



Elevated CO₂ Enhances Dynamic Photosynthesis in Rice and Wheat

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Crops developed under elevated carbon dioxide (eCO₂) exhibit enhanced leaf photosynthesis under steady states. However, little is known about the effect of eCO₂ on dynamic photosynthesis and the relative contribution of the short-term (substrate) and long-term (acclimation) effects of eCO₂. We grew an *Oryza sativa japonica* cultivar and a *Triticum aestivum* cultivar under 400 μmol CO₂ mol⁻¹ air (ambient, A) and 600 μmol CO₂ mol⁻¹ air (elevated, E). Regardless of growth [CO₂], the photosynthetic responses to the sudden increase and decrease in light intensity were characterized under 400 (a) or 600 μmol CO₂ mol⁻¹ air (e). The Aa¹, Ae², Ea³, and Ee⁴ treatments were employed to quantify the acclimation effect (Ae vs. Ee and Aa vs. Ea) and substrate effect (Aa vs. Ae and Ea vs. Ee). In comparison with the Aa treatment, both the steady-state photosynthetic rate (P_N) and induction state (IS) were higher under the Ae and Ee treatments but lower under the Ea treatment in both species. However, IS reached at the 60 sec after the increase in light intensity, the time required for photosynthetic induction, and induction efficiency under Ae and Ee treatment did not differ significantly from those under Aa treatment. The substrate effect increased the accumulative carbon gain (ACG) during photosynthetic induction by 45.5% in rice and by 39.3% in wheat, whereas the acclimation effect decreased the ACG by 18.3% in rice but increased it by 7.5% in wheat. Thus, eCO₂, either during growth or at measurement, enhances the dynamic photosynthetic carbon gain in both crop species. This indicates that photosynthetic carbon loss due to an induction limitation may be reduced in the future, under a high-CO₂ world.

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INTRODUCTION

According to IPCC (2014), “the atmospheric CO₂ concentration is projected to reach beyond 550 μmol CO₂ mol⁻¹ air by 2100 under high emissions scenarios.” increase in atmospheric CO₂ has been reported to enhance photosynthesis under constant light conditions, i.e., steady-state photosynthesis (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007). However, terrestrial plants in natural environments are exposed to fluctuating light

¹Aa, plants grown at 400 μmol mol⁻¹ CO₂ were measured at 400 μmol mol⁻¹ CO₂.

²Ae, plants grown at 400 μmol mol⁻¹ CO₂ were measured at 600 μmol mol⁻¹ CO₂.

³Ea, plants grown at 600 μmol mol⁻¹ CO₂ were measured at 400 μmol mol⁻¹ CO₂.

⁴Ee, plants grown at 600 μmol mol⁻¹ CO₂ were measured at 600 μmol mol⁻¹ CO₂.

(Percy, 1983; Tang et al., 1988; Percy et al., 1990), which increases leaf carbon gain more than steady light under long-term exposure to elevated CO₂ (eCO₂) (Leakey et al., 2002). Thus, knowledge about photosynthetic responses to fluctuating light, i.e., dynamic photosynthesis, at eCO₂ conditions helps improve our understanding of the future global carbon flux.

Plasticity in photosynthesis in response to long-term eCO₂ can be attributed to the short-term (substrate) effect, long-term (acclimation) effect, or both. The substrate effect is related to increased CO₂ supply, which is reported to enhance dynamic photosynthesis greatly (Tomimatsu and Tang, 2012; Tomimatsu et al., 2014, 2019; Kaiser et al., 2017b). The acclimation effect is related to variations in leaf morphological, anatomical, and biochemical traits, but little information is known about its role and relative contribution.

The activation of Calvin-Benson cycle enzymes and stomatal opening regulate the photosynthetic response to a sudden increase in light intensity (Way and Percy, 2012; Kaiser et al., 2015) and are affected by variations in leaf chemical and morphological traits. Leaves exposed to long-term eCO₂ have a lower Rubisco activase (Rca) content (Geiger et al., 1999; Aranjuelo et al., 2011; Tomimatsu et al., 2019) and develop small stomata (Maherali et al., 2002; Zhu et al., 2018; Zheng et al., 2019). The rate of Rubisco activation is proportional to Rca content (Woodrow and Mott, 1989; Yamori et al., 2012). Stomatal morphology affects the stomatal opening kinetics in some species (Drake et al., 2013; Kardiman and Ræbild, 2018; Zhang et al., 2019), although contradictory evidence has also been reported (Elliott-Kingston et al., 2016; McAusland et al., 2016). Whether and how acclimation to eCO₂ affects dynamic photosynthesis remains untested.

All studies listed in **Table 1** have investigated woody species, except for one study on C₄ grass (Knapp et al., 1994). Rice and wheat are important for the global population as direct sources of food (FAO, 2016); yet, there are no available reports on the effect of long-term eCO₂ on dynamic photosynthesis in rice, wheat, or any other C₃ crop plants. The light environments for crop plants are characterized by long periods of sunlight punctuated by shade flecks (Percy et al., 1990), whereas those for within-canopy woody plants are characterized by long periods of diffuse light punctuated by sunflecks (Tang et al., 1988; Chazdon and Percy, 1991). The slow photosynthetic induction in wheat costs at least 21% of its daily potential assimilation (Taylor and Long, 2017). Thus, it is of great importance to investigate whether acclimation to eCO₂ improves dynamic photosynthesis in C₃ crop plants.

In this study, we aimed to address: (1) how eCO₂ affects dynamic photosynthesis; (2) the relative contribution of the acclimation and substrate effects of eCO₂ on dynamic photosynthesis.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The study was conducted at the Agricultural Meteorology and Ecology Experimental Station, Nanjing University of Information Engineering, Nanjing, China (32°16′N, 118°46′E).

An *Oryza sativa japonica* cultivar (Nanjing 9108) and a *Triticum aestivum* cultivar (Yangmai 22) were used. There are many cultivars of rice and wheat since both are widely cultivated crop species with a very long cultivation history. Nanjing 9108 was nominated as “super rice” by the Chinese Ministry of Agriculture in 2015, whereas Yangmai 22 is a high-yield wheat cultivar. Currently, both are the major and most widely cultivated cultivars in the middle and lower reaches of the Yangtze River. The rice seeds were sown (rice, May 2017; wheat, October 2017) at 400 μmol CO₂ mol⁻¹ air. Afterward, the rice seedlings were transplanted into octagonal open-top chambers (OTCs) with 400 (ambient, denoted by A) or 600 μmol CO₂ mol⁻¹ air (elevated, denoted by E). The wheat seeds were directly sown in the soil in each OTC. The OTCs were 3 m high with a bottom area of ~12 m². Ventilation fans were installed on the inner walls of the OTCs to minimize the impact of heterogeneous temperature and CO₂ concentration on the height of the flag leaves. The plants grown within the core area of an ambient [CO₂] OTC and an elevated [CO₂] OTC were selected for the experiment. The soil was carefully plowed before transplanting the rice seedlings and sowing the wheat seeds. These practices allowed for fairly homogeneous soil conditions for the two CO₂ treatments (OTCs), which were only 8–10 m apart from each other. The soil nutrition content at a 0–20-cm depth before sowing the wheat seeds was 1.25 vs. 1.34 g N kg⁻¹, .84 vs. .83 g P kg⁻¹, and 18.1 vs. 18.08 g K kg⁻¹ for the ambient vs. elevated OTC, respectively. Atmospheric environments during the growth period were also similar between the two OTCs (**Supplementary Figure 1**). Furthermore, all plants in the two treatments received the same management practices, e.g., fertilization, irrigation, and pest control. All these conditions ensured that the major difference between the two treatments was growth CO₂ concentration. We then focused on the biological replication, i.e., the individual plants, for photosynthetic measurement replicates. The measurements were conducted during grain filling: rice, September 15–28, 2017; wheat, April 20–27, 2018.

Gas Exchange Measurement

In both species, flag leaves contribute an important portion of assimilates used for grain filling (Yoshida, 1981; Carmo-Silva et al., 2017). We then decided on all the measurements to be conducted with the flag leaves. Gas exchange parameters were measured on the south-facing, fully expanded flag leaves with portable infrared gas analyzers (Li-Cor 6400 and Li-Cor 6800, LI-COR Biosciences, Lincoln, NE, USA). Air temperature during grain filling was slightly higher for rice than for wheat; therefore, the block temperature was set to 32.5°C for rice and 30°C for wheat. Relative humidity was maintained at 60–65%. Measurements were made on three to four leaves from different plants (one leaf per plant).

To determine the responses of the net photosynthetic rate to the intercellular CO₂ concentration (P_N-C_i curves, equivalent to $A-C_i$ curves as in common usage), leaves were acclimated to a saturating light intensity of 1,500 μmol photons m⁻² s⁻¹ at a CO₂ concentration identical to growth [CO₂]. Then, the CO₂ concentration in the reference cell was varied from 50

TABLE 1 | Summaries of previous studies addressing the effect of long-term elevated carbon dioxide (eCO₂) on photosynthetic induction.

References	Species	Metabolism/ life form	CO ₂ treatment ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$)	Treatment duration	Percentage change per 100 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$ (%)				
					$T_{P50\%}$	$T_{P90\%}$	$T_{g50\%}$	$T_{g90\%}$	τ_R
Knapp et al. (1994)	<i>Andropogon gerardii</i> Vitman	C ₄ /grass	ambient vs. double ambient [†]	< 1 year					
Naumburg and Ellsworth (2000)	<i>Acer rubrum</i>	C ₃ /woody	365 vs. 569	14–15 years	U	U			
	<i>Liriodendron tulipifera</i>	C ₃ /woody	365 vs. 569	14–15 years	U	U			
	<i>Cornus florida</i>	C ₃ /woody	365 vs. 569	14–15 years	U	U			
	<i>Liquidambar styraciflua</i>	C ₃ /woody	365 vs. 569	14–15 years	U	U			
Leakey et al. (2002)	<i>Shorea leprosula</i> Miq.	C ₃ /woody	377 vs. 710	216 days	+29.71	−8.00	+14.00	+7.43	
Holišova et al. (2012)	<i>Fagus sylvatica</i>	C ₃ /woody	355 vs. 724	3 year		−1.43			
	<i>Picea abies</i>	C ₃ /woody	355 vs. 724	3 year		−8.00			
Tomimatsu and Tang (2012)	<i>Populus euramericana</i> cv. I-55	C ₃ /woody	380 vs. 720	60 days	−24.61	−15.62			
	<i>Populus koreana</i> × <i>trichocarpa</i> cv. Pea	C ₃ /woody	380 vs. 720	60 days	−11.14	−5.01			
Tomimatsu et al. (2019)	<i>Populus koreana</i> × <i>trichocarpa</i> cv. Pea	C ₃ /woody	380 vs. 1020	60 days	−7.16	−3.14			

Data were taken from tables and figures shown in the articles using WebPlotDigitizer version 4.2 (<https://automeris.io/WebPlotDigitizer/index.html>). Shown are the normalized effects of eCO₂, which were calculated as percentage changes in parameters divided by the differences in CO₂ concentrations applied. "U" indicates no changes in the parameter. Numbers in bold style indicate significant eCO₂ effects on the parameters at P = 0.05 level.

[†]Ambient CO₂ concentration was 330–340 $\mu\text{mol L}^{-1}$.

to 1,500 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air. Altogether, 14 different CO₂ concentrations were investigated.

To characterize dynamic photosynthesis, we measured the photosynthetic time course in response to a simulated increase and decrease in light intensity. The leaves were first acclimated at 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for over 30 min, then the light intensity within the leaf chamber was increased to 1,500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, and kept constant for 5 min. Afterward, the light intensity was decreased to 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and kept constant for 5 min. The net photosynthetic rate and stomatal conductance (g_s) were recorded every second for the entire measurement period. Photosynthetic characteristics in plants grown at 600 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air were influenced not only by the short-term but also the long-term effect of eCO₂, in comparison with plants grown at 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air. To distinguish both effects, we measured dynamic photosynthesis at 400 (denoted by *a*) and 600 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air (denoted by *e*), regardless of growth [CO₂]. The differences between the two growth conditions [CO₂] under the same measurement [CO₂] (i.e., *Ae* vs. *Ee* and *Aa* vs. *Ea*) were considered to be caused by the acclimation effect of eCO₂, while the differences between the two measurements [CO₂] from the same growth [CO₂] (i.e., *Aa* vs. *Ae* and *Ea* vs. *Ee*) were considered to be due to the instantaneous substrate effect of eCO₂.

Carbon and Nitrogen Analysis

The leaves used for the gas exchange measurements were collected, brought to the laboratory, and then oven-dried at 65°C for 48 h before grinding. The carbon and nitrogen concentrations of ground samples were determined using a CHNOS elemental analyzer (Vario EL III, Elementar Analysensysteme GmbH, Langensfeld, Germany).

Stomatal Anatomy

Five flag leaves other than those used for the gas exchange measurements from the same OTC were sampled and kept in a 2.5% glutaraldehyde solution. Images of the center of the abaxial leaf surface at $\times 800$ magnification for rice and $\times 400$ for wheat were acquired with a scanning electron microscope (FEI Quanta 200F, Thermo Fisher Scientific, Waltham, MA, USA). Stomatal density and guard cell length were determined using the ImageJ software (National Institute of Health, Bethesda, MD, USA) from 10–20 fields of view.

Data Analysis

To evaluate the rates of photosynthetic and stomatal induction responses, we calculated the time to reach 50 and 90% of the steady-state photosynthetic rate (P_N) ($T_{P50\%}$ and $T_{P90\%}$) and g_s ($T_{g50\%}$ and $T_{g90\%}$) at 1,500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Photosynthetic induction state (IS) was calculated after Chazdon and Pearcy (1986):

$$IS(t) = \frac{P_N(t) - P_{N,100}}{P_{N,1500} - P_{N,100}} * 100\% \quad (1)$$

where $P_{N,100}$ is the steady-state P_N reached at 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and $P_{N,1500}$ is the steady-state P_N reached at 1,500 μmol

$\text{photons m}^{-2} \text{ s}^{-1}$. Both were calculated by averaging single values over the last half-minute of each period.

We assumed that the transition between the Rubisco-limited state and the ribulose 1,5-bisphosphate (RuBP) regeneration-limited state occurs at a C_i of 200–400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air at ambient growth [CO₂] and a C_i of 400–600 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air at elevated growth [CO₂] during the induction response. Then, P_N - C_i curves were divided into two parts and fitted separately to obtain the apparent maximum Rubisco carboxylation rate ($V_{c,max}$), electron transport rate at 1,500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (J_{1500}), and CO₂ photo compensation point (Γ^*). Parameters estimated from the P_N - C_i curves were then used to correct transient P_N by removing stomatal limitations. Since RuBP regeneration limitation typically relaxes within 2 min (Taylor and Long, 2017) and triose phosphate use limitation barely occurs at the CO₂ concentration investigated in this study (Long and Bernacchi, 2003), the P_N was corrected only 2 min after the increase in light intensity. Under Rubisco limitation, P_N at time t can be calculated after Farquhar et al. (1980):

$$P_N(t) = V_{c,max}(t) \left[\frac{C_i(t) - \Gamma^*}{C_i(t) + K_m} \right] - R_L \quad (2)$$

where $V_{c,max}(t)$ and $C_i(t)$ is $V_{c,max}$, and C_i at time t , respectively, K_m is the Michaelis–Menten constants of Rubisco taken from Bernacchi et al. (2001), and R_L is mitochondrial respiration rate in the light and assumed to be 60% of dark respiration rate (Way et al., 2019), which was determined in a preliminary test. To remove the stomatal limitation, P_N was recalculated by replacing $C_i(t)$ with the final, steady-state C_i ($C_{i,t}$):

$$P_N^*(t) = V_{c,max}(t) \left[\frac{C_{i,f} - \Gamma^*}{C_{i,f} + K_m} \right] - R_L \quad (3)$$

where $P_N^*(t)$ is corrected $P_N(t)$. Combining Equations (1, 2) to eliminate the unknown variable $V_{c,max}(t)$:

$$P_N^*(t) = \frac{[P_N(t) + R_L](C_{i,f} - \Gamma^*)[C_i(t) + K_m]}{[C_i(t) - \Gamma^*](C_{i,f} + K_m)} - R_L \quad (4)$$

The time courses of P_N^* were then fitted to the model proposed by Woodrow and Mott (1989):

$$P_N^*(t) = P_{N,f}^* - (P_{N,f}^* - P_{N,i}^*) * e^{(-\frac{t}{\tau_R})} \quad (5)$$

where $P_{N,f}^*$ and $P_{N,i}^*$ are the final corrected and estimated initial photosynthetic rate, respectively; τ_R is the apparent time constant of Rubisco activation. Diffusional and biochemical limitations were estimated after Kaiser et al. (2017a).

Accumulative carbon gain (ACG) during a period of time was calculated as

$$ACG(t) = \int_{t_0}^t P_N(t) dt - P_{N,100} * (t - t_0) \quad (6)$$

where t_0 is the time when light intensity was increased. The eCO₂ may affect the steady-state P_N and photosynthetic

induction, both of which result in changes in transient P_N during photosynthetic induction and, thus, affect ACG (**Figure 1**). To distinguish the relative contribution of eCO₂ to the ACG via accelerated photosynthetic induction from the enhanced steady-state P_N , we decomposed ACG into ideal carbon gain (ICG) and induction efficiency (IE). The ICG during the same period of time was defined as

$$ICG(t) = (P_{N,1500} - P_{N,100}) * (t - t_0) \quad (7)$$

As Equation (7) shows, ICG is determined by steady-state P_N only. Thus, IE is calculated by dividing the ACG by the ICG, after Tang et al. (1994):

$$IE(t) = \frac{\int_{t_0}^t P_N(t) dt - P_{N,100} * (t - t_0)}{(P_{N,1500} - P_{N,100}) * (t - t_0)} \quad (8)$$

Induction efficiency is linearly correlated with $T_{P90\%}$ (**Supplementary Figure 2**); thus, the accelerated photosynthetic induction will result in a higher IE. The relative contribution of eCO₂ on ACG via accelerated photosynthetic induction is represented as the percentage difference in ACG under the *Aa* treatment and estimated using IE under the *Ae* and *Ee* treatments.

Additionally, post-illumination carbon gain (PICG) due to the simulated lightfleck was calculated as,

$$PICG = \int_{t_1}^t [P_N(t) - P_{N,50}] dt, \text{ when } P_N(t) > P_{N,50} \quad (9)$$

where t_1 is the time when light intensity was decreased.

Statistical Analysis

A two-way ANOVA was performed to evaluate the contribution of the acclimation and substrate effects and their interaction. When the requirement of the normality and homogeneity of variances were met, the differences in the means of different treatments (*Aa*, *Ae*, *Ea*, and *Ee*) were then assessed by a least significant difference test. All statistical analyses were carried out with SPSS Statistics Version 26 for Windows (IBM Corp., Armonk, NY, USA) at a significance level of 0.05.

RESULTS

Steady-State Photosynthesis Under Different CO₂ Treatments

In both species, the $P_{N,100}$ and $P_{N,1,500}$ in leaves from both growth [CO₂] treatments were higher when the measurements were conducted under eCO₂ (**Table 2**). The averaged $P_{N,1,500}$ under the *Ee* treatment was significantly higher than that under the *Aa* treatment by 30.5% in rice and 37.8% in wheat. The averaged $P_{N,50}$ did not differ significantly across treatments in rice. In wheat, $P_{N,50}$ was significantly lower under the *Ea* treatment compared with the other three treatments. In both species, $g_{s,1,500}$ did not differ significantly across treatments (**Table 2**). In rice,

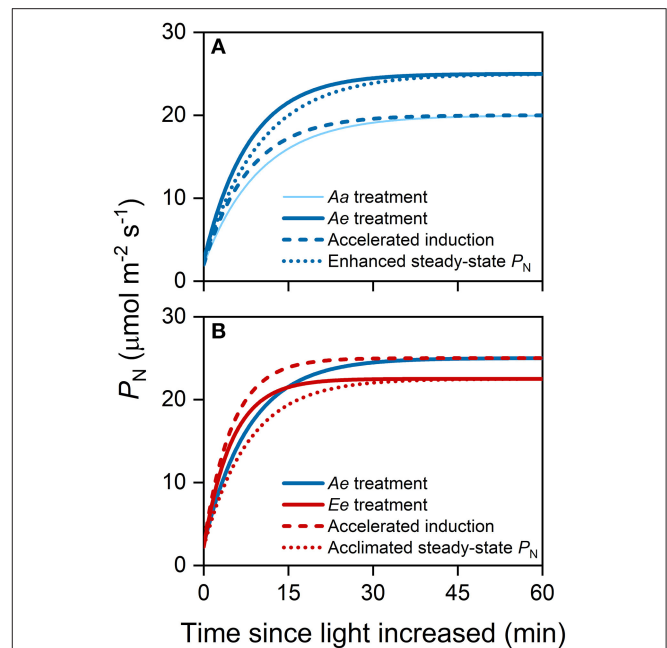


FIGURE 1 | An example illustrating the substrate effect of elevated carbon dioxide (eCO₂) (**A**) and the acclimation effect of eCO₂ (**B**) on accumulative carbon gain (ACG) via changes in steady-state photosynthetic rates (P_N) and photosynthetic induction. In this study, we assume that the acclimation to long-term eCO₂ accelerates photosynthetic induction but decreases the steady-state P_N at high light intensity. All lines were modeled with an exponential function. (**A**) The thin solid curve represents the time course of P_N following an increase in light intensity from 100 to 1,500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ under the *Aa* treatment, while the thick solid curve represents that under *Ae* treatment. The dashed blue curve represents the time course of P_N considering accelerated photosynthetic induction only, i.e., the rate of photosynthetic induction is the same as that under the *Ae* treatment. The dotted blue curve represents the time course of P_N considering changes in steady-state P_N only i.e., steady-state P_N are the same as that under the *Ae* treatment. (**B**) The solid red curve represents the time course of P_N under the *Ee* treatment. The dashed red curve represents the time course of P_N considering changes in photosynthetic induction only, i.e., the rate of photosynthetic induction is the same as that under the *Ee* treatment. The dotted red curve represents the time course of P_N considering changes in steady-state P_N only i.e., the final, steady-state P_N is the same as that under the *Ee* treatment.

$g_{s,100}$ did not differ significantly across treatments, however, $g_{s,50}$ was higher under the *Ae* and *Ea* than under the *Ee* and *Aa* treatments. In wheat, both $g_{s,100}$ and $g_{s,50}$ were higher under the *Aa* and *Ee* than under the *Ae* and *Ea* treatments. The averaged $C_{i,50}$, $C_{i,100}$, and $C_{i,1,500}$ were higher when measurements were conducted under eCO₂ than under ambient CO₂ in both species.

In rice, the differences in $P_{N,100}$ and $P_{N,1,500}$ were attributable to measurement [CO₂] only, whereas the differences in $g_{s,100}$ and $g_{s,1,500}$ were attributable to the interaction of growth and measurement [CO₂] only. The differences in $g_{s,1,500}$ were also, to a less extent, attributable to measurement [CO₂]. In wheat, the differences in P_N and g_s were attributable to measurement [CO₂]; the differences in $P_{N,100}$ and $g_{s,100}$ were also attributable to the interaction of growth and measurement [CO₂].

TABLE 2 | Steady-state photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO₂ concentration (C_i) reached under different light intensities in rice and wheat.

Parameter	CO ₂ treatment			
	Aa	Ae	Ea	Ee
Rice				
$P_{N,100}$	2.22 ± 0.38a	3.77 ± 0.38b	3.08 ± 0.40ab	3.57 ± 0.17b
$g_{s,100}^{\dagger}$	0.058 ± 0.006	0.094 ± 0.012	0.104 ± 0.021	0.080 ± 0.010
$C_{i,100}$	323 ± 4a	506 ± 17b	335 ± 6a	502 ± 10b
$P_{N,1,500}$	18.98 ± 0.73a	28.04 ± 1.59b	18.45 ± 0.61a	24.77 ± 1.01b
$g_{s,1,500}^{\dagger}$	0.393 ± 0.040	0.430 ± 0.022	0.551 ± 0.057	0.361 ± 0.030
$C_{i,1,500}$	272 ± 10a	424 ± 8b	299 ± 5c	423 ± 6b
$P_{N,50}$	0.21 ± 0.10	0.84 ± 0.18	1.09 ± 0.34	0.59 ± 0.10
$g_{s,50}$	0.036 ± 0.003a	0.082 ± 0.006b	0.069 ± 0.016bc	0.050 ± 0.004ac
$C_{i,50}$	378 ± 4a	564 ± 2b	363 ± 5c	560 ± 4b
Wheat				
$P_{N,100}$	4.41 ± 0.21a	4.78 ± 0.30a	3.46 ± 0.21b	5.07 ± 0.13a
$g_{s,100}$	0.268 ± 0.027a	0.130 ± 0.014b	0.175 ± 0.019bc	0.199 ± 0.024c
$C_{i,100}$	357 ± 3a	518 ± 8b	353 ± 3a	536 ± 5c
$P_{N,1,500}$	28.24 ± 0.65a	37.65 ± 1.05b	26.70 ± 1.34a	38.91 ± 1.39b
$g_{s,1,500}^{\ddagger}$	0.643 ± 0.043	0.503 ± 0.037	0.547 ± 0.047	0.523 ± 0.019
$C_{i,1,500}$	275 ± 4a	406 ± 6b	270 ± 1a	406 ± 4b
$P_{N,50}$	1.58 ± 0.15a	1.39 ± 0.15a	0.62 ± 0.13b	1.51 ± 0.07a
$g_{s,50}^{\dagger}$	0.427 ± 0.043a	0.195 ± 0.038b	0.249 ± 0.019ab	0.256 ± 0.011ab
$C_{i,50}$	382 ± 1a	571 ± 1b	385 ± 1a	574 ± 1b

Values are the means (± SE) of three to four biological replicates, i.e., individual plants for each species. Different letters following the means indicate significant ($P < 0.05$) differences across treatments within each species.

$P_{N,50}$, $P_{N,100}$, $P_{N,1,500}$, $g_{s,50}$, $g_{s,100}$, $g_{s,1,500}$, $C_{i,50}$, $C_{i,100}$, and $C_{i,1,500}$ were steady-state photosynthetic rate (unit $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance for H₂O (unit $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and intercellular CO₂ concentration (unit $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$) reached at 50, 100, and 1,500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, respectively, calculated by averaging single values over the last half-minute of each period. The uppercase letters A and E indicate ambient and elevated growth [CO₂], respectively, and the lowercase letters a and e indicate ambient and elevated measurement [CO₂] respectively.

[†]Statistical analysis using a Dunnett's T3 test.

[‡]Statistical analysis using a Kruskal–Wallis test.

Dynamic Photosynthesis Under Different CO₂ Treatments

In both species, P_N increased faster when measurements were conducted under eCO₂ after the sudden increase in light intensity (Figures 2A,B). During the photosynthetic induction and post-illumination period, transient P_N was higher under the Ae than under the Ee treatment in rice but was higher under the Ee than under the Ae treatment in wheat. Transient IS was highest under the Ee treatment and lowest under the Ea treatment in both species (Figures 2C,D). In rice, the IS was similar between the Aa and Ae treatments, whereas, in wheat, the IS was surprisingly higher under the Aa than under the Ae treatment over the first 5 min of photosynthetic induction. In rice, transient g_s was higher under the Ae and Ea than under the Aa and Ee treatments (Figure 2E). In wheat, transient g_s was higher under the Aa than under the Ae treatment but was similar between the Ea and Ee treatments (Figure 2F). During the first 10 min of photosynthetic induction, transient C_i was higher under the Ee than under the Ae treatment in both species (Figures 2G,H). Transient C_i was higher under the Ea than under the Aa treatment in rice but was higher under the Aa than under the Ea treatment in wheat. During

the post-illumination period, transient C_i was similar across treatments in both species.

In both species, the averaged $TP_{50\%}$ and $TP_{90\%}$ were lower under the Ee than under the Aa and Ae treatments, despite that such differences were insignificant (Figures 3A,B). In wheat plants grown at an ambient CO₂ concentration, $T_{g50\%}$ was significantly higher under the Ae than under the Aa treatment, whereas $T_{g90\%}$ was higher under the Aa than the Ae treatment (Figures 3C,D). In wheat plants grown at an eCO₂ concentration, both $T_{g50\%}$ and $T_{g90\%}$ were higher under the Ea than under the Ee treatment. However, $T_{g50\%}$ and $T_{g90\%}$ were similar in rice leaves from different growth conditions [CO₂] or between different measurements [CO₂] from the same growth [CO₂]. No significant differences in the IS_{60s} values across treatments were found in both species (Figure 3E). In both species, τ_R was shorter when measurements were conducted under eCO₂ (Figure 3F). In wheat, the differences in $TP_{90\%}$ were attributable to measurement [CO₂] and the interaction of growth and measurement [CO₂] (Table 3). The differences in $T_{g50\%}$ in both species were attributable to the interaction of growth and measurement [CO₂] only. In both species, the diffusional limitation was similar between the Aa and the Ea treatment, i.e.,

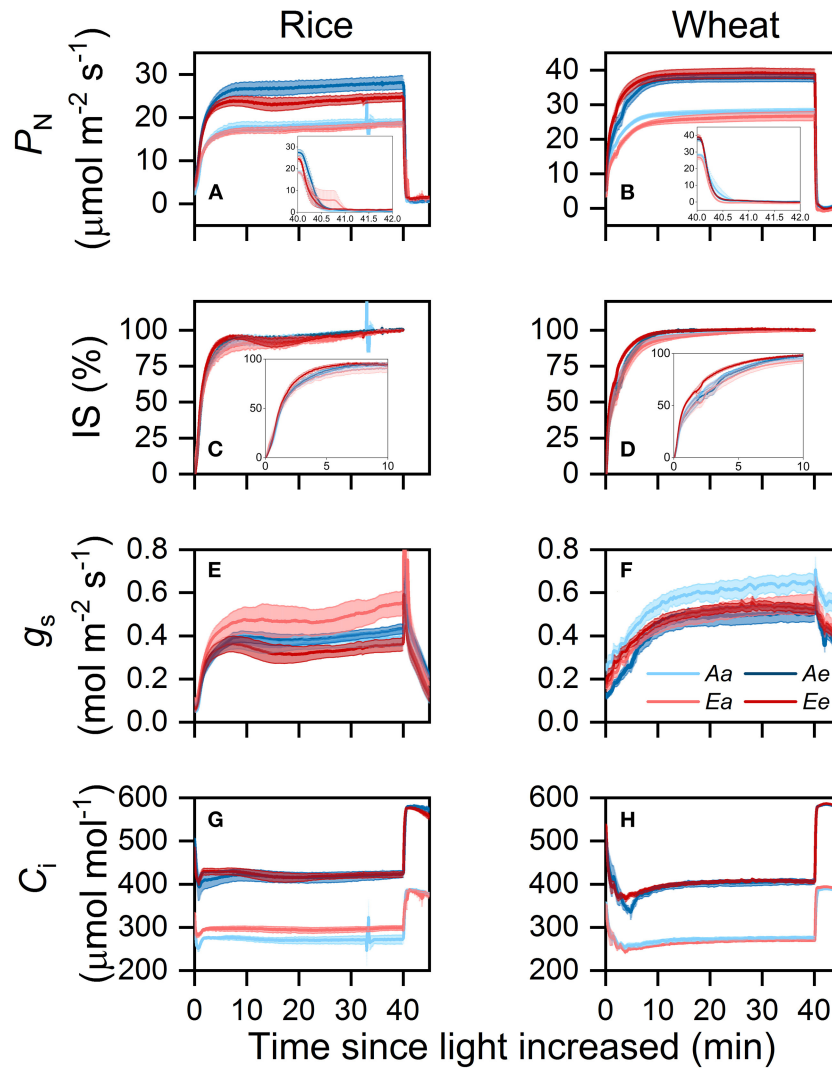


FIGURE 2 | Time courses of P_N (A,B), IS (C,D), g_s (E,F), and C_i (G,H) in rice (A,C,E,G) and wheat (B,D,F,H) leaves following an increase in light intensity from 100 to 1,500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and then a decrease in light intensity from 1,500 to 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Values are presented as the means \pm SE of three to four biological replicates, i.e., individual plants for each species. P_N , photosynthetic rate; IS, induction state; g_s , stomatal conductance; C_i , intercellular CO₂ concentration.

the limitation showed minimal significant differences in leaves from different growth conditions [CO₂] when measurements were conducted under ambient CO₂. However, the diffusional limitation was lower under the *Ee* than under the *Ae* treatment. The biochemical limitation tended to be lower when measurements were conducted under eCO₂, regardless of growth [CO₂] (Supplementary Figure 3). The percentage of diffusional limitation among the total photosynthetic limitation was lower than 10% during most of the time of the photosynthetic induction period (Supplementary Figures 3A,B). Biochemical limitation dominated over diffusional limitation for the first 3 min of induction in both species.

ACG and IE

In comparison with the *Aa* treatment, ACG_{30min} was higher by 45.5 (*Ae*) and 27.2% (*Ee*) in rice (Figure 4A) and by 39.3 (*Ae*) and

46.8% (*Ee*) in wheat (Figure 4B). However, ACG_{30min} was lower under the *Ea* than the *Aa* treatment by 10.9% in rice and by 4.7% in wheat. In comparison with the *Aa* treatment, PICG was higher by 45.4 (*Ae*) and 67% (*Ee*) in rice (Figure 4C) and by 4.7 (*Ae*) and 4.1% (*Ee*) in wheat (Figure 4D). In rice, PICG was higher under the *Ea* than the *Aa* treatment by 29.4% in rice but was lower by 26.3% in wheat.

Despite these changes in ACG_{30min}, the IE_{30min} did not differ significantly among the *Aa*, *Ae*, and *Ee* treatments in both species (Figures 5A,B). In both species, IE_{30min} was lowest under the *Ea* treatment. There were no significant differences observed in IE between the *Aa* and the *Ae* treatments in both species. In wheat, the averaged IE was highest under the *Ee* treatment throughout photosynthetic induction.

To further evaluate the contribution of accelerated photosynthetic induction under the *Ae* and *Ee* treatments

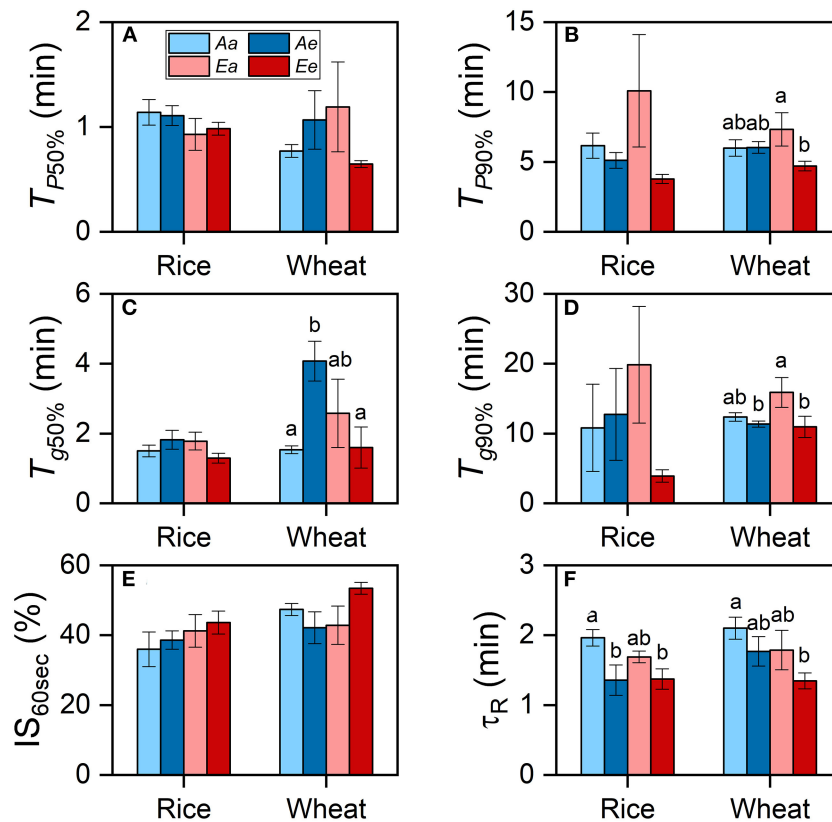


FIGURE 3 | The rates of photosynthetic induction and stomatal opening in rice and wheat leaves. **(A)** Time required for the photosynthetic rate to reach 50% of $P_{N,1,500}$ ($T_{P50\%}$). **(B)** Time required for the photosynthetic rate to reach 90% of $P_{N,1,500}$ ($T_{P90\%}$). **(C)** Time required for stomatal conductance to reach 50% of $g_{s,1,500}$ ($T_{g50\%}$). **(D)** Time required for stomatal conductance to reach 90% of $g_{s,1,500}$ ($T_{g90\%}$). **(E)** The IS reached 30 s following an increase in light intensity (IS_{60s}). **(F)** The apparent time constant of Rubisco activation (τ_R). Bars and vertical lines indicate the means and standard errors of three to four biological replicates, i.e., individual plants for each species, respectively. Different letters above error bars indicate significant differences between two treatments within each species. The absence of letters denotes the absence of significant difference.

to ACG_{30min}, we estimated the potential ACG_{30min}, assuming no effect of eCO₂ on steady-state P_N , which was equivalent to no changes in ICG. Compared with the Aa treatment, the increase in IE_{30min} under Ae alone increased ACG_{30min} by 0.6% in rice and by 0.9% in wheat, whereas the increase in IE_{30min} under Ee alone increased ACG_{30min} by 0.4% in rice and by 3.3% in wheat (Figures 6A,B). The changes in steady-state P_N under Ae alone increased ACG_{30min} by 44.5% in rice and 38.1% in wheat, whereas the changes in steady-state photosynthesis under Ae alone increased ACG_{30min} by 26.3% in rice and 42.1% in wheat.

Rubisco Carboxylation Capacity, Stomatal Anatomy, and Element Analysis

The Rubisco carboxylation capacity ($V_{c,max}$) was lower at elevated than at ambient growth [CO₂]; the difference was significant in wheat (Supplementary Table 1). Regardless of growth [CO₂], $V_{c,max}$ was higher in wheat than in rice. There were no significant differences in stomatal density at the two growth conditions [CO₂] (Supplementary Table 1). Guard cells were shorter at elevated than at ambient growth [CO₂]; the difference was significant in wheat. No significant differences in

carbon or nitrogen content were found between ambient and elevated growth [CO₂] (Supplementary Table 1).

DISCUSSION

Increased CO₂ Supply Enhances Leaf Dynamic Photosynthesis Without Accelerating Photosynthetic Induction in Crops

Several studies reported accelerated photosynthetic induction in woody species at eCO₂ (Leakey et al., 2002; Holíšová et al., 2012; Tomimatsu and Tang, 2012). In this study, the differences in T_p between the Aa and Ae treatments were small in two crop species (Figures 3A,B), and the differences in $T_{P50\%}$ were not related to different measurement [CO₂]. The modeling analysis also indicates that the changes in IE_{30min} under the Ae treatment alone increased ACG_{30min} by <1% in both species (Figure 6). On the contrary, the changes in steady-state P_N alone increased ACG_{30min} by more than one-third in both species (Figure 6). Acevedo-Siaca et al. (2020)

TABLE 3 | The influences of growth (G) and measurement CO₂ concentration (M) on the differences in the photosynthetic characteristics of rice and wheat.

Parameter	Factors		
	Growth [CO ₂]	Measurement [CO ₂]	G × M
Rice			
<i>P</i> _{N,50}	2.838	0.110	8.912**
<i>P</i> _{N,100}	1.249	12.005**	3.232
<i>P</i> _{N,1,500}	3.693	76.750***	1.432
<i>g</i> _{s,50}	0.000	2.531	15.160***
<i>g</i> _{s,100}	1.607	0.229	5.953*
<i>g</i> _{s,1,500}	1.727	4.973*	11.119**
<i>T</i> _{P50%}	2.970	0.015	0.190
<i>T</i> _{P90%}	0.457	3.689	1.874
<i>T</i> _{g50%}	0.399	0.193	4.363*
<i>T</i> _{g90%}	0.000	1.700	2.759
IS _{60s}	2.350	0.564	0.001
τ _R	0.606	7.724	0.770
IE _{30min}	0.546	1.095	0.395
ACG _{30min}	5.374*	43.884***	0.343
PICG	1.129	2.990	0.027
Wheat			
<i>P</i> _{N,50}	14.087***	9.541**	22.845***
<i>P</i> _{N,100}	2.882	26.314***	10.332**
<i>P</i> _{N,1,500}	0.020	118.795***	1.977
<i>g</i> _{s,50}	4.752*	17.685***	20.164***
<i>g</i> _{s,100}	0.374	9.378**	18.529***
<i>g</i> _{s,1,500}	1.360	6.140*	3.067
<i>T</i> _{P50%}	0.000	0.313	3.552
<i>T</i> _{P90%}	0.000	4.300*	4.582*
<i>T</i> _{g50%}	1.656	1.979	10.051**
<i>T</i> _{g90%}	1.753	6.336*	2.765
IS _{60s}	1.070	0.672	5.868*
τ _R	3.825	4.224	0.083
IE _{30min}	0.036	8.278**	4.200*
ACG _{30min}	0.121	132.070***	2.373
PICG	2.054	6.274*	1.838

Shown are Wald χ^2 statistics followed by significance symbols, which are **P* < 0.05, ***P* < 0.01, and ****P* < 0.001, respectively.

reported that, in rice, increases in the photosynthetic carbon gain in fluctuating light are smaller compared with those in steady state. If the contribution of improved transient *P*_N at eCO₂ on carbon gain were excluded in their study, the increased CO₂ supply may play a limited role in accelerating photosynthetic induction in crops. Thus, an increased CO₂ supply improves crop photosynthesis by improving steady-state and transient *P*_N (Table 2, Figures 2A,B), rather than accelerating photosynthetic induction.

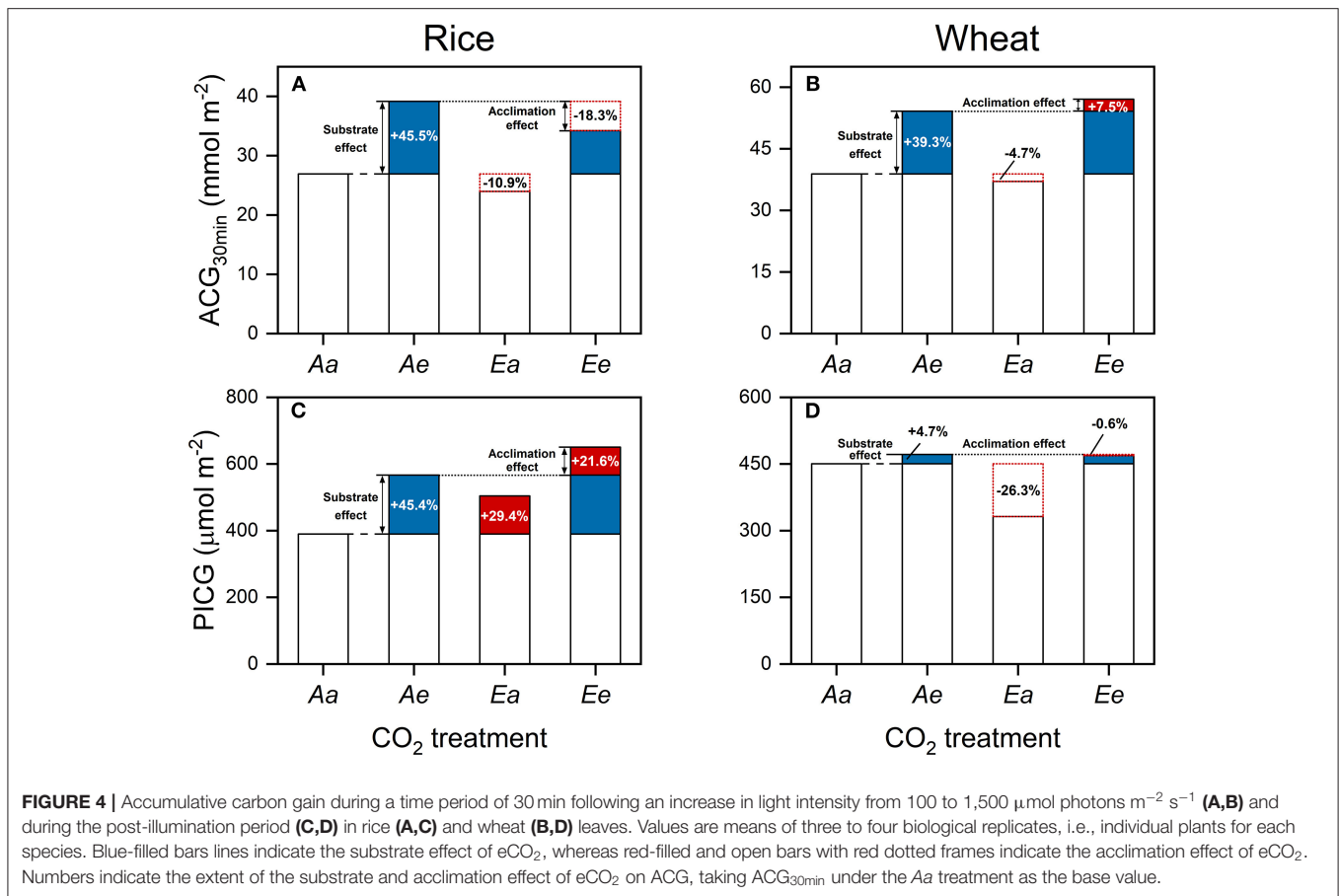
There are no relevant investigations on why an increased CO₂ supply imposes limited influences on the rate of photosynthetic induction in crop species, however, we hypothesized that dumbbell-shaped stomata, which respond to light faster than kidney-shaped stomata (Franks and Farquhar, 2007; McAusland

et al., 2016; Harrison et al., 2020), lower the diffusion limitation imposed on photosynthetic induction in the crop species. Therefore, crop species may exhibit little changes in the rates of photosynthetic induction when there is a minimal increase in CO₂ supply. The difference in measurement [CO₂] in our study (200 μmol CO₂ mol⁻¹ air) was smaller than that in the studies reporting significant effects of eCO₂ on photosynthetic induction (Table 1).

Differential Effect of Elevated CO₂ on Leaf Carbon Gain in Rice and Wheat

The effect of acclimation to eCO₂ has rarely been distinguished from the effect of increased CO₂ supply because the measurement [CO₂] was the same as the growth [CO₂] in previous studies. In comparison with the *Aa* treatment, the acclimation effect alone resulted in a 18.3% decrease in ACG_{30min} in rice but a 7.5% increase in ACG_{30min} in wheat (Figures 4A,B), suggesting the differential influences of the acclimation effect on the photosynthetic carbon gain between the two crop species. Such differences are likely to be related to the difference in the acclimation effect on *P*_{N,1,500}, because the high IE_{30min} under the *Ee* treatment led to a smaller increase in ACG_{30min} than high *P*_{N,1,500}, correspondingly ICG_{30min}, under the *Ee* treatment in both species (Figure 6). Previous eCO₂ experiments also show that the enhancement of photosynthesis is greater in wheat than in rice (Long et al., 2006). Wheat allocates less leaf nitrogen to Rubisco and a greater catalytic constant of Rubisco carboxylation than rice (Supplementary Table 1), and thus may have greater benefits from eCO₂ (Makino, 2011).

In this study, IS_{60s}, *T*_{P90%}, and IE did not differ significantly between *Ee* and *Ae*, or even between the *Ee* and *Aa* treatments (Figures 2C,D, 3B, 5), suggesting limited influence of acclimation effect on the rate of photosynthetic induction. At timescales of minutes, the rate of photosynthetic induction is mainly determined by the light-activation of Rubisco and stomatal opening (Way and Percy, 2012; Kaiser et al., 2015). To speed up the rate of Rubisco activation, more resources should be allocated to Rca, which is necessary for Rubisco activation (Yamori et al., 2012). This requirement is unlikely to be met at eCO₂, as previous studies reported the decreased content of Rca in rice (Chen et al., 2005) and wheat (Zhang et al., 2009; Aranjuelo et al., 2011). This is consistent with the finding that τ_R (Figure 3F) and biochemical limitation (Supplementary Figures 3C,D) did not differ significantly between the *Ae* and *Ee* treatments in both species. On the contrary, the differences in *T*_P between the *Ae* and *Ee* treatments may be related to the changes in the rate of stomata opening. Accumulating evidence demonstrates that species with fast stomatal opening show fast photosynthetic induction (Drake et al., 2013; Deans et al., 2019; Yamori et al., 2020). In comparison with the *Ae* treatment, the stomata tended to open faster (Figures 3C,D) and stomatal limitation tended to be lower (Supplementary Figures 3A,B) under the *Ee* treatment in both species. We hypothesized that the faster stomatal opening in the leaves acclimated to eCO₂ was achieved by a decrease in guard cell length (Supplementary Table 1), which is consistent



with previous studies (Maherali et al., 2002; Zhu et al., 2018; Zheng et al., 2019). In general, small dumbbell-shaped stomata open faster than large ones within the same genus (McAusland et al., 2016). However, a negative correlation between stomatal size and $T_{g50\%}$ in the genus *Oryza* was reported recently (Zhang et al., 2019). More research is needed to address how acclimation to eCO₂ in stomatal morphology affects photosynthesis.

Implications

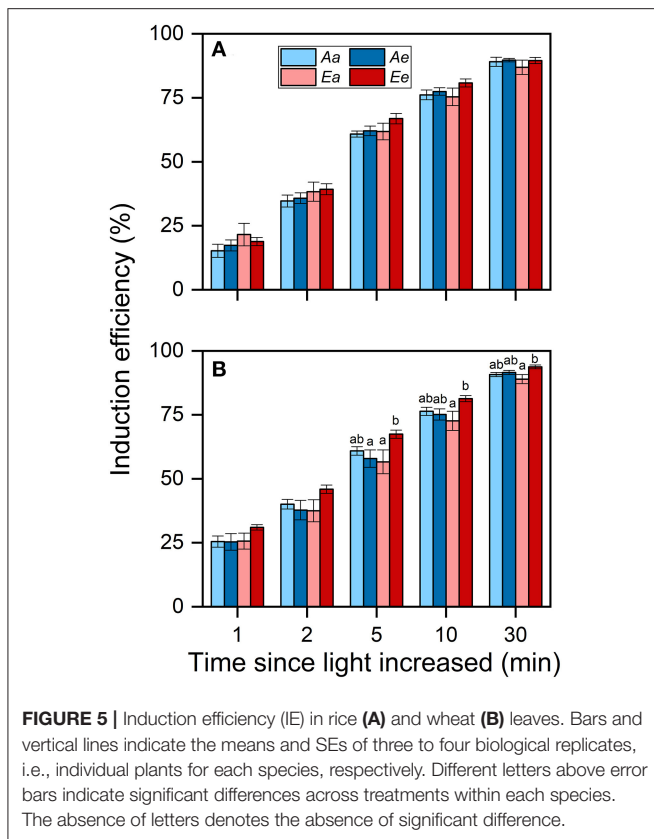
The effects of eCO₂ on the dynamic photosynthesis in crops have been rarely investigated until recently (Acevedo-Siaca et al., 2020; Ohkubo et al., 2020). This study showed that eCO₂ has limited influences on the rates of photosynthetic induction in two crop species but increased photosynthetic carbon gain greatly by improving steady-state and transient P_N . These findings indicate that photosynthetic carbon loss due to induction limitation may be reduced in the future, under a high CO₂ world. We acknowledge that the photosynthetic responses to increasing CO₂ concentration are complex. To project crop photosynthesis and yield in the future, both field experiments and *in silico* modeling, spanning a wide range of CO₂ concentrations, are urgently needed (Drag et al., 2020). The findings in this study, which clarified the contribution of the substrate and acclimation effects of eCO₂, imply that dynamic photosynthesis is likely to reduce photosynthetic induction limitation under

temporally changing light environments in the future, under a high CO₂ world.

In comparison with wheat, the photosynthetic acclimation to eCO₂ in rice compromised the beneficial effect of an increased CO₂ supply on ACG_{30min} but further improved PICG (Figure 4A). Such an increase in PICG may counterbalance the decrease in ACG if rice leaves receive many brief (<1 min) sunflecks. Nonetheless, photosynthetic characteristics vary greatly among rice and wheat accessions (Qu et al., 2017; Salter et al., 2019; Acevedo-Siaca et al., 2020), and the effects of eCO₂ on photosynthesis are likely to differ between them. The results presented here do not necessarily suggest an advantageous position for wheat in the future. Instead, this study may provide a potential reason for the lower enhancement of yield in rice than in wheat at eCO₂ (Long et al., 2006), though detailed assessments are needed because of the large variations in photosynthetic light utilization among them.

CONCLUSIONS

By examining dynamic photosynthesis under four different CO₂ treatments, this study showed that neither an increased CO₂ supply nor an acclimation to eCO₂ imposes large influences on the rates of photosynthetic induction in two crop species. But, an increased CO₂ supply enhances photosynthetic carbon gain



greatly *via* improving steady-state P_N . The acclimation effect of eCO₂ may compromise, or slightly strengthen, the beneficial effect of an increased CO₂ supply, depending on the species and the fluctuations in light intensity. Our study suggests that the photosynthetic carbon gain in the two crop species is likely to be enhanced in a CO₂-enriched future when photosynthetic induction limitation becomes significant for leaf carbon gain.

DATA AVAILABILITY STATEMENT

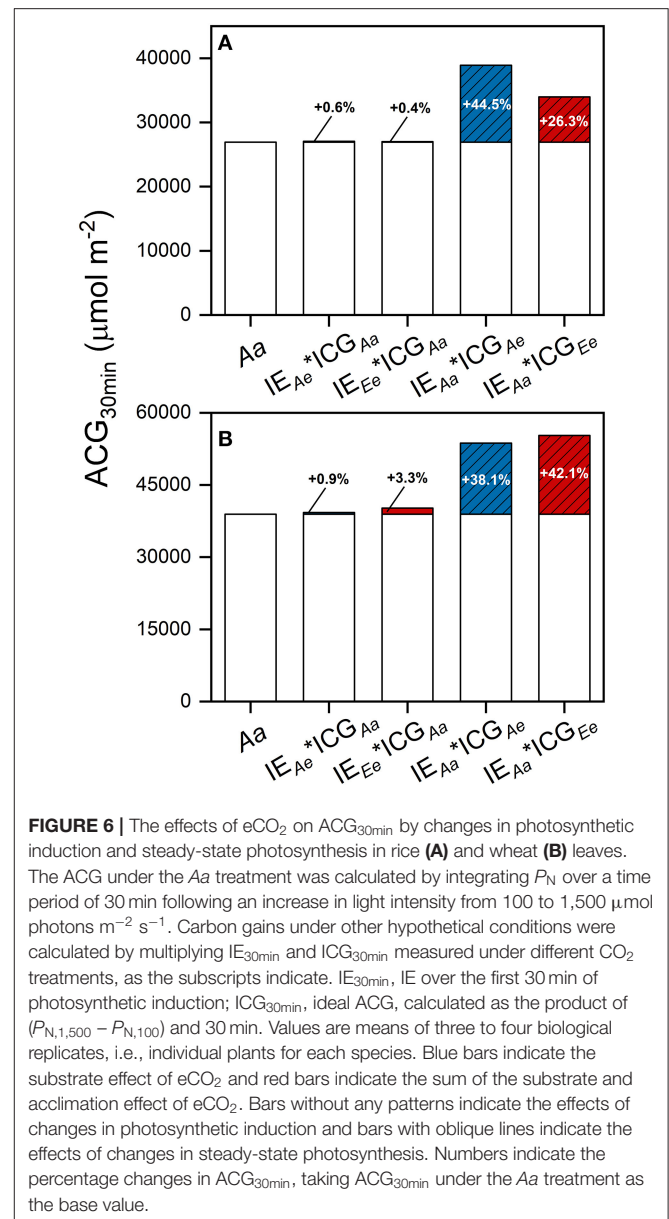
The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

YT and YH conceived and designed the experiment. WS and ZH provided the experimental materials. HK, TZ, YZ, XK, and HS conducted the experiment and collected data. HK, TZ, and YZ analyzed the data and drafted the manuscript. All authors contributed to the editing and revising of the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.727374/full#supplementary-material>

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