Dynamic Response of Photorespiration in Fluctuating Light Environments

Authors: Xinyu Fu[1], Berkley J. Walker[1,2*]

[1] Department of Energy-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA

[2] Department of Plant Biology, Michigan State University, East Lansing, MI 48824, USA

[*]Correspondence to: Berkley Walker, berkley@msu.edu

Highlight

Photorespiration is as dynamic as photosynthesis in fluctuating light, impacting photosynthetic induction, energy balancing and other interacting metabolic processes.

Abstract

Photorespiration is a dynamic process that is intimately linked to photosynthetic carbon assimilation. There is a growing interest in understanding carbon assimilation during dynamic conditions, but the role of photorespiration under these dynamic conditions is unclear. In this review, we discuss recent work relevant to the function of photorespiration under dynamic conditions, with a special focus on light transients. This work reveals that photorespiration is a fundamental component of the light induction of assimilation where variable diffusive processes limit CO_2 exchange with the atmosphere. Additionally, metabolic interactions between photorespiration and the C3 cycle may help balance fluxes under dynamic light conditions. We further discuss how the energy demands of photorespiration present special challenges to energy balancing during dynamic conditions. We finish the review with an overview of why regulation of photorespiration may be important under dynamic conditions to maintain appropriate fluxes through metabolic pathways related to photorespiration like nitrogen and one-carbon metabolism.

Introduction

Photorespiration is an essential component of plant central metabolism responsible for recycling byproducts of photosynthesis-related oxygen uptake (Husic *et al.*, 1987). Photorespiration is intrinsically coupled with photosynthesis due to the dual specificity of ribulose 1,5-bisphosphate (RuBP) carboxylase (rubisco) for CO_2 and oxygen. While the carboxylation of RuBP incorporates CO_2 to yield two 3-phosphoglycerate (3-PGA) that enter the C3 cycle, the oxygenation of RuBP leads to one 3-PGA and one inhibitory molecule 2-phosphoglycolate (2-PG) (Bowes *et al.*, 1971). The core photorespiratory pathway recycles 75% of the carbon comprising 2-PG through a series of reactions to 3-PGA back into the C3 cycle for RuBP regeneration and requires the coordination of several subcellular compartments (Berry *et al.*, 1978) (Box 1). This recycling process releases CO_2 and consumes ATP and NADPH, thus

reducing photosynthetic efficiency and affecting the energy balance of the plant (Walker *et al.*, 2016). The reduction of net carbon fixation by photorespiratory carbon loss is not constant and is strongly dependent on leaf physiology related to CO_2 diffusion (Valentini *et al.*, 1995). Additionally, many governing factors for photosynthesis also affect photorespiration, such as stomatal and mesophyll conductance and activation of rubisco, making photosynthesis and photorespiration dynamic under fluctuating environments (Sakoda *et al.*, 2021; Taylor *et al.*, 2022).

Despite the dynamic nature of photorespiration, most of our knowledge regarding photorespiratory metabolism comes from steady-state studies that probe the biochemistry of photorespiratory metabolism by exposing plants to various environmental changes (Timm *et al.*, 2012; Busch, 2020; Dellero *et al.*, 2021), applying stable-isotope tracers (Cegelski and Schaefer, 2006; Abadie *et al.*, 2016, 2018; Tcherkez *et al.*, 2017), and utilizing mutants with disrupted components in the photorespiratory pathway (Wingler *et al.*, 1999; Eisenhut *et al.*, 2017; Timm *et al.*, 2021). However, the current understanding of steady-state photorespiration is insufficient for evaluating its role under dynamic natural field conditions. Natural conditions are incredibly dynamic; for example, on a typical cloudless day, a wheat canopy can receive over 2,600 sunflecks that comprise 83% of total irradiance, indicating that for many instances dynamic conditions predominate over steady-state (Kaiser *et al.*, 2018). While the mechanisms of dynamic photosynthetic response to fluctuating light environments have been explored (Vialet-Chabrand *et al.*, 2017; Slattery *et al.*, 2018; Morales and Kaiser, 2020; Gjindali *et al.*, 2021), little is known about the effect of dynamic fluctuations in environments on photorespiratory metabolism and its impact on net assimilation (Huang *et al.*, 2015; Eisenhut *et al.*, 2017).

Many excellent reviews cover various aspects of photorespiration (Timm *et al.*, 2012; Obata *et al.*, 2016; Walker *et al.*, 2016; Hodges *et al.*, 2016; Busch, 2020; Fernie and Bauwe, 2020; Shi and Bloom, 2021). In this review, we argue that including the dynamic role of photorespiration is essential for fully understanding (and possibly improving) the dynamic response of net assimilation to light. In the next sections, we will summarize some recent key findings in the photosynthetic response to light and relate them to photorespiratory mechanisms. We will not be comprehensive to the response of net assimilation to light generally but instead focus on what is related most to photorespiration.

Photosynthesis is intimately linked to photorespiration

The intimate link between photorespiration and photosynthesis (net CO_2 assimilation) has been long recognized since the discovery that rubisco has both carboxylase and oxygenase activity (Bowes *et al.*, 1971). These activities are integral to the classic C3 photosynthetic model for the rate of net CO_2 assimilation, which uses the mass balance between the rate of rubisco carboxylation (V_c) from the rate of CO_2 releasing reactions stemming from rubisco oxygenation (V_o) and respiration in the light (R_l) (Farquhar *et al.*, 1980; von Caemmerer, 2013). Rates of photorespiratory CO_2 loss are represented as half of V_o because it is usually assumed that recycling 1 mol of 2-phosphoglycolate through the photorespiratory cycle results in the release of 0.5 mol of CO_2 in the mitochondria (von Caemmerer, 2013; Abadie *et al.*, 2016) (Box 1). The energy requirements and CO_2 loss from photorespiration are not trivial. There are ~2 rubisco oxygenation reactions for every 5 carboxylation reactions under ambient conditions, which

require 30-40% of leaf energy in the light and release CO_2 at ~25% the rate of net assimilation (Walker *et al.*, 2016). These values highlight that net CO_2 assimilation in C3 plants cannot be separated from rates of photorespiration. The rates of rubisco oxygenation and carboxylation under constant light could be significantly different than under the fluctuating light conditions (Huang *et al.*, 2015).

Net photosynthesis is directly affected by the absolute values and relative ratio of oxygenation to carboxylation rate (V_o/V_c) determined by the kinetic constants of rubisco and the chloroplastic concentrations of CO_2 and O_2 (von Caemmerer, 2013). The chloroplastic concentration of CO_2 depends on the conductance of CO_2 diffusion from ambient through intercellular airspace to the chloroplasts (Flexas *et al.*, 2008), making chloroplastic CO_2 a dynamic relationship with the environment. Experimentally manipulating the CO_2 and O_2 concentrations around the leaf can drastically change net CO_2 assimilation by influencing the V_o/V_c ratio (Abadie *et al.*, 2018). When O_2 concentrations are constant under field conditions, changes in chloroplastic CO_2 affect the V_o/V_c ratio, leading to changes in net CO_2 assimilation. Under normal atmospheric conditions, the V_o/V_c ratio is around 0.5 in C3 species but can be highly variable under transient changes in leaf physiology (Bellasio *et al.*, 2014). For example, transient changes in stomatal conductance result in decreased leaf internal CO_2 concentrations, imposing a variable V_o/V_c ratio that affects photosynthetic performance.

Photosynthetic and photorespiratory adjustments under fluctuating light

Light is the most dynamic environmental condition that directly affects photosynthesis and photorespiration. Light fluctuations occur in timescales from milliseconds to seasons due to changes in sunflecks, self-shading, cloud-shading, and diurnal and seasonal light intensity (Pearcy, 1990; Assmann and Wang, 2001; Slattery *et al.*, 2018; Morales and Kaiser, 2020). Leaves therefore must respond to short- and long-term light fluctuations. Understanding photosynthetic performance under dynamic light environments is gaining more attention, as exemplified by recent reviews (Vialet-Chabrand *et al.*, 2017; Slattery *et al.*, 2018; Morales and Kaiser, 2020; Gjindali *et al.*, 2021). Given that photorespiratory release of CO_2 is a major determinate of net assimilation, it is interesting that relatively limited studies have focused on how photorespiration specifically responds and interacts with photosynthesis under fluctuating light (Huang *et al.*, 2015; Shi *et al.*, 2022).

Photorespiration is activated over season-long time scales when plants acclimate to long-term increases in light. For example, plants grown under high light have a higher rate of rubisco oxygenation than leaves exposed to low light (Huang *et al.*, 2014). Since rates of rubisco oxygenation are related to total rubisco catalytic turnover, these increased rates are mostly due to the higher rubisco content and lower intercellular CO_2 concentration in the leaves acclimated to high light (Huang *et al.*, 2014). Long-term acclimation to fluctuating light involves changes in gene expression in timescales of days (Athanasiou *et al.*, 2010; Karim and Johnson, 2021; Gjindali *et al.*, 2021). For example, the expression of the photorespiratory gene *HPR1* is induced by high light intensity (Wang *et al.*, 2022). The long-term growth effects are also seen in the absolute rates of V_c and V_o which are higher in plants grown under high light than in low light, with an increased ratio V_o/V_c under high light conditions (Ma *et al.*, 2014; Walker *et al.*, 2020). While the content of rubisco and general photosynthetic capacity explains much of the response of photorespiration to growth under various light regimes, other factors constrain the response of

net photosynthesis and photorespiration to rapid fluctuations in light intensity, which occur at timescales too fast to be driven by changes in gene expression.

When leaves transition under short-term time scales from shade to full sunlight, net CO_2 assimilation does not immediately reach a maximum light-saturated value but instead gradually increases to a new steady-state level, a phenomenon known as "photosynthetic induction" (Pearcy, 1990). The delay in reaching the maximum A after a sudden increase in light intensity decreases daily photosynthetic carbon gain, which can further reduce crop productivity (Tanaka *et al.*, 2019). The speed of photosynthetic induction varies between and among species (Tanaka *et al.*, 2019). For example, the slow photosynthetic adjustment from shade to sun accounted for a 13% loss of carbon assimilation in cassava (De Souza *et al.*, 2020) and a 21% loss of net canopy CO_2 assimilation and productivity in wheat (Taylor and Long, 2017). These major losses of potential productivity beg the question: What are the physiological and biochemical mechanisms underlying the response of net assimilation to light, and does photorespiration play a larger role in this response than we realize?

Stomatal and mesophyll conductance effects rates of rubisco oxygenation and carboxylation

The diffusion of CO_2 from the atmosphere to the catalytic site of rubisco in the chloroplast is a major factor constraining the induction of assimilation in dynamic light environments. CO_2 diffusion is directly affected by the speed at which stomata open and close under changing environments (Vialet-Chabrand *et al.*, 2017). Stomatal responses under fluctuating light are often an order of magnitude slower than photosynthetic responses, like the light activation of C3 cycle enzymes, resulting in a temporal disconnect between the stomatal conductance and the biochemical CO_2 assimilation (Lawson *et al.*, 2010). There are substantial interspecific and intraspecific variations in the speed of stomatal responses to changes in light intensity, ranging from tens of minutes to over an hour (McAusland *et al.*, 2016; Faralli *et al.*, 2019). The limitation imposed on photosynthesis by CO_2 supply during the light induction period by stomatal conductance (g_s) is 10-15% across thirteen C3 and C4 crop species (McAusland *et al.*, 2016). When this limitation is relieved, plants perform higher rates of assimilation. For example, a rice cultivar with a faster photosynthetic induction response to light has a faster response of g_s and recovers faster from the drop of intracellular CO_2 concentration after the sudden increase in light intensity (Adachi *et al.*, 2019). Removal of the stomatal induction generally can lead to a faster photosynthetic induction in rice and Arabidopsis mutants with constitutively opened stomata, but this comes at the cost of high water loss (Kimura *et al.*, 2020; Yamori *et al.*, 2020).

The slow stomatal kinetics under fluctuating light also affects photorespiration in non-steady-state conditions due to the fundamental link between photorespiration and CO_2 availability. During a light induction, both absolute and relative rates of V_o increase. Absolute rates increase as total rubisco catalysis increases, resulting in higher rates of V_c and V_o. Relative rates of photorespiration also increase when CO_2 is limited by stomatal conductance, driven by the increase in the ratio of O_2/CO_2. The increase in O_2/CO_2 occurs when higher rates of CO_2 assimilation under high light increase the CO_2 drawdown in the intercellular airspace due to CO_2 fixation. Increases in absolute and relative rates of V_o are illustrated using data from McAusland et al. (2016), where we calculated V_o, V_c, and V_o/V_c in two species with distinct stomatal kinetics during the light induction (Fig. 1). In both species, there is an almost 10-fold increase in V_o during the light induction, with a slightly smaller increase in V_c. Concurrently, driven by a

decrease in the intercellular CO_2 concentration and an increase in the ratio of O_2/CO_2, there is an increase in relative rates of photorespiration as shown by increases in V_o/V_c.

Different stomatal kinetics between species further affect absolute and relative rates of photorespiration. For example, rice (*Oryza sativa*) has a faster response of stomatal conductance and net CO_2 assimilation than broad bean (*Vicia faba*) (Fig. 1). This faster response results in V_o and V_o/V_c rapidly reaching a plateau during the induction period. This plateau is maintained as stomatal conductance reaches its maximal value, and a new steady-state CO_2 concentration is present in the intercellular airspace. In contrast to rice, broad bean has a slow stomatal response (Fig. 1). This slow response results in initially higher V_o and V_o/V_c values, which decrease as stomata open and increase CO_2 delivery to rubisco. Both of these cases highlight how stomatal conductance directly shapes the absolute and relative response of V_o during a light induction.

Mesophyll conductance (g_m) describes the ease at which CO_2 diffuses from the intercellular airspace to the carboxylation site in the chloroplasts and is another factor constraining photosynthetic induction during fluctuating light (Slattery *et al.*, 2018). The limitation of g_m on photosynthesis under steady-state conditions is well known, but the extent of this limitation under fluctuating light is still under ongoing investigation due to the difficulty of accurately measuring the dynamics of g_m under non-steady-state conditions (Kaiser *et al.*, 2015). The response of g_m in light varies between plant species and the technique used to estimate g_m (Xiong *et al.*, 2018; Carriquí *et al.*, 2019; Sakoda *et al.*, 2021; Liu *et al.*, 2022). The response of g_m is slower than g_s but not faster than the maximum rate of RuBP carboxylation during light induction (Sakoda *et al.*, 2021; Liu *et al.*, 2022).

Like stomatal conductance, g_m also limits net photosynthesis during light induction and shapes the partitioning between V_o and V_c. For example, g_m limits net CO_2 assimilation by over 20% in Arabidopsis and tobacco when integrated over the entire light induction period (Sakoda *et al.*, 2021; Liu *et al.*, 2022). These studies showed g_m plays a significant role in restricting dynamic photosynthesis during the transient period of light induction. When CO_2 is limited by g_m during the light induction, V_o also increases relatively faster than V_c, increasing the V_o/V_c ratio (Fig. 2). These re-analyses of published data highlight that gas diffusional processes like stomatal conductance and g_m shape photorespiration as much as photosynthesis under fluctuating light.

Activation of rubisco constrains V_o as well as V_c

Once CO_2 enters the chloroplast, carbon fixation can be limited by the activation of a series of C3 cycle enzymes. The initial enzyme of C3 cycle, rubisco, must be fully activated by rubisco activase (Rca) to effectively overcome inhibition by catalytic misfire products and maintain maximum capacity to catalyze the carboxylation reaction (Portis *et al.*, 2008). During the low to high light transition, rubisco is quickly activated with a linear increase within the first 4 minutes, but the full activation requires more than 20 minutes to reach the steady state (Yamori *et al.*, 2012). Thus Rca plays an important role in regulating dynamic photosynthesis under fluctuating light conditions, evidenced by a positive correlation between Rca concentration and the speed of photosynthetic induction upon transition from low to high light (Hammond *et al.*, 1998; Yamori *et al.*, 2012).

Since rubisco catalyzes both carboxylation and oxygenation, the induction kinetics of rubisco activation also affect photorespiration under fluctuating light conditions. Interestingly, the photorespiratory intermediate glyoxylate has been shown to inhibit the activation state of rubisco in isolated chloroplasts (Chastain and Ogren, 1989; Campbell and Ogren, 1990). In addition, glycolate can inhibit rubisco activity. Increased glycolate levels in maize leaves induced by photorespiratory inhibitors accompanied decreased photosynthesis (Gonzalez-Moro *et al.*, 1997). The photosynthetic induction response of dark-adapted leaves is much slower in a maize mutant with disrupted glycolate oxidase activity than in the wild-type plant that can maintain low steady-state levels of glycolate (Zelitch *et al.*, 2009). Indeed, many other photorespiratory intermediates are involved in the crosstalk with the C3 cycle metabolism (Timm and Hagemann, 2020). The metabolic interactions between photorespiration and the C3 cycle may play a role in balancing the fluxes through photorespiration and carbon fixation under dynamic environments. For example, when rates of photorespiration become too high, the carbon released may negate the benefits of continued rubisco catalysis. Under these conditions, it may be beneficial to decrease total rates of rubisco activity until more favorable conditions are present.

Metabolic interactions between photorespiration and the C3 cycle under fluctuating light

The dynamics of photorespiratory and photosynthetic fluxes under fluctuating light also depend on enzyme activities and metabolite concentrations downstream from rubisco. In addition to the light activation of rubisco, the activity of several key enzymes in the C3 cycle is regulated by the thioredoxin-ferredoxin system in a light-dependent manner (Buchanan, 1980). Activating these enzymes during light induction is more rapid than for rubisco, taking up to 10 minutes (Sassenrath-Cole and Pearcy, 1994). The slow activation response of these enzymes contributes to the delayed photosynthetic activation under fluctuating light. Many of the C3 cycle enzymes are regulated by photorespiratory intermediates. For example, 2-PG inhibits several enzymes in the C3 cycle and starch metabolism, including triosephosphate isomerase (TPI), sedoheptulose-1,7-bisphosphate phosphatase (SBPase), and phosphofructokinase (PFK) (Anderson, 1971; Kelly and Latzko, 1976; Flügel *et al.*, 2017). These interactions present a regulatory feedback loop via 2-PG levels that may adjust carbon fluxes through the C3 cycle and balance carbon partitioning between RuBP regeneration and starch biosynthesis (Flügel *et al.*, 2017). This may be important in maintaining adequate C3 pool sizes when carbon is being lost from the cycle from photorespiration.

Besides interacting with the C3 cycle enzymes through regulatory interactions, photorespiratory metabolites can directly constrain the photosynthetic response under fluctuating light. Pool sizes of photorespiratory intermediates generally increase when switching plants from low to high light condition (Florez-Sarasa *et al.*, 2012; Adachi *et al.*, 2019; Bao *et al.*, 2021). Specifically, glycine and serine respond quickly to changes in light intensity in rice, with glycine peaking at 10 min and serine peaking at 30 min (Adachi *et al.*, 2019). Among all photorespiratory intermediates, glycine showed the strongest accumulation upon various photorespiratory pressures (Hitz and Stewart, 1980; Timm *et al.*, 2012; Abadie *et al.*, 2016, 2018; Eisenhut *et al.*, 2017). The increased glycine pools during the transient to high light hold carbon that is not passed through glycine decarboxylation and therefore contributes to greater gain of carbon during light induction since this carbon stays in reduced bonds rather than being released through

glycine decarboxylation. This increase in assimilation is temporary and ceases when glycine pool sizes reach their new steady-state concentrations.

Increased photorespiratory metabolite pools during light induction may additionally function as carbon reservoirs needed for photosynthetic induction. Compared to the rapid turnover (< 1s) and small pool size of C3 cycle intermediates, photorespiratory intermediates have a much larger pool size with turnover times of 10-15 minutes (Szecowka *et al.*, 2013; Ma *et al.*, 2014; Arrivault *et al.*, 2017; Xu *et al.*, 2021). Isotope labeling studies suggested that inactive pools of glycine, serine, and glycerate may be stored in vacuoles (Szecowka *et al.*, 2013; Ma *et al.*, 2014; Xu *et al.*, 2021). These carbon reservoirs could be used to replenish C3 cycle intermediate pools when transitioning to low light, where pools of RUBP and its precursors are lower (Borghi *et al.*, 2019). The buffering capacity of large pools of photorespiratory intermediates could also be important for C4 photosynthesis under fluctuating light (Schlüter and Weber, 2020). The final step of the photorespiratory pathway, glycerate kinase, is located in the mesophyll cells, where the resulting 3-phosphoglycerate (3PGA) is reduced to triose phosphate shuttles to the bundle sheath cells (Bräutigam *et al.*, 2008). Increasing pools of photorespiratory intermediates not only allows for a fast build-up of metabolite pools for the 3PGA/triose phosphate shuttle during the photosynthetic induction but also feeding additional substrate into the carbon-concentrating mechanism via the interconnection of 3PGA and phosphoenolpyruvate (Kromdijk *et al.*, 2014).

Maintaining a basal level of the C3 cycle intermediate pools in low light would facilitate the high flux to support both rapid RuBP regeneration and photorespiration during light induction (Stitt *et al.*, 2021). C3 cycle intermediate pools generally increase as irradiance increases, but many of them do not fall to very low levels at low light (Borghi *et al.*, 2019; Stitt *et al.*, 2021). For example, triose-phosphate at the light compensation point already reached about one-fifth (Arabidopsis) or one-third (rice) of the levels at high light. If C3 cycle metabolites are too low in low light, it would take longer to build up the metabolite pools to support high photorespiratory and C3 cycle enzyme activity when transitioning to high light.

The energy demand of photorespiration during light transients

Energy produced from the light reactions must be balanced with energy consumed during metabolism to prevent photodamage and reduce photosynthetic inefficiencies (Kramer and Evans, 2011; Walker *et al.*, 2020). This balancing needs to occur not just in absolute terms but also stoichiometrically, meaning that both ATP and reducing equivalents (NAD(P)H) need to be produced and consumed at the same rates. NAD(P)H includes all products of linear electron flow (LEF), including NADH, NADPH, and Ferredoxin). LEF produces ATP and NAD(P)H in a fixed stoichiometry, commonly accepted to be ~1.28 ATP/NAD(P)H (Kramer and Evans, 2011; Walker *et al.*, 2020). The biochemical requirements of the C3 cycle and photorespiration in the steady-state are 1.5 and 1.75 ATP/NAD(P)H, resulting in an ATP deficit (Edwards and Walker, 1983; von Caemmerer, 2000). This ATP deficit requires additional production to safely balance energy consumption and production. There have been many mechanisms proposed to meet this ATP deficit targeting energy production under steady-state conditions, including cyclic electron flow around photosystem I, the “malate valve” and the water-water cycle as discussed in previous reviews (Hangarter and Good, 1982; Asada, 1999; Scheibe, 2004; Li *et al.*, 2004). What is less clear is how ATP/NAD(P)H demand changes under non-steady-state conditions and what are the downstream consequences of this changing demand?

In this section, we will focus exclusively on how energy demand from photorespiration changes from the steady-state 1.75 ATP/NAD(P)H during transient conditions. The origin of this demand stoichiometry arises from the various reactions of the C3 cycle and photorespiration itself that are required to regenerate the Ribulose 5-phosphate (R5P) used to produce the substrate RuBP consumed per V_o (Box 1). Note that these demands assume the steady-state processing of photorespiratory intermediates, an assumption that is not true under fluctuating light when photorespiratory intermediates, especially serine and glycine, do not maintain constant pool sizes as discussed above.

During light induction, when glycine pools are increasing, photorespiration no longer requires energy stemming from reactions downstream from glycine decarboxylation, shifting the demands of photorespiration to two ATP and one NAD(P)H per glycine that is not decarboxylated. Not processing glycine through photorespiration results in an ATP/NAD(P)H demand of 2, greatly increasing the ATP deficit of increased relative rates of V_o during light induction (Box 2). This increase in glycine pool size therefore temporarily increases the ATP/NAD(P)H demand of photorespiration metabolically. It is unclear how plants manage this temporary increase in stoichiometric energy demand, but it appears that production-side mechanisms like cyclic electron flow around photosystem I, the "malate valve" and the water-water cycle are sufficient to minimize major photodamage.

Regulation of photorespiration and impacts on photosynthesis

Given its essential yet dynamic role, is photorespiration regulated? The large transients in the photorespiratory flux under fluctuating light outlined above suggest that this pathway may be highly regulated in response to dynamic environmental changes. The need to regulate photorespiration may come from its interaction with other metabolic pathways such as nitrogen assimilation, respiration, sulfur, and C1 metabolism has been extensively reviewed (Obata *et al.*, 2016; Hodges *et al.*, 2016; Abadie *et al.*, 2017; Busch, 2020), but how changes in photorespiratory flux affect interacting metabolic pathways remains unclear. Since photorespiration carries the second-largest carbon flux in plants, rapid shifts in the photorespiratory flux in dynamic light conditions would significantly affect fluxes to these interacting metabolic pathways. One hypothesis is that activation of photorespiration helps coordinate carbon and nitrogen metabolisms under non-steady-state conditions. Additionally, photorespiration may be regulated to maintain adequate flux to related metabolisms like C1 and sulfur metabolism. In this section, we will discuss the evidence that photorespiration is regulated and examine the hypotheses for why photorespiration might be regulated.

The accumulation of glycine when V_o increases indicate that the mitochondrial glycine to serine conversion via GDC is a bottleneck flux that needs to be adjusted dynamically. GDC is believed to have a high coefficient of control in the photorespiratory pathway (Timm and Hagemann, 2020). Elevated GDC activity increases the flux capacity through photorespiration, possibly by reducing the steady-state level of glycine and all other photorespiratory intermediates, resulting in increases in plant growth and net assimilation (Timm *et al.*, 2012). In contrast, impaired GDC activity reduces the photorespiratory flux and decreases photosynthesis and growth (López-Calcagno *et al.*, 2019). The importance of GDC to photorespiratory flux raises the question of how is GDC activity regulated under fluctuating light? One possible mechanism for regulating enzymatic activities is via thioredoxins (trx) which regulate several enzymes in the C3 cycle

under fluctuating light conditions (Nikkanen and Rintamäki, 2014). Two mitochondrial trxs, trx o1, and trx h2, downregulate GDC as shown by reverse genetics and biochemical assays (Reinholdt *et al.*, 2019*b*; da Fonseca-Pereira *et al.*, 2020). Moreover, the trx o1-mediated redox regulation is important for the rapid induction of photorespiration and photosynthesis in response to short-term light/dark changes (Reinholdt *et al.*, 2019*a*). In addition to GDC, other photorespiratory core enzymes such as SHMT1, HPR1 and GOX appear to be regulated by post-translational modifications (Liu *et al.*, 2019, 2020; Jossier *et al.*, 2020).

The regulation of photorespiratory flux may also enable plants to optimize N utilization during light induction. Leaf N content can limit the speed of photosynthetic induction under fluctuating light in several C3 and C4 species (Chen *et al.*, 2013; Liu *et al.*, 2021; Sun *et al.*, 2022). The link between *de novo* nitrogen assimilation and photorespiration is supported by the observations that rates of NO_3^- uptake and assimilation decrease as rates of photorespiration decrease (Rachmilevitch *et al.*, 2004; Bloom *et al.*, 2010). During light induction, the transient increase in photorespiratory flux could increase N assimilation to support the higher N demand for increased glycine pools under high light. This newly assimilated N may not stay in the photorespiratory cycle. When photorespiration is not limited by NO_3^- uptake, a large portion of photorespiration-derived amino acids (glycine and serine) may be exported from the photorespiratory pathway (Busch *et al.*, 2018). The exported photorespiratory glycine and serine can be used to synthesize proteins and various specialized metabolites (Noctor *et al.*, 1999; Dirks *et al.*, 2012; Benstein *et al.*, 2013).

Photorespiration may function to sense the changes in the metabolic demand for downstream metabolism under fluctuating light and respond accordingly to match the supply with demand. Photorespiration is thought to be the main biosynthetic pathway for providing the 1-C units for the synthesis of nucleic acids, proteins, vitamins, and methylated molecules in leaf tissue (Hanson and Roje, 2001; Li *et al.*, 2003). Plant sulfur assimilation is stimulated under high photorespiratory conditions (Abadie and Tcherkez, 2019; Abadie *et al.*, 2021). Regulating photorespiration flux could ensure adequate and consistent flux to interacting metabolic pathways such as C1 metabolism and sulfur metabolism despite fluctuating light and absolute rates of V_o.

Future Research

Given the importance and unknowns of the operation of photorespiration under fluctuating conditions, we feel that the field would benefit from several areas of increased research investment. Studies investigating the metabolic changes of photorespiratory intermediates during light transients would help resolve some of these questions, as would investigating the effects of fluctuating light under non-photorespiratory and photorespiratory conditions. Investigating non-steady-state behavior also requires analytical approaches developed for dynamic systems that integrate a time component to best compare and evaluate *in vivo* signals like net CO_2 assimilation of chlorophyll fluorescence. Engineering control theory provides apt tools for this type of analysis and has been already applied to control leaf temperature under dynamic light (Pare *et al.*, 2017). We feel that control theory approaches may be uniquely suited to bring new insight into the behavior of photorespiration and photosynthesis generally under fluctuating light.

Conclusion

While considerable progress has been made regarding the role and regulation of photorespiration in the steady-state, it is critical to better understand photorespiration under non-steady-state conditions. Many factors simultaneously constrain the response of net photosynthesis and photorespiration during light transients, including the diffusional conductance to CO_2 from the atmosphere to the catalytic site of rubisco in the chloroplasts and the activation of rubisco. The metabolic interactions between photorespiration and the C3 cycle and their energy demand are also dynamic under light transients. Considering the tight connection between photorespiration and other pathways such as nitrogen assimilation and C1 metabolism, transient changes in the photorespiratory flux can affect overall carbon and nitrogen partitioning. As photorespiration is involved in the induction of net assimilation under fluctuating light, exploiting the genetic manipulation of the transient responses of photorespiration has the potential to improve crop performance. Increasing our understanding of photorespiration regulation in transient presents an opportunity to improve net carbon assimilation and optimize nutrient uptake and partition in dynamic environments.

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Author contributions

XF and BJW: conceptualization; XF: writing - original draft; XF and BJW: writing - editing and approval of the final version.

Conflict of interest

The authors have no conflicts of interest to disclose.

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Figure legends

Fig. 1. Response of stomatal conductance to water vapour (g_s), net CO_2 assimilation (A), rate of rubisco carboxylation (V_c), rate of rubisco oxygenation (V_o), and the V_o/V_c ratio of rice (*Oryza sativa*) and broad bean (*Vicia faba*) to an increase in irradiance from 100 (shaded area) to 1000 (unshaded area) μmol m^{-2} s^{-1} followed by a decrease to 100 μmol m^{-2} s^{-1}. Data were taken or calculated from McAusland et al., 2016.

Fig. 2. Response of mesophyll conductance (g_m), stomatal conductance to water vapour (g_s), net CO_2 assimilation (A), rate of rubisco carboxylation (V_c), rate of rubisco oxygenation (V_o), and the V_o/V_c ratio of wild-type Arabidopsis to an increase in irradiance from 100 (shaded area) to 1200 (unshaded area) μmol m^{-2} s^{-1}. Data were taken or calculated from Liu et al., 2021.

Box 1. Schematic view of photorespiration and the interacting metabolic pathways.

The core photorespiratory pathway involves three subcellular compartments, chloroplasts, peroxisomes and mitochondria. Photorespiration is initiated by rubisco oxygenation (R0), yielding the inhibitory molecule 2-phosphoglycolate (P-glocate), which is dephosphorylated to glycolate. Glycolate is exported from the chloroplast into the peroxisomes, then reacts irreversibly with O_2 to form glyoxylate by glycolate oxidase (R1), producing H_2O_2 as a byproduct. Next, glyoxylate is aminated by either glutamate glyoxylate:aminotransferase (R2) or serine:glyoxylate transaminase (R4) to produce glycine. The glycine produced is moved from the peroxisomes to mitochondria, then decarboxylated to form serine releasing CO_2, NH_4, cycling tetrahydrofolate (THF) and methyl-THF (M-THF), and reducing NAD by a multienzyme complex, glycine–cleavage system (R3). Photorespiratory serine is moved back to the peroxisome and converted to hydroxypyruvate (Hpyr) while serine:glyoxylate transaminase (R4) is catalyzing the amino group transfer to glyoxylate. Hpyr is reduced to glycerate by hydroxypyruvate reductase (R5) and transported back to the chloroplast. Glycerate is phosphorylated to 3-phosphoglycerate (P-glycerate), which can then enter the C3 cycle. The H_2O_2 produced by R1 is decomposed to oxygen and water by catalase (R6). Nonenzymatic decarboxylations can occur either between glyoxylate and H_2O_2 (R7) or between HPyr and H_2O_2 (R8), releasing CO_2 in the process. The extra glycine and serine can be exported out of the photorespiratory pathway for protein synthesis or other metabolic processes (R9 and R10). Figure and caption are revised from Bao et al., 2021.

Box 2. Energy demand from photorespiration

To understand how the stoichiometry of energy demand from photorespiration changes during transient conditions, we first must understand where it originates. This energy demand is most easily followed by starting with the C3-cycle intermediate Ribulose 5-phosphate (R5P), the immediate precursor to RuBP. To follow the entire energy demand of photorespiration, we must account for all ATP and NAD(P)H needed to convert all downstream products of R5P through the various cycles back into R5P. R5P requires a single ATP to produce RuBP. To initiate photorespiration, RuBP is oxygenated and produces a single 3-phosphoglycerate (3-PGA) and 2-PG per V_o. Photorespiration recycles 2-PG to 0.5 3-PGA, which is processed in the C3 cycle at the cost of one ATP and one NADPH each back to R5P. Recycling of 2-PG by photorespiration first involves energy during glycine decarboxylation, which produces 0.5 NADH per V_o and releases NH_4^+ from glycine. This NH_4^+ is refixed in the chloroplast by the glutamine synthetase-glutamate synthase pathway at the cost of one ATP and two ferredoxin, or 0.5 ATP and 0.5 NAD(P)H per V_o. The downstream product of glycine decarboxylation requires 0.5 NADH to convert hydroxypyruvate to glycerate and one ATP to convert glycerate to 3-PGA, which can enter the C3 cycle and require an additional 0.5 ATP and 0.5 NAD(P)H per V_o.

Energy Demands of Steady-State Photorespiration		
	ATP Demands	NAD(P)H Demands
R5P to RuBP Conversion	1	--
3-PGA produced per V_o to R5P	1	1
NADH produced from GDC	--	-0.5
Refixation of NH_4^+ by GS-GOGAT	0.5	0.5
Reduction of hydroxypyruvate	--	0.5
Glycerate phosphorylation	0.5	--
3-PGA recycled from photorespiration to R5P	0.5	0.5
Total	3.5	2