Temperature dependence of growth, development, and photosynthesis in maize under elevated CO₂

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Abstract

Global atmospheric carbon dioxide concentrations (Cₐ) 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 Cₐ by the end of the century. Because changes in Cₐ and temperature are likely to occur concomitantly, it is important to evaluate how the temperature dependence of key physiological processes are affected by rising Cₐ in major crop plants including maize (Zea mays L.), a globally important grain crop with C₄ 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) Cₐ throughout the vegetative stages using sunlit controlled environmental chambers in order to test if the temperature dependence of these processes was altered by elevated Cₐ. Leaf and canopy photosynthetic rates, C₄ 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 Cₐ in maize. Biomass, leaf area, leaf appearance rate, and photosynthesis measured at growth Cₐ was not changed in response to CO₂ enrichment. Carboxylation efficiency and the activities of C₄ enzymes were reduced with CO₂ enrichment indicating possible photosynthetic acclimation of the C₄ 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 C₄ enzyme activities on growth temperatures was comparable between current and elevated Cₐ. The results of this study suggest that the temperature effects on growth, development, and photosynthesis may remain unchanged in elevated Cₐ compared with current Cₐ in maize.

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Keywords: Zea mays; C₄ plant; Photosynthesis; Temperature; Interaction; CO₂ enrichment; Acclimation; Global climate change; Stomatal conductance; C₄ enzymes; Sunlit growth chambers; Model parameters

1. Introduction

Global atmospheric carbon dioxide concentrations (Cₐ) 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 Cₐ (Cubasch et al., 2001). Since both Cₐ 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 Cₐ and air temperature on the growth and yield of key crop plants. Because changes in Cₐ 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).
In C3 plants, enhanced growth and photosynthesis are generally observed in response to elevated C4. However, plant responses to elevated C4 can be mitigated by various acclimation mechanisms (Moore et al., 1999; Stitt, 1991). Owing to the biochemical and anatomical specialization associated with the CO2 concentrating mechanism, changes in photosynthesis and growth in C4 plants in response to elevated C4 were thought to be minimal. However, several studies reported that both photosynthesis and plant growth of C4 species responded positively to elevated C4 (see review by Ghannoum et al., 2000). In general, C4 plants have higher temperature optima for photosynthesis and growth than C3 plants and thus are better adapted to warmer climates. It is unclear if the temperature dependence of growth and photosynthesis in C4 plants would remain unchanged in elevated C4 compared with current C4. In C3 plants, the temperature optima for photosynthesis were greater at elevated than at ambient C4 and the stimulation of photosynthesis by high C4 was greater at higher temperatures (Kim and Lieth, 2003; Kirschbaum, 1994; Long, 1991). The interactive effects of temperature and CO2 on the growth and photosynthesis of C4 plants may be similar to those of C3 plants but this requires further examination (Morison and Lawlor, 1999).

In *Amaranthus*, the CO2 saturation point was increased with temperature indicating that the sensitivity of photosynthesis to CO2 in C4 plants might be enhanced by elevated temperature (Sage, 2002). The temperature dependence of photosynthesis at low C4 (<36 Pa) was similar to that of C3 plants in *Amaranthus* (Sage, 2002). It is important to better understand the interaction between elevated C4 and higher temperatures in C4 plants in order to predict plant responses to future climate change.

Photosynthetic acclimation to long-term CO2 enrichment also has been reported in C4 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 CO2 saturated photosynthetic rate (Kim et al., 2006; Read et al., 1997; Watling et al., 2000), as well as a reduction in C4 enzyme activities (Maroco et al., 1999; Watling et al., 2000) in the enhanced C4 treatments. However, it is unclear how photosynthetic acclimation to high C4 in C4 plants is related to growth temperature.

The use of free-air CO2 enrichment (FACE) systems enabled scientists to assess the effects of elevated C4 and other climate variables (e.g., ozone) on various C3 and C4 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) chambers offer an alternative method for studying the interactive effects of C4 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, CO2 concentration, and humidity makes it possible to determine the temperature dependence of physiological processes under elevated C4 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 C4 species in the world. An accurate assessment of the effects of elevated C4 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 C4 and temperature on growth and photosynthesis have been investigated for many C3 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 C4 crops including maize. A primary reason for the pronounced interactive effects of high C4 and rising temperature on growth and photosynthesis of C4 plants can be that suppression of photorespiration by high C4 and subsequent increase in photosynthesis will be greatest at higher temperatures (Long, 1991). Unlike C3 plants, photorespiratory loss of carbon is greatly inhibited in C4 plants due to the CO2 concentrating mechanism. This suggests that elevated C4 may not alter the temperature dependence of growth and photosynthesis significantly in C4 plants unless the temperature response of the C4 cycle changes considerably. Quantification of the response of crop physiological and phenological processes to broad ranges of air temperature at both ambient and elevated C4 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 C4 and to test if the temperature dependence of these processes is altered by elevated C4. We also investigated C4 enzyme activities to determine if the interactive effect between temperature and C4 was significant in the C4 cycle, and to test if the degree of acclimation to high C4, 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 μmol mol−1) or elevated C4 (750 μmol 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) C4 values (mean ± S.D. of five chambers) were 374 ± 1.5 μmol mol−1 and 747 ± 0.5 μmol mol−1 for ambient
and elevated $C_a$, respectively. Corresponding nighttime $C_a$ values were $481 \pm 17.0 \text{mol m}^{-2} \text{mol}^{-1}$ and $684 \pm 5.0 \text{mol m}^{-2} \text{mol}^{-1}$, 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 (≥151 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$_4$Cl, 1.5; NH$_4$H$_2$PO$_4$, 1.0; Ca(NO$_3$)$_2$•4H$_2$O, 4.0; MgSO$_4$•7H$_2$O, 3.5; KCl, 2.5; KNO$_3$, 4.0; and K$_2$SO$_4$, 1.0. Micronutrient concentrations in μM were: (NH$_4$)$_6$Mo$_7$O$_{24}$•4H$_2$O, 0.07; H$_2$BO$_3$, 20.6; CuSO$_4$•5H$_2$O, 0.16; MnSO$_4$•H$_2$O, 5.0; ZnSO$_4$•7H$_2$O, 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 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$_2$O drawdown method (Baker et al., 2004).

### 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-CO$_2$, Omega Engineering, Inc., Stamford, 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$_2$O 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 μmol mol$^{-1}$ with PAR inside the leaf cuvette controlled to saturating levels of either 1800 or 2000 μmol m$^{-2}$ s$^{-1}$. The $A/Q$ response was determined at nine PAR levels between 0 and 2500 μmol m$^{-2}$ s$^{-1}$ at the respective growth $C_a$. Leaf temperature was maintained at growth air temperatures and relative humidity ranged 52 ± 9.7%.

The maximum photochemical efficiency of PSII was determined as relative variable chlorophyll fluorescence ($F_v/F_m$) 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. Pre-dawn 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).
2.4. Leaf pigments and enzyme assays

Samples for determining chlorophyll (Chl) and C₄ 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 cm² 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 N₂ 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 ice cold extraction buffer consisting of 50 mM Tris—HCl (pH 7.50), 10 mM MgCl₂, 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 cm²) 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. The samples were collected on 3rd, 11th, and 16th of July (26, 34, and 39 DAP) and averages were used for analyses. Four separate 0.5 ml centrifuge tubes on ice. The aliquots were quickly placed in liquid N₂ 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. Phosphoenolpyruvate carboxylase (PEPC) was measured in 1 ml solution containing 50 mM Tris—HCl (pH 8.0), 5 mM NaHCO₃, 5 mM MgCl₂, 10 mM NADH, 10 mM PEP (tricyclohexylamine 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 MgCl₂, 5 mM malic acid, 5 mM dithioerythritol, 0.5 mM NADP⁺ and 0.025 ml sample. Phosphoenolpyruvate dikinase (PPDK) was assayed in 1 ml solution containing 0.1 M Tris—HCl (pH 8.0), 0.1 M MgCl₂, 1 mM EDTA, 1.25 ml Na-pyruvate, 2.5 mM K₂HPO₄, 50 mM NaHCO₃, 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 C₄ photosynthesis by von Caemmerer (2000) was fitted to the A/Cᵢ 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/Cᵢ curves were used to estimate carboxylation efficiency (CE) and maximum PEPC activity (Vₑmax). In this region of the A/Cᵢ response, the rate of PEPC (Vₑ) was described by the Michaelis—Menten equation assuming the substrate, phosphoenolpyruvic acid (PEP), was saturating (von Caemmerer, 2000):

\[ V_p = V_{pmax} \frac{C_{m}}{C_{m} + K_p} \]  

where \( K_p \) is the Michaelis—Menten constant for CO₂ of PEPC and was set to 57.0 μmol mol⁻¹ (Pfeffer and Peisker, 1998). Mesophyll CO₂ concentration (Cₘₑ) was set equal to Cᵢ and mesophyll resistance to CO₂ diffusion was ignored. All CO₂ concentrations were converted to partial pressures for model predictions. The carboxylation limited A (Aₑ) at low CO₂ partial pressure (i.e., unsaturated region of the A/Cᵢ curve) is given by:

\[ A_c = \min \{ (V_p + g_{bs} C_{m} - R_m), (V_{cmax} - R_d) \} \]  

where ‘\( \min \{ \} \)’ denotes ‘minimum of’, \( g_{bs} \) the bundle-sheath conductance to CO₂ and was set to 3.0 mmol m⁻² s⁻¹. The product \( g_{bs} C_{m} \) is the inward diffusion of CO₂ into the bundle sheath (von Caemmerer, 2000). Mesophyll mitochondrial respiration (Rₘₑ) was set equal to 0.5 Rₗₑ where Rₗₑ 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 \( R_d \).

\[ f(T_k) = k_{25} \exp \left( \frac{E_a(T_k - 298)}{298RT_k} \right) \]  

\( T_k \) 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 k₂₅ 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 C₄ 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/Cᵢ 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 (Aₑ) was approximated by:

\[ A_j = \min \left\{ \left( \frac{xJ}{2} + g_{bs} C_{m} - R_m \right) \cdot \left( \frac{(1-x)J}{3} - R_d \right) \right\} \]  

where ‘\( \min \{ \} \)’ denotes ‘minimum of’ and \( x \) is a partitioning factor of electron transport rate between C₃ and C₄ cycles (=0.4). \( J \) was related to PAR absorbed by PSII (Iₑ) assuming leaf absorbance of 0.85 and \( J_{max} \) by:

\[ \theta J^2 - J(I_{2} + J_{max}) + I_{2} J_{max} = 0 \]  

where \( \theta \) is an empirical curvature factor (=0.7).
The temperature dependence of $J_{\text{max}}$ 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 $C_a (A_{\text{in}})$:

$$g(T_k) = k_{25} \exp \left[ \frac{E_a(T_k - 298)}{298RT_k} \right] \left[ 1 + \exp((298S - H)/298R) \right]$$

$$\times \left[ 1 + \exp((ST_k - H)/RT_k) \right]$$

where $T_k$ is the leaf temperature (K), $k_{25}$ here is the value of $J_{\text{max}}$ or $A_{\text{in}}$ 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):

$$g(T_k) = k_{\text{opt}} \frac{H \exp(E_a(T_k - T_{\text{opt}})/T_kRT_{\text{opt}})}{H - E_a[1 - \exp(H(T_k - T_{\text{opt}})/T_kRT_{\text{opt}})]}$$

This form has alternative parameters of $T_{\text{opt}}$ and $k_{\text{opt}}$ for optimal temperature in K and the rate at $T_{\text{opt}}$, 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 $J_{\text{max}}$ and $A_{\text{in}}$ 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 ($T_{\text{opt}}$) is then related to $S$ by:

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

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):

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

The rate of process is represented by $h(T)$ as a function of air temperature ($T$ in °C), $T_{\text{opt}}$ is the optimal temperature at which the maximal rate of the process ($R_{\text{max}}$) occurs. $T_{\text{ceil}}$ 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):

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

The rate of leaf-tip appearance is represented by $r$ (leaves days$^{-1}$). $D$ denotes the day temperature period in hours ($=16.0$ h). $T_{\text{day}}$ is the day temperature and $T_{\text{night}}$ 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 CO2 and temperatures. Values shown are means ± standard errors (n = 8) in each chamber.

<table>
<thead>
<tr>
<th>CO2 (μmol mol⁻¹)</th>
<th>Temperature (°C)</th>
<th>Aboveground dry matter (g plant⁻¹)</th>
<th>Leaf area (m² plant⁻¹)</th>
<th>SLA (cm² g⁻¹)</th>
<th>Days to silking (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Stalk</td>
<td>Leaves</td>
<td>Ear</td>
</tr>
<tr>
<td>370</td>
<td>19/13</td>
<td>149.1 ± 4.81</td>
<td>97.7 ± 3.53</td>
<td>48.1 ± 1.13</td>
<td>3.3 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>25/19</td>
<td>112.2 ± 3.02</td>
<td>72.0 ± 2.07</td>
<td>33.5 ± 1.33</td>
<td>6.7 ± 0.62</td>
</tr>
<tr>
<td></td>
<td>31/25</td>
<td>116.0 ± 3.19</td>
<td>69.5 ± 2.07</td>
<td>28.5 ± 0.53</td>
<td>18.0 ± 2.43</td>
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<td></td>
<td>35/29</td>
<td>102.2 ± 3.36</td>
<td>63.3 ± 2.31</td>
<td>27.2 ± 0.92</td>
<td>11.8 ± 1.23</td>
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<tr>
<td></td>
<td>38.5/32.5</td>
<td>75.1 ± 3.46</td>
<td>43.3 ± 2.42</td>
<td>27.4 ± 2.06</td>
<td>4.4 ± 1.38</td>
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<tr>
<td>750</td>
<td>19/13</td>
<td>159.2 ± 2.85</td>
<td>106.8 ± 2.15</td>
<td>49.3 ± 1.97</td>
<td>3.2 ± 1.16</td>
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<td>32.0 ± 0.60</td>
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<td>66.4 ± 2.03</td>
<td>31.9 ± 1.88</td>
<td>5.9 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>35/29</td>
<td>107.7 ± 7.63</td>
<td>67.4 ± 4.92</td>
<td>33.4 ± 3.43</td>
<td>6.9 ± 0.87</td>
</tr>
<tr>
<td></td>
<td>38.5/32.5</td>
<td>75.6 ± 4.85</td>
<td>42.4 ± 2.48</td>
<td>26.5 ± 2.01</td>
<td>6.7 ± 1.40</td>
</tr>
<tr>
<td>P CO2</td>
<td></td>
<td>0.991</td>
<td>0.652</td>
<td>0.303</td>
<td>0.341</td>
</tr>
<tr>
<td>P Temperature</td>
<td></td>
<td>0.002</td>
<td>0.001</td>
<td>0.003</td>
<td>0.393</td>
</tr>
<tr>
<td>P Non-additivity</td>
<td></td>
<td>0.484</td>
<td>0.215</td>
<td>0.904</td>
<td>0.059</td>
</tr>
<tr>
<td>LSD0.05</td>
<td>Temperature</td>
<td>16.7</td>
<td>10.5</td>
<td>6.3</td>
<td>–</td>
</tr>
</tbody>
</table>

Main effects of CO2 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 $C_a$ 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.

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 $C_a$ ($P = 0.08$) 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 ($A_{\text{in}}$) was similar between plants grown at ambient and elevated $C_a$ (Fig. 3a). The optimal temperature for $A_{\text{in}}$ was near 34 °C in either $C_a$ 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
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 $k_0$) 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 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

<table>
<thead>
<tr>
<th>CO$_2$ (μmol mol$^{-1}$)</th>
<th>Temperature (°C)</th>
<th>Carbon (% w/w)</th>
<th>Nitrogen (% w/w)</th>
<th>C/N ratio</th>
<th>Chlorophyll (g m$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>370</td>
<td>19/13</td>
<td>41.0 ± 0.39</td>
<td>4.8 ± 0.07</td>
<td>8.6 ± 0.09</td>
<td>0.22 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>31/25</td>
<td>41.8 ± 0.30</td>
<td>3.5 ± 0.13</td>
<td>11.9 ± 0.43</td>
<td>0.24 ± 0.023</td>
</tr>
<tr>
<td></td>
<td>38.5/32.5</td>
<td>40.5 ± 0.47</td>
<td>3.5 ± 0.01</td>
<td>11.5 ± 0.12</td>
<td>0.19 ± 0.011</td>
</tr>
<tr>
<td>750</td>
<td>19/13</td>
<td>39.5 ± 2.32</td>
<td>4.9 ± 0.30</td>
<td>8.1 ± 0.15</td>
<td>0.21 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>31/25</td>
<td>42.2 ± 0.69</td>
<td>3.9 ± 0.12</td>
<td>10.8 ± 0.22</td>
<td>0.21 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>38.5/32.5</td>
<td>40.8 ± 0.15</td>
<td>3.5 ± 0.03</td>
<td>11.6 ± 0.08</td>
<td>0.17 ± 0.007</td>
</tr>
</tbody>
</table>

$P$-value: Temperature – 0.66, 1.89 –

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.

### Table 3

Activities of C$_4$ enzymes in fully expanded young sunlit maize leaves in response to CO$_2$ enrichment and temperature

<table>
<thead>
<tr>
<th>CO$_2$ (μmol mol$^{-1}$)</th>
<th>Temperature (°C)</th>
<th>PEPC (μmol m$^{-2}$ s$^{-1}$)</th>
<th>MDH (μmol m$^{-2}$ s$^{-1}$)</th>
<th>NADP-ME (μmol m$^{-2}$ s$^{-1}$)</th>
<th>PPDK (μmol m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>370</td>
<td>19/13</td>
<td>68.3 ± 4.55</td>
<td>54.8 ± 5.03</td>
<td>63.1 ± 4.00</td>
<td>11.0 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>31/25</td>
<td>57.8 ± 4.38</td>
<td>31.8 ± 4.39</td>
<td>46.7 ± 4.47</td>
<td>7.6 ± 0.63</td>
</tr>
<tr>
<td></td>
<td>38.5/32.5</td>
<td>62.7 ± 2.79</td>
<td>30.9 ± 4.05</td>
<td>42.1 ± 2.49</td>
<td>5.8 ± 0.26</td>
</tr>
<tr>
<td>750</td>
<td>19/13</td>
<td>58.3 ± 3.32</td>
<td>44.2 ± 4.73</td>
<td>57.8 ± 2.46</td>
<td>9.3 ± 0.53</td>
</tr>
<tr>
<td></td>
<td>31/25</td>
<td>43.9 ± 3.83</td>
<td>24.2 ± 4.57</td>
<td>42.2 ± 2.39</td>
<td>6.3 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>38.5/32.5</td>
<td>51.3 ± 3.92</td>
<td>34.2 ± 4.56</td>
<td>38.6 ± 3.02</td>
<td>5.4 ± 0.51</td>
</tr>
</tbody>
</table>

$P$-value: CO$_2$ 0.009, Temperature 0.023, Non-additivity 0.108

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 CO₂ and temperature. (a) net photosynthesis (A<sub>m</sub>); (b) stomatal conductance (g<sub>s</sub>); (c) transpiration rate (E); (d) carboxylation efficiency (CE); (e) dark respiration rate (R<sub>d</sub>); (f) maximum photochemical efficiency of PSII (F<sub>v/Fm</sub>). 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<sup>−2</sup> s<sup>−1</sup>) with respective growth CO₂ and temperature. Shown are mean ± standard error of three to five plants except in F<sub>v/Fm</sub> where n = 16. Open and closed circles represent ambient (○) and elevated (●) C<sub>a</sub>, respectively.

Table 4
Temperature dependence of maximum PEPC activity (V<sub>max</sub>), leaf dark respiration rate (R<sub>d</sub>), maximum rate of whole-chain electron transport (J<sub>max</sub>), leaf photosynthetic capacity (A<sub>m</sub>), and leaf tip appearance rate (LAR) in relation to growth C<sub>a</sub>

<table>
<thead>
<tr>
<th>Model</th>
<th>Process</th>
<th>Parameter</th>
<th>Units</th>
<th>Ambient C&lt;sub&gt;a&lt;/sub&gt;</th>
<th>Elevated C&lt;sub&gt;a&lt;/sub&gt;</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eq. (3)</td>
<td>V&lt;sub&gt;max&lt;/sub&gt;</td>
<td>k&lt;sub&gt;25&lt;/sub&gt;</td>
<td>μmol m&lt;sup&gt;−2&lt;/sup&gt; s&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>62.5 ± 4.29</td>
<td>57.7 ± 3.67</td>
<td>60.4 ± 2.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E&lt;sub&gt;2&lt;/sub&gt;</td>
<td>kJ mol&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>77.9 ± 5.16</td>
<td>74.1 ± 4.66</td>
<td>75.1 ± 3.64</td>
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<tr>
<td></td>
<td>R&lt;sub&gt;d&lt;/sub&gt;</td>
<td>k&lt;sub&gt;25&lt;/sub&gt;</td>
<td>μmol m&lt;sup&gt;−2&lt;/sup&gt; s&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>2.7 ± 0.18</td>
<td>2.7 ± 0.21</td>
<td>2.7 ± 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E&lt;sub&gt;2&lt;/sub&gt;</td>
<td>kJ mol&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>39.7 ± 5.23</td>
<td>40.0 ± 5.72</td>
<td>39.8 ± 3.74</td>
</tr>
<tr>
<td>Eq. (6)</td>
<td>J&lt;sub&gt;max&lt;/sub&gt;</td>
<td>k&lt;sub&gt;25&lt;/sub&gt;</td>
<td>μmol m&lt;sup&gt;−2&lt;/sup&gt; s&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>234.6 ± 3.12</td>
<td>221.8 ± 2.65</td>
<td>228.2 ± 2.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E&lt;sub&gt;2&lt;/sub&gt;</td>
<td>kJ mol&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>33.3 ± 2.39</td>
<td>32.4 ± 1.92</td>
<td>32.8 ± 1.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>J mol&lt;sup&gt;−1&lt;/sup&gt; K&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>703.4 ± 1.05</td>
<td>701.9 ± 0.90</td>
<td>702.6 ± 0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>k&lt;sub&gt;opt&lt;/sub&gt;</td>
<td>μmol m&lt;sup&gt;−2&lt;/sup&gt; s&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>293.6 ± 3.30</td>
<td>281.8 ± 2.65</td>
<td>287.5 ± 2.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T&lt;sub&gt;opt&lt;/sub&gt;</td>
<td>°C</td>
<td>33.5 ± 0.24</td>
<td>34.1 ± 0.22</td>
<td>33.8 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>A&lt;sub&gt;m&lt;/sub&gt;</td>
<td>k&lt;sub&gt;25&lt;/sub&gt;</td>
<td>μmol m&lt;sup&gt;−2&lt;/sup&gt; s&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>35.5 ± 0.85</td>
<td>34.9 ± 0.65</td>
<td>35.2 ± 0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E&lt;sub&gt;2&lt;/sub&gt;</td>
<td>kJ mol&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>27.1 ± 4.08</td>
<td>26.3 ± 3.00</td>
<td>27.0 ± 2.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>J mol&lt;sup&gt;−1&lt;/sup&gt; K&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>701.0 ± 2.00</td>
<td>701.2 ± 1.45</td>
<td>701.2 ± 0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>k&lt;sub&gt;opt&lt;/sub&gt;</td>
<td>μmol m&lt;sup&gt;−2&lt;/sup&gt; s&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>42.9 ± 0.73</td>
<td>41.7 ± 0.63</td>
<td>42.4 ± 0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T&lt;sub&gt;opt&lt;/sub&gt;</td>
<td>°C</td>
<td>33.7 ± 0.46</td>
<td>33.5 ± 0.33</td>
<td>33.6 ± 0.29</td>
</tr>
<tr>
<td>Eq. (7)</td>
<td>LAR</td>
<td>R&lt;sub&gt;max&lt;/sub&gt;</td>
<td>leaves days&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>0.53 ± 0.03</td>
<td>0.52 ± 0.03</td>
<td>0.53 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T&lt;sub&gt;opt&lt;/sub&gt;</td>
<td>°C</td>
<td>32.2 ± 0.73</td>
<td>32.0 ± 0.75</td>
<td>32.1 ± 0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T&lt;sub&gt;ceil&lt;/sub&gt;</td>
<td>°C</td>
<td>44.0 ± 1.97</td>
<td>43.3 ± 1.99</td>
<td>43.7 ± 1.11</td>
</tr>
</tbody>
</table>

The Arrhenius equation (3) was used for V<sub>max</sub> and R<sub>d</sub>. The peaked model (the rate of declination parameter (H) was set to 220 kJ mol<sup>−1</sup>) (Eqs. (6a) and (6b)) was fitted with J<sub>max</sub> and A<sub>m</sub> 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<sub>a</sub>.

The Arrhenius equation (3) was used for V<sub>max</sub> and R<sub>d</sub>. The peaked model (the rate of declination parameter (H) was set to 220 kJ mol<sup>−1</sup>) (Eqs. (6a) and (6b)) was fitted with J<sub>max</sub> and A<sub>m</sub> 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<sub>a</sub>.
parameters indicating that the CO2 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 C3 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 \(^\circ\)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 \(^\circ\)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 \(^\circ\)C (Tollenaar, 1989b), were decreased by temperatures above 37 \(^\circ\)C and complete inhibition occurred near 45 \(^\circ\)C (Crafts-Brandner and Salvucci, 2002). Optimal leaf temperatures for CO2 assimilation at saturated PAR (\( A_m \)) were near 34 \(^\circ\)C in both CO2 treatments in the present study (Table 4). In a prior study, dry matter accumulation in maize was maximal near 19 \(^\circ\)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 \(^\circ\)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 \(^\circ\)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 CO2 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 CO2 treatments. Moreover, the dependence of photosynthetic and respiratory processes on leaf temperature also was conserved since the temperature response parameters were virtually unchanged for \( A_m, J_{max}, V_{max}, \) and \( R_d \) in response to CO2 enrichment (Table 4).

Foliage temperatures of both C3 and C4 plants are expected to increase because stomatal conductance and transpiration rates are decreased under elevated \( C_a \) (Bunce, 2004; Siebke et al.,

4. Discussion

4.1. CO2 and temperature effects

We observed little evidence of increased growth in response to CO2 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 CO2 enrichment in the measured day\(^{-1} \) which occurred at 32 \(^\circ\)C (\( T_{opt} \)) by fitting Eq. (8) to the pooled data.

Fig. 4. Canopy CO2 exchange rates (\( A_{can} \)) of maize plants in response to CO2 and temperature 29 DAP. (a) PAR and CO2 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 ((△), (▲)) \(^\circ\)C treatment; (c) canopy CER as a function of temperature under high PAR (>1800 \( \mu \)mol 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.
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 C4 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 CO2 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 C4 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 C4 productivity under climate change scenarios. Overall, high $C_a$ did not alter temperature responses of canopy photosynthesis or biomass accumulation in C4 maize. This may have significant ecological implications on the biogeography of C4 species in future climates because temperature is among the key variables determining the distribution of C4 species around the world (Sage and Kubien, 2003).

4.3. Temperature dependence of C4 photosynthesis in elevated C4, and its implications for modeling

The optimum temperature for leaf photosynthesis of C3 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 CO2 and O2 at different temperatures (Long, 1991). In C4 plants, the competition between photosynthetic carbon reduction and photorespiration is already greatly suppressed under current $C_a$ by concentrating CO2 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 C3 photosynthetic cycle significantly in C4 plants. Photosynthetic response to leaf temperature at high $C_a$ may be altered if the temperature dependence of the C4 cycle changes considerably. Our in vitro measurements of the C4 cycle enzymes suggested that the temperature dependence of C4 cycle was not altered in response to CO2 enrichment (Fig. 3; Table 3). In Amaranthus, the CO2 saturation point increased with temperature as the CO2 saturated $A$ increased while CE was relatively constant (Sage, 2002). This response indicates that C4 photosynthesis may also be sensitive to rising $C_a$ at high temperature particularly when operating C4 is low. At higher temperatures, the CO2 saturated $A$ is thought to be limited by RuBP regeneration rate or PEP regeneration rate while CE mainly controlled by PEPC activity in C4 plants (Sage, 2002). In the present study, the CO2 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 CO2 enrichment even at higher temperatures.

According to the current model of C4 photosynthesis, a reduction of CE due to changes in the initial slope of the $A/C_i$ response indicates decreased C4 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 C4 enzyme cycle in maize leaves acclimated to the CO2 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 C4 cycle enzyme assays agreed that photosynthetic acclimation occurred in response to CO2 enrichment in the current study. Despite the reduction in C4 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 3). 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 C4 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 CO₂ 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 C₄. Also note that, it may be important to account for reduced C₄ cycle activities (i.e., Vₚmax) when modeling the effects of elevated C₄ on maize.

5. Conclusions

Growth, development, and photosynthesis of maize plants were not changed in response to CO₂ enrichment but were significantly altered by growth temperatures. Also, the temperature dependence of measured growth, photosynthesis, and developmental parameters were not significantly altered by CO₂ enrichment. Temperature optima for leaf Aₚ and leaf appearance rate were near 34 and 31 °C, respectively. Carboxylation efficiency and estimated Vₚmax from leaf A/Cᵢ curves were reduced in response to CO₂ enrichment across temperatures. This was further supported by reduced in vitro activities of the C₄ enzymes. These results indicate that while many of the growth and photosynthesis parameters examined here were minimally responsive to elevated C₄, an acclimation process might occur in the C₄ cycle by way of reducing the activities of C₄ enzymes.

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References


