

# Genomic basis for stimulated respiration by plants growing under elevated carbon dioxide

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Photosynthetic and respiratory exchanges of CO<sub>2</sub> by plants with the atmosphere are significantly larger than anthropogenic CO<sub>2</sub> emissions, and these fluxes will change as growing conditions are altered by climate change. Understanding feedbacks in CO<sub>2</sub> exchange is important to predicting future atmospheric [CO<sub>2</sub>] and climate change. At the tissue and plant scale, respiration is a key determinant of growth and yield. Although the stimulation of C<sub>3</sub> photosynthesis by growth at elevated [CO<sub>2</sub>] can be predicted with confidence, the nature of changes in respiration is less certain. This is largely because the mechanism of the respiratory response is insufficiently understood. Molecular, biochemical and physiological changes in the carbon metabolism of soybean in a free-air CO<sub>2</sub> enrichment experiment were investigated over 2 growing seasons. Growth of soybean at elevated [CO<sub>2</sub>] (550 μmol·mol<sup>-1</sup>) under field conditions stimulated the rate of nighttime respiration by 37%. Greater respiratory capacity was driven by greater abundance of transcripts encoding enzymes throughout the respiratory pathway, which would be needed for the greater number of mitochondria that have been observed in the leaves of plants grown at elevated [CO<sub>2</sub>]. Greater respiratory quotient and leaf carbohydrate content at elevated [CO<sub>2</sub>] indicate that stimulated respiration was supported by the additional carbohydrate available from enhanced photosynthesis at elevated [CO<sub>2</sub>]. If this response is consistent across many species, the future stimulation of net primary productivity could be reduced significantly. Greater foliar respiration at elevated [CO<sub>2</sub>] will reduce plant carbon balance, but could facilitate greater yields through enhanced photoassimilate export to sink tissues.

climate change | elevated CO<sub>2</sub> | free air CO<sub>2</sub> enrichment | metabolic | soybean

The rate at which atmospheric CO<sub>2</sub> concentration ([CO<sub>2</sub>]) is rising and driving climate change is the net consequence of anthropogenic carbon emissions plus ecosystem processes that release or remove carbon from the atmosphere. Carbon emission to the atmosphere from fossil fuel burning, cement production and land use change has risen to ≈10 PgCy<sup>-1</sup> (1). Dark respiration from plants in terrestrial ecosystems is a much larger flux, emitting 50–60 PgCy<sup>-1</sup> (2). The change in plant respiration that will occur by the middle to end of this century in direct response to rising [CO<sub>2</sub>] has long been of interest and uncertainty (3, 4). Changes in respiration will combine with the well characterized stimulation of C<sub>3</sub> photosynthesis by elevated [CO<sub>2</sub>] to impact the net primary productivity of ecosystems and their capacity to act as sources or sinks of carbon. Key synthesis papers have variously concluded that elevated [CO<sub>2</sub>] will cause plant respiration to increase as much as 11%, decrease as much as 18%, or not change (5–8). This uncertainty corresponds to an increase or decrease in carbon release to the atmosphere similar in size to current anthropogenic carbon emissions. The primary reason for uncertainty is that the mechanisms of plant respiratory responses to elevated [CO<sub>2</sub>] have not been resolved (3, 5–8). This knowledge gap also restricts our understanding at the tissue and whole-plant scales of how elevated [CO<sub>2</sub>] impacts growth

and crop yield. Our research tested the hypothesis that plants respond to the greater carbon supply resulting from long-term growth at elevated [CO<sub>2</sub>] through acclimation for increased metabolic capacity and greater respiratory flux.

## Results and Discussion

The mechanisms by which field-grown plants respond to growth at elevated [CO<sub>2</sub>] were investigated in this study by combining genomic analysis with biochemical and physiological phenotyping of soybean in a free-air CO<sub>2</sub> enrichment (FACE) experiment. Soybean was grown over its entire lifecycle in 4 plots at ambient [CO<sub>2</sub>] (≈380 μmol·mol<sup>-1</sup>) and 4 plots at elevated [CO<sub>2</sub>] (≈550 μmol·mol<sup>-1</sup>). This model system featured: (i) the best possible simulation of growth at [CO<sub>2</sub>] projected for 2050, i.e., plant growth under field conditions without unwanted perturbation of the microclimate or plant growth volume (9, 10); (ii) low genetic and environmental variability among experimental units, which increased the statistical power to detect subtle treatment effects; and (iii) a subject species for which commercially available microarrays allowed genome-wide transcript profiling. Physiological, biochemical and molecular analyses were performed at 4 key developmental stages during 2005 and 3 of the same developmental stages in 2006.

The effect of growth at elevated [CO<sub>2</sub>] on the abundance of >37,000 RNA transcripts encoding metabolically active, regulatory and structural proteins in soybean was tested. A significantly greater fraction of transcripts associated with carbohydrate metabolism and respiration were consistently responsive to elevated [CO<sub>2</sub>] during both growing seasons, when compared with transcripts associated with other functions (25% compared with 10% in the total transcript sample; *P* < 0.001, 1-tailed Fisher's exact test) (Fig. S1). This principal response to elevated [CO<sub>2</sub>] involved greater abundance of >90 transcripts encoding many components of starch metabolism, sugar metabolism, glycolysis, the tricarboxylic acid (TCA) cycle and mitochondrial electron transport in 2005 (Figs. S2–S4 and Table S1) and 2006 (Figs. 1–3; Table S1). Simultaneously, there was greater carbohydrate substrate availability resulting from enhanced photosynthesis at elevated [CO<sub>2</sub>], and stimulated rates of respiratory O<sub>2</sub> uptake and CO<sub>2</sub> release (Figs. 1 and 3 and Figs. S2 and S4). Although posttranscriptional processes play an important role in regulating metabolism, the greater abundance of transcripts for the entire respiratory pathway, not just a few individual enzymes, provides unique evidence for a transcriptionally driven mechanism supporting stimulation of respiration at elevated [CO<sub>2</sub>].

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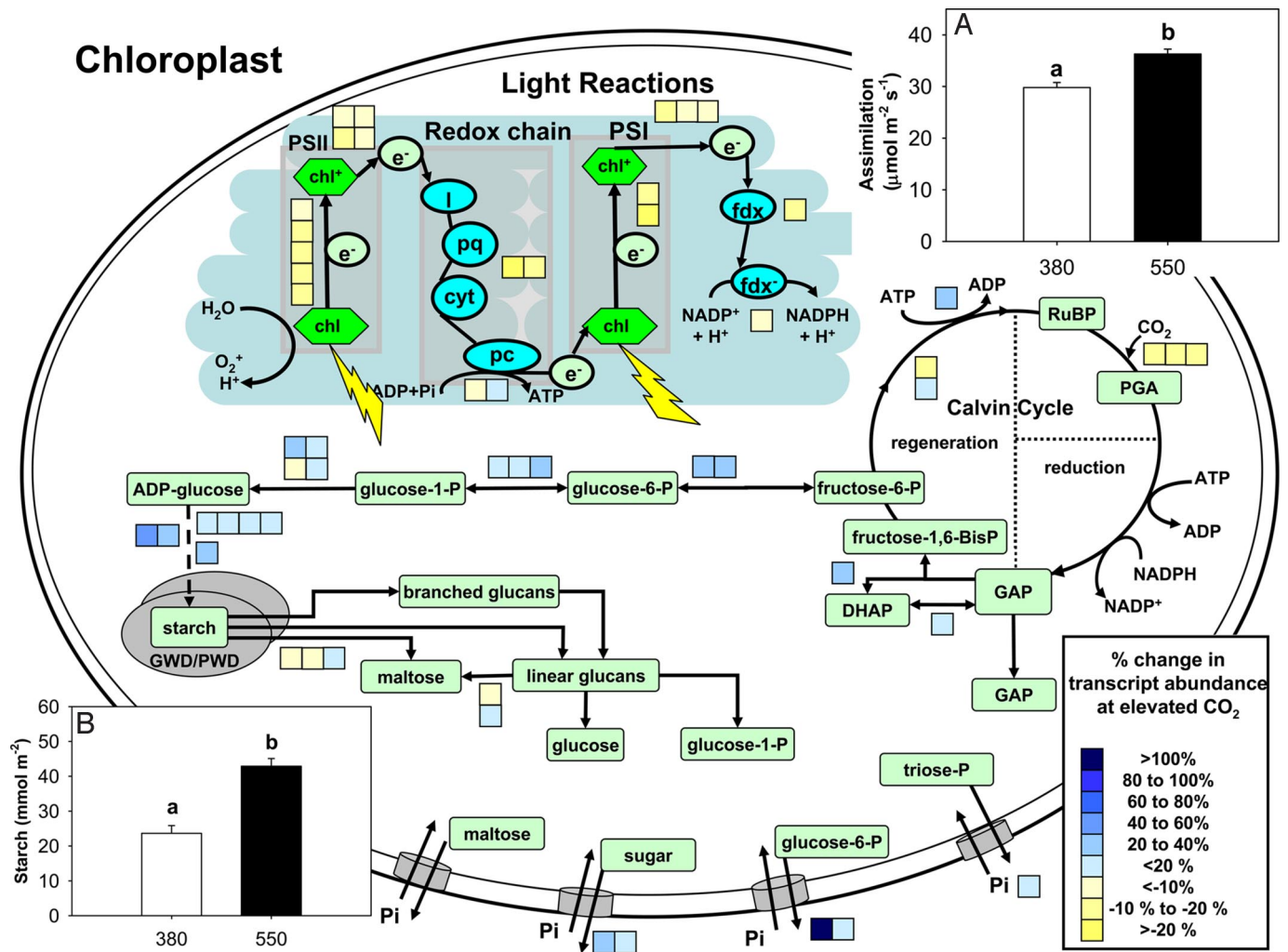
The authors declare no conflict of interest.

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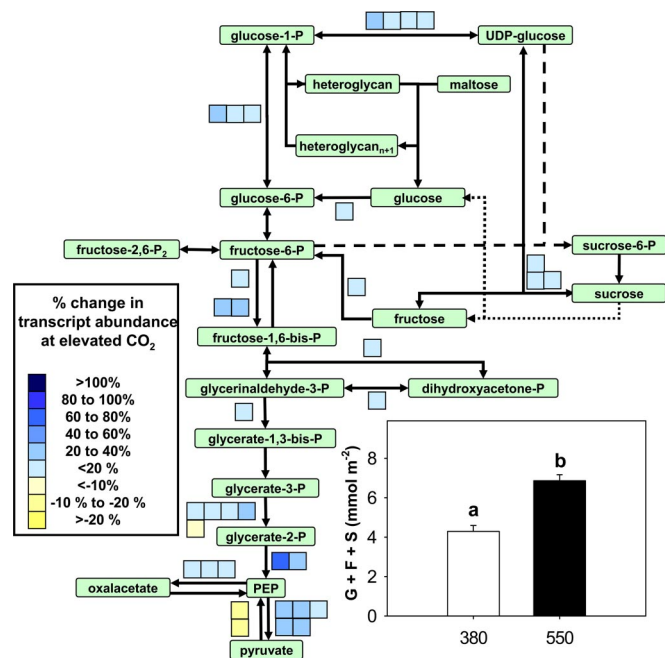


**Fig. 1.** Graphical representation of carbon metabolism at midday in the chloroplasts of mature, sun leaves of soybean grown at SoyFACE. Green boxes represent metabolites. Arrows represent metabolic steps. All data represent average responses to elevated [CO<sub>2</sub>] (550 μmol·mol<sup>-1</sup>) compared with ambient [CO<sub>2</sub>] (380 μmol·mol<sup>-1</sup>), across 3 developmental stages in 2006 (Table S3). Each blue or yellow box represents the statistically significant treatment response ( $P < 0.05$ ) of a unique transcript encoding an enzyme or protein structure (details of transcriptional response in Table S1; details about gene annotations in *S1 Materials and Methods*). (Insets) Mean treatment values (± SE) of the rate of photosynthesis (μmol·m<sup>-2</sup>·s<sup>-1</sup>) (A) and leaf starch content (mmol·m<sup>-2</sup>) (B). Means sharing a common letter are not statistically different.

Elevated [CO<sub>2</sub>] stimulated photosynthesis of soybean by 20% in 2005 (Fig. S2A) and 22% in 2006 (Fig. 1A), which is consistent with the observed response in soybean (11) and C<sub>3</sub> plants in general (12). This photosynthetic response is primarily biochemical, resulting from greater rates of carboxylation and reduced rates of oxygenation catalyzed by Rubisco. Foliar pool sizes of soluble sugars and starch were consequently greater under elevated [CO<sub>2</sub>] in 2005 [soluble +44% (Fig. S3A); starch +89% (Fig. S2B)] and 2006 [soluble +60% (Fig. 2 Inset); starch +81% (Fig. 1B)], as is typical for C<sub>3</sub> species (12). It is this carbohydrate accumulation and resultant sugar signaling that has been identified as the trigger for reduced expression of photosynthetic genes under elevated [CO<sub>2</sub>] (13). This drives photosynthetic acclimation to elevated [CO<sub>2</sub>], which can be a benefit when reallocation of N from the photosynthetic machinery to other pools reduces N limitation on growth under conditions of high C availability (12). Across the growing season in 2005 (Fig. S2) and 2006 (Fig. 1), very few transcripts that encode components of the photosynthetic apparatus were less abundant in elevated [CO<sub>2</sub>] compared with ambient [CO<sub>2</sub>]. This is consistent with: (i) small reductions in photosynthetic carboxylation capacity at

elevated [CO<sub>2</sub>] in soybean (14); and (ii) the ability of soybean to fix N and therefore a diminished benefit from reallocating N away from the photosynthetic machinery (12). Although the theory of optimality and mechanisms underlying photosynthetic responses to elevated [CO<sub>2</sub>] are well accepted, there is no such consensus on how and why respiration responds to elevated [CO<sub>2</sub>] (3, 5–8).

By comparison with changes in transcript abundance related to photosynthetic metabolism at elevated [CO<sub>2</sub>], there were many, larger, positive changes in transcript abundance at elevated [CO<sub>2</sub>] associated with the metabolism of photoassimilate (Figs. 1–3; Figs. S1–S4). This concerted response suggests that there is a transcriptionally driven acclimation, which increases respiratory capacity and drives the use of additional carbohydrate substrate available at elevated [CO<sub>2</sub>]. Under controlled environmental conditions, the rate of starch degradation in plants at night is regulated in response to changes in source and sink activities to support metabolism throughout the night while minimizing any excess starch remaining at dawn (15). Such mechanisms are consistent with greater photoassimilate supply in field-grown soybean under elevated [CO<sub>2</sub>], leading to greater



**Fig. 2.** Graphical representation of sugar metabolism and glycolysis in mature, sun leaves of soybean grown at SoyFACE. (Inset) Mean treatment values ( $\pm$  SE) of leaf glucose, fructose and sucrose (G+F+S) content ( $\text{mmol}\cdot\text{m}^{-2}$ ). Symbols and color coding are as in Fig. 1.

abundance of transcripts encoding the enzymes responsible for starch synthesis and starch degradation (Fig. 1 and Fig. S2). The export of carbon from the chloroplast into the cytosol through carbohydrate transporters is a potential bottleneck between stimulated photosynthesis at elevated  $[\text{CO}_2]$  and stimulated sink activity. An increased capacity to transport a range of sugars and sugar phosphates was suggested by greater abundance of transcripts encoding chloroplast transporters (Fig. 1 and Fig. S2). This may be particularly important because 132–146% greater abundance of transcript for the sugar transporter, GPT2 (16) was the second largest response to elevated  $[\text{CO}_2]$  in both years (Table S2).

The greater abundance of transcripts at elevated  $[\text{CO}_2]$  associated with starch metabolism was balanced with greater abundance of transcripts associated with many steps in sugar metabolism and glycolysis (Fig. 2 and Fig. S3). As in the chloroplast, there was greater abundance of transcripts encoding mitochondrial transport proteins (Fig. 3 and Fig. S4). Also, the abundance of transcripts encoding many components of the TCA cycle and mitochondrial electron transport chain was greater at elevated  $[\text{CO}_2]$  compared with ambient  $[\text{CO}_2]$ . This up-regulation of the entire respiratory pathway at elevated  $[\text{CO}_2]$  in soybean is more complete than that reported for *Drosophila*, yeast and plants in response to sugar feeding (17, 18). This may in part result from screening transcript profiles in soybean for average changes across 2 growing seasons, to reveal the most fundamental, long-term responses. It has been observed in chamber grown *Arabidopsis* that the diel cycles of activity for enzymes in carbohydrate metabolism are strongly dampened by comparison with variation in transcript abundance over the same period (19). However, the predominance of posttranslational control of carbohydrate fluxes over the diel cycle does not preclude the importance of transcriptional regulation to long-term changes in respiratory capacity. Most notably, the leaves of many species, including soybean, contain greater numbers of mitochondria when grown long-term under elevated  $[\text{CO}_2]$  compared with ambient  $[\text{CO}_2]$  (20). Additional organelle biogenesis would not

be possible without greater transcription of many genes encoding the respiratory machinery, such as observed in soybean.

The transcriptional and biochemical changes in carbohydrate metabolism and respiration at elevated  $[\text{CO}_2]$  were accompanied by greater respiratory flux. In 2005, this was quantified as a 22% greater rate of  $\text{O}_2$  uptake at elevated  $[\text{CO}_2]$  compared with ambient  $[\text{CO}_2]$  (Fig. S4). Greater respiratory  $\text{O}_2$  uptake is likely to be coupled to greater respiratory  $\text{CO}_2$  efflux, but the magnitude of the responses could easily differ. Therefore, in 2006, both fluxes were assessed (Fig. 3). Respiratory flux was stimulated at elevated  $[\text{CO}_2]$  in terms of  $\text{CO}_2$  efflux (+37%) in addition to  $\text{O}_2$  uptake (+28%). This meant that respiratory quotient ( $\text{CO}_2$  efflux/ $\text{O}_2$  uptake), was 7% greater at elevated  $[\text{CO}_2]$  compared with ambient  $[\text{CO}_2]$ . Because greater respiratory quotients indicate the use of more highly oxygenated substrates, these data provide additional evidence that carbohydrates were contributing in higher proportion to the substrate pool supplying respiration at elevated  $[\text{CO}_2]$ . Also, this suggests that the reported average stimulation of respiratory  $\text{O}_2$  uptake at elevated  $[\text{CO}_2]$  across many species and functional types (+11%) (5) underestimates the stimulation of  $\text{CO}_2$  efflux. In combination with the transcript profile and metabolite data, the flux data provide novel evidence that increased capacity for respiration at elevated  $[\text{CO}_2]$  is achieved through a transcriptionally driven acclimation process, which increases the capacity of respiration to use the greater amount of carbohydrate substrate available to meet demand for C skeletons and energy. This interpretation of our findings supports Williams and Farrar (21), who argued that the availability of substrates, primarily carbohydrates, determines the longer-term capacity for respiration, whereas the current demand for ATP controls respiratory flux in the shorter-term. Phloem loading accounts on average for  $\approx 30\%$  of respiratory energy demand in fully expanded leaves (22). Therefore, the greater photoassimilate export from source leaves necessary to support greater growth in other parts of plant at elevated  $[\text{CO}_2]$  is one of several factors likely to increase energy demand in the leaf to make use of the greater capacity for respiration.

Transcript profiling provided a novel view of the complex changes in the sinks for C skeletons and energy produced by respiration at elevated  $[\text{CO}_2]$  compared with ambient  $[\text{CO}_2]$  (Table S2). There were significant and consistent increases in the abundance of transcripts encoding enzymes involved in some biosynthetic pathways (e.g., cellulose synthase-like genes in cell wall synthesis) and decreases in others (e.g., CER1 in epicuticular wax synthesis). In general, metabolism-related transcripts displayed positive responses to elevated  $[\text{CO}_2]$ , which is consistent with greater overall metabolic activity. Meanwhile there were relatively few differences between ambient and elevated  $[\text{CO}_2]$  in the abundance of transcripts encoding components of the RNA transcription or protein synthesis machinery. Notably, in a previous study of developing soybean leaves, increased abundance of transcripts encoding respiratory machinery was associated with greater growth of the leaves themselves at elevated  $[\text{CO}_2]$  (23). The data presented here adds substantially to the evidence against the long-held view that respiration is inhibited by elevated  $[\text{CO}_2]$  (5, 20, 24). For example, the number of mitochondria in leaves is greater at elevated  $[\text{CO}_2]$  compared with ambient  $[\text{CO}_2]$ , without any observable change in mitochondrial size, across a wide range of species (20). This was difficult to rationalize when respiration was thought to be inhibited by elevated  $[\text{CO}_2]$  (3, 6), but is consistent with an increase in the abundance of transcripts encoding the respiratory machinery and a stimulation in respiratory flux, as shown here. It appears the false impression that respiration is instantaneously inhibited by elevated  $[\text{CO}_2]$  resulted from technical problems with some of the gas exchange techniques used to measure respiratory  $\text{CO}_2$  flux, which also impacted previous assessments



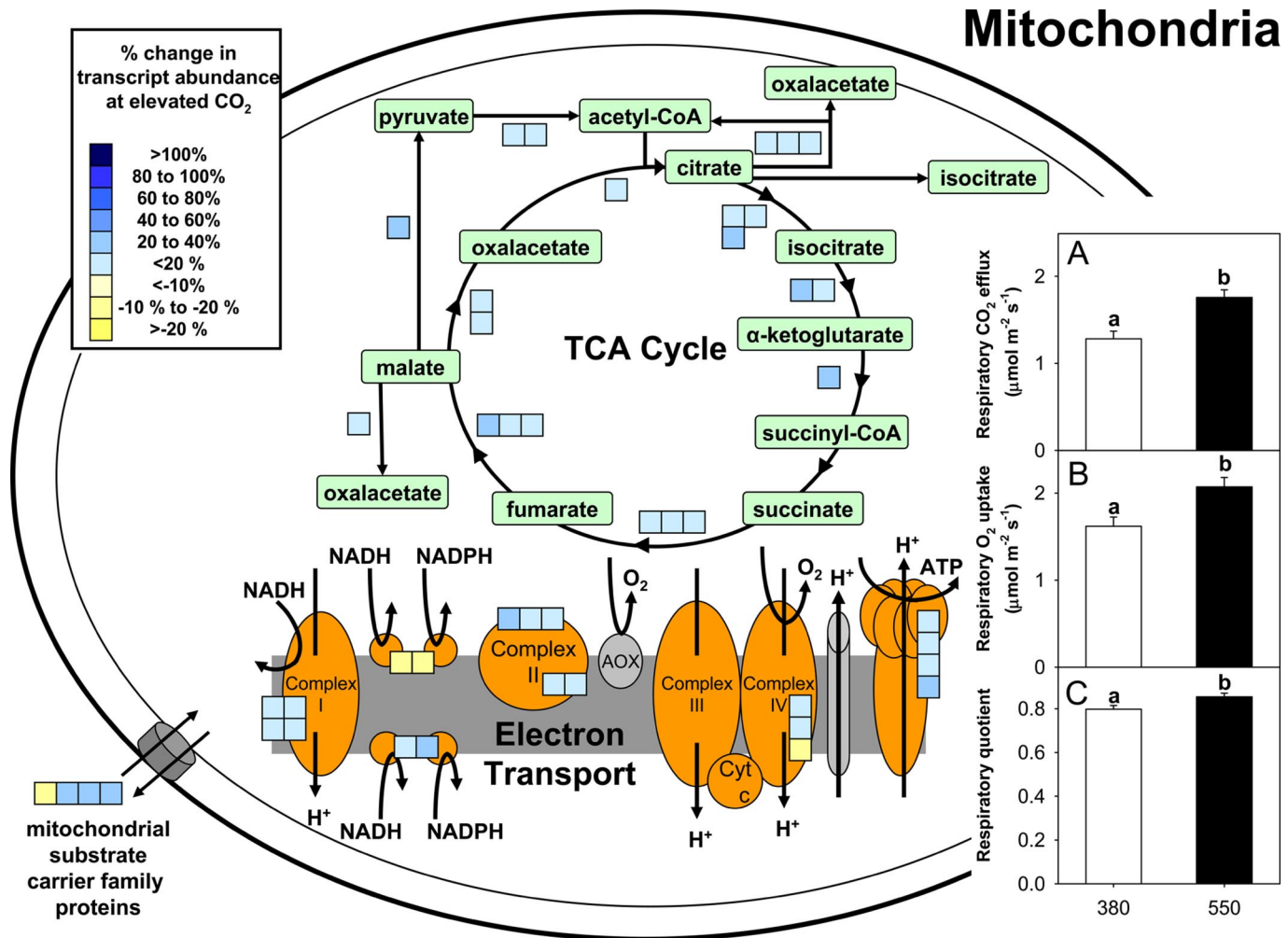


Fig. 3. Graphical representation of carbon metabolism in mitochondria of mature, sun leaves of soybean grown at SoyFACE. (Inset) Mean treatment values ( $\pm$  SE) of the nighttime rate of respiratory CO<sub>2</sub> efflux ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) (A), rate of respiratory O<sub>2</sub> uptake ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) (B), and respiratory quotient (C). Symbols and color coding are as in Fig. 1.

of long-term responses when respiration was measured under growth [CO<sub>2</sub>] (7, 25).

There is undoubtedly variation in the impact of growth at elevated [CO<sub>2</sub>] on plant respiration associated with genetic variation within and among species, tissue type, growth conditions and ontogenetic development. Understanding the mechanism that senses carbohydrate accumulation and up-regulates synthesis of the respiratory machinery will greatly aid efforts to assess and explain variation in the effects of elevated [CO<sub>2</sub>] on respiration. The new information presented here is particularly valuable for meeting this challenge because it evaluates the response to enhanced carbon supply over the whole lifecycle of field grown plants, whereas most previous research investigating the regulation of carbon metabolism has focused on responses to short-term sugar feeding or carbon shortages in controlled environments (15). The transcript profiling data from soybean provides new insight into potential components of the mechanism regulating acclimation to elevated [CO<sub>2</sub>], with 627 transcripts significantly responding to growth at elevated [CO<sub>2</sub>] in common across both growing seasons (Table S2). Although many of these responses are relatively subtle, treatment effects of 10–50% correspond closely with the magnitude of biochemical and physiological responses of plants to elevated [CO<sub>2</sub>]. In addition, the highly consistent magnitude of the CO<sub>2</sub> effect on transcript abundance between years ( $\Delta 2006 = 0.92 \times \Delta 2005 +$

2.4;  $r^2 = 0.7$ ;  $P < 0.001$ ) (Fig. S5) supports the veracity of the results. A number of these CO<sub>2</sub>-responsive transcripts encode proteins that have functions regulating carbon metabolism and growth in Arabidopsis. For example, T-DNA insertion and over-expressing lines have revealed that expression of the transcription factor GNC (GATA, Nitrate-inducible, Carbon metabolism-involved) up-regulates components of carbon-related metabolism, including sugar transporters and cellulose synthase (26). Transcript abundance of GNC was 16–32% greater at elevated [CO<sub>2</sub>] compared with ambient [CO<sub>2</sub>], making it a putative regulator of respiratory capacity (Table S2). Normal rates of starch degradation at night require expression of the putative protein phosphatase SEX4 (Starch EXcess4) (15). The abundance of SEX4 was 9% greater at elevated [CO<sub>2</sub>] in 2005 (Fig. S2), suggesting it may play a role in driving greater starch breakdown. Hexokinase is the best understood sugar sensor in plants (15) and has been implicated in regulation of photosynthetic acclimation to elevated [CO<sub>2</sub>] (13). The abundance of a transcript encoding hexokinase (HXK1) was  $\approx 10\%$  greater at elevated [CO<sub>2</sub>] in both years (Fig. 2 and Fig. S3). Meanwhile, trehalose-6-phosphate is an important signaling molecule in sugar sensing (13) and the abundance of transcript encoding isoform 5 of trehalose-6-phosphate synthase (TPS5) was  $\approx 30\%$  greater at elevated [CO<sub>2</sub>] in both growing seasons (Table S2). TPS5 binds a 14–3–3 protein upon phosphorylation by the

protein kinase SNRK1 (Sucrose Non-fermenting-1-Related protein Kinase 1), which has known involvement in sugar signaling (15, 27). Sugar transporters are involved in sugar signaling in yeast and probably plants (13, 15). The sugar transporter glucose 6-phosphate/phosphate translocator2 (GPT2) deserves further study because it displayed the second largest treatment response in both years (Table S2), and it was also a prominent element of transcriptional responses in *Arabidopsis* achieving enhanced carbon gain during acclimation to high light (28). It is important to identify regulatory elements such as those highlighted above as potential targets for biotechnological improvement of crop performance under future, elevated [CO<sub>2</sub>] conditions (29).

The results listed above strongly suggest that: (i) a number of mechanisms elucidated in *Arabidopsis* under controlled growth conditions are involved in regulating carbon metabolism of field-grown plants; and (ii) the uncharacterized transcripts responding to elevated [CO<sub>2</sub>] in soybean include previously unrecognized factors contributing to the coordination of carbon supply and carbon use in plants.

In conclusion, combining transcript profiling with biochemical and physiological analysis of soybean grown at elevated [CO<sub>2</sub>] under field conditions revealed the mechanistic basis for 37% greater nighttime respiration. Acclimation for increased respiratory capacity was driven by greater abundance of >90 transcripts encoding many components of pathways in carbohydrate metabolism and respiration, which would be needed to generate more mitochondria per cell, as observed in leaves of plants grown at elevated [CO<sub>2</sub>] (19). Greater respiratory quotient and leaf carbohydrate status at elevated [CO<sub>2</sub>] indicated that stimulated rates of respiration were supported through the use of the additional photoassimilate from enhanced photosynthesis at elevated [CO<sub>2</sub>]. Although the effects of elevated [CO<sub>2</sub>] on photosynthesis are well represented in models, the simulation of respiration has been hampered by our poor understanding of the mechanisms of response. This is an important source of uncertainty in models of plant carbon balance and crop yield (3), and ecosystem carbon balance and the global carbon cycle (7). At the leaf and plant scales, stimulated respiration at elevated [CO<sub>2</sub>] will reduce net carbon balance. However, it is possible that enhanced respiration could facilitate increased yield, by providing greater energy for export of photoassimilate from source leaves to sink tissues. Because leaf respiration is between 1/3 and half of global autotrophic respiration [20–30 PgC<sub>y</sub><sup>-1</sup> (6)], if many other species respond similarly to soybean, greater respiration at elevated [CO<sub>2</sub>] could offset the stimulation of photosynthesis and net primary productivity significantly. Although the mechanisms regulating the balance of carbon gain and carbon use in plants could be highly conserved across species, there will certainly be variation in the degree to which elevated [CO<sub>2</sub>] stimulates respiration. For example, photosynthesis in C<sub>4</sub> species is not consistently stimulated by elevated [CO<sub>2</sub>] (30, 31), suggesting that this functional group will not display enhanced respiration like soybean. There is also variation in the degree to which C<sub>3</sub> photosynthesis is stimulated among functional groups (12) and further work is needed to evaluate the impact this will have on respiration.

## Methods

**Field Site and Cultivation.** This experiment was done on soybean [*Glycine max* (L.) Merr. cv. 93B15 (Pioneer Hi-Bred International, Des Moines, IA)] grown at the SoyFACE facility in Champaign, IL ([www.soyface.uiuc.edu](http://www.soyface.uiuc.edu)) during 2005 and 2006. Detailed descriptions of the site (30, 32), agronomic practices (33) and FACE technology used for CO<sub>2</sub> fumigation (30, 34) have been published previously. The experiment was a randomized complete block design with 4 blocks ( $n = 4$  for all statistical tests). Within each block, 1 plot was maintained at current ambient [CO<sub>2</sub>] and 1 plot was fumigated during daylight hours to a target [CO<sub>2</sub>] of 550 μmol·mol<sup>-1</sup>, simulating conditions expected in the year 2050 (2). Details of fumigation timing and efficiency are available in *SI Materials and Methods*.

**Gas Exchange, Tissue Sampling, and Biochemical Analyses.** Mature, sun leaves of soybean were assessed on 6 dates in 2005 and 3 dates in 2006 corresponding to key developmental events in reproductive growth: full flowering, full pod, beginning seed and full seed (35) (Table S3). At midday, photosynthetic gas exchange under growth conditions was measured on 3 plants per plot using open gas-exchange systems (LI-6400; LICOR Inc.) as described in ref. 31. Directly after the photosynthetic measurements, tissue was excised from 3 plants per plot for determination of carbohydrate content, as described in ref. 31. Simultaneously, 6 whole leaflets from 6 separate plants were excised, immediately plunged into liquid nitrogen, and stored at -80 °C until extraction of RNA.

After sunset on the same dates, the petioles of 2 fully expanded leaves in each plot were cut near the stem. The petiole was recut under water, and kept in water while rates of dark respiration at 25 °C were measured in the laboratory. In 2005, respiratory O<sub>2</sub> uptake was measured with an open gas-exchange system incorporating a dual-cell differential oxygen analyzer (S104-DOX; Qubit Systems, Kingston, ON, Canada), as described in refs. 5 and 36. In 2006, respiratory O<sub>2</sub> uptake and CO<sub>2</sub> efflux were measured simultaneously with an open gas exchange system incorporating an IR CO<sub>2</sub>-analyzer (LI-7000, LI-COR Inc.) and the differential O<sub>2</sub> analyzer, as described in *SI Materials and Methods*.

## RNA Preparation, Genechip Hybridization, and Transcript Profile Data Analysis.

Total RNA was extracted using a guanidine thiocyanate acid phenol-based method (37). The quantity and quality of RNA samples was determined with a spectrophotometer (Nanodrop 1000, Thermo Fischer Scientific) and a microfluidic visualization tool (Bioanalyzer, Agilent Technologies). The cRNA labeling protocol, and subsequent steps leading to hybridization and scanning of the soybean genechip arrays were provided by the manufacturer (Affymetrix). The soybean genechips were hybridized and scanned by the Keck Center for Comparative and Functional Genomics at the University of Illinois ([www.biotech.uiuc.edu/centers/Keck](http://www.biotech.uiuc.edu/centers/Keck)). Data processing was performed as described in *SI Materials and Methods*.

**Construction of the Soybean Mapping File for MapMan.** The FASTA file containing the target sequences for each probe-set on the Affymetrix *Glycine max* genechip was obtained from Affymetrix. To fit these target sequences into the existing hierarchical categories (BINs) originally created in the Mapman visualization software for *Arabidopsis* (38), we constructed a database with the TAIR 7 release of the *Arabidopsis* proteome and used a BlastX search to find the best matches for each *G. max* probe set target sequence. This resulted in 21,363 target sequences with acceptable matches ( $E$  value  $<10^{-6}$ ). These matches were assigned to the appropriate MapMan BIN and SubBIN based on the best-matched *Arabidopsis* protein in the "Ath\_AGLTAIR7" mapping file (<http://gabi.rzpd.de/projects/MapMan>). To double check the assigned function of the *G. max* probe-sets and to assign function to the remaining unmatched probe-sets, we performed a second BlastX search against the National Center for Biotechnology Information non-redundant protein database. We parsed the resulting file to retrieve the top 5 hits ( $E$  value  $<10^{-6}$ ) for each target sequence. If a match from *Glycine*, *Phaseolus*, or *Medicago* was one of the top 5 hits, that annotation was used in place of the match from the *Arabidopsis* database. To determine the BIN placement, the bean annotation was compared with the annotation derived from the *Arabidopsis* search, and if they matched, the original BIN was kept. If the annotations did not match (or there were no hits in the *Arabidopsis* proteome), the appropriate BIN was chosen based on biological function. This search resulted in annotation of 4,712 target sequences, 1,159 of which had no hit in the *Arabidopsis* proteome database.

**Statistical Analysis.** Transcript abundance, photosynthesis, metabolite, and respiration data were tested with a randomized complete block mixed model ANOVA, using the Kenward-Rogers option (PROC MIXED, SAS 9.1 or JMP Genomics 3.0; SAS Institute). In all tests, CO<sub>2</sub> treatment and sampling date were fixed effects and a  $P$  value  $<0.05$  was the threshold for significance. The 2 years were tested separately. To determine if transcripts associated with a given functional category contributed significantly to altered transcript profiles at elevated [CO<sub>2</sub>], a Fisher's exact test was performed (PROC FREQ; SAS 9.1; SAS Institute).

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