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Temperature dependence of growth, development, and photosynthesis in maize under elevated CO_2

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Abstract

Global atmospheric carbon dioxide concentrations (C_a) are rising. As a consequence, recent climate models have projected that global surface air temperature may increase 1.4–5.8 °C with the doubling of C_a by the end of the century. Because, changes in C_a and temperature are likely to occur concomitantly, it is important to evaluate how the temperature dependence of key physiological processes are affected by rising C_a in major crop plants including maize (*Zea mays* L.), a globally important grain crop with C_4 photosynthetic pathway. We investigated the temperature responses of photosynthesis, growth, and development of maize plants grown at five temperature regimes ranging from 19/13 to 38.5/32.5 °C under current (370 μmol mol^{-1}) and doubled (750 μmol mol^{-1}) C_a throughout the vegetative stages using sunlit controlled environmental chambers in order to test if the temperature dependence of these processes was altered by elevated C_a. Leaf and canopy photosynthetic rates, C_4 enzyme activities, leaf appearance rates, above ground biomass accumulation and leaf area were measured. We then applied temperature response functions (e.g., Arrhenius and Beta distribution models) to fit the measured data in order to provide parameter estimates of the temperature dependence for modeling photosynthesis and development at current and elevated C_a in maize. Biomass, leaf area, leaf appearance rate, and photosynthesis measured at growth C_a was not changed in response to CO_2 enrichment. Carboxylation efficiency and the activities of C_4 enzymes were reduced with CO_2 enrichment indicating possible photosynthetic acclimation of the C_4 cycle. All measured parameters responded to growth temperatures. Leaf appearance rate and leaf photosynthesis showed curvilinear response with optimal temperatures near 32 and 34 °C, respectively. Total above ground biomass and leaf area were negatively correlated with growth temperature. The dependence of leaf appearance rate, biomass, leaf area, leaf and canopy photosynthesis, and C_4 enzyme activities on growth temperatures was comparable between current and elevated C_a. The results of this study suggest that the temperature effects on growth, development, and photosynthesis may remain unchanged in elevated C_a compared with current C_a in maize.
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1. Introduction

Global atmospheric carbon dioxide concentrations (C_a) are rising (367 μmol mol^{-1} in 1999) and are projected to reach between 540 and 970 μmol mol^{-1} by the end of the 21st century (Prentice et al., 2001). Recent climate model projections have also suggested that global surface air temperature may increase 1.4–5.8 °C in association with this doubling of C_a (Cubasch et al., 2001). Since both C_a and temperature are among the most important environmental variables that regulate physiological and phenological processes in plants, it is critical to evaluate the effects of C_a and air temperature on the growth and yield of key crop plants. Because changes in C_a and temperature are likely to occur concomitantly, it is of particular interest to quantify the interactions of these two climate variables (Morison and Lawlor, 1999).

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In C_3 plants, enhanced growth and photosynthesis are generally observed in response to elevated C_a. However, plant responses to elevated C_a can be mitigated by various acclimation mechanisms (Moore et al., 1999; Stitt, 1991). Owing to the biochemical and anatomical specialization associated with the CO_2 concentrating mechanism, changes in photosynthesis and growth in C_4 plants in response to elevated C_a were thought to be minimal. However, several studies reported that both photosynthesis and plant growth of C_4 species responded positively to elevated C_a (see review by Ghannoum et al., 2000). In general, C_4 plants have higher temperature optima for photosynthesis and growth than C_3 plants and thus are better adapted to warmer climates. It is unclear if the temperature dependence of growth and photosynthesis in C_4 plants would remain unchanged in elevated C_a compared with current C_a. In C_3 plants, the temperature optima for photosynthesis were greater at elevated than at ambient C_a and the stimulation of photosynthesis by high C_a was greater at higher temperatures (Kim and Lieth, 2003; Kirschbaum, 1994; Long, 1991). The interactive effects of temperature and CO_2 on the growth and photosynthesis of C_4 plants may be similar to those of C_3 plants but this requires further examination (Morison and Lawlor, 1999). In *Amaranthus*, the CO_2 saturation point was increased with temperature indicating that the sensitivity of photosynthesis to CO_2 in C_4 plants might be enhanced by elevated temperature (Sage, 2002). The temperature dependence of photosynthesis at low C_a (<36 Pa) was similar to that of C_3 plants in *Amaranthus* (Sage, 2002). It is important to better understand the interaction between elevated C_a and higher temperatures in C_4 plants in order to predict plant responses to future climate change.

Photosynthetic acclimation to long-term CO_2 enrichment also has been reported in C_4 plants (Kim et al., 2006; Maroco et al., 1999; Read et al., 1997; Watling et al., 2000). In these prior studies leaf gas exchange measurements displayed both lowered carboxylation efficiency and CO_2 saturated photosynthetic rate (Kim et al., 2006; Read et al., 1997; Watling et al., 2000), as well as a reduction in C_4 enzyme activities (Maroco et al., 1999; Watling et al., 2000) in the enhanced C_a treatments. However, it is unclear how photosynthetic acclimation to high C_a in C_4 plants is related to growth temperature.

The use of free-air CO_2 enrichment (FACE) systems enabled scientists to assess the effects of elevated C_a and other climate variables (e.g., ozone) on various C_3 and C_4 plants in open field settings (Kimball et al., 2002; Long et al., 2004). However, controlling air temperature in the field remains challenging. Soil–plant–atmosphere research (SPAR) units offer an alternative method for studying the interactive effects of C_a and air temperature on plant growth because of their ability to control air temperature and humidity under naturally sunlit conditions (Kim et al., 2004; Reddy et al., 2001). The ability of SPAR units to measure real-time canopy-level gas exchange rates while controlling air temperature, CO_2 concentration, and humidity makes it possible to determine the temperature dependence of physiological processes under elevated C_a at a wide range of growth temperatures (e.g., Prasad et al., 2003; Reddy et al., 1997; Vu et al., 1997).

Maize is the most cultivated C_4 species in the world. An accurate assessment of the effects of elevated C_a and temperature on plant growth and development is critical in order to forecast potential impacts of climate change on maize productivity. The interactive effects of C_a and temperature on growth and photosynthesis have been investigated for many C_3 plants including such major crops as rice, soybean, and wheat (Baker et al., 1992; Delgado et al., 1994; McKee and Woodward, 1994; Vu et al., 1997). This has not been the case with C_4 crops including maize. A primary reason for the pronounced interactive effects of high C_a and rising temperature on growth and photosynthesis of C_3 plants can be that suppression of photorespiration by high C_a and subsequent increase in photosynthesis will be greatest at higher temperatures (Long, 1991). Unlike C_3 plants, photorespiratory loss of carbon is greatly inhibited in C_4 plants due to the CO_2 concentrating mechanism. This suggests that elevated C_a may not alter the temperature dependence of growth and photosynthesis significantly in C_4 plants unless the temperature response of the C_4 cycle changes considerably. Quantification of the response of crop physiological and phenological processes to broad ranges of air temperature at both ambient and elevated C_a is also a key for developing process-based crop simulation models to be used for predicting crop and agricultural systems responses to global climate change.

The objectives of this study were to determine the temperature responses of growth, development, and photosynthesis of maize under current and elevated C_a and to test if the temperature dependence of these processes is altered by elevated C_a. We also investigated C_4 enzyme activities to determine if the interactive effect between temperature and C_a was significant in the C_4 cycle, and to test if the degree of acclimation to high C_a, if present, is influenced by growth temperature. In addition, we provide parameter estimates of the models for predicting the temperature dependence of photosynthetic and developmental processes that can be used in maize crop models.

2. Materials and methods

2.1. Plant culture

Maize plants (*Zea mays* L. cv. Pioneer hybrid 3733) were grown in naturally sunlit, controlled environment Soil–plant–atmosphere research (SPAR) chambers located at the Beltsville Agricultural Research Center, Beltsville, MD, USA. A physical description of these SPAR chambers and methods of operation and environmental control have been described previously (Baker et al., 2004). The maize cultivar used in the study has a relative maturity rating of 102 days (Stewart et al., 1998). Ten SPAR chambers were randomly assigned to ambient (370 $\mu mol\ mol^{-1}$) or elevated C_a (750 $\mu mol\ mol^{-1}$) treatments, and to one of five air temperature regimes (19/13, 25/19, 31/25, 35/29, and 38.5/32.5 °C) with 16 h of the higher temperature daily (i.e., from 05:00 to 21:00 h Eastern Standard Time). Measured daytime (06–18 h EST) C_a values (mean ± S.D. of five chambers) were $374 \pm 1.5\ \mu mol\ mol^{-1}$ and $747 \pm 0.5\ \mu mol\ mol^{-1}$ for ambient

and elevated C_a, respectively. Corresponding nighttime C_a values were $481 \pm 17.0\ \mu mol\ mol^{-1}$ and $684 \pm 5.0\ \mu mol\ mol^{-1}$, respectively. Air temperatures inside the chambers were maintained within 0.5 °C of the set-points throughout the experiment. Total photosynthetically active radiation (PAR) inside each SPAR chamber was mostly within 10% of the ambient levels (Kim et al., 2004). Relative humidity was 55% or greater in all chambers. This corresponds to VPD < 2.0 kPa except in 38.5/32.5 °C treatment. Plants were grown on a mix of sterilized sand and vermiculite (1:1 by volume) and each SPAR soil bin had an approximate volume of 1 m^3 (approximately 2 m length × 0.5 m width × 1 m depth). Maize seeds were sown on 7 June 2002. All chambers were maintained at 33/25 °C day/night temperatures to ensure uniform emergence. Air temperature treatments were initiated on 4 days after planting (DAP) after all seedlings emerged. The seedlings were thinned to 36 plants per chamber with equal row spacing (i.e., 9 rows with 25 cm between rows). Plants were fertigated four times per day (>15 l $chamber^{-1}\ day^{-1}$) using a drip irrigation system with a complete nutrient solution as described in Robinson (1984). The nutrient solution contained macronutrients in mM as follows: NH_4Cl, 1.5; $NH_4H_2PO_4$, 1.0; $Ca(NO_3)_2{\cdot}4H_2O$, 4.0; $MgSO_4{\cdot}7H_2O$, 3.5; KCl, 2.5; KNO_3, 4.0; and K_2SO_4, 1.0. Micronutrient concentrations in μM were: $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.07; H_3BO_3, 20.6; $CuSO_4{\cdot}5H_2O$, 0.16; $MnSO_4{\cdot}H_2O$, 5.0; $ZnSO_4{\cdot}7H_2O$, 0.34; and Fe-chelate, 107.4 as Sequestrene 330. Shade cloth, designed to simulate canopy spectral properties (Easy Gardener, Waco, TX, USA), was placed along the chamber walls inside each chamber, and raised to canopy height twice per week as the plants grew. Beginning 2 weeks after planting, plants per chamber were sampled weekly from row 1, 3, 5, 7 or 9 for the next 5 weeks (or 6 weeks for 19/13 °C chambers) for the determination of dry matter and leaf area. As a result, final plant density was 16 plants per chamber (i.e., 4 rows with 50 cm between rows). The remaining plants were harvested at silking stage (R1). Due to slower developmental rates, plants in 19/13 °C chambers were harvested when the flag leaves were fully expanded. The number of leaf-tips per plant was counted twice per week to obtain leaf appearance rate (LAR). Growing degree days with a base temperature of 8 °C (GDD_8) and phyllochron interval ($GDD_8\ leaf^{-1}$) were calculated according to Lizaso et al. (2003). At the completion of the experiment, each plant was detached at ground level and the leaves, stalk, and ears were separated. Total laminar area per plant was measured with a leaf area meter (LI-COR, LI-3000, Lincoln, NE, USA[1]). Harvested plant parts were dried in a forced-air oven at 70 °C for at least 72 h prior to determining dry weight. Eight plants per chamber from the final harvest were used for dry matter determination. Specific leaf area (SLA) was determined as leaf area divided by dried leaf mass ($cm^2\ g^{-1}$). Silking date was determined when one-half of the plants had silk visible on the outside of the husks and four recently, fully expanded sunlit leaves were sampled from each chamber 4 weeks after planting. The latter were oven-dried, ground, and used for determining total carbon and nitrogen concentrations by combustion (CHN-2000; LECO Corporation, St. Joseph, MI, USA).

2.2. Canopy gas exchange measurements

The [CO_2] of each chamber was measured continuously with a dedicated infrared gas analyzer (Model LI-6252, LI-COR, Inc., Lincoln, NE, USA). Chamber CO_2 was supplied as medical grade CO_2 from a compressed gas cylinder. Injection rates of CO_2 were regulated by mass flow controllers (FMA-766-V-CO2, Omega Engineering, Inc., Stanford, CT, USA) located in the air handling system of each chamber using a feed-forward, feed-back proportional-integral-differential (PID) control algorithm. Canopy CO_2 exchange rates (A_{can}) were calculated from mass balance equations, solved every 30 s, and averaged and recorded every 300 s. Photosynthetically active radiation (PAR), both outside and inside each chamber, was measured with quantum sensors (Model LI-190SB, LI-COR, Inc., Lincoln, NE, USA). In order to correct A_{can} for chamber leakage, chamber CO_2 leakage rates were determined daily using a N_2O drawdown method (Baker et al., 2004).

2.3. Leaf gas exchange and chlorophyll fluorescence measurements

A portable photosynthesis system (LI-6400; LI-COR, Inc., Lincoln, NE, USA) with a red/blue LED light source (LI6400-02B) mounted onto a 6 cm^2 clamp-on leaf chamber was used to determine the rate of net CO_2 assimilation (A). Three to five plants per chamber were selected, and fully expanded young leaves (leaf 5–14) of the main stem were used for CO_2 (A/C_i) and PAR (A/Q) response determinations from late June to early July (19–35 DAP) between 09:00 and 16:00 h. The A/C_i measurements were made at 10 CO_2 levels between 35 and 1500 $\mu mol\ mol^{-1}$ with PAR inside the leaf cuvette controlled to saturating levels of either 1800 or 2000 $\mu mol\ m^{-2}\ s^{-1}$. The A/Q response was determined at nine PAR levels between 0 and 2500 $\mu mol\ m^{-2}\ s^{-1}$ at the respective growth C_a. Leaf temperature was maintained at growth air temperatures and relative humidity ranged $52 \pm 9.7\%$.

The maximum photochemical efficiency of PSII was determined as relative variable chlorophyll fluroescence (F_v/F_m) using a pulse amplitude modulated chlorophyll fluorescence system (Model OS-500, Opti-Sciences Inc., Tyngsboro, MA, USA). The illumination and measurement signals were provided by a trifurcated light guide held at either 45° or 90° to the upper leaf surface. Red modulating light and saturating flashes of white light were from the fluorometer. Predawn fluorescence measurements were performed daily using the most recent, fully expanded leaf of all plants in each chamber over a 10-day period beginning July 2nd (25 DAP).

[1] Mention of this or other proprietary products is for the convenience of the readers only, and does not constitute endorsement or preferential treatment of these products by USDA-ARS.

2.4. Leaf pigments and enzyme assays

Samples for determining chlorophyll (Chl) and C_4 enzyme activities were collected on 3rd, 11th, and 16th of July (26, 34, and 39 DAP) and averages were used for analyses. Four 1.65 cm^2 leaf discs were removed from the lamina of the most recent and fully expanded leaf of three plants from each chamber. Samples were collected in full sunshine within 2 h of solar noon on the assigned dates. Leaf material was rapidly transferred to labeled envelopes and immediately immersed in liquid N_2 to quench metabolism. All samples were stored for a maximum of 1 month at −80 °C prior to analysis. One leaf disc from each plant was extracted with 1 ml 80% acetone, and Chl *a* and *b* were quantified from optical density measurements according to Lichtenthaler (1987). The remaining three leaf discs from each plant were extracted with 1.5 ml ice cold extraction buffer consisting of 50 mM Tris–HCl (pH 7.50), 10 mM $MgCl_2$, 1 mM EDTA, 1% (w/v) PVP-40, 5 mM Na^+-pyruvate and 10% glycerol. Immediately prior to extraction the solution was made to 1 μM leupeptin and 5 mM dithiothreitol. Leaf material (4.95 cm^2) was extracted at 0 °C with a ground glass tissue homogenizer and the homogenates were transferred to 2 ml plastic centrifuge tubes. The samples were spun in a Brinkmann model 5415C microfuge for 3 min at 14,000 × *g* and 0.23 ml aliquots of each supernatant was immediately transferred to four separate 0.5 ml centrifuge tubes on ice. The aliquots were quickly placed in liquid N_2 and remained at −80 °C until used for analysis.

Enzyme activity measurements were performed spectrophotometrically at 25 °C as described by Maroco et al. (1999). Briefly, NADP-malate dehydrogenase (MDH) was measured in 1 ml solution containing 50 mM Tris–HCl (pH 8.0), 1 mM EDTA, 100 mM oxalacetic acid, 10 mM NADPH and 0.025 ml leaf extract. Phosphoenol pyruvate carboxylase (PEPC) was measured in 1 ml solution containing 50 mM Tris–HCl (pH 8.0), 5 mM $NaHCO_3$, 5 mM $MgCl_2$, 10 mM NADH, 10 mM PEP (tricyclohexlamine salt), 1 unit malate dehydrogenase and 0.025 ml sample. NADP-malic enzyme (NADP-ME) was measured in 1 ml solution containing 50 mM Tris–HCl (pH 8.0), 5 mM EDTA, 22.5 mM $MgCl_2$, 5 mM malic acid, 5 mM dithioerythritol, 0.5 mM $NADP^+$ and 0.025 ml sample. Pyruvate Pi dikinase (PPDK) was assayed in 1 ml solution containing 0.1 M Tris–HCl (pH 8.0), 10 mM $MgCl_2$, 1 mM EDTA, 1.25 ml Na-pyruvate, 2.5 mM K_2HPO_4, 50 mM $NaHCO_3$, 5 mM DTT, 0.2 mM NADPH, 1.25 mM ATP, 2 units malate dehydrogenase, 2 units PEP carboxylase and 0.025 ml sample. All measurements were performed using a Shimadzu model 2101 spectrophotometer operated in the kinetic mode. Enzyme activities were calculated from the rate of change in optical density at 340 nm.

2.5. Determination of model parameters

A simplified version of the biochemical model of C_4 photosynthesis by von Caemmerer (2000) was fitted to the A/C_i and A/Q response curves using PROC NLIN of the SAS software (version 9.1, SAS Institute Inc., Cary, NC, USA). The initial slope and unsaturated region of the A/C_i curves were used to estimate carboxylation efficiency (CE) and maximum PEPC activity (V_{pmax}). In this region of the A/C_i response, the rate of PEPC (V_p) was described by the Michaelis–Menten equation assuming the substrate, phosphoenolpyruvic acid (PEP), was saturating (von Caemmerer, 2000):

$$V_p = V_{pmax}\frac{C_m}{C_m + K_p} \quad (1)$$

where K_p is the Michaelis–Menten constant for CO_2 of PEPC and was set to 57.0 μmol mol^{-1} (Pfeffer and Peisker, 1998). Mesophyll CO_2 concentration (C_m) was set equal to C_i and mesophyll resistance to CO_2 diffusion was ignored. All CO_2 concentrations were converted to partial pressures for model predictions. The carboxylation limited A (A_c) at low CO_2 partial pressure (i.e., unsaturated region of the A/C_i curve) is given by:

$$A_c = \min\left\{(V_p + g_{bs}C_m - R_m), (V_{cmax} - R_d)\right\} \quad (2)$$

where 'min {}' denotes 'minimum of', g_{bs} the bundle-sheath conductance to CO_2 and was set to 3.0 mmol m^{-2} s^{-1}. The product $g_{bs}C_m$ is the inward diffusion of CO_2 into the bundle sheath (von Caemmerer, 2000). Mesophyll mitochondrial respiration (R_m) was set equal to 0.5 R_d where R_d is leaf mitochondrial respiration rate. V_{cmax} denotes Rubisco capacity and was set to 60 μmol m^{-2} s^{-1} at 25 °C (Sage, 2002; von Caemmerer, 2000).

The Arrhenius equation was used to describe the temperature dependence of V_{pmax} and R_d.

$$f(T_K) = k_{25}\ \exp\left[\frac{E_a(T_K - 298)}{(298RT_K)}\right] \quad (3)$$

T_K denotes leaf temperature in degree K and R is the universal gas constant (8.314 J mol^{-1} K^{-1}). E_a represents the activation energy in kJ mol^{-1} and k_{25} is the rate of V_{pmax} or R_d at a leaf temperature of 25 °C. An experimentally determined value for the temperature dependence of K_p was not available from the literature. Here we used a Q_{10} of 2.0 for K_p (Chen et al., 1994; Collatz et al., 1992). We used E_a for V_{cmax} of 55.9 kJ mol^{-1} as determined in Sage (2002) for several C_4 species. Rubisco activity (V_{cmax}) has been found to limit C_4 photosynthesis mostly in low temperatures (<18 °C) (Kubien et al., 2003; Sage, 2002).

The data from A/Q curves and the saturated region of A/C_i curves were utilized to estimate the temperature dependence of the maximum rate of electron transport (J_{max}), assuming that the rate of whole chain electron transport (J) was a limiting factor of photosynthesis in these measurements. Following the simplified approach of von Caemmerer (2000), light and electron transport limited A (A_j) was approximated by:

$$A_j = \min\left\{\left(\frac{xJ}{2} + g_{bs}C_m - R_m\right), \left(\frac{(1-x)J}{3} - R_d\right)\right\} \quad (4)$$

where 'min {}' denotes 'minimum of' and x is a partitioning factor of electron transport rate between C_3 and C_4 cycles (=0.4). J was related to PAR absorbed by PSII (I_2) assuming leaf absorptance of 0.85 and J_{max} by:

$$\theta J^2 - J(I_2 + J_{max}) + I_2 J_{max} = 0 \quad (5)$$

where θ is an empirical curvature factor (=0.7).

The temperature dependence of J_{max} was approximated using the peaked Arrhenius equation in order to account for the rate inhibition at higher temperatures. This model was also applied to describe the rate of net CO_2 assimilation at saturating PAR in growth C_a (A_m):

$$g(T_K) = k_{25} \exp\left[\frac{E_a(T_K - 298)}{298RT_K}\right] \times \frac{[1 + \exp((298S - H)/298R)]}{[1 + \exp((ST_K - H)/RT_K)]} \quad (6a)$$

where T_K is the leaf temperature (K), k_{25} here is the value of J_{max} or A_m at 25 °C (μmol m^{-2} s^{-1}), E_a is the activation energy (kJ mol^{-1}) governing the rate of exponential increase below the optimum temperature, S represents the entropy factor (J mol^{-1} K^{-1}), and H describes the rate of decrease (kJ mol^{-1}) of the function above the optimum.

Alternatively, the peaked Arrhenius function can be written in the following form (Medlyn et al., 2002):

$$g(T_K) = k_{opt} \frac{H \exp(E_a(T_K - T_{opt})/T_K R T_{opt})}{H - E_a[1 - \exp(H(T_K - T_{opt})/T_K R T_{opt})]} \quad (6b)$$

This form has alternative parameters of T_{opt} and k_{opt} for optimal temperature in K and the rate at T_{opt}, respectively. This four-parameter peaked model (Eqs. (6a) or (6b)) requires sufficient data points at above optimum temperature for full parameterization (Medlyn et al., 2002). Estimates of E_a, S, and H for both J_{max} and A_m were highly variable in full parameterization partly because of insufficient data past the temperature optimum, where rates decreased rapidly. Here, we assumed H equal to 220 kJ mol^{-1} as used in C_3 plants (von Caemmerer, 2000) after determining that H from the full model was not significantly different between treatments. The optimum temperature (T_{opt}) is then related to S by:

$$T_{opt} = \frac{H}{S - R \ln(E_a/(H - E_a))} \quad (6c)$$

A reduced beta distribution model was used to evaluate the temperature dependence of leaf-tip appearance rate (LAR). The beta distribution function has been found useful for describing plant metabolic responses to temperature and yields biologically meaningful parameters (Yin et al., 1995). This reduced beta function is represented by the following equation (Yan and Hunt, 1999):

$$h(T) = R_{max}\left(\frac{T_{ceil} - T}{T_{ceil} - T_{opt}}\right)\left(\frac{T}{T_{opt}}\right)^{T_{opt}/(T_{ceil} - T_{opt})} \quad (7)$$

The rate of process is represented by $h(T)$ as a function of air temperature (T in °C). T_{opt} is the optimal temperature at which the maximal rate of the process (R_{max}) occurs. T_{ceil} is the ceiling temperature at which the process ceases. Using mean daily temperature as input variable for this nonlinear model could yield erroneous results in describing the instantaneous effects of temperature on developmental rates because of the differences in day and night temperatures in the experiment. Thus, the effect of day and night temperatures on LAR was implemented by the following relationship suggested by Yin et al. (1996):

$$r = \frac{D}{24}h(T_{day}) + \left(1 - \frac{D}{24}\right)h(T_{night}) \quad (8)$$

The rate of leaf-tip appearance is represented by r (leaves days^{-1}). D denotes the day temperature period in hours (=16.0 h). T_{day} is the day temperature and T_{night} is night temperature. Eq. (8) assumes that the nonlinear temperature response is the same between day and night.

2.6. Statistical analyses

A two-way ANOVA was used to analyze the results. Chambers were the experimental units and the individual plants were subsamples in this experiment. Since chambers were not replicated over the combinations of CO_2 and temperature, it was necessary to determine if an interaction was present prior to testing for the main effects. First, a heuristic test for interaction was performed which determined the homogeneity of variances of the main effects according to Milliken and Johnson (1989). Then, the presence of a multiplicative interaction was tested using Tukey's test for non-additivity (Milliken and Johnson, 1989; Sokal and Rohlf, 1995). Both tests failed to detect any significant interactions between CO_2 and temperature in the measured parameters indicating that using a two-way ANOVA was valid. As an additional test for interactions, the influence of C_a on the relationship between growth and temperature was examined by comparing linear regressions of biomass and leaf area as a function of mean growth temperatures between CO_2 treatments. ANOVA and regression analyses were carried out using SAS PROC GLM.

3. Results

3.1. Growth and development

All measured growth parameters including dry matter allocated to leaves, stalks, and ears at silking were similar between the elevated and ambient C_a treatments (Table 1). Conversely, these same growth parameters differed among temperature treatments, except for specific leaf area (SLA). The interaction between CO_2 and temperature was not significant for the measured growth parameters at 5% significance level. Leaf area development was decreased at lower temperatures but was similar between CO_2 treatments (Fig. 1a). Final leaf area per plant was negatively correlated with growth temperature indicating that final plant size was greater at lower than at higher temperatures (Fig. 1b). Regression lines, intercepts, and slopes of leaf area as a function of mean growth temperature were not different between CO_2 treatments (all P-values were greater than 0.75; Fig. 1). Above ground biomass accumulation and total leaf area responded similarly to temperature and CO_2 (data not shown). This result also indicated that the temperature responses of leaf area development and of biomass accumulation were not influenced by growth C_a. Final leaf number was greatest (=18.4) and least (=16.3) for the 35/29 °C and the 25/19 °C growth

Table 1
Biomass accumulation, leaf area, specific leaf area (SLA), and days to silking of maize plants in response to elevated CO_2 and temperatures. Values shown are means ± standard errors ($n = 8$) in each chamber

CO_2 (μmol mol^{-1})	Temperature (°C)	Aboveground dry matter (g plant^{-1})				Leaf area (m^2 plant^{-1})	SLA (cm^2 g^{-1})	Days to silking (days)
		Total	Stalk	Leaves	Ear			
370	19/13	149.1 ± 4.81	97.7 ± 3.53	48.1 ± 1.13	3.3 ± 0.43	1.04 ± 0.018	204.1 ± 5.76	>66
	25/19	112.2 ± 3.02	72.0 ± 2.07	33.5 ± 1.33	6.7 ± 0.62	0.74 ± 0.042	213.8 ± 5.98	48
	31/25	116.0 ± 3.19	69.5 ± 2.07	28.5 ± 0.53	18.0 ± 2.43	0.59 ± 0.018	203.1 ± 2.86	43
	35/29	102.2 ± 3.36	63.3 ± 2.31	27.2 ± 0.92	11.8 ± 1.23	0.54 ± 0.023	194.0 ± 3.79	45
	38.5/32.5	75.1 ± 3.46	43.3 ± 2.42	27.4 ± 2.06	4.4 ± 1.38	0.54 ± 0.046	190.8 ± 4.90	56
750	19/13	159.2 ± 2.85	106.8 ± 2.15	49.3 ± 1.97	3.2 ± 1.16	1.17 ± 0.042	206.1 ± 7.45	>66
	25/19	108.0 ± 3.02	68.5 ± 2.03	32.0 ± 0.60	7.4 ± 0.89	0.65 ± 0.021	193.3 ± 3.54	48
	31/25	104.3 ± 3.35	66.4 ± 2.03	31.9 ± 1.88	5.9 ± 0.54	0.64 ± 0.053	192.6 ± 5.73	45
	35/29	107.7 ± 7.63	67.4 ± 4.92	33.4 ± 3.43	6.9 ± 0.87	0.70 ± 0.093	200.6 ± 8.48	45
	38.5/32.5	75.6 ± 4.85	42.4 ± 2.48	26.5 ± 2.01	6.7 ± 1.40	0.48 ± 0.049	170.5 ± 5.82	56
P	CO_2	0.991	0.652	0.303	0.341	0.495	0.201	0.374
	Temperature	0.002	0.001	0.003	0.393	0.008	0.208	<0.001
	Non-additivity	0.484	0.215	0.904	0.059	0.479	0.484	0.422
$LSD_{0.05}$	Temperature	16.7	10.5	6.3	–	0.22	–	2.78

Main effects of CO_2 and temperature were tested with a two-way ANOVA assuming their interactive effects were additive. The interaction was tested using Tukey's non-additivity test.

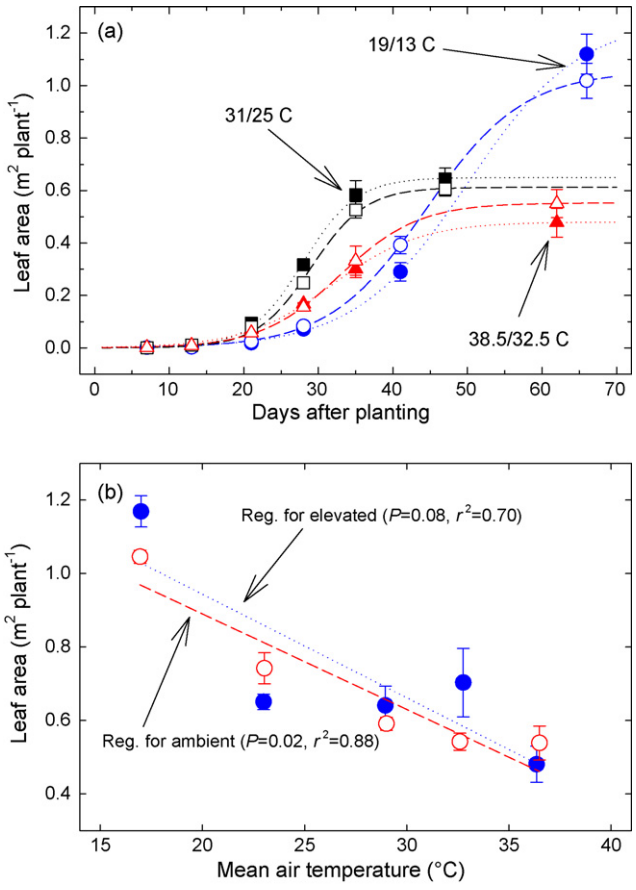

Fig. 1. Leaf area per plant in response to C_a and air temperature treatments. (a) Leaf area growth over time. Each point represents mean ± standard error ($n = 4$ or 8) for ambient ((◯), (□), (△)) or elevated ((●), (■), (▲)) C_a in combination with 19/13 ((◯), (●)), 31/25 ((□), (■)), or 38.5/32.5 ((△), (▲)) °C temperature treatment. Dashed and dotted lines represent best fit of the logistic function for ambient and elevated C_a, respectively, in each temperature treatment. (b) Final leaf area per plant as a function of daily mean air temperatures in relation to C_a. Shown are mean ± standard error of eight plants for ambient (◯) and elevated (●) C_a. Dashed and dotted lines are regression lines for ambient and elevated C_a, respectively.

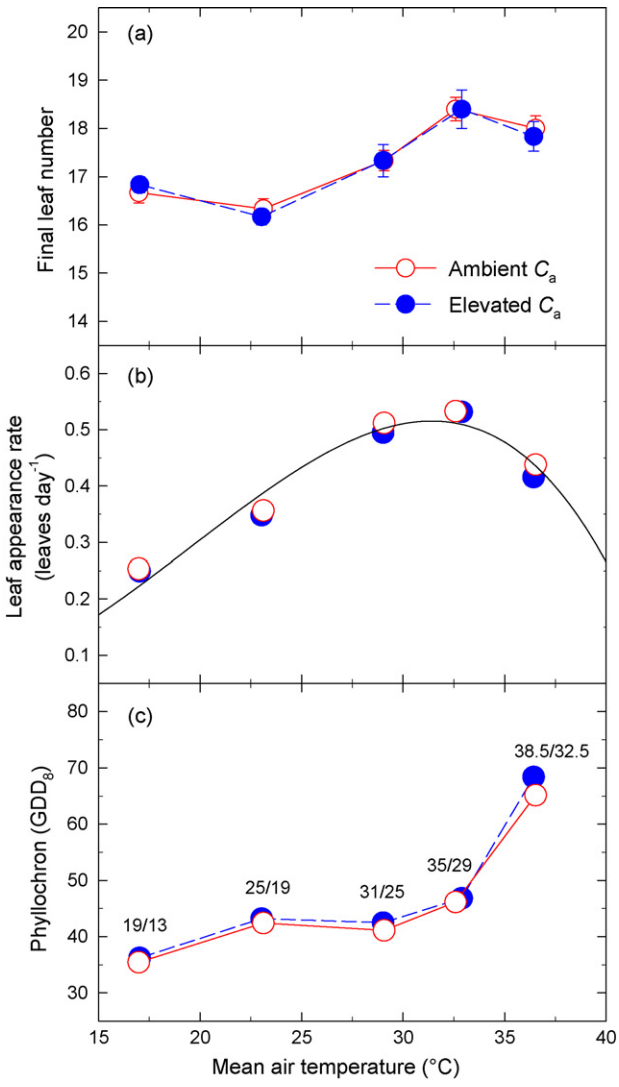

Fig. 2. Final leaf number (a), leaf tip appearance rate (b), and phyllochron (c) of maize plants in response to CO_2 and temperature. Line in (b) represents best fit of the beta function (Eq. (7)) as a function of daily mean air temperature. Open and closed circles represent ambient (◯) and elevated (●) C_a, respectively.

treatments, respectively (Fig. 2a). Leaf development rate represented by LAR was similar between CO_2 treatments (Fig. 2b) and was highest in 35/29 °C temperature treatment and lowest in 19/13 °C treatment (Fig. 2b). Days to silking was affected similarly by the growth temperature (Table 1). The optimal temperature (T_{opt}) and the ceiling temperature (T_{ceil}) for LAR were approximately 32 and 44 °C, respectively, at either C_a. Phyllochron intervals were similar for all temperature treatments below 35/19 °C and were maximal for plants in the 38.5/32.5 °C temperature treatment (Fig. 2c).

3.2. Leaf constituents and enzyme activities

Total leaf carbon and nitrogen concentrations, and their ratios were similar between ambient and elevated C_a (Table 2). Leaf nitrogen concentration was negatively correlated with growth temperatures ($r^2 = 0.79$; $P < 0.001$). Total chlorophyll content was affected by both CO_2 ($P = 0.08$) and growth temperature ($P = 0.05$; Table 2). The activities of PEPC and NADP-ME were decreased in the leaves of plants grown at elevated C_a compared with ambient C_a ($P < 0.01$; Table 3). These activities were also influenced by temperature ($P < 0.05$; Table 3). The activities of MDH and PPDK were not strongly influenced by C_a while PPDK activity was responsive to temperature ($P = 0.02$). No significant treatment interactions were found for measurements of leaf constituents and enzyme activities.

3.3. Leaf gas exchange rates and chlorophyll fluorescence

At high PAR (i.e., >1800 μmol m^{-2} s^{-1}) net leaf photosynthesis (A_m) was similar between plants grown at ambient and elevated C_a (Fig. 3a). The optimal temperature for A_m was near 34 °C in either CO_2 treatment (Fig. 3a). Leaves grown at elevated C_a exhibited an average reduction of 36% in stomatal conductance ($P < 0.01$) and 27% in transpiration ($P < 0.01$) compared

Table 2
Total leaf carbon, nitrogen, the carbon/nitrogen ratio (C/N), and chlorophyll content of fully expanded young sunlit maize leaves in response to CO_2 enrichment and temperature

CO_2 (μmol mol^{-1})	Temperature (°C)	Carbon (%, w/w)	Nitrogen (%, w/w)	C/N ratio	Chlorophyll (g m^{-2})
370	19/13	41.0 ± 0.39	4.8 ± 0.07	8.6 ± 0.09	0.22 ± 0.009
	31/25	41.8 ± 0.30	3.5 ± 0.13	11.9 ± 0.43	0.24 ± 0.023
	38.5/32.5	40.5 ± 0.47	3.5 ± 0.01	11.5 ± 0.12	0.19 ± 0.011
750	19/13	39.5 ± 2.32	4.9 ± 0.30	8.1 ± 0.15	0.21 ± 0.009
	31/25	42.2 ± 0.69	3.9 ± 0.12	10.8 ± 0.22	0.21 ± 0.009
	38.5/32.5	40.8 ± 0.15	3.5 ± 0.03	11.6 ± 0.08	0.17 ± 0.007
P	CO_2	0.700	0.321	0.285	0.077
	Temperature	0.252	0.023	0.029	0.051
	Non-additivity	0.494	0.866	0.972	0.766
$LSD_{0.05}$	Temperature	–	0.66	1.89	–

Shown are means ± standard errors ($n = 4$) in each chamber. Main effects of CO_2 and temperature were tested with a two-way ANOVA assuming their interactive effects were additive. The interaction was tested using Tukey's non-additivity test.

with ambient C_a across temperature treatments (Fig. 3b and c). Growth temperature affected A_m and transpiration rate (E) ($P < 0.01$) but not g_s ($P = 0.18$). Carboxylation efficiency (CE) determined by the initial slope of the A/C_i curves was reduced in response to CO_2 enrichment ($P < 0.01$) and was increased with temperature ($P < 0.01$; Fig. 3d). Maximum PEPC activity (V_{pmax}) estimated by fitting Eq. (1) with A/C_i measurements varied similarly to CE (data not shown). Dark respiration rates (R_d) did not differ between C_a treatments ($P = 0.74$) but increased with temperature ($P < 0.01$) and were maximal at 35/29 °C (Fig. 3e). There were no differences in F_v/F_m between ambient and elevated C_a ($P = 0.56$). On the other hand, F_v/F_m ratios ($P = 0.001$) were altered by temperatures and were maximal at 25/19 °C (Fig. 3f).

3.4. Canopy CO_2 exchange rates

Diurnal patterns of canopy CO_2 exchange rates measured 29 days after planting did not differ between C_a treatments but were affected by temperature (Fig. 4b). For these measurements plants were in the V6, V8, V9, V10, and V9 growth stages for the 19/13, 25/19, 31/25, 35/29, and 38.5/32.5 °C temperature treatments, respectively. Canopy CO_2 exchange rates at high PAR (>1800 μmol m^{-2} s^{-1}) were similar between elevated and ambient C_a ($P = 0.23$) and exhibited a nonlinear response to temperature (Fig. 4c). The optimum temperature for canopy CO_2 exchange rates at high PAR was 31.4 °C when estimated using the beta function (Eq. (7)).

3.5. Temperature dependence of model parameters

The activation energies (E_a) of V_{pmax} estimated using Eq. (3) were similar between CO_2 treatments with a pooled value of 75.1 kJ mol^{-1} (Table 4). Leaf dark respiration rate (R_d) at 25 °C was not affected by the CO_2 treatment and the E_a of R_d was also similar between CO_2 treatments resulting in a pooled value of 39.8 kJ mol^{-1} (Table 4). The optimal temperatures for J_{max} and A_m were near 34 °C in both the ambient and doubled C_a treatments (Table 4). Estimates of E_a and S as used in Eq. (6a) also were similar between CO_2 treatments for both J_{max} and A_m (Table 4). While the parameter estimates of the temperature dependence were unchanged, the rates (i.e., k_{25} and k_{opt}) of J_{max} and A_m were marginally reduced in elevated C_a (Table 4). The maximum rate (R_{max}) of leaf tip appearance was 0.53 leaves

Table 3
Activities of C_4 enzymes in fully expanded young sunlit maize leaves in response to CO_2 enrichment and temperature

CO_2 (μmol mol^{-1})	Temperature (°C)	PEPC (μmol m^{-2} s^{-1})	MDH (μmol m^{-2} s^{-1})	NADP-ME (μmol m^{-2} s^{-1})	PPDK (μmol m^{-2} s^{-1})
370	19/13	68.3 ± 4.55	54.8 ± 5.03	63.1 ± 4.00	11.0 ± 0.54
	31/25	57.8 ± 4.38	31.8 ± 4.39	46.7 ± 4.47	7.6 ± 0.63
	38.5/32.5	62.7 ± 2.79	30.9 ± 4.05	42.1 ± 2.49	5.8 ± 0.26
750	19/13	58.3 ± 3.32	44.2 ± 4.73	57.8 ± 2.46	9.3 ± 0.53
	31/25	43.9 ± 3.83	24.2 ± 4.57	42.2 ± 2.39	6.3 ± 0.32
	38.5/32.5	51.3 ± 3.92	34.2 ± 4.56	38.6 ± 3.02	5.4 ± 0.51
P	CO_2	0.009	0.359	0.014	0.107
	Temperature	0.023	0.094	0.002	0.020
	Non-additivity	0.108	0.665	0.253	0.286
$LSD_{0.05}$	Temperature	5.81	–	2.77	2.04

All measurements were performed at 25 °C. Shown are means ± standard errors ($n = 4$) in each chamber. Main effects of CO_2 and temperature were tested with a two-way ANOVA assuming their interactive effects were additive. The interaction was tested using Tukey's non-additivity test.

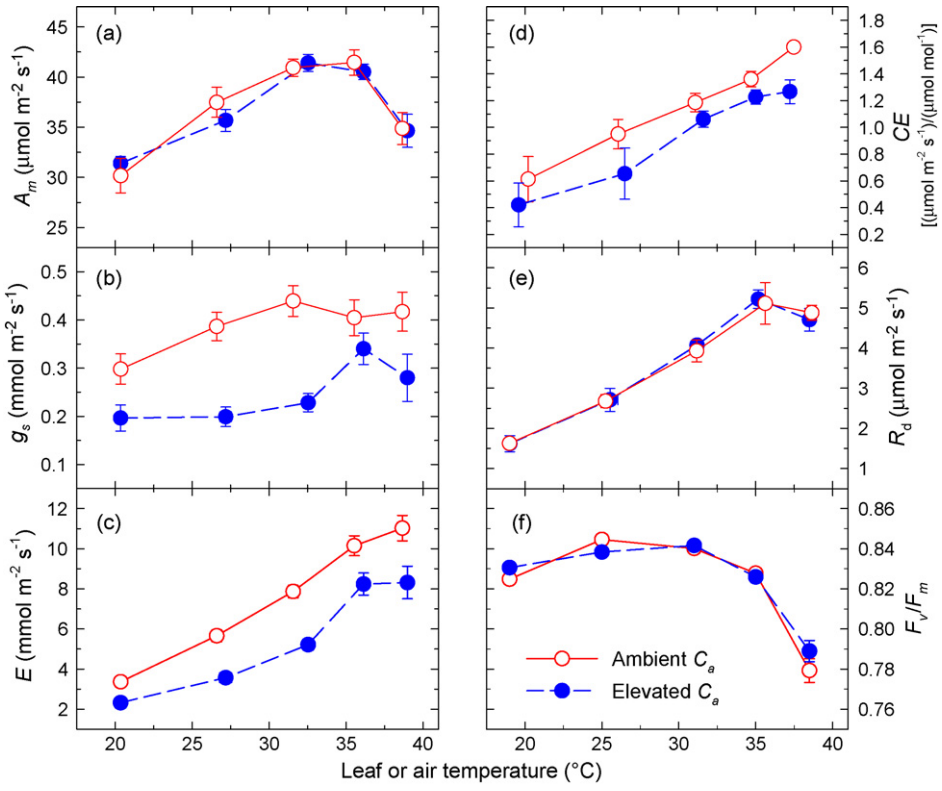

Fig. 3. Leaf gas exchange parameters measured 15–35 DAP and chlorophyll fluorescence between 25 and 35 DAP of fully expanded young leaves (leaf 5–14) in response to CO_2 and temperature. (a) net photosynthesis (A_m); (b) stomatal conductance (g_s); (c) transpiration rate (E); (d) carboxylation efficiency (CE); (e) dark respiration rate (R_d); (f) maximum photochemical efficiency of PSII (F_v/F_m). Horizontal axis represents leaf temperature (°C) except in (f) where air temperature is used. Leaf gas exchange rates were measured at high PAR (>1800 μmol m^{-2} s^{-1}) with respective growth CO_2 and temperature. Shown are mean ± standard error of three to five plants except in F_v/F_m where $n = 16$. Open and closed circles represent ambient (◯) and elevated (●) C_a, respectively.

Table 4
Temperature dependence of maximum PEPC activity (V_{pmax}), leaf dark respiration rate (R_d), maximum rate of whole-chain electron transport (J_{max}), leaf photosynthetic capacity (A_m), and leaf tip appearance rate (LAR) in relation to growth C_a

Model	Process	Parameter	Units	Ambient C_a	Elevated C_a	Pooled
Eq. (3)	V_{pmax}	k_{25}	μmol m^{-2} s^{-1}	62.5 ± 4.29	57.7 ± 3.67	60.4 ± 2.96
		E_a	kJ mol^{-1}	77.9 ± 5.16	74.1 ± 4.66	75.1 ± 3.64
	R_d	k_{25}	μmol m^{-2} s^{-1}	2.7 ± 0.18	2.7 ± 0.21	2.7 ± 0.13
		E_a	kJ mol^{-1}	39.7 ± 5.23	40.0 ± 5.72	39.8 ± 3.74
Eq. (6)	J_{max}	k_{25}	μmol m^{-2} s^{-1}	234.6 ± 3.12	221.8 ± 2.65	228.2 ± 2.06
		E_a	kJ mol^{-1}	33.3 ± 2.39	32.4 ± 1.92	32.8 ± 1.53
		S	J mol^{-1} K^{-1}	703.4 ± 1.05	701.9 ± 0.90	702.6 ± 0.69
		k_{opt}	μmol m^{-2} s^{-1}	293.6 ± 3.30	281.8 ± 2.65	287.5 ± 2.12
		T_{opt}	°C	33.5 ± 0.24	34.1 ± 0.22	33.8 ± 0.16
	A_m	k_{25}	μmol m^{-2} s^{-1}	35.5 ± 0.85	34.9 ± 0.65	35.2 ± 0.56
		E_a	kJ mol^{-1}	27.1 ± 4.08	26.3 ± 3.00	27.0 ± 2.63
		S	J mol^{-1} K^{-1}	701.0 ± 2.00	701.2 ± 1.45	701.2 ± 0.26
		k_{opt}	μmol m^{-2} s^{-1}	42.9 ± 0.73	41.7 ± 0.63	42.4 ± 0.50
		T_{opt}	°C	33.7 ± 0.46	33.5 ± 0.33	33.6 ± 0.29
Eq. (7)	LAR	R_{max}	leaves days^{-1}	0.53 ± 0.03	0.52 ± 0.03	0.53 ± 0.02
		T_{opt}	°C	32.2 ± 0.73	32.0 ± 0.75	32.1 ± 0.42
		T_{ceil}	°C	44.0 ± 1.97	43.3 ± 1.99	43.7 ± 1.11

The Arrhenius equation (3) was used for V_{pmax} and R_d. The peaked model (the rate of declination parameter (H) was set to 220 kJ mol^{-1}) (Eqs. (6a) and (6b)) was fitted with J_{max} and A_m and the reduced beta function (Eq. (7)) was applied for LAR. Shown are parameter estimate ± approximated standard error estimated using SAS NLIN (all non-linear regressions were significant with P-values <0.005). Pooled values represent parameters estimated using both C_a.

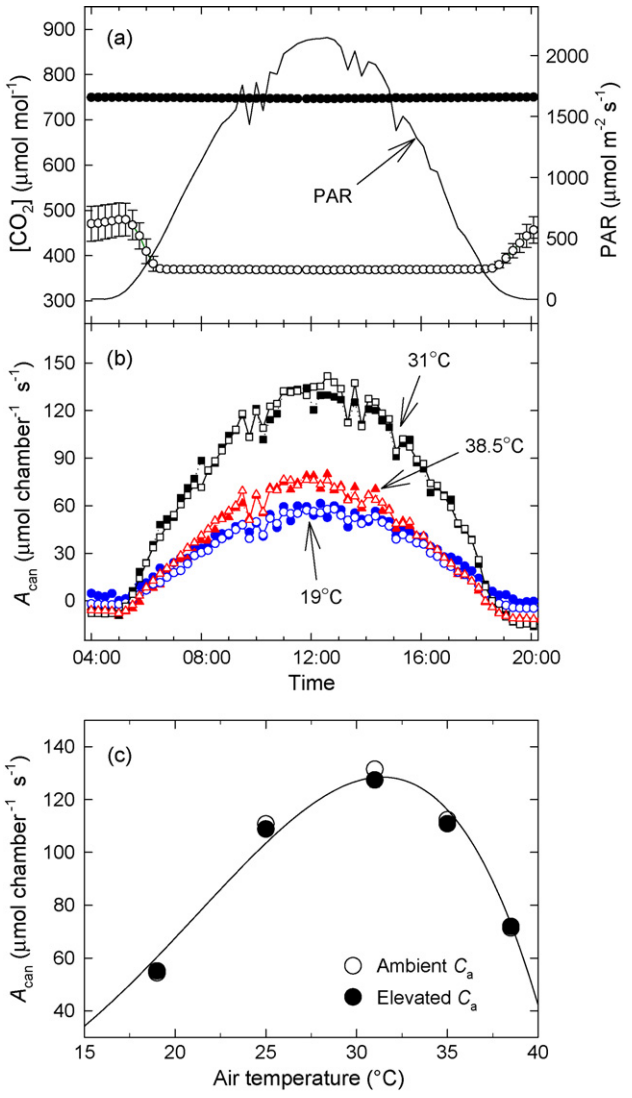

Fig. 4. Canopy CO_2 exchange rates (A_{can}) of maize plants in response to CO_2 and temperature 29 DAP. (a) PAR and CO_2 concentrations; (b) diurnal patterns of A_{can}. Each point represents 15 min average of A_{can} for ambient ((◯), (□), (△)) or elevated ((●), (■), (▲)) C_a in combination with 19/13 ((◯), (●)), 31/25 ((□), (■)), or 38.5/32.5 ((△), (▲)) °C treatment; (c) canopy CER as a function of temperature under high PAR (>1800 μmol m^{-2} s^{-1}). Line represents best fit of the beta function (Eq. (7)). Open and closed symbols represent ambient and elevated C_a, respectively.

day^{-1} which occurred at 32 °C (T_{opt}) by fitting Eq. (8) to the pooled data.

4. Discussion

4.1. CO_2 and temperature effects

We observed little evidence of increased growth in response to CO_2 enrichment in maize plants grown over a wide range of temperatures. This result was in agreement with data for both leaf and canopy photosynthetic rates. On the other hand, almost all of the measured parameters in this study responded significantly to temperature. The extent of this temperature response was not significantly altered by CO_2 enrichment in the measured parameters indicating that the CO_2 and temperature effects were additive except in ear weight for which the effects appeared marginally non-additive (Table 1). Note that the present study did not span over the entire reproductive stages. The temperature response of reproductive processes of maize in elevated C_a merits further research. In some C_3 crops, despite an increase in photosynthesis, the detrimental effect of high temperature on seed yield was not alleviated by elevated C_a (e.g., Prasad et al., 2003; Ziska et al., 1997). Prior studies have shown that maximal leaf developmental rates in maize occur near 31 °C at ambient C_a (Tollenaar et al., 1979; Warrington and Kanemasu, 1983a; Yan and Hunt, 1999). In the current study, estimates of air T_{opt} for LAR were near 32 °C under both ambient and elevated C_a (Table 4). This indicated that the maize temperature optimum for leaf development cited above is conserved under elevated C_a. Leaf net photosynthesis rates for maize reached maximal near 31 °C (Tollenaar, 1989b), were decreased by temperatures above 37 °C and complete inhibition occurred near 45 °C (Crafts-Brandner and Salvucci, 2002). Optimal leaf temperatures for CO_2 assimilation at saturated PAR (A_m) were near 34 °C in both CO_2 treatments in the present study (Table 4). In a prior study, dry matter accumulation in maize was maximal near 19 °C (Tollenaar, 1989a). In agreement with this, biomass and leaf area at silking in the present study decreased with growth temperature resulting in maximal values at 19/13 °C in both C_a treatments. The difference between the optima for leaf photosynthesis and dry matter accumulation was probably related to the findings that respiration rates, the duration of growth, and rates of dry matter partitioning varied with temperature (Tollenaar, 1989a,b). The temperature dependence of final leaf number at either C_a was similar to that of previous studies (e.g., Warrington and Kanemasu, 1983b). The discrepancy in phyllochrons between the highest temperature regime used here (38.5/32.5 °C) and the other temperature treatments emphasizes the need for the maize models to incorporate the curvilinear temperature response of leaf development above the temperature optimum (Fig. 2c).

4.2. Dependence on plant and air temperatures in elevated C_a

It is important to determine whether or not the temperature dependence of the various physiological parameters measured in this study remained unchanged under elevated C_a. Note that CO_2 enrichment may alter the dependence of various physiological and developmental processes with respect to either plant (leaf or tissue) temperature or air temperature. Our results indicated that the dependence of developmental responses on air temperature, estimated as the T_{opt} for leaf development, was unchanged between CO_2 treatments. Moreover, the dependence of photosynthetic and respiratory processes on leaf temperature also was conserved since the temperature response parameters were virtually unchanged for A_m, J_{max}, V_{pmax}, and R_d in response to CO_2 enrichment (Table 4).

Foliage temperatures of both C_3 and C_4 plants are expected to increase because stomatal conductance and transpiration rates are decreased under elevated C_a (Bunce, 2004; Siebke et al.,

2002). Canopy temperature of maize plants grown at 31/25 °C inside the SPAR chambers was increased up to 1 °C in response to doubled C_a and this increase was positively related to PAR (Kim et al., 2006). The rate of plant development is thought to be a function of meristem temperature (Guilioni et al., 2000; Poethig, 2003). Provided that the dependence of developmental and physiological processes on plant temperature is conserved under elevated C_a in C_4 plants, it is reasonable to infer that the optimum air temperature for these processes should decrease as a result of increased foliage temperature in response to high C_a. However, this inference was not confirmed in the present study. It is possible that the effects of increased leaf temperature on plant development due to elevated C_a were too small to detect. Conversely, reduction in stomatal conductance in response to high C_a may have had relatively little influence on the meristem temperature because buds in general have few or no stomata (Grace, 2006). In addition, changes in total conductance of water vapor in the apical whorl in response to an increase in C_a may be minimal because the apical region is likely to be shaded by the expanded leaves at the top of the canopy (Guilioni et al., 2000). Boundary layer conductance inside the apical whorl would be lower due to the longer diffusive path to the ambient air in comparison with that of the top expanded leaves. Overall, the latent heat flux for tissues inside the apical whorl could be relatively insensitive to changes of stomatal conductance in response to high C_a. In other words, the stomatal effect on meristem temperature may be minimal because convection from meristematic regions is less than previously thought (Grace, 2006).

Canopy CO_2 exchange rates and biomass accumulation were similar between current and elevated C_a across the temperature treatments in this study. The effects of potential leaf warming due to high C_a on canopy photosynthesis, growth, and biomass accumulation could be mitigated by low light conditions and shading within the canopy over the growing season (Siebke et al., 2002). This might partly explain why the temperature dependence of canopy processes remained unchanged under elevated C_a. It is critical to identify under what circumstances potential leaf warming due to high C_a may alter canopy processes. The use of coupled gas exchange models that mechanistically combine the C_4 photosynthesis model and its temperature dependence with the leaf energy budget and a stomatal conductance model can be a useful tool to quantify the extent of contribution of leaf warming to C_4 productivity under climate change scenarios. Overall, high C_a did not alter temperature responses of canopy photosynthesis or biomass accumulation in C_4 maize. This may have significant ecological implications on the biogeography of C_4 species in future climates because temperature is among the key variables determining the distribution of C_4 species around the world (Sage and Kubien, 2003).

4.3. Temperature dependence of C_4 photosynthesis in elevated C_a and its implications for modeling

The optimum temperature for leaf photosynthesis of C_3 plants is thought to increase with C_a due to the variable dependence of the carboxylation and oxygenation reactions of Rubisco on temperature and to the altered solubilities of CO_2 and O_2 at different temperatures (Long, 1991). In C_4 plants, the competition between photosynthetic carbon reduction and photorespiration is already greatly suppressed under current C_a by concentrating CO_2 into the bundle-sheath cells where Rubisco is localized (Sage, 1999). This means that doubling of C_a is not likely to alter the temperature dependence of the C_3 photosynthetic cycle significantly in C_4 plants. Photosynthetic response to leaf temperature at high C_a may be altered if the temperature dependence of the C_4 cycle changes considerably. Our *in vitro* measurements of the C_4 cycle enzymes suggested that the temperature dependence of C_4 cycle was not altered in response to CO_2 enrichment (Fig. 3; Table 3). In *Amaranthus*, the CO_2 saturation point increased with temperature as the CO_2 saturated A increased while CE was relatively constant (Sage, 2002). This response indicates that C_4 photosynthesis may also be sensitive to rising C_a at high temperature particularly when operating C_i is low. At higher temperatures, the CO_2 saturated A is thought to be limited by RuBP regeneration rate or PEP regeneration rate while CE mainly controlled by PEPC activity in C_4 plants (Sage, 2002). In the present study, the CO_2 saturation point did not appear to increase with temperature in either ambient or elevated C_a. This could be partly because CE was increased with temperature in both C_a (Fig. 3d). This can in part explain our observations that temperature responses of leaf photosynthesis were insensitive to CO_2 enrichment even at higher temperatures.

According to the current model of C_4 photosynthesis, a reduction of CE due to changes in the initial slope of the A/C_i response indicates decreased C_4 cycle enzyme activities. This is an important indicator of decreased PEPC activity if saturating substrate levels are assumed (Pfeffer and Peisker, 1998; von Caemmerer, 2000). In the current study average estimates of V_{pmax} across temperatures were reduced by 5.5% in elevated compared to ambient C_a. These observations suggested that the C_4 enzyme cycle in maize leaves acclimated to the CO_2 enriched treatment. A similar decrease in the initial slope of the A/C_i response was reported previously in sorghum and maize (Kim et al., 2006; Maroco et al., 1999; Watling et al., 2000). Our *in vitro* enzyme analyses revealed that PEPC activity and NADP-ME activity were decreased in response to elevated C_a across temperature treatments (Table 3). The gas exchange results and C_4 cycle enzyme assays agreed that photosynthetic acclimation occurred in response to CO_2 enrichment in the current study. Despite the reduction in C_4 enzyme activities due to high C_a, the apparent temperature dependence of photosynthesis was not altered (Fig. 3). Likewise, the temperature dependence of V_{pmax} remained similar between C_a treatments (Table 4). It should be noted that the temperature dependence determined in this study was derived from the plants that were acclimated to the static temperature regimes in which they were grown. The model parameter estimates determined in the present study should be adopted with caution when used for dynamic temperature responses. In a previous maize study, photosynthesis and the activities of C_4 enzymes, i.e., PPDK and NADP-ME, acclimated to growth temperatures at ambient C_a (Ward, 1987). In addition to PPDK and NADP-ME, PEPC activity was affected

by growth temperatures in the present study (Table 3). There were no apparent interactive effects of CO_2 and temperature on the occurrence of photosynthetic acclimation in maize. Present results suggest that for future modeling efforts the temperature dependence of growth, development, and photosynthesis in maize could be comparable under ambient and elevated C_a. Also note that, it may be important to account for reduced C_4 cycle activities (i.e., V_{pmax}) when modeling the effects of elevated C_a on maize.

5. Conclusions

Growth, development, and photosynthesis of maize plants were not changed in response to CO_2 enrichment but were significantly altered by growth temperatures. Also, the temperature dependence of measured growth, photosynthesis, and developmental parameters were not significantly altered by CO_2 enrichment. Temperature optima for leaf A_m and leaf appearance rate were near 34 and 31 °C, respectively. Carboxylation efficiency and estimated V_{pmax} from leaf A/C_i curves were reduced in response to CO_2 enrichment across temperatures. This was further supported by reduced *in vitro* activities of the C_4 enzymes. These results indicate that while many of the growth and photosynthesis parameters examined here were minimally responsive to elevated C_a, an acclimation process might occur in the C_4 cycle by way of reducing the activities of C_4 enzymes.

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