



Biochar effects on durum wheat (*Triticum durum*) under ambient and elevated atmospheric CO₂

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ABSTRACT

Wheat has a pivotal role in food chains and human diet, and the understanding of its productive and qualitative performance under elevated CO₂ (e[CO₂]) is therefore a primary research target. While e[CO₂] generally boosts wheat yield and biomass, major concerns remain about the impact on qualitative traits. The use of biochar as amendment, thanks to its well-known ability to improve soil fertility and crop production and quality, could be a suitable solution to cope with the negative effect of e[CO₂] on qualitative traits. To test this hypothesis, we present the results of an open field experiment investigating the combined effects of biochar application and e[CO₂] on quantitative and qualitative performance of two commercial durum wheat (*Triticum durum*) cultivars (Aureo and Claudio). The experimental design included plots treated without [B0] or with 30 t ha⁻¹ of biochar as amendment [B30] and grown at ambient a[CO₂] and e[CO₂] (570 ppm). Results indicated that biochar increased soil temperatures by +1 °C during winter, thus favouring an earlier phenology and average biomass (+10 % and +7.2 %) and yield (+13.2 % and +8.5 %) increase in both a[CO₂] and e[CO₂] treatments compared to the no biochar [B0] treatments. Biochar treatments also increased flag leaf chlorophyll and decreased flavonoids, and enhanced intrinsic water use efficiency due to increases in photosynthesis and decreases in stomatal conductance. Spike density, falling number and thousand kernel weight were positively and significantly altered by biochar amendment, whilst the remaining parameters mostly differed between cultivars only. By contrast, biochar application did not alleviate nitrogen reduction in grains and straw in both cultivars neither increase grain protein content, although it remained sufficient for pasta making under all treatment combinations. Accordingly, biochar use could be preferentially adopted for increasing the productivity of high-quality cultivars whose destination and industrial processing would not suffer from a decrease in quality parameters.

1. Introduction

The steady rise in atmospheric carbon dioxide (CO₂) concentration creates the need to improve the knowledge base on its effects within diverse cropping systems. A large effort is continuously dedicated to understanding the impact of increased CO₂ on both crop productivity and quality because of environmental, eco-physiological and phenological changes [1–5]. Among cereals, wheat is a preferential target of

investigation due to its importance in food chain and human diet, and therefore often investigated through Open Top Chambers (OTC) and Free Air Carbon Dioxide Enrichment (FACE) experiments [6,7] to evaluate its changes in structure, functioning, yields and grain quality in response of elevated CO₂ (e[CO₂]). Despite several studies that suggested an overall increase in wheat yield and biomass production under e[CO₂] [6,8–12], major concerns remain about the change in qualitative traits. Consistent detrimental effects were widely reported as reviewed

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by for grain nitrogen and protein content [13–15], other micro and macronutrients [14–19] and carotenoids [20]. Related changes in grain proteome were studied by Refs. [21–24]. The decrease in grain nitrogen content under $e[CO_2]$ and concomitant changes in protein composition led to negative effects on rheological and baking quality of bread wheat [22,25,26], as well as gluten and pasta quality of durum wheat [27].

The effect of $e[CO_2]$ on grain qualitative traits raises the question on which management strategies could be adopted to cope with the observed nutritional decline. Results of studies applying low and high nitrogen fertilizer levels [28–30] imply that higher doses of nitrogen fertilizer, eventually applied with late topdressings, compared to those applied at $a[CO_2]$ could compensate for the decrease in grain nitrogen content. However, N-based strategies, especially when over-applied, can produce detrimental environmental effects such as species richness reduction due to N deposition [31], leaching increase [32–34] and greenhouse gas emissions [35].

To cope with the negative effect of $e[CO_2]$ on crops qualitative traits and to reduce N inputs, the use of biochar could be an effective win-win strategy. Biochar, a carbon (C)-rich material obtained from the thermochemical conversion of biomass in oxygen-limited environments, is one of the most promising materials for C sequestration in soils, capable to maintain crop production and quality [36–42]. Mixing biochar with soil was found to change soil physical properties such as texture, particle size distribution, and porosity [43,44], increase micropores and the related root volume ratio [45], hydraulic conductivity and water retention [46], soil carbon pools [47], soil water content [48] even after many years [49,50].

Globally, biochar systems could deliver emission reductions of 3.4–6.3 PgCO₂e, half of which constitutes CO₂ removal [51] and is strictly related to its recalcitrance as once incorporated in soil biochar has a mean residence time that was estimated from the centennial to millennial time scale. The effects of biochar on wheat were widely investigated, reporting a general increase in above-ground biomass and yield, and no significant detrimental impacts on nutritional values [52–55]. Despite biochar application can provide benefits for agriculture and environment, to our knowledge, no study has been conducted to investigate the integrated effect of biochar application and $e[CO_2]$ on durum wheat quantitative and qualitative performances in open field conditions.

Based on these premises, the aim of this study was to compare and evaluate the quantitative and qualitative traits of two durum wheat commercial cultivars named Aureo and Claudio under none treatment [B0] and with 30 t ha⁻¹ of biochar as amendment [B30], grown at ambient ($a[CO_2]$ = 400 ppm) and elevated ($e[CO_2]$ = 570 ppm) atmospheric CO₂ concentration in a field experiment in northern Italy. We hypothesized that biochar could mitigate the detrimental effects of elevated CO₂ on quality traits while enhancing yield.

2. Materials and methods

2.1. Test-site characteristics and experimental layout

The experiment was made in the fields of the CREA-Research Centre for Genomics and Bioinformatics in the 2012–2013 cropping season. The site located in the Po Valley in northern Italy, at Fiorenzuola d'Arda (44.9278N, 9.8938E) is characterized by a warm continental climate, with cold winters and hot summers. The precipitation from August 2012 through July-2013 was 1059 mm (1027 mm during the crop growth cycle), about +19 % compared to the 1980–2010 long-term average (LTA = 772 mm), however most of the differences were observed in March (+140 mm compared to LTA), which also resulted to be the rainiest month (197 mm) of the 2012–2013 growing season (GS) (Fig. 1). Average minimum (8.3 °C) and maximum (18.3 °C) air temperatures were in line with LTA (8.1 and 18.1 °C, respectively), with major differences observed in August (Δ = +2.1 °C), November (Δ = +2.3 °C) and December (Δ = –2.3 °C) for minimum temperatures, and in August (Δ = +3.7 °C), November (Δ = +2.4 °C) and March (Δ = –3.6 °C) for maximum temperatures, (Fig. 1). In general, the 2012–2013 growing season was in line with LTA (Fig. S1), with no occurrence of extreme temperatures or damaging frost.

The soil is alkaline (pH 8.09), with a silty-clay texture (14 % sand, 50 % silt, 36 % clay) in the whole soil layer (0–100 cm). Soil properties also included total carbonate (10.19 %), total C (28.1 g kg⁻¹, of which 12.22 and 15.9 g kg⁻¹ of inorganic and organic, respectively), total N (0.10 %), C:N ratio (15.6), P₂O₅ (21.7 mg kg⁻¹) and the cation exchange capacity (6.85 cmol(+) kg⁻¹), as reported by Ref. [26] on the same field experiment.

Two durum wheat commercial cultivars (cv) Aureo and Claudio was

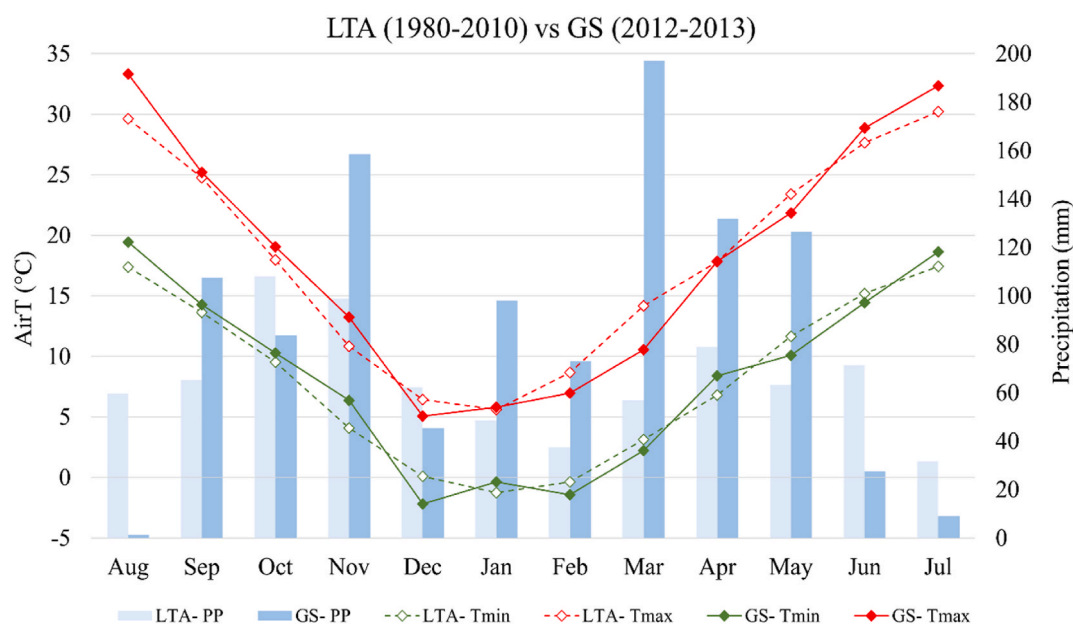


Fig. 1. Monthly minimum (green filled line) and maximum (red filled line) air temperatures (°C) and monthly cumulated precipitation (mm, cerulean histograms) for the growing season (GS) calculated from August 2012 through July 2013 at Fiorenzuola d'Arda, and their long-term average (LTA) calculated over the period 1980–2010 in the study area. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

employed in the experiment. Aureo is a high-quality commercial wheat used in industrial, mono-varietal pasta production, characterized by medium-early cycle, consistently high grain protein content and gluten quality, and medium grain yields. Claudio is characterized by a stable and high production in all the Mediterranean area with good resistance to drought.

The experimental design included the two durum wheat cultivars grown in a randomized block design with plots of 2.2×1.3 m, and two levels of CO₂ concentration (ambient: a[CO₂] at 400 ppm and elevated at 570 ppm: e[CO₂]), without biochar amendment [B0] and with 30 t ha⁻¹ of biochar as amendment [B30] in four replicates, resulting in a total of 32 plots. The control was defined as a[CO₂][B0]. Biochar was superficially applied before sowing and mechanically mixed into the soil to a depth of 30 cm using a chisel plow tiller in September 2012. Sowing, start of CO₂ fumigation and harvest dates were 24 October 2012, 9 November 2012, and 11 July 2013, respectively. Plots were fertilized using N-P-K at pre seeding (4 days prior to sowing: N:P:K 15:15:15; 30 kg N ha⁻¹) and two topdressings with ammonium nitrate (March 4, 2013: 78 kg N ha⁻¹ and April 18, 2013: 126 kg N ha⁻¹) for a total of 234 kg N ha⁻¹. The e[CO₂] condition was generated through a FACE (Free Air CO₂ Enrichment) system. To avoid unexpected effect from treated to no-treated plots (i.e., biochar particles movements and leakage), the plots were separated by light trenches and control plot was used as main reference to assure no-contamination value.

2.2. FACE system description

The FACE system was developed by CNR-IBE [56], formerly known as CNR-IBIMET, and included four octagonal CO₂ enrichment rings with a diameter of 14 m, each one consisting of eight vertical poles with eight horizontal cross sections in aluminium (Fig. 2). Polyvinyl chloride (PVC) fumigation tubes (Ø 2 cm) with laser-drilled holes (Ø 0.3 mm) facing outwards were fixed at the horizontal sections. The CO₂ concentration was measured in the centre of the FACE ring through a CO₂ probe (Vaisala Carbocup GMP343), whilst wind speed and direction (Gill wind sentry) were measured above the FACE ring at a height of 3.5 m. Based on the CO₂ concentration level recorded in the centre of the FACE and by wind speed and direction, CO₂ was continuously released to maintain the target concentration of 570 ppm. Fumigation with CO₂ was applied during daytime hours. For the whole study period, the height of the

fumigation tubes was regularly adjusted to be slightly above the plant canopy.

2.3. Biochar characteristics

Biochar was obtained from orchard pruning biomass through a slow pyrolysis process at temperature of 500 °C in a transportable ring kiln of 2.2 m in diameter and holding around 2 t of feedstock. The biochar (77.8 % of total carbon, 0.91 % nitrogen, 101 cmol(+)/kg CEC, and 2722 mm³ g⁻¹ porosity), at the end of the pyrolysis was crushed into particles smaller than 5 cm of diameter before application. Biochar bulk density was determined from oven dried cores as mass per volume of oven dried biochar using 260 mm × 220 mm cylindrical cores. Further information on chemical and physical properties of pure biochar applied in the field experiment were reported in supplementary (Table S1).

2.4. Field measurements and laboratory analysis

High-frequency (10 min) measurement of soil temperature at a depth of 5 cm was made in all plots using J-type thermocouples connected to CR10 dataloggers (Campbell Scientific, Inc.) from DAS 12 to DAS 133. Winter damage was scored with a scale ranging from 0 for healthy plants to 9 for dead plants [57]. Flag-leaf photosynthesis and chlorophyll fluorescence were measured at DAS (days after sowing) 195 and DAS 198 with a Licor 6400 instrument at 2000 µmol m⁻² s⁻¹ photosynthetic photon flux density (PPFD) and temperature of incoming air of 25 °C. Flow rates and consequently leaf to air vapor pressure deficit (VPD) differed between the two measurement dates. Therefore, ANOVA was performed on the deviation of the individual measurements from the average of all measurements at a given day.

Leaf chlorophyll and flavonoid contents were assessed using an optical detector, Dualex (Force A, Orsay, France) at DAS 162, 195 and 198, while spectral measurements of canopy were taken with an ASD handheld VNIR field spectroradiometer (ASD Inc. Boulder, Co, USA). The instrument recorded 10 spectra taken from about 1 m above the canopy which were averaged to obtain a single average spectra value. For the calculation of Normalized Difference Vegetation Index (NDVI), an index sensitive primarily to the green leaf area or green leaf biomass and serves as a proxy for the photosynthetically active biomass of plant



Fig. 2. The octagonal FACE installed at the Research Centre for Genomics and Bioinformatics (CREA-GB), Fiorenzuola d'Arda (44°56' N, 9°54' E; 80 m.a.s.l.) in the 2012–2013 cropping season.

canopies [58], 10 average spectra (i.e., 100 measurements) were taken walking along the plots in North-South direction during midday hours for each field campaign. This approach was repeated around every 20 days for a total of 11 field campaigns.

Average plant height per plot was measured during grain filling (DAS 220). Before the final harvest 1.5 m linear meter samples were manually taken from the central rows of the plots to avoid the edge effects. Plots were harvested manually on 11th June 2013, total above ground biomass determined and immediately threshed with a plot combine harvester (Nurserymaster, Wintersteiger, Austria). Straw was oven-dried and weighted. Wheat ears of the linear meter harvest were separated from the straw and the grains were separated from the ears using a laboratory thresher (LD 350, Wintersteiger, Ried, Austria). Then, spike density (SD), seed weight, and thousand kernel weight (TKW) were determined using an electronic balance. Number of grains per spike, peduncle length (PLE), the size of the internode below the spike, and spike length (SL) were measured. Grain mass, grain moisture, TKW, and test weight (TW) were determined for the main plot harvest and grain yield calculated as the sum of the whole plot and linear meter harvests.

Total Ca^{2+} , K^+ , Mg^{++} , Mn^+ , Na^+ , P , and Fe^{++} were determined on the oven-dried straw subsamples (105 °C for 24 h) from the harvest, according to the EPA method 3052 using an ICP-OES spectrophotometer (Varian Inc., Vista MPX). C tot. and N tot. concentrations were determined via total combustion in an elemental analyzer (EA Flash 1112 ThermoFinnigan). Isotopic ratios on the straw were determined with an Isotope Ratio Mass Spectrometer (Thermo Fischer Scientific, Delta V Plus). Natural abundances of stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes were determined.

Grain and straw carbon ([C]), nitrogen ([N]) and hydrogen ([H]) concentration were determined with an elemental analyser (TruSpec CHN, LECO, Michigan, USA) and expressed as % of total mass.

Grain samples from each subplot were ground to wholemeal using a 1 mm sieve Cyclotec mill (Foss Tecator AB, Höganäs, Sweden). Grain nitrogen concentration was converted to grain protein concentration (GPC) multiplying by the factor of 5.72 [59].

Grain samples (100 g) were tempered for 19 h at 16 % moisture and milled with a laboratory mill (Monza, Italy) that separates semolina from bran and shorts. The extraction rate ranged from 55 to 60 %. Dry gluten content and gluten index (GC and GI) were determined in triplicate on semolina according to Ref. [60]. For the determination of dry gluten, wet gluten was dried with Glutorex 2020 at 150 °C for 5 min and left to cool for 3 min. Yellow pigment content (YPC) was determined on 10–100 mg of semolina extracted in 250–500 µL of water-saturated 1-butanol for 15 min in an ultrasonic bath. The absorbance was read at 435 nm after manual injection of an aliquot of extract directly into a UV/VIS HPLC detector. The measures were repeated in triplicate and expressed as µg/g of β carotene on dry weight basis.

2.5. Statistical analysis

Statistical analysis including analysis of variance (ANOVA), principal component analysis (PCA), correlation analysis, multiple regression analysis and graphical presentation of data were performed using R software (R core team, 2014). ANOVA was used to test for equal means between CO_2 treatments, biochar treatments, cultivars and their interaction. PCA, a multivariate analysis technique, was used to reduce the dimensionality of a set of 23 random variables. The fraction of total variance in the data explained by the resulting projection on orthogonal axes (principal components) informs on the combined effect of correlated variables. The loadings of the single variables on the principal components were used to inspect uncorrelated effects on productive and qualitative traits and the clustering of the treatment combinations for separation of treatment and cultivar effects.

3. Results

3.1. Biochar impacts on soil temperature NDVI and phenology

Soil temperatures were collected at high-frequency (10-min) for four months and then aggregated at daily time step (Fig. 3a). Average soil temperatures were higher in the plots treated with biochar compared to untreated plots, with maximum differences in the range of +4 to +6 °C during November and December and an average total temperature difference of +1 °C along the whole period of measurements.

The seasonal course of NDVI (Fig. 3b) was characterized by a decrease during winter months followed by an increase in spring up to a plateau attained when flag leaves had expanded and lasting to the onset of leaf senescence. For cv Claudio the major differences were observed during tillering (about 80 days after sowing, DAS), where the plots treated with biochar showed a higher NDVI value (0.51) compared to the untreated plots (0.35). Also, at maturity stage (i.e., DAS 206, 212 and 226), NDVI for Claudio in the treated plots was found slightly higher, on average (0.9) compared to the untreated plot (0.85). By contrast, cv Aureo did not show a clear response to biochar application neither at the beginning (i.e., DAS: 23 to 140) nor at maturity (i.e., DAS: 206 to 226) stage, with similar NDVI values (i.e., 0.35 and 0.85 and 0.33 and 0.86 for untreated and treated plots, respectively) in both treatments.

At $\text{e}[\text{CO}_2]$, the NDVI pattern of both cultivars showed a slight response to biochar application compared to the untreated plots [B0]. For cv Claudio the major differences were observed in the early growing phase (DAS 20 to 105), where the plots treated with biochar showed slightly higher NDVI values (0.474, on average) compared to that of untreated plots (0.452), and at DAS 161, where the treated plots showed a considerably lower NDVI (0.48) compared to that of the untreated plots (0.77). At maturity stage the NDVI values of both treatments were comparable. By contrast, cv Aureo showed a continuously higher NDVI under [B30] compared to [B0], with the major differences found at the beginning (DAS 43, +13 %) and in the middle (DAS 140–161; +14.3 %, on average) of the growing season. As found for cv Claudio, at maturity stage the NDVI values of both treatments were in line. The effect of biochar on soil temperature was also evaluated through the spectral reflectance of bare soil and the two treatments (Fig. S2), which confirmed higher radiation absorption as lower spectral reflectance values (at wavelengths >550 nm) were observed in the treated plots, with a major development of the red edge at the seedlings stage.

In the very moist early growing season, snow fell onto the water-saturated soil during three periods, leading to waterlogging (Fig. 3b, Fig. S3). NDVI was lower after snow melt of the second and third snow cover period relative to before snow fall. This reduction in NDVI coincided with appearance of senescent leaves and single dead plants. The occurrence of senescence, mortality, and reduction in NDVI due to waterlogging were also reflected in winter visual damage ratings between 2 and 6 (on a 0 to 9 scale). Winter damage ratings were 0.7 units higher for Aureo than for Claudio (Table 1) and there was a trend for lower winter damage ratings by 0.6 units under [B30] ($p = 0.09$).

The biochar effect on phenological phases of the two cultivars was then further evaluated using BBCH (Biologische Bundesanstalt, Bundesortnamt und Chemische Industrie) scale, averaging observations between ambient and elevated conditions (Fig. 4).

On January the 10th (DAS 78) the phenological stage did not significantly differ between treatments, while cv Claudio had reached the fourth leaf stage earlier than Aureo. By contrast, at March 27th (DAS 154), a slight but non-significant effect of biochar on the phenology in Aureo (mean = 15.42 ± 0.51) and Claudio (mean = 15.48 ± 0.37) compared to that of the untreated plots (mean = 15.15 ± 0.47 and 15.32 ± 0.53 for Aureo and Claudio, respectively) was observed. The slight advance in phenology of Claudio relative to Aureo of on average 0.52 BBCH units was consistently observed at 7 dates between DAS 30 and DAS 154 (paired t -test, $p < 0.001$). In contrast to the slower phenological

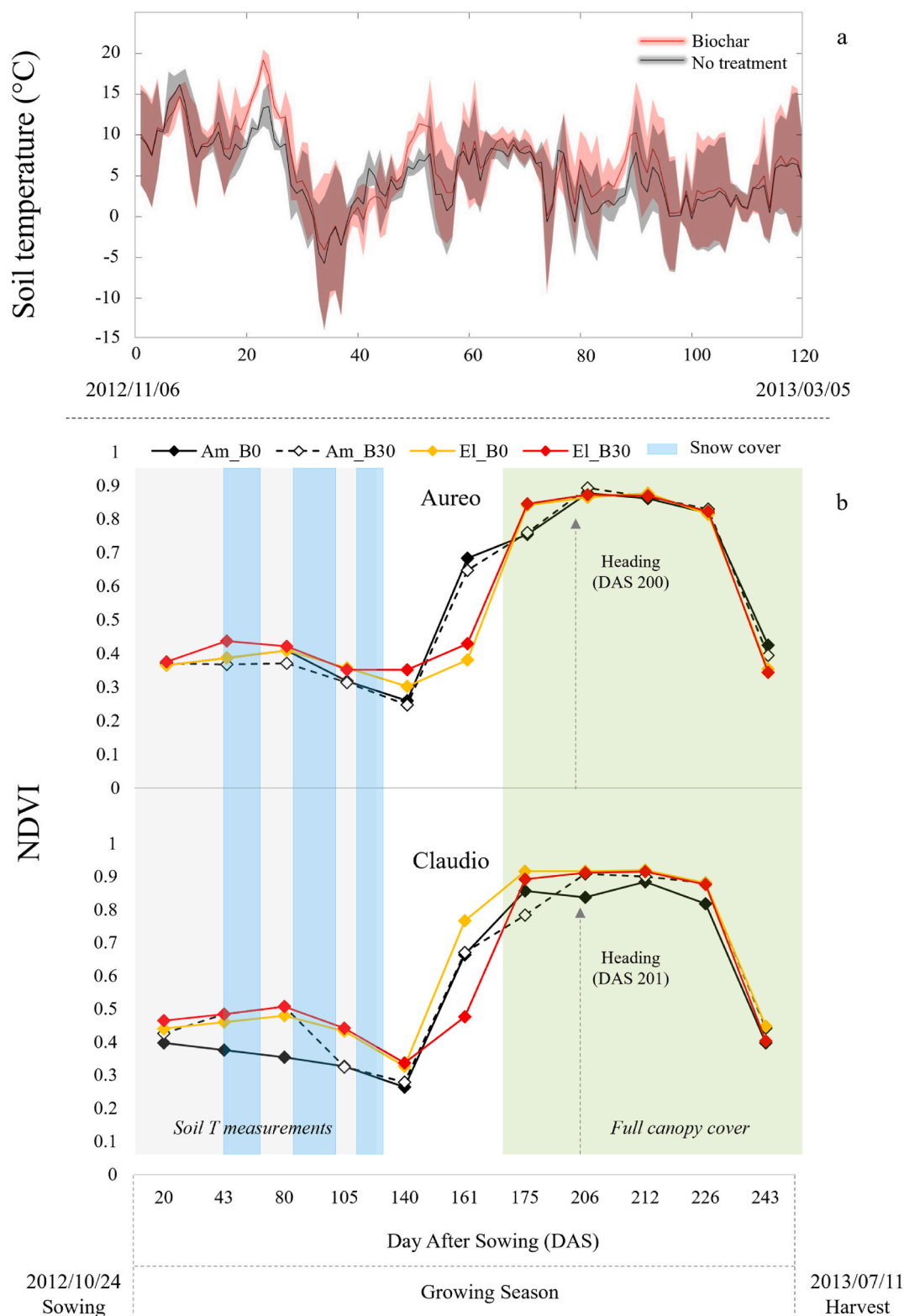


Fig. 3. (a) pattern of soil temperature for the period 6 November 2012 to 5 March 2013 (120 days) in plots with biochar amendment (red line) and without biochar amendment (black line) averaged for a[CO₂] and e[CO₂]. The red and grey areas represent the respective confidence intervals of the measurements; (b) NDVI pattern of cv Claudio and cv Aureo under all interactions during the whole growing season (2012/10/24 to 2013/07/11). The grey shaded area represents the days after sowing (DAS) at which soil temperature was measured while the green shaded area those in which full canopy cover was achieved. The cyan area represents the periods of snow cover in field. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1

ANOVA of productive, morphological, and quality traits and abiotic stress indicators. Significant codes are the following: p-value = ‘****’ [0, 0.001]; ‘***’ (0.001, 0.01]; ‘**’ (0.01, 0.05]; ‘.’ (0.05, 0.1]; ‘ns’ not significant.

Compartment	Trait	P CO ₂	F value	P Biochar	F value	P Cultivar	F value	P Interaction	F value
Photosynthesis and pigments	A _n	0.155 ns	2.109	0.176 ns	1.905	0.103 ns	2.79	<0.007 CO ₂ x C	8.355
	g _s	<0.001	263.8	0.883 ns	0.022	<0.008 **	8.115	<0.038 * CO ₂ x C	4.625
								<0.020 * CxDAS	5.944
	WUE _i	<0.001	360.5	<0.049	4.169	<0.002 **	11.12	<0.043 * CO ₂ x CxDAS	4.407
Productive and morphological parameters	Chlorophyll	0.483 ns	0.731	<0.070	3.48	<0.002 **	11.79	none	none
	Flavonoids	<0.010	99.81	<0.027 *	5.361	<0.001 ***	32.9	0.056 C x B	3.891
	NBI	0.248 ns	2.606	<0.053 ns	4.009	0.686 ns	0.166	0.069 CO ₂ x CxB	3.516
	Grain yield dry mass	0.071	4.78	0.041 *	4.838	<0.001 ***	18.43	none	none
	Grain number m ⁻²	0.024 *	8.986	0.078	3.493	<0.001 ***	16.67	none	none
	Grain nitrogen yield	0.485 ns	0.553	0.087	3.269	0.053	4.277	none	none
	Grain humidity	0.51 ns	0.49	0.34 ns	0.953	<0.001 ***	42.11	0.092 CO ₂ x C	3.169
	Aboveground dry mass	0.02 *	9.88	0.078	3.506	<0.001 ***	32.91	none	none
	Aboveground fresh mass	0.019 *	10.26	0.079	3.476	<0.001 ***	34.43	none	none
	Aboveground nitrogen yield	0.428 ns	0.723	0.171 ns	2.092	<0.027 *	5.838	none	none
	Straw dry mass	0.018 *	10.38	0.229 ns	1.551	<0.001 ***	39.44	none	none
	Straw nitrogen yield	0.421 ns	0.747	0.983 ns	0.0	<0.011	8.192	none	none
	Straw humidity	0.65 ns	0.23	0.37 ns	0.854	0.010 *	8.209	0.06 CO ₂ x BxC	3.923
	HI	0.23 ns	1.75	0.22 ns	1.641	0.003 **	11.87	none	none
	Spike density	0.007 **	15.67	<0.001 ***	24.08	0.15 ns	2.238	0.070 CO ₂ x C	3.699
	Test weight	0.89 ns	0.02	0.37 ns	0.86	<0.001 ***	36.88	none	none
	TKW	0.40 ns	0.83	0.023 *	6.231	0.052.	4.327	none	none
	Grain DM per spike	0.41 ns	0.8	0.54 ns	0.4	0.38 ns	0.829	none	none
	Grains per spike	0.76 ns	0.1	0.65 ns	0.207	0.35 ns	0.941	none	none
	Height	0.075.	4.63	0.607 ns	0.274	<0.001 ***	19.76	none	none
	Peduncle length	0.59 ns	0.33	0.052.	4.32	0.68 ns	0.172	0.089 CO ₂ x C	3.223
	Spike length	0.43 ns	0.72	0.70 ns	0.158	0.002 **	13.65	0.081 CO ₂ x C	3.411
	Heading date	0.056.	5.61	0.27 ns	1.296	<0.001 ***	17.42	none	none
	Gluten content	0.56 ns	0.39	0.40 ns	0.739	0.0012 **	14.696	0.0697 CO ₂ x B	3.719
	Carotenoid content	0.91 ns	0.02	0.42 ns	0.687	<0.001 ***	134.37	none	none
	Grain N content, GPC	0.007 **	16.01	0.37 ns	0.835	<0.001 ***	20.447	none	none
	Grain C content	0.025 *	8.87	0.014 *	7.425	0.61 ns	0.27	none	none
	Grain H content	0.105 ns	3.64	0.23 ns	1.499	0.019 *	6.591	0.0269 * CO ₂ x B	5.805
	SDS	0.97 ns	0	0.11 ns	2.75	<0.001 ***	27.36	0.0153 * CO ₂ x B	7.177
	Gluten index	0.09.	3.96	0.88 ns	0.023	0.02 *	6.507	0.0106 * CO ₂ x B	8.128
	Carotenoid index	0.40 ns	0.82	0.59 ns	0.296	<0.001 ***	47.607	none	none
	Grain δ ¹³ C	nd ^a	nd ^a	0.55 ns	0.378	0.007 **	9.448	none	none
	Grain δ ¹⁵ N	0.14 ns	2.81	0.004 **	10.55	0.92 ns	0.01	0.004 ** CO ₂ x C	10.55
	Straw N content	0.98 ns	0	0.20 ns	1.768	0.093.	3.139	none	none
	Straw C content	0.30 ns	1.3	0.052.	4.313	0.12 ns	2.607	none	none
	Straw H content	0.002 **	25.41	0.24 ns	1.496	0.074.	3.594	0.0463 * CO ₂ x BxC	4.581
	Straw δ ¹³ C	nd ^a	nd ^a	<0.014 *	13.94	0.76 ns	0.095	none	none
	Straw δ ¹⁵ N	0.283 ns	1.45	0.015 *	7.444	0.027 *	5.905	none	none
Abiotic stress	Winter damage rating	0.39 ns	0.85	0.09 ns	3.183	0.043 *	4.755	none	none

^a not determined because the difference between air δ¹³C of the a[CO₂] and e[CO₂] treatments is not known.

development observed in the vegetative growth phase, Aureo headed before Claudio (DAS 200 vs DAS 201, respectively).

3.2. Photosynthesis and leaf pigments

Biochar addition [B30] led to an increase in leaf chlorophyll content (trend at $p < 0.070$; Tables 1 and 2). Expressed as chlorophyll per unit leaf surface area using the equation reported by Ref. [61], the increase was 5.3 % from on average 30 $\mu\text{g cm}^{-2}$ under [B0] to 31.5 $\mu\text{g cm}^{-2}$

under [B30]. Leaf flavonoid content decreased ($p < 0.027$) by 6 % in Aureo [B30] relative to [B0], while it was virtually unaffected (-0.005 %) in Claudio and increased under e[CO₂] by 6 % ($p < 0.01$). On average the flavonoid content was higher in Claudio (2.86 Dualex units) than in Aureo (2.64). NBI tended to increase from 12.9 under B0 to 13.9 under B30 ($p < 0.053$). The increase was higher for Aureo than for Claudio.

Photosynthesis deviation from the daily mean measurements increased under e[CO₂] relative to a[CO₂] in Aureo, while it decreased in Claudio (Table 2, interaction cultivar x CO₂: $p < 0.007$). The

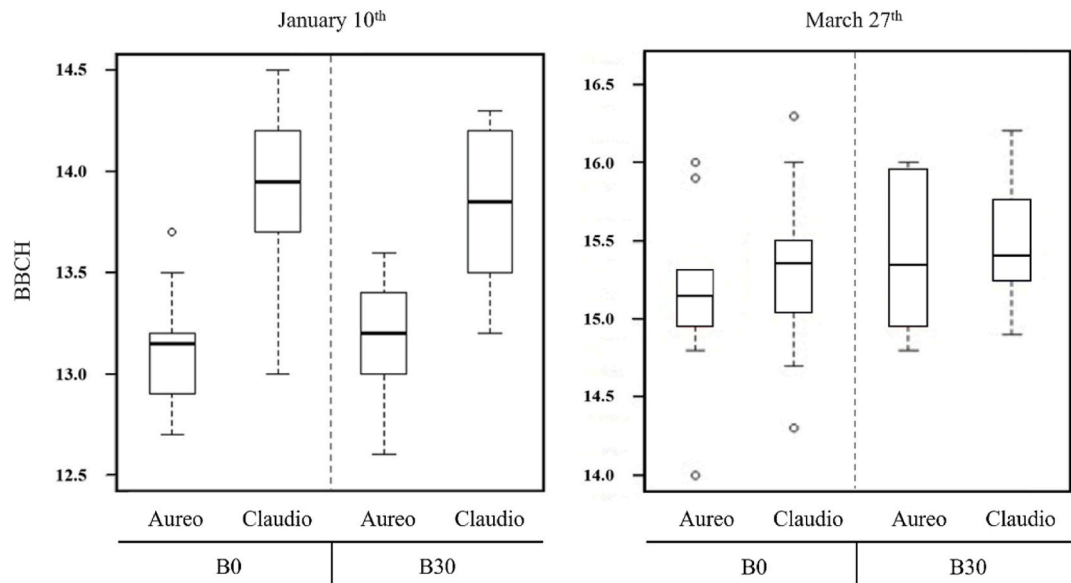


Fig. 4. Phenological phases of the two cultivars for untreated [B0] plots and plots amended with biochar [B30], averaging observations for $a[\text{CO}_2]$ and $e[\text{CO}_2]$ based on BBCH scale on January 10th (fourth leaf stage) and March 27th.

Table 2

Flag leaf pigments, nitrogen balance index (NBI), photosynthetic rate, stomatal conductance, and intrinsic water use efficiency (WUEi) at PPFD 2000 $\text{mmol m}^{-2} \text{s}^{-1}$ averaged across the two measurement dates.

Cultivar	Variable	Unit	$a[\text{CO}_2]$ B0	$e[\text{CO}_2]$ B0	$a[\text{CO}_2]$ B30	$e[\text{CO}_2]$ B30
Claudio	Leaf chlorophyll content	Dimensionless arbitrary units	39.9	36.5	38.5	40.1
Aureo			35.7	31.1	38.4	34.3
Claudio	Leaf flavonoid content		2.8	2.93	2.75	2.95
Aureo			2.68	2.77	2.45	2.68
Claudio	NBI		14.5	12.3	13.6	13.6
Aureo			13.1	11.6	15.8	12.7
Claudio	Net photosynthesis	$\text{mmol m}^{-2} \text{s}^{-1}$	30.7	30	32.4	30.9
Aureo			28.9	33.3	30.3	35.6
Claudio	Stomatal conductance	$\text{mol m}^{-2} \text{s}^{-1}$	0.55	0.29	0.53	0.26
Aureo			0.58	0.38	0.57	0.37
Claudio	WUEi	mmol mol^{-1}	56	104	61	123
Aureo			51	88	53	95

photosynthesis rate averaged across all treatments and the two cultivars was $31.6 \mu\text{mol m}^{-2} \text{s}^{-1}$. Stomatal conductance deviation from the daily mean did not change in response to biochar amendment and decreased under $e[\text{CO}_2]$ relative to $a[\text{CO}_2]$ stronger so in Claudio than in Aureo (quantitative interaction: $p < 0.038$). This effect augmented from the first to the second measurement date in Claudio (DAS 195 and 198), while it was attenuated in Aureo (interaction: $p < 0.02$). The average stomatal conductance was $0.435 \text{ mol m}^{-2} \text{s}^{-1}$, 0.554 under $a[\text{CO}_2]$ and 0.332 under $e[\text{CO}_2]$. The deviation of intrinsic water use efficiency (WUEi) from the daily mean increased under $e[\text{CO}_2]$ relative to $a[\text{CO}_2]$ ($p < 0.001$) with a quantitative interaction only between measurement dates. Biochar amendment led to an overall increase in intrinsic water use efficiency ($p < 0.049$), though with opposite trends between the cultivars at the two measurement days (interaction: $p < 0.043$). The mean WUEi was $79.9 \mu\text{mol mol}^{-1}$, 55.7 under $a[\text{CO}_2]$ and 100.8 under $e[\text{CO}_2]$.

3.3. Productive and morphological parameters

Aboveground dry biomass straw mass and yield significantly increased under $e[\text{CO}_2]$ relative to $a[\text{CO}_2]$ (Fig. 5). Biomass increased by 38.4 % and 34.1 %, whilst grain yield increased by 33.7 % and 30.7 %, for Claudio and Aureo, respectively (Fig. 5). Biochar amendment led to a significant increase in yield, while it resulted in non-significant trends

for increased aboveground dry biomass and straw yield ($p = 0.078$ and 0.229 , respectively, Table 1). The biomass of cv Claudio increased by 7.5 % in response to biochar amendment under $a[\text{CO}_2]$ and by 9.8 % under $e[\text{CO}_2]$, whilst that of Aureo increased by 13.5 % under $a[\text{CO}_2]$ and by 4.5 % under $e[\text{CO}_2]$ (Fig. 5). Grain yield of Claudio increased in response to biochar amendment by 10.6 % under $a[\text{CO}_2]$ and by 12.2 % under $e[\text{CO}_2]$, whilst grain yield of Aureo increased by 16.2 % under $a[\text{CO}_2]$ and by 4.8 % under $e[\text{CO}_2]$. In all treatments the mass fractions of Claudio were higher than those of Aureo (Fig. 5). Biochar amendment and $e[\text{CO}_2]$ did not change HI, that was higher in Aureo (0.54 averaged across treatments) than in Claudio (0.51). Claudio reached a greater canopy height than Aureo with a partial gap reduction in $e[\text{CO}_2]$ treatment (Table 3). Although there were no significant treatment effects on canopy height, $e[\text{CO}_2]$ led to a trend for height increase ($p = 0.075$).

A marked increase in spike density resulted in response to elevated CO_2 as well as to biochar amendment (Tables 1 and 3), increasing by 63.2 % and 37.3 % under $e[\text{CO}_2]$ [B30] compared to $a[\text{CO}_2]$ [B0].

Biochar amendment led to a slight but significant increase in thousand kernel weight (TKW). The highest TKW was observed under $a[\text{CO}_2]$ [B30] for both cultivars (50.5 and 51.2 g for Aureo and Claudio, respectively, Table 3). Neither $e[\text{CO}_2]$ nor biochar amendment caused a significant change in grain dry matter (DM) per spike and grains per spike (Table 3). Peduncle length and spike length did not significantly

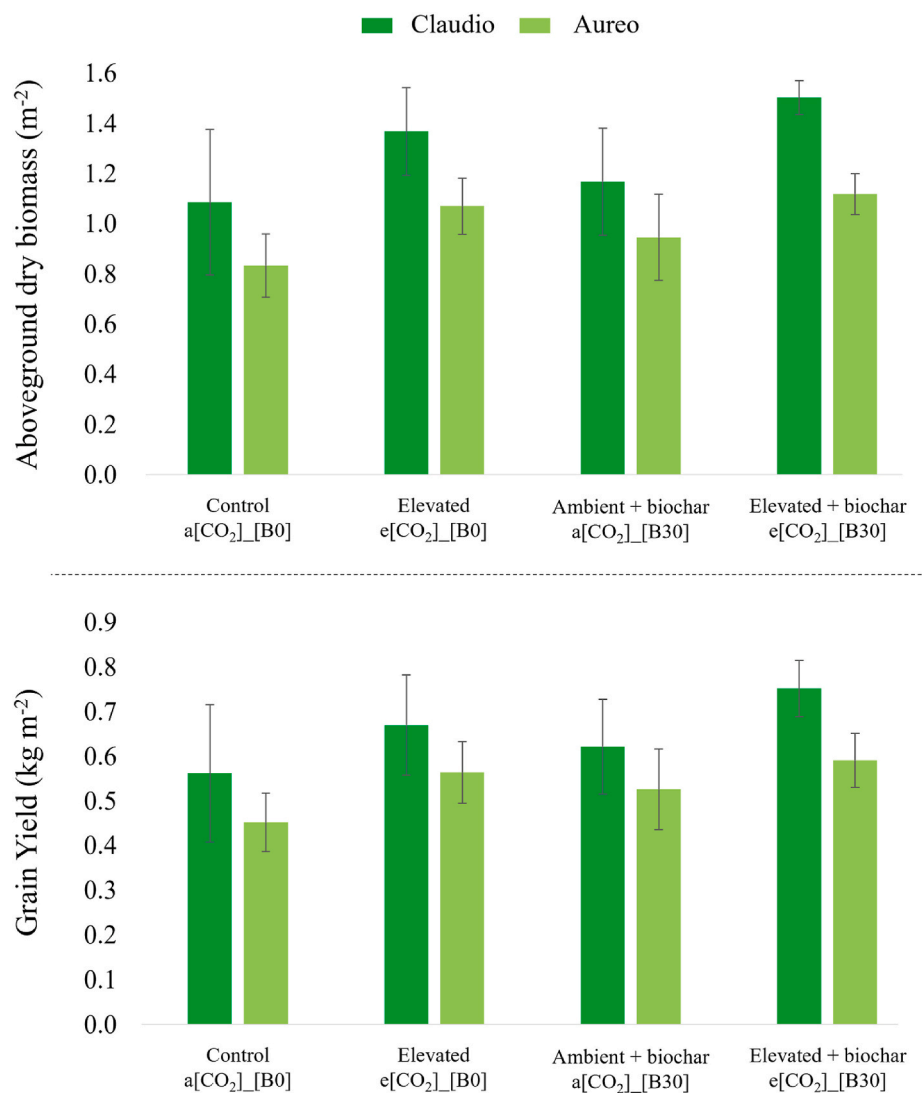


Fig. 5. Aboveground dry biomass and grain yield (kg m^{-2}) under all treatment combinations for Claudio (dark green histograms) and Aureo (light green histograms). Grey vertical bars represent standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3

Absolute values of morphological parameters and yield components under all four treatment combinations for Claudio and Aureo.

Cultivar	Variable	Unit	a[CO ₂] _[B0]	e[CO ₂] _[B0]	a[CO ₂] _[B30]	e[CO ₂] _[B30]
Claudio	Spike density	m ⁻²	293.7	371.9	360.7	479.3
Aureo			288.8	344.2	379.5	396.4
Claudio	Grain number	n° m ⁻²	11428	13804	12243	15367
Aureo			9226	11761	10487	12281
Claudio	HI	g g ⁻¹	0.52	0.49	0.53	0.5
Aureo			0.54	0.53	0.56	0.53
Claudio	TKW	g	49.7	49.2	51.2	49.5
Aureo			49.5	48.5	50.5	48.7
Claudio	Grain DM per spike		1.07	1.06	1.14	1.3
Aureo			1.05	1.1	0.83	1.2
Claudio	Grains per spike		37.9	34.9	36.2	37.8
Aureo			39.5	36.4	39.1	39.1
Claudio	Height	cm	88.5	93.7	89.5	92.7
Aureo			84	88.7	84.2	90.5
Claudio	Peduncle length		18.9	18.9	21.8	20.8
Aureo			18.4	21.9	20.3	20.9
Claudio	Spike lenght		5.5	5.8	5.3	5.8
Aureo			6.2	5.8	6	6.1

vary in response to the treatment factors. Spike length averaged across treatments was higher in Aureo (6 cm) compared to Claudio (5.6 cm) (Table 3). Grain number per unit area of Claudio was higher than that of Aureo in all treatment combinations. Biochar amendment and e [CO₂] led to increased grain numbers, significant only for the latter (p = 0.024, Table 1).

3.4. Grain quality

Overall, Aureo had superior quality traits relative to Claudio, with consistently higher grain nitrogen, protein, gluten, and carotenoid content, as well as gluten index and SDS sedimentation volume (Table 4).

Grain nitrogen content and Grain Protein Content (GPC%) significantly decreased under e[CO₂], and biochar amendment did not lead to significantly higher nitrogen and protein contents (Table 4). While nonsignificant main effects of e[CO₂] and biochar amendment were found for semolina gluten content, gluten index and SDS sedimentation volume, a significant interaction between CO₂ and biochar for gluten index and SDS as well as an interaction with p = 0.0697 for gluten content was observed (Table 1). In all these three cases the interaction was due to opposite effects of biochar amendment under e[CO₂] versus a [CO₂]. Biochar addition in a[CO₂] caused higher gluten content, higher SDS and lower gluten index, while the opposite was observed under e [CO₂]. Straw nitrogen content was always lower than 1 % and did not significantly vary between treatments and cultivars (Table 3). However, straw [N] was correlated with grain [N] (r = 0.87, p < 0.001) indicating a similar trend in response to the treatments (Table 1). Straw [C] tended to be lower due to biochar amendment (p = 0.052). More marked treatment effects were found for straw [H], that increased from 5.67 % on average under a[CO₂] to 5.91 % under e[CO₂].

The analysis of straw macro-element concentration revealed that biochar amendment did not lead to a significant change in macro-elements (p > 0.16 for all comparisons, n = 4, paired t-test) (Table 4). Iron and phosphorus concentrations were reduced under e[CO₂] by 18

and 47 % (p < 0.011, n = 4, paired t-test) while magnesium showed a non-significant trend (p = 0.099) for a reduction by 13 % (Tab 1 and S3). Calcium concentration in Claudio straw was 26 % lower than in Aureo straw (p < 0.002), potassium was 20 % lower (p < 0.004), while manganese was 32 % higher in Claudio as compared to Aureo (p < 0.001) (Tab. 1 and S3).

Biochar amendment did not lead to a change in grain $\delta^{13}\text{C}$ while it caused a slight increase in discrimination against $\delta^{13}\text{C}$ in straw. Straw $\delta^{13}\text{C}$ was more depleted in Claudio than in Aureo, while it did not differ between the cultivars in grains. Elevated CO₂ was associated with less negative $\delta^{15}\text{N}$ in straw as well as grains. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were more depleted in straw than in grains.

3.5. Multivariate analysis of traits related to production and quality

The first two axes of a principal component analysis (PCA) explained 48.34 % of the variability in 23 traits (Fig. 6) with major loadings of traits related to productivity on axis 1; while axis 2 was mainly determined by loadings of traits related to grain quality (Fig. S4).

The PCA separated e[CO₂] from a[CO₂] along a diagonal running from lower-left to upper-right except two plots of Aureo under e[CO₂] that overlapped with the polygons for a[CO₂]. Thus, the combination of productive and quality traits clearly separated the clusters of results for treatment/cultivar combinations. Claudio was separated from Aureo with higher and lower values on axis 1 and 2 respectively, consistent with the higher productivity and lower quality of Claudio relative to Aureo. Results indicated differences in the responses of productive and quality traits to the treatment combinations. Specifically, the productive traits showed overall additive effects (PC1), while qualitative traits resulted in an opposite way to biochar and [CO₂] (PC2).

3.6. Heatmap and cluster analysis

The clustering of response ratios of the treatment combinations including elevated CO₂ and/or biochar amendment relative to a[CO₂]

Table 4

Absolute values of qualitative parameters measured in straw and grain under all four treatment combinations for Claudio and Aureo.

Compartment	Cultivar	Variable	Unit	Control	Elevated	a[CO ₂][B30]	e[CO ₂][B30]
				a[CO ₂][B0]	e[CO ₂][B0]		
Grain and semolina	Claudio	GPC	%	13.4	12.8	14.2	12
	Aureo			15.8	13.9	15	13.4
	Claudio	Gluten content		8	7.8	8.8	7.5
	Aureo			9	8.9	9.7	8.6
	Claudio	Carotenoids		7.4	7.2	7.4	7.4
	Aureo			8.6	8.7	8.6	8.8
	Claudio	[N]		2.3	2.2	2.5	2.1
	Aureo			2.8	2.4	2.6	2.3
	Claudio	[C]		45.7	45.2	45.4	44.7
	Aureo			45.8	45.3	45.6	44.7
	Claudio	[H]		6.1	6.2	6	6.2
	Aureo			6.1	6.2	6.1	6.3
	Claudio	SDS	mL	3.7	3.7	3.85	3.5
	Aureo			3.8	4.3	4.5	4.2
	Claudio	Gluten	Index	91.5	87.8	81	97
	Aureo			94.3	95	92.3	97
	Claudio	$\delta^{13}\text{C}$		-27	-27.1	-27.3	-27.1
	Aureo			-27	-26.7	-27	-26.8
Straw	Claudio	$\delta^{15}\text{N}$		1.3	1.7	1.2	1.4
	Aureo			1.1	1.9	1	1.6
	Claudio	[N]	%	0.75	0.64	0.78	0.57
	Aureo			0.9	0.75	0.79	0.72
	Claudio	[C]		45.3	44.8	44.7	44.7
	Aureo			44.8	44.8	44.6	44.3
	Claudio	[H]		5.7	5.9	5.7	6
	Aureo			5.7	5.9	5.7	5.9
	Claudio	$\delta^{13}\text{C}$		-28.5	-28.2	-28.8	-28.5
	Aureo			-28.6	-28.5	-28.8	-28.3
	Claudio	$\delta^{15}\text{N}$		-0.67	-0.55	-0.9	-0.81
	Aureo			-0.3	-0.08	-0.9	-0.038

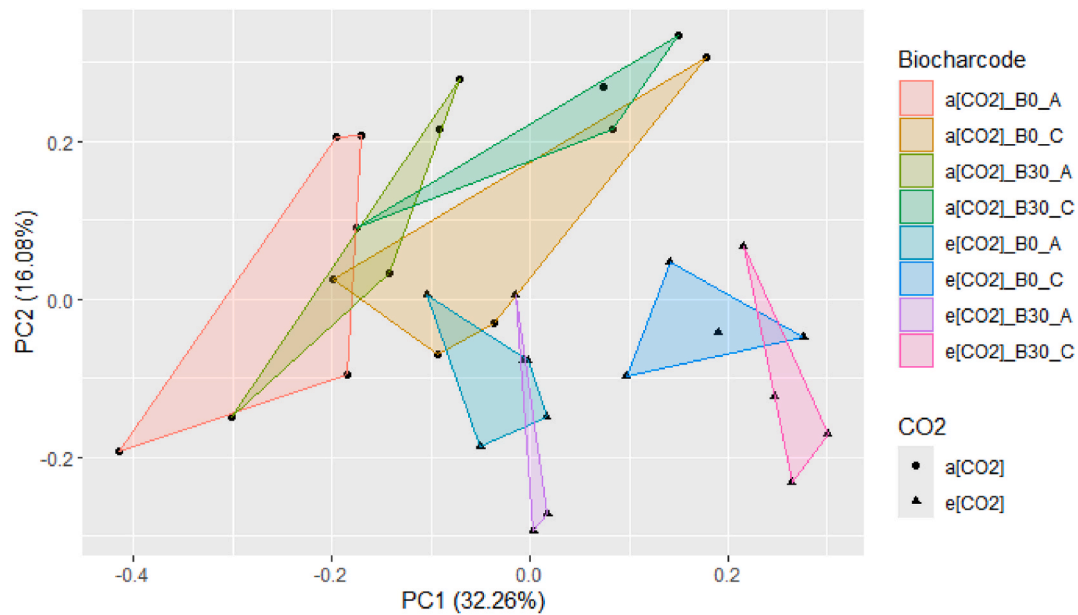


Fig. 6. Principal component analyses for 23 traits related to productivity and quality (see Fig. S4 for the traits used in these analyses and their loadings on PC1 and PC2). The abbreviations for the treatment combinations are made up by strings indicating ambient (a[CO₂]) or elevated (e[CO₂]) CO₂ treatment, biochar application (B30) or none (B0) and cultivar Aureo (A) or Claudio (C). Single data points derived from cultivation under a[CO₂] are shown with filled circles, while those derived from cultivation under e[CO₂] are shown with filled triangles. Clustering of the projections onto PC1 and PC2 separate the treatment combinations, shown with coloured polygons. Main contributions to PC1 come from biomass traits, height and plant density, while main contributions to PC2 come from stoichiometry, gluten content, gluten index and heading date.

_B0 was dominated by the contrast between ambient and elevated CO₂ (Fig. 7a) due to a general increase in biomass fractions. The allocation between straw and grain changed only marginally. Within the CO₂ treatments biochar amendment led to a further mass increase. Also, the conjoint analysis of response ratios of grain quality traits (Fig. 7b) showed a separate clustering of CO₂ treatments, mainly determined by decreased TKW, GPC and GC under e[CO₂], while biochar amendment mainly led to an increase of these quality traits under a[CO₂]. SDS and GI showed a more complex response pattern. The clusters for traits related to leaf gas exchange and pigments (Fig. 7c) were dominated by the decrease in stomatal conductance and the related increase in intrinsic water use efficiency under e[CO₂].

4. Discussion

The improvement of food crop resilience to climate change is one of the most important future challenges. In this study the agronomic performances in terms of production, morphological parameters and quality were assessed in two durum wheat cultivars, Claudio and Aureo, cultivated under open field conditions with and without biochar addition to soils at both ambient and elevated CO₂. Biochar addition affected surface soil temperatures during periods of partial canopy cover, leaf pigmentation, leaf gas exchange, growth and biomass production, as well as quality of grains and straw.

The vegetative growth period proceeded under exceptionally moist conditions. High soil moisture during wintertime, concomitant with three periods of snow cover lasting for several days, led to winter damage as traced by observations of senescent leaves, dead plants, and a decrease in NDVI (Fig. 3b). As no frost temperatures occurred below the level that can be expected to lead to frost damage [62], the most probable, putative cause of winter damage was anoxia due to waterlogging under snow cover (Fig. S3), with Aureo more affected by this winter stress than Claudio (Table 1). A trend for lower winter damage under B30 indicates that biochar addition might have mitigated the effect of waterlogging. Such an effect was also reported by Ref. [63] on four-week-old soybean plants under experimental waterlogging where a

reduced root and shoot mass losses was associated to biochar treatment. The beneficial effect of biochar under waterlogging may be related to the documented increase in saturated hydraulic conductivity in fine textured soils [64]. An elevated probability of waterlogging conditions in the western part of the Po valley has been shown by Ref. [65] who also reviewed experiments on waterlogging effects on wheat.

4.1. Productive and morphological parameters

Biochar amendment led to an increase in flag leaf chlorophyll and a decrease in flavonoids and in consequence an increase in NBI (Table 1). The change in chlorophyll content was in the same order of magnitude as that observed by Ref. [66] in combination with different fertilization regimes and [67] at different soil salinity levels. Again [68,69], found increased chlorophyll contents in drought stressed barley with biochar amendment. Increased chlorophyll content and NBI due to biochar amendment were also reported by Ref. [70] in soybean.

Light saturated flag leaf photosynthesis of the cultivar Claudio had completely acclimated to e[CO₂]; it was on average 4.5 % lower under e[CO₂] relative to a[CO₂]. As opposed to this net photosynthesis of the cultivar Aureo increased by 16.9 % on average in response to e[CO₂]. Thus, Aureo photosynthesis had only partially acclimated to e[CO₂] relative to an expected instantaneous response of ca. +30 %. These results corroborate the results obtained with a preceding FACE experiment at Fiorenzuola in which Claudio photosynthesis had completely acclimated and Aureo showed a stimulation above 10 % [62]. The most evident response in leaf gas exchange was a decrease in stomatal conductance and a corresponding increase in WUEi in response to e[CO₂] (Fig. 7c). Small, non-significant, increases in photosynthesis and decreases in stomatal conductance due to biochar amendment led to a further increase in WUEi.

Overall, the response of leaf pigmentation and gas exchange to the treatment combinations was dominated by [CO₂], as evidenced by the clustering shown with Fig. 7c. Biochar amendment slightly improved the leaf nitrogen balance and partially compensated for photosynthetic acclimation.

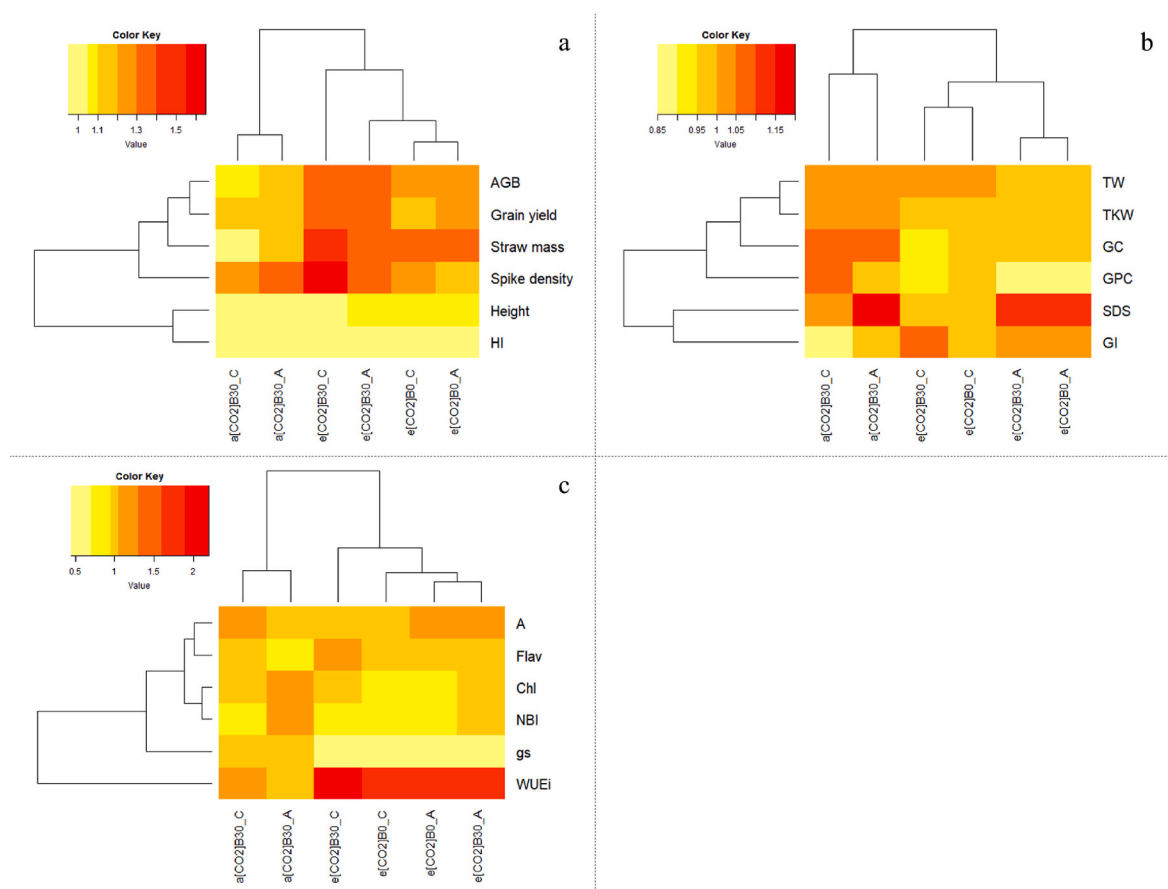


Fig. 7. Response ratios of (a) biomass and plant dimensions, (b) qualitative traits and (c) photosynthesis and leaf pigments. The labels on the x-axis stand for the response ratios, always relative to the treatment $a[CO_2]B0$ and the cultivar examined, e.g. $e[CO_2]B30_C$ labels the column with the response ratio $e[CO_2]B30/a[CO_2]B0$ for cultivar Claudio. Abbreviations: AGB - aboveground biomass, HI - harvest index, TW - test weight = hectoliter weight, TKW - thousand kernel weight, GC - gluten content, GPC - grain protein content, SDS - SDS sedimentation volume, GI - gluten index, A - net assimilation rate, Flav - leaf flavonoid content, Chl - leaf chlorophyll content, NBI - leaf nitrogen balance index, gs - stomatal conductance, WUEi - intrinsic water use efficiency.

Wheat biomass and grain yield were higher under $e[CO_2]$ (biomass: +27.0 % and +36.5 % for $e[CO_2]_B0$ vs control and $e[CO_2]_B30$ vs control, respectively; yield: +21.6 % and +32.4 % $e[CO_2]_B0$ vs control and $e[CO_2]_B30$ vs control, respectively), with the effect of biochar addition being higher under $a[CO_2]$ (+10.1 % and +13.1 % on average, for biomass and yield respectively) rather than under $e[CO_2]$ (+7.5 % and +8.8 % on average, for biomass and yield respectively). The increase in yield due to $e[CO_2]$ was higher than in the preceding year of FACE experimentation at Fiorenzuola, when it had increased by 10 and 15 % for Aureo and Claudio, respectively [71], corroborating the higher grain yield increase of Claudio compared to Aureo. The aboveground biomass of the bread wheat cultivar Bologna increased by 23 % and 27 % under $e[CO_2]$ in the two experimental years [72]. These results agreed with large part of literature [8–10,12,73]. Also [6], indicated an average grain-yield increase of 15 % in a meta-analysis on bread wheat grown at $e[CO_2]$ in FACE experiments. In their meta-analysis [12], reported an average wheat yield increase of 26 % under $e[CO_2]$ (605 ppm), whilst in a meta-analysis of the elevated CO_2 effects (450–800 ppm) on wheat ecophysiology [74], observed an average increase in grain yield by 24 %. These patterns were explained by the well-known plant growth enhancement due to elevated levels of CO_2 concentrations, which generally promote net CO_2 assimilation rate and improve water use efficiency (WUE) [28,74–76]. However, despite a generally observed CO_2 -driven increase in yield, crop response can vary depending on intrinsic genomic characteristics, soil properties and management [6, 77]. Again [11,78], reported that variation in the response of wheat yield to $e[CO_2]$ were due to environmental factors including air

temperature, solar radiation, water availability and nutrient supply. Other studies [12,74,79] further suggested that crop productivity increases in regions with low agronomic productivity but sufficient nutrient supply.

Timing of phenological phases and morphological data were found respectively slightly anticipated and higher in plots treated with biochar, where the surface soil temperatures resulted by approximately +1 °C compared to the untreated plot (Table S2), increasing the minimum night-time (19 p.m.–7 a.m.) soil temperature and the daytime soil temperature (Fig. S5). These results were primarily due to the reduced soil albedo following biochar application as previously reported by Refs. [64,80–85]. Higher soil temperature putatively influences absorption, transportation, and storage of water and mineral in roots, and lead to higher and earlier plant growth [54,86].

The effect of biochar addition to crop productivity is the result of physical-chemical-biological processes induced by the amendment within the soil-plant system. In the current study biochar application led to soil warming (Fig. 1), thus likely increasing soil microbial activity and indirectly enzyme activity and nutrient cycling [87,88].

Grain yield of both cultivars increased in response to biochar amendment under ambient as well as elevated CO_2 (Fig. 5), while straw yield did not significantly change. The sum of both resulted in a trend for increased aboveground biomass. The increase in crop productivity that follows biochar application is widely reported in the literature. For instance Ref. [52], reported a 10 % increase of durum wheat yield after 10 t ha⁻¹ of biochar application in Central Italy, and [53] observed an increase of up to 30 % on wheat biomass production and yield applying a

large volume of biochar (30 and 60 t ha⁻¹) [89], showed that biochar application increased water use efficiency, growth, and yield in wheat under drought stress conditions [90]; reported that biochar stimulated fine root proliferation in wheat, resulting in higher relative growth, aboveground biomass, and yield than in control plants [91]. found a plant growth improvement, especially in early growth stages, when biochar from wheat straw was applied because of higher electrical conductivity, available K and Na, and increased dissolved Na⁺, K⁺, and Cl⁻ ions in pore water [54]. indicated a 20–30 % increase in wheat grain yield mixing biochar with high mineralization rate to a nutrient-poor acidic loamy sand soil, whilst [92] showed that a relatively high volume of biochar (5–20 t/ha) increased wheat grain yield by 2.9–19.4 %.

In our experiment, the relative biomass allocation to seeds (i.e., harvest index, HI) significantly differed only between cultivars (Table 1). Though, it slightly decreased under e[CO₂] and slightly increased with B30 (Table 3). The lack of significant changes in HI as well as TKW in response to e[CO₂] in our experiment is consistent with the results of a meta-analysis for wheat [12]. A combined additive effect of biochar and e[CO₂] was observed for spike density and grain number, while TKW increased due to biochar amendment (Tables 1 and 3). The increase in grain number was also reported with the meta-analysis of [12]. [26] reported increased spike density and grain yield also for a bread wheat cultivar in 3 years of FACE experimentation at Fiorenzuola, while TKW decreased under e[CO₂]. The traits related to single spike dimensions did not significantly respond to the treatment combinations, suggesting that some intrinsic crop parameters were not affected by treatments.

Overall, the observed changes in NDVI (Fig. 2), flag leaf photosynthesis (Tables 1 and 2) and yield components (Table 3) indicate a major role of crop performance in the vegetative growth phase in determining the stimulation of biomass and grain production by elevated CO₂ and biochar. A higher number of stems (i.e. tillers) and grains per square meter testimony the enhanced mass accumulation in this growth phase, while the downregulation of photosynthesis in flag leaves due to e[CO₂] and its only partial compensation by biochar suggests a less important role of enhancement of leaf carbon assimilation in the phase of grain maturation. Note that downregulation was stronger in the more productive cultivar Claudio. In an agronomic year without marked high temperature or drought stresses during grain maturation, the plants kept pace with filling grains to nearly equal TKW in the diverse treatment combinations. A slight reduction in TKW under e[CO₂] was nearly compensated for by an increase due to biochar. Globally, several parameters differed significantly between the two cultivars, leaving space for a deeper exploration of cultivar-specific responses. This suggests the need to conduct multi-year experiments that would help assess the sustainability of biochar application and its cumulative effects on soil health and crop performance.

4.2. Wheat quality

The knowledge of effects of e[CO₂] on wheat quality is essential for coping with the threats of climate change but still incomplete, with scientific results showing discrepancies depending on soil properties, management, and cultivars.

The drop in GPC under e[CO₂] observed with the current study (Table 4) was in line with most of the results documented in the literature [14,26–28,93,94]. The decline in protein concentration observed in Aureo cv (>12 %) and Claudio cv (~5 %) at e[CO₂] fell into the range (3.5–14.3 %) reported by several studies [13,14,16,17,95–99]. Under all treatments the average protein concentrations of both Aureo cv (14.5 %) and Claudio cv (13.1 %) were higher than 11.5 %, which is the threshold suggested by Ref. [77] and similar studies [14,18] as adequate quality for bread-making, and above the suggested threshold (i.e., 12–13 %) known to ensure a good pasta quality [100]. However, in the highly productive, preceding year of FACE experimentation at Fiorenzuola GPC of Claudio had dropped below 11.5 % under e[CO₂] [71] that in turn,

had negatively affected the firmness value of cooked pasta [27].

Biochar amendment did not lead to a significant compensation of the decrease in GPC. Interestingly, for three further traits related to grain quality, i.e. GC, GI and SDS, a qualitative interaction was found (Table 1). GC and GI increased due to biochar amendment under a[CO₂] and decreased under e[CO₂], while the opposite was the case for SDS (Table 4). In summary, biochar amendment did not lead to a complete compensation of the negative effects of e[CO₂] on grain quality. However, the effects on gluten evidence quality improvements due to biochar under a[CO₂]. Separated ANOVAs per cultivar indicated significant CO₂ and biochar effects on grain [N] and GPC. However, when adding grain yield as an additional covariate the null hypothesis of equal trait values was not rejected any more, indicating that a dilution effect is a candidate for the most parsimonious hypothesis on the cause of decrease in [N] and GPC, as previously indicated by Ref. [27]. Specifically, higher biomass production and yield due to biochar application through the process of soil temperature increase [101], which contributed to enhance soil microbial activity and indirectly enzyme activity and nutrients availability, diluted the [N] and GPC content thus resulting in lower values of qualitative traits.

The differences in the responses of productive and quality traits to the treatment combinations mirror the result obtained with PCA (Fig. 6), with traits related to production showing overall additive effects preferentially loading on PC1, while traits related to quality often responded in opposite ways to biochar and [CO₂] and preferentially loaded on PC2 (Fig. S4). Although treatment effects on photosynthesis, production and quality were all dominated by the change in [CO₂], the clustering within e[CO₂] first separated B0 from B30 for productive traits, while it first separated cultivars for photosynthesis and quality traits (Fig. 7). In this study biochar showed its limitations to alleviate nitrogen reduction in grains and straw and increase grain protein content under e[CO₂], however, its potential has not been evaluated against all possible qualitative traits such as amino acid composition and gluten quality. In this perspective, future studies may include a wider range of qualitative traits to provide a more comprehensive assessment of biochar's impact on winter wheat cultivars. Also, comparative analyses across different geographic and climatic regions should be conducted, thus making more generalizable the findings of this study.

4.3. Nitrogen yield and agronomic nitrogen use efficiency

Although e[CO₂] had led to a decrease in [N], grain and above-ground nitrogen yield did not change. The increase in biomass due to both CO₂ and biochar was accompanied by a slight increase in the amount of nitrogen taken up by the crops (17.5–20.1 g m⁻² under e[CO₂] versus 15.9–19.5 g m⁻² under a[CO₂]). Although biochar did not completely compensate for N reduction under e[CO₂], biochar amendment tended to increase total grain N (totgrainN) and straw nitrogen yield (totABGN) by 8.7 and 6.7 %, respectively (Fig. S4). Also [92], observed an increase in N use efficiency by 5.2–37.9 % in wheat when high volumes of biochar (5–20 t/ha) were applied. They found a concomitant relatively higher total amino acid content (5.21–9.1 %). The nitrogen yield measured for the different treatment combinations in our experiment correspond to an agronomic nitrogen use efficiency between 68 % for Aureo under a[CO₂].B0 and 86 % for Claudio under e[CO₂].B30 calculated on aboveground biomass basis. Biochar amendment increased agronomic NUE by 6.6 % on average. These trends, together with the slight positive effects on grain quality, discussed above, indicate that biochar amendment led to the expected improvement in nitrogen status, although far from completely compensating the negative effects of e[CO₂] on grain quality. By contrast, as discussed in the last paragraph of section 4.1, a chain of biochar effects including improvement of flag leaf performance, with the expected effect of increased spike density and grain number, while single spike dimensions remain unchanged and better grain filling, translates the improved nutrient status due to biochar into biomass increases. This aspect of

biochar effects adds onto the $e[CO_2]$ effects. The increase in carbon yields under biochar amendment (Fig. S6) with increasing nitrogen yields confirms the stimulation of carbon assimilation. Furthermore, the increase in C/N ratios under biochar amendment, with exception for cultivar Claudio under $a[CO_2]$ confirm the dominance of translation of increased nitrogen uptake by the plants into a stimulation of biomass production. In this perspective, biochar appears to be a suitable solution for crop production increase, alternative to N-fertilization based strategies, while it only marginally improves crop quality. However, further studies are needed to explore biochar by CO_2 interactions under different environmental conditions (including high temperature and drought stress) and soil fertility regimes. While the study showed positive impacts on yield and some qualitative traits, the sole one-year of data did not allow to explore potential long-term effects of biochar application on soil microbial activity or carbon sequestration. Given that these processes play a key role for crop system performance at small and large scale (i.e., climate mitigation), incorporating mechanistic studies (i.e., soil nutrient cycling and plant physiological responses) would provide a deeper understanding of biochar's role under varying CO_2 conditions.

4.4. Biochar - mitigation and adaptation options

In the climate-change era, the transition towards a major sustainability of agriculture is needed to provide major benefits not only to agricultural farms, industries and related workers, but also civil society and natural environment. As a broad-based concept rather than a specific methodology, sustainable agriculture indeed emphasizes the integration of environmental health, economic profitability, and social equity [102]. However, the shift from conventional to sustainable agriculture not only relies on research and innovation of technologies and methods able to facilitate this change, but also on the increase of knowledge about the agricultural-environmental interactions. Sustainable agriculture indeed, must address a two-fold challenge, mitigate emissions while adapting production systems to changing climates and enhanced climate variability. In some way, these two aspects can appear in contrast to each other, since emissions reduction may suggest the need to decrease agricultural inputs that, however, are the main drivers for crop productivity. Also, climate mitigation includes soil carbon storage increase, which may suggest the need to change the typical management applied in crop systems, thus affecting the current balance of carbon and/or nitrogen cycles and in turn crop productivity. However, as suggested by Ref. [103], there is a space for synergies between the two measures, with some adaptation options having positive impacts on mitigation, such as measures that reduce and leaching of nitrogen and phosphorus, soil moisture conservation, use of organic amendment, minimum tillage, etc. Among these, the use of biochar has a considerable potential to improve soil fertility and crop productivity while increasing C sequestration in soils [104]. In the perspective of climate change adaptation options, biochar addition can enhance the efficiency of agricultural inputs, soil water use efficiency, and reduce nutrient leaching and runoff, thus improving soil structure and the related microbial and chemical properties, finally resulting in a generalized increase in the resilience of different agricultural systems. At the same time, biochar application can reduce soil degradation and N fertilizer use, avoid CH_4 emissions from biomass decay and increase stable C pools, thus reducing the overall GHG emissions from a system. The thorough evaluation of benefits of biochar application for agriculture and environment in Europe by Ref. [105] evidenced a potential to compensate for 15–35 % of agricultural GHG emissions. However, biochar application often is not profitable if external benefits are not internalized through market instruments [106]. The linkages between mitigation and adaptation adopting biochar are still not completely explored, leaving space for investigation to better quantify biochar effects on crop production and mitigative efficiency. Findings achieved in this study should be considered therefore as a starting point to develop

novel studies integrating $e[CO_2]$ with biochar application, so as to gather further knowledge to develop and implement specific agricultural practices aimed to provide environmental and crop productivity benefits.

5. Conclusions

In this study the use of biochar was tested as possible win-win strategy able to increase crop production and maintaining elevated qualitative parameters compared to the control. The novelty of this approach raises from the fact that, to our knowledge, no study was carried out by integrating biochar application with $e[CO_2]$ on wheat cultivation to assess changes in productive, morphological, and qualitative traits. Results indicated that biochar influenced soil properties, particularly increasing soil temperatures of $+1\text{ }^\circ\text{C}$ along the season, thus resulting in earlier phenology which can partly explain plant growth and higher biomass and grain production. For cv Claudio and Aureo, the highest biomass ($+38.4\%$ and $+34.1\%$) and yield ($+33.7\%$ and $+30.7\%$) compared to the control were found at $e[CO_2]$ [B30]. The biochar effect [B30] was clearly observed when compared with related untreated growing conditions (Ambient and Elevated), since biochar amendment led to an average increase in both biomass ($+10.5\%$ and $+7.2\%$) and yield ($+13.4\%$ and $+8.2\%$). Biochar amendment led to an increase in flag leaf chlorophyll and a decrease in flavonoids and in consequence an increase in NBI. Small, non-significant, increases in photosynthesis and decreases in stomatal conductance due to biochar amendment led to a further increase in WUEi. By contrast, biochar application did not influence morphological parameters, with the only exception for spike density, falling number and TKW, nor qualitative traits, resulting unable to increase/maintain GPC content or alleviate N reduction in grain yield and straw of both cultivars. Globally, biochar adoption showed a very limited effectivity in counteracting the decrease in qualitative traits that are typical of elevated atmospheric CO_2 concentration environments. Accordingly, biochar use could be preferentially adopted for increasing the productivity of those high-quality cultivars (i.e. grains with high GPC) whose destination and industrial processing would not suffer from a slight decrease in quality parameters.

CRedit authorship contribution statement

Lorenzo Brilli: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Franz-Werner Badeck:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lorenzo Genesio:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Silvia Baronti:** Writing – review & editing, Investigation. **Fulvia Rizza:** Writing – review & editing, Investigation. **Clara Fares:** Writing – review & editing, Investigation. **Luigi Cattivelli:** Writing – review & editing, Funding acquisition. **Franco Miglietta:** Methodology, Funding acquisition. **Anita Maienza:** Writing – review & editing, Investigation. **Alessandro Zaldei:** Methodology. **Federica Camin:** Writing – review & editing, Investigation. **Rosella Motzo:** Writing – review & editing, Investigation. **Piero Toscano:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jafr.2025.101719>.

Data availability

Data will be made available on request.

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