High atmospheric CO_2 concentration causes increased respiration by the oxidative pentose phosphate pathway in chloroplasts – Supporting information

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Notes S1. Recalculation of previously reported estimates of flux through the plastidial anaplerotic pathway at low C_a

In Wieloch *et al.* (2022), we expressed fractionation signals discussed here as

$$\delta D_i = \frac{D_i}{\Sigma D_{ME}/6} - 1 \qquad \text{Eqn S1}$$

where D_i and D_{ME} denote relative deuterium abundances at specific carbon-bound hydrogens of glucose and the six methyl-group hydrogens of the glucose derivative used for NMR measurements (3,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose), respectively. Here, I express these signals as

$$\delta D_1 = \frac{D_1}{D_{6S}} - 1 \qquad \text{Eqn S2}$$

and

$$\delta D_2 = \frac{D_2}{D_{6R}} - 1 \qquad \text{Eqn S3}$$

Based on equation S2, δD_1 is 66‰ at C_a = 280 ppm and 92‰ at C_a = 180 ppm (Figure S1A). Furthermore, δD_1 and thus flux through the plastidial anaplerotic pathway is significantly greater than zero at C_a = 180 ppm ($p < 0.05$, $n = 2$) while it comes close to being significantly greater than zero at C_a = 280 ppm ($p < 0.13$, $n = 2$). A previously published model describing deuterium fractionation by G6PD can be used to estimate the plastidial anaplerotic flux, and associated respiration (Wieloch *et al.*, 2022). Based on this model, ≈ 9.3% and 12.7% of the G6P entering the starch biosynthesis pathway is diverted into the anaplerotic pathway at C_a = 280 and 180 ppm, respectively. Assuming 50% of all net assimilated carbon becomes starch (Sharkey *et al.*, 1985), anaplerotic flux and associated respiration proceeds at ≈ 5% and ≈ 7% relative to the rate of net carbon assimilation at C_a = 280 and 180 ppm. These rates are probably strongly underestimated since, at low C_a, much of the fractionation signal introduced by G6PD can be expected to not arrive in starch (see biochemical explanation in Wieloch *et al.*, 2022).

Based on equation S3, δD_2 is ≈ -427‰ at $C_a \geq 450$ ppm, -273‰ at $C_a = 280$ ppm, and -15‰ at $C_a = 180$ ppm (Figure S1B). This indicates that the PGI reaction is on the side of F6P at $C_a \geq$ 450 ppm and shifts towards equilibrium with decreasing C_a below 450 ppm (Wieloch *et al.*, 2022).

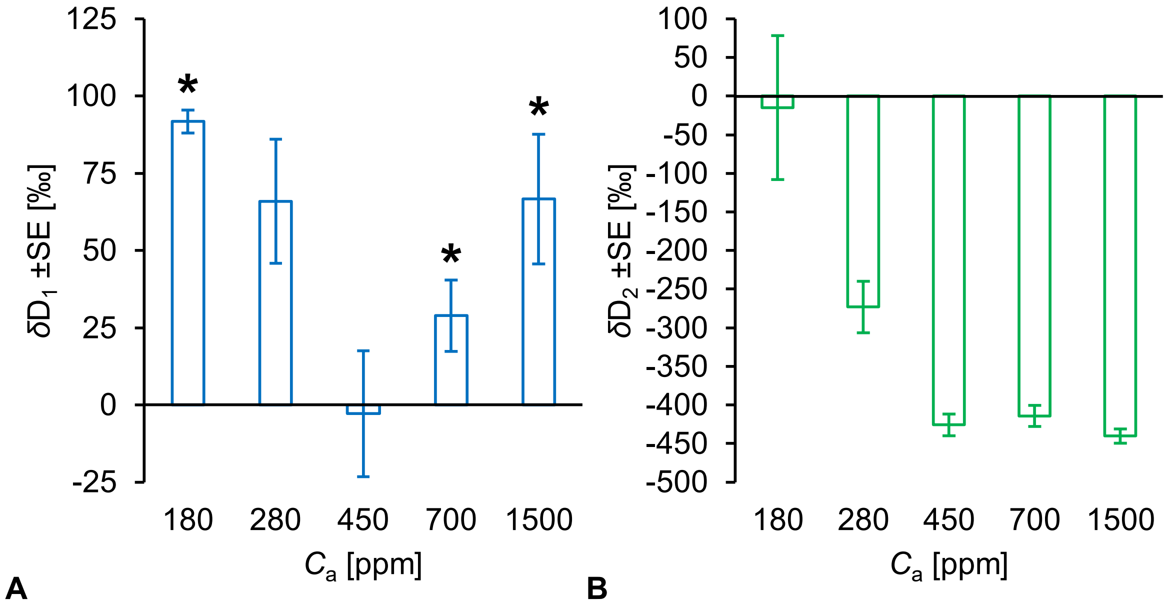

Figure S1 Deuterium abundance at glucose H[1] (**A**, blue bars), and H[2] (**B**, green bars) of sunflower leaf starch. Asterisks denote deuterium abundances that are significantly greater than zero (one-tailed one-sample t-test: $p < 0.05$, $n \geq 2$). At 280 ppm, δD_1 is close to being significantly greater than zero ($p < 0.13$, $n = 2$). The plants were raised in chambers over 7 to 8 weeks at $C_a = 450$ ppm. After a day in darkness to drain the starch reserves, the plants were grown for two days at different levels of C_a (180, 280, 450, 700, 1500 ppm) corresponding to different levels of C_i (140, 206, 328, 531, 1365 ppm). Data expressed as $\delta D_1 = D_1/D_{6S}-1$ and $\delta D_2 = D_2/D_{6R}-1$ where D_i denotes relative deuterium abundances at specific carbon-bound hydrogens of glucose. Deuterium abundances at glucose H^{6S} and H^{6R} are used as references because glucose H^1 and H^{6S} and H^2 and H^{6R} have the same precursors at the chloroplast triose-phosphate level, and H^{6S} and H^{6R} are not modified in the starch biosynthesis pathway (Wieloch *et al.*, 2022).

References

Sharkey TD, Berry JA, Raschke K. **1985**. Starch and sucrose synthesis in *Phaseolus vulgaris* as affected by light, CO_2, and abscisic acid. *Plant Physiology* **77**: 617–620.

Wieloch T, Augusti A, Schleucher J. **2022**. Anaplerotic flux into the Calvin-Benson cycle. Hydrogen isotope evidence for *in vivo* occurrence in C_3 metabolism. *New Phytologist* **234**: 405–411.