Supplementary Material

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

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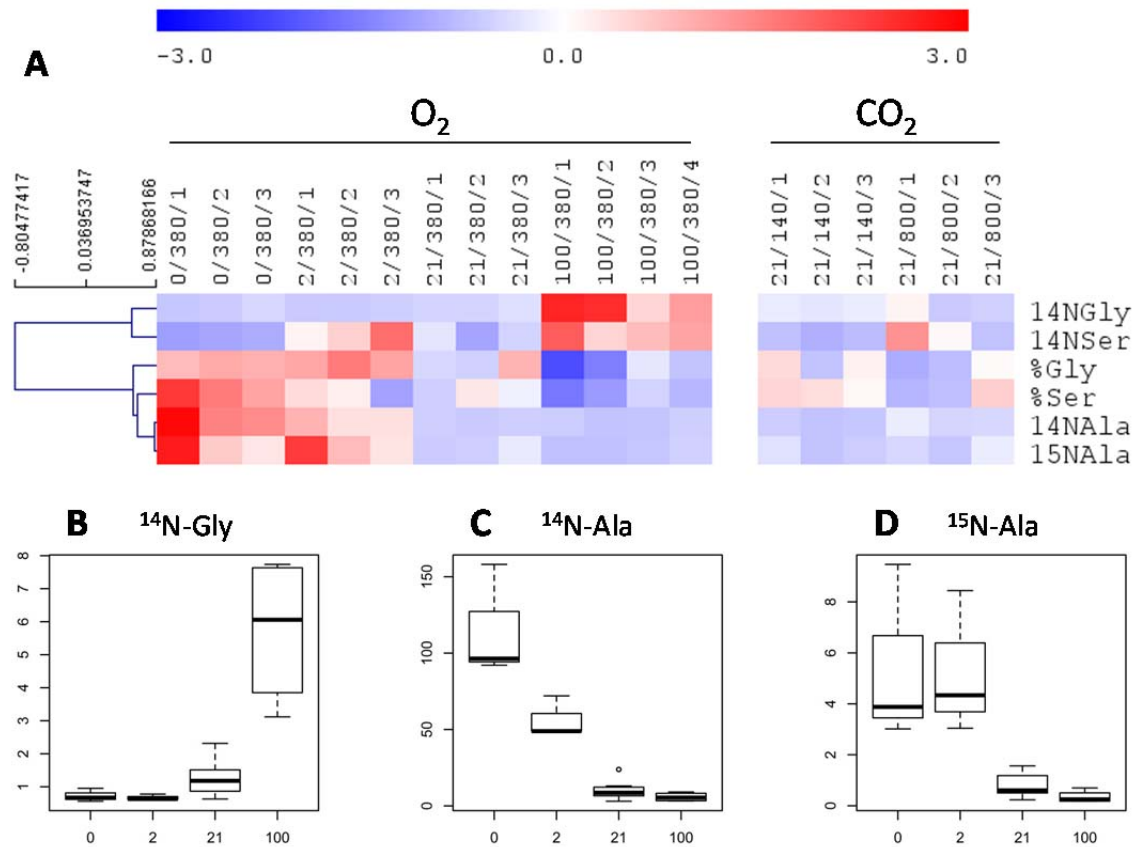[C]Corresponding author. Email: guillaume.tcherkez@anu.edu.au

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).
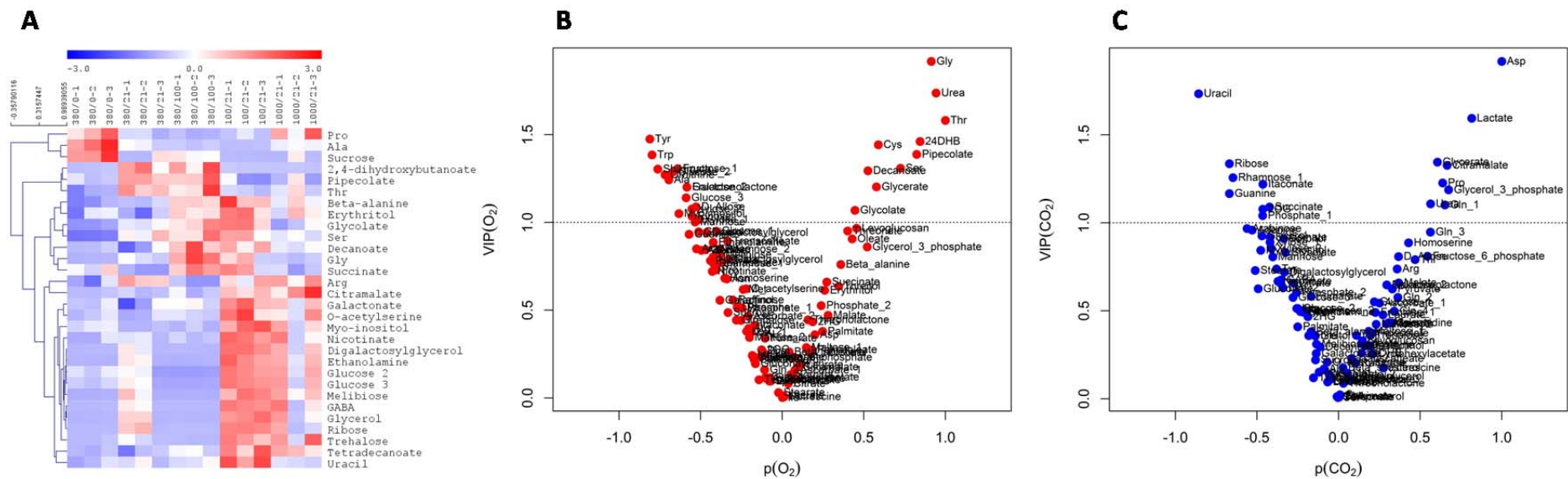
Figure S2. Univariate and multivariate analyses of leaf metabolome of illuminated Arabidopsis rosettes under different CO_2/O_2 conditions: **A**, heat map showing significant metabolites ($p<0.01$) along a one-way ANOVA. Conditions in each column are indicated with CO_2 (ppm)/O_2 (in %) (the last number is the replicate no.). **B-C**, volcano plots (VIP versus loading) associated with O_2 and CO_2 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 CO_2 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).
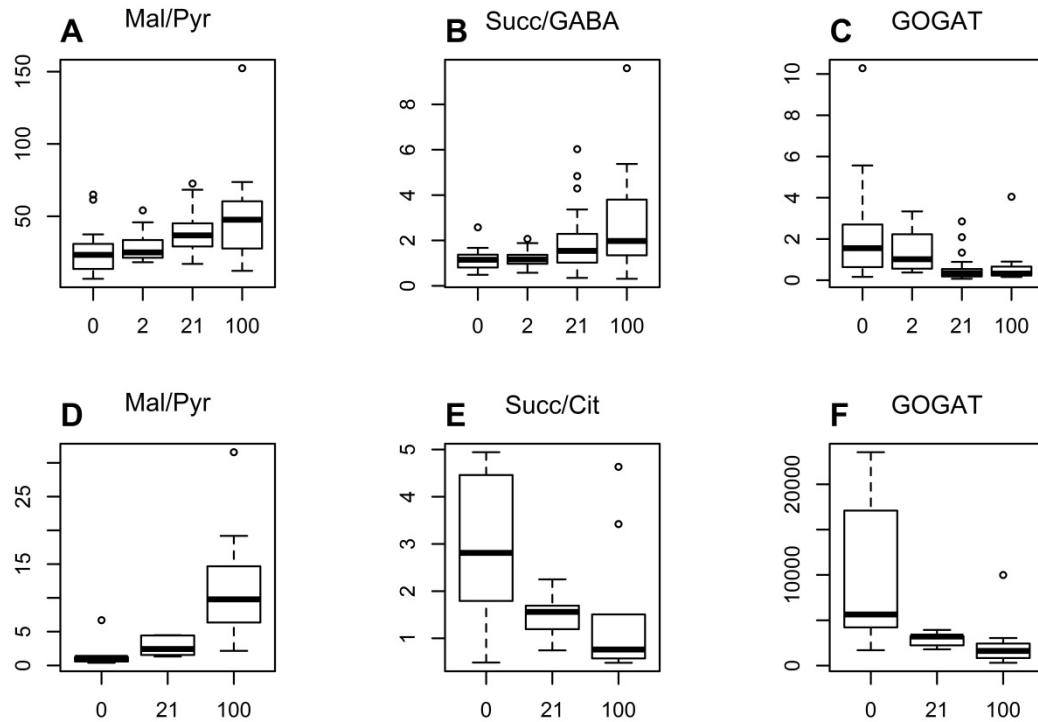
Figure S3. Metabolic ratios in sunflower (**A-C**) and Arabidopsis (**D-F**) illuminated leaves under different % O_2. In **A-C**, each box integrates all data obtained in the % O_2 considered regardless of time (all boxes) or CO_2 (at 21% O_2) thus $n = 16$ to 48. In **C-D**, $n = 6$. Mal/Pyr, malate-to-pyruvate 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 glutamate^2/[2-oxoglutarate·glutamine].
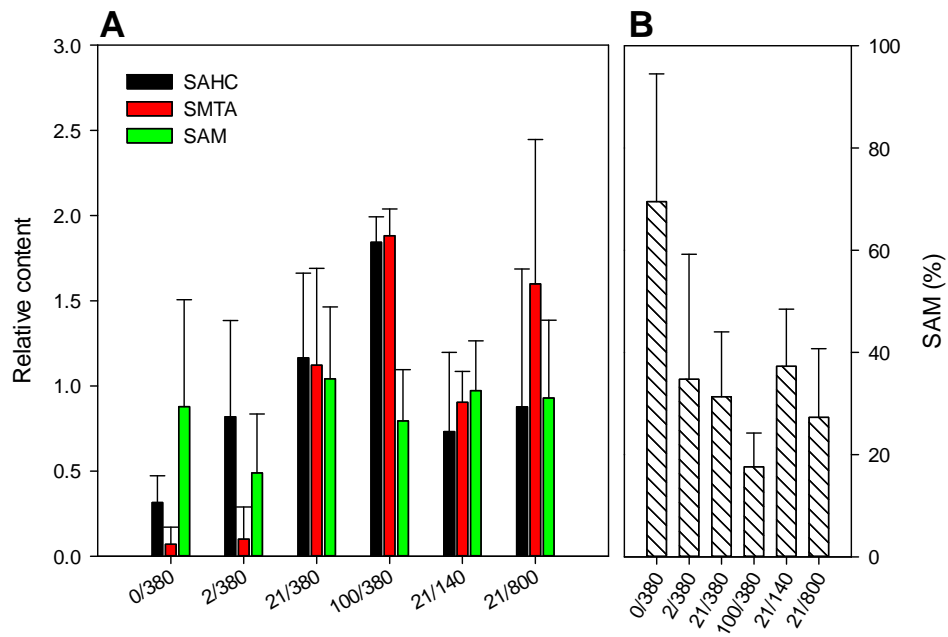
Figure S4. Relative content in S-adenosylhomocysteine (SAHC), S-methylthioadenosine (SMTA), S-adenosylmethionine (SAM) (**A**) and SAM content in % of all three species (**B**), in sunflower illuminated leaves under different O_2/CO_2 conditions. The only significant difference at the 0.05 level is between SMTA (or SAHC) and SAM under 100% O_2. Replotted from source data in Abadie *et al.* (2016a).

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