Acclimation to future atmospheric CO₂ levels increases photochemical efficiency and mitigates photochemistry inhibition by warm temperatures in wheat under field chambers

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Abstract
A study was conducted over two years to determine whether growth under elevated CO₂ (700 µmol mol⁻¹) and temperature (ambient + 4°C) conditions modifies photochemical efficiency or only the use of electron transport products in spring wheat grown in field chambers. Elevated atmospheric CO₂ concentrations increased crop dry matter at maturity by 12%-17%, while above-ambient temperatures did not significantly affect dry matter yield. In measurements with ambient CO₂ at ear emergence and after anthesis, growth at elevated CO₂ concentrations decreased flag leaf light-saturated carbon assimilation. The quantum yield of electron transport (Φₚₛᵢᵢ) measured at ambient CO₂ and higher irradiances increased at ear emergence and decreased after anthesis in plants grown at elevated CO₂. At higher light intensities, but not in low light, photochemical quenching (qP) decreased after growth in elevated CO₂ conditions. Growth under CO₂ enrichment increased dark- (Fv:Fm) and light-adapted (Fv’:Fm’) photochemical efficiencies, and decreased the chlorophyll a:b ratio, suggesting an increase in light-harvesting complexes relative to PSII reaction centres. A relatively higher decrease in carbon assimilation than the decrease in Φₚₛᵢᵢ pointed to a sink other than CO₂ assimilation for electron-transport products at defined growth stages. With higher light intensities, warmer temperatures increased Φₚₛᵢᵢ and Fv’:Fm’ at ear emergence and decreased Φₚₛᵢᵢ after anthesis; in ambient -but not elevated- CO₂, warmer temperatures also decreased qP after anthesis. CO₂ fixation increased or did not change with temperature, depending on the growth stage and year.

We conclude that elevated CO₂ decreases the carbon assimilation capacity, but increases photochemistry and resource allocation to light harvesting, and that elevated levels of CO₂ can mitigate photochemistry inhibition due to warm temperatures.
Keywords: acclimation; chlorophyll a:b ratio; chlorophyll fluorescence; carbon assimilation; elevated CO₂; elevated temperature; crop yield; wheat
Introduction

A rise in CO$_2$ concentration above current atmospheric levels will increase photosynthesis in C$_3$ plants because Rubisco is not substrate-saturated and oxygenation is inhibited (Long et al. 2004). Under elevated CO$_2$, photosynthetic electron transport destined for CO$_2$ assimilation will increase proportionately, while electron flux to oxygen will be inhibited. The net result of these changes will determine whether total electron flux increases (Hymus et al. 2001) if there is no effect of elevated CO$_2$ on alternative electron sinks (Habash et al. 1995; Hymus et al. 1999). An acclimatory decrease in Rubisco activity with growth under elevated CO$_2$ (Riviere-Rolland et al. 1996; Drake et al. 1997; Nakano et al. 1997; Farage et al. 1998; Moore et al. 1999; Geiger et al. 1999; Pérez et al. 2005) will restrict the increase in electron fluxes for CO$_2$ and O$_2$ fixation and, if the decrease in Rubisco is strong, will decrease electron transport relative to plants under ambient CO$_2$ conditions (Hymus et al. 2001).

Increases, or at least no decrease, in photochemical activity in plants grown and measured under elevated CO$_2$ conditions have been reported frequently (Habash et al. 1995; Faria et al. 1996; Gouk et al. 1999; Luomala et al. 2003; Usuda 2004; Centritto 2005; Zhang and Dang 2006). However, photosynthetic acclimation to elevated CO$_2$ has been shown to lead to a decrease in the electron transport rate (Roden and Ball 1996; Scarascia-Mugnozza et al. 1996; Kitao et al. 2005; Aranda et al. 2006; Bigras and Bertrand 2006). This has been reported by some authors using measurements at a common CO$_2$ concentration in plants grown in elevated and ambient CO$_2$ conditions (Spunda et al. 1998; Aranda et al. 2006), allowing a distinction to be made between the direct (short term) and acclimatory (long-term) effects of atmospheric CO$_2$ enrichment. In some (Bartak et al. 1996; Hogan et al. 1997; Usuda 2004; Kitao et al. 2005; Martinez-Carrasco et al. 2005), but not all (Spunda et al. 1998; Bigras and Bertrand
2006), the preceding reports, maximal photochemical efficiency in the dark adapted state (Fv:Fm) did not change with down-regulation of photosynthesis under elevated CO₂, suggesting that variations in electron transport would be due to the capacity to use its products rather than to changes in the photosynthetic apparatus. Modifications in the quantum yield of Photosystem II (PSII) electron transport (ΦPSII) induced by growth under elevated CO₂ have been related to changes in photochemical quenching (qP) rather than in maximum photochemical efficiency in light (Fv′:Fm′) (Hymus et al. 1999; Kitao et al. 2005; Aranda et al. 2006). In contrast, we have previously found (Martinez-Carrasco et al. 2005) that Fv′:Fm′, measured under ambient CO₂ in wheat plants grown under elevated CO₂, increased at ear emergence and decreased soon after anthesis, together with decreases in qP at ear emergence. After anthesis, qP did not decrease in one of the years studied, while it was lower under elevated than under ambient CO₂ late in grain growth in another year. The question remains as to whether decreases in qP may also occur soon after anthesis. Increases in Fv′:Fm′, accompanying decreases in qP, may involve an increased transfer of excitation energy from the pigment bed to a smaller fraction of open PSII centres, as has been reported for water stress (Giardi et al. 1996; Sánchez-Rodriguez et al. 1997). They would also suggest that growth under elevated CO₂ changes the relative size of the PSII light-harvesting complex. Habash et al. (1995) reported no effect of elevated CO₂ on Fv′:Fm′ or the chlorophyll a:b ratio in the second fully expanded leaves of wheat six weeks after sowing. In contrast, Bigras and Bertrand (2006) found a decrease in this ratio – consistent with a relative increase in the light-harvesting antenna - in association with a down-regulation of photosynthesis under elevated CO₂ in Picea mariana. Likewise, we have found (Pérez et al. 2007) a lower chlorophyll a:b ratio, together with increased
Φ_{PSII}, in wheat flag leaves after, but not before, anthesis. Our measurements, however, were conducted only at low light intensity and Fv':Fm’ and qP were not determined.

Above-ambient temperatures decrease Φ_{PSII} without affecting Fv:Fm in Lolium perenne, attributed to a down-regulation of carbon assimilation caused by the treatment (Bartak et al. 1996). Similarly, with mild drought or no water stress, growth at 32 ºC as compared to 26 ºC does not significantly affect Fv/Fm, but significantly decreases Φ_{PSII}, regardless of soil moisture in the perennial grass Leymus chinensis (Xu and Zhou 2006). qP was also reported to be decreased by a 4 ºC temperature increase in Lolium perenne (Nijs and Impens 1996). In a boreal environment, increasing the temperature by 2 to 6 ºC in field chambers increased Fv:Fm and chlorophyll a+b contents in Scots pine needles (Wang et al. 2003). In previous studies with wheat (Pérez et al. 2007), chlorophyll fluorescence measurements at low light intensities and ambient CO₂ revealed a negative effect of high temperatures on Φ_{PSII} in plants grown in ambient CO₂, but not under elevated CO₂. Whether this positive interaction of warm temperatures and elevated CO₂ occurs at high irradiance warrants further research. Growth in a CO₂-enriched atmosphere affords protection from the short-term effects of high temperature on net carbon uptake and Fv:Fm (Faria et al. 1996; Huxman et al. 1998); the beneficial effect of elevated CO₂ was also observed for Φ_{PSII} (Huxman et al. 1998). In contrast, Roden and Ball (1996) found a lower Fv:Fm during a heat stress treatment in Eucalyptus macrorhyncha grown in elevated as compared with ambient CO₂.

The aim of this work was to know whether high CO₂ and warmer temperatures modify the photochemical efficiency, or only the use of electron transport products, or both. Chlorophyll fluorescence and carbon assimilation were measured in flag leaves at ear emergence and 10-13 days after anthesis, and growth parameters were recorded at ear emergence and maturity. The photosynthetic performance of flag leaves in the
period after ear emergence is of paramount importance for grain yield. We determined chlorophyll fluorescence in the dark-adapted state and in response to a wide range of light intensities. Differing responses at low and high light intensity can help to distinguish between environmental effects on the photochemical efficiency and the use of photochemistry products. In addition, we analyzed chlorophyll contents and chlorophyll a:b ratios, as an index of changes with treatments in the balance between PSII reaction centres and antennae.
Materials and Methods

This study was conducted in two field experiments in different years. The experiment site, located on the farm of the Institute of Natural Resources and Agrobiology, CSIC, in Salamanca (40 ° 95 ‘ N, 5 ° 5 ‘ W, 800 m a.s.l.), has a clay-sand soil. The climate corresponds to a Mediterranean type. The long-term (20 year) average for the minimum temperature in the coldest month (January) is 0.0 °C and the maximum temperature of the warmest month (July) is 27.2 °C. Mean annual rainfall is 506 mm.

Spring wheat (*Triticum aestivum* L cv. Gazul) was sown at a rate of 180 kg ha⁻¹ and 0.13 row spacing on 29 January 2004 and 25 January 2006. Before sowing, 60 kg ha⁻¹ each of P and K, in both years, and 32 kg ha⁻¹ N in 2004 were applied. An application by hand of nitrogen fertilizer [Ca(NO₃)₂] as an aqueous solution was performed, at the two different amounts indicated below, on 21 April 2004 and 27 March 2006. Ten days after sowing, herbicides (clortoluron + diflufenican, 2.3 l ha⁻¹) were added; insecticides were applied as required. The crop was watered weekly with a drip irrigation system, providing the amount of water required to equal the average rainfall for each particular month (February, 24.4 mm; March, 25.6 mm; April, 49.7 mm; May, 57.8 mm, and June, 34.3 mm). This represented a low water supply at later growth stages.

After seedling emergence, six temperature-gradient chambers (Aranjuelo *et al.* 2005; Pérez *et al.* 2005), based on those described by Rawson *et al.* (1995), were mounted over the crop at different field sites each year. The chambers were 9m long, 2.2m wide and 1.7m high at the ridge. The chambers had transparent polycarbonate walls and polyethylene sheet roofing, and comprised three consecutive modules (each 3m long) separated by horizontally slotted polycarbonate septa to reduce the mixing of air between modules through convection. Inlet fans and outlet fans and heaters kept the inlet module at temperatures similar to those in the outside air and the final outlet
module at 4 °C higher temperatures; the central module was left as a spacer. Three chambers were kept at ambient CO₂ (370 μmol mol⁻¹) while in the other three atmospheric CO₂ was increased to 700 μmol mol⁻¹ (elevated CO₂) by injecting pure CO₂ at the two inlet fans during the light hours. Since there appear to be no, or only small, positive (Davey et al., 2004) direct effects of growth CO₂ on leaf dark respiration, reports of their presence in the literature seeming to refer to artefacts (Jahnke and Krewitt, 2002), the lack of CO₂ enrichment during the night is probably irrelevant. The CO₂ concentrations and temperatures recorded in the chambers were very close to set values, as in our preceding experiments with these temperature-gradient chambers (Fig. 1 in Del Pozo et al. 2005; Fig. 2 in Pérez et al. 2005). Two levels of nitrogen supply were established by adding 108 and 140 kg ha⁻¹ in 2004 and 2006, respectively, to one longitudinal half of the chambers, and none and 15 kg ha⁻¹ in 2004 and 2006, respectively, to the other half, affording total N amounts of 140 and 32 kg ha⁻¹ in 2004 and 140 and 15 kg ha⁻¹ in 2006. Less fertilizer was used in 2006 than 2004 because of the probability of a carry-over from 2004 and 2005 to 2006. Control systems were as described (Pérez et al. 2005). The CO₂ concentrations and temperatures recorded in the chambers were homogeneous within each chamber module and close to set values (data not shown). The crops achieved densities of 465 ± 13 (2004) and 367 ± 7 (2006) shoots m⁻², reaching ear emergence and anthesis four days earlier than the plants outside the chambers.

Chlorophyll fluorescence measurements

At ear emergence (24-28 May 2004 and 15-17 May 2006) and 10 to 13 days after anthesis (7-10 June 2004 and 29 May to 1 June 2006), chlorophyll fluorescence was measured with a modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany) in the
central segment of two flag leaves (subsamples) of each CO$_2$, temperature and nitrogen combination in the three replicate chambers (48 recordings), between 3 and 8 h after the start of the photoperiod. All the leaves were measured with the same, ambient CO$_2$ concentration. For this, CO$_2$ fumigation was temporarily suspended in the chamber where fluorescence was recorded. For measurements in the dark-adapted state, leaf sections were darkened for 20 min with leaf clips. Then, Fo was recorded and a saturating flash of light (several thousands $\mu$mol m$^{-2}$ s$^{-1}$) was applied for 0.8 s to determine Fm. Fo and Fm represent, respectively, the minimal and maximal fluorescence in the dark-adapted state. From these, $Fv:FM = [(FM-Fo)/FM]$, the maximal photochemical efficiency, was calculated. Light-adapted leaves were illuminated with the red actinic light source of the fluorometer and protected with a radiation shield to obtain an irradiance of 220 $\mu$mol m$^{-2}$ s$^{-1}$. Saturating light pulses were given every 20 s until steady-state chlorophyll fluorescence parameter values were obtained (about 6 min), recording the fluorescence values immediately before ($Fs$, steady-state fluorescence) and after ($Fm'$, maximal fluorescence in the light) each pulse. Then, the leaf was covered with a black cloth, the actinic light was switched off, and an infrared light was switched on for 3 s to quickly reoxidize the PSII centres and measure Fo’, the minimal fluorescence with a non-photochemical quenching similar to that found in the steady-state under light. The equipment determines $\Phi_{PSII} = [(FM'-Fs)/FM']$, the quantum yield of PSII electron transport. We calculated photochemical quenching $qP = [(FM'-Fs)/(FM'-Fo')]$ and $Fv':FM' = [(FM'-Fo')/FM']$, the photochemical efficiency under light. It should be noted that $\Phi_{PSII} = Fv':FM' \cdot qP$. Non-photochemical quenching (NPQ) equates to $(FM/FM')-1$.

In the second experiment (2006), rapid fluorescence – light responses were recorded. In addition to estimating the maximum rate of electron transport and the quantum yield
under saturating irradiance (Rascher et al. 2000), fluorescence-irradiance response curves without prior darkening of leaves allowed the maximum quantum yield, qP and Fv’:Fm’ to be calculated in light-adapted leaves. Leaves were placed in the clip (2030-B, Walz), were rapidly covered with a black cloth, and illuminated with the PAM-2000 light sources through fibre optics. The red LED was used to obtain irradiances up to about 150 µmol m⁻² s⁻¹, and the white halogen source for higher light intensities. The change of light source was justified because the LED light does not provide higher light intensities and the halogen light does not allow an adequate range of low-intensity irradiances to be obtained. Although red light might not be sufficient to induce stomatal opening and might thus lead to limiting CO₂ supplies, it is assumed that this was unlikely under the low light-intensity range in which the red LED was used. Light was increased in 11 20 s-steps separated by saturating pulses, from less than 10 to about 2500 µmol m⁻² s⁻¹. Subsequently, the leaf was darkened to determine Fo’ by the procedure described. The parameters ΦPSII and Fv’:Fm’ were obtained and from these qP was calculated (see above). Chlorophyll fluorescence parameters in dark-adapted leaves were obtained at ear emergence (not after anthesis) following the procedure described for the 2004 experiment.

Gas exchange measurements

Gas exchange of leaves was recorded on the same hours and dates and with the same sampling scheme as chlorophyll fluorescence. Measurements were carried out in the central segment of flag leaves with an air flow rate of 300 ml min⁻¹, 1500 µmol m⁻² s⁻¹ irradiance, and a 1.6 ± 0.23 kPa vapour pressure deficit, using a 1.7 cm²-window leaf chamber connected to a portable infrared gas analyzer (CIRAS-2, PP Systems, Hitchin, Herts., UK) with differential operation in an open system. Temperature was kept at 25
°C with the Peltier system of the analyzer. Photosynthesis was recorded at 370 and 700 µmol mol⁻¹ CO₂.

**Chlorophyll concentration**

At mid-morning on the dates of fluorescence and gas exchange measurements in the second year, two subsamples of flag leaves, each consisting of four leaves, were harvested from each treatment combination and replicate, and were rapidly plunged *in situ* into liquid nitrogen and then stored at -80 °C until analyzed. The fresh weight, leaf area (image analysis), and total chlorophyll, chlorophyll a and chlorophyll b in acetone extracts (Arnon 1949) of frozen subsamples were determined as described (Pérez *et al.* 2005). This allowed the results to be expressed on a leaf-area basis.

**Green area and dry matter**

At ear emergence and at maturity, samples were harvested to determine the area of green tissues and dry matter accumulation. At ear emergence, the number of shoots in 50 cm of two adjacent rows was counted and five consecutive shoots were harvested from each row. The green area of leaves, stems and ears was measured with an electronic planimeter (LI-3050A, Li-Cor, Lincoln, Nebraska, USA) and the dry weight recorded after drying in an oven at 60 °C for 48 h. At maturity, all shoots in 50 cm of two adjacent rows were harvested, separated into ears and straw, dried at 60 °C for 48 °C and weighed.

**Experimental design and statistical analyses**

The design of the experiment was a randomized-block strip-plot design with three blocks, the two atmospheric CO₂ concentrations (one chamber each) allocated to whole-
plots within blocks, temperature and nitrogen as rows and columns within whole-plots; the subsamples in subplots within rows and columns, and sampling dates in a stratum within subplots. Variance was estimated using the method of residual maximum likelihood (REML; Genstat 6.2). The effects of factors and factor interactions were tested with the Wald statistic, which corresponds to the factor or interaction sum of squares divided by the stratum mean square, and the respective $\chi^2$ probabilities were obtained.

Fluorescence-light responses in the 2006 experiment were analyzed through regressions (exponential curve of the shape $y=a+br^x$) fitted with Genstat 6.2. The curves fitted to each treatment were compared through an analysis of parallelism (Genstat 6.2) to assess whether common or different parameters (only the $a$ regression parameter; both $a$ and $b$ parameters; or all three parameters) should be fitted to each treatment. The significant regression model with greatest parameter separation should be selected. If only the $a$ parameter differs among treatments, then parallel curves are obtained which differ in the maximum value. With more differing parameters for each treatment, the curves also show differences in the slope of the light response. The change from red LED to white halogen light sources caused discontinuities in the response of the fluorescence parameters to irradiance. Accordingly, light responses under (LED) and above (halogen) about 150 µmol m$^{-2}$ s$^{-1}$ were analyzed separately.
Results

Chlorophyll fluorescence

Chlorophyll fluorescence measurements at ambient CO2 concentration in all treatments allowed the acclimatory changes induced by growth conditions to be estimated. The nitrogen supply had no significant effect on the chlorophyll fluorescence parameters or their response to light intensity in either of the two experiments reported here (data not shown). Therefore, only the CO2 and temperature effects will be described. In the 2004 experiment, the values for ΦPSII, Fv’:Fm’ and qP were generally higher, and those for Fv:Fm were lower after anthesis than at ear emergence (Table 1); the NPQ values decreased in ambient CO2, and increased in elevated CO2 from ear emergence to anthesis. The reasons for these changes over time are not clear. At ear emergence in 2004, growth in elevated CO2 significantly decreased ΦPSII (Table 1) as a consequence of decreased qP. In contrast, CO2 enrichment increased the maximal photochemical efficiency, Fv:Fm. After anthesis, the negative effect of elevated CO2 on qP was also significant, while Fv’:Fm’ and NPQ were higher in elevated than ambient CO2. The contrasting effects of elevated CO2 on qP and Fv’:Fm’ resulted in little effect on ΦPSII. CO2 enrichment increased Fv:Fm to a greater extent after anthesis than at ear emergence. Growth temperature had no significant effects on chlorophyll fluorescence parameters during either of the two growth stages.

To further explore whether CO2 and temperature modified the efficiency of photochemistry or the demand for the products of electron transport, in a new experiment we measured chlorophyll fluorescence quenching at a range of light intensities, since electron sinks are more likely to limit electron transport at high than low irradiance. In the 2006 experiment, with measurements at ambient CO2, increasing light intensities decreased the quantum yield of photosynthetic electron transport (ΦPSII)
throughout the range of irradiances (Figs. 1 a, b and 2 a, b). Fv’:Fm’ showed little change with light in the range under 150 µmol m⁻² s⁻¹ (Figs. 1 c and 2 c) and, in contrast, decreased with higher irradiance (Figs. 1 d and 2 d). At ear emergence, the increases in NPQ with light intensity (Fig. 1 h) were much greater than the decreases of Fv’:Fm’. The decrease in qP with irradiance (Figs. 1 e, f and 2 e, f) was greater than that of Fv’:Fm’, such that the decline in Φ<sub>PSII</sub> with light can mainly be attributed to the reduction in qP.

At ear emergence in the 2006 experiment, elevated CO₂ slightly, but significantly, (Table 2) increased Φ<sub>PSII</sub> (Fig. 1 a, b). After anthesis, Φ<sub>PSII</sub> at low light was also higher in elevated than ambient CO₂ (Fig. 2 a). With light intensities above about 150 µmol m⁻² s⁻¹ the difference was reversed, such that elevated CO₂ decreased Φ<sub>PSII</sub> (Fig. 2b). In both growth stages, the differences in Φ<sub>PSII</sub> between CO₂ levels were due to the maximum (parameter <i>a</i>) for the regression over light intensity <i>(y = a + bx)</i>, Table 2) rather than to changes in the rate of decrease with irradiance. Like Φ<sub>PSII</sub>, Fv’:Fm’ increased in elevated CO₂ (Figs. 1 c, d and 2 c, d) due to higher initial values (regression parameter <i>a</i>, Table 2) and, with low light intensity at ear emergence, to slower decreases with light in elevated than ambient CO₂ (Fig. 1 c; Table 2, different <i>a</i> and <i>b</i> regression parameters). Treatment differences in Φ<sub>PSII</sub> were therefore associated with higher Fv’:Fm’ values in elevated than ambient CO₂ throughout the range of light intensities both at ear emergence and after anthesis (Figs. 1 c, d and 2 c, d). At ear emergence, qP showed a significant difference between ambient and elevated CO₂ in the <i>a</i> and <i>b</i> regression parameters (Table 2). This implies that with low light intensities (Fig. 1 e) qP was higher for elevated CO₂, but underwent a greater decline with irradiance, in such a way that with higher light intensities (Fig. 1 f) qP was lower in elevated than ambient CO₂. After anthesis, the initial qP values under low light (Fig. 2 e) were no longer
different between ambient- and elevated-CO\textsubscript{2} plants. As at ear emergence, the CO\textsubscript{2} effect with light intensities above 150 µmol m\textsuperscript{-2} s\textsuperscript{-1} (Fig. 2 f) became significant and was reversed, qP being lower with elevated than ambient CO\textsubscript{2}; the difference was due to the \( a \) regression parameter (Table 2) and not to different slopes in the light response. Thus, after anthesis the higher Fv':Fm' in elevated than ambient CO\textsubscript{2} was combined with similar (low light) or lower (moderate or high light) qP in elevated CO\textsubscript{2}. This led to an enhancement (low light) or inhibition (high light) of \( \Phi_{PSII} \) in response to CO\textsubscript{2} enrichment. The increase with CO\textsubscript{2} enrichment in Fv:Fm (data not shown) was small and did not reach statistical significance at ear emergence. The NPQ response to light intensity at ear emergence displayed lower values (smaller \( a \) regression parameter) with elevated than ambient CO\textsubscript{2} and similar slopes (\( b \) and \( r \) parameters) with both CO\textsubscript{2} concentrations (Fig. 1 g, h). After anthesis Fv:Fm and NPQ were not recorded.

At ear emergence in the 2006 experiment, temperatures 4 ºC above ambient values increased \( \Phi_{PSII} \) (Fig. 1 a, b) through an increase in the \( a \) regression parameter (Table 2); namely, in its maximal value. After anthesis, the differences between growth temperatures in \( \Phi_{PSII} \) at low light did not reach statistical significance. With moderate or high light intensities after anthesis, warmer temperatures decreased \( \Phi_{PSII} \) in elevated CO\textsubscript{2}, while this effect did not reach significance in ambient CO\textsubscript{2} (Fig. 2 b). At ear emergence, Fv':Fm’ increased with warmer temperatures (Fig. 1 c, d), except at low light intensities in elevated growth CO\textsubscript{2}. Among ambient CO\textsubscript{2}-grown plants, the initial (below 150 µmol m\textsuperscript{-2} s\textsuperscript{-1} irradiance) decrease in Fv':Fm’ with light was lower at warmer temperatures (Fig. 1 c; significant difference in the \( a \) and \( b \) parameters of the regression, Table 2). After anthesis, Fv':Fm’ was not significantly affected by the growth temperature (Fig. 2 c, d). Above-ambient growth temperatures had no significant effect on qP at ear emergence (Fig. 1 e, f) or, under low light, after anthesis (Fig. 2 e). With
moderate and high light intensities after anthesis, warmer temperatures had a negative
effect on qP in plants grown in ambient CO₂ (Fig. 2 f), but no significant effect in
elevated CO₂-grown plants. At ear emergence, Fv:Fm was not significantly affected by
temperature. The NPQ light response at low irradiances (Fig. 1 g) showed no significant
change with the growth temperature. At higher irradiances (Fig. 1 h), warmer
temperatures decreased NPQ (lowered the a regression parameter, Table 2) both in
ambient and elevated CO₂. Fluorescence parameters in the dark-adapted state were not
determined after anthesis

*Photosynthetic carbon assimilation*

In both experimental years, when measured at the respective growth CO₂
concentrations, carbon fixation increased with elevated CO₂ by 62% to 72% (data not
shown). To assess the acclimatory effects of growth CO₂, comparisons were made at a
common CO₂ concentration of 370 μmol mol⁻¹, at which chlorophyll fluorescence
measurements were also performed. Photosynthesis declined between ear emergence
and 10-13 days after anthesis (Table 3). Growth in elevated CO₂ decreased carbon
fixation measured in ambient CO₂ at ear emergence in 2004 and 2006 and 10-13 days
after anthesis in 2004; the effect did not reach significance after anthesis in 2006. This
decrease in carbon fixation was not accompanied by decreases in intercellular CO₂
concentrations (Cᵢ, Table 3), pointing to a non-stomatal limitation to photosynthesis in
elevated growth CO₂, in agreement with the data for stomatal conductance (gₛ, Table 3).
In the first year, above-ambient temperatures increased photosynthesis and gₛ at ear
emergence, but not after anthesis, while increasing Cᵢ on both dates (Table 3). Thus,
warmer temperature-enhanced CO₂ diffusion through stomata resulted in faster
photosynthesis rates at ear emergence in 2004. Elevated temperatures had no significant effect on carbon assimilation, C_i or g_s in 2006.

_Clorophyll content and the chlorophyll a:b ratio_

From ear emergence to 10-13 days after anthesis in the 2006 experiment, total chlorophyll and chlorophyll a contents per unit leaf area (Table 4) decreased in ambient but not in elevated CO2; the decrease with time in chlorophyll b was similar in both growth CO2 concentrations. In turn, the chlorophyll a:b ratio increased after anthesis relative to ear emergence. Total chlorophyll and chlorophyll a and b contents per unit leaf area at ear emergence and after anthesis (Table 4) were higher in elevated than ambient CO2 in plants grown in ambient temperatures, and they showed no differences due to CO2 in those grown in warmer temperatures. Elevated CO2 decreased the chlorophyll a:b ratio. There were no effects of temperature on chlorophyll contents or ratios.

_Green area and total dry matter_

Growth in elevated CO2 increased total plant dry matter at ear emergence and maturity without significantly affecting the green area at ear emergence (Fig. 3) in both years. Warmer temperatures had no significant effect on the green area or dry matter.
Discussion

Measurements in ambient CO₂ and moderate or high irradiance have shown that photochemical quenching of fluorescence decreases in plants grown in elevated CO₂, in most cases leading to lower quantum yields of Photosystem II electron transport. Although the magnitude of these changes was only small, in the long term they could have an effect on carbon gain. When compared at the same CO₂ concentration, light-saturated carbon assimilation was also decreased by elevated growth CO₂ while Cᵢ increased (Table 3), indicating that photosynthetic capacity was down-regulated, as observed in many previous studies (Drake et al. 1997; Long et al. 2004; Del Pozo et al. 2005; Pérez et al. 2005). The amount and activity of Rubisco also decreased in elevated CO₂ in our earlier experiments under conditions similar to those of the present study (Pérez et al. 2005; Alonso et al. 2008) In contrast with the results of Aranda et al. (2006), however, elevated CO₂ did not decrease qP at irradiances under about 150 µmol m⁻² s⁻¹. The capacity to use electron transport products is known to progressively limit photochemistry as light intensity increases (Lawson et al. 2002; Baker and Oxbourgh 2004). Thus, the shift from higher, or similar, to lower qP in elevated than ambient CO₂ as irradiance increased implies that restrictions in the carbon assimilation capacity under CO₂ enrichment decreased the use in photochemistry of the light absorbed by the PSII antenna. While qP is generally much more affected by the effective rate constant for photochemistry than by the rate constant for non-radiative dissipation (Baker and Oxbourgh 2004), after anthesis in 2004 qP decreased and NPQ increased in elevated CO₂, suggesting that limitations in carbon assimilation decreased photochemical quenching and that the energy was dissipated non-photochemically (but see below for a discussion of the value of NPQ as an indicator of non-photochemical quenching). In contrast with our results, qP does not decrease in wheat leaves when growth in elevated
CO₂ causes no downward acclimation of carbon assimilation (Habash et al. 1995). In spite of photosynthetic acclimation, carbon assimilation (measured at the respective growth CO₂ concentration) and total dry matter (Fig. 4) were higher in elevated than ambient CO₂, as found in other studies (Long et al. 2004). The observed decreases in qP and Φₚₛᵢᵢ in elevated CO₂ are therefore likely to disappear in comparisons at the respective growth CO₂ concentrations.

Unlike qP, the maximum photochemical efficiency in light did not decrease, but increased with elevated CO₂ at all light intensities when measured in ambient CO₂. Fv′:Fm′ can be determined by the rate constants for both photochemistry and non-radiative decay in PSII (Baker and Oxbourgh 2004). NPQ was decreased by elevated CO₂ at ear emergence in 2006. However, NPQ does not allow direct evaluation of the proportion of the change in Φₚₛᵢᵢ that can be attributed to changes in non-photochemical quenching, whereas Fv′/Fm′ does (Baker et al. 2007). The fact that Fv′:Fm′ was also higher in elevated than ambient CO₂ in conditions (high light; decreased Φₚₛᵢᵢ after anthesis, Figs 1 and 2) that could enhance non-radiative energy dissipation, strongly suggests that growth in elevated CO₂ increases the photochemical efficiency of PSII. Measurements of Fv:Fm in the 2004 experiment support this conclusion. Reported responses to elevated CO₂ of photochemistry include: a) only small changes in chlorophyll fluorescence, in spite of the lowered Rubisco content in elevated CO₂ (Tezara et al. 2002); b) no alterations in Fv′:Fm′ (Habash et al. 1995; Hymus et al. (1999); and c) increases in photochemical efficiency, together with an enhanced carboxylation capacity (Zhang and Dang 2006). To our knowledge, the fact that growth in elevated CO₂ decreases the sink for electron transport products, but increases the maximal PSII photochemical efficiency, is a new finding.
It seems likely that the changes in the chlorophyll a:b ratio (Table. 4) are caused by changes in the PSII light-harvesting antenna rather than by changes in the PSII:PSI ratio. As a sensitive indicator of changes in the PSII light-harvesting complex (Habash et al. 1995), the decrease in chlorophyll a:b ratios caused by elevated CO₂ pointed to an increase in these complexes with respect to the PSII reaction centres, which could account for the enhancement of photochemical efficiency. Similar increases in light-harvesting complexes in elevated CO₂ have been found in shaded lower leaves of wheat after anthesis (Osborne et al. 1998; Adam et al. 2000). We have previously found decreased chlorophyll a:b ratios late in the development of wheat leaves in elevated CO₂ (Pérez et al. 2007), but Fv’/Fm’ and qP were not determined. In contrast to our findings, Habash et al. (1995) found no changes in chlorophyll a:b ratios caused by elevated CO₂. A decrease in carboxylation capacity, together with an increase in photochemical efficiency and the antenna complexes, suggests a shift towards resource allocation for photon capture in plants grown in elevated CO₂. Although a change in balance between Rubisco and electron transport with atmospheric CO₂ enrichment is not consistent with some reports (Sage et al. 1995, Nakano et al. 1997), it is in agreement with the results of Makino et al. (1997), Osborne et al. (1998), and Mitchell et al. (2000).

Notably, at ear emergence in 2006 there were greater decreases with elevated CO₂ in carbon fixation than in ΦPSII when measured in ambient CO₂, suggesting a sink other than CO₂ assimilation for electron transport products. Acclimatory decreases in Rubisco in elevated CO₂ should prevent increases in electron flow to the photorespiratory carbon oxidation cycle. Indirect estimates indicate significant water-water cycle (Mehler ascorbate peroxidase) activity when CO₂ assimilation is restricted (Ort and Baker 2002), but no effect of elevated CO₂ on alternative electron sinks has been found previously (Habash et al. 1995; Hymus et al. 1999). Further combined measurements of
chlorophyll fluorescence, carbon assimilation and activities of the enzymes involved in the Mehler pathway are required to gain (indirect) evidence of changes in alternative electron sinks in leaves acclimated to elevated CO$_2$.

In the 2006, though not in the 2004 experiment, warmer temperatures elicited an effect on chlorophyll fluorescence that was dependent on leaf age and, at later growth stages, on CO$_2$. At ear emergence, the increase in $\Phi_{\text{PSII}}$ with temperature in measurements with ambient CO$_2$ was due to an increase in Fv$'$/Fm$'$ at all light intensities, with no change in qP, suggesting that the use of electron transport products was not decreased by temperature. In agreement with this, carbon assimilation was not affected, or tended to increase, in ambient + 4 $^\circ$C temperatures. The decrease with temperature in NPQ (Fig 1) was consistent with a decrease in non-photochemical quenching. After anthesis, the positive effect of temperature on Fv$'$/Fm$'$ disappeared, tending to be reversed in plants under elevated CO$_2$, in which qP did not change and $\Phi_{\text{PSII}}$ decreased with temperature. This suggests an increase in non-radiative energy dissipation, which could protect PSII centres from over-reduction. In ambient CO$_2$ plants, the absence of effects of high temperatures on Fv$'$/Fm$'$ indicated that non-radiative energy dissipation did not change, and this was associated with an increased reduction of PSII reaction centres, as evidenced by the decrease in qP. In previous studies with low measurement light (Pérez et al. 2007), after anthesis we found a negative effect of high temperatures on $\Phi_{\text{PSII}}$ in ambient CO$_2$, but not in elevated CO$_2$, consistent with similar trends in the present experiments (Fig. 2 a). Under high light, by increasing the non-radiative energy dissipation in warmer temperature, elevated CO$_2$ can protect PSII from damage, as reported by Taub et al. (2000). Although above-ambient temperatures did not significantly affect dry matter production, a trend towards decreased growth at high temperatures was observed in both years. Warmer
temperatures are known to enhance growth but cause its earlier cessation (Ford and Thorne 1975). The loss over time in the positive effects of temperature on $\Phi_{\text{PSII}}$ is consistent with this growth response to warming.

We conclude that future increases in atmospheric CO$_2$ and temperature may have a positive effect on photochemical efficiency, in spite of the down-regulation of carbon assimilation in elevated CO$_2$. High CO$_2$ can mitigate the adverse effect of high temperatures on photochemistry observed in ambient CO$_2$ late in leaf growth. This work provides evidence that with air CO$_2$ enrichment a reallocation of resources favouring light capture may occur.
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Figure captions

Fig. 1. 2006 experiment. Responses to low (a, c, e, g) and high (b, d, f, h) light intensities of chlorophyll fluorescence parameters measured in ambient CO\(_2\) in flag leaves of wheat at ear emergence. Wheat was grown in ambient (open symbols) or elevated (closed symbols) CO\(_2\) combined with ambient (circles) or ambient + 4 °C (squares) temperatures in field chambers. Common or separate regressions were fitted to the treatments according to the analysis of parallelism in Table 2. Vertical bars represent standard errors. Each point is the mean of 12 replicates.

Fig. 2. 2006 experiment. Responses to low (a, c, e) and high (b, d, f) light intensities of chlorophyll fluorescence parameters measured in ambient CO\(_2\) in flag leaves of wheat 10-13 days after anthesis. Wheat was grown in ambient (open symbols) or elevated (closed symbols) CO\(_2\) combined with ambient (circles) or ambient + 4 °C (squares) temperatures in field chambers. Common or separate regressions were fitted to the treatments according to the analysis of parallelism in Table 2. Vertical bars represent standard errors. Each point is the mean of 12 replicates.

Fig. 3. Percentage change with growth in elevated CO\(_2\) (CO\(_2\)) and ambient + 4 °C temperatures (T4) of total green area (white bars) and dry matter (grey bars) at ear emergence and total dry matter at maturity (black bars) in wheat grown in field chambers in 2004 and 2006. Asterisks represent significant effects.
Fig. 1
Fig. 2