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Effects of Elevated CO_2 on the Photosynthesis, Chlorophyll Fluorescence and Yield of Two Wheat Cultivars (*Triticum aestivum* L.) under Persistent Drought Stress

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Abstract: The interactive effects of elevated [CO_2] and drought on leaf photosynthesis, physiology and yield in wheat (*Triticum aestivum* L.) are not well understood. This study evaluated the effects of persistent drought stress (35–45% of field water capacity) and elevated CO_2 (ambient concentration + 200 μmol mol^{-1}) on leaf photosynthesis, chlorophyll fluorescence, stress physiological indices, biomass, and grain weight (in g m^{-2}) in two wheat cultivars (large-spike cultivar Z175 and multiple-spike cultivar Triumph) at the open-top chamber (OTC) experimental facility in North China. We found that elevated [CO_2] enhanced the positive effects of drought on F_v/F_m and WUE but did not ameliorate the adverse effects of drought on P_N in the two cultivars. Moreover, as a large-spike cultivar, Z175 showed enhanced photosynthesis performance and sink capacity (spike number and kernel number per spike) compared with Triumph in the grain filling stage under elevated [CO_2], which helped counteract the adverse effects of drought. In contrast, although Triumph had more tillers and spikes at the current [CO_2] concentration, most of them were thin and had limited photosynthesis capacity. The photosynthesis capacity of leaves on the main shoot and the spike number did not significantly increase in Triumph under elevated [CO_2]. Hence, elevated [CO_2] mitigated drought-induced inhibition of grain weight in Z175 plants but not in Triumph plants under persistent drought stress.

Keywords: elevated [CO_2]; persistent drought; photosynthesis; yield; wheat

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1. Introduction

Rising atmospheric CO_2 concentrations ([CO_2]) and droughts are predicted to impact crop growth and yield [1–3]. Elevated [CO_2] generally reduces stomatal conductance (g_s) [4,5], increases CO_2 in chloroplasts, enhances the Rubisco carboxylation rate and, as a result, increases WUE and drought resistance [6–8]. Some studies have shown that elevated [CO_2] mitigates the impact of drought stress on maize [9,10], barley [11], *Arabidopsis thaliana* [8], grassland species [12] and bread wheat varieties [11,13,14]. However, elevated [CO_2] cannot compensate for water stress in some plants, such as soybean [3] and native and exotic riparian plants [15].

Wheat (*Triticum aestivum* L.) is one of world's most important food crops. Long-term FACE (Free Air CO_2 Enrichment, FACE) experiments in Arizona in the United States and in Beijing in China have shown that elevated [CO_2] could increase the yield of winter wheat by 25–28% under persistent moderate drought conditions [16,17]. This yield increase is more than the 11–19% increase reported to result from irrigation conditions [16,17]. However, the results from FACE experiments in Australia showed that elevated [CO_2] increased the winter wheat yield more under irrigation conditions than under drought conditions [18,19].

Therefore, the responses of plant growth to elevated [CO_2] and persistent drought are very distinct between the environment, different species, soil conditions, etc.

The wheat yield in China accounts for one-fifth of the world's total each year. Thus, variations in wheat productivity in China would cause world-wide fluctuations in wheat yield under future climates. Moreover, drought is one of the key factors limiting plant productivity in northern China and semiarid regions throughout the world. Currently, research on the interactive effects of elevated [CO_2] and persistent drought on wheat in North China is lacking. The first objective of the present study is to investigate the effects of elevated [CO_2] and persistent water-deficit conditions on the photosynthesis, antioxidative ability, chlorophyll fluorescence, WUE and yield of two wheat cultivars. The second aim is to investigate interspecies differences in large-spike and multi-spike wheat in response to increased [CO_2] and drought stress. This study will provide insights into wheat crop adaptation and management under future climate change.

2. Materials and Methods

2.1. Experimental Design

The growth experiments were carried out within an open-top chamber facility (OTC) at the experimental station of Shanxi Agricultural University (37.42° N, 112.55° E) in Taigu, Shanxi, China. Air temperature and relative humidity inside the chambers were monitored throughout the growing season. CO_2 concentrations of 400 and 600 µmol mol^{-1}, relating to the control and elevated [CO_2] level ($e$$CO_2$), respectively, were generally maintained within the two growth chambers from seedling emergence to harvest (Figure 1). We did not control the CO_2 concentration in the chambers when the daily minimum temperature was below zero (11 December 2013–15 February 2014 and 11 December 2014–9 February 2015). The facility operational procedures were described in Hao et al.'s [20] work. Two winter wheat cultivars, cv. Zhongmai 175 (Z175) and cv. Triumph, were selected for the experiment. Z175 was released in 2012 by the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences. As a large-spike cultivar, Z175 has a thick shoot diameter, reduced tiller number, shorter plants, and higher harvest index. Triumph, a multi-spike cultivar introduced from the USA 70 years ago, has taller and thin shoots, more tillers and lower harvest indices. A total of 60 seeds of wheat were sown on 8 October 2013 and 10 October 2014 in polyethylene containers (60 cm × 40 cm × 28 cm). There were nine replicates per treatment per year.

Polyethylene containers were filled with clay loam soil, surface soil (0–20 cm) of nearby cropland, with a pH (1:5 soil:water) of 8.1 and contained 1.38% organic carbon (C) and 0.11% total N. Fertilizers were applied at sowing at rates of 1.73 g N and 1.31 g P per pot. The two following soil water conditions were maintained in each chamber after 1 March 2014 and 2015 with nine replications in each growth chamber: well-watered (60–80% relative water content (RWC)) or droughted (35–45% RWC). The drought and genotype treatments were randomized within each growth chamber. The soil moisture content was measured with a wet sensor (TZS-1; Zhejiang TOP Cloud-agri Technology Co., Ltd., Zhejiang, China) and maintained at the targeted moisture regimes through daily irrigation during the growth period.

2.2. Gas Exchange Measurements

Leaf photosynthetic gas exchange measurements were carried out using an open gas exchange system (LI-COR 6400; Lincoln, NE, USA) at the booting stage and grain filling stage. Healthy flag leaves of the primary tillers were selected per pot. Measurements were conducted from 09:00 to 11:30 using the methods described by Hao et al. [20]. The photosynthetic photon flux density (PPFD) was set at the saturating light level (1500 µmol m^{-2} s^{-1}), and the leaf chamber temperature was constant, at 25 °C. The leaf chamber CO_2 concentration was set to 400 µmol mol^{-1} for CK OTC and 600 µmol mol^{-1} for HCO_2 OTC. The light-saturated net photosynthesis rate (P_N), stomatal conductance (g_s), and transpiration rate (E) were recorded automatically, and the water use efficiency (WUE) (WUE = P_N/E) was calculated.

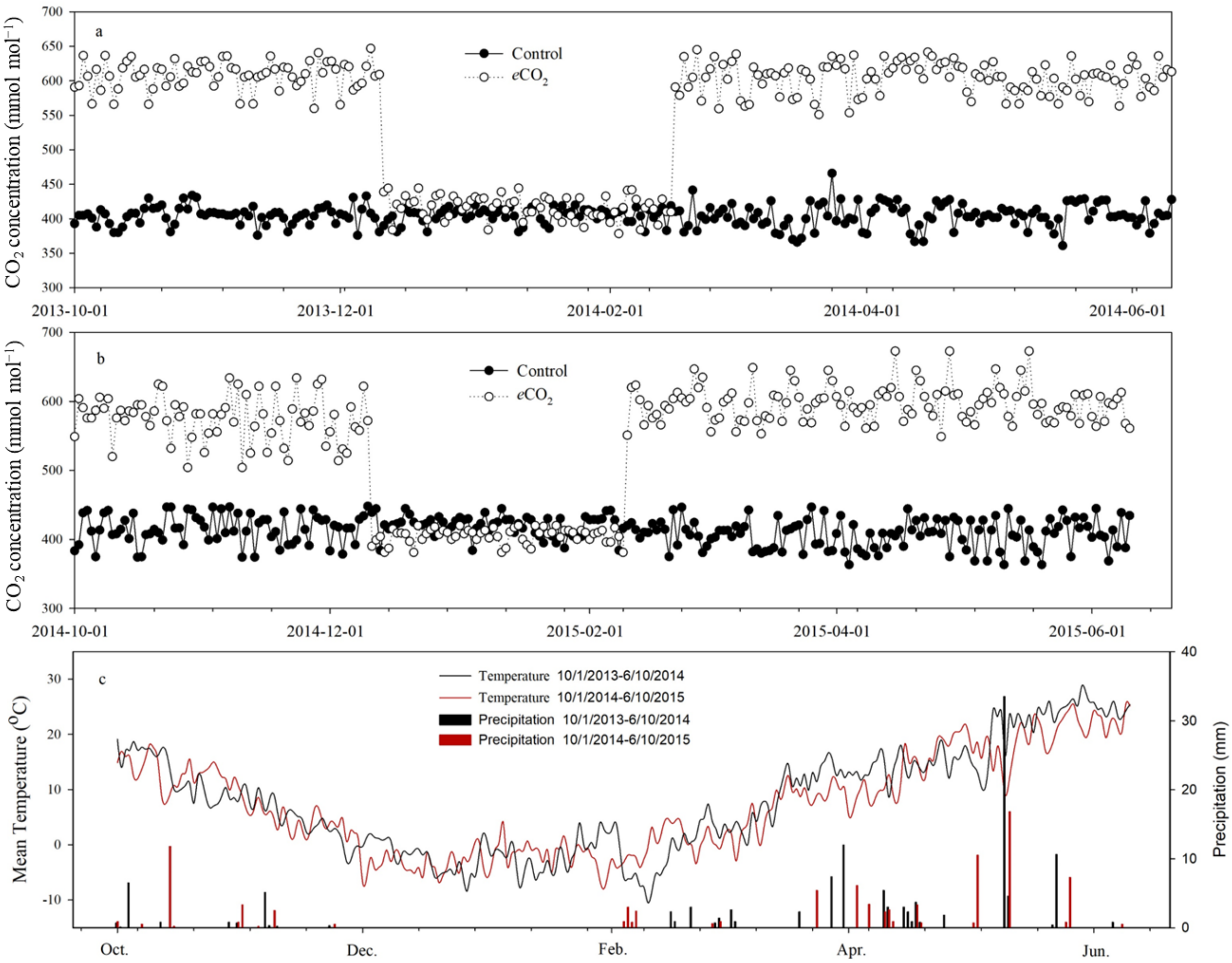

Figure 1. The CO_2 concentration (**a**,**b**) and air temperature (**c**) in open-top chamber of wheat growing season on 1 October 2013–1 June 2014 and 1 October 2014–1 June 2015. Control—ambient atmospheric CO_2 concentration; eCO_2—elevated atmospheric CO_2 concentration.

2.3. Chlorophyll Fluorescence

One flag leaf of the primary tillers per pot was selected for the measurement of chlorophyll fluorescence using a miniaturized pulse-amplitude modulated fluorescence analyzer (PAM-2000, Walz, Effeltrich, Germany) on the same day as the gas exchange measurements. The initial fluorescence yield of the light-adapted state (F_0'), maximal fluorescence yield of the light-adapted state (F_m'), steady-state fluorescence yield (F_s), initial fluorescence of the dark-adapted state when all reaction centers were open (F_0), and maximal fluorescence of the dark-adapted state when all reaction centers were closed after illumination (F_m) were measured using the methods described by Wang et al. [21]. The effective quantum yield of PSII photochemistry (Φ_{PSII}), maximal quantum yield of PSII photochemistry (F_v/F_m), intrinsic efficiency of PSII (F_v'/F_m'), photochemical quenching coefficient (qP), and nonphotochemical quenching of variable chlorophyll fluorescence (NPQ) were determined as described by Kramer et al. [22].

2.4. Determination of Photosynthetic Pigment Content, Malondialdehyde (MDA) and Peroxidase (POD)

Flag leaves of wheat plants from three pots (out of the nine replicate pots) in each chamber were cut and immersed in liquid nitrogen and kept in a freezer at −80 °C for the analysis of the photosynthetic pigment, MDA and POD contents at the booting stage and grain filling stage. Chlorophyll a, chlorophyll b, and carotenoid contents were estimated following the protocol provided by Arnon [23]. MDA content was estimated using the thiobarbituric acid (TBA) test following the methods of Heath and Packe [24]. The activity of POD was determined as enzyme units per gram fresh weight (U/g fw) according to Sakharov and Aridilla's method [25].

2.5. Harvesting

At maturity, wheat plants were harvested on 13 June 2014 (249 days after sowing) and 11 June 2015 (245 days after sowing). After air-drying, the spike number per square meter, number of kernels per spike, thousand seed weight, biomass and yield were determined.

2.6. Statistical Analysis

A randomized full block design was employed in a factorial arrangement with two CO_2 treatments, two cultivars, and two water treatments. The standard errors were calculated using the ANOVA table's pooled error term. Treatments were compared using Duncan's multiple range tests at $p = 0.05$ in SPSS software. Percentage was used to evaluate the impact of elevated [CO_2] or drought on gas exchange parameters, chlorophyll fluorescence, photosynthetic pigment content, MDA and POD concentrations, and biomass and yield. The denominator is the difference between the sum of two water statuses, two cultivars, and two growth stages under elevated [CO_2] and the sum of that under atmospheric [CO_2] (Control). The numerator is the sum of two water statuses, two cultivars, and two growth stages under atmospheric [CO_2].

3. Results

3.1. P_N and Gas Exchange Parameters

Elevated [CO_2] significantly increased P_N (by 20.4%) and WUE (by 65.6%) but decreased g_s (by 37.3%) and E (by 30.2%) in 2015. Drought significantly decreased P_N (by 45.7%), g_s (by 48.9%) and E (by 41.0%) but increased WUE by 57.7%. The interaction of CO_2 and drought on P_N and WUE was significant ($p < 0.05$), but not for g_s or E. Elevated [CO_2] enhanced the positive effects of drought on WUE and the negative effects of drought on P_N. Specifically, the decrease in P_N through the eCO_2 × drought interaction (−7.05 = 9.44 − 16.49) was significantly greater than that resulting from drought (−6.98 = 9.51 − 16.49) and eCO_2 (4.87 = 21.35 − 16.49). The increase in WUE through the eCO_2 × drought interaction (3.69 = 6.85 − 3.33) was significantly greater than that resulting from drought (1.01 = 4.34 − 3.33) and eCO_2 (1.92 = 5.25 − 3.33). Cultivar, CO_2 and drought had no interactive effects on P_N, g_s, E or WUE (Table 1). Therefore, elevated CO_2 had a positive effect on increasing WUE but had little effect on improving P_N. Compared with Triumph, Z175 had higher g_s and P_N under well-watered conditions at both the booting stage and grain filling stage. Moreover, under elevated [CO_2] and drought conditions, Z175 had a higher P_N than Triumph, even though the g_s was low, especially at the booting stage.

3.2. Chlorophyll Fluorescence

Elevated [CO_2] had no effect on the maximal quantum yield of PSII photochemistry (F_v/F_m), intrinsic efficiency of PSII (F_v'/F_m'), effective quantum yield of PSII photochemistry (Φ_{PSII}), photochemical quenching coefficient (qP) or nonphotochemical quenching (NPQ). Drought significantly decreased F_v'/F_m' (by 4.60%) and Φ_{PSII} (by 10.9%) but increased NPQ by 13.8%. CO_2 and drought had positive interactive effects on F_v/F_m ($p < 0.05$) but no interactive effects on F_v'/F_m', Φ_{PSII}, qP or NPQ. The increase in F_v/F_m through the eCO_2 × drought interaction (−0.0033 = 0.8081 − 0.8049) was significantly greater than that through drought (−0.0055 = 0.7994 − 0.8049) and $e$$CO_2$ (−0.0010 = 0.8039 − 0.8049). Cultivar, CO_2 and drought had interactive effects on F_v/F_m and F_v'/F_m' (Table 2). Therefore, although elevated [CO_2] had little effect on improving F_v/F_m in well-watered plants, it effectively increased F_v/F_m in drought-stressed plants. There was no difference between the two cultivars.

Table 1. Effects of elevated [CO_2] and drought on gas exchange parameters of two wheat cultivars in 2015.

Stage	Cultivar	Water Condition	Growth [CO_2]	P_N [μmol m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]	WUE [μmol (CO_2) mol(H_2O)$^{-1}$]
Booting stage	Z175	Normal	Control	20.7 ± 0.46 b	0.42 ± 0.03 a	7.35 ± 0.36 a	2.97 ± 0.11 d
			$e$$CO_2$	26.8 ± 0.42 a	0.23 ± 0.01 b	4.79 ± 0.10 b	5.62 ± 0.07 c
		Drought	Control	10.0 ± 0.83 d	0.12 ± 0.02 cd	2.70 ± 0.35 c	4.76 ± 0.26 c
			$e$$CO_2$	11.9 ± 0.65 d	0.06 ± 0.00 de	1.46 ± 0.10 d	8.34 ± 0.12 a
	Triumph	Normal	Control	16.6 ± 0.49 c	0.19 ± 0.01 b	4.20 ± 0.16 b	4.01 ± 0.05 cd
			$e$$CO_2$	23.8 ± 0.88 ab	0.20 ± 0.01 b	4.24 ± 0.24 b	5.91 ± 0.16 c
		Drought	Control	10.2 ± 0.48 d	0.08 ± 0.00 d	1.95 ± 0.11 cd	5.32 ± 0.10 c
			$e$$CO_2$	7.7 ± 0.39 e	0.04 ± 0.00 e	0.92 ± 0.05 e	8.40 ± 0.18 a
Filling stage	Z175	Normal	Control	18.6 ± 0.93 a	0.29 ± 0.02 a	7.37 ± 0.46 a	2.64 ± 0.07 d
			$e$$CO_2$	23.1 ± 1.14 a	0.20 ± 0.02 b	5.93 ± 0.35 b	3.93 ± 0.06 c
		Drought	Control	12.9 ± 0.99 b	0.14 ± 0.01 c	3.99 ± 0.27 c	3.14 ± 0.06 d
			$e$$CO_2$	10.4 ± 0.99 bc	0.04 ± 0.00 e	1.70 ± 0.10 d	4.84 ± 0.13 b
	Triumph	Normal	Control	10.1 ± 0.60 c	0.09 ± 0.01 d	2.85 ± 0.20 c	3.72 ± 0.12 cd
			$e$$CO_2$	11.7 ± 0.96 bc	0.06 ± 0.01 de	2.16 ± 0.19 cd	5.57 ± 0.14 ab
		Drought	Control	5.0 ± 0.26 d	0.04 ± 0.00 e	1.22 ± 0.07 d	4.16 ± 0.10 bc
			$e$$CO_2$	9.8 ± 0.89 c	0.05 ± 0.01 e	1.73 ± 0.19 d	5.83 ± 0.08 a
	cultivars			*	*	*	*
	CO_2			*	*	*	*
	drought			*	*	*	*
	cultivars × CO_2			ns	*	*	ns
	cultivars × drought			*	*	*	*
	CO_2 × drought			*	ns	ns	*
	cultivars × CO_2 × drought			ns	ns	ns	ns

Note: Values are means of eight replicates. Control—ambient atmospheric CO_2 concentration; $e$$CO_2$—elevated atmospheric CO_2 concentration. Means not sharing the same letters differ significantly at $p \leq 0.05$. * or ns indicates significant or nonsignificant at $p \leq 0.05$.

Table 2. Effects of elevated [CO_2] and drought on chlorophyll fluorescence parameters of two wheat cultivars in 2015.

Stage	Cultivars	Water Conditions	Growth [CO_2]	F_v/F_m	F_v'/F_m'	Φ_{PSII}	qP	NPQ
Booting stage	Z175	Normal	Control	0.83 ± 0.00 a	0.66 ± 0.03 a	0.39 ± 0.01 a	0.59 ± 0.02 b	0.89 ± 0.11 d
			$e$$CO_2$	0.84 ± 0.00 a	0.60 ± 0.02 a	0.37 ± 0.02 a	0.62 ± 0.02 ab	1.42 ± 0.19 c
		Drought	Control	0.82 ± 0.00 a	0.54 ± 0.02 ab	0.30 ± 0.02 bc	0.56 ± 0.02 bc	1.42 ± 0.31 c
			$e$$CO_2$	0.83 ± 0.00 a	0.48 ± 0.01 c	0.28 ± 0.01 c	0.58 ± 0.02 b	2.27 ± 0.29 a
	Triumph	Normal	Control	0.83 ± 0.00 a	0.54 ± 0.01 ab	0.33 ± 0.02 b	0.62 ± 0.02 ab	1.85 ± 0.21 ab
			$e$$CO_2$	0.83 ± 0.00 a	0.52 ± 0.02 bc	0.34 ± 0.02 ab	0.66 ± 0.02 a	1.68 ± 0.24 bc
		Drought	Control	0.83 ± 0.00 a	0.49 ± 0.01 c	0.26 ± 0.01 c	0.52 ± 0.02 c	1.70 ± 0.36 abc
			$e$$CO_2$	0.83 ± 0.00 a	0.49 ± 0.02 c	0.30 ± 0.03 bc	0.60 ± 0.03 ab	1.92 ± 0.34 a
Filling stage	Z175	Normal	Control	0.77 ± 0.00 b	0.52 ± 0.03 a	0.56 ± 0.06 a	1.11 ± 0.15 a	1.11 ± 0.14 b
			$e$$CO_2$	0.78 ± 0.00 ab	0.53 ± 0.04 a	0.54 ± 0.05 a	1.07 ± 0.18 a	1.14 ± 0.17 b
		Drought	Control	0.79 ± 0.00 a	0.49 ± 0.06 ab	0.56 ± 0.05 a	1.24 ± 1.20 a	1.36 ± 0.25 ab
			$e$$CO_2$	0.78 ± 0.00 ab	0.48 ± 0.05 ab	0.38 ± 0.06 b	0.86 ± 0.18 a	1.40 ± 0.21 ab
	Triumph	Normal	Control	0.78 ± 0.00 ab	0.51 ± 0.06 a	0.47 ± 0.05 a	1.02 ± 0.17 a	1.30 ± 0.26 ab
			$e$$CO_2$	0.77 ± 0.01 b	0.38 ± 0.07 b	0.31 ± 0.07 b	1.20 ± 0.44 a	1.68 ± 0.31 a
		Drought	Control	0.76 ± 0.00 b	0.39 ± 0.07 b	0.46 ± 0.08 ab	1.26 ± 0.23 a	1.52 ± 0.27 ab
			$e$$CO_2$	0.79 ± 0.00 a	0.53 ± 0.02 a	0.44 ± 0.08 ab	0.86 ± 00.17 a	1.24 ± 0.08 ab
p value	cultivars			ns	*	*	ns	ns
	CO_2			ns	ns	ns	ns	ns
	drought			ns	*	ns	ns	ns
	cultivars × CO_2			ns	ns	ns	ns	ns
	cultivars × drought			ns	ns	ns	ns	*
	CO_2 × drought			*	ns	ns	ns	ns
	cultivars × CO_2 × drought			*	*	ns	ns	ns

Note: Values are means of eight replicates. Control—ambient atmospheric CO_2 concentration; $e$$CO_2$—elevated atmospheric CO_2 concentration. Means not sharing the same letters differ significantly at $p \leq 0.05$. * or ns indicates significant or nonsignificant at $p \leq 0.05$.

3.3. Determination of Photosynthetic Pigment Content, MDA and POD Concentrations

Elevated [CO_2] significantly increased the content of chlorophyll a (by 7.6%) and carotenoids (by 14.0%) but decreased that of MDA (by 37.0%) and POD (by 20.0%). Drought significantly decreased chlorophyll *a* (by 5.1%), chlorophyll b (by 18.6%), POD (by 11.9%), and MDA (by 42.4%). CO_2 and drought had no interactive effects on chlorophyll a, chlorophyll b, carotenoid, MDA or POD. Cultivar, CO_2 and drought had interactive effects on chlorophyll a and carotenoids (Table 3). Z175 had higher contents of chlorophyll a and POD than Triumph at the booting stage, especially under elevated [CO_2] and drought conditions. This illustrated that more chlorophyll a and POD could be produced in Z175 than in Triumph under *e*[CO_2] and drought.

Table 3. Effects of elevated [CO_2] and drought on photosynthetic pigment content, POD and MDA at the booting stage and filling stage of two wheat cultivars in 2015.

Stage	Cultivar	Water Condition	Growth [CO_2]	C_a	C_b	$C_{x \cdot c}$	MDA [m mol/g Fw]	POD [mol/g Fw]
Booting stage	Z175	Normal	Control	2.39 ± 0.16 ab	1.49 ± 0.24 a	0.34 ± 0.03 bc	0.07 ± 0.00 a	0.52 ± 0.04 a
			eCO_2	2.28 ± 0.03 b	0.99 ± 0.05 b	0.41 ± 0.00 ab	0.06 ± 0.00 a	0.36 ± 0.05 bc
		Drought	Control	2.08 ± 0.42 b	1.66 ± 0.32 a	0.28 ± 0.09 c	0.09 ± 0.01 a	0.40 ± 0.02 ab
			eCO_2	2.63 ± 0.08 a	1.16 ± 0.09 ab	0.42 ± 0.01 a	0.06 ± 0.00 a	0.39 ± 0.03 ab
	Triumph	Normal	Control	1.98 ± 0.09 c	1.06 ± 0.06 b	0.33 ± 0.02 bc	0.06 ± 0.01 a	0.34 ± 0.06 bc
			eCO_2	2.09 ± 0.11 b	1.12 ± 0.06 b	0.33 ± 0.02 bc	0.04 ± 0.00 a	0.25 ± 0.03 c
		Drought	Control	2.53 ± 0.10 a	1.20 ± 0.08 ab	0.40 ± 0.01 ab	0.10 ± 0.05 a	0.37 ± 0.04 ab
			eCO_2	1.98 ± 0.21 bc	0.99 ± 0.08 b	0.31 ± 0.04 bc	0.04 ± 0.00 a	0.13 ± 0.01 d
Filling stage	Z175	Normal	Control	2.45 ± 0.09 ab	1.22 ± 0.08 b	0.37 ± 0.01 a	0.08 ± 0.00 a	0.26 ± 0.04 cd
			eCO_2	2.84 ± 0.08 a	1.62 ± 0.03 a	0.43 ± 0.02 a	0.06 ± 0.00 ab	0.39 ± 0.07 b
		Drought	Control	1.95 ± 0.17 bc	1.03 ± 0.12 b	0.35 ± 0.03 a	0.08 ± 0.02 a	0.25 ± 0.06 d
			eCO_2	1.93 ± 0.14 c	0.83 ± 0.08	0.40 ± 0.03 a	0.05 ± 0.00 ab	0.42 ± 0.07 ab
	Triumph	Normal	Control	2.38 ± 0.18 ab	1.27 ± 0.06 ab	0.39 ± 0.03 a	0.05 ± 0.01 ab	0.57 ± 0.04 a
			eCO_2	2.73 ± 0.12 a	1.50 ± 0.14 ab	0.43 ± 0.01 a	0.04 ± 0.00 b	0.36 ± 0.03 b
		Drought	Control	2.19 ± 0.13 b	1.55 ± 0.15 a	0.35 ± 0.03 a	0.06 ± 0.00 ab	0.34 ± 0.02 bc
			eCO_2	2.35 ± 0.05 ab	1.45 ± 0.10 ab	0.42 ± 0.00 a	0.04 ± 0.00 b	0.26 ± 0.01 cd
	cultivars			ns	*	ns	*	*
	CO_2			*	ns	*	*	*
	drought			*	*	ns	ns	*
		cultivars × CO_2		ns	ns	*	ns	*
		cultivars × drought		*	*	ns	ns	ns
		CO_2 × drought		ns	ns	ns	ns	ns
		cultivars × CO_2 × drought		*	ns	*	ns	ns

Note: Values are means of three replicates. Control—ambient atmospheric CO_2 concentration; *e*CO_2—elevated atmospheric CO_2 concentration. Means not sharing the same letters differ significantly at $p \leq 0.05$. * or ns indicates significant or nonsignificant at $p \leq 0.05$.

3.4. Biomass and Yield

Elevated [CO_2] significantly increased the main shoot diameter and biomass, spike number per square meter and kernel number per spike for two years. Elevated [CO_2] had no effect on thousand seed weight and harvest index. Drought decreased the kernel number per spike and thousand seed weight by 48.4 and 11.6%, respectively. CO_2 and drought had no interactive effects on spike number per square meter, kernel number per spike or thousand seed weight (Table 4). Therefore, CO_2 offsets the negative impact of drought on spike number per square meter and kernel number per spike.

Table 4. Effects of elevated [CO_2] and drought on plant morphological parameters, weight, and yield component of two wheat cultivars in 2014 and 2015.

Year	Cultivar	Water Condition	Growth [CO_2]	High [cm]	Main Shoot Diameter [cm]	Main Shoot Biomass [g]	Harvest Index	Spike Number per Square Meter [m^2]	Kernel Number per Spike	Thousand Seed Weight [g]
2014	Z175	Normal	Control	62.10 ± 1.10 d	2.61 ± 0.01 ab	1.48 ± 0.11 ab	0.32 ± 0.02 a	428.40 ± 19.22 c	15.48 ± 0.64 a	25.12 ± 0.38 a
			eCO$_2$	64.88 ± 1.09 cd	2.91 ± 0.07 a	1.70 ± 0.20 a	0.36 ± 0.03 a	610.09 ± 21.89 b	13.78 ± 4.64 ab	22.74 ± 1.48 ab
		Drought	Control	47.77 ± 1.44 e	1.84 ± 0.48 c	1.04 ± 0.09 b	0.25 ± 0.01 b	331.72 ± 19.22 d	10.53 ± 6.32 b	17.78 ± 1.29 b
			eCO$_2$	47.06 ± 2.06 e	2.86 ± 0.08 a	1.12 ± 0.07 b	0.25 ± 0.02 b	417.07 ± 19.54 c	15.45 ± 3.89 ab	20.42 ± 1.24 ab
	Triumph	Normal	Control	92.88 ± 4.54 a	2.31 ± 0.38 bc	1.15 ± 0.08 b	0.09 ± 0.04 c	713.04 ± 43.23 ab	12.81 ± 4.34 ab	18.01 ± 0.75 b
			eCO$_2$	91.42 ± 1.04 a	2.14 ± 0.04 c	1.28 ± 0.09 b	0.09 ± 0.06 c	743.51 ± 43.95 a	16.40 ± 0.83 a	15.91 ± 0.88 bc
		Drought	Control	74.14 ± 2.85 bc	2.09 ± 0.07 c	1.02 ± 0.08 b	0.07 ± 0.03 c	456.19 ± 38.99	15.57 ± 0.56 a	15.63 ± 0.80 c
			eCO$_2$	81.38 ± 2.05 ab	2.00 ± 0.05 c	1.26 ± 0.05 b	0.10 ± 0.08 c	741.65 ± 51.33 a	11.63 ± 3.02 ab	14.87 ± 0.49 c
2015	Z175	Normal	Control	68.48 ± 2.07 bc	2.57 ± 0.14 ab	1.25 ± 0.02 ab	0.25 ± 0.03 a	500.00 ± 29.04 a	15.99 ± 1.07 a	26.78 ± 5.44 b
			eCO$_2$	72.19 ± 2.39 b	2.72 ± 0.15 a	1.41 ± 0.03 a	0.29 ± 0.03 a	498.33 ± 43.91 a	18.26 ± 1.21 a	32.28 ± 0.68 a
		Drought	Control	64.36 ± 1.88 c	2.54 ± 0.10 ab	1.06 ± 0.20 b	0.20 ± 0.02 ab	413.88 ± 30.41 b	9.71 ± 1.01 b	27.66 ± 1.51 ab
			eCO$_2$	60.90 ± 2.52 c	2.69 ± 0.08 a	1.26 ± 0.09 ab	0.23 ± 0.02 a	429.88 ± 17.54 b	10.21 ± 0.58 b	26.81 ± 0.70 b
	Triumph	Normal	Control	89.00 ± 1.07 a	2.25 ± 0.06 bc	0.97 ± 0.02 bc	0.20 ± 0.02 ab	502.38 ± 30.45 a	4.66 ± 0.43 c	24.77 ± 1.13 bc
			eCO$_2$	90.99 ± 3.19 a	2.24 ± 0.10 bc	1.02 ± 0.03 b	0.20 ± 0.03 ab	455.96 ± 26.95 ab	5.96 ± 0.59 c	25.06 ± 0.79 bc
		Drought	Control	73.47 ± 2.69 b	2.17 ± 0.08 c	0.86 ± 0.04 c	0.20 ± 0.02 ab	312.50 ± 26.71 c	4.16 ± 0.54 c	22.33 ± 0.72 c
			eCO$_2$	70.96 ± 2.68 b	2.21 ± 0.04 bc	0.76 ± 0.06 c	0.14 ± 0.04 b	288.33 ± 19.96 c	7.79 ± 0.78 b	23.12 ± 0.38 c
p value		year		*	ns	*	ns	*	*	*
		cultivars		*	*	*	*	*	*	*
		CO_2		ns	*	*	ns	*	*	ns
		drought		*	*	*	*	*	*	*
		year × cultivars		*	*	*	*	*	*	ns
		year × CO_2		ns	ns	*	ns	ns	ns	ns
		year × drought		*	*	*	ns	*	*	ns
		cultivars × CO_2		*	*	*	*	*	ns	ns
		cultivars × drought		*	*	*	*	*	*	*
		CO_2 × drought		*	*	*	*	ns	ns	ns
		year × cultivars × CO_2		ns	ns	*	ns	*	ns	ns
		year × cultivars × drought		*	*	*	ns	ns	*	ns
		cultivars × CO_2 × drought		*	*	*	ns	ns	ns	ns
		year × cultivars × CO_2 × drought		*	*	*	ns	ns	ns	*

Note: Values are means of five replicates. Control—ambient atmospheric CO_2 concentration; eCO$_2$—elevated atmospheric CO_2 concentration. Means not sharing the same letters differ significantly at $p \leq 0.05$. * or ns indicates significant or nonsignificant at $p \leq 0.05$.

Elevated [CO_2] significantly increased aboveground biomass and grain weight in g m^{-2} by an average of 21.8 and 31.3%, respectively, for two years. Drought decreased aboveground biomass and grain weight by 39.8 and 49.6%, respectively. CO_2 and drought had a significant negative interactive effect on aboveground biomass ($p < 0.05$) and grain weight ($p < 0.05$). Specifically, the decrease in aboveground biomass through the eCO_2 × drought interaction (−253.16 = 647.93 − 901.09) was significantly greater than that of the individual effects of drought (−359.97 = 541.12 − 901.09) and eCO_2 (174.19 = 1075.28 − 901.09). The decrease in grain weight by the eCO_2 × drought interaction (−70.46 = 132.93 − 203.38) was significantly greater than that resulting from drought (−100.36 = 103.02 − 203.38) and eCO_2 (61.65 = 265.04 − 203.38). Cultivar, CO_2 and drought had no interactive effects on aboveground biomass and grain weight (Figures 2 and 3). Therefore, drought decreased aboveground biomass and grain weight to a greater extent under elevated CO_2 than under atmospheric CO_2. Nevertheless, the values of aboveground biomass and grain weight in the two cultivars (especially in Z175) were still significantly higher under elevated CO_2 than under atmospheric CO_2.

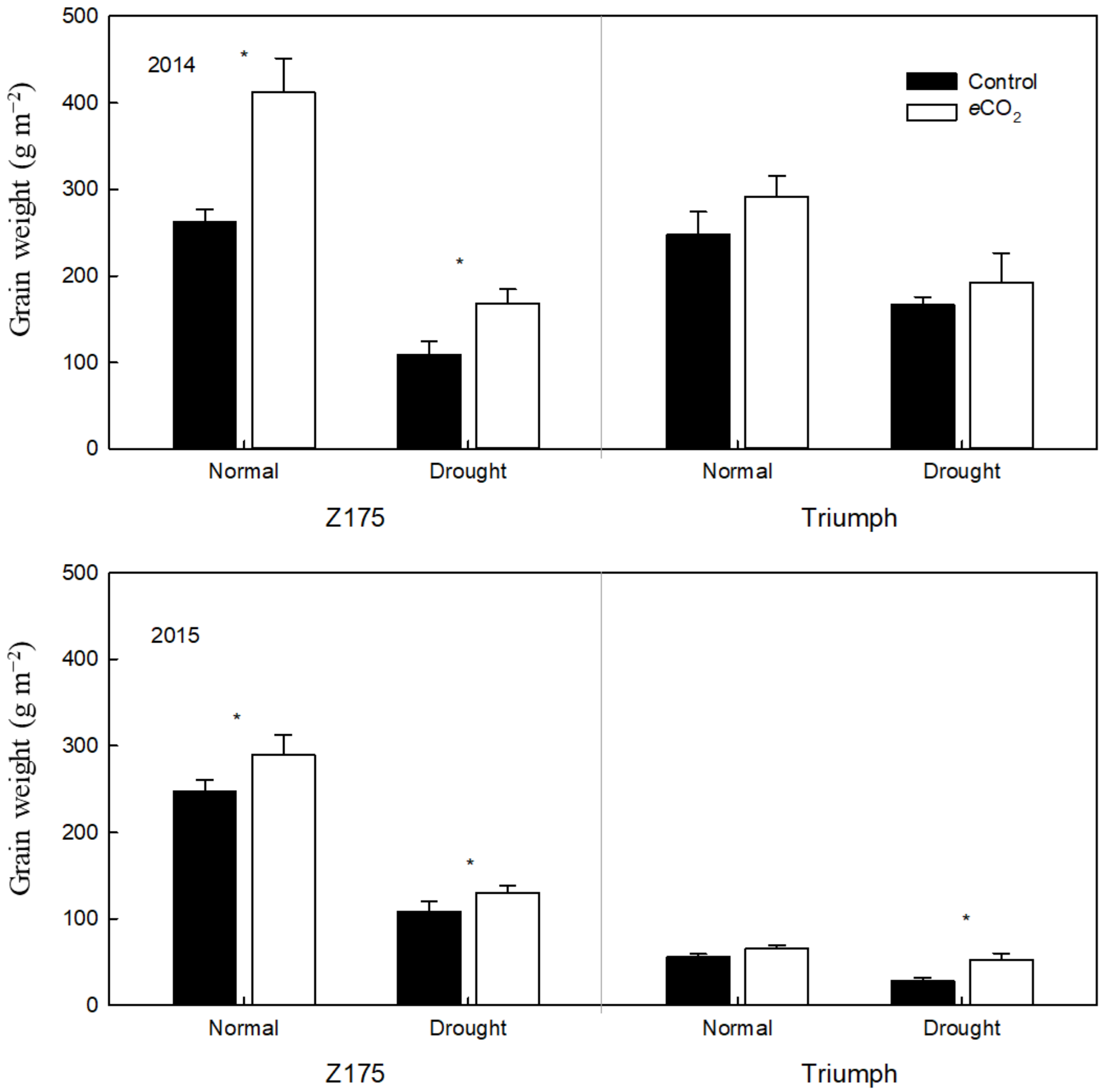

Figure 2. Effects of elevated [CO_2] and drought on the grain weight of two wheat cultivars in 2014 and 2015. Values are means of 5 replicates. Control—ambient atmospheric CO_2 concentration; eCO_2—elevated atmospheric CO_2 concentration. * indicates significance at $p \leq 0.05$.

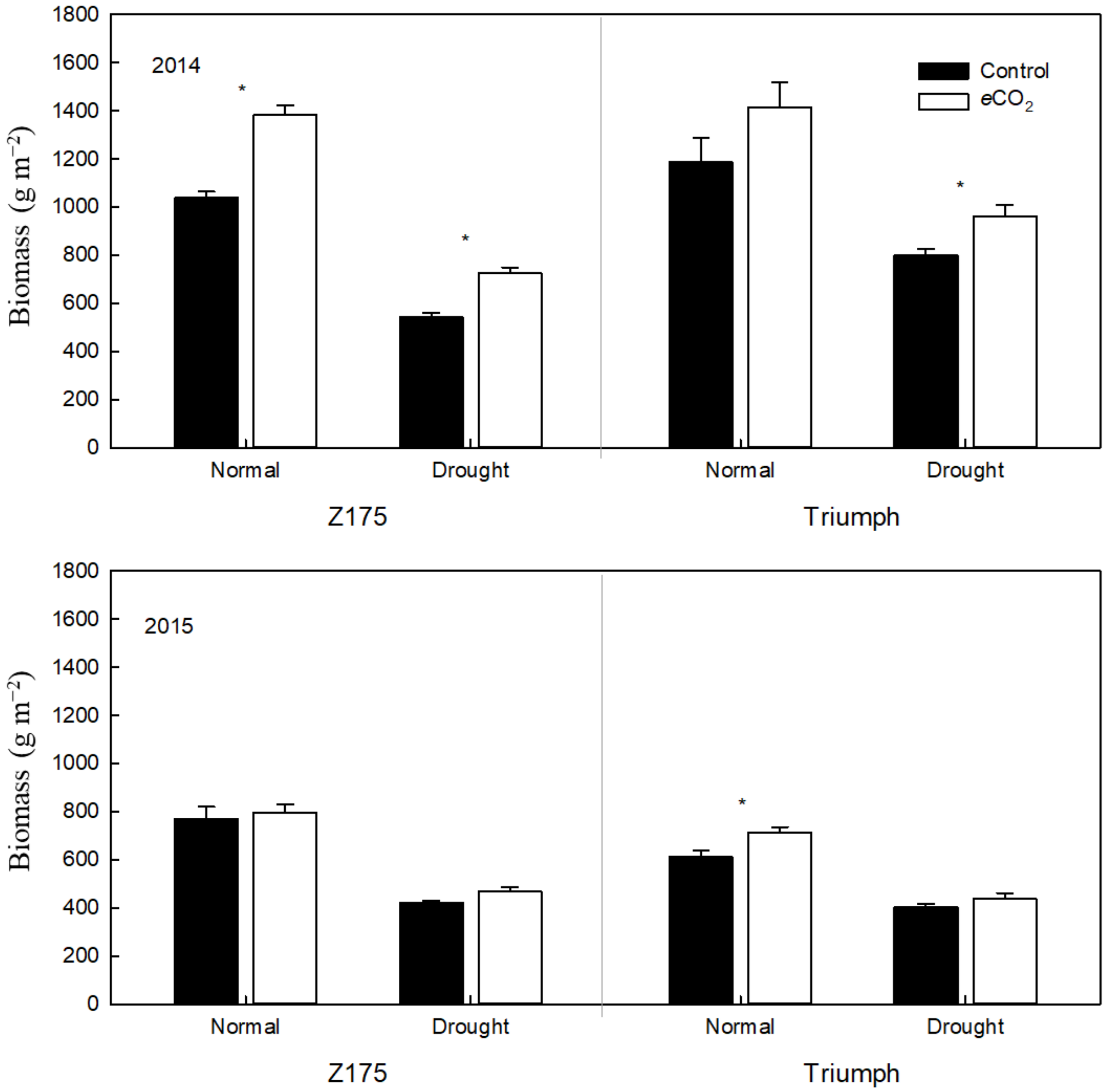

Figure 3. Effects of elevated [CO_2] and drought on the aboveground biomass of two wheat cultivars in 2014 and 2015. Values are means of 5 replicates. Control—ambient atmospheric CO_2 concentration; $e$$CO_2$—elevated atmospheric CO_2 concentration. * indicates significance at $p \leq 0.05$.

4. Discussion

Elevated [CO_2] has been widely reported to improve leaf WUE by increasing the photosynthesis rate and decreasing leaf transpiration (by reducing g_s) [4,5]. This effect is predicted to be helpful in alleviating drought stress on crops [26,27]. Stomatal closure, however, is widely recognized as the primary limitation of carbon assimilation [28]. In the current study, elevated [CO_2] increased WUE under drought by decreasing E (decreased g_s) but decreased P_N due to strong stomatal closure, which was in agreement with a previous study on wheat [14]. When comparing the two selected cultivars, the large-spike cultivar Z175 had a higher P_n than the multi-spike cultivar Triumph under elevated [CO_2]. This helped Z175 plants assimilate more carbon than Triumph under drought stress and elevated [CO_2]. Meanwhile, although elevated [CO_2] increased the content of chlorophyll a and carotenoids, CO_2 and drought had no interactive effects on chlorophyll a, chlorophyll b and carotenoids. Further analysis of PSII performance showed that CO_2 and drought also had no interactive effects on F_v'/F_m', Φ_{PSII}, qP and NPQ, which was consistent with studies on soybean [3] and basil [29] but in contrast to other studies on rice [30], sugarcane [31] and

Arabidopsis [8]. However, the two selected cultivars showed different response patterns in photosynthetic pigments to elevated [CO_2] and drought. Thus, [CO_2] and drought had positive interactive effects on chlorophyll a in Z175 and had negative effects on chlorophyll a in Triumph at the booting stage. However, the chlorophyll a in Z175 was not enough to improve PSII performance under elevated [CO_2] and drought. Although we did not measure the photochemical parameters, we speculate that, rather than PSII performance (F_v'/F_m' and Φ_{PSII}), photosynthesis biochemical features (such as substrate and energy partitioning between rubisco carboxylation and photorespiration) possibly contribute more to carbon assimilation at low g_s in Z175 under elevated [CO_2] [32]. Elevated [CO_2] is expected to reduce photorespiration; in contrast, however, drought has been proven to increase photorespiration. Changes in photosynthesis biochemical features would be influenced by the partitioning of substrate and photon flux between carboxylation and photorespiration and might be cultivar-dependent [27].

POD can eliminate the deleterious effects of H_2O_2 on an organism through the oxidation of co-substrates [33]. MDA may function as a biomarker to assess the level of oxidative stress in plants. MDA activity and content are unusually correlated with a wide range of plant physiological processes [34,35]. In the current study, elevated [CO_2] significantly decreased MDA at the booting stage and the grain filling stage, and decreased POD at the booting stage but did not affect MDA or POD when combined with drought. Barickman et al. [29] and Bencze et al. [14] also reported that no significant change was observed in POD under elevated [CO_2] and drought conditions. Moreover, the two selected cultivars showed different response patterns to elevated [CO_2]. Z175 had more POD than Triumph, which possibly contributed to alleviating ROS damage caused by drought under elevated [CO_2], especially at the booting stage. This could be another reason for the improved carbon assimilation in Z175 under elevated [CO_2] and drought.

Elevated [CO_2] stimulated more spikes for Z175 than for Triumph (Table 4), especially in 2014, which is consistent with previous studies [36,37]. The enhanced photosynthesis performance and sink capacity (spike number and kernel number per spike) at the grain filling stage contribute to the yield in Z175 plants under elevated [CO_2], which can help counteract the adverse effects of drought. Although Triumph had more tillers and spikes at the current [CO_2] concentration, most of them were thin and had limited photosynthetic capacity (Tables 1 and 4). Both the main shoot biomass accumulation and tiller and spike number of Triumph did not significantly increase under elevated [CO_2], irrespective of water status. Hence, elevated [CO_2] mitigated drought-induced inhibition of grain weight in Z175 plants but not in Triumph plants under persistent drought stress. Although the response patterns of Z175 plants to elevated [CO_2] and drought were the same between the two years, the magnitudes of these changes were smaller in 2015 than in 2014. According to the analysis of environmental elements in the two years, the mean daily temperature was higher (approximately 1.5–2 °C), and there were fewer precipitation days (air moisture may be lower) during March and April in 2015 than in 2014 (Figure 1). At that time, the winter wheat plants were at the elongation stage and heading stage. It was proven that plants under elevated [CO_2] tend to obtain a new balance of C and N metabolism, such as a decrease in foliar photorespiration, an increase in carbohydrate assimilation and belowground partitioning, a decrease in N concentration in leaves, and an increase in N accumulation at the whole plant level [27]. The increase in air temperature and decrease in moisture may have contributed to an increase in leaf respiration, the consumption of carbohydrates [38], and a further increase in carbohydrate partitioning belowground [39] in 2015, resulting in a decrease in aboveground biomass and grain weight. Therefore, the positive effects that elevated [CO_2] brought to drought-induced inhibition on grain weight in cv. Z175 diminished in the year with higher temperature and less precipitation at the key growth stages.

5. Conclusions

Elevated [CO_2] significantly increased chlorophyll a and carotenoids, decreased g_s and *E*, and, consequently, increased P_N and WUE in well-watered wheat plants. However, when plants were under drought stress, elevated [CO_2] only improved WUE and F_v/F_m and did not ameliorate the adverse effects of drought on P_N. Moreover, Z175 showed more improved photosynthesis performance and sink capacity (spike number and kernel number per spike) than Triumph at the grain filling stage under elevated [CO_2]. Elevated [CO_2] partially mitigated drought-induced inhibition of the grain weight of Z175 plants but not of Triumph plants. Therefore, to fully utilize the fertilization effect of future elevated [CO_2] on growth and yield in wheat, we may cultivate wheat cultivars that can adapt to drought stress in semi-arid regions.

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Abbreviations

[CO_2]—atmospheric CO_2 concentration; *E*—transpiration rate; HCO_2—high atmospheric CO_2 concentration; ETR—electron transport rate; F_0—minimal fluorescence yield of the dark-adapted state; F_0'—minimal fluorescence yield of the light-adapted state; F_m– maximal fluorescence yield of the dark-adapted state; F_m'—maximal fluorescence yield of the light-adapted state; FM—fresh mass; F_s—steady-state fluorescence yield; F_v/F_m—maximal quantum yield of PSII photochemistry; F_v'/F_m'—intrinsic efficiency of PSII; g_s–stomatal conductance; MDA–malondialdehyde; NPQ– nonphotochemical quenching; P_N—net photosynthetic rate; POD—peroxidase; qP—photochemical quenching coefficient; ROS—reactive oxygen species; WUE—water-use efficiency (=P_N/E); Φ_{PSII}—effective quantum yield of PSII photochemistry.

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