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Communication

Leaf Gas Exchange and Photosystem II Fluorescence Responses to CO_2 Cycling

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Abstract: Experimental systems to simulate future elevated CO_2 conditions in the field often have large, rapid fluctuations in CO_2. To examine possible impacts of such fluctuations on photosynthesis, the intact leaves of the field-grown plants of five species were exposed to two-minute cycles of CO_2 between 400 and 800 µmol mol^{-1}, lasting a total of 10 min, with photosynthesis, stomatal conductance and PSII fluorescence measured at the end of each half-cycle and also 10 min after the end of the cycling. Prior to the cyclic CO_2 treatments, the steady-state responses of leaf gas exchange and fluorescence to CO_2 were determined. In four of the five species, in which stomatal conductance decreased with increasing CO_2, the cyclic CO_2 treatments reduced stomatal conductance. In those species, both photosynthesis and the photochemical efficiency of PSII were reduced at limiting internal CO_2 levels, but not at saturating CO_2. In the fifth species, there was no change in stomatal conductance with CO_2 and no change in either photosynthesis or PSII efficiency at any CO_2 level with CO_2 cycling. It is concluded that in many, but not all, species, fluctuations in CO_2 may reduce photosynthesis at low CO_2, partly by decreasing the photochemical efficiency of photosystem II as well as by decreasing stomatal conductance.

Keywords: elevated CO_2; fluctuation; photosynthesis; stomatal conductance; photosystem II; cycling; fluorescence

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1. Introduction

With the continuing increase in CO_2 concentrations in the atmosphere [1], there has been considerable research examining the impacts of changes in CO_2 concentration on plant functions and growth [2–5]. As a substrate for photosynthesis, CO_2 is still currently a growth-limiting resource for plants that have C_3 metabolism. Experiments imposing different CO_2 concentrations on growing plants generally use CO_2 sensors to dynamically regulate the supply of CO_2 to the experimental system, while any removal of CO_2 required during daylight is usually accomplished by plant photosynthesis and/or wind. Concerns over the impacts of short-term variations in CO_2 concentration on plant function resulted primarily from the recognition of large-magnitude CO_2 fluctuations in free-air-carbon dioxide-enrichment facilities. Free-air-CO_2-enrichment (FACE) facilities were developed to provide elevated CO_2 treatments to plant ecosystems outdoors with a minimal disturbance from other environmental factors, such as wind, light, air temperature and humidity, and soil conditions [6]. However, in most FACE systems, CO_2 release is at the perimeter of the plot, while the CO_2 concentration sampled to control the CO_2 release is near the center of the plot, often many meters from the release points. Because air movement is needed to distribute CO_2 across the plot, there is a variable time lag between CO_2 release and the detection of the achieved concentration as well as the disturbance by air turbulence. A few papers documented large fluctuations in CO_2 concentrations over time within a given plot with FACE systems, using sampling systems that averaged CO_2 concentrations over about 5 s periods [7,8]. Surprisingly, despite the existence of rapid-response open-path CO_2 analyzers for about the last 25 years, rapid (seconds) CO_2 concentration measurements in

FACE plots have only recently been published [9,10]. Based on measurements in a FACE system of the Brookhaven National Laboratory design, Allen et al. [10] concluded that "due to the difficulty of controlling elevated CO_2 concentrations in turbulent air, the range of fluctuations of CO_2 in FACE experiments are more than 10-fold greater than plants experience in natural conditions". After reviewing experiments comparing plant responses to elevated CO_2 with different degrees of fluctuation, it was concluded that plant growth was suppressed by the larger CO_2 fluctuations in FACE systems, probably by reducing photosynthesis [10].

Because of the difficulty of reproducing fluctuations observed in FACE plots in controlled experiments, most experiments to assess the impacts of fluctuating CO_2 have used either regular cycles of CO_2 or brief pulses of high CO_2 [11–15]. Hendrey et al. [11] measured chlorophyll fluorescence responses to the short-term cyclic variation in CO_2 concentration of several frequencies. Holtum and Winter [12] measured responses of CO_2 uptake to the short-term cyclic variation in CO_2 concentration but did not measure stomatal conductance, and found that variations in CO_2 reduced photosynthesis in two tree species. Bunce [13] provided long-term cyclic CO_2 treatments compared with constant elevated CO_2 treatments at the same mean elevated CO_2 in open top chambers, and found that the cyclic CO_2 treatments reduced photosynthesis, stomatal conductance and plant growth in wheat and cotton. Short-term series of pulses of elevated CO_2 mimicking those observed in FACE plots reduced photosynthesis and stomatal conductance in wheat and rice leaves [14]. In indoor chambers, a larger magnitude of continuously applied fluctuations of CO_2 reduced photosynthesis, stomatal conductance and the growth of four herbaceous species compared with a smaller amplitude of CO_2 variation [15]. Although reduced stomatal conductance often occurs in response to CO_2 fluctuations, it is not the sole cause of reductions in photosynthesis, even if the stomatal closure is entirely "patchy" in nature [15,16].

This work examined whether a reduced photochemical efficiency of photosystem II occurred in response to CO_2 fluctuations and might cause some of the suspected reductions in photosynthesis in field-grown plants in FACE systems, in addition to reductions in stomatal conductance.

2. Results

Throughout the cycling of CO_2, four of the five species studied, *G. max*, *L. purpureus*, *L. tulipifera* and *S. lycopersicum*, had a reduced assimilation rate (A) and PSII efficiency (ΦPSII) at rate-limiting sub-stomatal CO_2 (C_i) values of about 250 to 300 μmol mol^{-1} occurring at 400 μmol mol^{-1} external CO_2 (Figure 1). At a higher C_i, occurring at 800 μmol mol^{-1} external CO_2, A was actually slightly increased in all of these species, except *L. tulipifera*, and the ΦPSII was the same as before the cycling of CO_2 in all four of these species (Figure 1). The reduction in ΦPSII and A to below steady-state values was evident at the end of the first 400 μmol mol^{-1} half-cycle and continued throughout the cycling of CO_2 in all of these four species. In *G. max*, the stomatal conductance decrease caused by cycling was nearly complete in the first half-cycle, while the other species had slower decreases in stomatal conductance, but stomatal conductance had stabilized before the end of the 10 min of cycling. All species were the same as *G. max* in terms of the speed of the ΦPSII decrease, i.e., it decreased by the end of the first half-cycle. The decrease in ΦPSII during CO_2 cycling, observed at the lower C_i, was accompanied by increased non-photochemical quenching. Ten minutes after the end of CO_2 cycling, a lower stomatal conductance remained at each CO_2 level in all four of these species (Table 1). Additionally, at ten minutes after the end of CO_2 cycling, ΦPSII and photosynthesis measured at 400 μmol mol^{-1} both remained lower than before the CO_2 cycling. However, the values of A and ΦPSII measured at 600 μmol mol^{-1} did not differ significantly from control values when measured at 600 μmol mol^{-1} (Table 2) in any species, despite a lower stomatal conductance in all species except *P. crispum*.

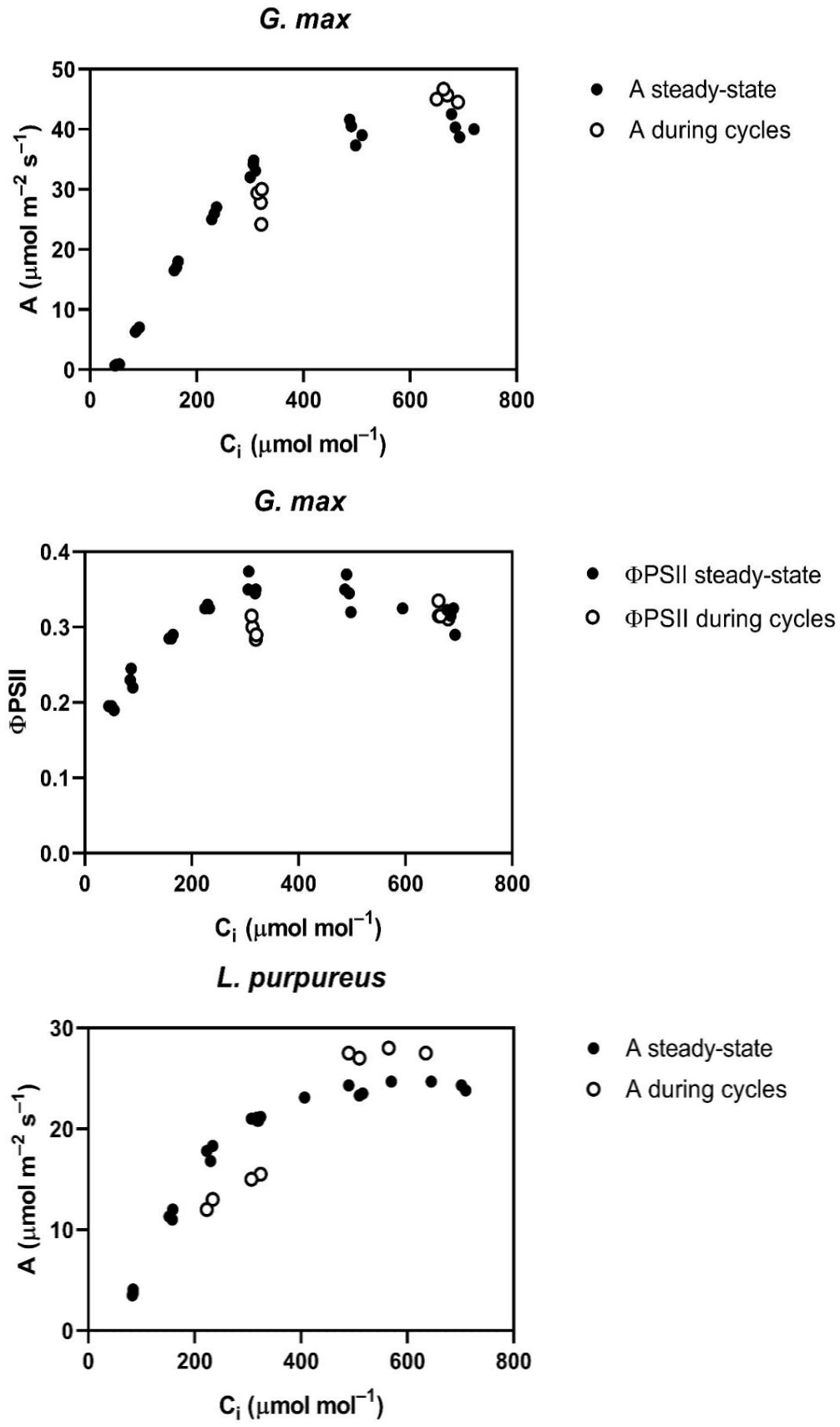
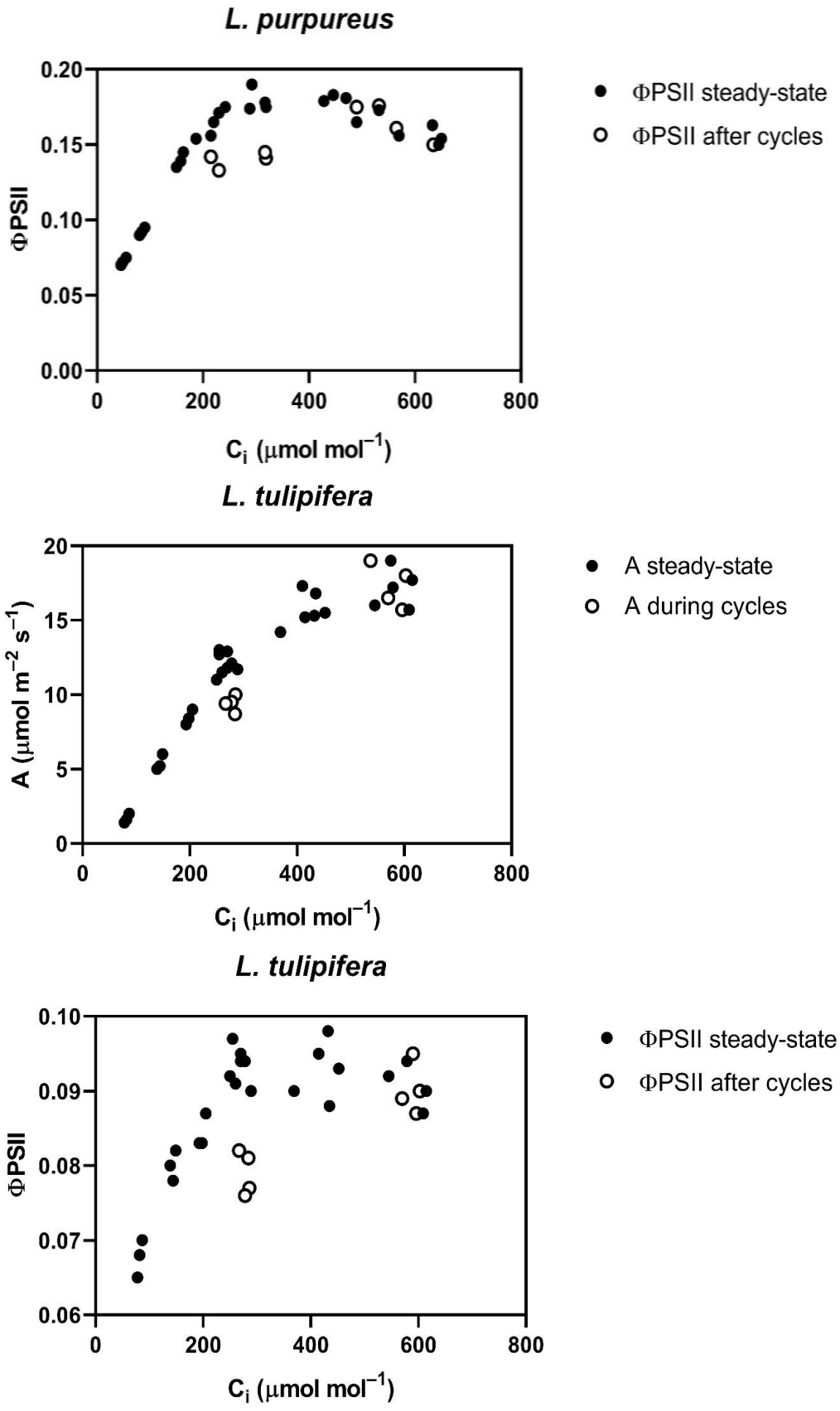


Figure 1. *Cont.*



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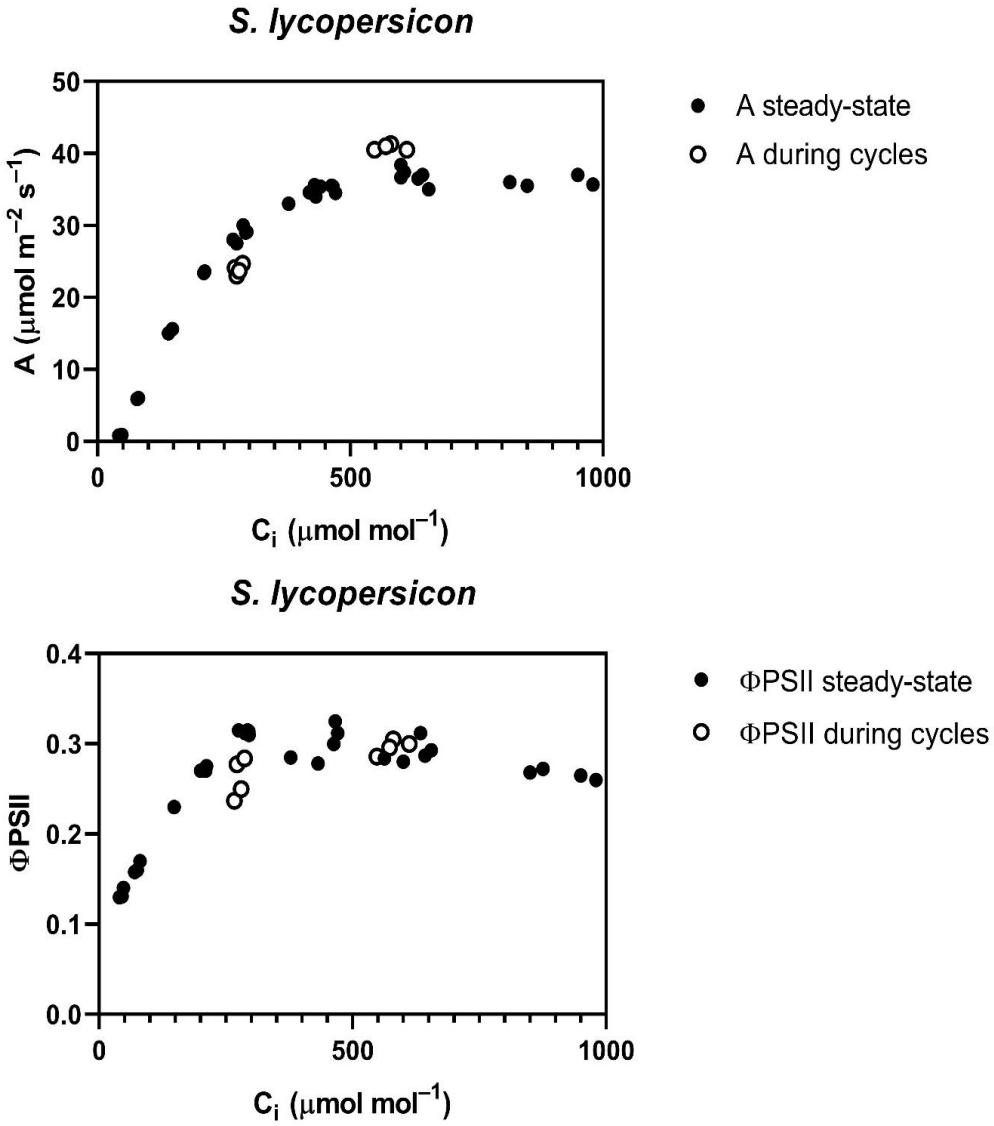

Figure 1. Responses of CO_2 assimilation rate (A) and PSII efficiency (ΦPSII) as a function of sub-stomatal CO_2 (C_i) before (steady-state) and during cycling of ambient CO_2 in four species. CO_2 was cycled between 400 and 800 μmol mol^{-1}, with one minute at each concentration before changing to the other concentration, for a total of 10 min. Each data point represents a measurement on a different plant taken after values had stabilized during the cycling.

Table 1. Mean values of stomatal conductance measured at 400 and 800 μmol mol^{-1} CO_2 before, during, and 10 min after cycling of CO_2 between 400 and 800 μmol mol^{-1}, with a full cycle length of 2 min, for a total of 10 min, in five species. Within rows, numbers followed by different letters are different at $p = 0.05$, using repeated measures ANOVA.

Species	Stomatal Conductance (mmol mol^{-1})				
	Before Cycling		During Cycling	After Cycling	
CO_2 (μmol mol^{-1}):	400	800	Both CO2s	400	800
G. max	1643 a	1465 b	1168 c	956 d	808 e
L. purpureus	652 a	437 b	269 c	280 c	240 d
L. tulipifera	205 a	183 b	159 c	152 c	144 c
S. lycopersicum	797 a	638 b	493 c	537 c	531 c
P. crispum	313 a	310 a	315 a	322 a	316 a

Table 2. Means values of A and ΦPSII at 600 μmol mol^{-1} CO_2 before and 10 min after the end of cycling of CO_2 between 400 and 800 μmol mol^{-1} CO_2 for 10 min. Within rows, numbers followed by different letters are different at $p = 0.05$, using repeated measures ANOVA.

Species	A (μmol m^{-2} s^{-1})		ΦPSII	
	Before	After	Before	After
G. max	37.1 a	36.5 a	0.333 a	0.313 a
L. purpureus	22.9 a	21.7 a	0.175 a	0.165 a
L. tulipifera	16.3 a	15.2 a	0.095 a	0.094 a
S. lycopersicum	34.5 a	33.7 a	0.310 a	0.308 a
P. crispum	35.1 a	35.3 a	0.255 a	0.257 a

The *P. crispum*, in contrast to the other four species, had no reduction in the A vs. C_i curve, or in ΦPSII after the cycling of CO_2 (Figure 2), and also showed no change in stomatal conductance with CO_2 (Table 1).

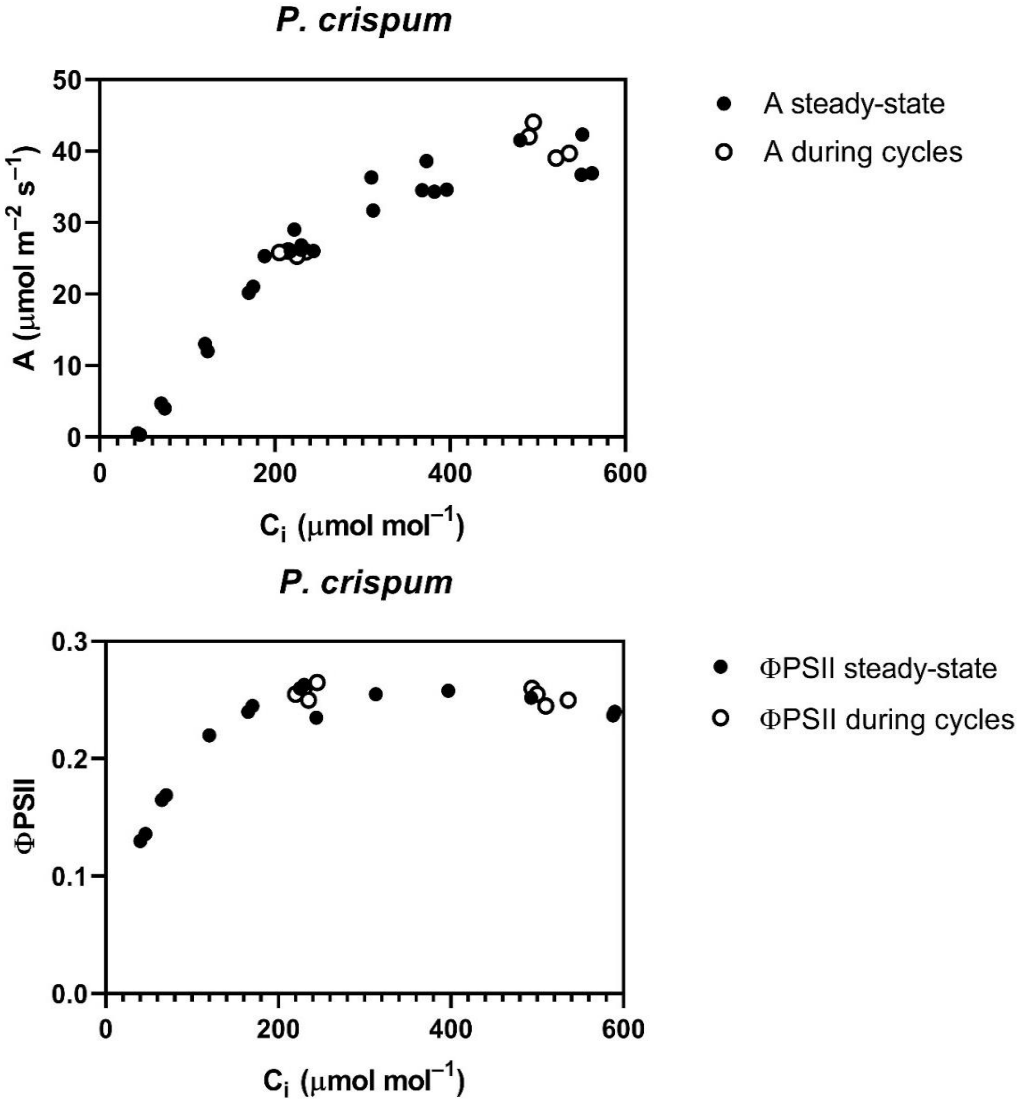

Figure 2. Responses of CO_2 assimilation rate (A) and PSII efficiency (ΦPSII) as a function of sub-stomatal CO_2 (C_i) before (steady-state) and during cycling of ambient CO_2 in *P. crispum*. CO_2 was cycled between 400 and 800 μmol mol^{-1}, with one minute at each concentration before changing to the other concentration, for a total of 10 min. Each data point represents a measurement on a different plant taken after values had stabilized during the cycling.

Stomatal conductance before the cycling of CO_2 was lower at 800 than at 400 μmol mol^{-1} CO_2 in all species except *P. crispum* (Table 1). Stomatal conductance during CO_2 cycling was reduced in all species, except *P. crispum* (Table 1). Ten minutes after cycling ended, the stomatal conductance remained lower than before cycling in all species, except *P. crispum*, in which the stomatal conductance was unchanged by all treatments (Table 1).

3. Discussion

All of these species had fairly typical A vs. C_i curves for C_3 species, with no decreases in A at the highest C_i values, which would be clear evidence of a limitation by triose phosphate utilization (TPU) [17]. However, all species had some decrease in ΦPSII at the highest C_i values, which McClain et al. [18] suggest is indicative of TPU limitation. A premature leveling off of A vs. C_i curves is more difficult to discern than reductions in ΦPSII as an indication of TPU limitation, except possibly by the fitting of a photosynthesis model that includes a TPU limitation to the observed data.

The reductions in the photochemical efficiency of PSII (ΦPSII) at 400 μmol mol^{-1} external CO_2 levels caused by the cycling of CO_2 concentration, which occurred in four of the five species examined, provide a new explanation of reduced photosynthesis rates for a given sub-stomatal CO_2 concentration, which has frequently been reported in CO_2 fluctuation experiments [12–15]. Prior suggestions that reduced photosynthesis might be the result of "patchy" stomatal closure [13,15] admittedly could not account for the lack of reduction in photosynthesis at elevated measurement CO_2 [15]. In the current experiments, the reduction in ΦPSII that occurred at the lower measurement CO_2 did not occur at the higher measurement CO_2. At the higher measurement CO_2, photosynthesis was also not inhibited by the cycling of CO_2 in these experiments, despite the continued lower stomatal conductance. The lack of decrease in A despite a lower stomatal conductance is to be expected at nearly saturating values of CO_2. Similar to the results presented here, in long-term cyclic CO_2 exposures in open top chambers, the relative reductions in photosynthesis in cotton were much larger for measurements made at the lower (near-ambient CO_2) than at the higher external CO_2 of the cycles [13].

McClain et al. [18] also reported reductions in ΦPSII in response to a large step increase in CO_2, which they proposed was related to a triose-phosphate limitation of photosynthesis at high CO_2. They provided no information on the stomatal conductance response to their treatments. However, in the fluctuating CO_2 experiments reported here, reduced ΦPSII only occurred at limiting CO_2 concentrations, not at elevated CO_2. This difference in plant response might be related to the much shorter duration of exposure to high CO_2 and lower elevated CO_2 concentrations in the present experiment (800 μmol mol^{-1}) compared with those of McClain et al. (1500 μmol mol^{-1}). In these experiments, leaves were actually at 800 μmol mol^{-1} during the cycling of CO_2 for less than five minutes.

I speculate that *P. crispum* had a qualitatively different photosynthetic response to the cyclic CO_2 treatment than the other four species studied here, because it had no response at all of stomatal conductance to CO_2 in the range of 400 to 800 μmol mol^{-1}, in contrast to all of the other species. Similar results for more species with stomates unresponsive to changes in CO_2 would be required to confirm this correlation. *L. tulipifera* was chosen for these experiments, based on the generally smaller response of stomatal conductance to CO_2 in tree species [19,20]. It did have a smaller relative response than the other three herbaceous species, but not a zero response, as occurred in *P. crispum*. It remains unclear how the presence or absence of changes in stomatal conductance during fluctuations in CO_2 could influence photochemical limitations on photosynthesis at low CO_2. However, the decrease in photosynthesis and ΦPSII observed in this tree species at the lower measurement CO_2 is consistent with the decreases in photosynthesis found by Holtum and Winter in two tropical tree species [12]. This suggests that FACE experiments may also not give the most accurate indication of tree responses to climate change.

Allen et al. [10] reviewed yield data in FACE and open top chambers (OTC) for several major C_3 crop species, and they concluded that the yield stimulation caused by the same

elevated CO_2 treatments was, in FACE, on average, only about 0.66× of that occurring in OTC. A smaller yield stimulation by elevated CO_2 in FACE than in OTC was documented for wheat and soybeans in the only side-by-side simultaneous FACE and OTC comparisons of crop yield [21] that exist to date. Allen et al. [10] tentatively attributed this smaller yield stimulation to a reduced stimulation of photosynthesis by elevated CO_2 in FACE than in OTC. The smaller stimulation of photosynthesis was thought to be caused by the much larger fluctuations in CO_2 in elevated CO_2 treatments in FACE than in OTC. Allen et al. [10] carefully documented larger CO_2 fluctuations in FACE with all of the available rapid CO_2 measurement data, and I am not aware of any more recent published data on CO_2 fluctuations in FACE. However, at the time that paper was written [10], reasons why rapid fluctuations in CO_2 would cause reduced photosynthesis were unclear, despite some documented cases of high-CO_2 pulses or the cycling of CO_2 reducing photosynthesis [12–14]. Deceases in photosynthesis caused by the pulses of elevated CO_2 or by the cycling of CO_2 have now been documented in many of the most important C_3 crop species, wheat [14], rice [14], soybean ([15] and this paper), and cotton [14], in two minor crop species, tomato and lablab [this paper], and also in three tree species ([12] and this paper). Up until the current work, the only clue about the reasons why fluctuations in CO_2 would inhibit photosynthesis were observations of a reduced stomatal conductance to water vapor [13–15].

The results presented here provide a new mechanism by which fluctuations in CO_2 around leaves can inhibit photosynthesis, a decrease in the photochemical efficiency of photosystem II. Of course, these results beg the question of why ΦPSII was decreased by the cycling of CO_2. Furthermore, the extent to which this decrease in ΦPSII at low CO_2 occurs in experiments exposing plants to a long-term elevation of CO_2, for example in FACE experiments, has not been determined. It is interesting to consider that reduced photosynthesis in FACE systems may primarily occur during those periods in which CO_2 fluctuations bring CO_2 levels down to near-ambient CO_2 levels, based on the results presented here. Most measurements of photosynthesis in FACE systems have been conducted at the targeted elevated CO_2 concentration, not at lower CO_2 concentrations. The only experiment to date that directly compared photosynthesis in plants grown simultaneously at elevated CO_2 in open top chambers and in FACE systems only measured leaf gas exchange at the elevated CO_2 [21], in the plants grown at elevated CO_2, and thus would have missed photosynthetic responses resembling those presented here.

4. Materials and Methods

Leaf gas exchange and chlorophyll fluorescence measurements were conducted on four species of herbaceous plants and one tree species grown outdoors at ambient CO_2. The species studied were *Glycine max* L. Merr. cv. Clark, *Lablab purpureus* L. Sweet, *Petroselinum crispum* Mill. Fuss var. *neopolitanum*, *Solanum lycopersicum* L. cv. Better Boy, and *Lireodendron tulipifera* L. The four herbaceous species were grown in Annapolis, Maryland in an unshaded plot with a sandy loam soil. Plants were grown from seed and planted in late April 2020. The plot was fertilized with a complete fertilizer containing 12% N, 4% P, and 8% K at 200 g of fertilizer per m^2, and it did not experience soil water stress. The *L. tulipifera* trees sampled were saplings, about 6 years old, growing at a south-facing forest edge in Annapolis, on a sandy loam soil. Leaf gas exchange and chlorophyll fluorescence measurements were conducted from mid-June through to the end of June 2020. The mean temperature in Annapolis in May 2020 was 16.0 °C, slightly below the long-term mean of 17.7 °C, and in June 2020 it was 23.3 °C, which equals the long-term mean temperature.

All leaf gas exchange and chlorophyll fluorescence measurements were conducted at 27 °C leaf temperature, 1500 μmol m^{-2} s^{-1} PPFD, with a leaf-to-air water vapor pressure difference of 1 to 1.5 kPa, using a Ciras-3 portable photosynthesis system with a PLC3 leaf chamber/fluorometer, with an air flow rate of 400 cm^3 min^{-1}. The "stored differential balance" function of the instrument was used to correct measurements for changes in calibration with background CO_2. The values of sub-stomatal CO_2 (C_i) were calculated from photosynthesis, stomatal and boundary layer conductances, and external CO_2 by the system

software. During the mornings of sunny days, a fully expanded upper-canopy leaf was selected for measurement. Steady-state responses of stomatal conductance, photosynthesis, and PS II chlorophyll fluorescence at CO_2 concentrations of 400, 600, and 800 µmol mol^{-1} were determined on a leaf, allowing sufficient time for the stomatal conductance to adjust to each CO_2 level, as observed on the graphical display of incoming data. Steady-state values were used to ensure that C_i values were accurate. The efficiency of PSII was assessed using multipulse fluorescence measurements at each CO_2 level. The CO_2 concentration was then returned to 400 µmol mol^{-1}, and cycles of CO_2 from 400 to 800 µmol mol^{-1} with a total cycle length of 2 min were then applied for 10 min, that is, one minute at 400 µmol mol^{-1}, one minute at 800 µmol mol^{-1}, one minute at 400 µmol mol^{-1}, etc., for a total of 10 min. Photosynthesis, stomatal conductance, and PSII efficiency were recorded at the end of each half-cycle. At the end of the cyclic CO_2 treatment, CO_2 was returned to 400 µmol mol^{-1}, and beginning ten minutes after the end of the CO_2 cycling, photosynthesis, stomatal conductance, and PSII efficiency were measured at 400, 600, and 800 µmol mol^{-1} CO_2. These measurements were made on at least four different plants of each species. On a few different leaves of each species, the responses of stomatal conductance, photosynthesis, and PS II chlorophyll fluorescence to CO_2 concentrations from 100 to 1200 µmol mol^{-1} were determined. There were nine steps of CO_2 (400, 300, 200, 100, 400, 600, 800, 1000, 1200 µmol mol^{-1}). Leaves were kept at each step of CO_2 for three to four minutes, waiting for the leaf gas exchange to stabilize, before measuring the photochemical efficiency of PSII using a multipulse measurement at each step in CO_2. The leaf-to-air water vapor pressure difference changed by less than 10% of its initial value of 1 to 1.5 kPa during the cycling of CO_2, which would have a minimal impact on the stomatal conductance.

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Data Availability Statement: Data are available from the author upon request.

Conflicts of Interest: The author declares no conflict of interest.

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