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Title

Elevated CO_2 impact on common wheat (*Triticum aestivum* L.) yield, wholemeal quality and sanitary risk.

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Abstract

The rising atmospheric CO_2, concentration is expected to exert a strong impact on crop production, enhancing crop growth, but threatening food security and safety. In this study, an improver wheat, a hybrid and its parents were grown at elevated CO_2, e[CO_2], in open field and their yield and rheological, nutritional and sanitary quality were assessed on wholemeal. An increase in both biomass (+19%) and grain yield (+16%) under e[CO_2] was observed, accompanied by a decrease in protein content (-1%) and a consistent reduction in dough strength for all cultivars. e[CO2] did not result in significant changes in phenolic acid content and composition, whereas it produced a significant increase in the deoxynivalenol content. All these findings highlight the need, in the upcoming wheat cropping systems, for agronomic practices and cultivar selection able to guarantee higher N responsiveness and minimization of sanitary risk.

KEYWORDS

Common wheat; carbon dioxide; FACE; grain yield; grain protein content; Glutopeak; antioxidant compounds; mycotoxins.

INTRODUCTION

The release of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) due to human activities is one of the major causes of climatic changes, with impacts on food security and safety for their effects on agricultural crops. The greenhouse gases are responsible for the increase in temperature, which will lead to higher drought stress for crops due to increased evapotranspiration combined with a more uneven distribution of rainfall events.[1] Contrariwise, the expected increasing levels of atmospheric CO_2 will increase photosynthesis, leading to a productive advantage for crops that exhibit C3 photosynthetic metabolism, such as wheat and other small grain cereals. Several field experiments on wheat carried out in different growing areas through the application of free-air CO_2 enrichment (FACE) led to a yield increase by 26% with an average CO_2 level of 602 ppm,[2] due mainly to the increase in grain number per unit surface area rather than to the increase in kernel weight. Although many experiments have highlighted the wheat productive response to elevated CO_2, the associated physiological mechanisms,[3,4] and the interactions with crop practices[5-7] and growing areas,[2,8] few FACE studies have considered the effects on wheat cultivars (cv) with specific productive and qualitative traits. In this context, since tillering was reported to be the most important factor influencing yield at elevated level of CO_2,[4] it would be interesting to investigate the response of cultivars with contrasting tillering capacity. Wheat hybrids, whose cultivation is expected to quickly increase in the near future, are planted at lower seeding rate compared to conventional varieties, but can overcome the apparent initial disadvantage by means of a higher tillering capacity.[9] Until now, only Yavad et al.,[10] have studied both a hybrid and a conventional wheat cultivar in a FACE experiment. However, this study was carried out in a sub-tropical climate, whereas no information is reported on the effect of FACE treatment on hybrid cultivars in temperate growing areas.

In addition to the yield effects, several FACE studies have highlighted the negative impact of elevated CO_2 on wheat grain protein content (GPC) and baking quality.[5,11,12] This negative effect could be even worse for high protein common wheat, which is classified, according to the specific

classification terminology of different countries, as improver wheat (in Italy), excellent or class E wheat (in France and Germany), or Hard Red wheat (in the U.S.A). Indeed, the optimum end-use quality and market price of these wheat categories are closely related to the protein content and to the dough rheological traits. ~~In addition, the effects of elevated CO_2 on bread-making quality of whole-grain flours is worthy of interest, since fibres negatively interact with the gluten network and a high gluten strength is necessary to assure adequate performance in production of bakery products.[13] This aspect needs to be considered also in view of the increased consumption of whole-grain bakery products in developed countries due to their high nutritional value.[14] The regular consumption of whole-grain foods helps regulate the blood glucose level and could contribute to reduce coronary heart diseases and several forms of cancer and to provide vitamins of B group and minerals. Moreover, several phytochemicals in wholemeal have been reported to exert antioxidant and anti-inflammatory effects.[15]~~ Several FACE experiments have highlighted the negative impact of an increased CO_2 concentration on macro-, meso- and microelements, and essential amminoacids in wheat grains,[12,10,16] resulting in a strong impoverishment of the diet for these nutrients. However, ~~to the best of the authors' knowledge,~~ no data are currently available on the effect of elevated CO_2 on bioactive compounds such as phenolic acids and carotenoids, which are responsible for the total antioxidant activity of wholemeal, and result in numerous beneficial effects for the consumers.[17]

As opposed to these beneficial effects, the consumption of whole-grain products is associated with a potential high intake of contaminants, such as pesticides, heavy metals and mycotoxins, all of which are more concentrated in the external kernel layers.[18] Among mycotoxins, deoxynivalenol (DON) and its modified forms (DON-3-G, 3-ADON, 15-ADON), that belong to type-B trichothecenes, are frequently detected at harvest in wheat grain in temperate growing areas.[19] These compounds are toxic for humans and animals and maximum admissible levels have been set up in several countries worldwide; therefore, it is useful to check the potential impact of elevated CO_2 on the mycotoxin contamination of cereal grains under open field conditions.

The objective was to provide further data on the response of common wheat in temperate areas under the future climatic scenarios, in order to suggest some main objectives for wheat breeding.

MATERIALS AND METHODS

Varieties studied

The winter wheat (*Triticum aestivum spp. aestivum* L.) cv Bologna (S.I.S. Società Italiana Sementi, San Lazzaro di Savena, BO, Italy) was studied in three experimental years. According to Italian bread wheat classification system,[20] it is an improver wheat. ~~and the most cultivated cultivar within the high protein supply chain in Italy. Characterized by medium-early precocity, it can be considered as a model of winter wheat cultivation in warm temperate and Mediterranean regions~~. In the third experimental year, the hybrid Hystar (Saaten-Union, Estrées St Denis, France, marketed in Italy by RV Venturoli, Pianoro, Italy) and its parents Apache (father, Limagrain, Saint Beauzire, France) and QH529 (mother, obtained from RV Venturoli, Pianoro, Italy) were also studied. All these cultivars are classified as ordinary bread-making wheat. ~~Apache, a medium-late cultivar with high productivity, is one of the major French wheat varieties grown in several European coun~~tries. ~~The hybrid Hystar is characterized by a precocity similar to that of its parental lines.~~

Experimental set up

Wheat plants were grown within the FACE facility of the Research Centre for Genomics and Bioinformatics (CREA-GB) at Fiorenzuola d'Arda (44.9278N, 9.8938E), Italy. The site is situated in the Po Valley, at an elevation of 70 m.a.s.l. and has a warm continental climate, classified as Cfa (Humid subtropical climate) in the Koeppen Geiger climate classification, i.e., temperate climate without dry seasons and hot summers. The soil is alkaline (pH 8.09), with total carbonate, 10.19%; total C, 28.1 g kg^{-1}; inorganic C, 12.22 g kg^{-1}; organic C, 15.9 g kg^{-1}; organic matter, 2.74%; total N, 0.10%; C:N ratio 15.6; P_2O_5, 21.7 mg kg^{-1}; K_2O 190 mg kg^{-1}; cation exchange capacity, 6.85 cmol(+) kg^{-1}. Two different experiments have been carried out in order to analyse the effect of elevated carbon dioxide (e[CO_2]) on wheat yield and qualitative traits compared to current ambient (a[CO_2]). With

115 the first experiment cv Bologna was cultivated in 3 different years (Y1, 2011-12; Y2, 2012-13, Y3,
116 2015-16). The second experiment compares the 4 previously reported cultivars in Y3, according to a
117 full factorial design. The experimental units for each cultivar were plots sized 2.2 m by 1.36 m. The
118 FACE treatment, with the e[CO_2] target value set at 570 ppm, was replicated in four octagons
119 inscribed in circles of 14 m diameter. The a[CO_2] controls were replicated four times in octagons
120 without FACE at ambient CO_2 (404 ppm). For sowing, start of fumigation and harvest dates refer to
121 Table 1. The FACE treatment was stopped when leaves were senescent and interrupted when the
122 plots were covered with snow. The agronomic technique applied in the experimental trials was in
123 accordance to the conventional farm management system in force in the experimental area. Briefly,
124 the preceding crops are detailed in table 1. The field was ploughed each year, incorporating the debris
125 in the soil, and this was followed by disk harrowing to prepare a proper seedbed. Planting was
126 conducted in 12 cm wide rows at a seeding rate of 350 seeds m^{-2}, except for the hybrid cultivar and
127 its parents, planted with a seeding rate of 200 seeds m^{-2}. The field experiment received 30, 13 and 25
128 kg ha^{-1} of N, P and K, respectively at pre-seeding in Y1 and Y2. In Y3 pre-seeding fertilization was
129 done with 45, 20 and 37 kg ha^{-1} of N, P and K, respectively. In spring, N was applied with two top-
130 dressings at the tillering and stem elongation stages, as ammonium nitrate in Y1 and Y2, while in Y3
131 ammonium nitrate and ammonium sulfate were used for the first and second top-dressing,
132 respectively. The total amount of nitrogen applied with the fertilizers was 149, 234 and 183 kg N ha^{-1}
133 in Y1, Y2 and Y3, respectively. No fungicide was applied at flowering to control Fusarium head
134 blight (FHB). Air temperature, precipitation, relative humidity, and global radiation were measured
135 and recorded at 10-min intervals with an automatic meteorological station located within the field site
136 of the FACE experiment at Fiorenzuola d'Arda.

137 *Morphological and productive traits*

138 Average plant height per plot was measured during maturation. Ear density, i.e. number of ears per
139 meter square was counted in the field (Y1) or determined on a 1.5 m linear meter harvest (Y2 and

Y3). After harvesting by plot combine harvester (Nurserymaster, Wintersteiger, Austria) in Y1 or manually in Y2 and Y3, grains were threshed with the plot combine harvester and aboveground dry biomass, grain yield, and harvest index were determined. Biomass data are reported at dry mass basis. For conversion to grain biomass, usually reported in production statistics at 13% humidity (FAO standards), the grain yield data needs to be multiplied by 1.149425.

Grain quality characterization

Test weight (TW) was determined by means of a Dickey-John GAC2000 grain analysis meter (Dickey-John Corp. Auburn, IL, USA), according to the supplied programme. Thousand kernel weight (TKW) was determined on two 100-kernel sets for each sample using an electronic balance. Grain samples (500 g) from each plot were ground to wholemeal using a 1-mm-sieve Cyclotec mill (Foss Tecator AB, Höganäs, Sweden). Protein content (PC) (N * 5.7, dry weight, AACC 39–1050),[21] and hardness (AACC 39–7050)[21] were determined by a NIR System Model 6500 (FOSS NIRSystems, Laurel, MD). The moisture content, determined in order to express all contents of bioactive compounds and mycotoxins on a dry weight (dw) basis, was obtained by oven-drying at 105 °C for 24 h.

Technological characterization

The SDS sedimentation volume (SSV) was determined according to Preston et al.[22]

The rheological properties were evaluated on wholemeal using GlutoPeak (Brabender GmbH and Co KG, Duisburg, Germany), according to the method reported by Marti et al.[23] Briefly, flour (9 g) was dispersed in distilled water (10 ml), scaling both water and flour weight on a 14% flour moisture basis in order to keep the liquid-to-solid ratio constant. During the test, the sample and water temperature were maintained at 35 °C by circulating water through the jacketed sample cup. The paddle was set to rotate at 3000 rpm and each test was run for 500 s. Curves were elaborated using the software provided with the instrument (Brabender GlutoPeak v 2.1.2) and the following indices were considered: i) Maximum Torque, expressed in Brabender Equivalents (BE) - corresponding to the

peak that occurs when gluten aggregates; ii) Peak Maximum Time (PMT), expressed in seconds, which corresponds to the peak torque time; iii) aggregation energy, expressed as the GlutoPeak Equivalent (GPE), which corresponds to the area under the portion of the curve 15 s before and 15 s after the peak. Each sample was analysed in duplicate.

Chemical analyses

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-*tert*-butyl-4-methylphenol (BHT, ≥99.0%), ethanol (CHROMASOLV®, 99.8%), ethylacetate (CHROMASOLV®, 99.8%), hexane (CHROMASOLV®, 97.0%), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%), hydrochloric acid (HCl, 37.0%), methanol (CHROMASOLV®, 99.9%), potassium hydroxide (KOH, 90.0%), sodium hydroxide (NaOH, ≥98.0%), *tert*-butyl methyl ether (MTBE, CHROMASOLV®, 99.9%), *trans*-β-Apo-8'-carotenal, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) and phenolic acid standards (caffeic acid ≥98%, *p*-coumaric acid ≥98%, *t*-ferulic acid ≥99%, *p*-hydroxybenzoic acid ≥99%, sinapic acid ≥98%, syringic acid ≥95% and vanillic acid ≥97%) were purchased from Sigma-Aldrich (St. Louis, Missouri, US). Xanthophylls standards (lutein ≥95% and zeaxanthin ≥98%) were purchased from Extrasynthese (Lyon, France).

Methanol (CH_3OH), acetonitrile (CH_3CN) and water (H_2O) were LC gradient grade or LC-MS grade, depending on their use during the extraction or the analytical phases, and were purchased from VWR (Milan, Italy). Glacial acetic acid (CH_3COOH) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Mycotoxin standards were dissolved in acetonitrile (CH_3CN), if not stated otherwise. Stock solutions of 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3-G) in CH_3CN/H2O 50/50, v/v, nivalenol (NIV) was purchased from Romer Labs Diagnostic GmbH (Tulln, Austria). Two composite standard working solutions were prepared by dissolving appropriate volumes of each analyte in a dilution phase mixture, CH_3CN/H_2O 50/50, v/v as follows: the first working solution contained DON

and DON-3-G, while the second one contained 3-ADON, 15-ADON and NIV. These two working solutions were then mixed in appropriate volumes and dissolved in $CH_3CN/H_2O/CH_3COOH$ 49.5/49.5/1 v/v/v in order to prepare the working solutions for the calibration. All the solutions were stored at -20°C in amber glass vials and were brought to room temperature before use.

Extraction of the soluble (SPAs) and cell wall-bound phenolic acids (CWBPAs) and quantification by means of RP-HPLC/DAD

The extraction and quantification of soluble (free and conjugated) and cell wall-bound phenolic acids was performed according to the procedure proposed by Nicoletti, et al.,[24] with some modifications as reported by Giordano, et al.[25] Quantifications were performed as reported in Giordano, et al.[26]

Extraction of xanthophylls and quantification by means of RP-HPLC/DAD

The extraction and quantification of xanthophylls was performed as previously reported by Giordano et al.[25]; *trans*-β-Apo-8'-carotenal was used as internal standard to ensure that losses due to the extraction method were accounted for.

Determination of DPPH radical scavenging activity (AC_{DPPH})

DPPH radical scavenging activity (QUENCHER procedure – direct measurement on solid sample) was carried out as reported by Giordano et al.[26] The DPPH radical scavenging activity was expressed as mmol of Trolox equivalents/kg of sample (dw).

Multi-mycotoxin LC-MS/MS analysis

The extraction and sample preparation were performed by applying the dilute-and-shoot method reported by Scarpino et al.[27] Briefly, 5 g of wheat flour was extracted by mechanical shaking at 300 rpm for 90 min with 20 mL of $CH_3CN/H_2O/CH_3COOH$ (79/20/1, v/v/v). The extract was filtered through Whatman® grade 1 filters (Brentford, UK) and subjected to dilution with the same volume of diluting solution ($CH_3CN/H_2O/CH3COOH$ 20/79/1, v/v/v). The diluted extract was vortexed and filtered through 15 mm diameter, 0.2 μm regenerated cellulose (RC) syringe filters (Phenex-RC,

214 Phenomenex, Torrance, CA, USA) and 20 µL were analyzed without any further pre-treatment. LC-
215 MS/MS analysis was carried out on a Varian 310 triple quadrupole (TQ) mass spectrometer (Varian,
216 Italy), equipped with an electrospray ionization (ESI) source, a 212 LC pump, a ProStar 410
217 AutoSampler and dedicated software. Liquid chromatography (LC) separation was performed on a
218 Gemini-NX C_{18} 100 × 2.0 mm i.d., 3 µm particle size, 110 Å equipped with a C_{18} 4 × 2 mm security
219 guard cartridge column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of two
220 eluents: water (eluent A) and methanol (eluent B), both of which were acidified with 0.1% v/v
221 CH_3COOH delivered at 200 µL min^{-1}. The chromatographic conditions of the runs and the mass
222 spectrometric parameters for the negative and positive ionization mode acquisitions were described
223 in detail by Scarpino et al.[27] The performance parameters of the method were reported for all the
224 analyzed mycotoxins by Scarpino et al.[27]

225 *Statistical analysis*

226 Normal distribution and homogeneity of variances were verified by performing the Kolmogorov–
227 Smirnov normality test and the Levene test, respectively. The analysis of variance (ANOVA) was
228 conducted separately for each experiment in order to evaluate the effect of elevated carbon dioxide
229 on grain yield, yield traits and qualitative traits on wholemeal using a completely randomized block
230 design, in which the concentration of carbon dioxide and the year (experiment 1) and the
231 concentration of carbon dioxide and the cultivar (experiment 2) were the independent variables.
232 SPSS, Version 25.0 statistical package (SPSS Inc., Chicago), was used for the statistical analysis.

233

234 **RESULTS**

235 *Meteorological conditions*

236 The three growing seasons were characterized by contrasting thermal and hydrologic conditions
237 (Table 1). The crops grown in Y1 experienced the coolest winter with the highest number of frost
238 days and minimum temperatures down to -18.7 °C. However, during the nights with more severe

239 frost the plants were protected by snow cover. With 54 frost days and minimum temperatures not
240 decreasing below -7.4 °C, Y3 was characterized by the warmest winter. Thermal conditions in Y2
241 were intermediate, with minimum temperatures reaching -11.2 °C, and plants were protected by snow
242 cover during periods with more intense frost. In particular, three snow cover periods lasting for
243 several days occurred during December, January and February. Growing seasons in Y1, Y2, and Y3
244 were moderately wet, extremely wet, and relatively dry, respectively, with potential
245 evapotranspiration not exceeding whole season precipitation in Y1 and Y2, while virtually identical
246 to precipitation in Y3.

247 *Grain yield and yield parameters*

248 Aboveground biomass production and grain yield of cv Bologna increased in e[CO_2] in the three
249 years of experiment 1 (Table 2). FACE X year interaction resulted significant for aboveground
250 biomass: the response ratios (trait in e[CO_2] / trait in a[CO_2]), were 1.230, 1.267, and 1.072 in Y1,
251 Y2 and Y3, respectively. Conversely, the interaction was not significant for grain yield and ear
252 density for which the average response ratio was 1.163 and 1.203, respectively. The increase in grain
253 yield and aboveground biomass is due to the higher plant density recorded in e[CO_2]. Instead, TKW
254 significantly decreased in e[CO_2], with an average response ratios of 0.970.

255 Plant height slightly increased under e[CO_2] and was lower in Y2 than Y1 and Y3. The stimulation
256 of height growth was most marked in Y2 with a response ratio of 1.06. Harvest index did not change
257 in response to e[CO_2], with a slight, non-significant decreasing trend. The length of the vegetative
258 growth period, i.e. the number of days from sowing to heading slightly increased, but non
259 significantly, by about 1 day in response to e[CO_2]. It was 201 to 202 days in Y1 and Y2, while it
260 was much shorter in Y3 (175 days). Aboveground biomass production, grain yield, harvest index and
261 plant height increased in e[CO_2] relative to a[CO_2] in experiment 2, while TKW significantly
262 decreased (Table 2). However the response to e[CO_2] varied between cultivars. The increase in yield
263 was significant in Apache, QH529 and Hystar (Figure 1). The response ratios for grain yield were

1.311, 1.285, 1.312 and 1.071, in Apache, Hystar, QH529 and Bologna, respectively. The ear density increased by 12.7%, 10.3%, 6.3% and 16.6% in Apache, Hystar, QH529 and Bologna, respectively.*Grain and wholegrain flour quality*

TW and grain hardness were not affected by FACE treatment in both experiments (Table 3). Conversely, GPC significantly decreased under e[CO_2] compared to a[CO_2] (-1.1 and -0.9 percentage points on average in experiment 1 and 2, respectively). Although the experiments were carried out in years and with cultivars characterized by different GPC, no significant interaction between CO_2 concentration and the considered factors was observed. SSV was not affected by FACE treatment in both experiments. Contrarily, CO_2 concentration significantly affected the gluten aggregation properties of cv Bologna (experiment 1). In particular, e[CO_2] promoted an increase in peak maximum time (PMT), indicating slower aggregation, and a decrease in both maximum torque (-12.5%) and aggregation energy (-10.7%), suggesting gluten weakening. As expected, also the year affected the gluten aggregation properties of wheat, with Y3, characterized by the lowest grain yield, being significantly different from the others. Indeed, Y3 exhibited a lower aggregation time and the highest maximum torque and energy, suggesting the highest gluten strength, confirmed also by the highest SSV value (71 mL). No significant interaction between CO_2 concentration and year was observed for gluten aggregation properties. The effect of e[CO_2] on gluten aggregation kinetics was confirmed in the 2nd experiment. QH529 and Hystar exhibited a similar GlutoPeak® profile with an intermediate behaviour between Bologna and Apache. The interaction between FACE treatment and cultivars was never significant, resulting in a similar impact on wholemeal rheological properties of wheat genotypes belonging to different qualitative market classes in both CO_2 treatments.

Bioactive compound content

In the 1st experiment, carried out on cv Bologna, the CO_2 concentration did not significantly affect the content of bioactive compounds, except for a slight but significant reduction of both zeaxanthin and antioxidant capacity in e[CO_2] (Table 4). The concentration of antioxidant compounds of the

289 wholegrain flour was significantly different between years. A significantly higher concentration of
290 soluble sinapic acid was observed in Y3. Similarly, SPAs were the highest in Y3, but no significant
291 difference was observed among the three growing seasons. An opposite trend was observed for
292 CWBPAs and bound ferulic acid. Both lutein and zeaxanthin resulted the lowest in Y2. A significant
293 interaction between FACE treatment and year was observed for SPAs, soluble sinapic acid, lutein,
294 zeaxanthin and for the antioxidant capacity (Figure 2). Zeaxanthin and AC showed significant
295 differences between a[CO_2] and e[CO_2] only in Y1. Otherwise, lutein decreased in Y1, while it
296 increased significantly both in Y2 and Y3. SPAs decreased in Y1 and increased in Y2, while no
297 significant change was observed in Y3. In the 2nd experiment, highly significant differences were
298 observed among the cultivars for all compounds. Lutein was the only compound significantly affected
299 by the FACE treatment: a higher concentration was observed in e[CO_2], but the increase amounted
300 to only 9%. The interaction between FACE treatment and cultivars was never significant. Regardless
301 the cultivar, the growing season and the FACE treatment, sinapic acid was the main soluble phenolic
302 acid (58.1%; Figure 1S), followed by ferulic acid (21.0%) and vanillic acid (8.8.%). As far as the
303 CWBPAs are concerned, ferulic acid was the predominant phenolic acid (89.0%), followed by sinapic
304 acid (5.9%) and *p*-coumaric acid (2.8%).

305 *Mycotoxin content*

306 The multi-mycotoxin LC–MS/MS analysis detected the trichothecenes DON and DON-3-G, while 3-
307 ADON, 15-ADON and NIV were under the limit of detection (LOD) for all samples. Y3 recorded
308 the highest content of total DON (sum of DON, DON-3-G, 3-ADON and 15-ADON), followed by
309 Y1 and Y2 (Table 5). The DON-3-G/DON ratio was significantly higher in Y3 compared to Y1 and
310 Y2. On average, e[CO_2] resulted in a significant increase in total DON (+120%), DON (+146%) and
311 DON-3-G (+64%). The DON-3-G/DON ratio was significantly reduced by 32% in e[CO_2] compared
312 to a[CO_2]. The interaction between FACE treatment and year was significant (Figure 3): a higher
313 increase in total DON content ($P<0.001$, +133%) and a significant reduction in DON-3-G/DON ratio

was observed in Y1 and Y3 under e[CO_2] in comparison to a[CO_2]. Conversely in Y2, the e[CO_2] treatment significantly increased the total DON (+84%), but the DON-3-G/DON ratio was not affected.

DISCUSSION

Wheat biomass and grain yield increased by 19% and 16%, respectively, as a consequence of the higher photosynthetic rate under e[CO_2] conditions.[1,3] In their meta-analysis of 95 FACE experiments, Broberg et al.[2] stated an average increase of 22% for grain yield, supported by an increase of aboveground biomass (+25%), grain number (+23%) and grain mass (+2%), while harvest index remained unaffected. Our study on cv Bologna during 3 experimental years highlights a significant interaction CO_2 x environmental conditions for aboveground biomass, while the increase in grain yield was consistent between growing seasons. A lower increase in plant biomass and yield under e[CO_2] was observed in Y3, which was characterized by the shortest vegetative growing period (175 days *vs* 201 days in Y1 and 202 days in Y2), and the shortest sowing to harvest period (245 days *vs* 257 days in Y1 and 259 days in Y2) due mainly to higher daily temperatures during winter and early spring. These data further highlight the key role of tillering capacity in the future CO_2 scenarios. Despite the shorter vegetative growth period, Y3 aboveground biomass production was intermediate between the ones obtained in Y1 and Y2, while grain yield was penalized by the low number of fertile tillers and by a lower harvest index. Conversely, a marked interaction CO_2 x cultivar was observed in Y3: cv Apache and the hybrid Hystar showed a higher grain yield than cv Bologna, related to both higher biomass production and higher harvest index. Furthermore, grain yield CO_2 responsiveness varied substantially between the cultivars, ranging from 1.072 for cv Bologna to 1.285 for Hystar, and 1.311 for Apache. The results corroborate data of Fares et al.[28] obtained on durum wheat in the same environments. Ziska[29] reported a higher response to e[CO_2] as a result of a greater tiller production and increase in ear density per unit surface area. Also for semi-arid conditions, Maphosa et al.[30] highlight that ear density may be the major determinant of cultivar response to CO_2.

339 Compared with conventional cultivars, hybrids exhibit higher speed of tiller occurrence, thus
340 relatively higher growth rate. Grain yield of the hybrid Hystar was 5% higher than that of the highly
341 productive parent Apache, likely due to an increase in biomass production at equal harvest index. ~~The~~
342 ~~yield advantage of Hystar was slightly less under e[CO_2] than under a[CO_2] as evidenced by the~~
343 ~~slightly higher response ratio of 1.311 for Apache versus 1.285 for Hystar. Nevertheless, yield~~
344 ~~improvement observed for the hybrid remained within the range of up to +10% usually encountered~~
345 ~~as effect of wheat hybrids.~~ In spite of the higher tillering capacity of Hystar, the experiment did not
346 result in indications for a higher responsiveness to e[CO_2] of the hybrid compared to the most
347 productive parent. Yadav et al.[10] reported that a hybrid and a conventional cultivar responded
348 similarly but to a different extent to CO_2 treatments, with the hybrid showing higher yield advantage
349 compared to the conventional cv (+19% vs +11%) because of a greater spike density. The experiment
350 was carried out in a sub-tropical environment, and, above all, the sowing density was low (67 seed
351 m^{-2}). Liu et al.[31] reported that hybrid rice appears to profit much more from e[CO_2] than conventional
352 rice, mainly for the significantly stronger effect on sink generation as indicated by a greater increase
353 in spikelet number per unit surface area. .
354 Despite the importance of wheat as food and the elevated number of studies focusing on the effects
355 of atmospheric CO_2 on nitrogen and other macro-, meso- and micronutrients, the knowledge of
356 probable consequences of rising CO_2 levels on its overall quality is still incomplete. Since quality
357 requirements depend on wheat end-uses, the possible qualitative impact needs to be evaluated
358 considering specific key parameters for the diverse supply chains (e.g., dough strength for improver
359 wheat, phytochemicals for wholegrain flour, contaminants for baby foods). The present experiment
360 resulted in the commonly observed drop of GPC under e[CO_2], while grain TW and hardness did not
361 change, maintaining unaltered the expected milling conditions and yield for common wheat. These
362 data are in agreement with results reported by Panozzo et al.,[11] while conflicting results for grain
363 hardness were reported for previous FACE experiments.[5,6] The discrepancy among the results might
364 be related to the environmental factors, e.g. temperature.[7] In our study, the average reduction of about

1 percentage point in GPC observed in the 3-year experiment for the improver wheat cv Bologna is consistent with the results obtained for ordinary bread-making cultivars in experiment 2, as well as with previous studies carried out in temperate growing areas.[5,12] Panozzo et al.[11] and Arachchige et al.[32] reported a lower GPC reduction for ordinary bread-making wheat (0.44%) but confirm the absence of CO_2 X genotype interaction in environments more prone to drought stress. Fernando et al.[7] did not find an intra-specific variation of GPC response within 8 cultivars with different quality characteristics. Comparing a wide number of old and modern durum wheat cultivars, Fares et al.[28] reported genotype differences in the extent of GPC reduction, with higher change in cultivars with higher grain yield gain. Högy and Fangmeier[33] hypothesized that at high CO_2 concentration GPC may decrease to values below the threshold for an adequate quality standard in bread-making (i.e., 11.5%). The present study highlights that the qualitative impact of near future air CO_2 increase could be more marked for high protein common wheats, which are used in baking products that require high protein and dough strength.[20] According to the established contracts characterizing these segregated marketing grades, the producers obtain a premium prize if they can satisfy and even surpass the GPC requirement of 14%. According to our study, the achievement of this qualitative target appears to be very challenging in a CO_2 enriched atmosphere. Högy et al.[34] reported a similar protein decrease (-1 percentage point), in an excellent baking quality spring cultivar. Because of the decrease in GPC, e[CO_2] has a significant negative effect on bread-making performance resulting in lower sedimentation volume, higher mixing time quantified on refined wheat flour, and lower bread volume.[12] In the present study the effect of CO_2 concentration on wholemeal quality was assessed using a new high shear-based approach, i.e. the GlutoPeak test, that has recently been proposed for the evaluation of gluten quality based on gluten aggregation kinetics.[35] Skipping the refinement process, the test has been successfully applied directly on wholemeal, whose rheological properties are often difficult to assess using the conventional tests (e.g., alveograph) due to the presence of fiber.[36] During the test, the increase in torque corresponds to the formation of the gluten network, whereas the decrease in torque, after reaching a maximum value, corresponds to the breakage of

391 gluten network due to prolonged mixing at high speed.[23] Usually, hard wheat flours (high protein)
392 exhibit longer aggregation time (i.e. PMT) and higher maximum torque than flours of soft (low
393 protein) wheat cultivars, as also found in our experiment 2 confronting cv Bologna with the hybrid
394 and its parents (Table 2). As regards the refinement level, whole grain flour showed a rapid buildup
395 in consistency to a sharply defined peak followed by a rapid break down compared to refined flours.[36]
396 The decrease in maximum torque under e[CO_2], observed consistently in all the considered years and
397 cultivars, coincides with the decrease in GPC. Fernando et al.[7] reported that the effect of e[CO_2] on
398 mixograph peak height, a surrogate for dough strength, varied between grains grown under different
399 environmental conditions, but not between cultivars. Also in the present study, e[CO_2] determined an
400 increase in PMT and a decrease in aggregation energy in all environments, suggesting a consistent
401 decrease in dough strength. In a recent study carried out on winter wheat varieties, gliadin content
402 was correlated to maximum torque, while glutenin content and the fraction with the highest molecular
403 weight (i.e., glutenin macropolymer) to the aggregation energy.[35] Changes in gluten aggregation
404 kinetics observed in the present study might be the result of the effects of e[CO_2] on quality-related
405 gluten protein fractions. Indeed, Wieser et al.[37] observed a decrease in gliadins (by 20%), glutenins
406 (by 15%), and glutenin macropolymer (by 19%) at increased atmospheric CO_2 concentration. the high
407 molecular weight (HMW) subunits of a high protein genotype were more affected than low molecular
408 weight (LMW) ones.[34] A higher decrease of HMW, compared to LMW glutenins, could contribute
409 to a further decline in dough strength especially for high protein cultivars, while this variation could
410 be beneficial for equilibrating an unbalanced P/L ratio, often characterised by excessive tenacity.[38]
411 At present, only few studies have investigated the effect of elevated atmospheric CO_2 on the
412 antioxidants of cereal grains and derived flour. The enrichment with [CO_2] may differentially affect
413 the concentration of phenolic compounds in leaves of cereals. Li et al.,[39] observed an increase in total
414 phenolics of wheat and maize leaves at e[CO_2] during both the vegetative and the ripening stage. In
415 rice grown at e[CO_2], a reduction of total phenolics of roots, stems and leaves was observed from the
416 seedling to the flowering stage; by contrast, at maturity phenolic compounds were higher under

417 e[CO_2] than under a[CO_2].[40] As far as antioxidants of rice grains are concerned, both free and bound
418 phenolic compounds were negatively affected by e[CO_2].[41] The authors hypothesized that in response
419 to CO_2 enrichment the sink capacity of the grain is enhanced and carbon is diverted from being used
420 in carbon-based secondary pathways. In fact, the increased protein demand of the developing grain
421 may cause a reduction of the availability of phenylalanine, which is a common precursor for the
422 synthesis of both proteins and phenolic compounds. Although in the present study the FACE
423 treatment did not result in a significant effect on SPAs and CWBPAs, the AC_{DPPH} of wholegrain flour
424 decreased significantly following the exposure to e[CO_2] in accordance to the results reported by
425 Goufo et al.[41] for brown rice.
426 At present the effect of e[CO_2] on xanthophylls of cereal grain is unknown. A recent meta-analysis[42]
427 showed that carotenoids of vegetables were not affected by e[CO_2]. On the contrary, a second meta-
428 analysis[43] evaluating a wide range of plants underlines that e[CO_2] decreases plant carotenoid
429 concentration by 15%. In the present study lutein and zeaxanthin were differently affected by the
430 FACE treatment. In the first experiment, carried out on cv Bologna, a slight but significant reduction
431 of zeaxanthin was observed under e[CO_2]. Otherwise, the concentration of lutein was higher under
432 e[CO_2], even if the difference was not significant. The second experiment was performed on 3 cv
433 highly different for both lutein and zeaxanthin contents, in fact lutein content varied from 0.63 to 2.2.
434 mg kg^{-1}, while zeaxanthin content varied from 0.19 to 0.27 mg kg^{-1}. Plants grown under e[CO_2]
435 showed a significantly higher concentration of lutein, while the decrease of zeaxanthin was not
436 significant. To the best of the authors' knowledge, this study is the first underlining an increasing risk
437 of higher DON contamination in wheat due to e[CO_2] in open field conditions with natural inoculum.
438 Previous investigations, such as those recently carried on by Bencze et al.[44] and Cuperlovic-Culf et
439 al.[45] were conducted in controlled conditions (greenhouse or phytotron) and on *F. graminearum* or
440 *F. culmorum*–inoculated wheat. Cuperlovic-Culf et al.[45] demonstrated that the effects of e[CO_2] on
441 FHB and DON contamination were dependent on both *F. graminearum* strain and wheat variety,
442 underlining that moderately resistant lines may become significantly more susceptible to mycotoxin

443 accumulation when infected by certain *F. graminearum* strains at e[CO_2]. Similarly, Bencze et al.[44]
444 and Váry et al.[46] observed variable effects of elevated CO_2 on head blight between wheat varieties
445 and suggested that CO_2 has the potential to directly affect not only the fungal pathogen or the host
446 plant, but also the plant–pathogen interactions. Conversely, Vaughan et al.[47] reported that e[CO_2]
447 increased maize susceptibility to *Fusarium verticillioides* proliferation, while fumonisin levels were
448 unaltered. Maize simultaneously exposed to e[CO_2] and drought was even more susceptible to *F.*
449 *verticillioides* proliferation and also prone to higher levels of fumonisin contamination, but the
450 amount of fumonisin produced in relation to pathogen biomass remained lower than in corresponding
451 plants grown at a[CO_2][48]. Therefore, the increase in fumonisin contamination in maize seemed to be
452 likely due to greater pathogen biomass rather than an increase in host-derived stimulants. As far as
453 the aflatoxin risk due to the rising CO_2 is concerned, Medina et al.[49] have studied the response of *A.*
454 *flavus* to climate change factors (water stress, temperature and exposure to e[CO_2]). Although growth
455 was not significantly affected by the three-way interactions, the relative expression of genes in the
456 biosynthetic pathway of aflatoxin production were stimulated by these interacting factors resulting in
457 an increase in phenotypic aflatoxin B_1 production. Unfortunately, the present study did not record the
458 FHB symptoms on ear during ripening, although the TW, qualitative kernel index strictly related to
459 the severity of the disease,[19] did not change in response to the CO_2 concentration. This suggests that
460 e[CO_2] impacted more directly on the toxigenic capacity of fungal species responsible for mycotoxin
461 contamination in grains, compared to the infection rate or the fungal development on wheat ears. As
462 far as the severity of disease (FHB) epidemics and accumulation of associated trichothecene
463 mycotoxins in wheat kernels are concerned Vaughan et al.[50] suggested that rates of wheat residue
464 decomposition, *F. graminearum* inoculum production and dispersal may be significantly altered by
465 changes in atmospheric carbon dioxide concentration, temperature and precipitation patterns, but the
466 impact may be much greater for regions where inoculum is more limited, such as temperate climates.
467 Thus, the results indicate that future environmental conditions, such as rising CO_2 levels, may
468 increase the threat of grain mycotoxin contamination. However, further studies are necessary to

469 understand the overall impact of the CO_2 increase on the development and the metabolism of fungal
470 species responsible of FHB, considering also other emerging and still not yet regulated mycotoxins
471 such as enniatins and moniliformin.[19]
472 In brief, our data underline that future wheat cultivation will require mitigation strategies in order to
473 guarantee an adequate N soil uptake and control of head diseases, while the effect of elevated CO_2
474 may only slightly impact on the content of bioactive compounds in warm temperate continental
475 climates. In order to counteract the negative effects of elevated CO_2 on grain quality, the upcoming
476 wheat cropping systems need to take into account all practices suited to maintain a higher soil fertility
477 in parallel with the management of previous crop residues on the soil surface and the application of
478 substances with high efficacy in controlling head fungal infection.[19] Furthermore, since the simple
479 use of more fertilizers and fungicides result in a further greenhouse gas emission, a more sustainable
480 way to limit impact of CO_2 on wheat quality is the selection of adapted genotypes and their
481 fundamental integration in cropping systems suitable to prevent the expected decline. Understanding
482 the traits that can confer better adaptability to elevated CO_2 is crucial for genetic improvement of
483 both wheat productivity and quality. First, breeding needs to focus on cultivars with higher tolerance
484 to FHB, in order to minimize the risk of DON contamination. In addition, it will be necessary to
485 consider other contaminants such as emerging or new mycotoxins or heavy metals. As previously
486 discussed, the major negative impact of elevated CO_2 could compromise particularly the cultivation
487 and commercialization of high protein improver wheat. Particularly for this market category it is
488 necessary to develop cultivars with a higher responsiveness to N, due to greater soil uptake, with a
489 more extensive root system, and superior sink capacity, also in qualitative terms as far as the gluten
490 composition is concerned. The heterotic effects of wheat hybridization need to be explored for these
491 potential qualitative benefits, in addition to the higher tiller and biomass production. More research
492 considering the interaction of different genotypes with growing conditions and agricultural practise
493 is needed to correctly address the priority for breeding selection in order to maintain existing wheat
494 quality standards and assure global food security and safety.

ABBREVIATIONS

3-ADON, 3-acetyldeoxynivalenol; 15-ADON, 15-acetyldeoxynivalenol; a[CO_2], atmospheric carbon dioxide concentration; ANOVA, Analysis of variance; BE, Brabender equivalent; BHT, 2,6-di-tert-butyl-4-methylphenol; CH_3CN, acetonitrile; CH_3COOH, glacial acetic acid; CH_3OH, methanol; CH_4, methane; CO_2, carbon dioxide; CREA-GB, Research Centre for Genomics and Bioinformatics; CV, cultivar; CWBPAs: cell wall-bound phenolic acids; DON, deoxynivalenol; DON-3-G, deoxynivalenol-3-glucoside; DPPH: 2,2-diphenyl-1-picrylhydrazyl; DM, dry matter; DW: dry weight; e[CO_2], elevated carbon dioxide concentration; EC, European Commission; EFSA, European Food Safety Authority; ESI, electrospray ionization; FACE, free-air CO_2 enrichment; FHB, Fusarium Head Blight; GPC, Grain protein content; GPE, GlutoPeak equivalent; GS, Growth stage; HCl, hydrochloric acid; H_2O, water; KOH, potassium hydroxide; LC-MS/MS, Liquid chromatography coupled with tandem mass spectrometry detection; LOD, limit of detection; MTBE, tert-butyl methyl ether; NaOH, sodium hydroxide; N_2O, nitrous oxide; NIV, nivalenol; PMT, peak maximum time; REGW-Q test, Ryan/Einot and Gabriel/Welsch test; SDS: sodium dodecyl sulphate; S.I.S., Società Italiana Sementi; SPAs, soluble phenolic acids; SSV, SDS sedimentation volume; TE, Trolox equivalents; TKW, thousand kernel weight; TPTZ, 2,4,6-tris(2-pyridyl)-*s*-triazine; Trolox, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; TW, test weight.

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665 **FIGURE CAPTIONS**

666 **Figure 1.** Effect of FACE treatment on grain yield of different common wheat cultivars.

667 **Figure 2.** Effect of FACE treatment on the content of bioactive compounds and antioxidant capacity

668 (AC) in common wheat wholemeal.

669 **Figure 3.** Effect of FACE treatment on total deoxynivalenol (DON) contamination in common wheat

670 wholemeal.

Table 1.

Meteorological and agronomic information of 3-year experiment

Variable	Y1[a]	Y2	Y3
Average daily mean temperature, sowing to heading [°C]	5.	6.1	7.0
Average daily mean temperature, heading to harvest [°C]	20.6	19.5	19.6
Number of frost days	93	83	54
Precipitation sum during the growth cycle [mm]	482	1027	347
Precipitation sum sowing to heading [mm] [b]	399	837	320
Precipitation sum heading to harvest [mm] [b]	83	191	27
Potential evapotranspiration / precipitation	0.66	0.30	0.96
Climatic water balance from heading to harvest [mm]	-94	2	-178
Cumulative water stress index from heading to harvest	0	0	27
Preceding crop[c]	Onion	Wheat	Wheat, Oat, Triticale
Sowing date	Oct. 19, 2011	Oct. 24, 2012	Nov. 9, 2015
Start of fumigation	Nov. 16, 2011	Nov. 9, 2012	Dec. 4, 2015
Harvest date	Jul. 2, 2012	Jul. 11,2013	Jul. 12, 2016

[a] Y1: 2011-12; Y2: 2012-12; Y3: 2015-16

[b] calculated using the heading date of cv Bologna

[c] in Y3 there were different preceding crops at the locations of the octagons

Table 2.

Effect of FACE treatment on productive parameters of common wheat **Experiment**	**Factor**	**Source of variation**	**Aboveground biomass (t ha^{-1} d.m.)**	**Plant height (cm)**	**Grain yield (t ha^{-1} d.m.)**	**Harvest index**	**Ear density (n° m^{-2})**	**TKW (g)**	**GNY (Kg N ha^{-1})**
1[a]	FACE[c]	a[CO_2]	14.0 b	82.0 b	6.7 b	0.49 a	693 b	37.5 a	167 a
		e[CO_2]	16.7 a	84.1 a	7.8 a	0.47 a	874 a	36.4 b	172 a
		P (F)[d]	< 0.001	< 0.001	< 0.001	0.073	< 0.001	0.006	0.388
		sem[e]	0.181	2.6	0.09	0.024	218	1.7	26.0
	year	Y1	19.3 a	86.9 a	8.7 a	0.45 b	784 a	33.9 c	173 a
		Y2	12.1 c	75.4 b	7.0 b	0.58 a	913 a	36.7 b	166 a
		Y3	14.6 b	86.9 a	6.0 c	0.41 c	558 b	40.4 a	166 a
		P (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.560
		sem	0.222	3.19	0.11	0.03	267	2.1	32.0
	FACE X year	*P* (F)	0.026	0.012	0.099	0.227	0.653	0.403	0.899
		sem	0.314	4.51	0.16	0.042	378	3.0	45.0
2[b]	FACE	a[CO_2]	15.0 b	91.2 b	6.3 b	0.42 b	546 b	47.3 a	156 b
		e[CO_2]	17.7 a	94.3 a	7.9 a	0.44 a	606 a	45.5 b	184 a
		P (F)	< 0.001	< 0.001	< 0.001	0.013	0.017	0.008	< 0.001
		sem	1.7	2.52	0.67	0.027	87	2.4	17.0
	cultivar (cv)	Bologna	14.6 b	86.9 c	6.0 b	0.41 b	579 a	40.4 d	166 b
		Apache	17.8 a	98.3 b	8.0 a	0.45 a	588 a	50.8 b	187 a
		QH529	14.3 b	82.0 d	5.8 b	0.41 b	579 a	44.7 c	138 c

	Hystar	18.7 a	103.9 a	8.4 a	0.45 a	559 a	55.6 a	189 a
	P (F)	< 0.001	< 0.001	< 0.001	< 0.001	0.842	< 0.001	< 0.001
	sem	2.4	3.56	0.95	0.038	107	3.4	24.0
FACE X cv	*P* (F)	0.107	0.072	0.014	0.220	0.882	0.420	0.031
	sem	3.4	5.04	1.35	0.054	151	4.8	34.0

[a] Experiment carried out in 3 growing seasons on cv Bologna

[b] a[CO_2] = atmospheric carbon dioxide concentration, e[CO_2] = elevated carbon dioxide concentration

[c] Means followed by different letters are significantly different (the level of significance is shown in the table). Reported values are based on 4 replications.

[d] sem: standard error of mean

[e] Y1: 2011-12; Y2: 2012-13; Y3: 2015-16

[f] Experiment carried out in the 2015-16 growing season

TKW, Thousand Kernel Weight;

Table 3.

Effect of FACE treatment on grain qualitative trait and rheological parameters of common wheat wholemeal

Experiment	Factor	Source of variation	TW (kg hl^{-1})	Grain hardness	GPC (%)	SSV (ml)	Glutopeak parameters		
							PMT (s)	Maximum torque (BE)	Aggregation energy (GPE)
1[a]	FACE[b]	a[CO_2]	81.9 a	70.3 a	14.6 a	61 a	90 b	64 a	1422 a
		e[CO_2]	82.2 a	68.2 a	13.5 b	59 a	114 a	56 b	1270 b
		P (F)[c]	0.566	0.056	< 0.001	0.314	< 0.001	< 0.001	< 0.001
		sem[d]	2.2	4.9	0.7	6.8	19	5	87
	year[e]	Y1	82.7 a	70.0 a	12.7 c	54 b	108 a	56 b	1313 b
		Y2	83.0 a	69.7 a	13.5 b	53 b	115 a	54 b	1257 c
		Y3	80.6 b	68.3 a	15.8 a	71 a	86 b	68 a	1458 a
		P (F)	< 0.001	0.406	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		sem	2.7	6.0	0.8	8.3	23	6	107
	FACE X year	*P*(F)	0.892	0.291	0.765	0.224	0.470	0.309	0.830
		sem	3.8	8.5	1.1	11.8	32	8	151
2[f]	FACE	a[CO_2]	78.5 a	53.2 a	14.7 a	64 a	66 b	59 a	1281 a
		e[CO_2]	78.8 a	52.8 a	13.8 b	61 a	80 a	53 b	1134 b
		P (F)	0.608	0.944	< 0.001	0.074	< 0.001	< 0.001	0.003
		sem	2.4	4.2	0.7	4.7	11	4	203
	cultivar (cv)	Bologna	80.6 a	68.3 a	15.8 a	71 a	86 a	68 a	1458 a
		Apache	79.5 a	54.1 b	13.3 bc	65 b	58 b	54 b	1184 b
		QH529	73.0 b	33.1 d	13.5 b	48 c	68 b	43 c	1014 c
		Hystar	79.7 a	41.3 c	12.8 c	55 d	65 b	45 c	926 c
		P (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		sem	3.4	5.9	1.0	6.6	16	6	288
	FACE X cv	*P* (F)	0.712	0.400	0.438	0.117	0.387	0.224	0.409
		sem	4.8	8.3	1.4	9.4	22	9	407

[a] Experiment carried out in 3 growing seasons on cv Bologna

[b] a[CO_2] = atmospheric carbon dioxide concentration, e[CO_2] = elevated carbon dioxide concentration

[c] Means followed by different letters are significantly different (the level of significance is shown in the table). Reported values are based on 4 replications.

[d] sem: standard error of mean

[e] Y1: 2011-12; Y2: 2012-13; Y3: 2015-16

[f] Experiment carried out in 2015-16 growing season

TW, Test Weight; GPC, Grain Protein Content; SSV, SDS-Sedimentation Volume; PMT, Peak Maximum Time; BE, Brabender Equivalent; GPE, GlutoPeak Equivalent.

Table 4.

Effect of FACE treatment on bioactive compounds and antioxidant capacity (AC) in common wheat wholemeal

Experiment	Factor	Source of variation	Phenolic acids				Xanthophylls		AC_{DPPH}
			SPAs[a] (mg kg^{-1})	Soluble sinapic acid (mg kg^{-1})	CWBPAs[a] (mg kg^{-1})	Bound ferulic acid (mg kg^{-1})	Lutein (mg kg^{-1})	Zeaxanthin (mg kg^{-1})	(mmol Trolox eq kg^{-1})
1[b]	FACE[c]	a[CO_2]	32.1 a	17.5 a	503.6 a	446.1 a	0.59 a	0.18 a	3.43 a
		e[CO_2]	32.6 a	17.3 a	516.3 a	456.8 a	0.61 a	0.17 b	3.29 b
		P (F)[d]	0.594	0.736	0.434	0.459	0.441	0.005	0.002
		sem[e]	5.4	2.3	90.4	65.5	0.07	0.02	0.19
	year[f]	Y1	31.1 a	16.4 b	516.7 a	455.0 a	0.62 a	0.18 a	3.31 a
		Y2	32.2 a	16.8 b	539.6 a	479.8 a	0.56 b	0.16 b	3.36 a
		Y3	33.8 a	19.1 a	473.5 b	419.5 b	0.63 a	0.19 a	3.41 a
		P (F)	0.081	< 0.001	0.006	0.005	0.003	0.000	0.123
		sem	7.7	2.9	127.8	80.2	0.09	0.02	0.23
	FACE X year	*P* (F)	< 0.001	0.003	0.564	0.476	< 0.001	< 0.001	0.016
		sem	10.8	4.1	180.7	113.5	0.13	0.03	0.33
2[g]	FACE	a[CO_2]	41.3 a	25.4 a	542.4 a	483.0 a	1.2 b	0.23 a	3.41 a
		e[CO_2]	42.0 a	25.3 a	562.9 a	503.2 a	1.3 a	0.23 a	3.39 a
		P (F)	0.294	0.986	0.412	0.363	0.012	0.952	0.931
		sem	3.6	2.8	84.3	75.6	0.16	0.02	0.18
	cultivar (cv)	Bologna	33.8 d	19.1 c	473.5 b	419.5 b	0.63 d	0.19 b	3.41 ab
		Apache	37.8 c	21.2 c	611.0 a	548.6 a	2.20 a	0.26 a	3.27 b
		QH529	55.3 a	37.1 a	627.4 a	561.5 a	1.22 c	0.25 a	3.55 a
		Hystar	47.4 b	30.1 b	577.8 a	516.5 a	1.54 b	0.27 a	3.34 b
		P (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		sem	5.1	3.9	119.2	107.0	0.23	0.04	0.26
	FACE X cv	*P* (F)	0.823	0.956	0.510	0.521	0.630	0.449	0.173
		sem	7.2	5.6	168.6	151.3	0.32	0.05	0.37

[a] sum of the soluble phenolic acids (SPAs) and cell wall-bound phenolic acids (CWBPAs) determined by means of the RP-HPLC/DAD

[b] Experiment carried out in 3 growing seasons on cv Bologna

[c] a[CO_2] = atmospheric carbon dioxide concentration, e[CO_2] = elevated carbon dioxide concentration

[d] Means followed by different letters are significantly different (the level of significance P is shown in the table). Reported values are based on 4 replications. Data are expressed on a dw basis.

[e] sem: standard error of mean

[f] Y1: 2011-12; Y2: 2012-13; Y3: 2015-16

[g] Experiment carried out in 2015-16 growing season

Table 5.

Effect of FACE treatment on mycotoxin content in common wheat wholemeal

Factor	Source of variation	Total DON ($\mu g\ kg^{-1}$)	DON ($\mu g\ kg^{-1}$)	DON-3-G ($\mu g\ kg^{-1}$)	DON-3-G/DON (molar ratio %)
FACE[a]	a[CO_2]	274 b	185.1 b	89.1 b	31.7 a
	e[CO_2]	602 a	456.1 a	146.3 a	21.5 b
	P (F)[b]	< 0.001	< 0.001	< 0.001	< 0.001
	sem[c]	84.5	80.0	24.6	6.5
year[d]	Y1	450 b	339.5 a	111.0 a	23.9 b
	Y2	364 c	264.1 b	99.7 b	24.9 b
	Y3	533 a	382.9 a	150.1 a	30.9 a
	P (F)	0.001	0.008	0.001	0.005
	sem	89.6	84.9	26.1	6.9
FACE X year	*P* (F)	0.024	0.008	0.941	< 0.001
	sem	146.4	138.6	42.7	11.2

[a] a[CO_2] = atmospheric carbon dioxide concentration, e[CO_2] = elevated carbon dioxide concentration. Experiment carried out in 3 growing seasons on cv Bologna

[b] Means followed by different letters are significantly different (the level of significance P is shown in the table). Reported values are based on 4 replications. Data are expressed on a dw basis.

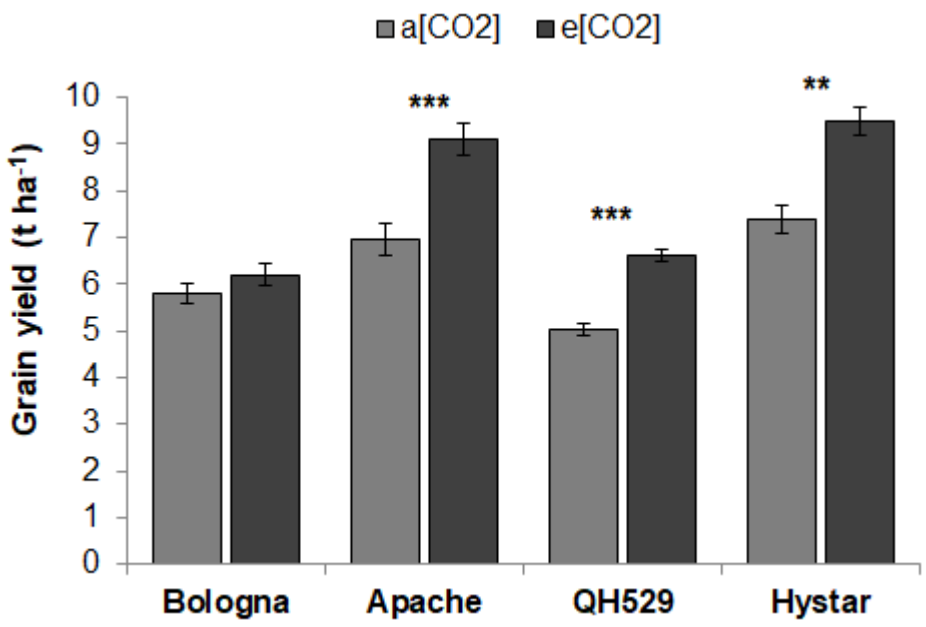
[c] sem: standard error of mean

[d] Y1: 2011-12; Y2: 2012-13; Y3: 2015-16

DON, deoxynivalenol; DON-3-G, deoxynivalenol-3-glucoside.

FIGURE GRAPHICS

Figure 1

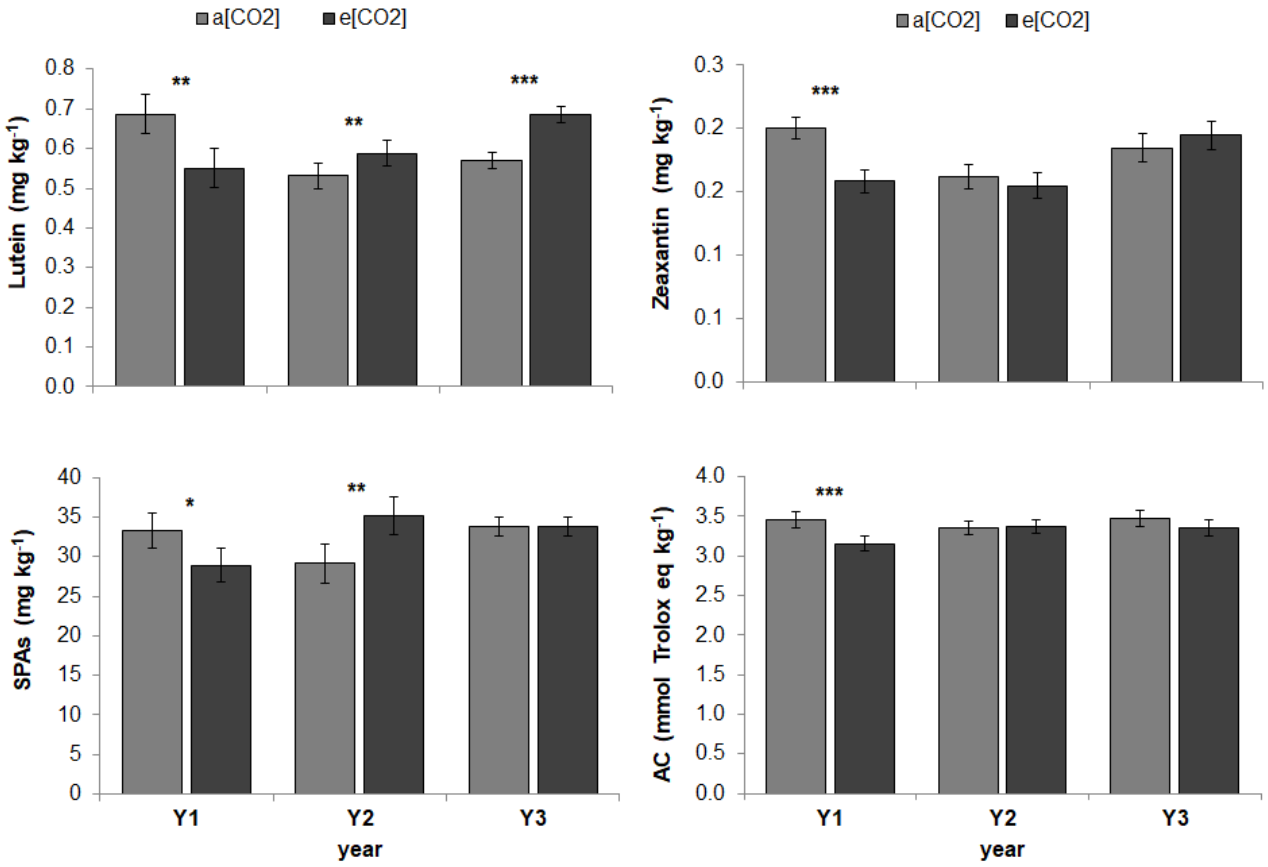


Experiment carried out in 2015-16 (Y3) on different cultivars (experiment 2).

a[CO_2] = atmospheric carbon dioxide concentration, e[CO_2] = elevated carbon dioxide concentration

Bars with asterisks are significantly different: *** P<0.001; ** P< 0.01; * P<0.05. The error bars represent the standard error of means.

Figure 2



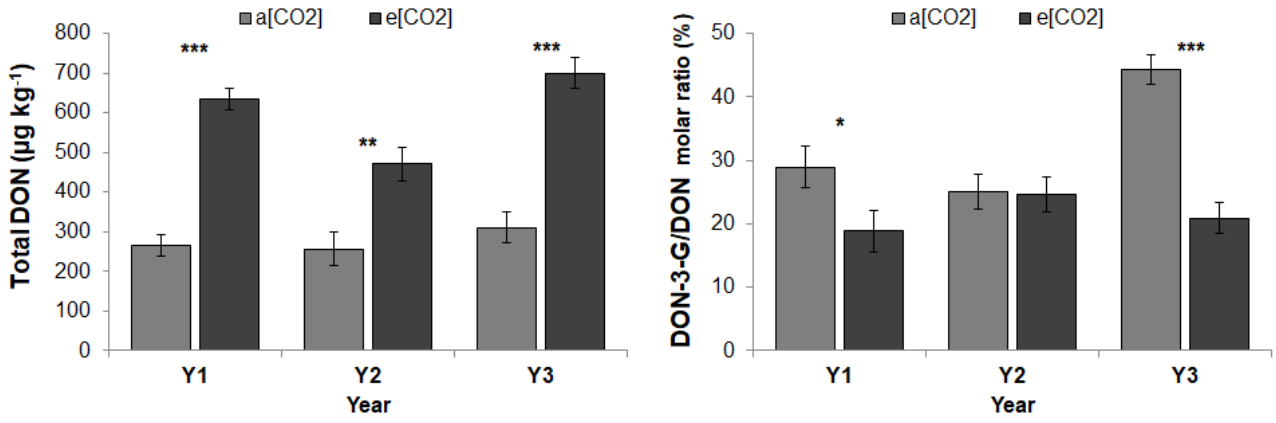
Experiment carried out in 3 years (2011-12, 2012-13 and 2015-16) on cv Bologna (experiment 1).

a[CO_2] = atmospheric carbon dioxide concentration, e[CO_2] = elevated carbon dioxide concentration

Bars with asterisks are significantly different: *** P<0.001; ** P< 0.01; * P<0.05.

Data are expressed on a dw basis. The error bars represent the standard error of means.

Figure 3



Experiment carried out in 3 growing seasons (2011-12, 2012-13 and 2015-16) on cv Bologna (1st experiment).
a[CO_2] = atmospheric carbon dioxide concentration, e[CO_2] = elevated carbon dioxide concentration
Bars with asterisks are significantly different: *** P<0.001; ** P< 0.01; * P<0.05. The error bars represent the standard error of means.

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