

Multi-year [CO₂] elevation improved the efficiency of PSII in a Japonica rice

Chunhua Lv¹, Yao Huang², Kai Yang³, Jingrui Yang³, Peipei Cao³, Zhenhua Hu⁴, Wenjuan Sun², and Lingfei Yu³

¹Affiliation not available

²Chinese Academy of Sciences

³Institute of Botany Chinese Academy of Sciences

⁴Nanjing University of Information Science and Technology School of Applied Meteorology

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Abstract

The response of crop photosystem activity to a certain level of [CO₂] elevation have been widely concerned. However, long-term effects of elevated [CO₂] over multi-generations of crops received little attention. Using open-top chambers, we set up two treatments of elevated [CO₂] from 2016 to 2019 in rice (*Oryza sativa*) growing seasons. One treatment was stepwise increase (SI) of +40 $\mu\text{mol mol}^{-1}$ per season, the other was constant increase (CI) of +200 $\mu\text{mol mol}^{-1}$. Rice seeds harvested in each [CO₂] environment was planted successively. Seeds from ambient [CO₂] were also planted in SI and CI OTCs in next year to study the short-term effect. We measured the diurnal change in PSII functionality of leaf in 2019. Results showed that both SI and CI promoted PSII function. SI was more beneficial to improve efficiency of electron trapping and transporting in PSII and performance index of leaf. The maximal photochemical efficiency of PSII decreased linearly with increasing photosynthetic photon flux density throughout the day. Predawn efficiency decreased dramatically with the development of growth stages. Together, we found PSII efficiency benefit from multi-year [CO₂] elevation, which could help to better understand the response of electron transport to elevated [CO₂].

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Running head : rice response to multi-year [CO₂] elevation

Chunhua Lv^{1,2}, Yao Huang^{1,2*}, Kai Yang^{1,2}, Jingrui Yang^{1,2}, Peipei Cao^{1,2}, Zhenghua Hu³, Wenjuan Sun¹, Lingfei Yu¹

¹State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing, China

²University of Chinese Academy of Sciences, Beijing, China

³School of Applied Meteorology, Nanjing University of Information Science and Technology, Nanjing, China

*Correspondence

Yao Huang, State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

Tel.: +86 10 82593955; Fax: +86 10 82596146; Email: huangyao@ibcas.ac.cn

ORCID: <https://orcid.org/0000-0002-0192-1421>

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Abstract

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Keywords

multi-year $[\text{CO}_2]$ elevation, PSII efficiency, rice, F_v/F_m , diurnal change, stepwise increase, constant increase, short-term

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1. Introduction

Atmospheric carbon dioxide concentration ($[\text{CO}_2]$) increased to $411.4 \pm 0.1 \mu\text{mol mol}^{-1}$ in 2019, which was ~47% higher than pre-industrial level. Meanwhile, the annual average growth rate of $[\text{CO}_2]$ has accelerating in the past ten years, from $1.61 \mu\text{mol mol}^{-1}$ in 2009 to $2.6 \mu\text{mol mol}^{-1}$ in 2019 (<https://www.esrl.noaa.gov/>).

Photosynthesis is the process by which plants, algae, cyanobacteria and photosynthetic bacteria convert light energy into chemical energy that supports their activities. Rising $[\text{CO}_2]$ promoted plant photosynthesis and thereby enhanced biomass production and grain yield of crops (Ainsworth & Long, 2004; Ainsworth & Rogers, 2007; Leakey *et al.*, 2009). Photosystem II (PSII) is the first integral membrane protein complex in the light-dependent reactions that executes the initial reaction of photosynthesis in plants (Taiz & Zeiger, 2010). Within PSII, antenna complexes absorbed photons of light to power the transfer of electron from the reaction center to the primary quinone acceptor of PSII, QA (Kalaji *et al.*, 2014; Nobel, 2020). Electron transport through PSII was most susceptible to environment changes (Taub *et al.*, 2000). As a result, changes of activities of light-harvesting, electron transport and energy-transduction altered metabolic potential for photosynthesis (Lawlor & Tezara, 2009).

The efficiency of an absorbed photon trapped by PSII reactor center could be an estimate of the maximum photochemical efficiency of PSII (F_v/F_m) (Baker & Oxborough, 2004). The mean value of F_v/F_m was 0.83~0.84 for most C3 species (Björkman & Demmig, 1987; Pfündel, 1998), with lowered values indicating stresses on plants (Maxwell & Johnson, 2000; Lichtenthaler *et al.*, 2005), which may be caused by the decrease in the fraction of PSII active reaction centers that are capable of photochemistry and/or the increase in non-photochemical quenching (Baker & Oxborough, 2004). The trapped exciton moved electron into the electron transport chain further QA⁻ and ultimately led to CO_2 fixation (Baker & Oxborough, 2004; Kalaji *et al.*, 2017). Quantum yield of electron transport (ϕE_o) expressed the probability that an absorbed

photon led to an electron transport further QA⁻ (Kalajiet al ., 2011; Holland et al ., 2016). Those processes were at the beginning of the electron flow in photosynthesis (Strasser et al ., 2004), and higher efficiency promoted electron flow from H₂O to NADP⁺. Performance index (PI_{ABS}) was a powerful and integrative expression to investigate plants' overall photosynthetic performance, which covered energy conservation from the absorption of photons by PSII to the reduction of intersystem electron acceptors (Lepedus et al ., 2012; Kalaji et al ., 2018; Faseela et al ., 2019). Those parameters mentioned above, calculated or inferred from chlorophyll fluorescence measurements, were considered to be non-destructive and effective tools and were widespread used to detect the response of PSII efficiency to various environmental stresses (Strasser et al ., 2000; Baker, 2008; Kalaji et al ., 2011; Faseela et al ., 2019), such as high temperature, drought, salt and chemical influences (e.g. Fricke & Peters, 2002; Feng et al ., 2014; Gao et al ., 2016; Zhou et al ., 2018; Y. Li et al ., 2019).

Elevated [CO₂] directly or indirectly impacted the PSII performance (Zong et al ., 2014), and researchers (e.g. Naumburg et al ., 2004; Ruhil et al ., 2015) were increasingly concerned about that. Wheat grown under elevated [CO₂] maintained higher F_v/F_m at tillering and booting stages (Shanmugam et al ., 2013), and elevated [CO₂] induced F_v/F_m reduction at 25 days after anthesis (Martínez-Carrasco et al ., 2005), and Robredo et al . (2010) found that F_v/F_m of barley in well-irrigated was not affect by elevated [CO₂]. However, Zhu et al . (2018) reported that elevated [CO₂] had no effect on wheat F_v/F_m, but decreased the probability that trapped exciton moves electron into the electron transport chain further QA⁻ (ψ_o) and φE_o. Another study suggested that elevated [CO₂] had positive effect on F_v/F_m of aged rice leaves (Ibarakiet al ., 2005).

Those researches have well studied the response of crop photosystem activity to short-term (one growing season) increased [CO₂] by 200~400 μmol mol⁻¹, and the fact that [CO₂] stepwise increases in a long-term was not taken into consideration. For example, the annual average increment of [CO₂] in 2019 was 2.6 μmol mol⁻¹ even if the growth rate has been accelerated (<https://www.esrl.noaa.gov/>). Klironomos (2005) observed significant difference in below-ground plant production between abrupt and stepwise [CO₂] increase treatments. Moreover, abundant researches have evidenced that long-term elevation of [CO₂] often reduce or remove the initial stimulation of photosynthesis resulted from short-term increase [CO₂] (Ainworth & Long, 2004; Long et al ., 2004, Ainsworth & Rogers 2007; Albert et al ., 2011). Furthermore, long-term elevation of [CO₂] led to larger enhancement in grain yield and more reduction in grain quality than short-term increase [CO₂] (X. Li et al ., 2019). However, long-term effects of elevated [CO₂] over multi-generations on PSII functionality remain largely unknown, and similarities and differences of the PSII response to stepwise and constant increase of [CO₂] are unclear.

In addition, effects of elevated [CO₂] on PSII efficiency was observed at different times of a day. For example, F_v/F_m was detected at noon (12:00) (Shanmugam et al ., 2013), during the afternoon (13:00-16:00) (Ziska & Teramura, 1992), and at an hour after sunset (Ibaraki et al ., 2005). The F_v/F_m of rice showed obvious diurnal variation (Li et al ., 2002; Panda. 2011). It is possible that non-photochemical quenching can be completely relaxed and photoinhibition can be reversed after overnight dark adaptation (Logan et al ., 1999; Demmig-Adams et al ., 2006). Therefore, predawn observation was more appropriate if researchers focus on long-term responses of plant to treatment (Kalaji et al ., 2014). A meta-analysis showed that a small decrease in F_v/F_m occurred when measured during the diurnal period than predawn (Poorter et al ., 2019). However, it was not well understood whether the effect of elevated [CO₂] on PSII efficiency in crop was consistent from predawn to dusk in crop.

Rice is one of the three major food crops in the world, which feeds more than half of the world's population. Increased food demand in the future requires more attention to the study of the impact of elevated [CO₂] on rice growth and production.

Therefore, we set up two long-term treatments of elevated [CO₂] from 2016 to 2019 in rice growing seasons. One treatment was a stepwise increase of +40 μmol mol⁻¹ per season, the other was a constant increase of +200 μmol mol⁻¹ for all seasons. We report the difference of the effects between short-term and multi-generations [CO₂] elevation on rice PSII efficiency.

2. Material and Methods

2.1 Study site description

This study was conducted at the agrometeorological experimental station of Nanjing University of Information Science and Technology, Jiangsu Province, China (32.16° N, 118.86° E). This site was located in a subtropical monsoon climate zone, with average annual temperature 15.6°C and average annual rainfall of 1100 mm. The average sunshine hours exceed 1900 hours, and the frost-free period was 237 days. The soil was silty loam texture with the content of sand, silt and clay of 5.3%, 85.2% and 9.5% in tillage soil (0-20 cm). The basic properties of the tillage soil: pH (H₂O) 6.1, bulk density 1.51 g cm⁻³, soil organic carbon 8.8 g kg⁻¹, and total nitrogen 1.2 g kg⁻¹.

2.2 OTC system

Open-top chambers (OTCs) were established to simulate atmospheric CO₂ concentration [CO₂] enrichment from 2016-2019. Two treatments of elevated [CO₂] were set up. One treatment was a stepwise increase (SI) of +40 µmol mol⁻¹ per season, the other was a constant increase (CI) of +200 µmol mol⁻¹ for all seasons. The target [CO₂] in SI was CK+160µmol mol⁻¹ in 2019. Four OTCs was designated as replicates for each treatment and ambient [CO₂] (CK).

The regular octagonal prismatic OTCs (height 3 m and have a bottom area ~10 m²) were lined with aluminum alloy frame and transparent glass, with the top opening slopes inclined inward by 45°. Pure CO₂ was stored in several cylinders and sprayed from emission tubes surrounding the glass wall of OTCs. CO₂ concentration in the OTCs were monitored by sensors (GMM 222, Vaisala Inc., Helsinki, Finland) handing in the center of chamber and regulated by computer program in real time. Temperature sensor was applied to detect and record temperature in OTCs. The photosynthetic photon flux density (PPFD) was monitored in real time by micro meteorological station (AWS800; Campbell Scientific, Inc., USA) installed in experiment field. Information on the CO₂ concentration increment, temperature and PPFD over the entire growth season was summarized in Table 1.

2.3 Rice cultivation

A conventional japonica rice (*Oryza sativa* L.) cv. Nanjing 9108 was planted in three [CO₂] environments: CK, SI and CI in 2016. Rice seeds harvested in each [CO₂] environment was planted in their respective conditions in the next year. Seeds from CK were also planted in SI and CI OTCs to study short-term effect of abrupt increase of [CO₂] by 160µmol mol⁻¹ (AI₁₆₀) and 200 µmol mol⁻¹ (AI₂₀₀) in 2019.

In 2019, seeds were sown under ambient air conditions on May 20. Seedlings were manually transplanted into OTCs at a density of three seedling per hill on June 20. Rice matured on October 28.

Total N was applied at a rate of 25.0 g N m⁻² with basal dressing (60%), one day before transplanting, and top dressing at tillage stage (20%) and booting stage (20%) in 2019. The basal dressing was compound fertilizer (N: P₂O₅: K₂O = 15%: 15%: 15%) and top dressing was urea. Moisture and other field management measures were in accordance with local practices.

2.4 Leaf chlorophyll fluorescence measurements

In 2019, diurnal change in PSII functionality was detected using Plant Efficiency Analyzer (*Pocket-PEA*, Hansatech Instrument, King's Lynn, Norfolk, UK). Three first fully expanded leaves in each type of seedling were randomly selected at jointing (August 7), heading (September 8) and grain filling stages (September 24), respectively. The central part of the selected leaves was dark-adapted for 0.5 hour using leaf clips before measuring at predawn, 7:00, 9:00, 11:00, 13:00, 15:00, 17:00 and 19:00. On the day of observation during each growth period, the dawn time was 4:57, 5:19 and 5:29, and the dark time was 19:24, 18:46 and 18:24, respectively. The temperature and PPFD at each observation time were shown in Fig.1. The saturating light pulse 3500 µmol m⁻²s⁻¹ was applied in *Pocket PEA*.

2.5 Parameter definition and calculation

The parameter F_v/F_m represented the maximum photochemical efficiency of PSII. The probability that trapped exciton by PSII moves an electron further than QA^- was expressed as ψ_o . The parameter ϕE_o stood for electron transport quantum yield. PI_{ABS} was the performance index which integrated different phenomena related to PSII activity.

A relative change (RC_X) was used to determine the variation of parameters from predawn to dusk as follows:

$$RC_X (\%) = \left(\frac{X_o - X_p}{X_p} \right) \times 100\% (1)$$

where X_p and X_o were the values of F_v/F_m , ψ_o , ϕE_o and PI_{ABS} observed at predawn and other time points in a day, respectively.

2.6 Statistical analysis

One-way ANOVA was conducted to test the differences in variables between short-term and multi-year elevation of $[CO_2]$ at each observation time, and the differences in parameters measured at predawn and other time points, and the differences in values among growth periods. Two-way ANOVA was performed to investigate the effects of $[CO_2]$ increase treatments, observation time points and their interactions on PSII efficiency. Pearson correlation was applied to inquiry the relationship between parameters of PSII efficiency. Statistical analysis was completed in IBM SPSS 21.0 (IBM Corp., Armonk, NY, USA).

3. Results

Overall, compared with short-term effect (one growing season) of abrupt increase of $[CO_2]$ by 200 $\mu\text{mol mol}^{-1}$ (AI_{200}), constant increase of $[CO_2]$ (four generations) by 200 $\mu\text{mol mol}^{-1}$ (CI) significantly increased the maximum photochemical efficiency of PSII (F_v/F_m) at jointing stage (Table 2, Fig. 2a), while this promotive effect was not observed at heading and grain filling stages (Table 2, Fig. 2b, c). Stepwise increase of $[CO_2]$ (four generations) by 160 $\mu\text{mol mol}^{-1}$ (SI) dramatically improved F_v/F_m than short-term (one growing season) abrupt increase of $[CO_2]$ by 160 $\mu\text{mol mol}^{-1}$ (AI_{160}) during three growth periods (Table 2, Fig. 2d-f). Moreover, the probability that trapped exciton moves an electron further than QA^- (ψ_o), electron transport quantum yield (ϕE_o) and performance index (PI_{ABS}) under SI were all prominently higher than that under AI_{160} at grain filling stage (Table 2, Figs. 4f, 5f, 6f), but under CI there was only higher PI_{ABS} than under AI_{200} at jointing stage with no significant difference in ψ_o and in ϕE_o (Table 2, Fig 6a).

The F_v/F_m , ψ_o , ϕE_o and PI_{ABS} all showed significant diurnal variation, and the p -values of them among observation time points were all < 0.001 (Table 2). F_v/F_m gradually decreased to the minimum value at midday, and then increased progressively back to predawn level (Fig. 2). That was opposite to the changing trend of PPFD in a day (Fig. 1). F_v/F_m declined with the increase of PPFD ($p < 0.001$) and air temperature ($p < 0.1$) over three growth stages (Fig. 3).

The daily trend of ψ_o and ϕE_o both showed the shape of letter “M”, with the two peak values both appeared at 7:00 and 17:00 at jointing and heading stages, and at 9:00 and 15:00 during grain filling stage in all treatments (Fig. 4, 5). The PI_{ABS} changed during three phases: started rising from predawn to 7:00, then decreased to minimum value at midday, and then rising again (Fig. 6). There was no obvious correlation between the diurnal change of ψ_o , ϕE_o and PI_{ABS} and PPFD or air temperature (correlation coefficient was not shown).

Significant effects of the interaction between $[CO_2]$ increase treatments and observation time points on F_v/F_m , ϕE_o and PI_{ABS} were detected, indicating that effect of SI and AI_{160} on F_v/F_m , ϕE_o and PI_{ABS} were influenced by observation time in a day (Table 2). SI promoted F_v/F_m by reducing midday depression at jointing stage (Fig. 2d). The relative change (RC) of F_v/F_m was 6.5% from predawn to 11:00 under SI, lower than 8.7% under AI_{160} , and F_v/F_m recovered to 93.0%, 95.4% and 97.2% of that predawn observation at 13:00, 15:00 and 17:00, all were higher than that under AI_{160} which were 91.2%, 94.3% and 96.5% (Table 3). When it came to heading stage, SI only reduced the down-regulation of F_v/F_m at midday, with the RC of F_v/F_m from predawn to 11:00 being approximately 2/3 of that in AI_{160} (Table 3). The relative levels of PI_{ABS} between AI_{160} and SI fluctuated over time at jointing and heading stage, which may result in an overall

insignificant difference between SI and AI₁₆₀ (Fig. 6d, e). At grain filling stage, SI improved F_v/F_m , ψ_o , φE_o and PI_{ABS} at all observation time in a day, overall increased by 1.0%, 4.6%, 5.5% and 12.2%, respectively (Fig. 2-5). No significant interaction was found between effects of AI₂₀₀ and CI and observation time points (Table 2).

The largest midday depression of F_v/F_m occurred at heading stage, reduced by 9.0%-14.1% relative to predawn value (Table 3). F_v/F_m under all treatments rebounded to 99% of predawn value until 19:00 when PPFD was 0.4 $\mu\text{mol m}^{-2}\text{s}^{-1}$ during jointing stage, and was fully restored to predawn level at 19:00 during heading stage when the PPFD dropped to 0 (Table 3). At grain filling stage, F_v/F_m under AI₂₀₀ and AI₁₆₀ measured at 17:00 showed no significant difference compared with those observed at predawn, while F_v/F_m under CI and SI were still 1% lower than the predawn values (Table 3).

There was almost no significant difference between the midday ψ_o and the predawn value at jointing and heading stages, with the maximum RC being 13.2% - 14.6% and 9.5% - 12.2% across all treatments, respectively (Table S1). While at grain filling stage, all ψ_o were dramatically greater than those measured at predawn, and the highest RC was 21.5% - 28.8% (Table S1), and the φE_o in a day was obviously higher than that at predawn, except at 13:00 (Table S2). From predawn to 7:00, PI_{ABS} under AI₂₀₀ and CI increased 11%, but AI₂₀₀ and SI caused no marked change at jointing stage, and RC at heading and grain filling stages was enhanced, while CI and SI led to lower RC than AI₂₀₀ and AI₁₆₀, respectively (Table S3). The largest midday attenuation of PI_{ABS} was up to 63.4% on average, which occurred during jointing period (Table S3).

The φE_o was positively correlated with ψ_o and F_v/F_m at jointing and heading stages under all treatments, while there was insignificant correlation with F_v/F_m at grain filling stage under AI₂₀₀ and SI (Table 4). Correlation coefficient between φE_o and ψ_o was greater than that between φE_o and F_v/F_m (Table 4). Moreover, PI_{ABS} was found to be positively correlated with F_v/F_m , φE_o and ψ_o and those three correlations were weaker than the former in sequence, except that no significant correlation was tested between PI_{ABS} and ψ_o under AI₁₆₀ during heading stage (Table 4).

The predawn F_v/F_m at jointing stage was significantly higher than that at heading and grain filling stages, but no significant difference was observed between the latter two stages (Table 5). The predawn F_v/F_m under CI and SI were correspondingly higher than those under AI₂₀₀ and AI₁₆₀ during grain filling stage (Fig. 2c, f). Moreover, ψ_o , φE_o and PI_{ABS} measured at predawn reduced dramatically with the advance of growth periods in all treatments (Table 5). The reduction of F_v/F_m , ψ_o , φE_o and PI_{ABS} from jointing to grain filling stage were 2.6%, 19.9%, 21.9% and 51.9% under CI, all were lower than those under AI₂₀₀, which were 3.3%, 21.4%, 24.1% and 55.7%, respectively (Table 5). SI caused less decrease (2.8%, 20.9%, 23.3%, 54.8%) than AI₁₆₀ (3.2%, 26.3%, 28.6%, 63.4%) in F_v/F_m , ψ_o , φE_o and PI_{ABS} , but slightly more than those under CI (Table 5).

4. Discussion

Diurnal variation of rice F_v/F_m in this study was consistent with what reported by Wu *et al.* (2007) and Panda *et al.* (2011) (Fig. 2). The decrease of F_v/F_m at midday was thought to be photoprotection under high-light intensity and high-temperature rather than photodamage to PSII (Roden & Ball, 1996; Huang *et al.*, 2006), resulted from reversible inactivation of PSII reaction centers, which was responsible for the midday depression in photosynthesis (Roden & Ball, 1996; Panda *et al.*, 2011). The negative correlation between F_v/F_m and PPFD and temperature supported photo-protection theory (Fig. 3).

Elevated [CO₂] enhanced PSII thermotolerance showed by the greater F_v/F_m under high temperature (Taub *et al.*, 2000; Pan *et al.*, 2018). Stepwise increase of [CO₂] (SI) further enhanced high-light and heat resistance than short-term abrupt increase of [CO₂] (AI₁₆₀), demonstrated by alleviated midday depression in F_v/F_m across three growth stages (Table 2; Fig. 2). However, constant increase of [CO₂] (CI) did not show this advantage than short-term abrupt increase of [CO₂] (AI₂₀₀). Different effects of the two treatments of elevated [CO₂] suggested that simulations closer to the actual pattern of [CO₂] increment may be more effective in reflecting crop responses to elevated [CO₂]. Consistent with conjecture of Taub *et al.* (2000), increased PSII thermotolerance under elevated [CO₂] may be associated with lower initial (F_o) at midday,

while Pan *et al.* (2018) reported that elevated $[\text{CO}_2]$ protected PSII by reducing the heat stress-induced reactive oxygen species accumulation. The different results remind us that more mechanism researches should be carried out.

Regardless of different treatment of $[\text{CO}_2]$ increase, the midday decrease in F_v/F_m of rice were 8%, 11% and 7% at jointing, heading and grain-filling stages, respectively (Table 2), lower than 37% at booting stage reported by Wu *et al.* (2007). In addition to the differences in growth periods, it may be related to the different cultivar of rice used in our study (japonica) and the experiment of Wu *et al.* (2007) (indica). When it came to the tolerance to high light, as reported by Li *et al.* (2002) that the decrease of F_v/F_m in high-light resistant japonica rice cv. 9516 was the least, while the depression in strong-light-sensitive indica rice cv. shanyou 63 was the most. Even for two photosensitive indica rice, the F_v/F_m of rice cv. IR42 reduced more than cv. FR13A at midday (Panda *et al.*, 2001). Planting high-light and high-temperature tolerated varieties of rice may help to maintain higher PSII efficiency at noon, thus reducing the midday downregulation of photosynthesis.

Short-term elevation of $[\text{CO}_2]$ (one growing season) enhanced F_v/F_m in japonica rice cv. Fujiyama-5 (Ziska & Teramura, 1992). Multi-year $[\text{CO}_2]$ elevation (four generations) caused higher F_v/F_m than short-term increase of $[\text{CO}_2]$, whether it was a constant increase or a stepwise increase of $[\text{CO}_2]$ (Fig. 2), indicating that long-term increase of $[\text{CO}_2]$ enhanced beneficial changes to improve photosynthesis in rice. As reported by X. Li *et al.* (2019), multi-generational exposure to elevated $[\text{CO}_2]$ could reinforce the response occurred in short-term exposure, so that long-term response of crop to increasing $[\text{CO}_2]$ would not be completely predicted by short-term response to elevated $[\text{CO}_2]$.

The SI treatment, consistent with actual increases pattern of atmospheric $[\text{CO}_2]$, was more conducive to strengthen the advantage mentioned above. It can be indicated by the result that the higher F_v/F_m occurred under SI than AI_{160} across all three growth stages, while F_v/F_m in CI was only higher than AI_{200} at jointing stage (Table 2; Fig. 2). Roden & Ball (1996) showed that the depression in F_v/F_m was associated with the reduced reaction center as reflected by an increase in the minimum fluorescence (F_o) and the increased levels of nonstructural carbohydrates. Since the corresponding nonstructural carbohydrates were not determined, we could not derive the relationship between them, but the reduced F_o corresponded to the increased F_v/F_m under SI and CI (Fig. S1), suggesting that long-term exposure to higher $[\text{CO}_2]$ increased rice reaction center, which helps PSII capture more light quantum for electron transfer.

Significant interactions between $[\text{CO}_2]$ treatments and observation time points on F_v/F_m ($p = 0.003$ at heading and $p = 0.087$ at jointing), PI_{ABS} ($p = 0.046$) and ϕE_o ($p = 0.066$) suggested that attention should be paid to the observation time points in studying the effects of increased $[\text{CO}_2]$ on PSII efficiency (Table 2). Measurements that span a long time in a day may amplify or mask treatment effects due to diurnal variation. Predawn observations of plants that have been dark-adapted overnight may be more effectively to reflect the effects of treatments (Kalaji *et al.*, 2014). Values observed at sunset were almost completely restored to those in the state of predawn, which can be used for analysis. In addition to the treatment effects of elevated $[\text{CO}_2]$, the observations at noon and afternoon may also involve the response of plants to high-light and high-temperature, and the ability to recover.

Contrary to the results of Zhu *et al.* (2018) that short-term elevation of $[\text{CO}_2]$ decreased ϕ_o of wheat, in this study, the marked increase of ϕ_o under SI than AI_{160} at grain filling stage reflected that SI increased plastoquinone pool, thus reduced the accumulation of QA, along with the unhindered donor side of PSII and electron transfer chain (Fig. 4; Fig. S2). This attribution was supported by previous studies revealing that the accumulated QA⁻ and reduced PQ pool caused higher fluorescence of OJ-phase (Kalaji *et al.*, 2011; Tsimilli-michael, 2019). While, CI did not affect ϕ_o , speculating that long-term increment of $[\text{CO}_2]$ mitigated adverse effects of short-term elevation of $[\text{CO}_2]$, and the stepwise increase would be more beneficial to PSII. On the other hand, this might the difference between wheat and rice in response to increased $[\text{CO}_2]$.

The ϕE_o , which was the product of ϕ_o and F_v/F_m , showed stronger correlation with ϕ_o than F_v/F_m (Table 4), indicating that elevated $[\text{CO}_2]$ -induced changes in electron transport had more to do with the changes

in ψ_o than in F_v/F_m . The process of transferring electrons by captured photons in the reaction center may be the rate-limiting step of the initial photochemistry. The dominant role of ψ_o was also reported by Jiang *et al.* (2008). The correlation coefficient between PI_{ABS} and F_v/F_m was the highest, suggesting that F_v/F_m contributed the biggest fraction to the increase in PI_{ABS} due to elevated $[CO_2]$ (Table 4).

The significant decrease in ψ_o , ϕE_o and PI_{ABS} along with the growth periods indicated that rice regulate the PSII efficiency according to growth needs, which may lead to a reduction in photosynthesis (Table 5). The lower attenuation under CI and SI indicated that long-term increase of $[CO_2]$ slowed down the reduction of PSII efficiency from jointing to grain filling stage, which may be a strategy to maintain higher photosynthesis.

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Author contributions

Y.H. and C.L. designed the study. C.L. undertook the experiments with the contribution of K.Y., J.Y. and P.C. C.L. performed analyses and wrote the paper. All authors commented on and approved this manuscript.

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Tables

Table 1 Summary of CO₂ concentration [CO₂] increment, temperature and photosynthetic photon flux density

	2016	2017	2018	2019
CO ₂ concentration increment relative to CK (μmol mol ⁻¹)				
SI	33±7	62±12	107±14	134±18
CI	175±30	165±28	178±23	180±22
Daily air temperature (°C)				
T_{mean}	27.8±3.9	27.6±4.2	27.7±3.6	27.2±3.1
T_{max}	31.7±4.2	31.2±4.9	33.7±4.1	33.6±3.8
T_{min}	24.4±3.9	24.4±4.0	23.3±4.1	22.5±3.4
Mean daily photosynthetic photon flux density (μmol m ⁻² s ⁻¹)	700±312	665±282	656±251	683±233

CK, SI and CI stand for ambient [CO₂], stepwise increase of [CO₂] by ~40 μmol mol⁻¹ in each season, and constant increase of [CO₂] by ~200 μmol mol⁻¹ for all seasons, respectively. T_{mean} , T_{max} and T_{min} are daily mean air temperature, daily maximum air temperature and daily minimum air temperature. Data is mean±SD.

Table 2 Effects of different treatments of [CO₂] elevation, observation time, and their interactions on F_v/F_m (maximum photochemical efficiency of PSII), ψ_o (the probability that trapped exciton moves an elec-

tron further than QA), ϕE_o (electron transport quantum yield) and PI_{ABS} (performance index) during different growth stages.

Models	Growth stages	Factors	F_v/F_m	ϕ_o	ϕE_o	PI_{ABS}
AI ₊₂₀₀ & CI	Jointing	CO ₂	0.005	0.513	0.216	0.011
		time	0.000	0.000	0.000	0.000
		CO ₂ × time	0.202	0.712	0.604	0.425
	Heading	CO ₂	0.565	0.295	0.527	0.733
		time	0.000	0.000	0.000	0.000
		CO ₂ × time	0.448	0.620	0.626	0.727
	Grain filling	CO ₂	0.273	0.718	0.962	0.243
		time	0.000	0.000	0.000	0.000
		CO ₂ × time	0.625	0.588	0.536	0.280
AI ₊₁₆₀ & SI	Jointing	CO ₂	0.000	0.774	0.213	0.811
		time	0.000	0.000	0.000	0.000
		CO ₂ × time	0.087	0.195	0.066	0.028
	Heading	CO ₂	0.031	0.883	0.275	0.536
		time	0.000	0.000	0.000	0.000
		CO ₂ × time	0.003	0.414	0.153	0.046
	Grain filling	CO ₂	0.000	0.000	0.000	0.000
		time	0.000	0.000	0.000	0.000
		CO ₂ × time	0.487	0.322	0.328	0.625

AI₊₂₀₀ and AI₊₁₆₀ were short-term (one growing season) abrupt increase of [CO₂] by ~200 $\mu\text{mol mol}^{-1}$ and ~160 $\mu\text{mol mol}^{-1}$, respectively. CI: constant increase of [CO₂] by ~200 $\mu\text{mol mol}^{-1}$ based on ambient [CO₂] for four generations. SI: stepwise increase of [CO₂] for four generations with the [CO₂] being ~40, 80, 120 and 160 $\mu\text{mol mol}^{-1}$ higher than ambient in each generation. Values are probability levels for significant differences.

Table 3 Relative change in maximum photochemical efficiency of PS II (F_v/F_m) between predawn and other observation time points under different treatment of elevated CO₂ concentration [CO₂].

	Observation time	Observation time	Observation time	Observation time	Observation time	Observation time
	7:00	9:00	11:00	13:00	15:00	17:00
Jointing						
AI ₊₂₀₀	-1.9	-4.5	-9.2	-9.5	-6.1	-3.2
CI	-2.0	-4.6	-8.9	-7.1	-4.6	-3.1
AI ₊₁₆₀	-2.0	-4.3	-8.7	-8.8	-5.7	-3.5
SI	-2.1	-4.1	-6.5	-7.0	-4.6	-2.8
Heading						
AI ₊₂₀₀	-0.4	-5.2	-9.5	-4.8	-3.1	-0.8
CI	-0.5	-4.3	-11.8	-5.7	-4.2	-1.5
AI ₊₁₆₀	-0.2	-4.6	-14.1	-6.9	-4.1	-1.3
SI	-0.6	-5.6	-9.0	-7.1	-3.9	-1.4
Grain filling						
AI ₊₂₀₀	-0.8	-2.2	-5.8	-6.7	-3.4	0.0
CI	-0.7	-3.9	-6.5	-8.0	-3.8	-0.9
AI ₊₁₆₀	-0.8	-3.1	-7.5	-8.3	-4.3	-0.6
SI	-0.9	-2.9	-6.6	-7.2	-3.7	-1.1

AI₊₂₀₀ and AI₊₁₆₀ were short-term (one growing season) abrupt increase of [CO₂] by ~200 $\mu\text{mol mol}^{-1}$ and ~160 $\mu\text{mol mol}^{-1}$, respectively. CI: constant increase of [CO₂] by ~200 $\mu\text{mol mol}^{-1}$ based on ambient [CO₂] for four generations. SI: stepwise increase of [CO₂] for four generations with the [CO₂] being ~40, 80, 120 and 160 $\mu\text{mol mol}^{-1}$ higher than ambient in each generation. Data is the relative change of F_v/F_m (n=12) from predawn to other moments calculated from equation 1.

Table 4 Correlation coefficient between quantum yield for electron transport (ϕE_o) and maximum photochemical efficiency PS II (F_v/F_m) and probability that trapped exciton moves an electron further than QA⁻(ψ_o) under different treatments of elevated CO₂ concentration [CO₂].

Models	Parameters	Jointing	Jointing	Jointing	Heading	Heading	Heading	Grain filling	Grain filling
		F_v/F_m	ψ_o	ϕE_o	F_v/F_m	ψ_o	ϕE_o	F_v/F_m	ψ_o
AI ₊₂₀₀	ϕE_o	0.663 ***	0.944 ***	1	0.497 ***	0.877 ***	1	0.158	0.953 ***
	PI _{ABS}	0.866 ***	0.561 ***	0.772 ***	0.788 ***	0.417 ***	0.746 ***	0.750 ***	0.425 ***
CI	ϕE_o	0.528 ***	0.940 ***	1	0.485 ***	0.704 ***	1	0.313 **	0.936 ***
	PI _{ABS}	0.849 ***	0.534 ***	0.766 ***	0.801 ***	0.178	0.748 ***	0.742 ***	0.452 ***
AI ₊₁₆₀	ϕE_o	0.686 ***	0.944 ***	1	0.685 ***	0.804 ***	1	0.219 *	0.946 ***
	PI _{ABS}	0.868 ***	0.598 ***	0.800 ***	0.775 ***	0.454 ***	0.808 ***	0.770 ***	0.413 ***
SI	ϕE_o	0.387 ***	0.917 ***	1	0.576 ***	0.782 ***	1	0.012	0.942 ***
	PI _{ABS}	0.844 ***	0.390 ***	0.698 ***	0.822 ***	0.342 **	0.789 ***	0.792 ***	0.204 *

AI₊₂₀₀ and AI₊₁₆₀ were short-term (one growing season) abrupt increase of [CO₂] by ~200 $\mu\text{mol mol}^{-1}$ and ~160 $\mu\text{mol mol}^{-1}$, respectively. CI: constant increase of [CO₂] by ~200 $\mu\text{mol mol}^{-1}$ based on ambient [CO₂] for four generations. SI: stepwise increase of [CO₂] for four generations with the [CO₂] being ~40, 80, 120 and 160 $\mu\text{mol mol}^{-1}$ higher than ambient in each generation. Significance levels are indicated by *, ** and *** for $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Table 5 Predawn values of F_v/F_m (maximum photochemical efficiency of PS II), ψ_o (probability that trapped exciton moves an electron further than QA⁻), ϕE_o (electron transport quantum yield) and PI_{ABS} (performance index) and results of analysis of variance among growth stages under different treatment of CO₂ concentration [CO₂] elevation.

Models	Growth stages	F_v/F_m	ψ_o	ϕE_o	PI _{ABS}
AI ₊₂₀₀	Jointing	0.851±0.001 a	0.654±0.005 a	0.557±0.004 a	12.15±0.47 a
	Heading	0.827±0.002 b	0.616±0.010 b	0.510±0.009 b	7.57±0.52 b
	Grain filling	0.823±0.003 b	0.514±0.006 c	0.423±0.006 c	5.38±0.34 c
CI	Jointing	0.854±0.001 a	0.654±0.005 a	0.558±0.004 a	12.66±0.36 a
	Heading	0.830±0.001 b	0.634±0.003 b	0.526±0.003 b	8.35±0.27 b
	Grain filling	0.832±0.001 b	0.524±0.007 c	0.436±0.006 c	6.09±0.25 c
AI ₊₁₆₀	Jointing	0.852±0.001 a	0.653±0.001 a	0.556±0.004 a	12.29±0.44 a
	Heading	0.829±0.002 b	0.627±0.004 b	0.519±0.004 b	7.68±0.37 b
	Grain filling	0.825±0.001 b	0.481±0.008 c	0.397±0.007 c	4.50±0.28 c
SI	Jointing	0.854±0.001 a	0.641±0.004 a	0.548±0.004 a	11.59±0.31 a
	Heading	0.831±0.002 b	0.615±0.005 b	0.512±0.005 b	7.59±0.33 b
	Grain filling	0.830±0.001 b	0.507±0.009 c	0.421±0.008 c	5.24±0.24 c

AI₊₂₀₀ and AI₊₁₆₀ were short-term (one growing season) abrupt increase of [CO₂] by ~200 $\mu\text{mol mol}^{-1}$ and ~160 $\mu\text{mol mol}^{-1}$, respectively. CI: constant increase of [CO₂] by ~200 $\mu\text{mol mol}^{-1}$ based on ambient [CO₂]

for four generations. SI: stepwise increase of $[\text{CO}_2]$ for four generations with the $[\text{CO}_2]$ being ~ 40 , 80 , 120 and $160 \mu\text{mol mol}^{-1}$ higher than ambient in each generation. Data is mean \pm SE ($n=12$). Different letters in the same CO_2 treatment indicate significant difference among growth stages ($p < 0.05$).

Figure legends

Fig. 1. Air temperature and photosynthetic photon flux density (PPFD) at each observation time during jointing, heading and grain filling stages.

Fig. 2 Diurnal variation of maximum photochemical efficiency of PSII (F_v/F_m) under different treatments of CO_2 concentration $[\text{CO}_2]$ elevation. (a) and (d) were measured at jointing stage, (b) and (e) at heading stage, and (c) and (d) at grain filling stage. AI₊₁₆₀ and AI₊₂₀₀ were short-term (one growing season) abrupt increase of $[\text{CO}_2]$ by $\sim 160 \mu\text{mol mol}^{-1}$ and $\sim 200 \mu\text{mol mol}^{-1}$, respectively. CI: constant increase of $[\text{CO}_2]$ by $\sim 200 \mu\text{mol mol}^{-1}$ based on ambient $[\text{CO}_2]$ for four generations. SI: stepwise increase of $[\text{CO}_2]$ for four generations with the $[\text{CO}_2]$ being ~ 40 , 80 , 120 and $160 \mu\text{mol mol}^{-1}$ higher than ambient in each generation. Data is mean \pm SE ($n=12$). Significance levels of F_v/F_m between two treatments at the same time points are indicated by +, * and ** for $p < 0.1$, $p < 0.05$ and $p < 0.01$.

Fig. 3 Correlation between maximum photochemical efficiency of PSII (F_v/F_m) and photosynthetic photon flux density (PPFD) and air temperature ($n=32$) at jointing (a, d), heading (b, e) and grain filling stage (c, f). AI₊₁₆₀ and AI₊₂₀₀ were short-term (one growing season) abrupt increase of $[\text{CO}_2]$ by $\sim 160 \mu\text{mol mol}^{-1}$ and $\sim 200 \mu\text{mol mol}^{-1}$, respectively. CI: constant increase of $[\text{CO}_2]$ by $\sim 200 \mu\text{mol mol}^{-1}$ based on ambient $[\text{CO}_2]$ for four generations. SI: stepwise increase of $[\text{CO}_2]$ for four generations with the $[\text{CO}_2]$ being ~ 40 , 80 , 120 and $160 \mu\text{mol mol}^{-1}$ higher than ambient in each generation. Values are probability levels for significant differences.

Fig. 4 Diurnal variation of efficiency that trapped exciton moves an electron further than QA⁻ in rice under different treatment of CO_2 concentration $[\text{CO}_2]$ elevation. (a) and (d) were measured at jointing stage, (b) and (e) were measured at heading stage, and (c) and (d) were observed at grain filling stage. AI₊₁₆₀ and AI₊₂₀₀ were short-term (one growing season) abrupt increase of $[\text{CO}_2]$ by $\sim 160 \mu\text{mol mol}^{-1}$ and $\sim 200 \mu\text{mol mol}^{-1}$, respectively. CI: constant increase of $[\text{CO}_2]$ by $\sim 200 \mu\text{mol mol}^{-1}$ based on ambient $[\text{CO}_2]$ for four generations. SI: stepwise increase of $[\text{CO}_2]$ for four generations with the $[\text{CO}_2]$ being ~ 40 , 80 , 120 and $160 \mu\text{mol mol}^{-1}$ higher than ambient in each generation. Data is mean \pm SE ($n=12$). Significance levels of ψ_o between two models at the same time are indicated by +, * and ** for $p < 0.1$, $p < 0.05$ and $p < 0.01$.

Fig. 5 Diurnal variation of electron transport quantum yield (ϕE_o) in rice under different treatment of CO_2 concentration $[\text{CO}_2]$ elevation. (a) and (d) were measured at jointing stage, (b) and (e) were measured at heading stage, and (c) and (d) were observed at grain-filling stage. AI₊₁₆₀ and AI₊₂₀₀ were short-term (one growing season) abrupt increase of $[\text{CO}_2]$ by $\sim 160 \mu\text{mol mol}^{-1}$ and $\sim 200 \mu\text{mol mol}^{-1}$, respectively. CI: constant increase of $[\text{CO}_2]$ by $\sim 200 \mu\text{mol mol}^{-1}$ based on ambient $[\text{CO}_2]$ for four generations. SI: stepwise increase of $[\text{CO}_2]$ for four generations with the $[\text{CO}_2]$ being ~ 40 , 80 , 120 and $160 \mu\text{mol mol}^{-1}$ higher than ambient in each generation. Data is mean \pm SE ($n=12$). Significance levels of ϕE_o between two models at the same time are indicated by +, * and ** for $p < 0.1$, $p < 0.05$ and $p < 0.01$.

Fig. 6 Diurnal variation of performance index (PI_{ABS}) in rice under different treatment of CO_2 concentration $[\text{CO}_2]$ elevation. (a) and (d) were measured at jointing stage, (b) and (e) were measured at heading stage, and (c) and (d) were observed at grain-filling stage. AI₊₁₆₀ and AI₊₂₀₀ were short-term (one growing season) abrupt increase of $[\text{CO}_2]$ by $\sim 160 \mu\text{mol mol}^{-1}$ and $\sim 200 \mu\text{mol mol}^{-1}$, respectively. CI: constant increase of $[\text{CO}_2]$ by $\sim 200 \mu\text{mol mol}^{-1}$ based on ambient $[\text{CO}_2]$ for four generations. SI: stepwise increase of $[\text{CO}_2]$ for four generations with the $[\text{CO}_2]$ being ~ 40 , 80 , 120 and $160 \mu\text{mol mol}^{-1}$ higher than ambient in each generation. Data is mean \pm SE ($n=12$). Significance levels of PI_{ABS} between two models at the same time are indicated by +, * and ** for $p < 0.1$, $p < 0.05$ and $p < 0.01$.

Supporting information

Table S1 Significance levels of difference and relative change in probability that trapped exciton moves an

electron further than QA⁻ (ψ_o) between predawn and other observation time points under different treatments of elevated CO₂ concentration [CO₂].

Table S2 Significance levels of differences and relative change in the electron transport quantum yield (ϕE_o) between predawn and other observation time points under different treatments of elevated CO₂ concentration [CO₂].

Table S3 Significance levels of differences and relative changes in the performance index (PI_{ABS}) between predawn and other observation time points under different treatments of elevated CO₂ concentration [CO₂].

Fig. S1 Diurnal variation of minimum fluorescence (F_o) in rice under different treatments of elevated CO₂ concentration [CO₂]. (a) and (d) were jointing stage, (b) and (e) were heading stage, and (c) and (d) were grain filling stage. AI₊₁₆₀ and AI₊₂₀₀ were short-term (one growing season) abrupt increase of [CO₂] by ~160 $\mu\text{mol mol}^{-1}$ and ~200 $\mu\text{mol mol}^{-1}$, respectively. CI: constant increase of [CO₂] by ~200 $\mu\text{mol mol}^{-1}$ based on ambient [CO₂] for four generations. SI: stepwise increase of [CO₂] for four generations with the [CO₂] being ~40, 80, 120 and 160 $\mu\text{mol mol}^{-1}$ higher than ambient in each generation. Data is mean \pm SE (n=12). Significance levels of F_o between two treatments at the same time were indicated by +, * and ** for p<0.1, p<0.05 and p<0.01.

Fig. S2 Diurnal variation of fluorescence at J-step (F_j) in rice under different treatments of elevated CO₂ concentration [CO₂]. (a) and (d) were measured at jointing stage, (b) and (e) were measured at heading stage, and (c) and (d) were observed at grain filling stage. AI₊₁₆₀ and AI₊₂₀₀ were short-term (one growing season) abrupt increase of [CO₂] by ~160 $\mu\text{mol mol}^{-1}$ and ~200 $\mu\text{mol mol}^{-1}$, respectively. CI: constant increase of [CO₂] by ~200 $\mu\text{mol mol}^{-1}$ based on ambient [CO₂] for four generations. SI: stepwise increase of [CO₂] for four generations with the [CO₂] being ~40, 80, 120 and 160 $\mu\text{mol mol}^{-1}$ higher than ambient in each generation. Data is mean \pm SE (n=12). Significance levels of F_j between two models at the same time were indicated by +, * and ** for p<0.1, p<0.05 and p<0.01.

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