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Canopy photosynthesis, evapotranspiration, leaf nitrogen, and transcription profiles of maize in response to CO_2 enrichment

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Abstract

The effects of CO_2 enrichment on the growth and physiology of maize were investigated at the molecular, biochemical, leaf, and canopy levels. Maize plants were grown in sunlit soil–plant–atmosphere research (SPAR) chambers at ambient (370 μmol mol^{-1}) or elevated (750 μmol mol^{-1}) atmospheric carbon dioxide concentration (C_a) under well-watered and fertilized conditions. Canopy gas exchange rates and leaf temperatures were monitored continuously during the growing season. CO_2 enrichment did not enhance the growth or canopy photosynthesis of maize plants. However, canopy evapotranspiration rates decreased by 22% and daytime leaf temperatures were increased about 1 °C in response to CO_2 enrichment. Leaf carboxylation efficiency and leaf nitrogen concentration also decreased at elevated C_a. Transcription profiling using maize cDNA microarrays revealed that approximately 5% of tested genes responded to CO_2 enrichment. Of the altered transcripts, several were known to encode proteins involved in stomatal development or photosynthesis. For the majority of the altered transcripts, however, it was difficult to link their functions with specific physiological factors partly because many of these genes encoded unknown proteins. We conclude that maize did not exhibit enhanced growth or photosynthesis in response to CO_2 enrichment but a number of molecular and physiological processes including those involved in stomatal relations were affected by growth in elevated C_a.

Keywords: C_4 photosynthesis, canopy gas exchange, cDNA microarrays, CO_2 enrichment, leaf temperature, stomata, sunlit growth chambers, transpiration, *Zea mays* L.

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Introduction

Global atmospheric CO_2 concentrations (C_a) are rising (367 μmol mol^{-1} in 1999) and are projected to reach 540–970 μmol mol^{-1} by the end of the 21st century (Prentice *et al*., 2001). While a number of studies dealt with the effects of elevated C_a on the physiological and biochemical processes in plants, the majority of prior studies focused on C_3 species that are known to be most sensitive to changes in C_a (see reviews by Kimball *et al*., 2002; Long *et al*., 2004; Nowak *et al*., 2004). About 18% of global primary productivity is contributed by C_4 plants (Ehleringer *et al*., 1997). Hence, any significant changes in C_4 productivity in response to rising C_a and other climatic variables are likely to have a substantial impact on global primary productivity (Wand *et al*., 1999). Although the photosynthetic and growth responses to elevated C_a in C_4 plants were expected to be minimal owing to the biochemical and anatomical specialization associated with the CO_2 concentrating mechanism based on C_4 acid metabolism, enhanced photosynthesis and growth have been observed in response to elevated C_a in C_4 species (Wand *et al*., 1999; Ghannoum *et al*., 2000). Maize, a NADP-ME-type C_4 species, is the most widely cultivated C_4 crop ranking as the third most produced food crop in the world (Young & Long, 2000). Any effects of elevated C_a on

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maize are likely to have significant consequences in terms of global food production (Leakey *et al*., 2004). Thus, it is critical to accurately assess how elevated C_a will affect growth and physiology of maize.

Enhanced plant growth at high C_a in C_4 species could be partly attributable to improved water use efficiency (WUE) under water-limited conditions (Ghannoum *et al*., 2000). Studies of C_4 plants (e.g. Wall *et al*., 2001; Leakey *et al*., 2004) showed that photosynthesis and/or growth were improved under elevated C_a when plants were subjected to transient or moderate drought stress. Whether CO_2 enrichment enhances growth and photosynthesis of C_4 plants under well watered and fertilized conditions remains controversial (Wong, 1979; Hocking & Meyer, 1991; Samarakoon & Gifford, 1996; Maroco *et al*., 1999). A key limitation in most prior studies of C_4 plant responses to elevated C_a was that photosynthetic rates have been measured at the single leaf level over the short term. Short-term analyses of steady-state leaf photosynthesis (A_{leaf}) of mature leaves do not fully account for season long whole plant carbon dynamics, which is further complicated by such factors as respiration, leaf expansion, and root-shoot partitioning (LeCain & Morgan, 1998; Ghannoum *et al*., 2000). Leaf photosynthesis measurements are often poorly correlated with either biomass accumulation or crop yield (Nelson, 1988), whereas there is generally good correlation between season long canopy photosynthesis measurements and yield (Zelitch, 1982). It is also possible that enhanced rates of A_{leaf} under elevated C_a may be apparent only during early developmental stages in C_4 plants (Cousins *et al*., 2001). Enhanced growth at high C_a in C_4 plants might result from changes in diurnal CO_2 assimilation patterns (Ghannoum *et al*., 2000). Therefore, the most accurate method to relate photosynthesis and growth responses to elevated C_a is to quantify diurnal variations in canopy CO_2 assimilation rates from early development to a fully closed canopy.

Elevated C_a has been shown to reduce leaf nitrogen in C_3 plants (Conroy & Hocking, 1993; Stitt & Krapp, 1999; Gifford *et al*., 2000) and the interaction between elevated C_a and nitrogen was seen to be closely related to acclimation of photosynthesis (Stitt & Krapp, 1999). Leaf nitrogen also decreased by 6% under elevated C_a in several C_4 grass species (Wand *et al*., 1999). Reduced nitrogen uptake because of lowered transpiration rates at high C_a may reduce leaf nitrogen level in response to CO_2 enrichment (Conroy & Hocking, 1993; McDonald *et al*., 2002). Growth in elevated C_a may result in morphological changes such as lower stomatal density (Maroco *et al*., 1999; Lake *et al*., 2001), increased tillering (Hocking & Meyer, 1991), or enhanced leaf area (Wand *et al*., 1999) without concomitant changes in leaf photosynthetic rates. For example, stomatal development of new leaves was down-regulated by CO_2 sensing of mature leaves exposed to high C_a in *Arabidopsis* (Lake *et al*., 2001). Specific genes may regulate phenotypic responses of plants to elevated C_a with or without the direct involvement of photosynthesis. The cDNA microarray technology is a useful tool for studying plant responses to such environmental treatment as high C_a or drought at the gene level (e.g. Yu & Setter, 2003; Miyazaki *et al*., 2004).

In the present study, we tested if CO_2 enrichment altered growth and physiology of C_4 maize at the molecular, biochemical, leaf, and canopy scales. We investigated transcriptome profiles of maize leaf tissue to identify genes that are responsive to CO_2 enrichment. We monitored leaf temperature and gas exchange of maize plants at the canopy level throughout the experiment. We also measured leaf gas exchange rates, leaf carbon and nitrogen content, and C_4 enzyme activities to examine the physiological and biochemical mechanisms underlying the canopy responses to elevated C_a.

Materials and methods

Plant culture

Maize plants (*Zea mays* L. cv. Pioneer 3733) were grown from individual seeds in sunlit 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 monitoring have been described previously (Baker *et al*., 2004). Six SPAR chambers were randomly assigned to ambient (370 μmol mol^{-1}) or elevated C_a (750 μmol mol^{-1}) treatment. Plants were grown on a mix of sterilized sand and vermiculite (1 : 1 by volume) in a soilbin with a volume of 1 m^3 (2 m × 0.5 m × 1 m). Thirty-two seeds per chamber were sown on 20 August 2002. All chambers were controlled at 31/25 °C temperatures with 16 h of 31 °C from 05:00 to 21:00 hours eastern standard time. The seedlings were thinned to 16 plants per chamber with a 25 cm × 25 cm spacing 8 days after planting (DAP). Plants were fertigated four times daily for 5–15 min depending on plant size using a drip irrigation system with half-strength nutrient solution as described in Robinson (1984). Soil moisture tension at 25 cm from the surface was monitored using tensiometers and was near 2 kPa throughout the experiment. Shade curtains to simulate border closure were not used in this experiment resulting in greater sunlit leaf area than in closed canopy studies (Kim *et al*., 2004). Photosynthetically active radiation (PAR) was measured with quantum sensors (LI-190SB; LI-191, LI-COR Inc., Lincoln,

Table 1 Environmental conditions inside SPAR units during the growing season

Treatment	Time	C_a (μmol mol^{-1})	PAR (μmol m^{-2} s^{-1})	Air T (°C)	Soil T (°C)	RH (%)
Ambient C_a	Dawn	563.7 ± 71.52	79.2 ± 7.74	29.6 ± 0.22	24.2 ± 0.47	70.9 ± 8.27
	Day	375.7 ± 5.32	716.4 ± 10.83	30.8 ± 0.19	26.0 ± 0.27	72.5 ± 7.11
	Dusk	456.1 ± 26.12	92.3 ± 5.95	31.0 ± 0.24	26.2 ± 0.25	70.5 ± 7.31
	Night	580.3 ± 75.07	0.14 ± 0.020	25.6 ± 0.16	24.3 ± 0.41	79.1 ± 6.07
Elevated C_a	Dawn	747.8 ± 3.25	71.7 ± 1.72	29.6 ± 0.24	23.8 ± 0.10	60.0 ± 5.97
	Day	736.3 ± 2.05	696.7 ± 22.27	30.8 ± 0.12	25.3 ± 0.60	61.8 ± 7.97
	Dusk	754.4 ± 1.71	97.1 ± 5.28	30.9 ± 0.14	25.4 ± 0.45	60.2 ± 6.42
	Night	747.9 ± 2.72	0.16 ± 0.031	25.8 ± 0.55	23.8 ± 0.30	69.3 ± 5.21

Time of day was grouped to dawn (4:00–8:00 hours), day (8:00–16:00 hours), dusk (16:00–20:00 hours), and night (0:00–4:00 and 20:00–24:00 hours). Shown are mean and standard deviations ($n = 3$) of CO_2 concentration (C_a), photosynthetically active radiation (PAR), air temperature (Air T), soil temperature averaged over four depths (Soil T), and relative humidity (RH).
SPAR, soil–plant–atmosphere research.

NE, USA)[1] located at the top and bottom of the canopy, and outside the chambers. Soil temperatures were measured at four depths using thermocouples. Environmental conditions inside SPAR chambers are summarized in Table 1. Plants were harvested between 69 and 71 DAP when the plants were at developmental stage R3 (i.e. the milky ripe stage), according to the system developed by Ritchie *et al*. (1997). At the completion of the experiment, shoots were detached at ground level and the leaves, tillers, and ears were separated. Total laminar area per plant was measured with a leaf area meter (LI-3000, LI-COR Inc.). Harvested plant parts were dried in a forced-air oven at 70 °C for at least 72 h prior to determining dry weight. Leaf ligule appearance was monitored on five plants per chamber four to six times per week to determine leaf developmental rates. Senescence was determined based on the number of dropped, withered, or brown leaves. Silking date was defined as the day that one-half of the plants had silk visible on the outside of the husks. Three fully expanded leaves near the top of the canopy were sampled from each chamber 45 DAP, and total carbon and nitrogen were analyzed by combustion (CHN-2000, LECO Corporation, St Joseph, MI, USA).

Canopy gas exchange measurements

The C_a of each chamber was measured continuously with a dedicated infrared gas analyzer (Model LI- 6252, LI-COR Inc.). Chamber CO_2 was supplied as medical grade CO_2 from a compressed gas cylinder (Potomac Airgas Inc., Linthicum, MD, USA). Injection rates of CO_2 were regulated by mass flow controllers (FMA-766-V-CO_2, 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, averaged, and recorded every 300 s. 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). Canopy evapotranspiration rates (ET_{can}) were determined by collecting the condensate from cooling coils used to control air temperature and relative humidity and passing the condensate through a conduit connected to pressure transducers. Canopy temperature was measured using an infrared thermometer (Model 4000.4ZL, Everest Interscience Inc., Tucson, AZ, USA) located above the canopy in each chamber.

Leaf gas exchange and chlorophyll (Chl) fluorescence measurements

A portable photosynthesis system (LI-6400, LI-COR Inc.) with a red/blue LED light source (LI6400-02B) mounted onto a 6 cm^2 clamp-on leaf chamber was used to determine the responses of CO_2 assimilation to light (A/Q) and CO_2 (A/C_i). Two or three plants per chamber were selected, and recently developed fully expanded young leaves (11, 12, or 13th leaves of the main stem) were used for A/C_i and A/Q response determinations on 35, 36, or 38 DAP between 09:00 and 16:00 hours. The A/C_i measurements were made at 10 CO_2 levels between 50 and 1200 μmol mol^{-1} air with PAR inside the leaf cuvette of 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 respective growth C_a. Leaf temperature was kept at 31.1 ± 0.25 °C and vapor pressure deficit was at 2.1 ± 0.30 kPa.

The photochemical efficiency of PSII was determined as the relative variable Chl fluorescence (F_v/F_m) using

[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.

a pulse amplitude modulated Chl fluorescence system (Model OS-500, Opti-Sciences Inc., Tyngsboro, MA, USA) about 1 h before dawn. 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. Fluorescence measurements were performed daily using the most recent, fully expanded leaf of all plants in each chamber over a 10 day period beginning 25 DAP.

Leaf pigments and enzyme assays

Samples for determining Chl content and C_4 enzyme activities were collected 30, 35, and 42 DAP within 2 h of solar noon on the assigned dates. Four 1.65 cm^2 leaf discs were removed from the lamina of the most recent, fully expanded leaf of three plants from each chamber. The disks were rapidly transferred to labeled envelopes, immediately immersed in liquid N_2 to quench metabolism. All samples were stored for a maximum of 1 month at −80 °C prior to use. 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 centrifuge (Model 5415C, Brinkmann, Westbury, NY, USA) 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 until used for analysis.

Enzyme activities were determined 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 U MDH, 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. All measurements were performed using a spectrophotometer (Model 2101, Shimadzu Scientific Instruments, Columbia, MD, USA) operated in the kinetic mode. Enzyme activities were calculated from the rate of change in optical density at 340 nm. Enzyme activity and Chl content results were averaged across dates for analyses.

Determination of C_4 photosynthesis model parameters and statistical analysis

The biochemical model of C_4 photosynthesis by von Caemmerer & Furbank (1999) was fitted to the A/C_i and A/Q response curves using SAS NLIN (SAS ver 8.12, SAS Institute Inc., Cary, NC, USA). The initial slope and rate saturated region of the A/C_i curves were used to estimate carboxylation efficiency, maximum PEPC activity (V_{pmax}), and maximum Rubisco activity (V_{cmax}). The initial slope and light saturated asymptote values of A/Q curves were used to estimate apparent quantum efficiency and maximum electron transport rate (J_{max}). Other parameters were used as described by von Caemmerer & Furbank (1999).

Each chamber was an experimental unit and treated as a replication, such that $n = 3$. All measurements on different plants within each chamber were regarded as subsamples. Statistical differences in growth, leaf gas exchange rates, enzyme activities, and other characteristics of plants grown at ambient or elevated C_a chambers were determined using a Student's *t*-test method assuming equal variances using SAS TTEST. Daily canopy gas exchange rates and diurnal canopy temperatures were analyzed as repeated measures using SAS MIXED procedure as measurements were repeated over time for each chamber (Littell *et al*., 1996). Linear regressions of hourly mean of canopy minus air temperature (T_{dif}) as a function of PAR in relation to CO_2 treatment were also analyzed using SAS MIXED.

RNA extraction and hybridization

Leaf tissue (~ 10 cm^2) for RNA extraction and DNA microarray analysis was removed from the central portions (excluding the mid-rib) of the most recent, fully expanded leaves of two plants in each chamber during the middle of the photoperiod on 35 DAP. Samples from each chamber were rapidly transferred to labeled envelopes and immediately frozen in liquid N_2 to stop metabolism. The samples were then held under liquid N_2 until analysis. Prior to extraction, equal amounts of leaf material from two plants within the same chamber were combined. Frozen leaf material was ground to a powder with liquid N_2 in a mortar and pestle and total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Maize DNA microarrays were obtained from the Maize Gene Discovery Project at

Table 2 Biomass and other growth parameters of maize plants grown under ambient and elevated C_a

Parameter	Unit	Ambient C_a	Elevated C_a	SE_{diff}	P
Above ground biomass	g plant^{-1}	219.6	226.7	18.7	0.726
Stem biomass	g plant^{-1}	57.7	64.2	4.1	0.191
Leaf biomass	g plant^{-1}	20.4	20.5	1.4	0.931
Ear biomass	g plant^{-1}	60.6	51.4	5.4	0.164
Tiller biomass	g plant^{-1}	81.0	90.5	11.0	0.436
Tillers	ea	1.52	2.06	0.23	0.080
Leaf area	m^2 plant^{-1}	0.760	0.878	0.071	0.183
Specific leaf area	cm^2 g^{-1}	193.7	199.1	7.2	0.495
Days to silking	days	45.0	46.4	1.02	0.387

SE_{diff} represents one standard error of the difference between the treatments. P is the probability that the difference between treatments is not significantly different from zero.

the University of Arizona (array type: Unigene 1.02.02, http://www.maizegdb.org/microarray.php). RNA extraction and other procedures were based on protocols recommended by the University of Arizona (http://www.maizegdb.org/documentation/mgdp/microarray/protocols. php). A total of 7442 expressed sequence tags (EST) were printed on each microarray and each spot was replicated at least once generating a total of 16 926 spots including blanks. Each slide was replicated and one replicated pair of microarray slides was used for each sample. Labeling of total RNA with 5′ Cy3 or 5′ Cy5 random 9-mers, hybridization of the labeled probes to the slides and imaging procedures were performed at the University of Maryland, DNA Microarray Core Facility located in College Park, Maryland, USA (http://www.umbi.umd.edu/~ cbr/macore/macorestart.htm).

Microarray data analysis

Scanned images of microarray slides were analyzed using ScanAlyze software (version 2.50) from Stanford University (http://genome-ww5.stanford.edu/resources/restech.shtml). Spots with high background were filtered and the data were normalized with a mixed linear model by Wolfinger *et al*. (2001) using the SAS MIXED procedure (Littell *et al*., 1996). This normalization procedure removed random variability because of chambers, slides, or spots and corrected for any detectable dye effects. The data were then transformed with a logarithm to the base 2 and the effects of elevated C_a treatment on gene expression were modeled and evaluated by determining the experimentwise error rate with the Bonferroni adjustment (Wolfinger *et al*., 2001). The critical P-value after the Bonferroni adjustment for pairwise comparison was 1.34e−6 (= 0.01/7442). This fixed the experimentwise error rate at $P = 0.01$. To be conservative, only those genes with greater than twofold change and a P-value less than the critical value were marked significant and selected for functional classification. All significantly induced or repressed genes with known functions were classified into groups based on gene ontology (GO) information obtained from the TIGR Maize Gene Index Database (http://www.tigr.org/tdb/zmgi/). GenBank accession numbers and homologue information corresponding to the microarrays used in this study were obtained from Maize Genetics and Genomics Database (http://www.maizegdb.org/).

Results

Biomass, growth, and development

Dry matter allocated to leaves, stems, tillers, and ears did not differ between treatments (Table 2). Total shoot dry mass was 220 and 227 g plant^{-1} ($P = 0.73$) for plants grown in the ambient and elevated C_a treatments, respectively. Leaf area per plant and specific leaf area (SLA) also did not respond to elevated C_a. The number of tillers per plant was 1.52 and 2.06 ($P = 0.08$) in the ambient and elevated C_a treatments, respectively. Days to silking did not differ between the CO_2 treatments ($P = 0.39$). Rates of ligule appearance and the development of senescent leaves also were similar between treatments (data not shown).

Canopy gas exchange rates and canopy temperatures

Diurnal patterns of canopy gas exchange rates are shown 21, 32, and 39 DAP when plants were at the V6, V10, and V14 growth stages, respectively (Fig. 1). At these growth stages, CO_2 enrichment did not enhance canopy photosynthesis ($P = 0.62$, 0.32, and 0.46, respectively). Plants grown at elevated C_a consistently exhibited lower canopy evapotranspiration rates than those

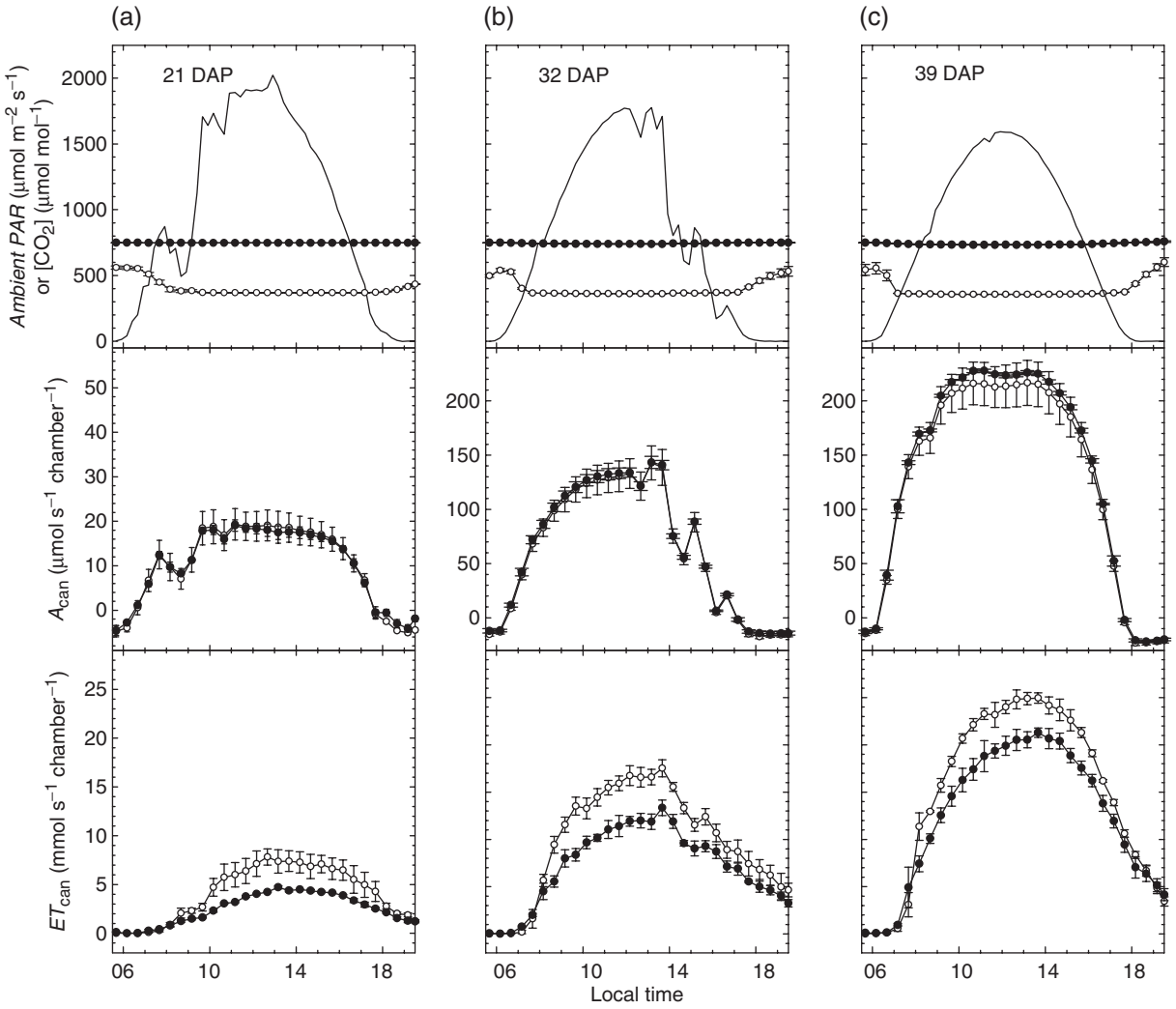

Fig. 1 Diurnal patterns of canopy photosynthesis (A_{can}) and evapotranspiration (ET_{can}). Shown are from three selected days: (a) 21 days after planting (DAP), (b) 32 DAP, and (c) 39 DAP. Ambient photosynthetically active radiation and C_a inside the soil–plant–atmosphere research chambers are shown in the top row. Each point represents the mean (± SE) of three chamber replicates for ambient (○) or elevated (●) C_a.

grown at ambient C_a across the three growth stages ($P = 0.05$, 0.03, and 0.06, respectively). Diurnal canopy CO_2 uptake (6:00–18:00 hours) was similar between treatments (Fig. 2a; $P = 0.37$) whereas diurnal canopy evapotranspiration rates in elevated C_a treatment were reduced by 21.5% compared with the values in ambient C_a (Fig. 2b; $P = 0.03$) from emergence to silking. Canopy temperatures were usually greater in the elevated compared with the ambient C_a treatments during the day (Fig. 3a). Canopy temperature minus air temperature (T_{dif}) between 10:00 and 15:00 hours was increased by 0.92 °C in response to CO_2 enrichment ($P = 0.09$). T_{dif} was similar between treatments when PAR is low (i.e. dawn, dusk, and night) (Fig. 3b inset). T_{dif} was positively correlated to PAR near noon while relatively insensitive to PAR during dawn and dusk in both treatments (Fig. 3b). The slopes for linear regression of T_{dif} on PAR both near noon and during dawn–dusk were heterogeneous between ambient and elevated C_a ($P < 0.05$).

Leaf gas exchange rates and Chl fluorescence

At saturating PAR (i.e. 2000 μmol m^{-2} s^{-1}) and at growth C_a, A_{leaf} of plants grown at ambient and elevated C_a were 48.7 and 50.6 μmol m^{-2} s^{-1}, respectively ($P = 0.41$; Table 3). Leaves grown at elevated C_a exhibited over 50% reduction in stomatal conductance (g_s) and transpiration (E_{leaf}) (Table 3). Intercellular CO_2 concentration (C_i) values were 169 and 312 μmol mol^{-1} ($P = 0.003$) for leaves grown under ambient and elevated C_a, respectively. This resulted in similar C_i/C_a

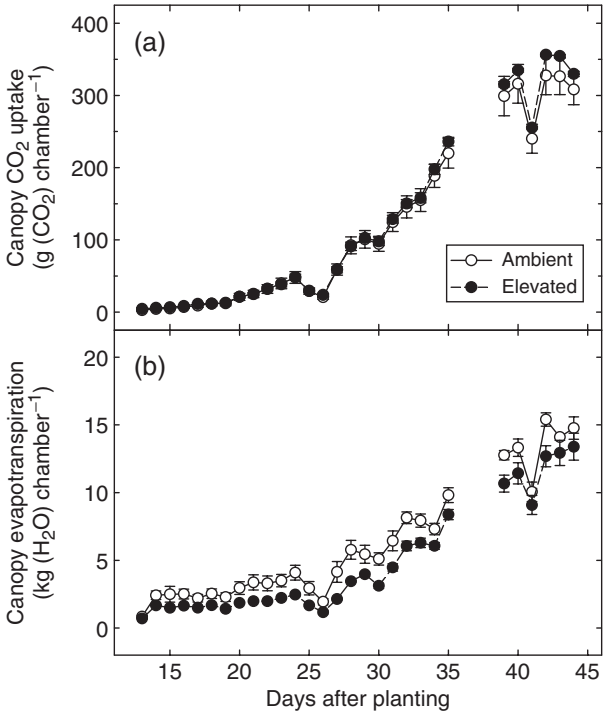

Fig. 2 Total daytime canopy gas exchange rates between 6:00 and 18:00 hours during vegetative stage: (a) canopy CO_2 uptake, (b) canopy evapotranspiration. Each point represents the mean (± SE) of three chamber replicates for ambient (○) or elevated (●) C_a.

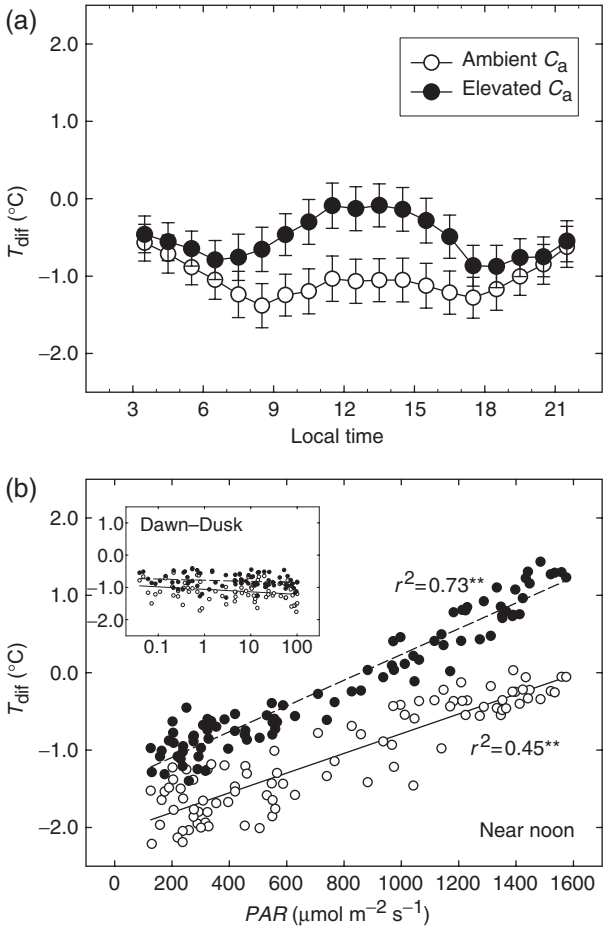

Fig. 3 Canopy temperature minus air temperature (T_{dif}): (a) hourly means of T_{dif} during the growing season. T_{dif} was greater at elevated C_a than at ambient C_a from 10:00 to 15:00 hours ($P < 0.10$). (b) T_{dif} vs. photosynthetically active radiation (PAR) near noon (10:00–14:00 hours) during the growing season. Inset represents T_{dif} vs. PAR at dawn (5:00–7:00 hours) and dusk (17:00–20:00 hours) with the horizontal axis in common log scale. Each point represents the mean (± SE in (a)) of three chamber replicates for ambient (○) or elevated (●) C_a. Solid and dashed lines are regression lines of canopy temperature minus air temperature as a function of PAR for ambient and elevated C_a, respectively. All regressions were significant ($P < 0.05$).

ratios of 0.46 and 0.42 for the ambient and elevated C_a treatments, respectively ($P = 0.44$). As a result of reduced g_s and E_{leaf}, instantaneous WUE measured as A_{leaf}/E_{leaf} was greater for leaves of plants grown under elevated compared to ambient C_a ($P = 0.001$).

Light response curves measured at growth C_a were similar for leaves grown at either C_a treatment (Fig. 4a). However, the A/C_i response curves revealed that A_{leaf} determined at low C_i generally was lower for plants grown at elevated C_a compared with ambient C_a (Fig. 4b). Conversely, A_{leaf} measured at high C_i was similar between treatments (Fig. 4b). The carboxylation efficiency estimated as the initial slope of A/C_i response was lower ($P = 0.04$) under elevated C_a than under ambient C_a (Table 3). Estimates of V_{pmax} of the C_4 photosynthesis model were 120.4 and 91.9 μmol m^{-2} s^{-1} for ambient and elevated C_a ($P = 0.05$), respectively. Estimates of V_{cmax} and J_{max} were similar between the treatments. There were no differences in F_v/F_m between ambient and elevated C_a (Table 3).

Leaf constituents and enzyme activities

The activities of three key enzymes of the C_4 cycle: PEPC, MDH, and NADP-ME were similar for leaves of ambient and elevated C_a grown plants (Table 3). Leaf concentrations of Chl *a*, Chl *b*, and total Chl (*a* + *b*) did not differ for plants grown under ambient and elevated C_a. However, the ratio of Chl *a*/*b* was lower under elevated compared with ambient C_a ($P = 0.08$; Table 3). Total carbon was approximately 45% of the total dry mass for leaves from the ambient and elevated C_a treatments. Leaf tissue contained 3.0% and 3.3% N (w/w) ($P = 0.04$) for plants grown in the elevated compared with the ambient C_a treatments, respectively. Consequently, the leaf C/N ratio was 14.8 under elevated C_a and this compared with a value of 13.7 under ambient C_a ($P = 0.02$).

Table 3 C_4 cycle enzyme activities, chlorophyll content, leaf nitrogen and carbon content, leaf gas exchange rates at PAR = 2000 μmol m^{-2} s^{-1}, and C_4 photosynthesis model parameters of maize plants grown under ambient and elevated C_a

Parameters	Unit	Ambient C_a	Elevated C_a	SE_{diff}	P
Biochemical properties					
PEPC	μmol m^{-2} s^{-1}	43.54	42.32	2.72	0.676
MDH	μmol m^{-2} s^{-1}	23.99	24.01	2.26	0.993
NADP-ME	μmol m^{-2} s^{-1}	39.51	39.44	1.96	0.972
Chl *a*	g m^{-2}	0.244	0.230	0.010	0.253
Chl *b*	g m^{-2}	0.053	0.052	0.002	0.528
Chl *a*/*b*	–	4.57	4.42	0.063	0.084
Leaf total carbon	% in w/w	45.07	44.67	0.445	0.420
Leaf total nitrogen	% in w/w	3.29	3.03	0.089	0.040
Leaf C/N ratio	–	13.71	14.81	0.311	0.024
Leaf gas-exchange rates and chlorophyll fluorescence					
A_{leaf}	μmol m^{-2} s^{-1}	48.72	50.63	2.07	0.409
g_s	mol m^{-2} s^{-1}	0.525	0.207	0.079	0.016
E_{leaf}	mmol m^{-2} s^{-1}	8.61	4.05	0.78	0.004
C_i	μmol mol^{-1}	168.5	312.0	21.98	0.003
C_i/C_a	–	0.455	0.417	0.044	0.437
WUE	μmol (CO_2)/mmol (H_2O)	6.05	12.58	0.806	0.001
F_v/F_m	–	0.8104	0.8097	0.0016	0.710
C_4 photosynthesis model parameters					
Quantum efficiency	mol(CO_2)/mol(quanta)	0.073	0.071	0.011	0.868
Carboxylation efficiency	(μmol m^{-2} s^{-1})/(μmol mol^{-1})	0.804	0.610	0.064	0.038
V_{pmax}	μmol m^{-2} s^{-1}	120.4	91.9	10.50	0.054
V_{cmax}	μmol m^{-2} s^{-1}	67.3	71.6	4.03	0.347
J_{max}	μmol m^{-2} s^{-1}	349.2	354.2	18.2	0.796

SE_{diff} represents one standard error of the difference between treatments. P is the probability that the difference is not significantly different from zero. C_4 photosynthesis model parameters were estimated by fitting the initial slope and saturated rates of the A/C_i and A/Q curves for each treatment using the photosynthesis model described by von Caemmerer & Furbank (1999).
PEPC, phosphoenol pyruvate carboxylase; MDH, malate dehydrogenase; NADP-ME, NADP-malic enzyme; WUE, water use efficiency.

DNA microarray analysis

Out of 7442 ESTs printed on each slide there were a total of 387 transcripts that were differentially expressed in the ambient and elevated C_a treatments. A significance plot displaying the relationship between the $\log_2$ of the estimated expression change and the $-\log_{10}$ of P-values for each transcript is shown in Supplemental Fig. S1. There were 227 and 160 maize transcripts that were induced or repressed, respectively, in response to CO_2 enrichment. It should be noted that the maize genome has not been completely sequenced or annotated. As a result, 279 out of the 387 transcripts identified in this experiment were unclassified and had no known GO. The remaining 108 transcripts were classified into functional groups (Fig. 5). Of these only 15 transcripts have been completely sequenced and have known functions to date. A list of genes altered by CO_2 enrichment is supplemented in Table S1.

A total of 51 maize transcripts were induced by CO_2 enrichment that also had some degree of annotation in the Maize Genome Database. Notably, two induced transcripts, AW011661 and AI657231, were putative calcium dependent protein kinases; calcium, or calmodulium regulated protein kinases that are linked to salinity or drought stress (Patharkar & Cushman, 2000). Other potentially important induced transcripts included AI670650 encoding S-adenosylmethionine decarboxylase proenzyme (AdoMetDC), AI665712 encoding Brittle-1, and AI586416 encoding ferredoxin. AdoMetDC is a key regulatory enzyme in polyamine biosynthesis (Hanfrey *et al*., 2003). Brittle-1 functions as a proposed adenylate translocator in maize amyloplasts (Shannon *et al*., 1998). Ferredoxins are crucial, soluble, low molecular weight, iron-sulfur proteins that function as electron carriers in various redox reactions (Hirohashi *et al*., 2001).

There were 57 annotated maize transcripts that were repressed by CO_2 enrichment. Notably, several putative and known genes involved in photosynthesis or in light capture in the chloroplast were repressed. These included AI947633, AI649549, two transcripts encoding a photosystem-II 10K protein [*Oryza sativa*], AI600810, a photosystem-I assembly protein ycf3 (IRF170) [*Zea*

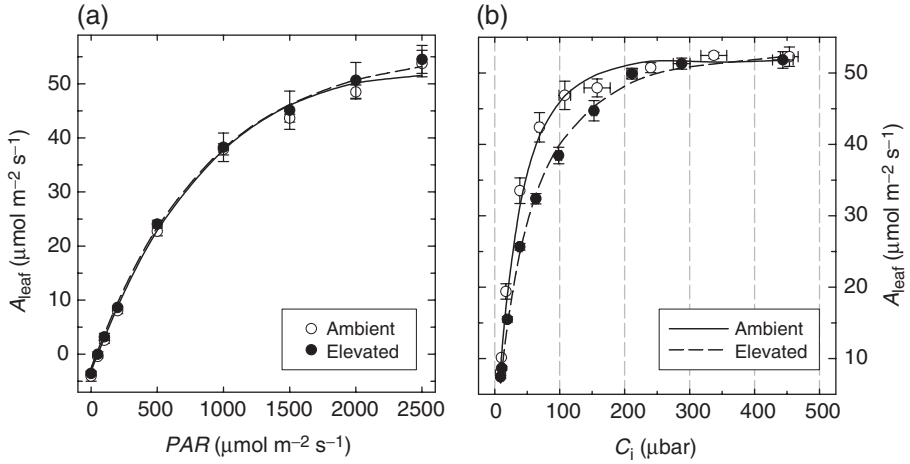

Fig. 4 Net photosynthetic rates (A_{leaf}) of maize leaves grown at ambient (○) or elevated (●) C_a: (a) A/Q response, (b) A/C_i response. Each point represents the mean (± SE) of three chamber replicates. Solid and dashed lines represent the best fit of the C_4 photosynthesis model (von Caemmerer & Furbank, 1999) for measured values of ambient and elevated C_a, respectively.

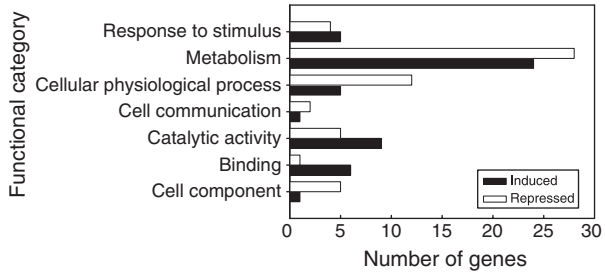

Fig. 5 Summary of significantly induced or repressed genes with known function. These genes were categorized into functional groups based on the classification by gene ontology. Total of 227 and 160 genes were induced or repressed, respectively, in response to elevated C_a, among which 51 and 57 were annotated with known classification. The rest were unclassified, unknown, hypothetical or putative proteins and were not included in the summary chart.

mays], and AI834658, a Chl *a*/*b*-binding protein (CP26) precursor [*Zea mays*]. Also repressed were AW331243 and AW438072, two transcripts for the precursor of the Rubisco small subunit protein [*Zea mays*]; AI737746 and AI881961, encoding the cytosolic isoform of glyceraldehyde 3-phosphate dehydrogenase (G3PDH); and AI739968 and AI603703, encoding fructose-1,6-bisphosphate aldolase and a putative fructose-1,6-bisphosphatase, respectively. A potentially important repressed transcript was AI676939 that encodes leucine-rich repeat (LRR) protein (Table 4). LRR protein was shown to be closely related to control of stomatal distribution in *Arabidopsis* (Nadeau & Sack, 2002). Other potentially important maize genes that were repressed by CO_2 enrichment included AI770450, a major intrinsic protein, AI677017, a cyclophilin, and AI674006 a high mobility group protein-d1 (HMGd1). Major intrinsic proteins are a family of periplasmic membrane proteins located on the plasma membrane and tonoplast that includes water channel proteins (Chaumont *et al*., 2000). Cyclophilins, also known as rotamases or peptidyl-prolyl *cis–trans* isomerases, are present throughout the plant and are involved in protein folding (Romano *et al*., 2004). Cyclophilins also may function in numerous important cellular processes including mRNA processing, protein degradation and in signal transduction. HMG proteins are thought to function in chromatin architecture (Renner *et al*., 2000).

Discussion

The response of plants possessing the C_4 photosynthetic pathway to elevated C_a is complicated by the presence of a CO_2 concentrating mechanism and to date results have been variable and difficult to explain. Although several previous studies reported that CO_2 enrichment enhanced the growth of maize under well watered and fertilized conditions, in the current study there was little evidence of increased biomass accumulation. This was further supported by a lack of response in leaf and canopy photosynthetic rates to the elevated C_a. Elevated C_a may increase the growth of maize via increases in C_i or by changes in diurnal CO_2 fixation patterns. Our results indicate that although C_i was increased neither A_{leaf} nor A_{can} responded to the elevated C_a. Diurnal canopy CO_2 fixation patterns also were similar between ambient and elevated C_a regardless of growth stages (Fig. 1a). Reduced C_4 cycle capacity normally results in a decrease in the initial slope of A/C_i curve and an increase in the CO_2 saturation point if saturating substrate levels are assumed (Sage, 1994; von Caemmerer & Furbank, 1999). We observed a decrease of carboxylation efficiency in the

Table 4 Expression changes of maize transcripts that are potentially linked to stomatal development and distribution in response to CO_2 enrichment

Accession no	Description	Fold change	*P*	Gene*
AI676939	Leucine-rich repeat protein	0.46	0.005	*TMM*
AI622721	Putative beta-ketoacyl-CoA synthase	0.54	0.017	*HIC*
AI737399	Putative subtilisin serine protease	0.31	0.098	*SDD1*
AI978071	Mitogen-activated protein kinase kinase	1.71	0.011	*YODA*

Description of potential gene products was obtained from the MaizeGDB website (http://www.maizegdb.org/). *P* is the probability representing the experimentwise error rate. Fold expression changes were expressed as the ratio of elevated C_a/ambient C_a.
*The *Arabidopsis* genes known to be involved in stomatal development with corresponding function.

plants grown at elevated C_a (see also Maroco *et al.*, 1999; Watling *et al.*, 2000). However, this decreased carboxylation efficiency was not accompanied by decreased PEPC activity *in vitro* or repression of the transcripts encoding PEPC. Reasons for this disparity were not obvious. On the other hand, elevated C_a significantly reduced water loss at the single leaf and canopy levels. Moreover, instantaneous leaf transpiration rate at high PAR was reduced over 50% and cumulative canopy evapotranspiration rates were decreased by 22% under elevated C_a. Consequently, reduced ET_{can} at elevated C_a resulted in increased canopy WUE ($P = 0.07$). The difference between leaf and canopy relative reductions in evapotranspiration probably resulted from the fact that a portion of leaf area was shaded in the canopy because the difference in g_s between ambient and elevated C_a would diminish as PAR decreases. This reduction in ET_{can} is comparable to findings from other studies of C_4 plants (Samarakoon & Gifford, 1996; Conley *et al.*, 2001; Triggs *et al.*, 2004). It should be noted that physiological responses of C_4 plants to high C_a may depend on species or C_4 subgroups so that a caution should be given when comparing results between species.

Measured leaf temperatures at the top of the canopy were greater in the elevated than in ambient C_a treatment probably because evapotranspiration rates and g_s were decreased by CO_2 enrichment. A similar increase in canopy temperature minus air temperature (T_{dif}) was observed previously in two C_4 species exposed to elevated C_a and this increase was attributed to reduced g_s (Siebke *et al.*, 2002). In the present study T_{dif} was positively correlated with PAR and was consistently greater at elevated C_a when PAR was relatively high ($> 100\,\mu mol\,m^{-2}\,s^{-1}$) (Fig. 3b). It is also suggested that leaf warming because of increasing PAR may be greater under elevated C_a compared with ambient C_a as the slope of the regression between PAR and T_{dif} was higher at high C_a. In general C_4 plants have higher optimal temperatures for photosynthesis and for growth in comparison with C_3 plants (Long, 1999). The lack of an effect of increased leaf temperature on A_{can} in the present study was probably because plants were grown near the temperature optimum for maize photosynthesis (Tollenaar, 1989).

The DNA microarray analyses performed here identified 387 out of a total 7442 genes (5.2%) that displayed statistically significant, twofold or greater expression changes in response to elevated C_a. Approximately 59% of these altered transcripts were induced by CO_2 enrichment. Note that 73% of the maize transcripts that responded to elevated C_a lacked annotation. This is a common problem in species with an incompletely sequenced genome. In the present study, transcript levels were altered by elevated C_a without concomitant changes in the rates of photosynthesis, C_4 cycle enzyme activities, growth, or development. Although leaf temperature was greater at elevated than at ambient C_a, less than a 1 °C difference in temperature near the growth temperature optimum was unlikely to have caused the large changes in transcript expression levels observed here. Therefore, it is difficult to fully explain the mechanisms underlying the observed changes in transcription profiles in response to CO_2 enrichment. One possible mechanism to account for the large number of altered transcript levels in the present study can be linked to decreased leaf nitrogen level. Inorganic nitrogen and cellular constituents containing organic nitrogen are among the powerful signaling metabolites in plant cells. Nitrogen and carbon metabolism are carefully integrated processes that are coordinated at the level of transcription (Stitt & Krapp, 1999). Many of the effects of elevated C_a on nitrate metabolism, photosynthate allocation, photosynthetic acclimation, and growth were due to a shift in N status in tobacco plants (Geiger *et al.*, 1999). Total nitrogen content in young leaves was about 9% lower in plants grown under elevated compared to ambient C_a although it was not at deficient level in either treatment in the present study. Nitrate flux from the soil to the shoot was controlled by both transpiration rate and the delivery rate of nitrate to the transpiration stream by the roots in maize (Shaner & Boyer, 1976). Hence, the reduced transpiration rates at

elevated C_a may lead to reduced nitrogen flux from the soil to the shoots (Conroy & Hocking, 1993; McDonald *et al*., 2002). This could potentially decrease the rates of N assimilation and partly account for the lower nitrogen content in the leaves grown in elevated C_a. The mechanism by which leaf nitrogen was decreased in the present study remains uncertain. Whether the reduction in leaf nitrogen was due to reduced nitrogen flux tied to reduced water uptake or was a result of acclimation which lowered nitrogen demand merits further investigation. In either case, the changes in nitrogen metabolism might have triggered signaling cascades that resulted in altered transcription profiles without notable changes in photosynthesis or growth. Hence, altered transcription profiles in CO_2 enriched maize plants could be a response to altered nitrogen metabolism in comparison to plants from the ambient C_a.

Results for several transcripts that responded to the elevated C_a were in agreement with prior findings. Two repressed maize transcripts (AW331243; AW438072) were orthologous to Rubisco small subunit genes (*rbcS*) from other species. Rubisco activity was reduced in previous maize studies using elevated C_a treatments (Wong, 1979; Maroco *et al*., 1999) and Rubisco protein and mRNA encoding both Rubisco small and large subunits declined significantly in response to long-term exposure to elevated C_a in *Arabidopsis* (Cheng *et al*., 1998). Two other related transcripts that were repressed in this study were AI739968 and AI603703. These encoded maize fructose-1,6-bisphosphate aldolase and a putative fructose-1,6-bisphosphatase, respectively. Two transcripts that encoded cytosolic G3PDH in maize (AI737746; AI881961) also were down-regulated in this study. These findings suggested that the genes encoding the key maize enzymes involved in glycolytic metabolism were repressed by the elevated C_a treatment. The activity of maize G3PDH also was decreased in response to elevated C_a in a prior study (Maroco *et al*., 1999). This merits further investigation as the effects of elevated C_a on inhibiting dark respiration rate per dry weight is still in debate (Drake *et al*., 1999; Bunce 2004; Davey *et al*., 2004; Gonzalez-Meler *et al*., 2004).

Stomatal development is controlled by C_a during early leaf development (Gray *et al*., 2000; Lake *et al*., 2001). A potentially important transcript (AI676939) encoding LRR protein which might be closely linked to stomatal development was altered by high C_a in the present study (Table 4; Table S1). In *Arabidopsis*, LRR protein is encoded by the gene *TOO MANY MOUTHS* (*TMM*) which is involved in the regulation of stomatal initiation and distribution (Nadeau & Sack, 2002). LRR-kinase may act as a receptor for signals related to stomatal patterning but its mechanism has yet to be identified (Fleming, 2005). Interestingly, another potentially related transcript (AW120176) encoding LRR transmembrane protein kinase was induced in response to high C_a (Table S1). Other genes that are known to be involved in stomatal development include *HIC* (Gray *et al*., 2000) and *SDD1* (Berger & Altmann, 2000). *HIC* encodes a putative 3-keto acyl coenzyme A synthase involved in the synthesis of very-long-chain fatty acids and acts as a regulator of stomatal development in response to C_a (Gray *et al*., 2000). A potentially related transcript (AI622721) encoding putative β-ketoacyl coenzyme A synthase was marginally down-regulated in response to high C_a (Table 4). This also suggests possible negative regulation of stomatal development. *SDD1* encodes a subtilisin-like serine protease and is involved in signal processing that leads to guard cell formation (Berger & Altmann, 2000). Partially similar to the gene encoding subtilisin-like serine protease was AI737399 which was marginally repressed in the present study. These three genes (i.e. *TMM, HIC,* and *SDD1*) were down-regulated in leaves that lacked guard cells as a result of a constitutive activation of *YODA* in *Arabidopsis* (Bergmann *et al*., 2004). *YODA* encodes a mitogen-activated protein kinase kinase (MAPKK) kinase, which functions in determining guard cell identity and formation (Bergmann *et al*., 2004). In the present study, all three transcripts, AI676939, AI622721, and AI737399, exhibiting functional similarities to *TMM*, *HIC*, and *SDD1* were down-regulated by elevated Ca (Table 4). In addition, the transcript (AI978071) encoding MAPKK was up-regulated indicating potential changes in guard cell formation in response to high C_a (Table 4). CO_2 enrichment reduced both stomatal opening and density in *Arabidopsis* (Lake *et al*., 2001) and maize (Maroco *et al*., 1999). The above findings suggest that, in agreement with the findings in *Arabidopsis*, the genes involved in stomatal development in C_4 plants may be altered under elevated C_a and that responses to high CO_2 in C_4 plants may be channeled by sensing of CO_2 in regulation of stomatal development and distribution.

In conclusion, elevated C_a did not enhance growth or A_{can} of well-watered and fertilized maize plants. Stomatal conductance and transpiration rates, both on a single leaf and on a canopy basis, were decreased in response to elevated C_a, thereby indicating increased WUE. Leaf temperature was increased and nitrogen content in young sunlit leaves was decreased at elevated C_a. The above findings suggested that most physiological responses to elevated C_a in maize may stem from altered water balance because of lowered stomatal conductance. This was further supported by down-regulation of the genes potentially linked to stomatal development at high C_a. Although more than 5% of the maize transcripts tested by DNA microarray

techniques were significantly altered in response to elevated C_a, it was difficult to fully associate the changes in transcription profiles with physiological responses partly because many of the altered genes had unknown function or were poorly annotated to date. While the responses of C_4 plants to elevated C_a may be species specific, the list of those transcripts that were responsive to elevated C_a in this study may serve as a useful resource for studying molecular mechanisms of physiological responses to elevated C_a in C_4 plants. Future studies may investigate the protein expression levels using such techniques as two-dimensional gel electrophoresis to determine the relationships among protein levels, transcripts profiles, and metabolic changes in response to CO_2 enrichment.

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References

Baker JT, Kim S-H, Gitz DC *et al*. (2004) A method for estimating carbon dioxide leakage rates in controlled-environment chambers using nitrous oxide. *Environmental and Experimental Botany*, **51**, 103–110.

Berger D, Altmann T (2000) A subtilisin-like serine protease involved in the regulation of stomatal density and distribution in *Arabidopsis thaliana*. *Genes and Development*, **14**, 1119–1131.

Bergmann DC, Lukowitz W, Somerville CR (2004) Stomatal development and pattern controlled by a MAPKK kinase. *Science*, **304**, 1494–1497.

Bunce J (2004) A comparison of the effects of carbon dioxide concentration and temperature on respiration, translocation and nitrate reduction in darkened soybean leaves. *Annals of botany*, **93**, 665–669.

Chaumont F, Barrieu F, Jung R *et al*. (2000) Plasma membrane intrinsic proteins from maize cluster in two sequence subgroups with differential aquaporin activity. *Plant Physiology*, **122**, 1025–1034.

Cheng S-H, Moore BD, Seemann JR (1998) Effects of short- and long-term elevated CO_2 on the expression of ribulose-1, 5-bisphosphate carboxylase/oxygenase genes and carbohydrate accumulation in leaves of *Arabidopsis thaliana* (L.) Heynh. *Plant Physiology*, **116**, 715–723.

Conley MM, Kimball BA, Brooks TJ *et al*. (2001) CO_2 enrichment increases water-use efficiency in sorghum. *New Phytologist*, **151**, 407–412.

Conroy J, Hocking P (1993) Nitrogen nutrition of C_3 plants at elevated atmopsheric CO_2 concentrations. *Physiologia Plantarum*, **89**, 570–576.

Cousins AB, Adam NR, Wall GW *et al*. (2001) Reduced photorespiration and increased energy-use efficiency in young CO_2-enriched sorghum leaves. *New Phytologist*, **150**, 275–284.

Davey PA, Hunt S, Hymus GJ *et al*. (2004) Respiratory oxygen uptake is not decreased by an instantaneous elevation of [CO_2], but is increased with long-term growth in the field at elevated [CO_2]. *Plant Physiology*, **134**, 520–527.

Drake BG, Dijkstra P, Azcon-Bieto J *et al*. (1999) Does elevated atmospheric CO_2 concentration inhibit mitochondrial respiration in green plants? *Plant, Cell and Environment*, **22**, 649–657.

Ehleringer JR, Helliker BR, Cerling TE (1997) C_4 photosynthesis, atmospheric CO_2, and climate. *Oecologia*, **112**, 285–299.

Fleming AJ (2005) The control of leaf development. *New Phytologist*, **166**, 9–20.

Geiger M, Haake V, Ludewig F *et al*. (1999) The nitrate and ammonium nitrate supply have a major influence on the response of photosynthesis, carbon metabolism, nitrogen metabolism and growth to elevated carbon dioxide in tobacco. *Plant, Cell and Environment*, **22**, 1177–1199.

Ghannoum O, von Caemmerer S, Ziska LH *et al*. (2000) The growth response of C_4 plants to rising atmospheric CO_2 partial pressure: a reassessment. *Plant, Cell and Environment*, **23**, 931–942.

Gifford RM, Barrett DJ, Lutze JL (2000) The effects of elevated [CO_2] on the C : N and C : P mass ratios of plant tissues. *Plant and Soil*, **224**, 1–14.

Gonzalez-Meler MA, Taneva L, Trueman RJ (2004) Plant respiration and elevated atmospheric CO_2 concentration: cellular responses and global significance. *Annals of botany*, **94**, 647–656.

Gray JE, Holroyd GH, van der Lee FM *et al*. (2000) The *HIC* signalling pathway links CO_2 perception to stomatal development. *Nature*, **408**, 713–716.

Hanfrey C, Franceschetti M, Mayer MJ *et al*. (2003) Translational regulation of the plant S-adenosylmethionine decarboxylase. *Biochemical Society Transactions*, **31**, 424–427.

Hirohashi T, Hase T, Nakai M (2001) Maize non-photosynthetic ferredoxin precursor is mis-sorted to the intermembrane space of chloroplasts in the presence of light. *Plant Physiology*, **125**, 2154–2163.

Hocking PJ, Meyer CP (1991) Effects of CO_2 enrichment and nitrogen stress on growth, and partitioning of dry matter and nitrogen in wheat and maize. *Australian Journal of Plant Physiology*, **18**, 339–356.

Kim S-H, Reddy VR, Baker JT *et al*. (2004) Quantification of photosynthetically active radiation inside sunlit growth chambers. *Agricultural and Forest Meteorology*, **126**, 117–127.

Kimball BA, Kobayashi K, Bindi M (2002) Responses of agricultural crops to free-air CO_2 enrichment. *Advances in Agronomy*, **77**, 293–368.

Lake JA, Quick WP, Beerling DJ *et al*. (2001) Signals from mature to new leaves. *Nature*, **411**, 154.

Leakey ADB, Bernacchi CJ, Dohleman FG *et al*. (2004) Will photosynthesis of maize (*Zea mays*) in the US Corn Belt increase in future [CO_2] rich atmospheres? An analysis of diurnal courses of CO_2 uptake under free-air concentration enrichment (FACE). *Global Change Biology*, **10**, 951–962.

LeCain DR, Morgan JA (1998) Growth, gas exchange, leaf nitrogen and carbohydrate concentrations in NAD_ME and NADP_ME C_4 grasses grown in elevated CO_2. *Physiologia Plantarum*, **102**, 297–306.

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic membranes. *Methods in Enzymology*, **148**, 350–382.

Littell RC, Milliken GA, Stroup WW *et al*. (1996) *SAS System for Mixed Models*. SAS institute Inc., Cary, NC, USA.

Long SP (1999) Environmental responses. In: *C_4 Plant Biology* (eds Sage RF, Monson RK), pp. 173–211. Academic Press, Toronto, Canada.

Long SP, Ainsworth EA, Rogers A *et al*. (2004) Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology*, **55**, 591–628.

Maroco JP, Edwards GE, Ku MSB (1999) Photosynthetic acclimation of maize to growth under elevated levels of carbon dioxide. *Planta*, **210**, 115–125.

McDonald EP, Erickson JE, Kruger EL (2002) Can decreased transpiration limit plant nitrogen acquisition in elevated CO_2? *Functional Plant Biology*, **29**, 1115–1120.

Miyazaki S, Fredricksen M, Hollis KC *et al*. (2004) Transcript expression profiles of *Arabidopsis thaliana* grown under controlled conditions and open-air elevated concentrations of CO_2 and of O_3. *Field Crops Research*, **90**, 47–59.

Nadeau JA, Sack FD (2002) Control of stomatal distribution on the arabidopsis leaf surface. *Science*, **296**, 1697–1700.

Nelson CJ (1988) Genetic associations between photosynthetic characteristics and yield: review of the evidence. *Plant Physiology and Biochemistry*, **26**, 543–554.

Nowak RS, Ellsworth DS, Smith SD (2004) Functional responses of plants to elevated atmospheric CO_2- do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist*, **162**, 253–280.

Patharkar OR, Cushman JC (2000) A stress-induced calcium-dependent protein kinase from Mesembryanthemum crystallinum phosphorylates a two-component pseudo-response regulator. *Plant Journal*, **24**, 679–691.

Prentice IC, Farquhar GD, Fasham MJR *et al*. (2001) Chapter 3. The carbon cycle and atmospheric carbon dioxide. In: *Climate Change 2001. The Scientific Basis* (eds Houghton JT, Ding Y, Griggs DJ *et al*.), pp. 183–237. Cambridge University Press, Cambridge, UK.

Renner U, Ghidelli S, Shafer MA *et al*. (2000) Alterations in titer and distribution of high mobility group proteins during embryonic development of *Drosophila melanogaster*. *Biochimica et Biophysica Acta*, **1475**, 99–108.

Ritchie SW, Hanway JJ, Benson GO (1997) *How a Corn Plant Develops*. Iowa State University of Science and Technology, Cooperative Extension Service, Ames, IA.

Robinson JM (1984) Photosynthetic carbon metabolism in leaves and isolated chloroplasts from spinach plants grown under short and intermediate photosynthetic periods. *Plant Physiology*, **75**, 397–409.

Romano PGN, Horton P, Gray JE (2004) The Arabidopsis Cyclophilin gene family. *Plant Physiology*, **134**, 1268–1282.

Sage RF (1994) Acclimation of photosynthesis to increasing atmospheric CO_2: the gas exchange perspective. *Photosynthesis Research*, **39**, 351–368.

Samarakoon AB, Gifford RM (1996) Elevated CO_2 effects on water use and growth of maize in wet and drying soil. *Australian Journal of Plant Physiology*, **23**, 53–62.

Shaner DL, Boyer JS (1976) Nitrate reductase activity in maize (*Zea mays* L.) leaves. I. Regulation by nitrate flux. *Plant Physiology*, **58**, 499–504.

Shannon JC, Pien F-M, Cao H *et al*. (1998) Brittle-1, an adenylate translocator, facilitates transfer of extraplastidial synthesized ADP-glucose into amyloplasts of maize endosperms. *Plant Physiology*, **117**, 1235–1252.

Siebke K, Ghannoum O, Conroy JP *et al*. (2002) Elevated CO_2 increases the leaf temperature of two glasshouse-grown C_4 grasses. *Functional Plant Biology*, **29**, 1377–1385.

Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell and Environment*, **22**, 583–621.

Tollenaar M (1989) Response of dry matter accumulation in maize to temperature: II. Leaf photosynthesis. *Crop Science*, **29**, 1275–1279.

Triggs JM, Kimball BA, Pinter JPJ *et al*. (2004) Free-air CO_2 enrichment effects on the energy balance and evapotranspiration of sorghum. *Agricultural and Forest Meteorology*, **124**, 63–79.

von Caemmerer S, Furbank RT (1999) Modelling C_4 photosynthesis. In: *C_4 Plant Biology* (eds Sage RF, Monson RK), pp. 173–211. Academic Press, Toronto, Canada.

Wall GW, Brooks TJ, Adam NR *et al*. (2001) Elevated atmospheric CO_2 improved Sorghum plant water status by ameliorating the adverse effects of drought. *New Phytologist*, **152**, 231–248.

Wand SJE, Midgley GF, Jones MH *et al*. (1999) Responses of wild C_4 and C_3 grass (*Poaceae*) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions. *Global Change Biology*, **5**, 723–741.

Watling JR, Press MC, Quick WP (2000) Elevated CO_2 induces biochemical and ultrastructural changes in leaves of the C_4 cereal sorghum. *Plant Physiology*, **123**, 1143–1152.

Wolfinger RD, Gibson G, Wolfinger ED *et al*. (2001) Assessing gene significance from cDNA microarray expression data via mixed models. *Journal of Computational Biology*, **8**, 625–637.

Wong SC (1979) Elevated atmospheric partial pressure of CO_2 and plant growth. I. Interactions of nitrogen nutrition and photosynthetic capacity of C_3 and C_4 plants. *Oecologia*, **44**, 68–74.

Young KJ, Long SP (2000) Crop ecosystem responses to climatic change: maize and sorghum. In: *Climate Change and Global Crop Productivity* (eds Reddy KR, Hodges HF), pp. 107–131. CABI International, Oxon, UK.

Yu L-X, Setter TL (2003) Comparative transcriptional profiling of placenta and endosperm in developing maize kernels in response to water deficit. *Plant Physiology*, **131**, 568–582.

Zelitch I (1982) The close relationship between net photosynthesis and crop yield: soybean, wheat, barley, sorghum, maize, tobacco. *BioScience*, **32**, 796–802.

Supplementary material

The following material is available for this article online:
http://www.ars.usda.gov/sp2UserFiles/Place/12755100/KimSH_GCB(2006)_Supplementary.pdf