season making this the driest grain FACE experimental site in the world. Furthermore, high temperature (22–30 °C) often dominates during grain filling and even short period of 1–4 days above 32 °C can have detrimental effects on yield (Fischer, 2011). In the Australian wheat-belt, high temperatures and drought episodes occur frequently during the growing season (Nicolas et al., 1984), and are predicted to increase in the near future (Mpelasoka et al., 2008).

Using two widely grown Australian wheat cultivars (cv. "Yitpi" and "Janz") classified as bread-wheat quality we tested the following questions: (i) How do bread wheat cultivars respond to $e[CO_2]$ in terms of grain physical, nutritional and rheological properties (ii). How do different growing conditions affect grain quality responses to $e[CO_2]$. (iii) Can changes in major grain quality traits be explained by physiological or environmental indices?

2. Materials and method

2.1. Experiment and CO_2 exposure

The experiment was conducted under field conditions in the AGFACE facility at Horsham, Victoria, Australia (36°45′07″S, 142°06′52″E; 128 m above sea level) during the 2007, 2008 and 2009 growing seasons. The climate in the region is Mediterranean with an average annual rainfall at the experimental site of 427 mm (1981–2010), where around 250–300 mm rainfall is received in the winter crop growing season, between May and November (Australian Bureau of Meteorology, 2012). The soil type of the experimental site is Vertosol according to the Australian Soil Classification (Isbell, 1996). Soil consists of ~35% clay at the soil surface, increasing up to 60% at 1.4 m depth.

Sixteen plots were arranged into four blocks with four replications in each year; 8 plots ("rings") with $e[CO_2]$ (~550 ppm) and 8 $a[CO_2]$ plots (~384 ppm). CO_2 was injected during daylight hours from crop emergence to maturity. The experimental design for all three growing seasons was a complete randomized split-split block design with two $[CO_2]$ treatments ($a[CO_2]$ and $e[CO_2]$) as the main plot (fixed factor). In 2007, plots were split for TOS, while in 2008 and 2009 they were split for water supply (rain-fed and irrigated). The two cultivars of wheat (*Triticum aestivum* L. cv. Yitpi and cv. Janz) were randomized within each half plot (TOS in 2007 and water supply in 2008 and 2009). In 2007 and 2008 ring diameters were 12 m, and in 2009 16 m. TOS_1 was a 'normal' sowing date according to local agricultural practice and the TOS_2 treatment was a late sowing date designed to push the flowering period later into a warmer time of year. In 2007, sowing and harvest dates, respectively, were: TOS_1, 18 June and 12 December; TOS_2, 23 August and 2 January 2008. In 2008, sowing and harvest dates, respectively, were: TOS_1, 4 June and 8 December; TOS_2, 5 August and 15 December 2008. In 2009 sowing and harvest dates, respectively, were: TOS_1, 23 June and 4 December; TOS_2, 19 August and 15 December 2009. Plots were hydrologically split to provide the two levels of irrigation using a buried polythene barrier to 0.8 m depth to avoid the water seepage in both 2008 and 2009 growing seasons. Agronomic practices in this experiment were similar to normal farming practices of the region.

2.2. Climatic and growth conditions

Growth conditions of normal sowing time and late sowing time of 2007, 2008 and 2009 growing seasons are illustrated in Fig. 1(a–c). Plants were sown at TOS_1 experienced cooler, wetter growing conditions during pre-anthesis (from sowing to 50% anthesis, DC65) than those sown at TOS_2 in each year (Fig. 1a–c). Average maximum air temperatures seven days before anthesis were for TOS_1 (23 °C in 2007 and 22 °C in 2009) compared with 33 °C in 2007 and 35 °C in 2009 for the TOS_2 treatment. During the 2008 growing season, average maximum air temperature seven days before anthesis was 18 °C in TOS_1 and 24 °C in TOS_2, showing only a 6 °C difference and lower temperatures than the other two years. The 2009-TOS_2 grown plants experienced two consecutive days of 40 °C immediately after anthesis. Accumulated growing degree days (GDD) were calculated by summing daily degree days according to (Darroch and Baker, 1990). Daily degrees days were calculated as $T_n = (T_{max} + T_{min})/2 - T_b$, where T_{max} and T_{min} are the maximum and minimum daily temperatures, respectively, and T_b is the base temperature (5 °C). Post-anthesis average GDD (from 50% anthesis, DC65 to grain maturity) differed between seasons: 2007-TOS_1 (4.6 °C), 2007-TOS_2 (6.4 °C) 2008-TOS_1 (6.1 °C), 2008-TOS_2 (7.4 °C), 2009-TOS_1 (7.8 °C), and in 2009-TOS_2 (9.8 °C).

2.3. Plant material

To bread wheat cultivars (*T. aestivum* L.) cv. Yitpi and cv. Janz, widely grown in South-eastern Australia were sown. Yitpi is a moderate to high yielding cultivar with high tillering capacity and categorised as Australian hard (AH) quality wheat. Cultivar Yitpi has the pedigree Champlein/II 8156//Mengavi/Siete Cerros T 66/4/Champlein/II 8156//Heron/3/Mengavi/Siete Cerros T 66/5/Dagger/Molineux//2*RAC111/Insignia and was developed by Waite Institute, University of Adelaide, Australia. Cultivar Janz is reported as a high yielding cultivar under drier conditions, classified as Australian hard (AH) wheat and has good milling and dough mixing properties. Cultivar Janz has the pedigree 3 Ag3/4* Condor//Cook and was developed by Queensland Department of Primary Industries in 1989 (Brennan et al., 1991). Overall flour and dough quality of cv. Yitpi is very similar to Janz.

2.4. Grain sample preparation, analysis of grain physical properties and protein

Wheat spikes harvested at maturity were dried at room temperature and grains were separated and aspirated (Vacuum separator, Kimseed, Australia) to remove the remaining husk and dust, and stored at 20 °C in plastic containers to avoid moisture absorption until further analysis. Grain size classes (>2.00 mm, 2 mm–1.8 mm, 1.8 mm–1.5 mm, <1.5 mm) were analysed using a Sortimat (Type K3, Pfeuffer, Germany). Thousand-grain weight (TGW) was measured. Test weight (kg hl^{-1}) was determined using chrondrometer according to approved method 55–10 (AACC International, 2000). The grain hardness index and single grain diameter were measured

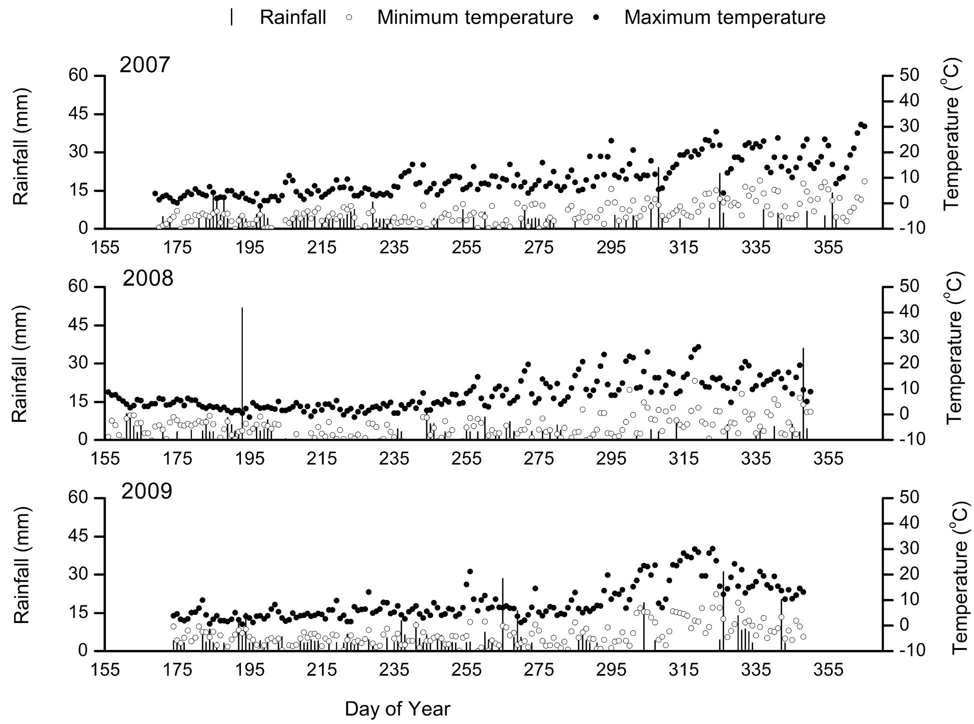

Fig. 1. Rainfall received, maximum and minimum temperature during pre-anthesis and post-anthesis of 2007-TOS_1, 2007-TOS_2, 2008-TOS_2, 2008-TOS_2, 2009-TOS_1 and 2009-TOS_2.

using the Single Kernel Characterization System (SKCS) (Perten Instruments North America, Springfield, IL, USA). Total protein content in whole-grain was determined by Near Infrared Reflectance Spectroscopy (NIR, Foss, Sweden) according to approved method 39–25 (AACC International, 2000) and was expressed on a grain dry weight basis.

2.5. Grain mineral analysis

Grain samples were analysed for mineral nutrient concentrations by inductively-coupled plasma atomic emission spectrometry (ICP–AES) (Applied Research Laboratories, 3580B, Switzerland) after digestion in concentrated nitric acid (HNO_3) following the procedure of (Zarcinas et al., 1987). Briefly, this entailed cold-digestion of 0.8 g of ground wheat kernels with ultrapure concentrated HNO_3 (9 ml) overnight in the tubes before heating. Hydrochloric acid (HCl) was added during two stages of digestion (0.4 ml initially + 0.2 ml at completion) under a controlled regime in an open vessel (75 ml borosilicate glass tube) and the final volume was made up to 25 ml. Digestion was conducted for 5–6 h with the maximum temperature not exceeding 140 °C. Mineral concentration (g kg^{-1} or mg kg^{-1}) was expressed on a grain dry weight basis.

2.6. Grain milling and flour mixing properties

Grain samples (30 g) were tempered for 24 h at 13.5% moisture and milled on a Quadrumat Junior Mill (Brabender, OHG Duisburg, Germany). Flour was separated from the bran through a 0.2 mm size sieve and flour yield was calculated [flour (g)/total grain (g) × 100]. Mixograph analysis was conducted using 10 g flour samples to obtain mixing properties (Reologica Instruments, Sweden) as described (Fernando et al., 2012b). Some of these reomixer parameters are directly related to the basic rheological aspects of the mixing properties (Bohlin, 2007). For example, "peak time" describes the mixing requirements, "peak height" is a measure of dough strength, "breakdown" reflects dough stability, and "peak width" measures extensibility (Bohlin, 2007).

2.7. Statistical analysis

Statistical differences among dependent variables were tested using the General Linear Models of MINITAB ver. 16 (Minitab, Sydney, NSW, Australia). A four way ANOVA was conducted testing effects of CO_2 ($e[CO_2]$ vs. $a[CO_2]$), growing environment conditions (TOS and year combined: 2007-TOS_1, 2007-TOS_2, 2008-TOS_1, 2008-TOS_2, 2009-TOS_1, 2009-TOS_2), water supply (rain-fed vs. irrigated) and cultivar (Janz vs. Yitpi) as well as their interactions on grain quality parameters followed by comparison of means with Student's paired t-test. Homogeneity of variances was checked with the Levene's test. Statistical effects were regarded as significant at $P \leq 0.05$.

3. Results

3.1. Grain physical properties

The effect of $e[CO_2]$ on TGW was dependent on the growing environment (Fig. 2a): At 2007-TOS_2 (by 5%), 2009-TOS_1 (by 5%) and 2009-TOS_2 (by 15%) TGW increased under $e[CO_2]$, but did not change at other conditions (Table 1, Fig. 2a). Similar to TGW, $e[CO_2]$ significantly increased the percentage of >2 mm size grains at 2009-TOS_1 (by 3%) and 2009-TOS_2 (by 6%) (Fig. 2b). TGW decreased at TOS_2 compared to TOS_1 for the 2007 and 2008 growing seasons, but increased at TOS_2 compared to TOS_1 in 2009 (Fig. 3a). Grain test weight response to $e[CO_2]$ was dependent on growing conditions (2c and 3b). Grain hardness index (GHI) increased significantly under $e[CO_2]$ at 2007-TOS_1 (by 4%) and 2009-TOS_2 (by 9%), while GHI was not changed under $e[CO_2]$ at other growing conditions (Table 1, Fig. 2d). In 2008 grain hardness increased in TOS_2 compared to TOS_1, which is a different pattern compared to the trends in TGW and test weight (Fig. 3d).

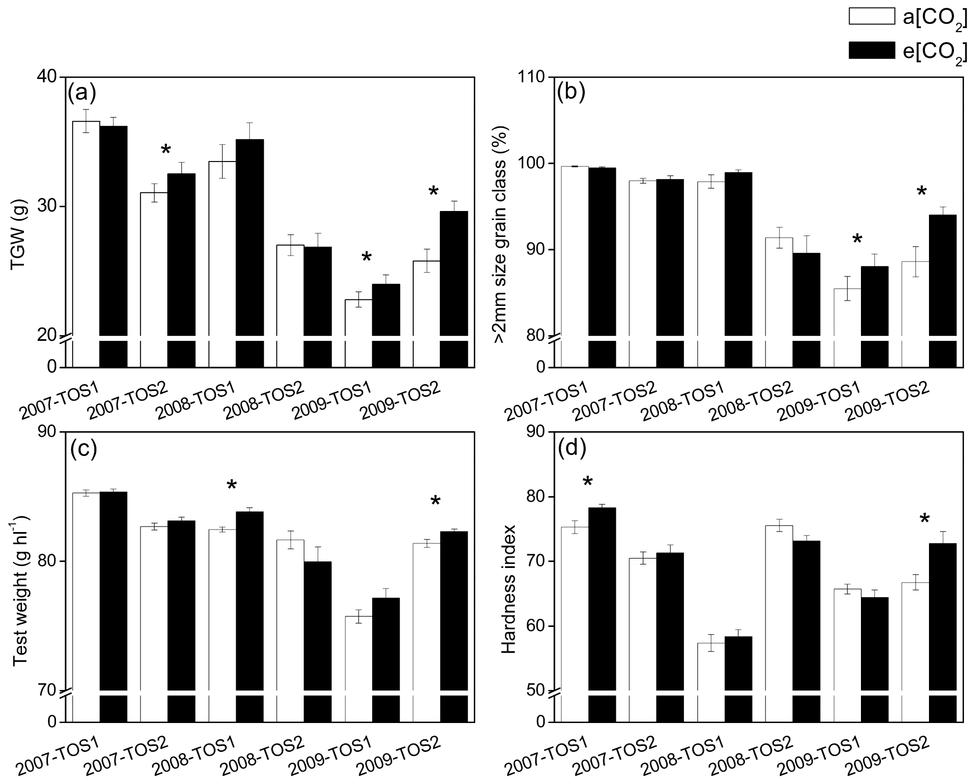

Fig. 2. Elevated [CO_2] effect on (a) TGW (thousand grain weight) (b) >2mm size grain weight class % (c) test weight (d) grain hardness index of two wheat (*Triticum aestivum* L.) cultivars (Janz and Yitpi) grown at six different environment conditions (2007-TOS_1, 2007-TOS_2, 2008-TOS_1, 2008-TOS_2, 2009-TOS_1, 2009-TOS_2). Data presented are the means from two water treatments (rain-fed only and supplemental irrigation). Each bar represent the mean ± Standard Error of n = 8 replicates. Ambient [CO_2] (~384 μmol mol^{-1}) (a[CO_2], open bars) and elevated [CO_2] (~550 μmol mol^{-1}) (e[CO_2], closed bars).

Table 1
A summary of results for TGW (Thousand-grain weight), grain protein, flour yield, GHI (grain hardness index) and some flour rheological properties of *Triticum aestivum* L. cv. Yitpi and cv. Janz grown under two [CO_2] (ambient and elevated [CO_2]; ~550 μmol mol^{-1}), two water levels (irrigated and rain-fed) and six environments (2007-TOS_1, 2007-TOS_2, 2008-TOS_1, 2008-TOS_2, 2009-TOS_1, 2009-TOS_2). Main ANOVA effect values are the mean of flour replicates.

	TGW	Grain protein % (db)	Flour yield % (db)	GHI	Bread volume (cm^3)	Peak time (min)	Peak height (au)	Peak width (au)	Breakdown (au)
a[CO_2]	29.0 ± 0.4	17 ± 0.2	71 ± 0.2	68 ± 0.8	175 ± 1	5.2 ± 0.05	6.0 ± 0.05	2.16 ± 0.01	0.85 ± 0.03
e[CO_2]	30.8 ± 0.4	16 ± 0.2	72 ± 0.3	70 ± 0.9	171 ± 2	5.0 ± 0.03	5.7 ± 0.08	2.19 ± 0.01	0.87 ± 0.02
2007-TOS_1	36.4 ± 0.6	15.1 ± 0.2	73.7 ± 0.2	77 ± 0.8	173 ± 4	4.6 ± 0.1	5.8 ± 0.07	2.12 ± 0.02	1.02 ± 0.03
2007-TOS_2	31.8 ± 0.6	15.2 ± 0.2	73.5 ± 0.2	71 ± 0.7	179 ± 3	5.3 ± 0.1	6.0 ± 0.05	2.21 ± 0.01	0.92 ± 0.04
2008-TOS_1	34.4 ± 01	17.4 ± 0.3	71.1 ± 0.4	58 ± 1	172 ± 4	5.5 ± 0.1	5.8 ± 0.09	2.17 ± 0.01	0.79 ± 0.02
2008-TOS_2	26.9 ± 0.7	17.8 ± 0.2	69.0 ± 0.4	74 ± 0.7	184 ± 3	5.6 ± 0.1	6.4 ± 0.08	2.26 ± 0.02	0.74 ± 0.05
2009-TOS_1	23.4 ± 0.5	16.2 ± 0.1	69.8 ± 1.5	65 ± 0.7	146 ± 3	5.3 ± 0.1	4.8 ± 0.09	2.0 ± 0.01	0.61 ± 0.05
2009-TOS_2	27.7 ± 0.7	17.5 ± 0.4	71.9 ± 1.5	70 ± 1.5	186 ± 2	4.5 ± 0.1	6.2 ± 0.05	2.28 ± 0.02	1.06 ± 0.04
Irrigated	29.6 ± 0.6	16.5 ± 0.2	71.8 ± 0.3	70 ± 0.8	172 ± 3	5.3 ± 0.06	5.8 ± 0.05	2.18 ± 0.02	0.85 ± 0.02
Rain-fed	30.6 ± 0.6	16.6 ± 0.1	71.2 ± 0.2	68 ± 0.9	173 ± 3	5.0 ± 0.02	5.9 ± 0.05	2.17 ± 0.02	0.87 ± 0.02
cv. Janz	27.9 ± 0.5	16.5 ± 0.2	71.4 ± 0.3	69 ± 0.8	185 ± 2	5.1 ± 0.1	6.4 ± 0.05	2.2 ± 0.02	0.95 ± 0.02
cv. Yitpi	32.3 ± 0.6	16.6 ± 0.2	71.6 ± 0.2	69 ± 0.7	162 ± 2	5.1 ± 0.1	5.3 ± 0.05	2.1 ± 0.02	0.77 ± 0.03
a[CO_2] × Janz	27.4 ± 0.1	17.1 ± 0.3	71.0 ± 0.2	68.5 ± 0.2	187 ± 3	5.3 ± 0.05	6.6 ± 0.09	2.2 ± 0.02	0.93 ± 0.04
a[CO_2] × Yitpi	31.6 ± 0.3	16.9 ± 0.2	71.7 ± 0.3	68.5 ± 0.5	164 ± 3	5.2 ± 0.06	5.4 ± 0.07	2.1 ± 0.02	0.77 ± 0.04
e[CO_2] × Janz	28.6 ± 0.4	16 ± 0.3	72.1 ± 0.1	70 ± 0.3	183 ± 3	4.9 ± 0.03	6.3 ± 0.06	2.3 ± 0.01	0.97 ± 0.03
e[CO_2] × Yitpi	32.9 ± 0.5	16.1 ± 0.2	71.4 ± 0.2	69 ± 0.4	160 ± 2	5.0 ± 0.1	5.3 ± 0.05	2.1 ± 0.02	0.77 ± 0.04
ANOVA results									
[CO_2]	**	***	ns	*	**	*	**	ns	ns
Environment	***	***	***	***	***	**	***	***	***
Water	*	ns	*	**	ns	**	ns	Ns	ns
Cultivar	***	ns	ns	ns	***	ns	***	***	***
[CO_2] × Envt	*	*	*	**	ns	ns	*	Ns	ns
[CO_2] × Cul	ns	ns	*	ns	ns	ns	ns	Ns	ns
Cul × Envt	*	***	*	**	***	***	***	***	**

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.005$.
ns $P \geq 0.05$.
Envt—environment.

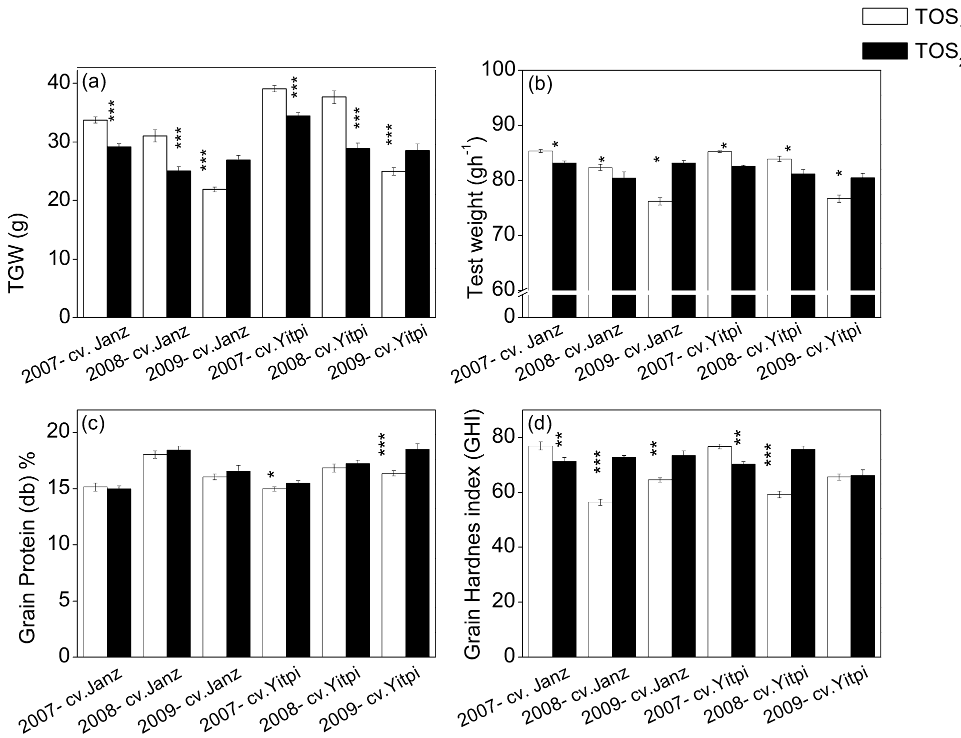

Fig. 3. Grain (a) TGW (thousand grain weight) (b) test weight (c) grain protein concentration (d) grain hardness index of two wheat (*Triticum aestivum* L.) cultivars (Janz and Yitpi) grown at six different environment conditions (2007-TOS_1, 2007-TOS_2, 2008-TOS_1, 2008-TOS_2, 2009-TOS_1, 2009-TOS_2). Data presented are the means from two [CO_2] treatments (ambient [CO_2] (~384 μmol mol^{-1}) and elevated [CO_2] (~550 μmol mol^{-1}) and two water treatments (rain-fed only and supplemental irrigation). Each bar represent the mean ± Standard Error of n = 8 replicates. Normal sowing time (TOS_1, open bars) and late sowing time (TOS_2, closed bars).

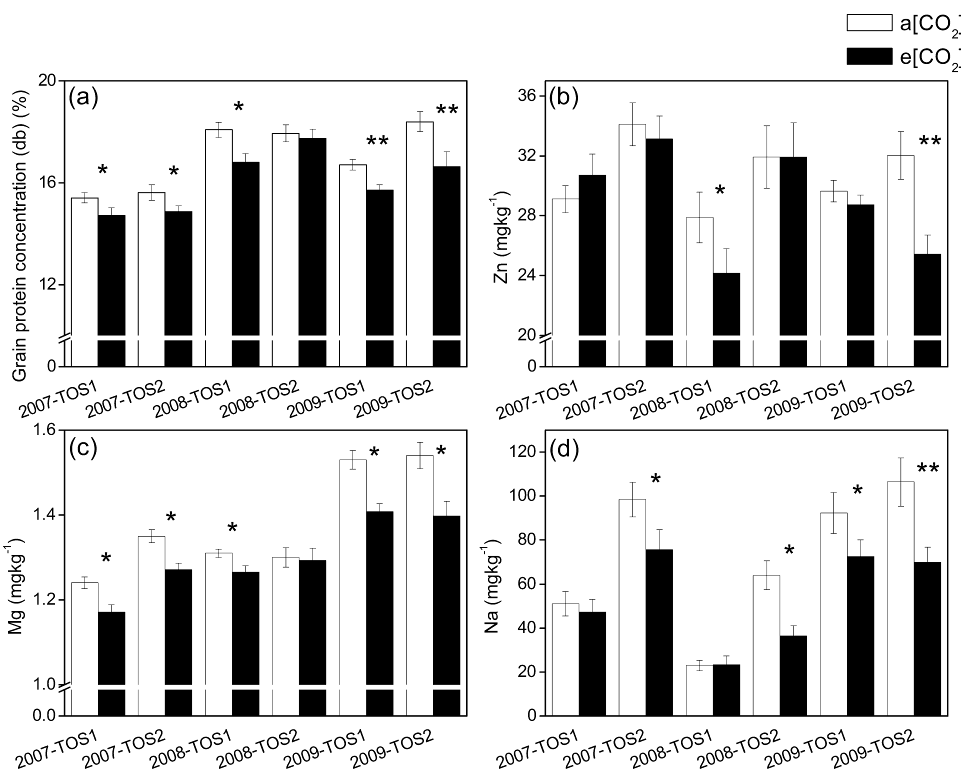

Fig. 4. Elevated [CO_2] effect on grain concentration of (a) protein (b) Zn (c) Mg and (d) Na of two wheat (*Triticum aestivum* L.) cultivars (Janz and Yitpi) grown at six different environment conditions (2007-TOS_1, 2007-TOS_2, 2008-TOS_1, 2008-TOS_2, 2009-TOS_1, 2009-TOS_2). Data presented are the means from two water treatments (rain-fed only and supplemental irrigation). Each bar represent the mean ± Standard Error of n = 8 replicates. Ambient [CO_2] (~384 μmol mol^{-1}) (a[CO_2], open bars) and elevated [CO_2] (~550 μmol mol^{-1}) (e[CO_2], closed bars).

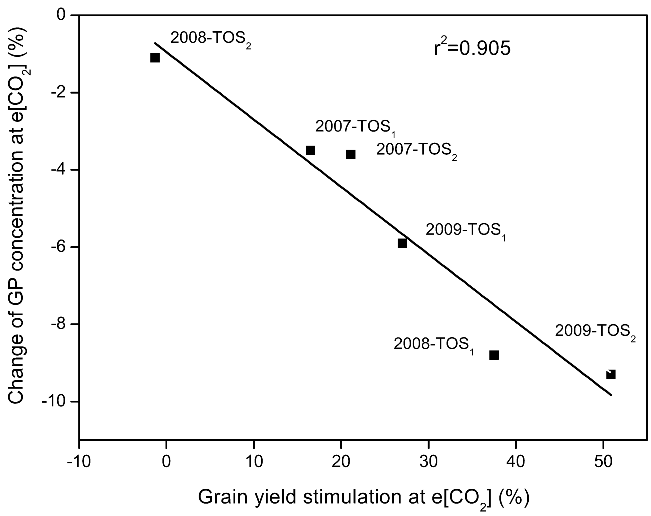

Fig. 5. Relationship between percentage decrease of grain protein concentration at elevated [CO_2] compared to the ambient [CO_2] and percentage yield stimulation at elevated [CO_2]. Relative effects were calculated from mean values of two water treatments (rain-fed only and supplemental irrigation) and two wheat (*Triticum aestivum* L.) cultivars (Janz and Yitpi).

3.2. Grain protein concentration

Grain protein concentrations decreased under e[CO_2] and the magnitude of the reductions were depended on the prevailing environmental condition; for example, 2007-TOS_1 (by 3.5%), 2007-TOS_2 (by 3.6%), 2008-TOS_1 (by 8.8%), 2009-TOS_1 (by 5.9%) and 2009-TOS_2 (by 9.3%) (Fig. 4a). No such reduction of grain protein concentrations was observed under 2008-TOS_2 growing conditions (Fig. 4a). For grain protein concentrations, cultivar Janz and Yitpi showed varied response to TOS treatment in each year (Fig. 3c). Grain protein concentration in cv. Yitpi increased at TOS_2 compared to respective TOS_1 and the increase was 2% and 8% in 2007 and 2009, respectively, (Fig. 3c). However, additional water supply had no significant effect on grain protein concentration (Table 1).

The relative decrease of grain protein at e[CO_2] (difference between e[CO_2] and a[CO_2] in %) was strongly correlated with grain yield stimulation by e[CO_2] in each environmental conditions ($r^2 = 0.905$) (Fig. 5). Further, we attempted to explain reduction of grain protein concentration at e[CO_2] using a number of environmental indices (Crop-water stress index, maximum temperature × evaporation, moisture index, GDD calculated for the growing season, for pre-anthesis and for post-anthesis), but none of these indices explained the differences in e[CO_2] effects on grain protein across the range of environmental conditions tested in this experiment. Grain protein concentrations of cultivars Yitpi and Janz were not statistically different (Table 1).

3.3. Grain mineral concentration

Concentrations of most of the grain mineral nutrients were significantly decreased under e[CO_2] (Fig. 6), irrespective of the cultivar. Grain Cu concentration decreased at e[CO_2] only in cv. Yitpi (3.4%), while grain P concentration decreased only in cv. Janz (3.2%) (Fig. 6c and f). Effects of e[CO_2] on grain Zn, Mg and Na concentrations were only dependent on the growing environment (Fig. 3b–d). Grain Zn concentration significantly decreased under e[CO_2] at 2008-TOS_1 (by 13%) and 2009-TOS_2 (by 20%), while grain Mg concentration decreased under all growing conditions except 2008-TOS_2 (Fig. 3b and c).

Relative reduction of grain S concentration at e[CO_2] was strongly correlated with relative grain yield stimulation of e[CO_2] ($r^2 = 0.9$) (Fig. 7). Relative decreased of other grain mineral nutrients (Fe, Zn, Mg, P, K) were also significantly correlated with yield stimulation of e[CO_2] (Fig. 7). However, the strength of the correlation was varied and dependent on the individual nutrients.

3.4. Flour rheological properties

Flour yield of cv. Janz increased at e[CO_2] whereas that of cv. Yitpi showed no differences (the [CO_2] × cultivar interaction was significant, Table 1). Flour rheological properties of both cultivars were significantly different under e[CO_2] (Table 1). Estimated bread volume from dough properties and dough mixing time were significantly decreased at e[CO_2], compared to a[CO_2] irrespective of growth environment or cultivar (Table 1). The three way interaction ([CO_2] × cultivar × environment) was significant for dough-mixing peak height, a measure of dough strength ($P = 0.01$) (Fig. 8). Peak height of cv. Janz was significantly lower at e[CO_2] in 2008-TOS_2 (by 13%) 2009-TOS_1 (by 7%) and peak height of cv. Yitpi was lower at e[CO_2] in 2008-TOS_1 (by 6%) and 2009-TOS_1 (by 8%) (Fig. 8). However, peak width (a measure of dough extensibility) and dough breakdown (a measure of dough stability) were not influenced by e[CO_2] in these cultivars (Table 1).

4. Discussion

Growing conditions of each year (2007-TOS_1, 2007-TOS_2, 2008-TOS_1, 2008-TOS_2, 2009-TOS_1 and 2009-TOS_2) were different based on total water received, average GDD during growing season, grain filling duration and growing season length. Despite their different genetic make-up, the two cultivars used showed similar responses to increasing [CO_2] for most physical and chemical quality characteristics of grains. Results from this study demonstrate that some of the grain physical, nutritional and flour rheological properties responses to e[CO_2] vary under different growing conditions of Mediterranean climate. Therefore, we attempted to explain whether these changes in one of major grain quality traits, namely grain protein concentration response to e[CO_2], can be explained by physiological or environmental indices, but found no clear relationship of relative effects of e[CO_2] with any of the environmental indices.

4.1. grain physical properties

Previous studies reported increased TGW (Li et al., 2001; Högy and Fangmeier, 2008), no changes in TGW (Kimball et al., 2001), or even decreased TGW at e[CO_2] (Högy et al., 2009). Our results, which show significant interactions of e[CO_2] and environment, indicate this discrepancy may be due to environmental growing conditions. In this experiment, TGW increased under e[CO_2], only when plants experienced heat or drought stress during the grain filling period, as in 2007-TOS_2, 2009-TOS_2 and 2009-TOS_1 (Fig. 2a). High temperature and/or drought stress decreases endosperm cell division, thus reduce cell number and starch granule size in the endosperm (Stone and Nicolas, 1998). It has also been reported that exposure to a temperature of 35 °C for one day during post-anthesis could cause reduction in kernel size which also contributes to 18–35% yield reduction (Alexander et al., 2010; Talukder et al., 2010). High CO_2 may avoid the reduction of cell number and/or starch granule size in the endosperm under stressful conditions, most likely associated with improved plant water relation, which could evade negative effects of drought and heat stress on grain size.

TGW is an important grain quality trait which determines milling quality and hence economic value of bread wheat cultivars

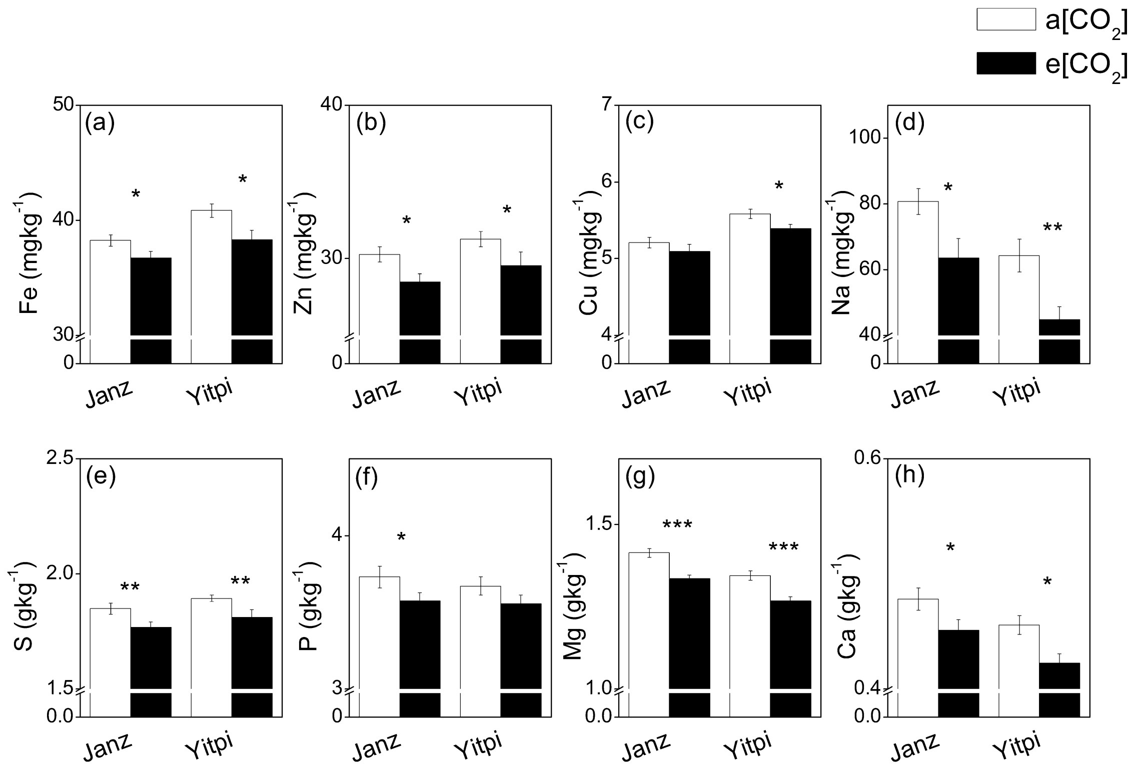

Fig. 6. Elevated [CO_2] effect on grain concentration of (a) Fe (b) Zn (c) Cu (d) Na (e) S (f) P (g) Mg and (h) Ca of wheat (*Triticum aestivum* L.) cv. Janz and Yitpi. Data presented are the means from two water treatments (rain-fed only and supplemental irrigation) and six growing environment conditions (2007-TOS_1, 2007-TOS_2, 2008-TOS_1, 2008-TOS_2, 2009-TOS_1, 2009-TOS_2). Each bar represent the mean ± Standard Error of $n = 48$ replicates. Ambient [CO_2] (~384 μmol mol^{-1}) (a[CO_2], open bars) and elevated [CO_2] (~550 μmol mol^{-1}) (e[CO_2], closed bars).

(Ohm et al., 1998). It has been previously reported that large grained bread wheat cultivars have better milling yield (Marshall et al., 1986). However, despite the increase in TGW at e[CO_2] for both cultivars, flour yield increased only in cv. Janz indicating that TGW alone does not determine changes in flour yield under e[CO_2] (Table 1). These results also suggest that genetic differences can play a role in determining milling quality traits under high [CO_2]. Regardless of TGW, the significant correlation of grain test weight and flour yield in both cultivars (data not shown) suggests that flour yield is not only determined by grain size (endosperm to surface area) but also test weight (grain density), as suggested previously (Schuler et al., 1995). Increased grain hardness index (GHI) at e[CO_2] suggests that e[CO_2] will be another environmental factor which determines the GHI, irrespective of cultivar. Grain hardness is used as a grading factor and a key determinant for classification of wheat and end product quality (Campbell et al., 1999; Morris, 2002; Pasha et al., 2010). Grain hardness has been shown to significantly impact on milling, baking and qualities of wheat (Bettge et al., 1995).

4.2. Grain protein

Results of our experiment suggest that e[CO_2] effect on grain protein concentration depends on growing environmental conditions (Fig. 4). We attempted to provide a better understanding of how grain protein responds to e[CO_2] by testing relationships with a number of environmental indices that were proposed to relate to grain protein: Crop-water stress index, maximum temperature × evaporation, moisture index and GDD). There was no clear relationship of the effects of e[CO_2] on grain protein with any of the tested indices. Grain protein can be inversely correlated with grain yield, a relationship most commonly found across cultivars, but sometimes also across growing environments, depending on what the main variables are (Barraclough et al., 2010). In our dataset, we did not find such a direct relationship between grain yield and grain N across the tested environments. Rather, a strong relationship exists between relative effects of e[CO_2] (as compared to a[CO_2] in the otherwise identical growing environment) on grain N and grain yield. This would be consistent with a "dilution effect" of N due to e[CO_2] stimulated accumulation of biomass, as suggested previously (Kuehny et al., 1991; Gifford et al., 2000), but may also point to a common underlying mechanism stimulating grain yields and restricting grain N under the low rainfall conditions tested here. In a review (Taub and Wang, 2008) proposed that the biomass a dilution effect does not completely account for the reduction of plant tissues N, even though it is the most frequently mentioned hypothesis (Taub and Wang, 2008). A recent meta-analysis also suggested that negative effects of e[CO_2] on grain protein accumulation can exist independently of yield stimulation (Pleijel and Uddling, 2012). However, in the present dataset which comprised a wide range of low rainfall growing conditions, the e[CO_2] effect on grain protein concentration for the tested wheat cultivars is closely correlated to yield stimulation by e[CO_2], as this relationship accounts for more than 90% of the data variance across environmental conditions. However, it is unclear why carbohydrates are seemingly translocated into the grains more efficiently than N (Taub and Wang, 2008). It is necessary to increase both shoot and root N acquisition to match the carbon gain to maintain the grain protein concentration in future e[CO_2] environment. Understanding of physiological role of shoot and root N acquisition can be exploited to reverse the decline in grain protein through breeding or management techniques.

4.3. Grain minerals

This experiment corroborated reports of reduction of micronutrients in wheat by several authors under FACE conditions:

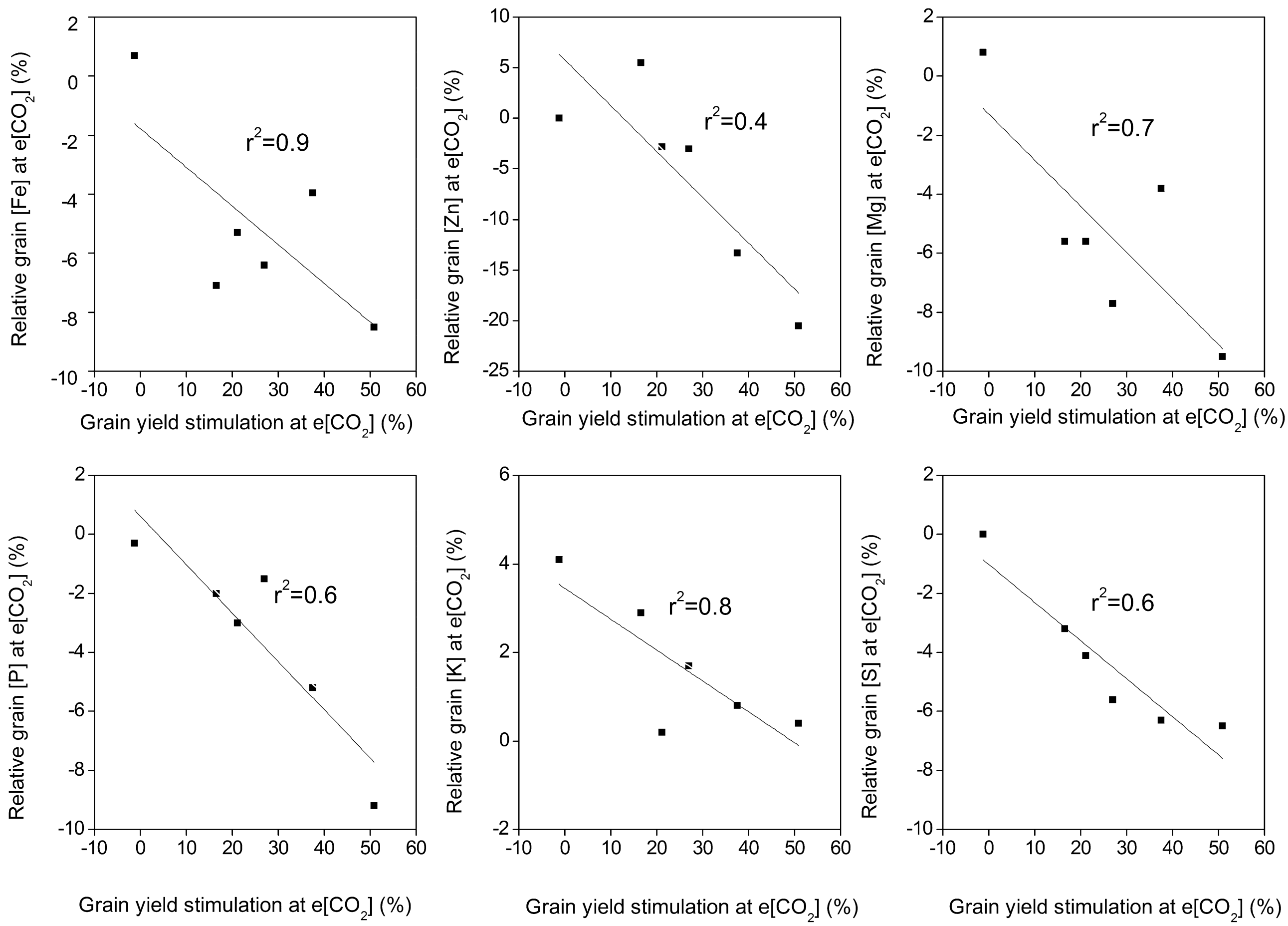

Fig. 7. Relationship between percentage decrease of grain S, Fe Zn Mg P and K concentration at elevated [CO_2] compared to the ambient [CO_2] and percentage yield stimulation at elevated [CO_2]. Relative effects were calculated from means values of two water treatments (rain-fed only and supplemental irrigation) and two wheat (*Triticum aestivum* L.) cultivars (Janz and Yitpi).

reduction of grain S (Erbs et al., 2010; Fernando et al., 2012a; Fernando et al., 2012b), Fe (Högy et al., 2009; Fernando et al., 2012a; Fernando et al., 2012b) P, Mg, Zn, Ca (Fernando et al., 2012b) and a decreasing trend of grain P, Mg, and Zn (Högy et al., 2009). In the current experiment, the magnitude of the reduction of some of macro and micronutrient concentrations under *e*[CO_2] were influenced by environmental conditions, particularly for Zn, Mg and Na concentrations (Fig. 3b–d). Across the literature, reduction of micronutrient concentration at *e*[CO_2] is varied. As an example, (Fangmeier et al., 1997) report impacts of *e*[CO_2] under low N fertilization on wheat grains elemental composition with decreases in Ca, S, and Fe but also an increase in Zn. In another study (Erbs et al., 2010) report *e*[CO_2] caused a reduction of grain Ca and Zn under low N fertilization and Fe tended to decrease, while Cu concentrations

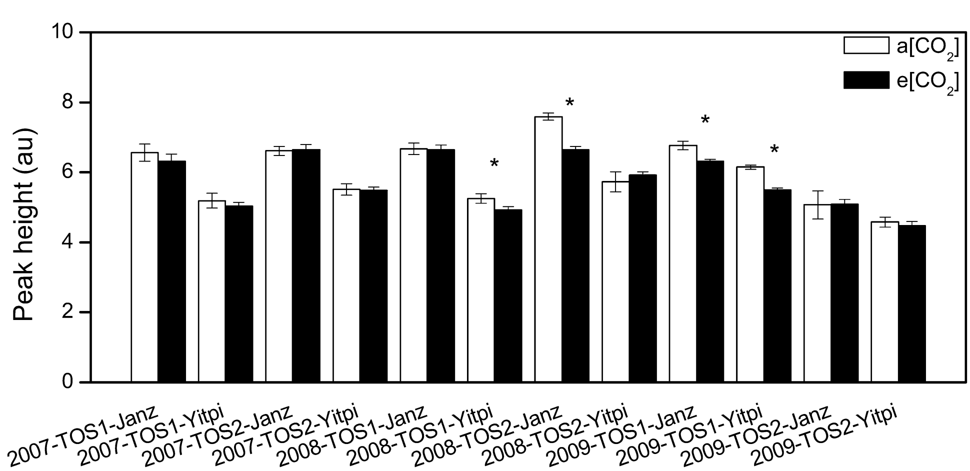

Fig. 8. Elevated [CO_2] effect on peak height of wheat (*Triticum aestivum* L.) cv. Janz and Yitpi grown at six growing environment conditions (2007-TOS_1, 2007-TOS_2, 2008-TOS_1, 2008-TOS_2, 2009-TOS_1, 2009-TOS_2). Data presented are the means from two water treatments (rain-fed only and supplemental irrigation). Each bar represent the mean ± Standard Error of $n = 4$ replicates. Ambient [CO_2] (~384 μmol mol^{-1}) (*a*[CO_2], open bars) and elevated [CO_2] (~550 μmol mol^{-1}) (*e*[CO_2], closed bars).

tended to be increased. Current experimental results suggest that these heterogeneous results observed in previous $e[CO_2]$ studies may be partly due to prevailing environmental conditions in each experiment.

Relative grain yield increase and relative reduction of grain S concentration at $e[CO_2]$ was significantly correlated, with yield increase accounting for more than 90% of the variation in S decrease ($r^2 = 0.9$) (Fig. 7). This relationship is similar to the one linking yield increase and grain protein concentration. In the current experiment, grain protein and grain S concentration were also highly correlated ($r^2 = 0.9$) and this is a relationship commonly observed independent of $e[CO_2]$ (Zhao et al., 1999). Because of the strong inter-dependence of N and S metabolism (Dijkshoorn and Van Wijk, 1967), it is not surprising that wheat grains decrease grain S concentration in association with decreasing protein concentrations, which helps to maintain a relatively constant ratio of organic N to organic S. In wheat grains approximately 80% of S is invested in proteins and the N/*S* ratio is around 15/1 (Zhao et al., 1999). Relative grain yield stimulation and relative reduction of grain Fe, Zn, Mg, P and K concentration at $e[CO_2]$ were also significantly correlated (Fig. 7), but the relationship explained only 50–70% of the variation confirming these changes; perhaps pointing towards a greater contribution by mechanisms other than biomass dilution. Among all tested growing conditions, the highest relative yield stimulation and highest relative reduction of grain S, Fe, Zn, Mg and P concentrations in $e[CO_2]$ treatments were reported at 2009-TOS_2. However, highest absolute values of grain mineral concentration was reported in $a[CO_2]$ of 2009-TOS_2, and this may also be associated with the lower grain yield reported under 2009-TOS_2 conditions.

Current literature does not contain sufficient data to understand inter-dependency of N metabolism and most of other macro and micronutrient metabolism (Waters et al., 2009). Therefore, further studies are required to explore the mechanisms and develop adaptive interventions to improve grain micronutrient concentrations under $e[CO_2]$.

4.4. Flour rheological properties

In this experiment, bread volume (estimated from flour rheological properties) and dough strength decreased under $e[CO_2]$ and reduction of bread volume was common under all the tested environmental conditions. Such reduction of estimated bread volume under $e[CO_2]$ is reported elsewhere, and concerns about lower end-product quality under high $[CO_2]$ have been raised (Högy et al., 2009; Fernando et al., 2012b). In this paper, we provide furthermore evidence that response of some flour rheological properties to $e[CO_2]$ were modified by growing conditions (significant $[CO_2] \times$ environment interaction for peak height, a surrogate for dough strength). On the other hand, we had little evidence of cultivar differences in the $e[CO_2]$ response of dough properties, as both cultivars showed similar responses (no $[CO_2] \times$ cultivar interaction, for the flour rheological properties except peak height).

From tested growing environment conditions, bread volume was greatest for both cultivars under 2009-TOS_2 growing conditions, when yield was lowest. However, under similar conditions, the highest grain protein and N concentration was observed for cv. Yitpi but it was not the case for cv. Janz (highest grain protein and N concentration for Janz observed in 2008-TOS_2) suggesting that highest bread volume was not only determined by the protein quantity. It has been reported that irrespective of grain protein content, gluten protein composition also associated with bread making properties (Shewry and Halford, 2002). Changes in protein quality under $e[CO_2]$ is predominantly due to a reduction of gluten fractions (Wieser et al., 2008; Högy et al., 2009). These findings suggest that responses of flour rheological property to $e[CO_2]$ are also associated with modification of protein composition (which we did not investigate here) and not only protein concentration at $e[CO_2]$.

5. Conclusions

Elevated $[CO_2]$ had a variety of effects on grain properties: Most grain mineral concentrations decreased, and rheological properties changed in a way that suggested lower product quality. Effects of $e[CO_2]$ on grain physical properties, protein, some minerals (Zn, Mg, Na) and flour rheological properties varied with environmental conditions, but not between the investigated cultivars. We could however not identify a single environmental index that would explain changes of grain quality responses to $e[CO_2]$ among different growing conditions, yet a strong relationship between the relative effects of $e[CO_2]$ on grain yield and grain protein suggested contribution of biomass dilution, i. e. greater grain biomass without similar increases in N translocation into grains. Whether biomass dilution is the only mechanism, or whether it is caused by reduced availability of N for translocation or some inhibition to translocation cannot be determined with the present experiment.

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