*Viewpoints*

# Net photosynthetic $CO_2$ assimilation: more than just $CO_2$ and $O_2$ reduction cycles


## Summary

Net photosynthetic assimilation in $C_3$ plants is mostly viewed as a simple balance between $CO_2$ fixation by Rubisco-catalyzed carboxylation and $CO_2$ production by photorespiration (and to a lower extent, by day respiration) that can be easily manipulated during gas exchange experiments using the $CO_2$ : $O_2$ ratio of the environment. However, it now becomes clear that it is not so simple, because the photosynthetic response to gaseous conditions involves 'ancillary' metabolisms, even in the short-term. That is, carbon and nitrogen utilization by pathways other than the Calvin cycle and the photorespiratory cycle, as well as rapid signaling events, can influence the observed rate of net photosynthesis. The potential impact of such ancillary metabolisms is assessed as well as how it must be taken into account to avoid misinterpretation of photosynthetic $CO_2$ response curves or low $O_2$ effects in $C_3$ leaves.


## Introduction

Photosynthesis of $C_3$ plants is the net result of concurrent processes in which $CO_2$ is fixed (carboxylation) and released (photorespiration, day respiration). Gas exchange measurements are essential for the characterization of leaf photosynthetic properties, including stomatal conductance, carboxylation rate, or water use efficiency. The cornerstone of photosynthesis is the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), that can fix either $CO_2$ (carboxylation) or $O_2$ (oxygenation). Oxygenation is the starting point of photorespiration, in which $CO_2$ is liberated in mitochondrial glycine-to-serine conversion by the glycine decarboxylase–serine hydroxymethyl transferase (GDC–SHMT) complex. Rubisco's bi-functionality is visible in photosynthetic Rubisco-limited models of net assimilation ($A$) based on Michaelis kinetics as follows (Farquhar *et al.*, 1980; Von Caemmerer & Farquhar, 1981; Von Caemmerer, 2013):

$$A = v_c \times \left(1 - \frac{\Gamma*}{c_c}\right) - R_d \qquad \text{Eqn 1}$$

where $v_c$ is ribulose 1,5-bisphosphate (RuBP) carboxylation rate, $\Gamma*$ the compensation point in the absence of day respiration, $c_c$ the $CO_2$ mole fraction at carboxylation sites, and $R_d$ day respiration (nonphotorespiratory $CO_2$ evolution). Here, $\Gamma*$ reflects the intrinsic $O_2$ : $CO_2$ partitioning of Rubisco fixation and subsequent photorespiratory $CO_2$ evolution. Hence, $\Gamma*$ can vary slightly if the stoichiometry of photorespiratory $CO_2$ release with respect to $O_2$ fixation changes from the theoretical value of one half (Cousins *et al.*, 2011; Keech *et al.*, 2012; Abadie *et al.*, 2016a). Since Rubisco is not the sole limiting factor under a range of $CO_2$ : $O_2$ conditions, the more general expression of assimilation is given by:

$$A = w \times \left(1 - \frac{\Gamma*}{c_c}\right) - R_d \qquad \text{Eqn 2}$$

where $w$ stands for the minimum of Rubisco carboxylation, RuBP regeneration and triose phosphates utilization. It can be seen in Eqn 2 that if there are alternative sinks (that may or may not be directly associated with $CO_2$ fixation or release), $A$ would be impacted because electron or triose phosphates consumption affect $w$. In addition, Eqns 1 and 2 neglect the contribution of non-Rubisco carboxylation events (e.g. anaplerotic $CO_2$ fixation), overlooks the contribution of processes other than (photo) respiration in $CO_2$ release, and assumes that $R_d$ is constant. Still, they are extremely efficient in modeling photosynthesis in the vast majority of cases, because alternative $CO_2$ influx or efflux processes (or alternative electron consumptions) are associated with rather modest fluxes. That is, measurements are often performed using unstressed plants under conditions where photosynthesis is sufficiently high, e.g. at ambient $CO_2$ and saturating light. However, it becomes problematic when photosynthesis is examined at extreme $CO_2$ mole fractions, typically near or below the compensation point (where $A$ has low values), because usually minor processes can become proportionally large. It can also be of importance when very fine measurements (such as internal conductance) are performed because even small numerical errors have pervading consequences (Harley *et al.*, 1992; Hanson *et al.*, 2016). Furthermore, day respiration ($R_d$) encompasses all sorts of $CO_2$-producing events, from the tricarboxylic acid pathway (TCAP) to the oxidative pentose phosphate pathway. The assumption that $R_d$ remains constant is currently believed to be incorrect, considering direct measurements or metabolic studies that all suggest $R_d$ varies with light intensity, $CO_2$ and $O_2$ (Tcherkez *et al.*, 2017).

Despite these limitations, $CO_2$ response curves of net photosynthesis have been used worldwide for > 30 years to compute maximal carboxylation capacity or maximal electron fluxes and also estimate internal conductance and day respiration. As a result, the seminal paper describing mathematical relationships in gas exchange (Von Caemmerer & Farquhar, 1981) has been cited > 4400 times. Recent studies have further discussed how photosynthetic models and $CO_2$ response curves might be exploited

using Eqn 2 to infer information on photorespiratory nitrogen metabolism (Busch *et al.*, 2018) (as has been done before for phosphate utilization (Harley & Sharkey, 1991)), or suggested alternative mechanisms for $CO_2$ efflux (Cousins *et al.*, 2011; Bloom & Lancaster, 2018). More generally, as we get more precision in photosynthetic measurements (including online isotopic analyses), it is tempting to infer as many parameters as possible (e.g. internal conductance).

However, to interpret photosynthetic response curves appropriately, metabolic imperatives of nonphotosynthetic and nonphotorespiratory pathways (that is, metabolisms other than simply photosynthesis and photorespiration) which both depend on $CO_2$ (or $O_2$) mole fraction in the short-term and influence leaf net $CO_2$ exchange, have to be taken into account. In fact, even though their associated fluxes are generally small, there might be particular situations where they have a significant impact on the photosynthesis rate and/or parameters derived therefrom.

In this Viewpoint, we intend to examine such nonphotosynthetic and nonphotorespiratory metabolic processes and their dependence on $CO_2$ (or $O_2$), using recent results that have provided molecular or metabolic insights on short-term response to $CO_2$ and $O_2$, with associated experimental flux measurements in $C_3$ plants (summarized in Fig. 1). Here, we will not discuss photochemistry (and associated redox control) or day respiration themselves, which have been shown to be influenced by $CO_2 : O_2$ as explained elsewhere (Heber *et al.*, 1992; Laisk *et al.*, 1997; Tcherkez *et al.*, 2017). As will become apparent later, quantitative data on metabolic fluxes associated with nonphotorespiratory and nonphotosynthetic processes in illuminated leaves are relatively scarce and the present article should be viewed as a call for more research in this area.

## Alternative carboxylation activity

In $C_3$ plants, Rubisco is the $CO_2$-fixing enzyme of photosynthesis (as reflected in photosynthetic models, see earlier). However, there are other carbon-fixing enzymes, in different metabolic pathways: phospho*enol*pyruvate carboxylase (PEPC), carbamoyl phosphate synthase (CPS), acetyl-CoA carboxylase, etc. Amongst these, PEPC is certainly the most significant considering both its protein abundance and carbon flux (Chollet *et al.*, 1996). However, it has been recently suggested that the reverse reaction catalyzed by chloroplastic NADP-dependent malic enzyme ($CO_2$ + pyruvate + NADPH → malate + NADP) is of importance (Bloom & Lancaster, 2018). This is extremely unlikely, considering the very high $K_m$ (Michaelis constant) of the enzyme (in mM) compared to the very low concentration of stromal pyruvate (in μM) (Wheeler *et al.*, 2008; Krueger *et al.*, 2011), the unfavorable NADPH : NADP ratio in the light (≈ 1 in the steady-state) (Takahama *et al.*, 1981), and the low carbon-13 ($^{13}C$) enrichment in C-atom position of malate other than C-1 and C-4 during $^{13}CO_2$ labeling (Tcherkez *et al.*, 2009). In fact, if the chloroplastic malic enzyme synthesized malate, it would consume chloroplastic pyruvate. Therefore, malate molecules would inherit the $^{13}C$-pattern of chloroplastic pyruvate in C-1 to C-3 atom positions, while it would inherit the $^{13}C$ signature of dissolved $CO_2$ in C-4. Since chloroplastic pyruvate is highly $^{13}C$-labeled (Abadie *et al.*, 2018), there would be little difference in the positional $\%^{13}C$ between

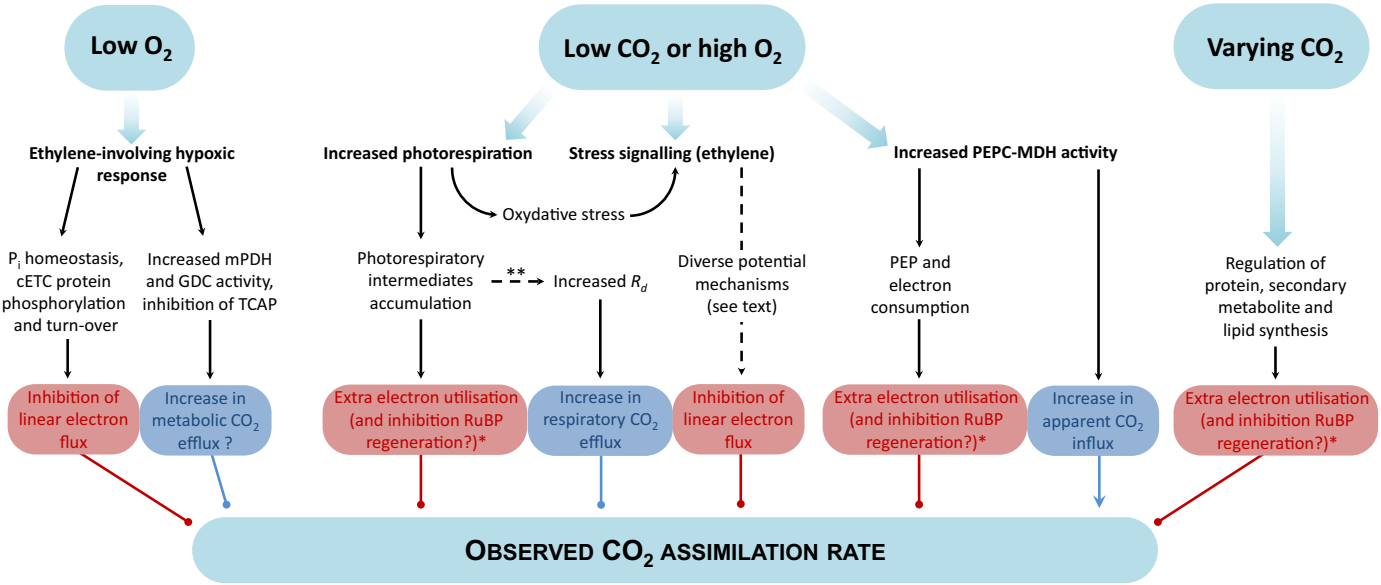


**Fig. 1** Summary of metabolic pathways affected by $CO_2 : O_2$ in the short-term and possibly affecting observed $CO_2$ assimilation rate. For simplicity, effects on photosynthesis are shown as falling into two categories: effect on chloroplastic electron transfer chain and/or ribulose 1,5-bisphosphate (RuBP) regeneration (red) and on $CO_2$ production or consumption (blue). See the text for more details on numerical flux values. The asterisk (*) stands for possible variation in the net effect due to possible decrease on redox pressure (e.g. under high light) and re-utilization of chloroplastic phosphate to facilitate RuBP regeneration (Harley & Sharkey, 1991). The double asterisks (**) represent the link between the nitrogen demand by photorespiration and the stimulation of day respiration, further discussed elsewhere (Tcherkez *et al.*, 2017). cETC, chloroplastic electron transport chain; GDC, glutamate decarboxylase; mPDH, mitochondrial pyruvate dehydrogenase; PEP, phospho*enol*pyruvate; Pi, free phosphate; $R_d$, day respiration; TCAP, tricarboxylic acid pathway.

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C-atom positions in malate synthesized *de novo*. Experimental evidence is to the contrary (Abadie & Tcherkez, 2019).

PEPC fixes bicarbonate onto phospho*enol*pyruvate and forms oxaloacetate in the cytosol, which can be then reduced to malate (by malate dehydrogenases, MDHs) in various cellular compartments. Isotopic labeling has demonstrated that this sequence of two reactions does occur in the illuminated leaf (Tcherkez *et al.*, 2009; Abadie *et al.*, 2017b). PEPC-derived oxaloacetate is also essential to sustain aspartate production, as demonstrated by both intramolecular natural $^{13}C$ abundance in leaf aspartate (Melzer & O'Leary, 1987) and isotopic labeling (Abadie *et al.*, 2017b). Nevertheless, it is generally assumed that PEPC activity is of little importance for steady-state $C_3$ photosynthesis (Von Caemmerer, 2013) and it is commonly not included in photosynthetic models while it is recognized to contribute to lowering the observed $^{12}C/^{13}C$ isotope fractionation during photosynthesis (Raven & Farquhar, 1990).

PEPC-MDH activity is extremely relevant for photosynthesis measurement since it leads to $CO_2$ fixation (via the rapid equilibrium between $CO_2$ and bicarbonate) thus potentially, can increase apparent photosynthesis rates. It also consumes two electrons per $CO_2$ to reduce oxaloacetate to malate, thereby impacting slightly the quantum yield of photosynthesis. In addition, PEPC-MDH activity is directly linked to (1) photorespiration via the utilization of reductive power generated by the GDC–SHMT complex and (2) TCAP replenishment to sustain glutamate and glutamine metabolism which is stimulated by photorespiration. In fact, mutants affected in MDH show an alteration in photorespiratory metabolism (Cousins *et al.*, 2008; Tomaz *et al.*, 2010) and mutants affected in glutamine-2-oxoglutarate amino transferase do exhibit an increase in PEPC activity that correlates to leaf glutamine content (Ferrario-Mery *et al.*, 2002). Quite critically, PEPC activity does vary with $CO_2$ and $O_2$ mole fraction. First, the enzyme appears to be slightly less phosphorylated at high $CO_2$ : $O_2$ ratio, suggesting a decline in activity. Second, phospho*enol*pyruvate provision by pyruvate Pi dikinase (PPDK) varies, due to a significant loss in PPDK phosphorylation and increase in activity at low $CO_2$ (Abadie *et al.*, 2016b). Third, direct *in vivo* PEPC-MDH activity measurements by NMR have shown that while being rather small at high photosynthesis (0.3 μmol $m^{-2}$ $s^{-1}$), PEPC activity increases to 1 μmol $m^{-2}$ $s^{-1}$ or more at low $CO_2$ (140 μmol $mol^{-1}$) or high oxygen (100% $O_2$), representing up to 40% of observed net assimilation under high $O_2$ mole fraction (Abadie & Tcherkez, 2019). Taken as a whole, anaplerotic $CO_2$ fixation is not a negligible actor in $CO_2$ response curves, and rather counterintuitively, it decreases as $CO_2$ increases.

## Electron and carbon utilization by anabolism

It is well-known that nitrogen (or sulfur) assimilation, amino acid and protein synthesis, lipid production and secondary metabolism represent significant carbon and electron sinks, thereby potentially inhibiting sucrose synthesis or RuBP regeneration and thus $CO_2$ assimilation. The extra electron consumption causes an 'offset' between total electron flux (for example measured by fluorescence or $O_2$ evolution), and electron consumption for $CO_2$ (and $O_2$) fixation only. Experimentally, it could be desirable to ensure that such an offset is 'real' by comparing fluorescence-derived electron transport rate (ETR) and maximal electron transport computed from photosynthetic response curves ($A/c_c$ curves). We note, however, that such a comparison can be complicated by (1) variations in the coefficient used to convert the yield of PSII photochemistry (ΦPSII) to ETR due to changes in, e.g. pseudo-cyclic electron flux, and (2) the fact that alternative fluxes are relatively small and possibly within the experimental error of ETR estimates. Regardless of these technical issues, several studies have taken advantage of the offset to estimate the electron flux to nitrate (nitrite) reduction using the assimilatory quotient (Rachmilevitch *et al.*, 2004; Bloom *et al.*, 2010; Eichelmann *et al.*, 2011). They have suggested that nitrogen assimilation is promoted under photorespiratory conditions (this conclusion being also supported by other measurements such as enzyme activities, and natural nitrogen-15 ($^{15}N$) abundance) (Rachmilevitch *et al.*, 2004; Bloom *et al.*, 2010). However, the other earlier-cited electron-consuming biosynthetic processes should not be overlooked. In fact, in Arabidopsis, cytosolic protein synthesis has been shown to depend on photosynthesis, with much higher polysome abundance at light (compared to dark) (Piques *et al.*, 2009) and a positive effect of $CO_2$ mole fraction on the phosphorylation of the ribosomal protein RPS6 and the translation initiation factor eIF4B, and a negative effect on the translation initiation inhibitor eIF4A (Boex-Fontvieille *et al.*, 2013). Accordingly, isotopic labeling of peach leaves with $^{14}CO_2$ along a $A/CO_2$ curve has shown that protein synthesis increases proportionally with photosynthesis (at about 1% of photosynthesis) as $CO_2$ mole fraction increases (Escobar-Gutiérrez & Gaudillère, 1997). These results clearly indicate that protein production is not constant and when expressed in absolute units (μmol $m^{-2}$ $s^{-1}$), increases with photosynthesis.

Chloroplastic fatty acid synthesis also strictly occurs in the light, and requires NADPH as well as acetyl-CoA formed from pyruvate by chloroplastic pyruvate dehydrogenase (pPDH) (Bao *et al.*, 2000). Contrary to its mitochondrial counterpart (mPDH), pPDH is not down-regulated by phosphorylation in the light, allowing efficient conversion of pyruvate to acetyl-CoA in the chloroplast (Tovar-Méndez *et al.*, 2003). It is generally believed that acetyl-CoA carboxylase, which represents the committed step of fatty acid synthesis, is activated by redox power to match NADPH availability in the chloroplast (Rawsthorne, 2002). That said, increasing $CO_2$ mole fraction has little effect on the flux to fatty acid synthesis (Williams & Harwood, 1997) and in fact, $^{14}C$-labelling has suggested that lipids represent about 1% of net fixed carbon regardless of $CO_2$ mole fraction (Escobar-Gutiérrez & Gaudillère, 1997). Fatty acid synthesis with $^{13}C$-labeling has also shown that the flux represents about 0.1 μmol carbon $m^{-2}$ $s^{-1}$ in Arabidopsis in a slightly $CO_2$-enriched atmosphere (≈ 450 μmol $mol^{-1}$ $CO_2$), i.e. ≈ 1% of photosynthesis (Bao *et al.*, 2000). However, the scarcity of quantitative measurements of lipid and protein synthesis should be noted, and the way the associated flux in electron, carbon or nitrogen equivalents varies with photosynthetic conditions is not so well-known. In addition, there is currently little quantitative knowledge on reserve remobilization

that may contribute to electron and ATP utilization but would remain invisible upon labeling.

Secondary compounds can also represent a small, but significant part of carbon utilization. At the leaf level, the synthesis of isoprenoids has been shown to be generally $< 0.1$ μmol $m^{-2}$ $s^{-1}$ and to depend on $CO_2$ and $O_2$ mole fraction (Rasulov *et al.*, 2009). In general, isoprene emission decreases at high $CO_2$ or low $O_2$ and this has been related to pools of intermediates and phosphate, reductant supply as well as phospho*enol*pyruvate utilization (Monson & Fall, 1989; Loreto & Schnitzler, 2010; Rasulov *et al.*, 2016). Interestingly, isoprene emission has also been shown to be modulated by nitrogen nutrition, with lower values at high nitrogen due to the competition for electrons and phospho*enol*pyruvate (Rosenstiel *et al.*, 2004). The carbon flux to phenylpropanoids has been suggested to be large because they can ultimately represent up to 30% of plant biomass (Maeda & Dudareva, 2012). But at the leaf level, chlorogenate synthesis has been measured precisely and found to be small (< 1% of photosynthesis) in sunflower and remarkably, has a bell-shaped pattern (i.e. decreased at low photosynthesis, and in 0.03% $O_2$, maximal under 'standard' $CO_2 : O_2$ conditions) (Abadie *et al.*, 2018). Such compounds are of interest because their synthesis starts in the chloroplast and requires NADPH, phospho*enol*pyruvate and erythrose-4-phosphate. The latter is directly abstracted from the Calvin cycle and therefore depends on RuBP regeneration metabolism.

## Photosynthesis and stress signaling

Changing the $CO_2 : O_2$ environment, in particular low $CO_2$ or high $O_2$, represents stressful conditions for photosynthesizing leaves. There is now substantial evidence that photorespiration is a source of reactive oxygen species (ROS, such as peroxisomal $H_2O_2$) that have to be detoxified (Noctor & Foyer, 1998; Foyer & Noctor, 2005) and trigger a rapid gene expression response (Eisenhut *et al.*, 2017). In addition, stress-related signaling events involving ethylene are believed to be of considerable importance.

Ethylene has long been known for its potential (and controversial) stimulation of stomatal closure, with considerable species-specific variations (Kays & Pallas, 1980; Pallas & Kays, 1982; Tanaka *et al.*, 2005; Iqbal *et al.*, 2011). Conversely, ethylene can antagonize abscisic acid (ABA)-induced stomatal closure under water deficit (Tanaka *et al.*, 2005), and in the long-term, might stimulate plant growth (Pierik *et al.*, 2006). Ethylene stimulates antioxidants (flavonols) synthesis in guard cells and thus counteracts ROS accumulation thereby down-regulating the ABA response in Arabidopsis (Watkins *et al.*, 2014) and tomato (Watkins *et al.*, 2017). In fact, this effect was not observed in Arabidopsis *ethylene-overproducing1* (*ein1*) and tomato *Neverripe* (*Nr*) mutants affected in ethylene signal transduction (Watkins *et al.*, 2014, 2017); accordingly, mutants affected in flavonol synthesis have a higher ROS content in guard cells and are more sensitive to ABA.

Unlike stomatal aperture, the photosynthetic machinery is negatively impacted by ethylene. Using photosynthetic $CO_2$ response curve after a brief pre-exposure to ethylene, a decline in maximal chloroplastic electron transport rate has been found (Taylor & Gunderson, 1986; Wullschleger *et al.*, 1992). Other studies have further shown a decrease in Rubisco activity itself (Tholen *et al.*, 2008) or carbon export from leaves (Woodrow *et al.*, 1988). Phosphoproteomics analyses have shown that when $CO_2 : O_2$ conditions vary, there are rapid changes in a number of signaling pathways (Abadie *et al.*, 2016b) and amongst them, ethylene response is the most represented. This includes ACC synthase and the central transducer EIN2, with a close correlation with the $CO_2 : O_2$ ratio, suggesting that both ethylene synthesis and perception are enhanced at low photosynthesis (Supporting Information Table S1). Although the specific effect of gaseous conditions on ethylene synthesis is less documented than in fruits (where it is well-known that $CO_2$ inhibits ethylene synthesis), available evidence indicates that in leaves, minimal carbon assimilation is required for carbon provision to ethylene synthesis (Grodzinski *et al.*, 1982; Guy & Kende, 1984) but increased $CO_2$ mole fraction (but not super-high $CO_2$) inhibits ethylene synthesis (Guo *et al.*, 2014). ROS-generating conditions (high photorespiration, low $CO_2 : O_2$ ratio) interact with ethylene signaling, as suggested by (1) the increased expression at low $CO_2$ of 2-cysteine peroxiredoxin A (2CP A) within a few hours and (2) the fact that 2CP B contains a strong ethylene response element in its promoter (Heiber *et al.*, 2007). In addition, in *Chlamydomonas* the retrograde ROS-signaling protein TER23 (ethylene-dependent EGY1 in Arabidopsis) participates in the fast phosphorylation response and thus on cyclic vs linear electron flux in the chloroplast under stressful conditions (Schönberg *et al.*, 2017). Accordingly, in nonsenescing leaves, ethylene has been shown to cause changes in antioxidant contents (Hodges & Forney, 2000) and a considerable increase in biosynthesis of phenolic compounds (Tomás-Barberán *et al.*, 1997; Liu *et al.*, 2016), thereby consuming sugar phosphates and reductive power in the chloroplast. Ethylene also perturbs polyamine synthesis and S-adenosylmethionine metabolism (Aziz *et al.*, 1997) and a beneficial effect on nitrogen and sulfur assimilation has been proposed (Iqbal *et al.*, 2012). Taken as a whole, ethylene-mediated stress signaling under unfavorable photosynthetic gaseous conditions (low $CO_2 : O_2$) has direct or indirect effects on photosynthetic electron transfer chain and this should in principle alter the quantum yield of photosynthesis in the short-term.

## Specific effects of low oxygen

It is common practice to use low $O_2$ mole fraction (typically 2% $O_2$ or less) in gas exchange experiments to eliminate oxygenation and thus infer the contribution of photorespiration to net $CO_2$ exchange. However, in addition to variable consequences on stomatal conductance, low $O_2$ is associated with strong metabolic effects that may change carboxylation activity.

First, low $O_2$ causes a well-known alteration of (sugar) phosphate homeostasis in the chloroplast (and thus ATP generation) that can lead to a decrease in photochemical quenching and photosynthesis rate (Sharkey & Vassey, 1989). In addition, hypoxia has been demonstrated to reconfigure the STN7-dependent phosphorylation of proteins of the chloroplastic electron transfer chain (Bergner *et al.*, 2015; Shapiguzov *et al.*, 2016), typically LHC proteins that

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are responsible for the photosynthetic state shift (state I/state II). This directly impacts on the balance between the cyclic electron flux around photosystem I and the linear electron flux in the short term, and thus the quantum yield of photosynthesis.

Second, despite the production of oxygen by photosynthesis, the low-$O_2$ atmosphere triggers a hypoxic response even in the short-term (here, 'short-term' means a time frame of 1 to 2 h, that is, the typical time required to carry out a photosynthetic response curve). From a metabolic perspective, hypoxia is associated with a reorganization of glycolysis, TCAP and nitrogen assimilation, with considerable accumulation of biomarkers metabolites such as the nitrogenous compounds alanine, β-alanine, glutamate or γ-aminobutyrate (Limami *et al.*, 2014). Such changes are associated with an increase in nitrogen demand (and thus nitrate assimilation using electrons of the chloroplastic electron chain) as shown by the decrease in phosphorylation of nitrate reductase under hypoxia (Lothier *et al.*, 2018). Hypoxia is also associated with nitric oxide production from nitrite produced by nitrate reductase (for a review, see Planchet *et al.*, 2017). Also, there is a potential change in $CO_2$ evolution: a substantial decrease in mPDH phosphorylation (Lothier *et al.*, 2018) and thus an increase in pyruvate decarboxylation activity, alanine synthesis (that abstracts pyruvate from catabolism) and γ-aminobutyrate synthesis by glutamate decarboxylation (Abadie *et al.*, 2017a). It has been suggested that under 0.03% $O_2$, the extra nitrogen demand represents about 0.2 μmol $m^{-2}$ $s^{-1}$, i.e. about 2 μmol electrons $m^{-2}$ $s^{-1}$ (Abadie *et al.*, 2018). However, the overall effect of hypoxia on day respiratory $CO_2$ efflux is not very well-known (for a specific discussion, see Tcherkez *et al.*, 2012).

Hypoxia may also lead to changes in the expression of genes encoding proteins that play key roles in photosynthesis (summarized in Table S2). A comparison of the translatome of various tissues of Arabidopsis photosynthetic tissues subjected to oxygen (and $CO_2$) deprivation showed that there is a rapid (within 2 h) change in the translation of mRNAs encoding photosystem II proteins CP22 (involved in nonphotochemical quenching) and OHP2 (involved in PSII assembly) as well as Rubisco chaperonins, suggesting an increase in turn-over of the photosynthetic machinery (Mustroph *et al.*, 2009). In the longer term, it is believed there is an increase in the expression of genes encoding chlorophyll binding proteins (LHCBs), as suggested by the comparison of transcriptome profiles under normoxia or hypoxia (for 2 h), in wild-type and mutants affected in the N-end-rule pathway and exhibiting a constitutive response to hypoxia (including the typical group VII ethylene response factors) (Gibbs *et al.*, 2011).

Taken as a whole, hypoxia directly affects photosynthesis via rapid changes in metabolism and synthesis of the photosynthetic machinery. Consequently, gas exchange experiments carried out at low oxygen are not simply representative of photorespiration-free conditions.

## The problem of photorespiration and nitrogen demand

Photorespiration does not only exist in a metabolic cycle whereby $O_2$ fixation (oxygenation) leads to glycine synthesis, subsequent $CO_2$ release and recycling of serine to 3-phosphoglycerate (PGA). In fact, photorespiration has been shown to involve alternative pathways (such as formate oxidation) (Peterhansel *et al.*, 2012; Obata *et al.*, 2016), and at high oxygenation rates, to be associated with glycine and serine accumulation (Abadie *et al.*, 2016a). Also, metabolic comparisons using gas chromatography-mass spectrometry (GC-MS) profiles (Carroll *et al.*, 2015) has shown that high photorespiration leads to a metabolic phenotype similar to some photorespiratory mutants (Abadie *et al.*, 2016a). It is worth noting that despite the amino acid build-up, photorespiration *per se* does not represent a nitrogen assimilation pathway since it strictly depends on the glutamine synthetase/glutamine-2-oxoglutarate amino transferase cycle (Wingler *et al.*, 2000). Photorespiratory recycling (or lack thereof) of glycerate, glycine and serine has been controversial for a long time (Harley & Sharkey, 1991; Dirks *et al.*, 2012; Tcherkez, 2013; Busch *et al.*, 2018). The abstraction of serine and glycine molecules from the photorespiratory cycle might be detrimental for photosynthesis because it leads to an extra-demand in electrons (for nirogen assimilation in glyoxylate amination to glycine) and PGA, potentially impeding RuBP regeneration. Using a modeling exercise that neglects (changes in) internal conductance ($g_m$), glycine and serine build-up has been suggested to be of considerable importance, with about 30% of serine not recycled to PGA, thus shaping the $CO_2$ response curve of photosynthesis (Busch *et al.*, 2018) due to serine accumulation rate of up to 0.9 μmol $m^{-2}$ $s^{-1}$ and nitrogen assimilation rates up to 1.2 μmol $m^{-2}$ $s^{-1}$. The nonquantitativity of glycine-to-serine conversion has been demonstrated experimentally (Abadie *et al.*, 2016a), but metabolite accumulation represents only a few percent of oxygenation rate. In effect, using both metabolite kinetics and isotopomer distribution, it has been shown that glycine and serine accumulation rates taken together never exceed 0.09 μmol $m^{-2}$ $s^{-1}$ regardless of the $CO_2$ : $O_2$ ratio in sunflower, and often represent < 0.05 μmol $m^{-2}$ $s^{-1}$ (Abadie *et al.*, 2016a, 2018). Therefore, glycine and serine nonrecycling increase the diversion of electrons to assimilate nitrogen, and maybe contribute to shaping $CO_2$ assimilation curves, but the impact is numerically very small.

Furthermore, leaf nitrogen assimilation (from nitrate) is unlikely to be as high as 1 μmol $m^{-2}$ $s^{-1}$ as suggested by modeling (Busch *et al.*, 2018). It should be recognized that measuring instantaneous leaf nitrogen assimilation is, perhaps surprisingly, more complicated than one would think. The assimilatory quotient method, which leads to high values, can be compromised by alternative electron utilization (e.g. sulfur assimilation, PEPC-MDH activity, anabolism), as outlined earlier. Elemental analysis of plant organic matter generally shows that nitrogen represents 2–5% of total organic matter (while carbon is about 40%), and therefore suggests that the nitrogen assimilation flux falls within $1/20^{th}$ and $1/8^{th}$ of net $CO_2$ assimilation. However, such an estimate is flawed simply because (1) roots can assimilate nitrogen in many species, (2) leaf-assimilated nitrogen is redistributed to other organs (and a small amount is lost as gaseous $NH_3$), and (3) a considerable amount of carbon is lost by respiration. Using isotopic labeling (e.g. using $^{15}N$) is also challenging because of the isotopic dilution by endogenous nitrates, the utilization of 'new' nitrogen atoms to synthesize less easily extractible compounds (like proteins) and nitrogen redistribution to other organs. However, available

evidence suggests that nitrogen assimilation is much lower than 1 μmol $m^{-2}$ $s^{-1}$, and more likely about 0.1 μmol $m^{-2}$ $s^{-1}$. In fact, with $^{13}C$ labeling, the flux to *de novo* glutamate synthesis has been found to be < 0.01 μmol glutamate $m^{-2}$ $s^{-1}$ regardless of $CO_2$ : $O_2$, meaning a total ≤ 0.1 μmol $m^{-2}$ $s^{-1}$, accounting for carbon remobilization for total glutamate synthesis (Abadie *et al.*, 2017b, 2018). Using $^{15}N$-labeling, total glutamate synthesis accounting for $^{15}N$ isotopic dilution has also been found to be about 0.1 μmol $m^{-2}$ $s^{-1}$ (Gauthier *et al.*, 2010). In these experiments, this value of 0.1 μmol $m^{-2}$ $s^{-1}$ was probably representative because short-term labeling was used so it is unlikely a very high proportion of the label was allocated to proteins or nucleotides and glutamate was the major product of nitrogen assimilation. Still, if we assume that proteins represent about 1% of net assimilated carbon, this would mean an extra nitrogen demand of about 0.07 μmol $m^{-2}$ $s^{-1}$ (assuming net photosynthesis at 20 μmol $m^{-2}$ $s^{-1}$). A recent estimation of fluxes in Arabidopsis leaves based on mass-balance (flux balance analysis, FBA) has also shown that in mature leaves, nitrogen assimilation from nitrate represents about 0.2% of gross photosynthesis (Shaw & Cheung, 2018), that is, about 0.04 μmol $m^{-2}$ $s^{-1}$. Taken as a whole, instantaneous leaf nitrogen assimilation is an important actor of photosynthetic leaf metabolism but absolute flux-values are important to keep in mind. Nitrogen assimilation is equal to, or < 0.2 μmol $m^{-2}$ $s^{-1}$ in most cases, meaning an electron consumption of about 2 μmol $m^{-2}$ $s^{-1}$. This value probably changes to higher values when particular circumstances increase the nitrogen demand considerably despite low photorespiration, such as hypoxia (see above). Also, it would be interesting to disentangle the metabolism associated with nitrogen assimilation in genetically modified plants where photorespiration has been bypassed and plant biomass increases (Kebeish *et al.*, 2007; South *et al.*, 2019).

## Under what conditions are these metabolic pathways of particular importance?

We have discussed the potential importance of metabolisms other than photosynthesis and photorespiration ('ancillary' metabolism such as PEPC fixation, nitrogen assimilation, etc.) when $CO_2$ and $O_2$ conditions vary and thus can have an impact on observed net $CO_2$ assimilation. It is presently difficult to provide precise guidelines to account for such 'ancillary' metabolisms in observed $CO_2$ exchange rate, simply because the numerical values of fluxes are not known with sufficient certainty. That said, considering their rather low flux values, the impact is numerically small under standard conditions (400 μmol $mol^{-1}$ $CO_2$, 21% $O_2$) or more generally, when photosynthesis (and electron utilization to $CO_2$ fixation itself) is sufficiently high (Table 1). In other words, their potential effect is more likely visible when photosynthesis is low, at low $CO_2$ or high $O_2$ because their relative impact on the $CO_2$ flux might be large. For example, when low $CO_2$ conditions are used to carry out Laisk curves so as to determine the compensation point in the absence of day respiration (Γ*) and day respiration ($R_d$), the change in assimilation caused by PEPC fixation (which itself varies with $CO_2$ mole fraction) could cause an error in estimated Γ* (Abadie & Tcherkez, 2019).

The alternative utilization of electrons in the chloroplast (for nitrogen and sulfur assimilation, malate synthesis, or secondary metabolism) is also relatively small, but can have subtle consequences on photosynthetic parameters. Typically, most calculations of internal conductance based on fluorescence assume that total electron flux ($J_t$) is partitioned between oxygenation ($J_o$) and carboxylation ($J_c$) and thus $J_t = J_o + J_c$. If the alternative consumption of electrons is not negligible, the relationship becomes $J_t - J_a = J_o + J_c$ where $J_a$ is the consumption of electrons to alternative pathways. For instance, based on the total computed in Table 1, when $J_t$ (ETR) is larger than 50 μmol $m^{-2}$ $s^{-1}$, potential errors in partitioning and conductance calculations must be small. By contrast, under ambient oxygen (21%), when net $CO_2$ assimilation is < 8 μmol $m^{-2}$ $s^{-1}$ and thus $J_t$ likely < 50 μmol $m^{-2}$ $s^{-1}$, potential errors are large. In particular, internal conductance calculations are quite sensitive to potential errors in electron flux, and thus can be affected by $J_a$. In fact, an error of 1 μmol $m^{-2}$ $s^{-1}$ in electron flux (that is, of about 1%) can lead to an offset of at least 0.01 mol $m^{-2}$ $s^{-1}$ in internal conductance (for a detailed sensitivity analysis, see Harley *et al.*, 1992). Unfortunately, as it is apparent in this Viewpoint, the way $J_a$ varies with $O_2$ and $CO_2$ is not known with precision. We also note that day respiration (not discussed here) can also impact on internal conductance (further discussed in Harley *et al.*, 1992 and Tcherkez *et al.*, 2017).

## Conclusions and perspectives

In these perspectives, we have emphasized the need to have a more integrative view of leaf metabolism, which goes beyond $CO_2$ and $O_2$ reduction cycles themselves. That is, even in the short-term, leaf metabolism reacts not only via the $CO_2$ : $O_2$ partitioning of Rubisco kinetics and activation, but also through other pathways that may have direct or indirect effects on observed $CO_2$ assimilation rate, summarized in Fig. 1. Unfortunately, quantitative data on these aspects are scarce and instantaneous metabolic flux analysis when $CO_2$ and $O_2$ conditions vary is insufficiently documented. It must be recognized that it is technically difficult and expensive to carry out detectable isotopic labeling in metabolites, or to apply omics techniques when gaseous conditions change rapidly and are coupled to precise photosynthesis (and transpiration) measurements. That said, recent studies (cited here) have provided experimental estimates of fluxes in some pathways and new metabolomics and fluxomics technologies now offer outstanding capabilities to use fluxomics and thus get more precision in flux determination, remobilization and nonquantitativity of photosynthetic and photorespiratory reactions.

Also, most available data were obtained in common $C_3$ plants (such as Arabidopsis), and more data are required in $C_3$ and $C_4$ crops as well as $C_3$ species that exhibit particular nitrogen metabolism (i.e. that can affect relationships between photosynthesis and photorespiration) such as legumes. A better knowledge of concurrent, nonphotosynthetic metabolisms affected by gaseous conditions would be instrumental to define important regulations and possibly, improve models of $CO_2$ capture by leaves in particular (1) when net assimilation is low and thus the impact of nonphotosynthetic metabolism may be proportionally large, and

**Table 1** Summary of nonphotorespiratory and nonphotosynthetic pathways discussed here and linked to net photosynthesis under varying $CO_2$ : $O_2$ conditions in illuminated leaves, in the short term.

| Process | Gas exchange conditions where it prevails | Key metabolic pathway | Consumes electrons or Calvin cycle intermediates? | Electron flux ($\mu mol\ m^{-2}\ s^{-1}$) | Produces or fixes $CO_2$? | $CO_2$ flux ($\mu mol\ m^{-2}\ s^{-1}$) |
|---|---|---|---|---|---|---|
| Anaplerotic fixation[a] | Increases at low $CO_2$ : $O_2$ ratio | PEPC-MDH activity | Yes (reduction of oxaloacetate to malate) | 0.6–2.2 | Fixes | +0.3 to +1.1 |
| Nitrogen assimilation | Increases at low $CO_2$ : $O_2$ | GS/GOGAT cycle (which is mobilized by recycling of photorespiratory $NH_3$) | Yes (ferredoxin-dependent GOGAT activity) | 0.1–1 | Produces, potentially (*de novo* 2-oxoglutarate synthesis) | −0.01 to −0.1 |
| Sulfur assimilation | Increases at high $O_2$? | Asp pathway (Met) and photorespiratory or nonphotorespiratory Ser synthesis (Cys) | Yes (sulfate reduction) | Unknown (probably ≈0.2) | In principle, produces (Asp and folate metabolism) | Unknown |
| Lipid synthesis[a] | Variations with $CO_2$ : $O_2$ poorly known | Mostly chloroplastic glycolysis (pyruvate synthesis) and cPDH for fatty acid synthesis | Yes (NADPH and triose phosphates utilization) | ≈0.4 | Produces (cPDH activity) | ≈−0.1 |
| Protein synthesis | Increases as photosynthesis increases? | Cytosolic (and chloroplastic) mRNA translation | Indirectly (ATP and nitrogen assimilates utilization) | 1? | No (but coupled to respiratory $CO_2$ production via ATP and nitrogen requirement) | |
| Secondary metabolism[a] | Complicated, poorly-known pattern | Various (mevalonate pathway, methylerythritol pathway, phenylpropanoids, etc.) | Yes (triose phosphates, erythrose-4-phosphate, etc.) | ≈0.1 | Sometimes (depends on synthesized metabolites) | ≈−0.1 |
| Hypoxic metabolism | Low $O_2$ | Fermentative or alternative electron-consuming processes | Maybe (increased nitrogen assimilation) | | Produces, potentially (GABA synthesis, pyruvate conversion to ethanol, . . .) | Unknown |
| Stress response | High $O_2$ or low $CO_2$ (high photorespiration) | Oxidative stress and photosynthetic downregulation mediated by ethylene | Yes (ROS detoxification) | ? | Possibly produces, via changes in polyamine and $C_1$ metabolisms | ? |
| Day respiration | Increases at high photorespiration | Glycolytic pyruvate production, TCAP, OPPP | No (unless recycles organic acids from the chloroplast) | | Produces | Usually within −0.2 and −1 |
| Total | | | | Up to 5 | | −1 to +0.7 |

The $CO_2$ flux is shown as negative when it is an efflux, and positive when it is an influx. Note the significant number of question marks (?), demonstrating the need in more quantitative analyses. See the text for further details on flux values. [a]Electron consumption and $CO_2$ exchange for anaplerosis, lipid synthesis and secondary metabolism only account for NADPH utilization and $CO_2$ fixation/generation from key precursors (pyruvate/phospho*eno*/pyruvate) and thus does not account for electrons needed to synthesize precursors themselves. The flux indicated for secondary metabolism here only accounts for isoprene + chlorogenate synthesis as an example. Abbreviations: cPDH, chloroplastic pyruvate dehydrogenase; GABA, γ-aminobutyrate; GOGAT, glutamine 2-oxoglutarate aminotransferase; GS, glutamine synthetase; OPPP, oxidative pentose phosphate pathway; PEPC-MDH, phospho*eno*/pyruvate carboxylase-malate dehydrogenase; ROS, reactive oxygen species; TCAP, tricarboxylic acid pathway.

(2) at low $O_2$ which causes hypoxic metabolism, and thus specific catabolic pathways and changes in nitrogen assimilation.

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## References

**Abadie C, Bathellier C, Tcherkez G. 2018.** Carbon allocation to major metabolites in illuminated leaves is not just proportional to photosynthesis when gaseous conditions ($CO_2$ and $O_2$) vary. *New Phytologist* **218**: 94–106.

**Abadie C, Blanchet S, Carroll A, Tcherkez G. 2017a.** Metabolomics analysis of postphotosynthetic effects of gaseous $O_2$ on primary metabolism in illuminated leaves. *Functional Plant Biology* **44**: 929–940.

**Abadie C, Boex-Fontvieille ER, Carroll AJ, Tcherkez G. 2016a.** *In vivo* stoichiometry of photorespiratory metabolism. *Nature Plants* **2**: 15220.

**Abadie C, Lothier J, Boex-Fontvieille E, Carroll A, Tcherkez G. 2017b.** Direct assessment of the metabolic origin of carbon atoms in glutamate from illuminated leaves using $^{13}$C-NMR. *New Phytologist* **216**: 1079–1089.

**Abadie C, Mainguet S, Davanture M, Hodges M, Zivy M, Tcherkez G. 2016b.** Concerted changes in the phosphoproteome and metabolome under different $CO_2$/$O_2$ gaseous conditions in *Arabidopsis* rosettes. *Plant and Cell Physiology* **57**: 1544–1556.

**Abadie C, Tcherkez G. 2019.** *In vivo* phospho*enol*pyruvate carboxylase activity is controlled by $CO_2$/$O_2$ and represents a major flux at low assimilation rates. *New Phytologist* **221**: 1843–1852.

**Aziz A, Martin-Tanguy J, Larher F. 1997.** Plasticity of polyamine metabolism associated with high osmotic stress in rape leaf discs and with ethylene treatment. *Plant Growth Regulation* **21**: 153–163.

**Bao X, Focke M, Pollard M, Ohlrogge J. 2000.** Understanding *in vivo* carbon precursor supply for fatty acid synthesis in leaf tissue. *The Plant Journal* **22**: 39–50.

**Bergner SV, Scholz M, Trompelt K, Barth J, Gäbelein P, Steinbeck J, Xue H, Clowez S, Fucile G, Goldschmidt-Clermont M *et al.* 2015.** STATE TRANSITION7-dependent phosphorylation is modulated by changing environmental conditions, and its absence triggers remodeling of photosynthetic protein complexes. *Plant Physiology* **168**: 615–634.

**Bloom AJ, Burger M, Asensio JSR, Cousins AB. 2010.** Carbon dioxide enrichment inhibits nitrate assimilation in wheat and *Arabidopsis*. *Science* **328**: 899–903.

**Bloom AJ, Lancaster KM. 2018.** Manganese binding to Rubisco could drive a photorespiratory pathway that increases the energy efficiency of photosynthesis. *Nature Plants* **4**: 414.

**Boex-Fontvieille E, Daventure M, Jossier M, Zivy M, Hodges M, Tcherkez G. 2013.** Photosynthetic control of *Arabidopsis* leaf cytoplasmic translation initiation by protein phosphorylation. *PLoS ONE* **8**: e70692.

**Busch FA, Sage RF, Farquhar GD. 2018.** Plants increase $CO_2$ uptake by assimilating nitrogen via the photorespiratory pathway. *Nature Plants* **4**: 46.

**Carroll AJ, Zhang P, Whitehead L, Kaines S, Tcherkez G, Badger MR. 2015.** PhenoMeter: a metabolome database search tool using statistical similarity matching of metabolic phenotypes for high-confidence detection of functional links. *Frontiers in Bioengineering and Biotechnology* **3**: 106.

**Chollet R, Vidal J, O'Leary MH. 1996.** Phospho*enol*pyruvate carboxylase: a ubiquitous, highly regulated enzyme in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**: 273–298.

**Cousins AB, Pracharoenwattana I, Zhou W, Smith SM, Badger MR. 2008.** Peroxisomal malate dehydrogenase is not essential for photorespiration in *Arabidopsis* but its absence causes an increase in the stoichiometry of photorespiratory $CO_2$ release. *Plant Physiology* **148**: 786–795.

**Cousins AB, Walker BJ, Pracharoenwattana I, Smith SM, Badger MR. 2011.** Peroxisomal hydroxypyruvate reductase is not essential for photorespiration in *Arabidopsis* but its absence causes an increase in the stoichiometry of photorespiratory $CO_2$ release. *Photosynthesis Research* **108**: 91–100.

**Dirks RC, Singh M, Potter GS, Sobotka LG, Schaefer J. 2012.** Carbon partitioning in soybean (*Glycine max*) leaves by combined $^{11}$C and $^{13}$C labeling. *New Phytologist* **196**: 1109–1121.

**Eichelmann H, Oja V, Peterson R, Laisk A. 2011.** The rate of nitrite reduction in leaves as indicated by $O_2$ and $CO_2$ exchange during photosynthesis. *Journal of Experimental Botany* **62**: 2205–2215.

**Eisenhut M, Bräutigam A, Timm S, Florian A, Tohge T, Fernie AR, Bauwe H, Weber APM. 2017.** Photorespiration is crucial for dynamic response of photosynthetic metabolism and stomatal movement to altered $CO_2$ availability. *Molecular Plant* **10**: 47–61.

**Escobar-Gutiérrez AJ, Gaudillère JP. 1997.** Carbon partitioning in source leaves of peach, a sorbitol-synthesizing species, is modified by photosynthetic rate. *Physiologia Plantarum* **100**: 353–360.

**Farquhar GD, von Caemmerer S, Berry JA. 1980.** A biochemical model of photosynthetic $CO_2$ assimilation in leaves of $C_3$ species. *Planta* **149**: 78–90.

**Ferrario-Mery S, Hodges M, Hirel B, Foyer CH. 2002.** Photorespiration-dependent increases in phospho*enol*pyruvate carboxylase, isocitrate dehydrogenase and glutamate dehydrogenase in transformed tobacco plants deficient in ferredoxin-dependent glutamine–α-ketoglutarate aminotransferase. *Planta* **214**: 877–886.

**Foyer CH, Noctor G. 2005.** Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell & Environment* **28**: 1056–1071.

**Gauthier PG, Bligny R, Gout E, Mahé A, Nogués S, Hodges M, Tcherkez Guillaume GB. 2010.** *In folio* isotopic tracing demonstrates that nitrogen assimilation into glutamate is mostly independent from current $CO_2$ assimilation in illuminated leaves of *Brassica napus*. *New Phytologist* **185**: 988–999.

**Gibbs DJ, Lee SC, Isa NM, Gramuglia S, Fukao T, Bassel GW, Correia CS, Corbineau F, Theodoulou FL, Bailey-Serres J. 2011.** Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. *Nature* **479**: 415–418.

**Grodzinski B, Boesel I, Horton RF. 1982.** Ethylene release from leaves of *Xanthium strumarium* L. and *Zea mays* L. *Journal of Experimental Botany* **33**: 344–354.

**Guo H, Sun Y, Li Y, Liu X, Zhang W, Ge F. 2014.** Elevated $CO_2$ decreases the response of the ethylene signaling pathway in *Medicago truncatula* and increases the abundance of the pea aphid. *New Phytologist* **201**: 279–291.

**Guy M, Kende H. 1984.** Ethylene formation in *Pisum sativum* and *Vicia faba* protoplasts. *Planta* **160**: 276–280.

**Hanson DT, Stutz SS, Boyer JS. 2016.** Why small fluxes matter: the case and approaches for improving measurements of photosynthesis and (photo) respiration. *Journal of Experimental Botany* **67**: 3027–3039.

**Harley PC, Loreto F, Di Marco G, Sharkey TD. 1992.** Theoretical considerations when estimating the mesophyll conductance to $CO_2$ flux by analysis of the response of photosynthesis to $CO_2$. *Plant Physiology* **98**: 1429–1436.

**Harley PC, Sharkey TD. 1991.** An improved model of $C_3$ photosynthesis at high $CO_2$: reversed $O_2$ sensitivity explained by lack of glycerate reentry into the chloroplast. *Photosynthesis Research* **27**: 169–178.

**Heber U, Neimanis S, Siebke K, Schönknecht G, Katona E. 1992.** Chloroplast energization and oxidation of P700/plastocyanin in illuminated leaves at reduced levels of $CO_2$ or oxygen. *Photosynthesis Research* **34**: 433–447.

**Heiber I, Ströher E, Raatz B, Busse I, Kahmann U, Bevan MW, Dietz K-J, Baier M. 2007.** The *redox imbalanced* mutants of *Arabidopsis* differentiate signaling pathways for redox regulation of chloroplast antioxidant enzymes. *Plant Physiology* **143**: 1774–1788.

**Hodges DM, Forney CF. 2000.** The effects of ethylene, depressed oxygen and elevated carbon dioxide on antioxidant profiles of senescing spinach leaves. *Journal of Experimental Botany* **51**: 645–655.

**Iqbal N, Khan NA, Nazar R, Silva JATd. 2012.** Ethylene-stimulated photosynthesis results from increased nitrogen and sulfur assimilation in mustard types that differ in photosynthetic capacity. *Environmental and Experimental Botany* **78**: 84–90.

**Iqbal N, Nazar R, Syeed S, Masood A, Khan NA. 2011.** Exogenously-sourced ethylene increases stomatal conductance, photosynthesis, and growth under optimal and deficient nitrogen fertilization in mustard. *Journal of Experimental Botany* **62**: 4955–4963.

**Kays SJ, Pallas JE. 1980.** Inhibition of photosynthesis by ethylene. *Nature* **285**: 51.

**Kebeish R, Niessen M, Thiruveedhi K, Bari R, Hirsch H-J, Rosenkranz R, Stäbler N, Schönfeld B, Kreuzaler F, Peterhänsel C. 2007.** Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in *Arabidopsis thaliana*. *Nature Biotechnology* **25**: 593–601.

**Keech O, Zhou W, Fenske R, Colas-des-Francs-Small C, Bussell JD, Badger MR, Smith SM. 2012.** The genetic dissection of a short-term response to low $CO_2$ supports the possibility for peroxide-mediated decarboxylation of photorespiratory intermediates in the peroxisome. *Molecular Plant* **5**: 1413–1416.

**Krueger S, Giavalisco P, Krall L, Steinhauser M-C, Büssis D, Usadel B, Flügge U-I, Fernie AR, Willmitzer L, Steinhauser D. 2011.** A topological map of the compartmentalized *Arabidopsis thaliana* leaf metabolome. *PLoS ONE* **6**: e17806.

**Laisk A, Oja V, Rasulov B, Eichelmann H, Sumberg A. 1997.** Quantum yields and rate constants of photochemical and nonphotochemical excitation quenching (experiment and model). *Plant Physiology* **115**: 803–815.

**Limami AM, Diab H, Lothier J. 2014.** Nitrogen metabolism in plants under low oxygen stress. *Planta* **239**: 531–541.

**Liu J, Liu Y, Wang Y, Zhang Z-H, Zu Y-G, Efferth T, Tang Z-H. 2016.** The combined effects of ethylene and MeJA on metabolic profiling of phenolic compounds in *Catharanthus roseus* revealed by metabolomics analysis. *Frontiers in Physiology* **7**: 217.

**Loreto F, Schnitzler J-P. 2010.** Abiotic stresses and induced BVOCs. *Trends in Plant Science* **15**: 154–166.

**Lothier J, De Paepe R, Tcherkez G. 2018.** Mitochondrial complex I dysfunction increases $CO_2$ efflux and reconfigures metabolic fluxes of day respiration in tobacco leaves. *New Phytologist* **221**: 750–763.

**Maeda H, Dudareva N. 2012.** The shikimate pathway and aromatic amino acid biosynthesis in plants. *Annual Review of Plant Biology* **63**: 73–105.

**Melzer E, O'Leary MH. 1987.** Anapleurotic $CO_2$ fixation by phosphoenolpyruvate carboxylase in $C_3$ plants. *Plant Physiology* **84**: 58–60.

**Monson RK, Fall R. 1989.** Isoprene emission from aspen leaves: influence of environment and relation to photosynthesis and photorespiration. *Plant Physiology* **90**: 267–274.

**Mustroph A, Zanetti ME, Jang CJ, Holtan HE, Repetti PP, Galbraith DW, Girke T, Bailey-Serres J. 2009.** Profiling translatomes of discrete cell populations resolves altered cellular priorities during hypoxia in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* **106**: 18843–18848.

**Noctor G, Foyer CH. 1998.** Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Biology* **49**: 249–279.

**Obata T, Florian A, Timm S, Bauwe H, Fernie AR. 2016.** On the metabolic interactions of (photo)respiration. *Journal of Experimental Botany* **67**: 3003–3014.

**Pallas JE, Kays SJ. 1982.** Inhibition of photosynthesis by ethylene: a stomatal effect. *Plant Physiology* **70**: 598–601.

**Peterhansel C, Blume C, Offermann S. 2012.** Photorespiratory bypasses: how can they work? *Journal of Experimental Botany* **64**: 709–715.

**Pierik R, Tholen D, Poorter H, Visser EJW, Voesenek LACJ. 2006.** The Janus face of ethylene: growth inhibition and stimulation. *Trends in Plant Science* **11**: 176–183.

**Piques M, Schulze WX, Höhne M, Usadel B, Gibon Y, Rohwer J, Stitt M. 2009.** Ribosome and transcript copy numbers, polysome occupancy and enzyme dynamics in *Arabidopsis*. *Molecular Systems Biology* **5**: 314.

**Planchet E, Lothier J, Limami AM. 2017.** Hypoxic respiratory metabolism in plants: reorchestration of nitrogen and carbon metabolisms. In: Tcherkez G, Ghashghaie J, eds. *Plant respiration: metabolic fluxes and carbon balance*. Berlin, Germany: Springer, 209–226.

**Rachmilevitch S, Cousins AB, Bloom AJ. 2004.** Nitrate assimilation in plant shoots depends on photorespiration. *Proceedings of the National Academy of Sciences, USA* **101**: 11506–11510.

**Rasulov B, Hüve K, Välbe M, Laisk A, Niinemets Ü. 2009.** Evidence that light, carbon dioxide, and oxygen dependencies of leaf isoprene emission are driven by energy status in hybrid aspen. *Plant Physiology* **151**: 448–455.

**Rasulov B, Talts E, Niinemets Ü. 2016.** Spectacular oscillations in plant isoprene emission under transient conditions explain the enigmatic $CO_2$ response. *Plant Physiology* **172**: 2275–2285.

**Raven JA, Farquhar GD. 1990.** The influence of N metabolism and organic acid synthesis on the natural abundance of isotopes of carbon in plants. *New Phytologist* **116**: 505–529.

**Rawsthorne S. 2002.** Carbon flux and fatty acid synthesis in plants. *Progress in Lipid Research* **41**: 182–196.

**Rosenstiel TN, Ebbets AL, Khatri WC, Fall R, Monson RK. 2004.** Induction of poplar leaf nitrate reductase: a test of extrachloroplastic control of isoprene emission rate. *Plant Biology* **6**: 12–21.

**Schönberg A, Rödiger A, Mehwald W, Galonska J, Christ G, Helm S, Thieme D, Majovsky P, Hoehenwarter W, Baginsky S. 2017.** Identification of STN7/STN8 kinase targets reveals connections between electron transport, metabolism and gene expression. *The Plant Journal* **90**: 1176–1186.

**Shapiguzov A, Chai X, Fucile G, Longoni P, Zhang L, Rochaix J-D. 2016.** Activation of the Stt7/STN7 kinase through dynamic interactions with the cytochrome $b_6f$ complex. *Plant Physiology* **171**: 82–92.

**Sharkey TD, Vassey TL. 1989.** Low oxygen inhibition of photosynthesis is caused by inhibition of starch synthesis. *Plant Physiology* **90**: 385–390.

**Shaw R, Cheung CYM. 2018.** A dynamic multi-tissue flux balance model captures carbon and nitrogen metabolism and optimal resource partitioning during *Arabidopsis* growth. *Frontiers in Plant Science* **9**: 884–884.

**South PF, Cavanagh AP, Liu HW, Ort DR. 2019.** Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. *Science* **363**: eaat9077.

**Takahama U, Shimizu-Takahama M, Heber U. 1981.** The redox state of the NADP system in illuminated chloroplasts. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **637**: 530–539.

**Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S. 2005.** Ethylene inhibits abscisic acid-induced stomatal closure in *Arabidopsis*. *Plant Physiology* **138**: 2337–2343.

**Taylor GE, Gunderson CA. 1986.** The response of foliar gas exchange to exogenously applied ethylene. *Plant Physiology* **82**: 653–657.

**Tcherkez G. 2013.** Is the recovery of (photo) respiratory $CO_2$ and intermediates minimal? *New Phytologist* **198**: 334–338.

**Tcherkez G, Gauthier P, Buckley TN, Busch FA, Barbour MM, Bruhn D, Heskel MA, Gong XY, Crous KY, Griffin K. 2017.** Leaf day respiration: low $CO_2$ flux but high significance for metabolism and carbon balance. *New Phytologist* **216**: 986–1001.

**Tcherkez G, Mahé A, Gauthier P, Mauve C, Gout E, Bligny R, Cornic G, Hodges M. 2009.** *In folio* respiratory fluxomics revealed by $^{13}C$ isotopic labeling and H/D isotope effects highlight the noncyclic nature of the tricarboxylic acid "cycle" in illuminated leaves. *Plant Physiology* **151**: 620–630.

**Tcherkez G, Mahé A, Guérard F, Boex-Fontvieille E, Gout E, Lamothe M, Barbour MM, Bligny R. 2012.** Short-term effects of $CO_2$ and $O_2$ on citrate metabolism in illuminated leaves. *Plant, Cell & Environment* **35**: 2208–2220.

**Tholen D, Pons TL, Voesenek LACJ, Poorter H. 2008.** The role of ethylene perception in the control of photosynthesis. *Plant Signaling & Behavior* **3**: 108–109.

**Tomás-Barberán FA, Loaiza-Velarde J, Bonfanti A, Saltveit ME. 1997.** Early wound- and ethylene-induced changes in phenylpropanoid metabolism in harvested lettuce. *Journal of the American Society for Horticultural Science* **122**: 399–404.

**Tomaz T, Bagard M, Pracharoenwattana I, Lindén P, Lee CP, Carroll AJ, Ströher E, Smith SM, Gardeström P, Millar AH. 2010.** Mitochondrial malate

dehydrogenase lowers leaf respiration and supports photorespiratory carbon flux and plant growth in Arabidopsis. *Plant Physiology* **154**: 1143–1157.

**Tovar-Méndez A, Miernyk JA, Randall DD. 2003.** Regulation of pyruvate dehydrogenase complex activity in plant cells. *European Journal of Biochemistry* **270**: 1043–1049.

**Von Caemmerer S. 2013.** Steady-state models of photosynthesis. *Plant, Cell & Environment* **36**: 1617–1630.

**Von Caemmerer S, Farquhar GD. 1981.** Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**: 376–387.

**Watkins J, Chapman JM, Muday GK. 2017.** Abscisic acid-induced reactive oxygen species are modulated by flavonols to control stomata aperture. *Plant Physiology* **175**: 1807–1825.

**Watkins JM, Hechler PJ, Muday GK. 2014.** Ethylene-induced flavonol accumulation in guard cells suppresses reactive oxygen species and moderates stomatal aperture. *Plant Physiology* **164**: 1707–1717.

**Wheeler MCG, Arias CL, Tronconi MA, Maurino VG, Andreo CS, Drincovich MF. 2008.** *Arabidopsis thaliana* NADP-malic enzyme isoforms: high degree of identity but clearly distinct properties. *Plant Molecular Biology* **67**: 231–242.

**Williams M, Harwood JL. 1997.** Effects of carbon dioxide concentration and temperature on lipid synthesis by young wheat leaves. *Phytochemistry* **45**: 243–250.

**Wingler A, Lea PJ, Quick WP, Leegood RC. 2000.** Photorespiration: metabolic pathways and their role in stress protection. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **355**: 1517–1529.

**Woodrow L, Thompson RG, Grodzinski B. 1988.** Effects of ethylene on photosynthesis and partitioning in tomato, *Lycopersicon esculentum* Mill. *Journal of Experimental Botany* **39**: 667–684.

**Wullschleger SD, Hanson PJ, Gunderson CA. 1992.** Assessing the influence of exogenous ethylene on electron transport and fluorescence quenching in leaves of *Glycine max*. *Environmental and Experimental Botany* **32**: 449–455.

## Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Table S1** Best phosphopeptides involved in signaling in Arabidopsis rosettes that correlate with gaseous conditions.

**Table S2** Hypoxia-responding genes related to photosynthesis in wild-type Arabidopsis.

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