Supplementary Material

Metabolomics analysis of postphotosynthetic effects of gaseous O2 on primary metabolism in illuminated leaves

Cyril Abadie\textsuperscript{A}, Sophie Blanchet\textsuperscript{A,B}, Adam Carroll\textsuperscript{A} and Guillaume Tcherkez\textsuperscript{A,C}

\textsuperscript{A}Research School of Biology, College of Medicine, Biology and Environment, Australian National University, Canberra, ACT 2601, Australia.

\textsuperscript{B}Institute of Plant Science Paris-Saclay, UMR Université Paris-Sud-CNRS-INRA-Université Paris-Diderot-UEVE 1403, 91405 Orsay, France.

\textsuperscript{C}Corresponding author. Email: guillaume.tcherkez@anu.edu.au
Figure S1. $^{15}$N-enrichment in metabolites upon labelling with $^{15}$N-glycine in different CO$_2$/O$_2$ gaseous conditions in illuminated sunflower leaves. Here, data shown for $^{14}$N or $^{15}$N signals are that obtained by GC-MS and are semi-quantitative. % corresponds to the percentage in $^{15}$N calculated from the isotopic pattern in the mass spectrum. A, heat map showing significant features along a one-way ANOVA ($p<0.01$). Conditions in each column are indicated with O$_2$ (in %)/CO$_2$ (ppm) (the last number is the replicate no.). B-D, boxplots showing $^{14}$N-glycine, $^{14}$N-alanine and $^{15}$N-alanine in different O$_2$ mole fraction (in %) in the background gas. Data redrawn from Abadie et al. (2016a).
Figure S2. Univariate and multivariate analyses of leaf metabolome of illuminated Arabidopsis rosettes under different CO2/O2 conditions: A, heat map showing significant metabolites \((p<0.01)\) along a one-way ANOVA. Conditions in each column are indicated with CO2 (ppm)/O2 (in %) (the last number is the replicate no.). B-C, volcano plots (VIP versus loading) associated with O2 and CO2 effects, respectively, in the O2PLS analysis. The O2PLS analysis was associated with very good regression coefficient \(R^2\) of 0.91, but a cross-validated regression coefficient \(Q_{cum}^2\) of 0.29 only, due to the limited response to CO2 in terms of total variance. However, the statistical O2PLS model was significant and not the result of chance, since the \(Q_{perm}^2\) coefficient upon the permutation test was negative (-0.17).
Figure S3. Metabolic ratios in sunflower (A-C) and Arabidopsis (D-F) illuminated leaves under different % O₂. In A-C, each box integrates all data obtained in the % O₂ considered regardless of time (all boxes) or CO₂ (at 21% O₂) thus n = 16 to 48. In C-D, n = 6. Mal/Pyr, malate-to-pyruvate ratio; Succ/Cit, succinate-to-citrate ratio; Succ/GABA, succinate-to-γ-aminobutyrate ratio; GOGAT, apparent mass action ratio of glutamine oxoglutarate amino transferase calculated as glutamate²/[2-oxoglutarate·glutamine].
Figure S4. Relative content in S-adenosylhomocysteine (SAHC), S-methylthioadenosine (SMTA), S-adenosylmethionine (SAM) (A) and SAM content in % of all three species (B), in sunflower illuminated leaves under different O₂/CO₂ conditions. The only significant difference at the 0.05 level is between SMTA (or SAHC) and SAM under 100% O₂. Replotted from source data in Abadie et al. (2016a).

Figure S5 (next page). Magnification of Fig. 4.