

Canopy warming caused photosynthetic acclimation and reduced seed yield in maize grown at ambient and elevated [CO₂]

URSULA M. RUIZ-VERA¹, MATTHEW H. SIEBERS¹, DAVID W. DRAG¹, DONALD R. ORT^{1,2} and CARL J. BERNACCHI^{1,2}

¹Department of Plant Biology, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA, ²Global Change and Photosynthesis Research Unit, USDA-ARS, 1201 W. Gregory Drive, 196 ERML, Urbana, IL 61801, USA

Abstract

Rising atmospheric CO₂ concentration ([CO₂]) and attendant increases in growing season temperature are expected to be the most important global change factors impacting production agriculture. Although maize is the most highly produced crop worldwide, few studies have evaluated the interactive effects of elevated [CO₂] and temperature on its photosynthetic physiology, agronomic traits or biomass, and seed yield under open field conditions. This study investigates the effects of rising [CO₂] and warmer temperature, independently and in combination, on maize grown in the field throughout a full growing season. Free-air CO₂ enrichment (FACE) technology was used to target atmospheric [CO₂] to 200 μmol mol⁻¹ above ambient [CO₂] and infrared heaters to target a plant canopy increase of 3.5 °C, with actual season mean heating of ~2.7 °C, mimicking conditions predicted by the second half of this century. Photosynthetic gas-exchange parameters, leaf nitrogen and carbon content, leaf water potential components, and developmental measurements were collected throughout the season, and biomass and yield were measured at the end of the growing season. As predicted for a C₄ plant, elevated [CO₂] did not stimulate photosynthesis, biomass, or yield. Canopy warming caused a large shift in aboveground allocation by stimulating season-long vegetative biomass and decreasing reproductive biomass accumulation at both CO₂ concentrations, resulting in decreased harvest index. Warming caused a reduction in photosynthesis due to down-regulation of photosynthetic biochemical parameters and the decrease in the electron transport rate. The reduction in seed yield with warming was driven by reduced photosynthetic capacity and by a shift in aboveground carbon allocation away from reproduction. This field study portends that future warming will reduce yield in maize, and this will not be mitigated by higher atmospheric [CO₂] unless appropriate adaptation traits can be introduced into future cultivars.

Keywords: elevated CO₂, free-air CO₂ enrichment, global warming, maize, photosynthesis, yields

Received 24 April 2015 and accepted 30 May 2015

Introduction

The atmospheric concentration of CO₂ ([CO₂]) is increasing at an annual average rate of over 2 μmol mol⁻¹ and is the main driver of global warming (Hartmann *et al.*, 2013). The C₄ photosynthetic pathway is generally insensitive to increases in [CO₂] as the concentration inside the bundle sheath cells is maintained many times higher than atmospheric effectively saturating Rubisco and minimizing the competing oxygenation reactions (Furbank & Hatch, 1987; Hatch, 1992, 2002; He & Edwards, 1996; Kiirats *et al.*, 2002; von Caemmerer & Furbank, 2003). Plants utilizing the C₄ photosynthetic pathway are, however, sensitive to temperature and in general are predicted to exhibit a thermal optimum well above that observed for C₃ species (Sage & Kubien, 2007). *Zea mays* (maize), a C₄ crop, is

the most highly produced crop worldwide (FAOSTAT, 2014). In the United States, the largest maize producer globally (USDA-FAS, 2014), an area of ~35 million Ha of harvested maize produces ~274 million metric tons of grain per annum (2012 data; USDA-NASS, 2013). Given the global importance of maize, it is critical to understand the interactive effects of rising [CO₂] and warming temperature on photosynthetic physiology, biomass, and seed yield.

Results from free-air CO₂ enrichment (FACE) experiments on maize confirm the prediction that elevated [CO₂] does not enhance photosynthetic carbon assimilation (*A*), aboveground biomass (AGB), or yield in maize under nonwater limited conditions (Leakey *et al.*, 2004, 2006; Markelz *et al.*, 2011). Evidence from experiments that tested the photosynthetic responses of maize to increases in temperature (e.g., Labate *et al.*, 1990; Crafts-Brandner & Salvucci, 2002; Kim *et al.*, 2007; Ben-Asher *et al.*, 2008; Zheng *et al.*, 2013) has demonstrated that photosynthesis of maize has a thermal optimum

Correspondence: Carl J. Bernacchi, tel. 217-333-8048, fax +1 (217) 244-6267, e-mail: bernacch@illinois.edu

near 34 °C (Edwards & Baker, 1993; Kim *et al.*, 2007; Hatfield *et al.*, 2011). However, the high photosynthetic rate for maize at warmer temperatures may not necessarily result in increased yield because warming can shorten the grain filling period (from ~18 days after silking until leaf senescence, Badu-Apraku *et al.*, 1983) and because some reproductive processes can be more sensitive to temperature than photosynthesis (Hatfield *et al.*, 2011). Previous research addressing warming effects on maize yield using modeling and time series techniques predict negative effects of warming on growth and yields (Kucharik & Serbin, 2008; Schlenker & Roberts, 2009; Urban *et al.*, 2012). Schlenker & Roberts (2009), assuming a cumulative effect of temperature over time, showed a temperature threshold of ~29 °C, well below the thermal optimum of photosynthesis, above which maize yields began to decline. At high water availability, models predict yield reductions of up to 8% for every 2 °C rise in growing season mean temperature in the Corn Belt central region, currently around 23 °C (Hatfield *et al.*, 2011). Warming coupled with drought can result in greater yield decreases given the influence of higher vapor pressure deficits (D) on productivity (Lobell *et al.*, 2014). Previous research in maize using growth chambers showed a curvilinear response of A and a negative correlation for AGB with increasing temperatures regardless of $[\text{CO}_2]$ (Kim *et al.*, 2007). In that study, elevated $[\text{CO}_2]$ did not affect A or AGB but growth at elevated $[\text{CO}_2]$ decreased the foliar concentration and the maximum activity of phosphoenolpyruvate carboxylase (PEPCase; V_{pmax}), which is the limiting biochemical parameter of C_4 photosynthesis at low C_i . In contrast, results from FACE experiments found no CO_2 effect on V_{pmax} or foliar concentration of PEPCase (Leakey *et al.*, 2006; Markelz *et al.*, 2011). Currently, no open-air manipulative studies exist testing the interactive effects of rising $[\text{CO}_2]$ and temperature on field-grown maize.

In this study, a temperature by free-air $[\text{CO}_2]$ enrichment (T-FACE) experiment was conducted during the 2010 growing season in Champaign, Illinois, USA, to understand and quantify the effects of increasing $[\text{CO}_2]$ (target of 200 $\mu\text{mol mol}^{-1}$ above ambient) and/or temperature (target of 3.5 °C above ambient) on maize photosynthesis, biomass, and yield. Both the $[\text{CO}_2]$ and temperature targets are within the range predicted for the second half of the century (Rowlands *et al.*, 2012; Collins *et al.*, 2013). Although 3.5 °C is at the higher end of predictions (Rowlands *et al.*, 2012), global anthropogenic carbon emissions are presently surpassing the higher end emissions scenarios suggesting greater warming potential (Peters *et al.*, 2013). We predicted that (i) elevated $[\text{CO}_2]$ would not affect photosynthetic rate, aboveground biomass, or yield within a

temperature treatment; (ii) despite model predictions that show increases in C_4 photosynthesis with instantaneous increases in temperature (e.g., von Caemmerer, 2000; Sage, 2002; Sage & Kubien, 2007), maize grown at elevated temperature would have lower photosynthetic rates driven by the down-regulation of key photosynthetic biochemical parameters such as V_{pmax} and the $[\text{CO}_2]$ -saturated rate of A (V_{max}); and (iii) the lower photosynthetic rates associated with higher temperatures would decrease both aboveground biomass and seed yield. Additional gas-exchange parameters, including the quantum efficiency of photosystem II (Φ_{PSII}), g_s , intercellular $[\text{CO}_2]$ (C_i) and additional biomass and yield variables were taken to help decipher the mechanisms and implications of the results.

Materials and methods

Site description and experimental design

This experiment was conducted during the 2010 growing season on maize (*Zea mays* cv. 34b43, Pioneer Hi-Bred International) grown at the soybean free-air CO_2 enrichment (SoyFACE) research facility (Champaign, Illinois, USA: 40°2'30.5"N, 88°13'58.8"W, 230 m a.s.l.). Nested within the 16 Ha field were four ambient and four elevated $[\text{CO}_2]$ octagonal plots, each 20 m across. Heated subplots, coupled with adjacent nonheated reference areas, were located within each of the ambient and elevated $[\text{CO}_2]$ plots to provide a full factorial $[\text{CO}_2]$ by temperature interaction study. The ambient $[\text{CO}_2]$ was ~390 $\mu\text{mol mol}^{-1}$, and the elevated $[\text{CO}_2]$ was ~585 $\mu\text{mol mol}^{-1}$. The treatments were as follows: control (ambient $[\text{CO}_2]$ & nonheated), eT (ambient $[\text{CO}_2]$ & heated), eC (elevated $[\text{CO}_2]$ & nonheated), and eT+eC (elevated $[\text{CO}_2]$ & heated). Site and management descriptions of the FACE experiment have been published previously (Ainsworth *et al.*, 2004; Leakey *et al.*, 2004; Rogers *et al.*, 2004; Bernacchi *et al.*, 2006).

The design of the heating array was similar to that used in Ruiz-Vera *et al.* (2013) over soybean (*Glycine max*) except that the current experiment used a telescoping mast system to orient the heaters 1.2 m above the much taller maize canopy (Fig. S1). Heating occurred continuously throughout the growing season except due to occasional technical problems or during precipitation events when the heaters were programmed to maintain ~10% output to minimize energy wasted on evaporating water. Because of technical issues and precipitation events, the daytime mean canopy temperature for the treatments was lower than the target set point: 22.7 ± 1.6 °C for control, 25.4 ± 1.6 °C for eT, 22.8 ± 1.7 °C for eC, and 25.5 ± 1.6 °C for eT+eC, which gave a mean temperature increment of 2.64 ± 0.33 °C ($n = 4$) for eT vs. control and of 2.73 ± 0.43 °C ($n = 3$) for eT+eC vs. eC (Fig. S2).

Meteorological data

Hourly temperature, humidity, and solar radiation data were collected from a station located ~10 km (40°30'0"N, 88°22'12"

W) from the experimental site using the Surface Radiation Network (<http://www.srrb.noaa.gov/surfrad/index.html>) and processed as described in Vanloocke *et al.* (2010). Vapor pressure deficit (D ; kPa) was calculated from the temperature and humidity data. The precipitation for 2010 was taken from the University of Illinois Willard Airport weather station, which is located ~1 km from SoyFACE (40°2'24"N, 88°16'12"W, <http://cdo.ncdc.noaa.gov/qclcd/QCLCD>). Temperature and precipitation monthly means for historical records (1978–2007) and for the 2010 growing season (2010) in Champaign were obtained from the Midwestern Regional Climate Center (MRCC, <http://mrcc.isws.illinois.edu/CLIMATE/>).

Diurnal photosynthesis measurements

Leaf level gas-exchange measurements were collected using a total of four open gas-exchange systems (LI-6400; LI-COR, Inc., Lincoln, NE, USA) with integrated chlorophyll fluorometers (LI-6400-40 Leaf Chamber Fluorometer; LI-COR, Inc.). At the beginning of the growing season, each gas-exchange system was calibrated and routine checks were performed as in Bernacchi *et al.* (2006). The gas-exchange system software calculates leaf gas-exchange fluxes and concentrations using the von Caemmerer & Farquhar (1981) equations and Φ_{PSII} using the Genty *et al.* (1989) equations.

Diurnal photosynthesis measurements were collected on five dates during the 2010 growing season at approximately 2-week intervals, thus representing the full range of developmental stages (Table S1). The physiological variables measured were A ($\mu\text{mol m}^{-2} \text{s}^{-1}$), g_s ($\text{mol m}^{-2} \text{s}^{-1}$), C_i ($\mu\text{mol mol}^{-1}$), the ratio of C_i to the atmospheric $[\text{CO}_2]$ (C_i/C_a), and Φ_{PSII} . Measurements on diurnal days were collected at 2-h intervals from 0800 to 1800 h. On most days, six time points were obtained but occasional inclement weather or instrument problems resulted in fewer diurnal time points. The measurements were taken from the youngest fully expanded leaves of three plants per plot. Four gas-exchange systems were used simultaneously, one per experimental block. The blocks and order of treatments to be measured for each gas-exchange instrument were randomly assigned for each time point. At the beginning of each measurement period, the values for the photosynthetically active radiation (PAR) and the chamber block temperature in the gas-exchange systems were set to the prevailing photosynthetic photon flux density (PPFD; LI-190; LI-COR, Inc.) and to a thermometer (HMP-45C; Campbell Scientific, Inc.) mounted in the aspirated temperature shield model (076B; Met One Instruments, Grants Pass, OR, USA) at the SoyFACE facility. The block temperature was set 3.5 °C higher than ambient for the heated plots. The reference $[\text{CO}_2]$ in the gas-exchange system was set to 400 $\mu\text{mol mol}^{-1}$ for control and eT, and 600 $\mu\text{mol mol}^{-1}$ for eC and eC+eT. The daily means for the relative humidity in the leaf sample chamber (H_s) ranged between 55 and 66%, and the daily means for the vapor pressure deficit calculated from the measured leaf temperature (D_L) ranged between 1.6 and 2.5 kPa. The season mean values for D_L were as follows: 1.9 kPa for control, 2.2 kPa for eT, 2.1 kPa for eC, and 2.2 kPa for eT+eC. The intrinsic water use efficiency (iWUE) was calculated as A/g_s .

The percent deviation of A and of Φ_{PSII} between all the pairwise treatment comparisons were calculated using the hourly plot means data from all the diurnals. The percent deviations of A were plotted with their corresponding percent deviations of Φ_{PSII} .

Photosynthetic response curves to changes in intercellular $[\text{CO}_2]$ concentrations

Photosynthetic $[\text{CO}_2]$ (A/C_i) response curves were measured four times throughout the growing season within 2 days of the diurnal measurements (Table S1). Beginning at dawn, four gas-exchange systems with integrated chlorophyll fluorometers were used in each block to measure A/C_i curves on the youngest fully expanded leaf on one plant per plot. PAR was maintained at 1500 $\mu\text{mol mol}^{-1}$ and the leaf temperature at 25 °C. The conditions within the chamber were held constant throughout the measurements with the exception of the reference $[\text{CO}_2]$, which was incremented sequentially from 400, 300, 200, 100, 75, 50, 25, 400, 600, 800, and 1200 $\mu\text{mol mol}^{-1}$. The daily means for H_s ranged between 59 and 77%, and D_L ranged between 1.1 and 2.1 kPa. V_{pmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) was obtained by fitting the measured data below a C_i of 50 $\mu\text{mol mol}^{-1}$ to the kinetic equation representing the rate of PEP carboxylation (von Caemmerer, 2000):

$$A_c = \frac{C_m V_{\text{pmax}}}{C_m + K_p} - R_m + (g_{\text{bs}} C_m) \quad (1)$$

where A_c ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is the Rubisco-limited rate of CO_2 assimilation, C_m (μbar) is the mesophyll CO_2 partial pressure, K_p (μbar) is the Michaelis–Menten constant of PEPcase for CO_2 , R_m ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is the mitochondrial respiration in the mesophyll cells, and g_{bs} ($\text{mol m}^{-2} \text{s}^{-1}$) is the bundle sheath conductance to CO_2 . In this study, the $g_{\text{bs}} C_m$ term in Eqn (1) was ignored because g_{bs} is low (von Caemmerer, 2000). V_{max} , which can represent the maximum carboxylation rate of Rubisco and/or the regeneration of PEP, was calculated from the horizontal asymptote of a nonrectangular hyperbola with four parameters as indicated in Markelz *et al.* (2011):

$$V_{\text{max}} = R_m + \frac{aC_i + A_{\text{max}} - \sqrt{(aC_i + A_{\text{max}})^2 - 4\theta aC_i A_{\text{max}}}}{2\theta} \quad (2)$$

where a is the initial slope, A_{max} is the horizontal asymptote, and θ is the curvature factor. Equation (2) was fit using values from the A/C_i curves. Because Eqn (2) extrapolates values to infinite C_i , which can inflate estimates of key parameters due to small random variation in measured data, these parameters were constrained to a maximum value of $C_i = 2000 \mu\text{mol mol}^{-1}$, symbolized as $V_{\text{max}(2000)}$. For both Eqns (1) and (2), the data were fit using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA).

Plant development and harvesting

Maize was planted on April 28, day of year (DOY) 118. Plant developmental stage was recorded every 2–3 days beginning with emergence of the first leaf (V1) and until physiological maturity (R6), following the descriptions outlined in Ritchie

et al. (1993). Aboveground biomass (AGB; stem + leaves + husk + cob + kernels), stem biomass (SB; stem +leaves), and seed yield (SY; kernels) were obtained from two adjacent 1 m rows (representing 0.73 m²) per plot after the R6 reproductive stage and when kernels were dry enough for harvesting (DOY 257). Plants were cut at the base of the stalk ~0.5 cm aboveground. Plant material was dried for 1 week in a forced air oven at 65 °C, after which the biomass components were recorded. The individual kernel weight was calculated as the mean of 200 randomly selected seeds per plot. Cob length, number of kernels per cob, and harvest index (HI, HI = SY/AGB) were recorded.

Plant tissue sampling and volumetric soil moisture content measurements

Plant tissue was collected midday on five plants per plot from the youngest fully expanded leaves on gas-exchange measurement days to obtain leaf percentage of nitrogen (%N) and carbon (%C) and leaf water potential components. Two disks (~1.9 cm diameter) per plant were collected for the N and C content analysis, were oven-dried at ~60 °C for 96 h, ground to a fine powder and ~2.0 mg of each sample processed in an elemental analyzer (Elemental Combustion System CHNS-O, Costech ECS 4010, Valencia, CA, USA). Three leaf tissue disks per plant of ~1.2 cm diameter were collected to determine water potential (WP). Immediately after collection, these disks were enclosed within psychrometric chambers (C-30; Wescor, Inc., Logan, UT, USA), transported to the laboratory and placed in a controlled environmental growth chamber for 2–3 h at 25 °C to achieve thermal equilibrium. Thereafter, the psychrometer chambers were connected to a dew point microvoltmeter (HR-33T; Wescor), and WP was measured as described previously (Leakey *et al.*, 2006). Osmotic potential (OP) was subsequently measured after the chambers were submerged into liquid nitrogen to disrupt cell structure. Turgor potential (TP) was calculated as the difference between WP and OP. The measurements were calibrated using similar WP data using sucrose solutions (0–1.50 M) as standards.

Soil volumetric water content (M_{soil} ; in units of H₂O%_{v/v}) was measured using a capacitance probe (Diviner-2000; Sentek Sensor Technologies, Stepney, SA, Australia) two to three times per week in 10 cm increments from 5 cm to 105 cm depths beginning after seedling emergence and continuing until physiological maturity. Measurements were taken between 1200 and 1500 h from four access tubes (two within planting rows and two between rows) installed in each plot. Raw probe data were calibrated against gravimetric data as in Paltineanu & Starr (1997). Data were divided into three layers for analysis, surface (5–25 cm), middle (35–55 cm), and deep (65–105 cm).

Statistical analysis

The photosynthetic variables (A , g_s , C_i , C_i/C_a , Φ_{PSII} , $i\text{WUE}$, V_{pmax} , $V_{\text{max}(2000)}$), leaf %N and %C, water potential variables (WP, OP, TP), and M_{soil} for each layer were analyzed as a complete block design using a mixed model repeated mea-

asures analysis of variance (ANOVA; PROC MIXED, SAS System 9.3, SAS Institute, Cary, NC, USA) with the Kenward–Roger option for the degrees of freedom calculation. For the seasonal analysis, the fixed effects were DOY, [CO₂], temperature (Temp), and the pair-wise interactions. Block was treated as a random effect. The analysis for the biomass and yield variables (AGB, SB, SY, HI, kernels per cob, individual kernel weight, and cob length) was similar to the analysis above except that repeated measurements and DOY fixed effect were absent. For the daily analysis of the variables with multiple time points, time of day replaced DOY and each day was analyzed separately. Pair-wise comparisons were generated with the differences of least square means (*t*-test). Pair-wise comparisons of M_{soil} between the treatments were conducted on data collected within 1 day of the diurnal measurements to assess any influence that changes in M_{soil} might have on leaf gas exchange. The relation between the percent deviation of A and Φ_{PSII} was fitted using a first-order linear regression. Differences between the slopes and the Y-intercept were statistically analyzed by comparing regression lines in STATGRAPHICS Centurion XVI.I (StatPoint Technologies, Inc., Warrenton, VA, USA). Statistical significance was set *a priori* at $P \leq 0.1$.

Results

The 2010 growing season was warmer than average with progressively declining precipitation

Growing season mean air temperature during the 2010 growing season (from May to August) was ~1.6 °C warmer than the 30-year mean (Table S2). The largest deviation from long-term monthly means occurred in August, which was 2.2 °C warmer than average. Monthly, total rainfall deviated from the long-term mean over much of the growing season. Precipitation amounts were substantially higher than the long-term mean during May and June, but July and August received only about half of normal rainfall. The intensity of high-precipitation events was similar throughout the whole growing season; however, the frequency of storms was notably greater in the first 2 months than in the last 2 months (Fig. S3a). This resulted in daily accumulated solar radiation being more variable in May and June than in July and August (Fig. S3a). The days in which measurements were collected encompassed the variable environmental conditions that occurred throughout the growing season (Fig. S3; Table S3).

Elevated temperature decreased photosynthesis in maize whereas elevated [CO₂] had no effect

Maize grown in heated treatments (eT and eT+eC) showed lower mean photosynthetic rates by 5% compared to nonheated treatments (control and eC;

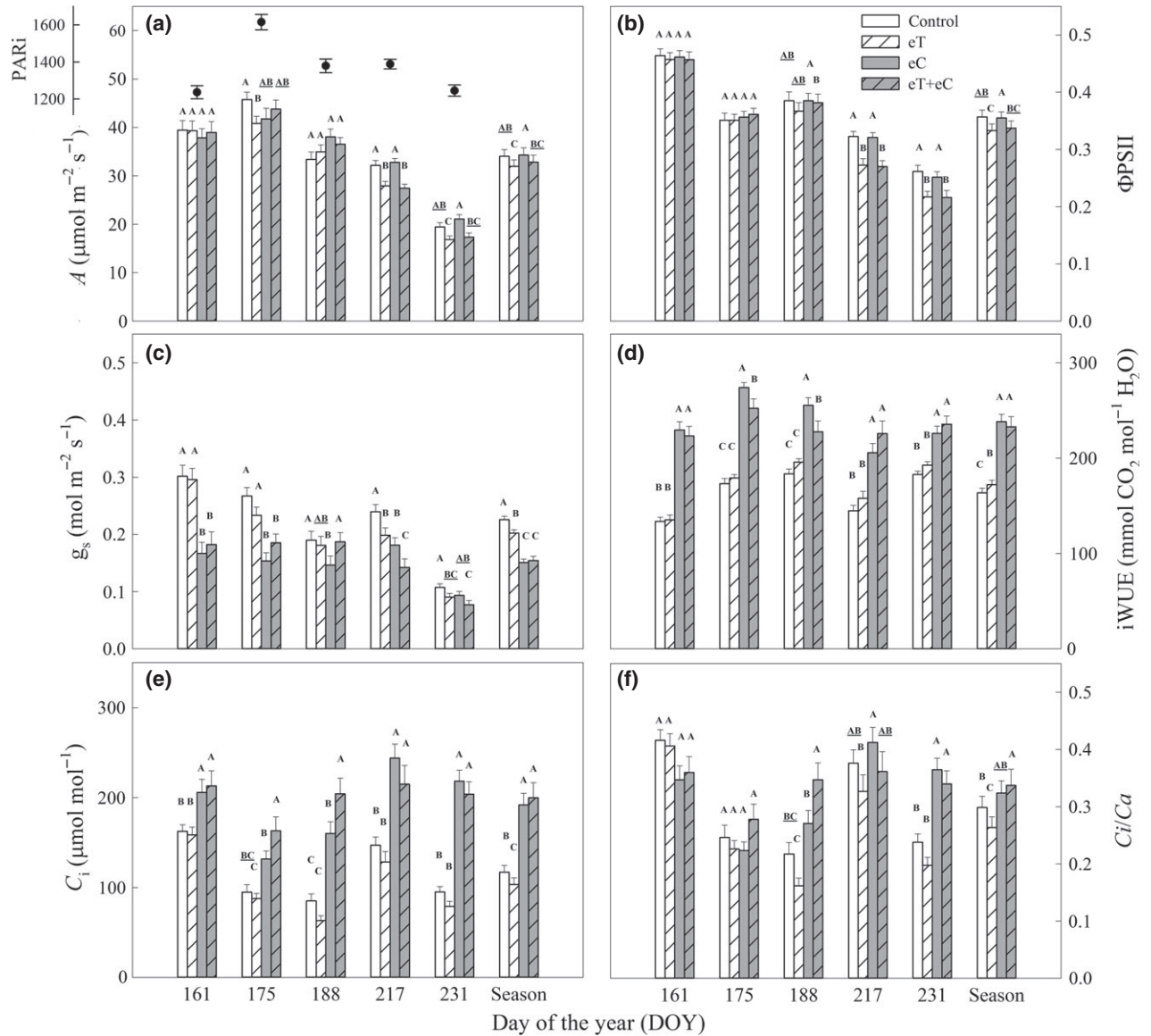


Fig. 1 Photosynthetic carbon uptake (A ; a), quantum yield efficiency of the photosystem II (Φ_{PSII} ; b), stomata conductance (g_s ; c), intrinsic water use efficiency ($i\text{WUE}$; d), intercellular [CO_2] (C_i ; e), and the rate of C_i to the atmospheric [CO_2] (C_i/C_a ; f) for each measurement day and as seasonal means. Bars represent the daily mean values ($n = 4$ for all treatments except the eT+eC treatment where $n = 3$) for each treatment. Also included in panel (a) is mean daily photosynthetically active radiation supplied to the leaves in gas-exchange chambers (PAR_i , $\mu\text{mol m}^{-2} \text{s}^{-1}$, black dots). Error bars are ± 1 standard error (SE) of the mean. Treatments with different letters represent statistically significant differences.

Fig. 1a; Table 1), when analyzed for the full growing season. However, this response was driven by the greater decreases in A at the end of the growing season. Consequently, the DOY by temperature interaction was statistically significant (Table 1) with the decreases in A due to warming observed on DOY 217 and 231 (Fig. 1a; Table S4). There were no resolvable differences in A between the control and elevated [CO_2] plots at any point during the growing season (Fig. 1a; Table S4). Similar trends were observed for

Φ_{PSII} , which led to a strong correlation between the treatment responses on both A and Φ_{PSII} (Figs 1a,b and 2; Table 1). There were no differences in the relationship of percentage change in A and Φ_{PSII} for any of the heated treatments relative to the nonheated treatments (Fig. 2). Season-long V_{pmax} was reduced by 14% and $V_{\text{max}(2000)}$ by 10%, in the heated relative to the nonheated treatments (Fig. 3; Table 1). There were no observable differences in either V_{pmax} or $V_{\text{max}(2000)}$ for eC relative to the control (Fig. 3).

Table 1 Complete block analysis of variance (ANOVA; with or without repeated measures) for the seasonal data of photosynthetic carbon uptake (A), quantum yield efficiency of the photosystem II (Φ_{PSII}), maximum PEP carboxylation rate (V_{pmax}), $[\text{CO}_2]$ -saturated rate of A ($C_i = 2000 \mu\text{mol mol}^{-1}$, $V_{\text{max}(2000)}$), stomatal conductance (g_s), intercellular $[\text{CO}_2]$ (C_i), the rate of C_i to the atmospheric $[\text{CO}_2]$ (C_i/C_a), intrinsic water use efficiency (iWUE), percentage of leaf nitrogen (%N) and carbon (%C), water and osmotic potential (WP, OP), turgor pressure (TP), soil moisture (M_{soil}) at three layers, aboveground biomass (AGB), stem biomass (SB), seed yield (SY), harvest index (HI), kernels per cob, individual (ind) kernel weight, and cob length from maize. The main effects are as follows: $[\text{CO}_2]$, temperature (Temp), and day of the year (DOY). Statistically significant differences ($P < 0.1$) and nonstatistical significance (ns) are shown in the Table 1

	Main effects						
	$[\text{CO}_2]$	Temp	Temp \times $[\text{CO}_2]$	DOY	DOY \times $[\text{CO}_2]$	DOY \times Temp	DOY \times Temp \times $[\text{CO}_2]$
2010							
A	ns	<0.008 (−5.2)	ns	<0.001	ns	<0.08	ns
Φ_{PSII}	ns	<0.003 (−5.9)	ns	<0.001	ns	<0.03	ns
V_{pmax}	ns	<0.007 (−14.0)	ns	<0.001	ns	ns	ns
$V_{\text{max}(2000)}$	ns	<0.003 (−10.2)	ns	<0.001	ns	ns	ns
g_s	<0.001 (−28.3)	ns	<0.04	<0.001	<0.001	<0.1	ns
C_i	<0.001 (77.8)	ns	<0.06	<0.001	<0.001	ns	ns
C_i/C_a	<0.001 (17.5)	ns	<0.04	<0.001	<0.001	ns	ns
iWUE	<0.001 (40.3)	ns	<0.05	<0.001	<0.001	ns	ns
%N	<0.09 (−3.9)	<0.003 (−3.4)	<0.02	<0.001	ns	ns	ns
%C	ns	<0.001 (−2.7)	ns	<0.001	ns	ns	ns
WP	ns	<0.006 (−11.5)	ns	<0.001	ns	ns	ns
OP	ns	<0.001 (−10.8)	ns	<0.001	ns	<0.005	ns
TP	ns	ns	ns	<0.001	ns	ns	<0.09
M_{soil} - surface	ns	ns	ns	<0.001	ns	<0.001	ns
M_{soil} - middle	ns	ns	ns	<0.001	ns	<0.001	ns
M_{soil} - deep	ns	ns	ns	<0.001	ns	<0.05	ns
AGB	ns	ns	ns	—	—	—	—
SB	ns	<0.09 (16.3)	ns	—	—	—	—
SY	ns	<0.007 (−13.7)	ns	—	—	—	—
HI	ns	<0.001 (−12.5)	<0.005	—	—	—	—
kernels per cob	ns	<0.008 (−12.8)	<0.07	—	—	—	—
ind kernel weight	ns	ns	ns	—	—	—	—
cob length	<0.03 (21.9)	<0.04 (19.9)	ns	—	—	—	—

The percent deviation for the $[\text{CO}_2]$ and Temp main effects that are significant are showed between parentheses. Main effects not tested are indicated with a line (—).

Elevated $[\text{CO}_2]$ decreased seasonal g_s and both elevated $[\text{CO}_2]$ and warming had a variable effect depending on the measurement day

Averaged across the growing season elevated $[\text{CO}_2]$ reduced g_s during diurnal measurements by ~28%. There was also a significant season-long temperature by $[\text{CO}_2]$ interaction on g_s (Table 1). This interaction effect was driven by g_s decreases in eT+eC that were significantly lower than eT but similar to eC (Fig. 1c). The effects of temperature and $[\text{CO}_2]$ on g_s varied based on the DOY (Table 1). For the elevated $[\text{CO}_2]$ treatments, the DOY interaction was driven by large reductions in g_s on three of the five measurement days, DOY 161, 175, and 217 (Fig. 1c; Table S4). The significant

DOY by temperature interaction was accompanied by increased g_s on 1 day (DOY 188) even though reduced g_s was observed on two other days (DOY 217, 231; Table S4).

Similar to g_s , the seasonal effects of $[\text{CO}_2]$, $[\text{CO}_2] \times$ temperature, and DOY \times $[\text{CO}_2]$ were significant for C_i , C_i/C_a , and iWUE (Table 1). Elevated $[\text{CO}_2]$ increased C_i on all measurement days (Fig. 1e; Tables 1 and S4). Throughout the growing season, eC and eT+eC had higher C_i than control and eT+eC vs. eC was not significantly different from one another. In contrast, eT resulted in a significantly lower C_i than the control by ~12% (Fig. 1e). While C_i responses were dominated by the increase in atmospheric $[\text{CO}_2]$ for the eC and the eT+eC treatments, normalizing C_i based on C_a (C_i/C_a)

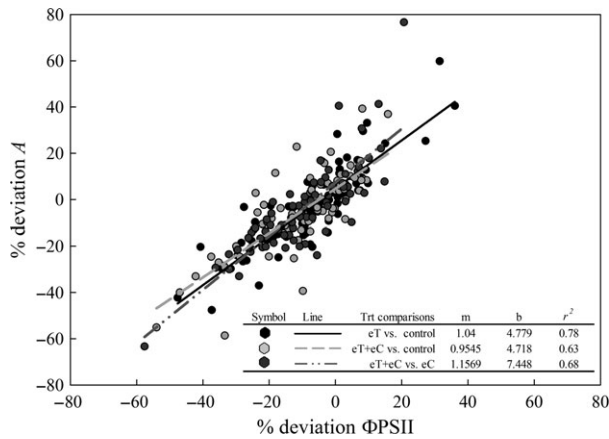


Fig. 2 Linear regressions for the percent deviation between heated and nonheated treatments, as indicated in the legend, in A and Φ_{PSII} . Each symbol represents the hourly plot mean on days in which diurnal measurements were collected. The inset table indicates the symbol and line format for the treatment comparisons and the slope (m), Y-intercept (b), and r^2 for the fitted lines. The linear regression constants between eC vs. control (the not heated treatments; data not shown) are as follows: $m = 0.9988$, $b = 0.995$, $r^2 = 0.50$; and between eT+eC vs. eT (the heated treatments) are as follows: $m = 0.8442$, $b = 1.510$, $r^2 = 0.52$.

resulted in smaller percentage differences between the elevated vs. nonelevated [CO₂] treatment comparisons (Fig. 1f). The degree of reduction in g_s for eT vs. control was reflected in the reductions for C_i and C_i/C_a (Fig. 1c,e,f). All treatments had higher iWUE than control. Seasonally, eT had 5% higher iWUE than control and eC together with eT+eC averaged a 43.9% higher iWUE than control (Fig. 1d; Table 1).

Leaf %N was reduced by both warming and [CO₂] while leaf %C was reduced by only warming

Increases in [CO₂] and temperature individually caused reduction in leaf %N with mean decreases of 3.7% through the season (Table 1). Consequently, all treatments had a lower %N than control (Fig. S4a). There was a statistically significant temperature by [CO₂] interaction, which was driven by warming resulting in lower leaf %N at ambient [CO₂] (control vs. eT) but not at elevated [CO₂] (eC vs. eT+eC). Warming resulted in a slight but significant decrease in leaf %C that averaged ~3% across the full season (Fig. S4b; Table 1).

Leaf water and osmotic potential were reduced by warming

Elevated [CO₂] had no effect on leaf water potential (WP), osmotic potential (OP), or turgor pressure (TP)

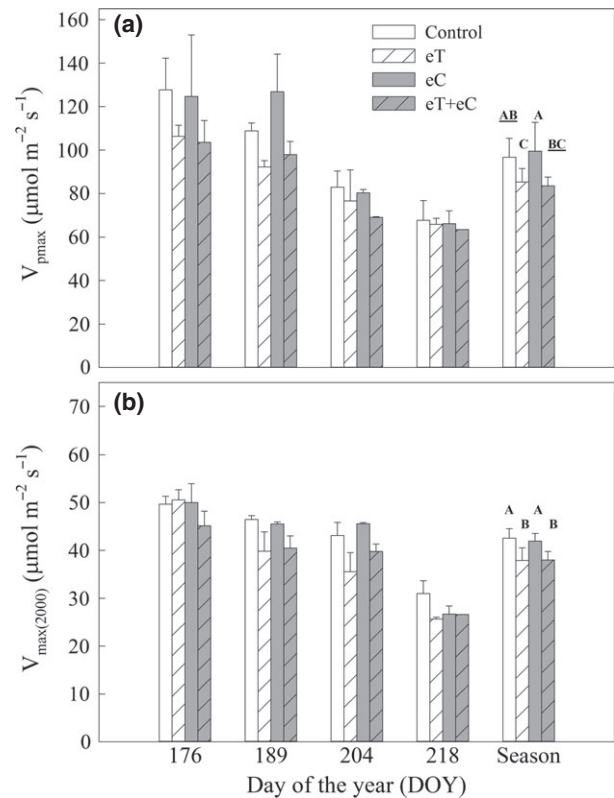


Fig. 3 The maximum PEP carboxylation rate (V_{pmax} ; a) and the [CO₂]-saturated rate of A ($C_i = 2000 \mu\text{mol mol}^{-1}$, $V_{max(2000)}$; b) calculated from the A/C_i curves taken at 4 days during the growing season. Each bar represents the mean for each treatment each day. Seasonal means for each variable are also presented at the right of each graph. Bars, error bars, and letters above each bar are as in Fig. 1.

throughout the season (Fig. 4a–c; Table 1). Warming treatments on the other hand significantly reduced seasonal averages of WP by 12% and OP by 11% (Fig. 4a,b; Table 1). Although there was no significant [CO₂] by temperature interaction, eT+eC vs. eC showed the greatest difference in WP and OP (14% lower in both cases; Fig. 4a,b). OP was significantly reduced by temperature on DOY 188 by 16% and in DOY 217 by 21% (Fig. 4b). On those same days, WP had similar large reductions in the heated treatments. Thus, the decline in WP appears to have been driven by a change in OP, rather than TP, which was not observed to change for any treatment (Fig. 4a–c; Table 1).

Soil moisture was depleted progressively throughout the season and had a variable response to warming

At the start of the growing season, soil moisture (M_{soil}) was at field capacity and gradually declined throughout the season. This response was consistent at all three

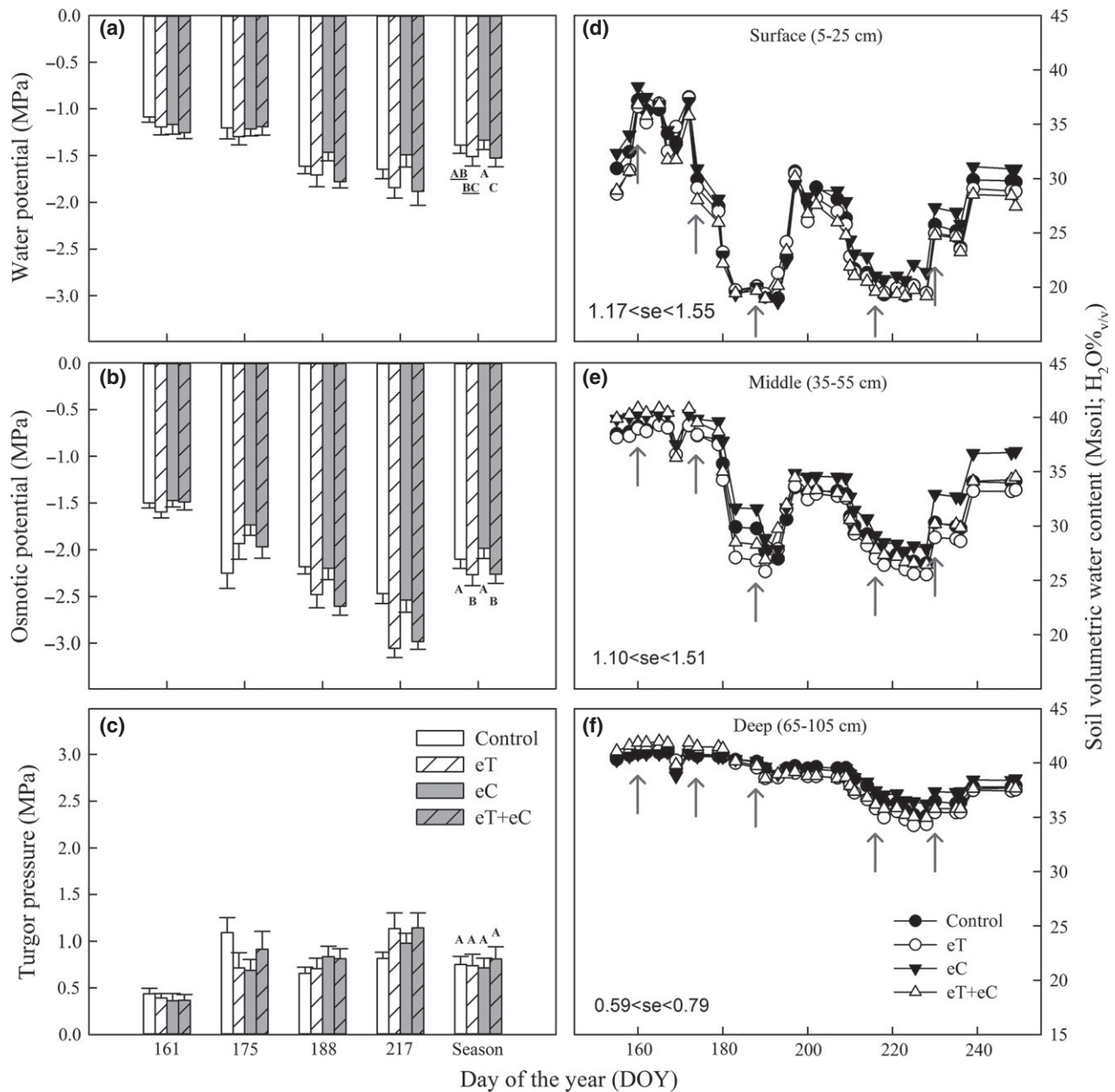


Fig. 4 Daily and seasonal mean water potential (WP), osmotic potential (OP), and turgor pressure (TP) (panels a–c). Error bars and letters above each bar are as in Fig. 1. Soil moisture (M_{soil}) for the surface, middle, and deep layers at multiple days for each treatment throughout the season (panels d–f). Days corresponding to gas-exchange measurements were analyzed with *t*-test pair-wise comparisons and are indicated by arrows. Values for SE are presented at the lower left corner of each graph.

soil depths measured, with the greatest drawdown at the surface layer and diminishing with depth (Fig. 4d–f). Rainfall in the middle of the season caused a short-lived recovery in M_{soil} at the surface and middle layers (Figs S3a and 4d–e). The deep layer M_{soil} declined continuously throughout the season with a more rapid decline late in the season (Fig. 4f) when deeper roots were likely extracting water. The day by temperature

interaction on M_{soil} was significant in all three layers (Table 1). The M_{soil} measured within 1 day of diurnal gas-exchange measurements, and 2 days of A/C_i curve measurements (DOY 160, 174, 188, 202, 216, 230) revealed no temperature effect in the surface layer. However, M_{soil} was lower in the high temperature treatments in the middle layer on 1 day (3% of $H_2O\%_{v/v}$ reduction on DOY 188, $P = 0.001$) and in the deep layer

on 2 days (~1% of H₂O%_{v/v} reduction for both DOY 216, $P = 0.023$, and 230, $P = 0.071$; Fig. 4e,f).

Warming reduced yield but stimulated vegetative biomass

Neither elevated [CO₂] nor warming altered total AGB or individual kernel weight (Fig. 5a,g; Table 1). How-

ever, warming did alter how the AGB was partitioned between vegetative vs. reproductive biomass. Warming increased SB by 16% and decreased SY by 14% compared to nonheated treatments (Fig. 5b,c; Table 1). The changes in AGB and SY led to decreases in HI for all the treatments relative to the control, and both treatments with higher temperatures did not differ from

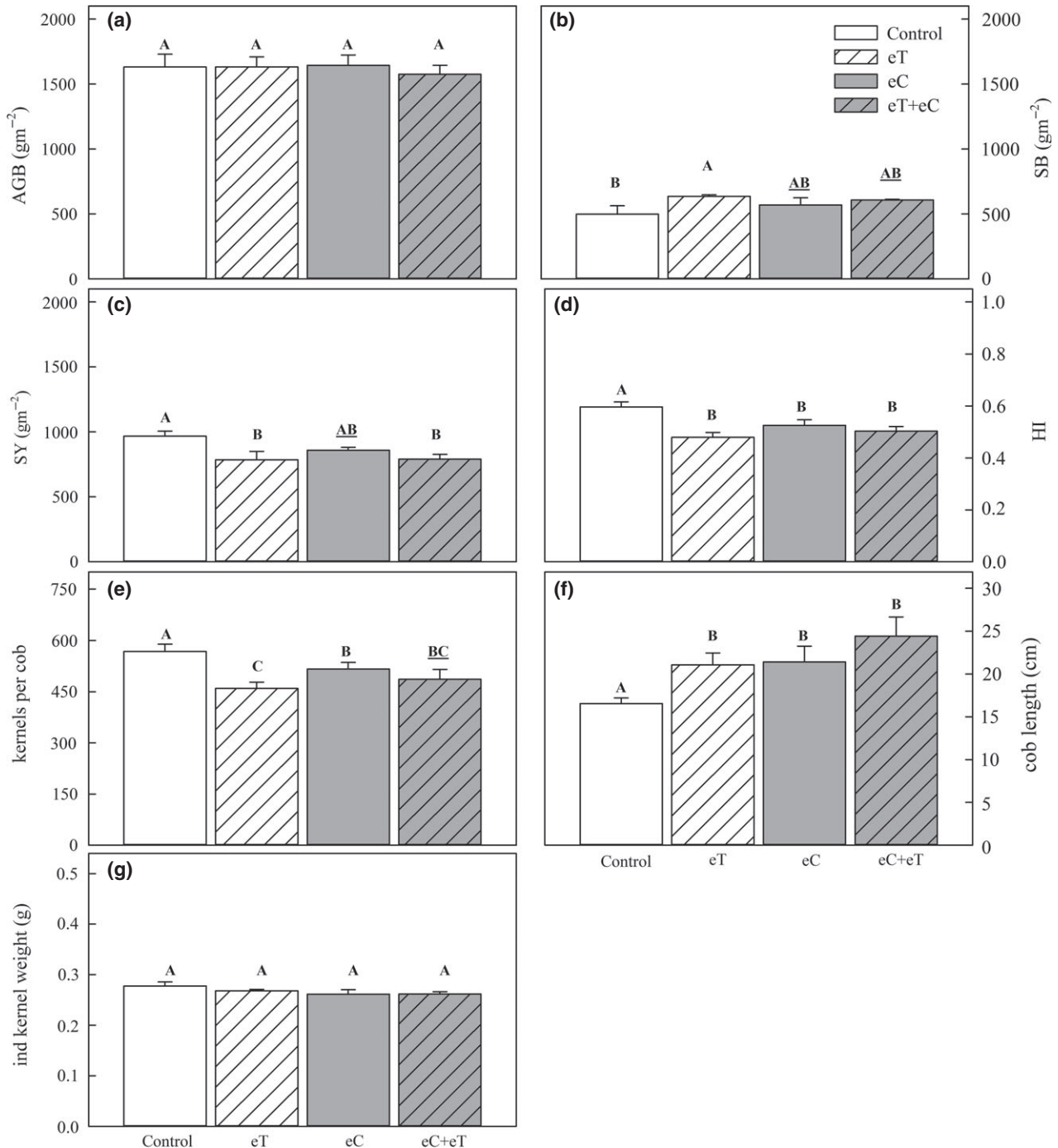


Fig. 5 Aboveground biomass (AGB; a), stem biomass (SB, b), seed yield (SY; c), harvest index (HI; d), kernels per cob (e), cob length (f), and individual (ind) kernel weight (g). Error bars, and letters above each bar are as in Fig. 1.

one another (Fig. 5d; Table 1). Despite no statistically significant difference in SB or SY between the control and eC treatment, there was a significant decrease in HI (Fig. 5d). The longer cob length in maize at elevated temperature and/or [CO₂] did not contribute to more kernels as there were fewer kernels per cob instead (Fig. 5e,f; Table 1).

Discussion

This study tested the predictions that (1) growth of maize in elevated [CO₂] will have no impact on photosynthesis, aboveground biomass, or yields regardless of growth temperature; (2) maize grown under warmer temperatures will have lower photosynthetic rates at both ambient and elevated [CO₂]; and (3) warming will decrease aboveground biomass and yield at both ambient and elevated [CO₂]. Elevated [CO₂] did not impact photosynthesis, aboveground ground biomass, or yield (Figs 1a and 5; Table 1), supporting prediction (1). The second and third predictions were only partially supported by the results. In the heated treatments, there were reductions in V_{pmax} , $V_{max(2000)}$, and Φ_{PSII} , which were reflected in lower A during the second half of the season (Fig. 3; Table S4). Although there was a reduction in SY with warming, AGB did not change with any of the treatments. Additionally, heated plots resulted in greater partitioning into vegetative and less into reproductive biomass than nonheated plots, leading to a decrease in harvest index (Fig. 5; Table 1). While limited to only one growing season, these results suggest that future warming will reduce yield in maize and will not be mitigated by higher atmospheric [CO₂] unless future cultivars are adapted to these conditions.

The lack of [CO₂] effect on A was constant throughout the season and consistent with previous results for maize (Patterson & Flint, 1980; Cure & Acock, 1986; Ziska & Bunce, 1997; Leakey *et al.*, 2004, 2006; Kim *et al.*, 2007; Markelz *et al.*, 2011) and other C₄ species (e.g., Patterson & Flint, 1980; Ziska & Bunce, 1997; von Caemmerer *et al.*, 2001; Wall *et al.*, 2001; Hatfield *et al.*, 2011). In contrast, canopy warming resulted in lower A , although this effect was limited to the second half of the growing season (Fig. 1a) when air temperatures were warmer and rainfall was reduced relative to the first half of the growing season. The lower A in the heated treatments could be driven by reductions in V_{pmax} and $V_{max(2000)}$, suggesting reductions in Rubisco activity or/and the regeneration of PEP (Table 1; Fig. 3). These data also suggest that the declines in V_{pmax} had a lesser effect on A than the declines in V_{max}

(2000). This is supported by the strong temperature-induced decreases in V_{pmax} without similar decrease in A at the beginning of the season and by the correlation between temperature-induced decreases in V_{max} (2000) and A during the second half of the growing season (Fig. 3; Table S4).

In C₄ species, photorespiration is minimized in most circumstances through the establishment of high [CO₂] in the bundle sheath. This results in a close relationship between Φ_{PSII} and A (Oberhuber & Edwards, 1993). Indeed, comparing the percent deviation of A and of Φ_{PSII} between heated and nonheated treatments (e.g., eT vs. control, eT+eC vs. control, and eT+eC vs. eC) resulted in strong correlations (Fig. 2). Additionally, both A and Φ_{PSII} declined in relatively similar patterns during the second half of the growing season when the weather conditions were less favorable than the first half (Fig. 1a,b).

Leaf nitrogen content is shown to correlate strongly with leaf photosynthetic capacity (Evans, 1989; Ghanoum & Conroy, 1998; Ghanoum *et al.*, 2005), consistent with the observed decline of both photosynthesis and leaf nitrogen as maize matured (Figs 1a and S4a). However, there was also lower photosynthesis in heated treatments vs. nonheated treatments at the end of the season (Fig. 1a). Whether the reductions in the leaf %N due to warming were sufficiently large to account for lower A is unlikely because similar leaf %N reductions were observed in eC, and these decreases did not result in lower A than control throughout the season (Figs 1a and S4a; Table 1).

Consistent with previous FACE studies (Leakey *et al.*, 2006; Markelz *et al.*, 2011), maize under elevated [CO₂] (eC) had lower g_s (seasonal reduction of 33%), higher C_i but no change in the C_i/C_a ratio relative to control (Fig. 1c,e,f; Table 1). However, under warming conditions, elevated [CO₂] drove differences between the eT+eC and eT treatments. High C_i/C_a was maintained in the combined treatment (eT+eC) while the warming treatment alone (eT) had the lowest C_i/C_a overall (Fig. 1f). During the growing season, the differences in g_s , C_i , and C_i/C_a between eT+eC and eT did not appear to affect A , consistent with the active maintenance of high CO₂ concentrations surrounding Rubisco.

Previously measured responses of A and g_s to rising temperature for maize have shown different responses with, for example, observed increases in both A and g_s (Zheng *et al.*, 2013), decrease of A but increase of g_s (Crafts-Brandner & Salvucci, 2002), and changes in A with no changes in g_s (Kim *et al.*, 2007). Thus, it is difficult to formulate an *a priori* prediction of how iWUE will be influenced by increases in temperature. Despite the variable results that elevated temperature had on

both A and g_s throughout the season, $iWUE$ had a slight increase at elevated temperature when comparing eT vs. control (+5%; Table S2), but this response was not observed when comparing eC vs. $eT+eC$ (Fig. 1d). These results suggest that higher $iWUE$ associated with rising temperature alone will not occur with concomitant rise in $[CO_2]$.

Warming had a direct and positive effect on SB regardless of growth $[CO_2]$ relative to the nonheated plants (Fig. 5b; Table 1). A shift in biomass allocation from below- to aboveground could potentially explain the observed response of SB to warming as has been suggested previously (Poorter *et al.*, 2012). However, minirhizotron data from within the experimental plots suggest no statistically resolvable differences in root biomass among any of the treatments (Gray *et al.*, in prep.). The loss in photosynthetic potential later in the growing season was unlikely to impact SB as it had already plateaued (Tables S1 and S4).

The decrease in SY, driven by fewer kernels per cob (Fig. 5e; Table 1), is likely driven by unsuccessful fertilization of the ovules and/or abortion of fertilized ovules (Westgate & Boyer, 1986; Zinselmeier *et al.*, 1999; Nielsen, 2013). While it is not clear from the current study which factors influenced the unsuccessful kernel formation, previous research indicates that higher temperatures can promote desiccation of silks making them less receptive to pollen (Heslop-Harrison, 1979; Schoper *et al.*, 1987). An increase in cob length (e.g., Fig. 5f; Table 1) has been shown to delay the emergence of silks and the duration of exposure to pollen (Nielsen, 2013) which can reduce the number of kernels per cob (Carcova & Otegui, 2001). High temperatures can also reduce pollen viability (Schoper *et al.*, 1987; Fonseca & Westgate, 2005) although in this study even a massive reduction in pollen from within the heated plots would be compensated by pollen from the surrounding, and unheated, field. Abortion of fertilized ovules has also been linked to reduced photosynthetic activity by biotic or abiotic factors including heat stress (Westgate & Boyer, 1986; Zinselmeier *et al.*, 1999; Setter *et al.*, 2001; Westgate & Hatfield, 2011; Nielsen, 2013) suggesting that the reduction of photosynthesis by warming could have contributed to the lower SY. Warmer temperatures can also shorten the grain filling developmental stage, leading to reduced kernel mass (Badu-Apraku *et al.*, 1983), although we were unable to resolve any differences in individual kernel weight among any of the treatments (Fig. 5; Table 1).

Despite the gradual reduction in precipitation (Fig. S3) and M_{soil} (Fig. 4d–f) as the season progressed, the Palmer crop moisture index (PCMI, an index linked

to soil water availability) for the region remained above a value of -1 throughout the 2010 growing season suggesting nondrought conditions (Hussain *et al.*, 2013). While PCMI does not account for the increased temperatures imposed through the heating arrays, other factors support the conclusion that water stress did not occur as a result of high D in the heated treatments. While the heated plants most likely used more water (Kimball, 2005) and the plants grown under heated conditions had lower leaf WP (Fig. 4a; Table 1), the values were within the range reported for field-grown maize in 2004 when water limitation was clearly not present (Leakey *et al.*, 2006). Previous research also indicates that CO_2 -induced increases in A and biomass are likely to occur when C_4 crops experience drought conditions (e.g., Ghannoum *et al.*, 2000; Ottman *et al.*, 2001; Wall *et al.*, 2001; Leakey *et al.*, 2004, 2006; Markelz *et al.*, 2011). In this experiment, CO_2 -induced increases in photosynthesis were not observed at either ambient or elevated temperature (eC vs. control and $eT+eC$ vs. eT ; Fig. 1a; Table 1), suggesting that the higher water demand in the heated treatments did not induce significant water stress.

Acknowledgements

The authors acknowledge Elizabeth A. Ainsworth and Stephen P. Long for helpful comments on an early draft of the manuscript. Funding for this research was provided by the United States Department of Agriculture (USDA) Agriculture Research Service (ARS) and by the Office of Science (BER), US Department of Energy, through the Midwestern Center of the National Institute for Climate Change Research (NICCR).

References

- Ainsworth EA, Rogers A, Nelson R, Long SP (2004) Testing the "source-sink" hypothesis of down-regulation of photosynthesis in elevated $[CO_2]$ in the field with single gene substitutions in *Glycine max*. *Agricultural and Forest Meteorology*, **122**, 85–94.
- Badu-Apraku B, Hunter RB, Tollenaar M (1983) Effect of temperature during grain filling on whole plant and grain yield in maize (*Zea mays* L.). *Canadian Journal of Plant Science*, **63**, 357–363.
- Ben-Asher J, Garcia Y, Garcia A, Hoogenboom G (2008) Effect of high temperature on photosynthesis and transpiration of sweet corn (*Zea mays* L. var. *rugosa*). *Photosynthetica*, **46**, 595–603.
- Bernacchi CJ, Leakey ADB, Heady LE *et al.* (2006) Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown at future CO_2 and ozone concentrations for 3 years under fully open-air field conditions. *Plant, Cell and Environment*, **29**, 2077–2090.
- von Caemmerer S (2000) *Biochemical Models of Leaf Photosynthesis*. CSIRO, Collingwood, Australia.
- von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, **153**, 376–387.
- von Caemmerer S, Furbank RT (2003) The C_4 pathway: an efficient CO_2 pump. *Photosynthesis Research*, **77**, 191–207.
- von Caemmerer S, Ghannoum O, Conroy JP, Clark H, Newton PCD (2001) Photosynthetic responses of temperate species to free air CO_2 enrichment (FACE) in a grazed New Zealand pasture. *Australian Journal of Plant Physiology*, **28**, 439–450.
- Carcova J, Otegui ME (2001) Ear temperature and pollination timing effects on maize kernel set. *Crop Science*, **41**, 1809–1815.

- Collins M, Knutti R, Arblaster J (2013) Long-term climate change: projections, commitments and irreversibility. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Stocker TF, Qin D, Plattner G-K *et al.*), pp. 1029–1136. Cambridge University Press, Cambridge, UK and New York, NY, USA.
- Crafts-Brandner SJ, Salvucci ME (2002) Sensitivity of photosynthesis in a C₄ plant, maize, to heat stress. *Plant Physiology*, **129**, 1773–1780.
- Cure JD, Acock B (1986) Crop responses to carbon dioxide doubling: a literature survey. *Agricultural and Forest Meteorology*, **38**, 127–145.
- Edwards GE, Baker NR (1993) Can CO₂ assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? *Photosynthesis Research*, **37**, 89–102.
- Evans J (1989) Photosynthesis and nitrogen relationships in leaves of C₃ plants. *Oecologia*, **78**, 9–19.
- Fonseca AE, Westgate ME (2005) Relationship between desiccation and viability of maize pollen. *Field Crops Research*, **94**, 114–125.
- Food and Agricultural Organization of the United Nations (FAO). The Statistic Division of the FAO (FAOSTAT) (2014) Production. Available at: <http://faostat-fao.org/site/291/default.aspx> (accessed 2 February 2015).
- Furbank RT, Hatch MD (1987) Mechanism of C₄ photosynthesis. *Plant Physiology*, **85**, 958–964.
- Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta*, **990**, 87–92.
- Ghannoum O, Conroy JP (1998) Nitrogen deficiency precludes a growth response to CO₂ enrichment in C₃ and C₄ *Panicum* grasses. *Australian Journal of Plant Physiology*, **25**, 627–636.
- Ghannoum O, von Caemmerer S, Ziska LH, Conroy JP (2000) The growth response of C₄ plants to rising atmospheric CO₂ partial pressure: a reassessment. *Plant, Cell and Environment*, **23**, 931–942.
- Ghannoum O, Evans J, Chow W (2005) Faster Rubisco is the key to superior nitrogen-use efficiency in NADP-malic enzyme relative to NAD-malic enzyme C₄ grasses. *Plant Physiology*, **137**, 638–650.
- Hartmann DL, Klein TankAMG, Rusticucci M (2013) Observations: atmosphere and surface. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. (eds Stocker TF, Qin D, Plattner G-K *et al.*), pp. 159–254. Cambridge University Press, Cambridge, UK and New York, NY, USA. doi:10.1017/CBO9781107415324.008
- Hatch MD (1992) C₄ Photosynthesis: an unlikely process full of surprises. *Plant and Cell Physiology*, **33**, 333–342.
- Hatch MD (2002) C₄ photosynthesis: discovery and resolution. *Photosynthesis Research*, **73**, 251–256.
- Hatfield JL, Boote KJ, Kimball BA, Izaurrealde RC, Ort DR, Thomson A, Wolfe DW (2011) Climate impacts on agriculture: implications for crop production. *Agronomy Journal*, **103**, 351–370.
- He D, Edwards GE (1996) Estimation of diffusive resistance of bundle sheath cells to CO₂ from modeling of C₄ photosynthesis. *Photosynthesis Research*, **2**, 195–208.
- Heslop-Harrison J (1979) An interpretation of the hydrodynamics of pollen. *American Journal of Botany*, **66**, 737–743.
- Hussain M, VanLoocke A, Siebers MH *et al.* (2013) Future carbon dioxide concentration decreases canopy evapotranspiration and soil water depletion by field-grown maize. *Global Change Biology*, **19**, 1572–1584.
- Kiirats O, Lea PJ, Franceschi VR, Edwards GE (2002) Bundle sheath diffusive resistance to CO₂ and effectiveness of C₄ photosynthesis and refixation of photorespired CO₂ in a C₄ cycle mutant and wild-type *Amaranthus edulis*. *Plant Physiology*, **130**, 964–976.
- Kim S-H, Gitz DC, Sicher RC, Baker JT, Timlin JD, Reddy VR (2007) Temperature dependence of growth, development, and photosynthesis in maize under elevated CO₂. *Environmental and Experimental Botany*, **61**, 224–236.
- Kimball BA (2005) Theory and performance of an infrared heater for warming ecosystems. *Global Change Biology*, **11**, 2041–2056.
- Kucharik CJ, Serbin SP (2008) Impacts of recent climate change on Wisconsin corn and soybean yield trends. *Environmental Research Letters*, **3**, 1–10.
- Labate CA, Adcock MD, Leegood RC (1990) Effects of temperature on the regulation of photosynthetic carbon assimilation in leaves of maize and barley. *Planta*, **181**, 547–554.
- Leakey ADB, Bernacchi CJ, Dohleman FG, Ort DR, Long SP (2004) Will photosynthesis of maize (*Zea mays*) in the US Corn Belt increase in future [CO₂] rich atmospheres? An analysis of diurnal courses of CO₂ uptake under free-air concentration enrichment (FACE). *Global Change Biology*, **10**, 951–962.
- Leakey ADB, Uribealarea M, Ainsworth EA, Naidu SL, Rogers A, Ort DR, Long SP (2006) Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO₂ concentration in the absence of drought. *Plant Physiology*, **140**, 779–790.
- Lobell DB, Roberts MJ, Schlenker W, Braun N, Little BB, Rejesus RM, Hammer GL (2014) Greater sensitivity to drought accompanies maize yield increase in the U.S. Midwest. *Science*, **344**, 516–519.
- Markelz RJC, Strellner RS, Leakey ADB (2011) Impairment of C₄ photosynthesis by drought is exacerbated by limiting nitrogen and ameliorated by elevated [CO₂] in maize. *Journal of Experimental Botany*, **62**, 3235–3246.
- Nielsen RL (2013) Effects of stress during grain filling in corn. Corny News Network, Purdue Univ. Available at: <http://www.agry.purdue.edu/ext/corn/news/timeless/GrainFillStress.html> (accessed 6 May 2015).
- Oberhuber W, Edwards GE (1993) Temperature dependence of the linkage of quantum yield of photosystem II to CO₂ fixation in C₄ and C₃ plants. *Plant Physiology*, **101**, 507–512.
- Ottman MJ, Kimball BA, Pinter PJ *et al.* (2001) Elevated CO₂ increases sorghum biomass under drought conditions. *New Phytologist*, **150**, 261–273.
- Paltineanu IC, Starr JL (1997) Real-time soil water dynamics using multisensor capacitance probes: laboratory calibration. *Soil Science Society of America Journal*, **61**, 1576–1585.
- Patterson DT, Flint EP (1980) Potential effects of global atmospheric CO₂ enrichment on the growth and competitiveness of C₃ and C₄ weeds and crop plants. *Weed Science*, **28**, 71–75.
- Peters GP, Andrew RM, Boden T *et al.* (2013) The challenge to keep global warming below 2 degrees C. *Nature Climate Change*, **3**, 4–6.
- Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist*, **193**, 30–50.
- Ritchie SW, Hanway JJ, Benson GO (1993) How a corn plant develops. Special Report No 48. Iowa State University of Science and Technology, Ames, IA.
- Rogers A, Allen DJ, Davey PA *et al.* (2004) Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under Free-Air Carbon dioxide Enrichment. *Plant, Cell and Environment*, **27**, 449–458.
- Rowland DJ, Frame DJ, Ackerley D *et al.* (2012) Broad range of 2050 warming from an observationally constrained large climate model ensemble. *Nature Geoscience*, **5**, 256–260.
- Ruiz-Vera UM, Siebers M, Gray SB *et al.* (2013) Global warming can negate the expected CO₂ stimulation in photosynthesis and productivity for soybean grown in the Midwest United States. *Plant Physiology*, **162**, 410–423.
- Sage RF (2002) Variation in the k_{cat} of Rubisco in C₃ and C₄ plants and some implications for photosynthetic performance at high and low temperature. *Journal of Experimental Botany*, **53**, 609–620.
- Sage RF, Kubien DS (2007) The temperature response of C₃ and C₄ photosynthesis. *Plant, Cell and Environment*, **30**, 1086–1106.
- Schlenker W, Roberts MJ (2009) Nonlinear temperature effects indicate severe damages to U.S. crop yields under climate change. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 15594–15598.
- Schooper JB, Lambert RJ, Vasilas RL, Westgate ME (1987) Plant factors controlling seed set in maize. *Plant Physiology*, **83**, 121–125.
- Setter TL, Flannigan BA, Melkonian J (2001) Loss of kernel set due to water deficit and shade in maize: carbohydrate supplies, abscisic acid, and cytokinins. *Crop Science*, **41**, 1530–1540.
- United States Department of Agriculture, Foreign Agricultural Service (USDA-FAS) (2014) World Agricultural Production. Available at: <http://apps.fas.usda.gov/psdonline/circulars/production.pdf> (acc) (accessed 6 May 2015).
- United States Department of Agriculture, National Agricultural Statistics Service (USDA-NASS) (2013) Crop Production. Available at: <http://usda.mannlib.cornell.edu/usda/nass/CropProd//2010s/2013/CropProd-11-08-2013.pdf> (accessed 6 May 2015).
- Urban D, Roberts MJ, Schlenker W, Lobell DB (2012) Projected temperature changes indicate significant increase in interannual variability of U.S. maize yields. *Climatic Change*, **112**, 525–533.
- Vanloocke A, Bernacchi CJ, Twine TE (2010) The impacts of *Miscanthus* × *giganteus* production on the Midwest US hydrologic cycle. *Global Change Biology Bioenergy*, **2**, 180–191.
- Wall GW, Brooks TJ, Adam NR *et al.* (2001) Elevated atmospheric CO₂ improved *Sorghum* plant water status by ameliorating the adverse effects of drought. *New Phytologist*, **152**, 231–248.
- Westgate ME, Boyer JS (1986) Reproduction at low silk and pollen water potentials in maize. *Crop Science*, **26**, 951–956.

- Westgate ME, Hatfield JL (2011) Genetic adjustment to changing climates: maize. In: *Crop Adaptation to Climate Change* (eds Yadav SS, Redden RJ, Hatfield JL, Lotze-Campen H, Hall AE), Chapter 13, pp. 314–325. JohnWiley & Sons, Ltd., Oxford, UK.
- Zheng Y, Xu M, Shen R, Qiu S (2013) Effects of artificial warming on the structural, physiological, and biochemical changes of maize (*Zea mays* L.) leaves in northern China. *Acta Physiologiae Plantarum*, **35**, 2891–2904.
- Zinselmeier C, Jeong BR, Boyer JS (1999) Starch and the control of kernel number in maize at low water potentials. *Plant Physiology*, **121**, 25–36.
- Ziska LH, Bunce JA (1997) Influence of increasing carbon dioxide concentration on the photosynthetic and growth stimulation of selected C₄ crops and weeds. *Photosynthesis Research*, **54**, 199–208.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Photograph of heated T-FACE (Temperature by Free Air CO₂ Enrichment) plot that is positioned within an elevated CO₂ plot.

Figure S2. Difference in surface temperature due to heating (ΔT ; a) and daily mean canopy temperature (T ; b) during the 2010 growing season.

Figure S3. Daily values for the meteorological conditions in the 2010 growing seasons.

Figure S4. Percentage of leaf nitrogen (%N; a) and carbon (%C; b) in maize, during the 2010 growing season.

Table S1. Calendar date and day of year (DOY) when the gas exchange (5 days) and A/C_i curves (4 days, indicated in bold and inside parenthesis) measurements were done in maize at the T-FACE (Temperature by Free Air CO₂ Enrichment) plots.

Table S2. Mean temperature (°C) and precipitation (mm) at annual, 4-month and monthly ranges during a 30-year period and in 2010.

Table S3. Day of year (DOY), mean, minimum, and maximum temperatures (Mean Temp, Min. Temp, Max. Temp), vapor pressure deficit (D), solar radiation and precipitation during the days of gas exchange and A/C_i curves measurements in 2010.

Table S4. Analysis of variance with complete block repeated measures for the daily data of: photosynthetic carbon uptake (A), quantum yield efficiency of the photosystem II (Φ_{PSII}), stomatal conductance (g_s), intercellular [CO₂] (C_i), the rate of C_i to the atmospheric [CO₂] (C_i/C_a), and the intrinsic water use efficiency (iWUE) from maize grown in a [CO₂] by temperature interaction.