

Effects of high CO₂ levels on dynamic photosynthesis: carbon gain, mechanisms, and environmental interactions

Hajime Tomimatsu² · Yanhong Tang^{1,2}

Received: 21 February 2016 / Accepted: 6 March 2016 / Published online: 19 April 2016
© The Botanical Society of Japan and Springer Japan 2016

Abstract Understanding the photosynthetic responses of terrestrial plants to environments with high levels of CO₂ is essential to address the ecological effects of elevated atmospheric CO₂. Most photosynthetic models used for global carbon issues are based on steady-state photosynthesis, whereby photosynthesis is measured under constant environmental conditions; however, terrestrial plant photosynthesis under natural conditions is highly dynamic, and photosynthetic rates change in response to rapid changes in environmental factors. To predict future contributions of photosynthesis to the global carbon cycle, it is necessary to understand the dynamic nature of photosynthesis in relation to high CO₂ levels. In this review, we summarize the current body of knowledge on the photosynthetic response to changes in light intensity under experimentally elevated CO₂ conditions. We found that short-term exposure to high CO₂ enhances photosynthetic rate, reduces photosynthetic induction time, and reduces post-illumination CO₂ burst, resulting in increased leaf carbon gain during dynamic photosynthesis. However, long-term exposure to high CO₂ during plant growth has varying effects on dynamic photosynthesis. High levels of CO₂ increase the carbon gain in photosynthetic induction in some species, but have no significant effects in other species. Some studies have shown

that high CO₂ levels reduce the biochemical limitation on RuBP regeneration and Rubisco activation during photosynthetic induction, whereas the effects of high levels of CO₂ on stomatal conductance differ among species. Few studies have examined the influence of environmental factors on effects of high levels of CO₂ on dynamic photosynthesis. We identified several knowledge gaps that should be addressed to aid future predictions of photosynthesis in high-CO₂ environments.

Keywords Carbon dioxide · Dynamic photosynthesis · Elevated CO₂ · Fluctuating irradiance · Lightfleck · Sunfleck

Introduction

There is growing concern over elevated atmospheric CO₂ concentrations (Pachauri et al. 2014). Terrestrial plant photosynthesis is a key process of models constructed for predicting the increase in atmospheric CO₂ or for assessment of its mitigating role in suppression of CO₂ increase. Most of these models are based on knowledge obtained under steady-state conditions, including constant light intensity, CO₂ concentration, temperature, and other air conditions such as airflow rate and water vapor density.

However, plant photosynthesis in nature, particularly in terrestrial ecosystems, never occurs under steady-state conditions. Photosynthetic activity changes over various time scales due to temporal variation in photosynthetic resources and environmental conditions. For example, light intensity is often referred to as the photosynthetic photon flux density (PPFD or PFD) and varies temporally in nature, fluctuating 100s of times within seconds in plant canopies or at forest floors (e.g. Pearcy 1999; Pearcy et al. 1996;

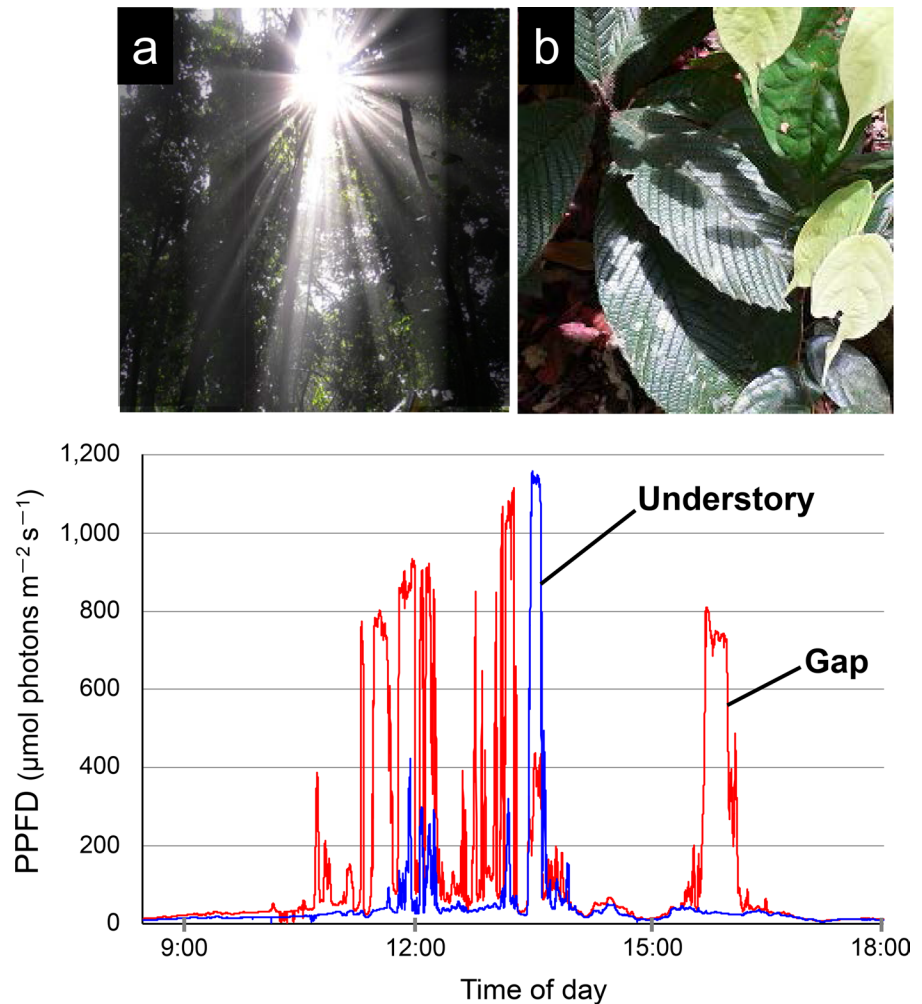
✉ Hajime Tomimatsu
tomimatsu.hajime@nies.go.jp

✉ Yanhong Tang
tangyh@nies.go.jp; tangyh@pku.edu.cn

¹ Present Address: Department of Ecology, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China

² Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies, Onogawa 16-2, Tsukuba 305-0053, Japan

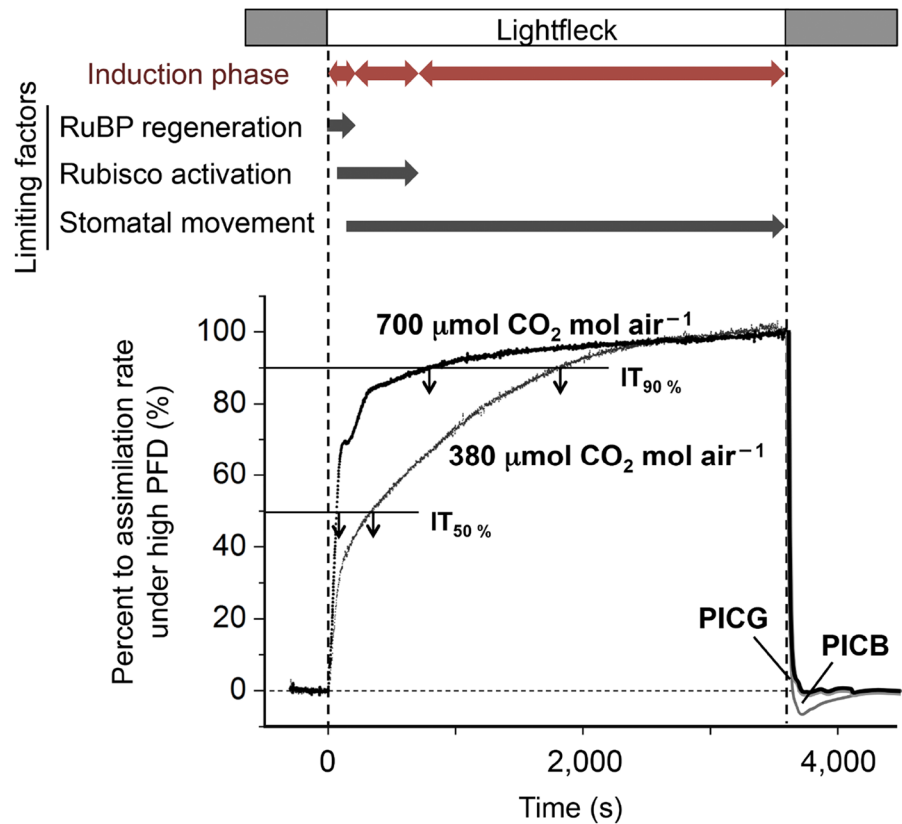
Fig. 1 Sunlight penetrating through forest canopy (a), sunflecks on leaves (b) and diurnal change of photosynthetic photon flux density (PPFD) in a gap (red color) and the understory (blue color) on the forest floor in Malaysia's Pasoh Forest Reserve (2°59'N, 102°18'E). PFD was measured 0.5 m above the ground at an interval of 1 s 15 April 2014 (color figure online)



Tang et al. 1988, see Fig. 1 for example). The photosynthetic machinery of plants senses and responds to variation in PFD at all temporal scales, from milliseconds to years (e.g. Baldocchi et al. 2001; Katul et al. 2001; Pearcy and Pfitsch 1995). It is important to understand how such temporal variation in photosynthesis will change in the future as global CO_2 levels increase. The recent rapid increase in atmospheric CO_2 has shifted academic focus towards understanding photosynthesis in the natural environment. This has led to the publication of many studies on photosynthesis, expanding from traditional steady-state models to more realistic dynamic models (e.g. Kaiser et al. 2015; Rascher and Nedbal 2006). The processes, mechanisms, and ecological consequences of dynamic photosynthesis in response to high levels of CO_2 could differ from those of steady-state photosynthesis (Kaiser et al. 2015); therefore, it is necessary to understand dynamic photosynthesis under high- CO_2 conditions to reliably assess and predict future photosynthesis in natural environments with high levels of CO_2 . However, the current understanding of dynamic photosynthesis related to high CO_2 levels is limited.

Dynamic photosynthesis can be viewed as two independent processes: first, photosynthetic rates increase after an increase in PFD, and second, photosynthetic rates decrease after a decrease in PFD. The increase in photosynthetic rate after a sudden increase in PFD is called the photosynthetic induction response (see details in “Introduction” section; Fig. 2), which has been of increasing interest in recent decades (e.g. Pearcy et al. 1994; Way and Pearcy 2012). The induction response limits light-use efficiency, and the degree of limitation varies depending on the physiology and biochemistry of the leaf photosynthetic system, photosynthetic resources, and environmental conditions such as CO_2 concentration. The photosynthetic induction response can be divided into three different but highly interactive sub-processes: electron transport, changes in the stomata system, and the photosynthetic enzyme system (Chazdon 1988; Pearcy et al. 1994; Pearcy and Way 2012; Tikhonov 2015). These sub-processes greatly depend on CO_2 concentration and are expected to differ in high- CO_2 environments (e.g. Chazdon and Pearcy 1986; Knapp et al. 1994; Naumburg and Ellsworth 2000). When PFD

Fig. 2 Representative responses of CO_2 assimilation rate of *P. euramericana* cv. *I-55* grown at 380 and 700 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$. Double-headed arrows show three photosynthetic induction phases. Three arrows indicate major biochemical processes limiting induction response at different periods. PFD was suddenly increased from 20 to 800 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Following the high PFD for 60 min, PFD was suddenly decreased to 20 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Redrawn from Tomimatsu and Tang (2012). *PICG* and *PICB* indicate the postillumination carbon gain and post-illumination CO_2 burst, respectively. Photosynthetic induction time required for the photosynthetic rate to reach 50 and 90 % of the steady-state rate under high PFD (IT_{50} and IT_{90}), were indicated down arrows (color figure online)



decreases, the photosynthetic rate generally decreases rapidly, but not instantaneously. The photosynthetic CO_2 uptake process after PFD decrease is often referred to as post-illumination CO_2 assimilation, which is also highly affected by environmental CO_2 .

We reviewed studies that have experimentally examined elevated CO_2 levels on photosynthesis in response to temporal variation in PFD within seconds–hours. For a broader review of dynamic photosynthesis, we recommend two excellent papers (Kaiser et al. 2015; Way and Pearcy 2012). We focused on the effects of experimentally high levels of CO_2 on the physiological effects, acclimation changes, and leaf carbon gain in dynamic photosynthesis, as well as the major mechanisms involved in and eco-physiological effects of environmental factors on the dynamic photosynthetic response to high levels of CO_2 .

Time course and carbon gain of dynamic photosynthesis under high CO_2

Time course studies provide important observations of dynamic systems. There are three types of time course studies that have been used to observe CO_2 gas exchange in dynamic photosynthesis: photosynthetic induction after increases in light, post-illumination CO_2 assimilation after

decreases in light, and response to a series of short light-flecks or fluctuating light. High levels of CO_2 are expected to affect all of these responses, but information on post-illumination assimilation and the photosynthetic response to short light-flecks is much more limited than that on the induction response.

Typically, the time course of photosynthetic induction after an increase of PFD can be arbitrarily divided into three phases (Fig. 2). The first phase, thought depending on leaf induction state, is very short and often completes within the first 1–2 min (Percy 2007). The second phase includes mainly Rubisco activation, often taking 5–10 min. The third phase typically takes 10–30 min, which is dominated by stomata opening. There are three major limiting processes involved in the induction time course, which are RuBP regeneration, Rubisco activation and stomatal movement.

In general, high- CO_2 conditions have previously been used in two ways: to measure photosynthesis only or to both grow plants and assess their photosynthesis. For convenience, the former is herein referred to as “short-term eCO_2 ” and the latter is referred to as “long-term eCO_2 ”. Short-term eCO_2 exposure usually lasts minutes–hours, while long-term exposure occurs for months–years, depending on the experimental design. Short-term eCO_2 exposure only stimulates the physiological responses of

Table 1 Characteristics of dynamic photosynthesis under “short-term” high CO₂ conditions

Species	Herbaceous or tree	[CO ₂] (ppm)	Induction time of A (min) ^a		A (μmol CO ₂ m ⁻² s ⁻¹)		Induction time of gs (min) ^a		gs (mol H ₂ O m ⁻² s ⁻¹)		LUE (%)	References
			IT _{50%}	IT _{90%}	Low-light (PFD = 10)	High-light (PFD = 500)	IT _{50%}	IT _{90%}	Low-light (PFD = 10)	High-light (PFD = 500)		
<i>A. macrorrhiza</i>	Herbaceous	360	1.1	19.0	–	1.1	–	–	–	–	–	Chazdon and Pearcy (1986)
		360	5.3	23.2	–	2.0	–	–	–	–	–	
		360	4.6	14.7	–	6.0	–	–	–	–	–	
		360	5.9	13.3	–	5.8	–	–	–	–	–	
		360	2.0	11.7	–	7.5	–	–	–	–	–	
<i>D. sublamellatus</i>	Tree	Ambient	0.8	7.3	1.9	4.6	8.1	17.4	0.07	0.10	91.9	Tomimatsu et al. (2014)
		Ambient	0.7	6.0	2.9	8.5	8.8	18.5	0.05	0.08	95.2	

LUE lightfleck (or sunfleck) use efficiency

^a IT_{50%} and IT_{90%} indicate induction time required to reach 50 and 90 % of the steady-state in photosynthetic rate (A) and stomatal conductance (gs) following an increase of PFD

dynamic photosynthesis, while long-term exposure is expected to cause photosynthetic machinery to acclimate. There is a large body of knowledge on the effects of short- and long-term CO₂ exposure on steady-state photosynthesis (Ainsworth and Long 2005; Ainsworth and Rogers 2007; Long et al. 2004), but very little on dynamic photosynthesis (Kaiser et al. 2015).

Effects of short-term elevated CO₂ on dynamic photosynthesis

Chazdon and Pearcy (1986) reported a dataset on the effects of short-term CO₂ on photosynthetic induction response in the herbaceous species *Alocasia macrorrhiza* (Table 1). To the best of our knowledge, this is the first study that showed the short-term response of photosynthetic induction to different CO₂ levels. In addition, two points should be taken from this report. First, despite the fact that the steady-state assimilation rate at high PFD increased with increasing ambient CO₂, the time course of photosynthetic induction differed depending on the measurement CO₂ level ranging from 130–1,284 μmol CO₂ mol⁻¹ [Table 2 in Chazdon and Pearcy (1986)]. At the CO₂ concentration below 200 μmol mol⁻¹, photosynthetic rate increased slowly and continuously with time. However, under CO₂ concentrations above 200 μmol mol⁻¹, the change in photosynthetic rate showed two phases: an early rapid increasing phase followed by a slow and sigmoidal increasing phase. This biphasic nature of the induction was more pronounced at the highest CO₂ concentration (Chazdon and Pearcy 1986). This is notable, as it suggests that physiological events at different time points during the induction process may be associated with different mechanisms. For example, Kirschbaum and Pearcy (1988a) indicated that high levels of CO₂ enhanced only the second phase of photosynthetic induction in the same species. Second, the full photosynthetic induction reported in Chazdon and Pearcy (1986) occurred quicker under higher CO₂ concentration. These two studies suggest that short-term eCO₂ results in higher carbon uptake in photosynthetic induction response than ambient and low CO₂ levels in herbaceous plants.

Tomimatsu et al. (2014) conducted the first experiment on the effects of short-term eCO₂ on tree species in natural environments. Comparing the effects of 350 and 700 μmol mol⁻¹ CO₂ on photosynthesis under a simulated sunfleck in *Dipterocarpus sublamellatus* seedlings in a tropical forest floor in Malaysia confirmed that high levels of CO₂ reduced the induction time and increased leaf carbon gain in seedlings. Both the initial and late induction phases occurred more rapidly under high levels of CO₂ than under low levels.

Although time course studies have examined post-illumination assimilation and the CO₂ burst period under

different CO₂ concentrations, often lower than ambient CO₂, to evaluate photorespiration (e.g. Doehrlert et al. 1979), there is little information on how high levels of CO₂ affect post-illumination assimilation. Tomimatsu et al. (2014) found that short-term eCO₂ exposure enhanced photosynthetic light-use efficiency in post-illumination CO₂ fixation, although the relative contribution was small, partly because the simulated sunfleck lasted for a long time (30 min) and only one sunfleck was applied. It is expected that short-term eCO₂ should enhance light-use efficiency and increase leaf carbon gain in post-illumination CO₂ fixation under short, frequent sunflecks.

There is no data available on the effects of short-term eCO₂ on photosynthetic responses to a series of light-flecks. Marin et al. (2014) found no significant differences in the net photosynthetic carbon gain under 260–490 µmol CO₂ mol⁻¹ air in their study examining leaf energy balance in response to dynamic light. In the morning, atmospheric CO₂ within plant canopies or on the forest floor varies considerably diurnally and seasonally. For example, in the morning, CO₂ concentration near the forest floor can be higher than the average CO₂ outside the forest by as much as about 100 µmol CO₂ mol⁻¹ air (Bazzaz and Williams 1991; Buchmann et al. 1996; Day et al. 2002). Therefore, it would be useful to know the contribution of short-term eCO₂ to leaf carbon gain in understory plants where PFD is often very limited (Tomimatsu et al. 2014). This research group examined CO₂ gas exchange in response to an artificial light-fleck in *D. sublamellatus* seedlings under 350 and 700 µmol CO₂ mol⁻¹ air in a tropical rain forest. The total photosynthetic carbon gain from the light-fleck was about two times greater under high levels of CO₂ than under low levels. The increase in light-fleck use efficiency contributed to 7 % of the increased carbon gain, most of which was due to a reduction in photosynthetic induction time under high levels of CO₂. This suggests that short-term eCO₂ increase photosynthetic light use efficiency under both steady-state and fluctuating light conditions, which should be considered when assessing the leaf carbon gain of understory plants.

Effects of long-term elevated CO₂ on dynamic photosynthesis

Several studies have examined the effects of CO₂ levels on dynamic photosynthesis using long-term eCO₂ exposure (Table 2) (Holisova et al. 2012; Knapp et al. 1994; Kosvancova et al. 2009; Leakey et al. 2002; Naumburg and Ellsworth 2000; Tomimatsu and Tang 2012). Knapp et al. (1994) conducted the first experiment to examine dynamic photosynthetic responses under long-term eCO₂ exposure, which is perhaps the only study to have examined the dynamic photosynthesis of C4 grass in relation to high

levels of CO₂. In a grassland dominated by C4 grasses in Kansas, USA, *Andropogon gerardii* Vitman was exposed to high levels of CO₂ (double the ambient level) continuously throughout the growing season of about 3 months. Stomatal and photosynthetic responses to different temporal PFD patterns were measured in plants under high and ambient CO₂. The study found that C4 grass appeared to have a fast stomatal response to elevated CO₂, due to both a high initial stomatal conductance and rapid stomata opening under high levels of CO₂. Because the leaves exposed to high levels of CO₂ developed and grew under such conditions, the change in initial stomatal conductance and opening speed was possibly due to acclimation. Stomatal response to high levels of CO₂ may be relatively more important in dynamic photosynthesis in C4 plants than C3 plants, but few studies have examined the effects of high levels of CO₂ on photosynthesis under fluctuating light in C4 plants. In this study, the maximum photosynthetic rate and carbon gain during induction response did not differ significantly between plants exposed to high and ambient levels of CO₂.

The effects of high levels of CO₂ on dynamic photosynthesis in C3 plants are not consistent in the literature. Naumburg and Ellsworth (2000) conducted the first study to examine the effects of long-term eCO₂ on dynamic photosynthesis in C3 plants. In a free air CO₂ enrichment (FACE) experiment in the understory of a *Pinus taeda* plantation, two shade-tolerant species (*Acer rubrum* and *Cornus florida*) and two shade-intolerant species (*Liquidambar styraciflua* and *Liriodendron tulipifera*) were grown under ambient and ambient + 200 ppm CO₂ conditions to assess potential differences in dynamic photosynthesis between the tree types under high levels of CO₂. High CO₂ levels had no significant effects on induction gain on any of the species throughout the day but extended the post-illumination assimilation period in all species after light decrease. Hence, high levels of CO₂ would be expected to increase the leaf carbon gain under fluctuating light.

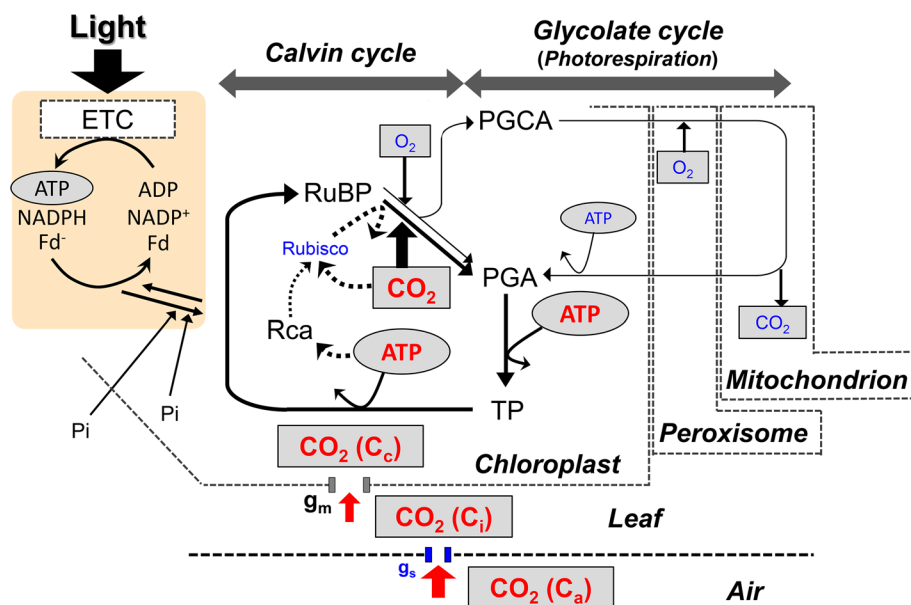
After growing seedlings of the tropical tree *Shorea leprosula* in 711 and 376 ppm of CO₂ for 216 days, Leakey et al. (2002) examined the effects of high levels of CO₂ on both steady-state and dynamic photosynthesis. As expected, long-term treatment increased carbon gain in leaves under both steady-state and fluctuating light environments. Interestingly, the gain was greater under dynamic light conditions. This was ascribed to the greater light fleck use efficiency under high-CO₂ conditions (Leakey et al. 2002, 2005). This high efficiency was due to a combination of faster carbon gain, slower carbon loss, increased post-illumination CO₂ fixation, and decreased post-irradiance CO₂ burst (Leakey et al. 2005).

Kosvancova et al. (2009) examined dynamic photosynthesis in the broadleaved *Fagus sylvatica* and coniferous *Picea abies* after exposure to 385 and 700 µmol CO₂ mol⁻¹

Table 2 Characteristics of dynamic photosynthesis under “long-term” high CO₂ conditions

Species	Herbaceous or tree	[CO ₂] (ppm)	Measurement		Induction time of A (min) ^a		A (μmol CO ₂ m ⁻² s ⁻¹)		Induction time of gs (min) ^a		gs (mol H ₂ O m ⁻² s ⁻¹)		LUE (%)	References	
			Growth	Measurement	IT _{50%}	IT _{90%}	Low-light	High-light	IT _{50%}	IT _{90%}	Low-light	High-light			
<i>A. rubrum</i>	Tree	Ambient +200	Ambient +200	Ambient	–	4.0	1.25	6.6	–	11.2	0.02	0.12	–	Naumburg and Ellsworth (2000)	
<i>C. florida</i>	Tree	Ambient +200	Ambient +200	Ambient	–	8.8	1.25	4.7	0.0	14.5	0.03	0.07	–		
<i>L. styraciflua</i>	Tree	Ambient +200	Ambient +200	Ambient	–	7.2	0.9	6.4	–	17.5	0.03	0.11	–		
<i>L. tulipifera</i>	Tree	Ambient +200	Ambient +200	Ambient	–	5.0	1.5	5.8	–	13.5	0.05	0.12	–		
		Ambient +200	Ambient +200	Ambient	–	–	1.9	7.1	–	–	0.04	0.09	–		
							(PFD = 30)	(PFD = 525)			(PFD = 30)	(PFD = 525)			
<i>S. leprosula</i>	Tree	376 711	350 700	1.2 2.4	13.6 9.8	0.7 1.4	4.9 8.2	2.5 13.1	3.8 9.8	0.04 0.04	0.12 0.10	90.5 95.1		Leakey et al. (2002)	
							(Fully dark)	(PFD = 1400)			(Fully dark)	(PFD = 1400)			
<i>F. sylvatica</i>	Tree	385 700	385 700	– –	27.0 (35.0) 22.0 (32.0)	– –	8.5 (9.6) 10.4 (11.2)	– –	– –	– –	– –	0.1 (0.15) 0.06 (0.08)	–	Kosvancova et al. (2009) ^d	
<i>P. abies</i>	Tree	385 700	385 700	– –	38.0 (40.0) 28.0 (32.0)	– –	7.2 (9.7) 10.7 (14.8)	– –	– –	– –	– –	0.16 (0.19) 0.08 (0.13)	–		
							(PFD = 20)	(PFD = 800)			(PFD = 200)	(PFD = 800)			
<i>P. euramericana</i> cv. I-55	Tree	380 700 1020	380 700 1020	5.8 1.2 0.9	27.0 12.9 10.6	0.6 0.9 0.8	14.9 20.3 17.4	28.9 25.4 23.7	50.7 49.0 49.8	0.08 0.17 0.10	0.48 0.35 0.20	– – –	–	Tominatsu and Tang (2012) ^b	
<i>P. koreana</i> × <i>trichocarpa</i> cv. Peace	Tree	380 700 1020	380 700 1020	1.6 1.2 0.9	6.4 5.5 4.2	0.7 1.1 0.8	15.5 19.0 16.2	– – –	– – –	0.20 0.24 0.28	0.24 0.27 0.33	– – –	–		

Fig. 3 Major components and processes of dynamic photosynthesis in relation to CO_2 . The relative increase and decrease under high CO_2 concentration are shown with red color (bold font) and blue color, respectively. *PGA* 3-phosphoglycerate, *PGCA* 2-phosphoglycolate, *TP* triose-phosphate, *Rca* Rubisco activase, *Rubisco* ribulose-1,5-bisphosphate carboxylase oxygenase, *RuBP* ribulose-1,5-bisphosphate, *ETC* electron transport chain, *Fd* ferredoxin, *Pi* phosphoric acid. *Italic letters* indicate the location of the organization and space (color figure online)



machinery (Müller et al. 2001), particularly in plants in shaded environments. Photosynthetic electron transport (PET) is important in regulating photosynthetic machinery to use fluctuating light while avoiding light stress (e.g. Horton et al. 1996; Kono and Terashima 2014; Rochaix 2011). However, there is little information on the effects of high levels of CO_2 on PET under fluctuating light.

Based on current knowledge of the regulation of PET under steady-state conditions (Foyer et al. 2012), one can expect that both linear electron transport and cyclic electron transport should play a role in dynamic photosynthesis under high levels of CO_2 . For example, high levels of CO_2 stimulate Calvin-Benson cycle turnover and increase electron flow through PSII, which affects the photosynthetic response to changes in light (Tikhonov 2015). In addition, high levels of CO_2 alter the cyclic PET performed by the thylakoid Ndh complex (Martin et al. 2015). For example, examining the functional relevance of this complex in relation to dynamic photosynthesis under ambient and high levels of CO_2 in tobacco plants showed that Ndh-defective plants (due to an *ndh* gene defect) showed slower photosynthetic responses to increases in light at high levels of CO_2 compared to ambient levels.

RuBP regeneration

In C3 plants, RuBP serves as a CO_2 receptor, and the size of the RuBP pool is a key factor determining the rate of photosynthesis. The amount of RuBP available for photosynthesis depends on the extent of RuBP regeneration, which is mainly affected by the rates of electron transport and the Calvin cycle. Changes in PFD duration and temporal

patterns, CO_2 concentration, and other environmental factors can affect RuBP regeneration. Under constant PFD conditions, high levels of CO_2 promote CO_2 fixation and stimulate the Calvin cycle, thereby increasing RuBP regeneration (Ainsworth and Rogers 2007).

There is very little information on the effects of high CO_2 levels on RuBP regeneration in dynamic photosynthesis. In one study, after an increase in PFD, the photosynthetic rate showed an initial increase within seconds and then increased again for several minutes, which was possibly limited by RuBP regeneration. This rapid increase in CO_2 uptake was accelerated by high levels of CO_2 (Kirschbaum and Percy 1988a). In addition, we previously found that the initial photosynthetic rate is greatly increased when CO_2 levels are high (Tomimatsu and Tang 2012).

Rubisco activase and Rubisco activation

Rubisco activase catalyzes the release of inhibitors from Rubisco and affects Rubisco activity. This process requires ATP and is influenced by temperature and CO_2 levels. Rubisco activase may be one of the most important enzymes in dynamic photosynthesis. Yamori et al. (2012) compared the photosynthetic induction response among plants with different levels of Rubisco activase (i.e., over-expressed, normal levels in wild-type plants, and reduced levels in antisense plants). They found that plants with higher levels had a more rapid induction response, and concluded that Rubisco activase has a more important role regulating dynamic photosynthesis than steady-state photosynthesis. Another study found that manipulating Rubisco activase regulation enhanced dynamic photosynthesis (e.g. Carmo-Silva and Salvucci 2013; Soleh et al. 2016).

Because the evidence from steady-state photosynthesis studies suggests that a decrease in Rubisco activation under high levels of CO₂ is due to the reduced activity of Rubisco activase (Crafts-Brandner and Salvucci 2000), the contribution of this enzyme to dynamic photosynthesis in high-CO₂ environments should be investigated. We previously found that induction limitations after long-term eCO₂ exposure seemed to be at least partially attributable to an increased Rubisco activase:Rubisco ratio in one *Populus* species (unpublished data, Tomimatsu and Tang).

Plants grown under long-term high-CO₂ environments tend to have lower Rubisco levels or activity (Sage et al. 1989; Sage 1994) compared to plants grown in low-CO₂ environments. If the rates of increase are constant, a smaller maximum Rubisco activity or lower maximum stomatal conductance should be reached more rapidly in plants exposed to high levels of CO₂ than those exposed to low CO₂. This leads to the hypothesis that plants exposed to high levels of CO₂ should have a faster photosynthetic induction response than those exposed to low CO₂ levels.

Post-illumination CO₂ assimilation

After a decrease in PFD, the photosynthetic rate drops quickly, usually within seconds (Fig. 2). The contribution of post-illumination CO₂ fixation to carbon gain in leaves is often not significant, but it may play a substantial role in light environments with many short sunflecks and in high-CO₂ environments (Percy 1994; Sharkey et al. 1986). CO₂ fixation immediately after a decrease in PFD is due to biochemical intermediates, including Calvin cycle intermediates, NADPH, and ATP (Laisk et al. 1984; Sharkey et al. 1986). The amount of these intermediates depends on the duration and intensity of previous light conditions, photosynthetic rate, and other environmental conditions such as CO₂ concentration. The data on the effects of high levels of CO₂ on post-illumination CO₂ fixation are not consistent in the literature (e.g. Laisk and Edwards 1997; Tomimatsu et al. 2014; Vines et al. 1983, see discussion and citations in Leakey et al. 2002). Further studies are needed to clarify this issue and improve estimations of carbon gains in leaves under fluctuating light environments and to improve plants themselves.

The leaves of C3 plants often show a CO₂ burst after a decrease in light (Fig. 2). This burst is due to photorespiratory metabolites (Fig. 3). The burst size depends on environmental conditions, including light intensity, CO₂ concentration, photosynthetic metabolites such as RuBP, photosynthetic enzymes such as Rubisco, and subsequent photorespiratory metabolism (for details, see Percy 1990). Because photorespiration is suppressed by CO₂, high levels of this gas should reduce the post-illumination burst. Several studies have confirmed this, under both short-term (Laisk and Edwards 1997; Tomimatsu et al. 2014; Vines

et al. 1983) and long-term CO₂ exposure (Leakey et al. 2002).

Effects of high levels of CO₂ on stomatal conductance and dynamic photosynthesis

Understanding stomatal conductance in response to fluctuations in light is key to understanding dynamic photosynthesis. Both long- and short-term eCO₂ exposure are expected to result in a decrease in stomatal conductance under constant light and increase the opening rate in response to changes in PFD (e.g. Ainsworth and Long 2005; Kosvancova et al. 2009; Tomimatsu et al. 2014; Tomimatsu and Tang 2012), although we found that high CO₂ increased stomatal conductance under low PFD in our unpublished data. However, the mechanisms involved may differ somewhat between long- and short-term exposures. In general, stomatal size and density decrease with long-term increases in CO₂ concentration (Franks and Beerling 2009; Woodward and Kelly 1995). These morphological changes, which are due to acclimation to high-CO₂ environments, can result in lower stomatal conductance. Instantaneous increases in CO₂ also reduce stomatal conductance, but this is only due to a decrease in stomatal aperture without any changes in quantity or size.

The rapid increase in stomatal conductance after an increase in PFD under high levels of CO₂ may be ascribed to the rapid opening of stomata, a high initial stomatal conductance before the increase in PFD, and a decreased maximum stomatal conductance at the higher PFD.

Knapp et al. (1994) reportedly observed rapid opening of stomata during photosynthetic induction in a C4 plant (*A. gerardii*) growing under high levels of CO₂. However, there is no further evidence to confirm the finding in either C4 or C3 plants. Increased stomatal conductance under low PFD or decreased conductance under high PFD could indicate rapid stomatal opening. However, observations in relation to these speculations are conflicting. In *F. sylvatica* L. and *P. abies*, a reduction in the maximum stomatal conductance was reported in plants under high levels of CO₂, which has been ascribed to contribute to the observed improvements in rapid induction (Kosvancova et al. 2009). We have shown that the effects of long-term eCO₂ on stomatal conductance differ among plant species and PFD conditions. In *Populus euramericana* cv. I-55, high levels of CO₂ increased stomatal conductance under low PFD but reduced conductance under high PFD (Tables 1 and 2). However, in *Populus koreana* × *trichocarpa* cv. Peace, high levels of CO₂ increased stomatal conductance under all experimental PFD conditions (Tomimatsu and Tang 2012). Conversely, some studies have found that stomatal conductance does not significantly change under high levels of CO₂ during photosynthetic induction, despite an

increase in induction speed (Leakey et al. 2005; Naumburg and Ellsworth 2000).

In conclusion, it is currently unclear how long-term exposure to high levels of CO₂ affects stomatal conductance in dynamic photosynthesis, as there are several knowledge gaps. For example, it is not known how stomatal acclimation to long-term eCO₂ exposure and the instantaneous response to different PFDs contribute to dynamic photosynthesis. Similarly, long-term eCO₂ reduces maximum stomatal conductance, which should enhance rapid changes in stomatal conductance during induction responses; however, long-term eCO₂ also makes stomata larger, which should lead to a slower stomatal response (Drake et al. 2013), as larger stomata take longer to open and close (Raven 2014). It is unknown how these two opposite forces affect the overall speed of stomatal conductance. Stomatal performance is critical to the dynamic response of photosynthesis in natural environments, therefore, studies are needed to clarify their contributions to dynamic photosynthesis under high-CO₂ conditions.

Biotic and abiotic factors related to the effects of high CO₂ levels on dynamic photosynthesis

Effects of isoprene emissions on dynamic photosynthesis under high levels of CO₂

Some plants emit isoprene from leaves, which has received increasing interest in the last two decades (Sharkey et al. 2008). Isoprene emissions may help maintain dynamic photosynthesis by protecting the photosynthetic system from heat and light stress induced by high PFD sunflecks, thereby benefiting the carbon gain by leaves under fluctuating light and temporally high temperature conditions (Sharkey et al. 2008; Way et al. 2011).

Based on the thermotolerance hypothesis of isoprene emissions, it was expected that leaves in high-CO₂ environments should emit more isoprene as a protection from heat stress, as high levels of CO₂ reduces stomatal conductance, increasing leaf temperature (Sharkey et al. 2008; Sun et al. 2013; Wilkinson et al. 2009). Therefore, dynamic photosynthesis in isoprene-emitting plants such as understory plants in humid tropical forests should benefit from high-CO₂ conditions. However, many observations do not support this hypothesis and show that high levels of CO₂ reduce isoprene emissions (Sharkey et al. 2008). Way et al. (2011) reported that electron transport rates recovered better under sunfleck conditions in isoprene-emitting plants. However, no evidence showed that high levels of CO₂ enhanced isoprene-related tolerance of heat- and light-stressed photosynthesis. These results suggested that isoprene biosynthesis may have evolved at low CO₂ concentrations, where its

physiological effect is greatest, and that elevated CO₂ will reduce the functional benefit of isoprene in the near future. Other hypotheses have been presented (Sharkey et al. 2008) to explain the inconsistencies. Further field studies on the effects of isoprene emissions on dynamic photosynthesis should be conducted to clarify this topic.

Relationship between water and dynamic photosynthesis under high levels of CO₂

Water levels in leaves and in the environment, including in soil and in air, should affect dynamic photosynthesis, mainly because these factors affect stomatal responses to fluctuating light (e.g. Tinoco-Ojanguren and Pearcy 1993). Water stress may shorten the photosynthetic induction time by decreasing stomatal conductance. Under high levels of CO₂, stomatal conductance generally decreases, which should in turn reduce water loss during dynamic photosynthesis (Tang and Liang 2000). However, few studies have assessed the effects of high CO₂ levels on water use during dynamic photosynthesis. In a CO₂ enrichment experiment in grasslands, Knapp et al. (1994) found that a C4 grass had significantly lower water loss under elevated levels of CO₂, due to a rapid stomatal response to fluctuating light and reduced stomatal conductance under low light. However, Marin et al. (2014) reported that under fluctuating light, tobacco leaves showed a significant increase in net photosynthesis but little change in transpiration. They speculated that future increases in atmospheric CO₂ would increase carbon gain in leaves but not change leaf transpiration. In another C3 plant species, *D. sublamellatus*, we found that accumulated water loss decreased during a single sunfleck, although not significantly, in 700 μmol CO₂ mol⁻¹ air compared to 350 μmol CO₂ mol⁻¹ air (Tomimatsu et al. 2014). However, there are no reports that address whether water loss is reduced more during dynamic photosynthesis than during steady-state photosynthesis in high-CO₂ environments.

Effects of high levels of CO₂ on dynamic photosynthesis in relation to plant fitness

FACE experiments have suggested that elevated CO₂ should affect photosynthesis and growth to different extents among plant species (Ainsworth and Long 2005); therefore, dynamic photosynthesis under high levels of CO₂ should also differ among species. For example, Rubisco content decreases under long-term acclimation to elevated CO₂ (Ainsworth and Rogers 2007), which may affect dynamic photosynthesis.

Naumburg and Ellsworth (2000) examined whether shade-tolerant and shade-intolerant species had different photosynthetic responses to sunflecks under high levels of

CO₂. They did not find a distinct difference but concluded that high levels of CO₂ enhanced photosynthetic induction during both dynamic and steady-state photosynthesis (see Tables 1 and 2). Although independent studies have shown that plant species differ in their induction time and stomatal conductance responses after long-term eCO₂ exposure, there is insufficient data to show a systematic difference in dynamic photosynthesis among different functional groups.

Minor physiological changes in individual species can result in large ecological consequences in ecosystems. Changes in dynamic photosynthesis could potentially influence the structure and function of ecosystems; some species grow better and have higher fitness than others, which could lead to further changes in the species composition of plant communities in a world with high levels of CO₂. K  lheim et al. (2002) provided evidence that rapid regulation of dynamic photosynthesis to variation in light contributed to plant fitness. Future studies should clarify the effects of minor physiological differences, such as dynamic photosynthetic response to high levels of CO₂, on plant fitness and species diversity in natural environments.

Conclusions

The global climate is changing not only in terms of increasing atmospheric CO₂ but also in terms of increasing temperature, changes in spatiotemporal precipitation patterns and nitrogen deposition, along with other environmental changes. The effects of high levels of CO₂ on dynamic photosynthesis will be affected by all of these environmental factors. However, there are few studies on how these factors in combination may affect dynamic photosynthesis. Furthermore, the effects of high levels of CO₂ on dynamic photosynthesis have been observed only in a limited number of plant species. Compared to studies on steady-state photosynthesis associated with high levels of CO₂, there are few mechanistic studies on dynamic photosynthesis under high-CO₂ conditions. As a result, the current body of knowledge on dynamic photosynthesis under high levels of CO₂ is insufficient to make reliable assessments and predictions of global climate change, and additional information on photosynthesis in natural environments is necessary. Finally, from an ecological perspective, future studies should aim to understand how dynamic photosynthesis under high-CO₂ conditions will contribute to species coexistence in light-limited ecosystems such as tropical forests.

Acknowledgments We thank Dr. Ichiro Terashima and Dr. Hiroyuki Muraoka for their invitation to this special issue, and their warm encouragement and patience during the preparation of this review paper.

References

- Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. *New Phytol* 165:351–371
- Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions. *Plant Cell Environ* 30:258–270
- Baldocchi D, Falge E, Wilson K (2001) A spectral analysis of biosphere–atmosphere trace gas flux densities and meteorological variables across hour to multi-year time scales. *Agric For Meteorol* 107:1–27
- Bazzaz F, Williams WE (1991) Atmospheric CO₂ concentrations within a mixed forest: implications for seedling growth. *Ecology* 72:12–16
- Buchmann N, Weny KAO, Ehleringer JR (1996) Carbon dioxide concentrations within forest canopies—variation with time, stand structure, and vegetation type. *Glob Chang Biol* 2:421–432
- Carmo-Silva AE, Salvucci ME (2013) The regulatory properties of Rubisco activase differ among species and affect photosynthetic induction during light transitions. *Plant Physiol* 161:1645–1655
- Chazdon RL (1988) Sunflecks and their importance to forest understorey plants. *Adv Ecol Res* 18:1–63
- Chazdon RL, Pearcy RW (1986) Photosynthetic responses to light variation in rain-forest species. 1. Induction under constant and fluctuating light conditions. *Oecologia* 69:517–523
- Crafts-Brandner SJ, Salvucci ME (2000) Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO₂. *Proc Natl Acad Sci* 97:13430–13435
- Day TA, Gober P, Xiong FS, Wentz EA (2002) Temporal patterns in near-surface CO₂ concentrations over contrasting vegetation types in the Phoenix metropolitan area. *Agric For Meteorol* 110:229–245
- Doehrlert DC, Ku MS, Edwards GE (1979) Dependence of the post-illumination burst of CO₂ on temperature, light, CO₂, and O₂ concentration in wheat (*Triticum aestivum*). *Physiol Plant* 46:299–306
- Drake PL, Froend RH, Franks PJ (2013) Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. *J Exp Bot* 64:495–505
- Foyer CH, Neukermans J, Queval G, Noctor G, Harbinson J (2012) Photosynthetic control of electron transport and the regulation of gene expression. *J Exp Bot* 63:1637–1661
- Franks PJ, Beerling DJ (2009) Maximum leaf conductance driven by CO₂ effects on stomatal size and density over geologic time. *Proc Natl Acad Sci USA* 106:10343–10347
- Holiso  va P, Z  itova M, Klem K, Urban O (2012) Effect of elevated carbon dioxide concentration on carbon assimilation under fluctuating light. *J Environ Qual* 41:1931–1938
- Horton P, Ruban A, Walters R (1996) Regulation of light harvesting in green plants. *Annu Rev Plant Biol* 47:655–684
- Kaiser E, Morales A, Harbinson J, Kromdijk J, Heuvelink E, Marcelis LF (2015) Dynamic photosynthesis in different environmental conditions. *J Exp Bot* 66:2415–2426
- Katul G, Lai C-T, Sch  fer K, Vidakovic B, Albertson J, Ellsworth D, Oren R (2001) Multiscale analysis of vegetation surface fluxes: from seconds to years. *Adv Water Resour* 24:1119–1132
- Kirschbaum MU, Pearcy RW (1988a) Gas exchange analysis of the fast phase of photosynthetic induction in *Alocasia macrorrhiza*. *Plant Physiol* 87:818–821
- Kirschbaum MU, Pearcy RW (1988b) Gas exchange analysis of the relative importance of stomatal and biochemical factors in photosynthetic induction in *Alocasia macrorrhiza*. *Plant Physiol* 86:782–785

- Knapp A, Fahnestock J, Owensby C (1994) Elevated atmospheric CO₂ alters stomatal responses to variable sunlight in a C4 grass. *Plant Cell Environ* 17:189–195
- Kono M, Terashima I (2014) Long-term and short-term responses of the photosynthetic electron transport to fluctuating light. *J Photochem Photobiol B Biol* 137:89–99
- Kosvancova M, Urban O, Sptova M, Hrstka M, Kalina J, Tomaskova I, Spunda V, Marek MV (2009) Photosynthetic induction in broadleaved *Fagus sylvatica* and coniferous *Picea abies* cultivated under ambient and elevated CO₂ concentrations. *Plant Sci* 177:123–130
- Külheim C, Ågren J, Jansson S (2002) Rapid regulation of light harvesting and plant fitness in the field. *Science* 297:91–93
- Laisk A, Edwards GE (1997) Post-illumination CO₂ exchange and light-induced CO₂ bursts during C4 photosynthesis. *Funct Plant Biol* 24:517–528
- Laisk A, Kiirats O, Oja V (1984) Assimilatory Power (Postillumination CO₂ Uptake) in Leaves. *Plant Physiol* 76:723–729
- Leakey A, Press M, Scholes J, Watling J (2002) Relative enhancement of photosynthesis and growth at elevated CO₂ is greater under sunflecks than uniform irradiance in a tropical rain forest tree seedling. *Plant Cell Environ* 25:1701–1714
- Leakey AD, Scholes JD, Press MC (2005) Physiological and ecological significance of sunflecks for dipterocarp seedlings. *J Exp Bot* 56:469–482
- Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants face the future. *Annu Rev Plant Biol* 55:591–628
- Marin D, Martin M, Serrot PH, Sabater B (2014) Thermodynamic balance of photosynthesis and transpiration at increasing CO₂ concentrations and rapid light fluctuations. *Biosystems* 116:21–26
- Martin M, Noarbe DM, Serrot PH, Sabater B (2015) The rise of the photosynthetic rate when light intensity increases is delayed in ndh gene-defective tobacco at high but not at low CO₂ concentrations. *Front Plant Sci* 6:34
- Müller P, Li X-P, Niyogi KK (2001) Non-photochemical quenching. A response to excess light energy. *Plant Physiol* 125:1558–1566
- Naumburg E, Ellsworth DS (2000) Photosynthetic sunfleck utilization potential of understory saplings growing under elevated CO₂ in FACE. *Oecologia* 122:163–174
- Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R, Church JA, Clarke L, Dahe Q, Dasgupta P, Dubash NK, Edenhofer O, Elgizouli I, Field CB, Forster P, Friedlingstein P, Fuglestedt J, Gomez-Echeverri L, Hallegatte S, Hegerl G, Howden M, Jiang K, Jimenez Cisneros B, Kattsov V, Lee H, Mach KJ, Marotzke J, Mastrandrea MD, Meyer L, Minx J, Mulugetta Y, O'Brien K, Oppenheimer M, Pereira JJ, Pichs-Madruga R, Plattner GK, Pörtner HO, Power SB, Preston B, Ravindranath NH, Reisinger A, Riahi K, Rusticucci M, Scholes R, Seyboth K, Sokona Y, Stavins R, Stocker TF, Tschakert P, van Vuuren D, van Ypersele JP (2014) Climate Change 2014: Synthesis Report. In: Pachauri R, Meyer L (eds) Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Geneva, Switzerland, IPCC, p 151
- Pearcy RW (1990) Sunflecks and photosynthesis in plant canopies. *Annu Rev Plant Biol* 41:421–453
- Pearcy RW (1999) Responses of plants to heterogeneous light environments. In: Pugnaire FI, Valladares F (eds) Handbook of functional plant ecology, Marcel Dekker Inc., New York Basel, pp 269–314
- Pearcy RW (2007) Responses of plants to heterogeneous light environments. In: Pugnaire FI, Valladares F (eds) Functional plant ecology. CRC Press, Boca Raton, pp 213–257
- Pearcy R, Pfitsch WA (1995) The consequences of sunflecks for photosynthesis and growth of forest understory plants. In: Schulze ED, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer, Berlin, pp 343–359
- Pearcy RW, Way DA (2012) Two decades of sunfleck research: looking back to move forward. *Tree Physiol* 32:1059–1061
- Pearcy RW, Chazdon RL, Gross LJ, Mott KA (1994) Photosynthetic utilization of sunflecks: a temporally patchy resource on a time scale of seconds to minutes. In: Caldwell MM, Pearcy RW (eds) Exploitation of environmental heterogeneity by plants. Academic Press, San Diego, pp 175–208
- Pearcy RW, Krall JP, Sassenrath-Cole GF (1996) Photosynthesis in fluctuating light environments. In: Baker NR (ed) Photosynthesis and the environment. Springer, Berlin, pp 321–346
- Rascher U, Nedbal L (2006) Dynamics of photosynthesis in fluctuating light. *Curr Opin Plant Biol* 9:671–678
- Raven JA (2014) Speedy small stomata? *J Exp Bot* 65:1415–1424
- Rochaix JD (2011) Reprint of: regulation of photosynthetic electron transport. *Biochim Biophys Acta* 1807:878–886
- Sage RF (1994) Acclimation of photosynthesis to increasing atmospheric CO₂: The gas exchange perspective. *Photosynth Res* 39:351–368
- Sage RF, Sharkey TD, Seemann JR (1989) Acclimation of photosynthesis to elevated CO₂ in five C3 species. *Plant Physiol* 89:590–596
- Sharkey TD, Seemann JR, Pearcy RW (1986) Contribution of metabolites of photosynthesis to postillumination CO₂ assimilation in response to lightflecks. *Plant Physiol* 82:1063–1068
- Sharkey TD, Wiberley AE, Donohue AR (2008) Isoprene emission from plants: why and how. *Ann Bot* 101:5–18
- Soleh MA, Tanaka Y, Nomoto Y, Iwahashi Y, Nakashima K, Fukuda Y, Long AP, Shiraiwa T (2016) Factors underlying genotypic differences in the induction of photosynthesis in soybean [*Glycine max* (L.) Merr.]. *Plant Cell Envi* 39:685–693
- Sun Z, Niinemets U, Huve K, Rasulov B, Noe SM (2013) Elevated atmospheric CO₂ concentration leads to increased whole-plant isoprene emission in hybrid aspen (*Populus tremula* × *Populus tremuloides*). *New Phytol* 198:788–800
- Tang Y, Liang N (2000) Characterization of the photosynthetic induction response in a *Populus* species with stomata barely responding to light changes. *Tree Physiol* 20:969–976
- Tang Y, Washitani I, Tsuchiya T, Iwaki H (1988) Fluctuation of photosynthetic photon flux density within a *Miscanthus sinensis* canopy. *Ecol Res* 3:253–266
- Tikhonov AN (2015) Induction events and short-term regulation of electron transport in chloroplasts: an overview. *Photosynth Res* 125:65–94
- Tinoco-Ojanguren C, Pearcy RW (1993) Stomatal dynamics and its importance to carbon gain in two rainforest *Piper* species. I. VPD effects on the transient stomatal response to lightflecks. *Oecologia* 94:388–394
- Tomimatsu H, Tang YH (2012) Elevated CO₂ differentially affects photosynthetic induction response in two *Populus* species with different stomatal behavior. *Oecologia* 169:869–878
- Tomimatsu H, Iio A, Adachi M, Saw LG, Fletcher C, Tang Y (2014) High CO₂ concentration increases relative leaf carbon gain under dynamic light in *Dipterocarpus sublamellatus* seedlings in a tropical rain forest, Malaysia. *Tree Physiol* 34:944–954
- Vines HM, Tu Z-P, Armitage AM, Chen S-S, Black CC (1983) Environmental responses of the post-lower illumination CO₂ burst as related to leaf photorespiration. *Plant Physiol* 73:25–30
- Way DA, Pearcy RW (2012) Sunflecks in trees and forests: from photosynthetic physiology to global change biology. *Tree Physiol* 32:1066–1081
- Way DA, Schnitzler JP, Monson RK, Jackson RB (2011) Enhanced isoprene-related tolerance of heat- and light-stressed photosynthesis at low, but not high, CO₂ concentrations. *Oecologia* 166:273–282

- Wilkinson MJ, Monson RK, Trahan N, Lee S, Brown E, Jackson RB, Polley HW, Fay PA, Fall RAY (2009) Leaf isoprene emission rate as a function of atmospheric CO₂ concentration. *Glob Chang Biol* 15:1189–1200
- Woodward F, Kelly C (1995) The influence of CO₂ concentration on stomatal density. *New Phytol* 131:311–327
- Yamori W, Masumoto C, Fukayama H, Makino A (2012) Rubisco activase is a key regulator of non-steady-state photosynthesis at any leaf temperature and to a lesser extent, of steady-state photosynthesis at high temperature. *Plant J* 71:871–880