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Responses of cotton and wheat photosynthesis and growth to cyclic variation in carbon dioxide concentration

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Abstract

The carbon dioxide concentration in free air carbon dioxide enrichment (FACE) systems typically has rapid fluctuations. In our FACE system, power spectral analysis of CO_2 concentration measured every second with an open path analyzer indicated peaks in variation with a period of about one minute. I used open-top chambers to expose cotton and wheat plants to either a constant elevated CO_2 concentration of 180 μmol mol^{-1} above that of outside ambient air, or to the same mean CO_2 concentration, but with the CO_2 enrichment cycling between about 30 and 330 μmol mol^{-1} above the concentration of outside ambient air, with a period of one minute. Three short-term replicate plantings of cotton were grown in Beltsville, Maryland with these CO_2 concentration treatments imposed for 27-day periods over two summers, and one winter wheat crop was grown from sowing to maturity. In cotton, leaf gas-exchange measurements of the continuously elevated treatment and the fluctuating treatment indicated that the fluctuating CO_2 concentration treatment consistently resulted in substantial down-regulation of net photosynthetic rate (P_N) and stomatal conductance (g_s). Total shoot biomass of the vegetative cotton plants in the fluctuating CO_2 concentration treatment averaged 30% less than in the constantly elevated CO_2 concentration treatment at 27 days after planting. In winter wheat, leaf gas-exchange measurements also indicated that down-regulation of P_N and g_s occurred in flag leaves in the fluctuating CO_2 concentration treatment, but the effect was not as consistent in other leaves, nor as severe as found in cotton. However, wheat grain yields were 12% less in the fluctuating CO_2 concentration treatment compared with the constant elevated CO_2 concentration treatment. Comparison with wheat yields in chambers without CO_2 addition indicated a nonsignificant increase of 5% for the fluctuating elevated CO_2 concentration treatment, and a significant increase of 19% for the constant elevated treatment. The results suggest that treatments with fluctuating elevated CO_2 concentrations could underestimate plant growth at projected future atmospheric CO_2 concentrations.

Additional key words: acclimation; down-regulation; stomatal conductance.

Introduction

FACE systems have some advantages over other enrichment systems for exposing crop plants to anticipated future atmospheric concentrations of CO_2. One advantage is the absence of enclosures which alter wind speed, radiation, temperature, and humidity. The long-term average CO_2 concentration enrichment achieved in FACE systems can be very consistent, and 1-min averages of daytime CO_2 concentration in FACE systems are generally within 10% of the target CO_2 concentration 80 to 90% of the time. However, large rapid fluctuations in CO_2 concentration often occur (Hendrey *et al*. 1999, Okada *et al.* 2001, Bunce 2011). The importance of these rapid fluctuations in CO_2 concentration to plant function remains uncertain. From leaf chlorophyll fluorescence measurements on wheat leaves Hendrey *et al.* (1997) concluded that fluctuations in CO_2 concentration with periods of less than one minute were unlikely to affect photosynthesis. However, Holtum and Winter (2003) measured P_N and found significantly lower mean rates when the CO_2 concentration varied with a period of 40 s compared to rates measured at a constant mean CO_2 concentration. In the experiments described here I tested whether the long-term growth and P_N of cotton and wheat plants were affected by 1-min cycles of CO_2 concentration.

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Abbreviations: C_i – CO_2 concentration in the substomatal (intercellular) airspace; FACE – free air carbon dioxide enrichment; g_s – stomatal conductance; P_N – net photosynthetic rate.
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Materials and methods

Design criteria: An open path CO_2 analyzer (*LI-7500, LI-Cor, Inc*., Lincoln, NE, USA) operating at 5 Hz mounted at canopy height near the center of an area distributed FACE plot (Bunce 2011) was used to record CO_2 concentration once per second for two hour periods on two days. Four independent sequences of 1,000-s duration were randomly selected from this data set, and time series analysis (*JMP v. 5.1, SAS Institute*, NC, USA) was used to develop power spectra for each 1,000-s sequence. All four power spectra had distinct peaks at periods of approximately 20 to 80 s, and all samples had a large peak very near 60 s (Fig. 1). Based on these observations, it was decided to use a period of 60 s for the fluctuating CO_2 concentration treatments. The daytime CO_2 concentration in our FACE system, when operated with a mean enrichment of 1.4 times ambient, was frequently little enriched above ambient, but was only seldom enriched to more than twice the ambient concentration (Bunce 2011, Fig. 3). For this study, a system was designed to expose plants to CO_2 concentrations ranging from about 30 to 330 μmol mol^{-1} above the concentration of outside air, with a period of one minute. This "fluctuating" elevated CO_2 concentration treatment was compared with a "constant" enrichment of 180 μmol mol^{-1} above the concentration of the outside air, which averaged 370 μmol mol^{-1} in the daytime.

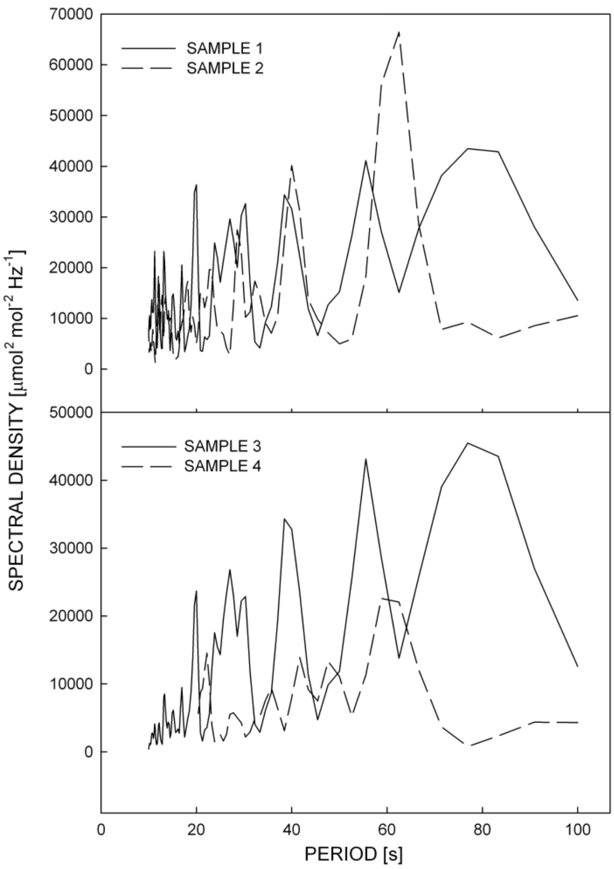

Fig. 1. Power spectra of CO_2 concentrations for four randomly selected 1,000-s periods for an area distributed free air carbon dioxide enrichment system (Bunce 2011). CO_2 concentrations were recorded at 1 Hz from an open path analyzer operating at 5 Hz.

CO_2 control: A solenoid valve was placed in the line supplying CO_2 to an open-top chamber equipped with a blower injecting air and CO_2 into the bottom of the chamber through a 10 cm diameter perforated plastic pipe running the whole length of the center of the chamber. For the fluctuating CO_2 concentration treatment, the flow rate of CO_2 to the inlet of the blower was twice the normal rate, and the solenoid valve was turned off for the first 30 s of every minute with an electronic timer. The range of CO_2 concentration achieved in the chamber was dependent on the air-volume turnover time of the chamber, which was nominally 0.6 min in both sizes of open-top chambers used in these experiments. Representative time courses of CO_2 concentration measured with the open path analyzer in "fluctuating" and "constant" elevated CO_2 concentration chambers, and in chambers with no CO_2 added are presented in Fig. 2. The sensor of the open path analyzer was mounted horizontally near the centers of the chambers, at the height of the upper canopy leaves, when plants were about 50 cm in height. This pattern of exposure with CO_2 concentration gradually oscillating between minimum and maximum values is similar to that of Holtum and Winter (2003), and is different from the pattern of sharp transitions between minimum and maximum exposures of Cardon *et al*. (1994) and Hendrey *et al*. (1997).

Cotton (*Gossypium hirsutum* L., var. Delta Pine 555) seeds were planted in four square open-top chambers each covering 1.9 m^2 of ground. The chambers were 2 m in height, and the walls were clear acrylic plastic. Two of the chambers had constantly elevated CO_2 concentrations and two had fluctuating elevated CO_2 concentrations, with CO_2 added 24 h per day. These treatments were rotated among chambers in three replicate runs over two summers. In the third replicate run, an additional two chambers were planted with cotton, but were operated with no CO_2 addition. Cotton was planted on days 197 and 236 in 2010, and 201 in 2011. The soil of the field plot containing the chambers was a Codorus silt loam, a fine-loamy, mixed, mesic Fluvaquentic Dystrochrept soil, and the prior crop was *Phaseolus vulgaris* grown with a 10-10-10, N, P and K fertilizer. No fertilizer was added to the soil for the cotton. Cotton seedlings were thinned to 24 plants per chamber, in two border and two interior rows. Plants sampled for leaf gas exchange and biomass were from the center of the interior rows of each chamber, *i.e*. bordered by other cotton plants on all sides.

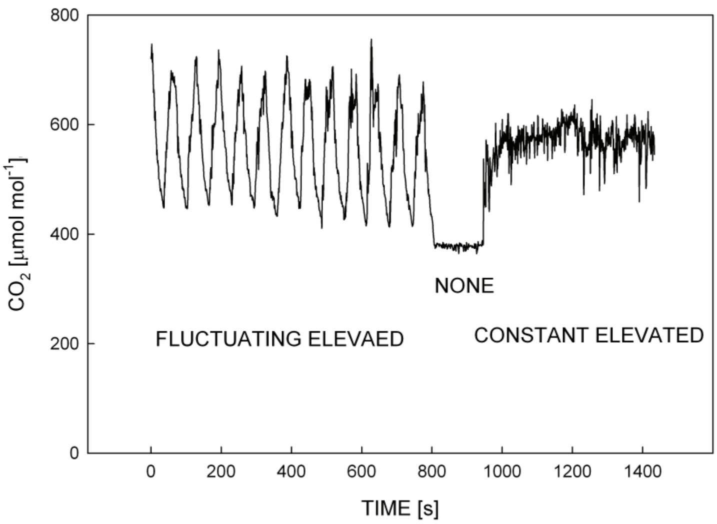

Fig. 2. Time sequences of measurements of CO_2 concentration in three CO_2 treatment chambers: fluctuating elevated, no added CO_2, and constant elevated. CO_2 concentrations were recorded at 1 Hz from an open path analyzer operating at 5 Hz. Mean concentrations were 559, 378, and 569 µmol mol^{-1} for the three treatments, respectively.

Air samples from each chamber were pumped to an infrared CO_2 analyzer in a nearby shelter, and mean CO_2 concentration of air from each chamber, and the CO_2 concentration of outside air were each recorded once per hour. CO_2 flow rates to each chamber were adjusted daily, as necessary. Mean air temperatures for the three replicate experiments were 26.5, 21.4, and 26.3°C. The plot was not irrigated, but no significant soil water deficits occurred.

Leaf gas exchange was measured on four dates, one date for each of the two 2010 crops, and on two dates in the 2011 crop. In 2010, leaf gas-exchange measurements were made 26 d after planting. In 2011, leaf gas-exchange measurements were made at 23 and 26 d after planting. Leaf gas exchange was measured near mid-day on clear days, using a *CIRAS-1* portable photosynthesis system (*PP-Systems*, Amesbury, Massachusetts, USA) with CO_2 concentration control. Two mature upper canopy leaves from each open-top chamber were measured at the ambient air temperature and water vapor content, in full sunlight on each occasion. Leaves from chambers with constant elevated CO_2 concentration were measured only at the growth CO_2 concentrations, but leaves from the fluctuating elevated CO_2 concentration were measured at the average minimum, mean, and maximum CO_2 concentration during growth. In the 2011 crop, leaves from chambers without added CO_2 were measured at the same three CO_2 concentrations as leaves from the fluctuating elevated CO_2 concentration chambers. In cases where measurements were to be made at three CO_2 concentrations, the first measurement was made at the mean growth CO_2 concentration. No significant change in stomatal conductance (g_s) with measurement CO_2 concentration occurred during these measurements, probably because leaves were kept at the different CO_2 concentration only long enough (about a minute) for P_N to become stable. Whole shoots of eight plants per chamber were harvested 27 d after sowing to determine shoot dry mass.

Winter wheat (*Triticum aestivum* L., var. Choptank) was planted on day of year 282 in 2010 in twelve rectangular open-top chambers, each covering 2.8 m^2 of ground. The chamber height was 2.5 m, and the chamber walls were clear acrylic plastic. There were four rows of plants per chamber, with 30 cm between rows. The soil was the same as described for the cotton experiments, and 60 g of urea was added to each chamber when wheat growth resumed in the spring. Three CO_2 concentration treatments, constant elevated CO_2 concentration, fluctuating CO_2 concentration, and no added CO_2 were randomly assigned to each of four chambers. CO_2 was added 24 h per day, except when the ground was covered with snow. Air from each chamber was pumped to an infrared CO_2 analyzer and mean CO_2 concentration from each chamber was recorded once an hour, with CO_2 flow rates adjusted daily, as necessary.

Leaf gas-exchange measurements, as described for the cotton experiment, were conducted on ten days with wheat, once in the fall of 2010, and nine times in spring, which included four days of measurements on flag leaves. A harvest of two plants from border rows in each of the four corners of each chamber was made 44 d after sowing in 2010, before shoots were damaged by low winter temperatures. At crop maturity in June 2011, 3 m of interior rows were harvested from each chamber to determine shoot and seed dry mass.

Statistics: Chambers were treated as the experimental units. Treatments were compared using *ANOVA*, except that leaf gas exchange in wheat was analyzed using repeated measures *ANOVA*, because the same experimental units were measured on multiple occasions.

Results

Cotton: For comparisons of the two elevated CO_2 concentration treatments, the measurement date affected P_N and g_s, but there was no significant interaction between measurement date and CO_2 concentration treatment for P_N or g_s. Mean values for the four measurement dates (Table 1) indicated substantially lower P_N and g_s, but similar substomatal carbon dioxide concentrations (C_i) for the plants grown with fluctuating elevated CO_2 concentration compared with constant elevated CO_2 concentration.

For the 2011 data on leaf gas exchange, plants grown without added CO_2 had higher rates of P_N than plants grown with fluctuating elevated CO_2 concentration when measured at the lower, but not at the higher CO_2 concentration, and low g_s when measured at both CO_2 concentrations (Table 2). C_i during these measurements did not differ significantly with growth conditions (Table 2). When measured at the mean elevated CO_2 concentration, plants grown without added CO_2 and those grown with constant elevated CO_2 concentration had mean P_N of 35.3 and 34.0 mmol m^{-2} s^{-1}, respectively, and g_s of 668 and 574 mmol m^{-2} s^{-1}, neither of which was different at P=0.05.

Averaged over the three replicate runs, shoot dry mass per plant averaged 1.31 g for the constant elevated CO_2 concentration treatment and 0.92 g for the fluctuating elevated CO_2 concentration treatment at 27 days after planting. These means were statistically different at P=0.05. In 2011, shoot dry mass for the constant elevated CO_2 concentration treatment was 1.47 times that of the treatment without added CO_2, compared with 1.25 times for the fluctuating elevated CO_2 concentration treatment. Mean masses in the three treatments were all different from each other at P=0.05 in 2011.

Table 1. Mean net photosynthetic rate (P_N), stomatal conductance (g_s), and substomatal concentration of carbon dioxide (C_i) of leaves of cotton plants grown with fluctuating and constant elevated carbon dioxide concentrations. Values are averaged over four measurement dates, and leaves were measured at the mean daytime elevated CO_2 concentration of 550 μmol mol^{-1}. Values within columns followed by *different letters* differed between CO_2 concentration treatments at P=0.05, using *ANOVA*.

Treatment	P_N [μmol m^{-2} s^{-1}]	g_s [mmol m^{-2} s^{-1}]	C_i [μmol mol^{-1}]
Constant	41.8^A	1180^A	418^A
Fluctuating	34.5^B	745^B	413^A
Ratio (F/C)	0.83	0.69	0.99

Table 2. Mean net photosynthetic rate (P_N), stomatal conductance (g_s), and substomatal concentration of carbon dioxide (C_i) of cotton plants grown with no added CO_2 or with a fluctuating elevated CO_2 concentration. Values are averaged over two 2011 measurement dates, and leaves were measured at two CO_2 concentrations. Values within columns followed by *different letters* differed between growth CO_2 concentration treatments at P=0.05, using *ANOVA*.

	P_N [μmol m^{-2} s^{-1}]		g_s [mmol m^{-2} s^{-1}]		C_i [μmol mol^{-1}]	
Measurement CO_2 [μmol mol^{-1}]	370	730	370	730	370	730
Treatment						
None	25.0^A	42.1^A	683^A	633^A	249^A	533^A
Fluctuating	19.9^B	38.8^A	493^B	456^B	248^A	537^B
Ratio (F/N)	0.80	0.92	0.72	0.72	1.00	1.01

Table 3. Mean photosynthetic rate (P_N), stomatal conductance (g_s), and substomatal concentration of carbon dioxide (C_i) of flag leaves of wheat plants grown without added CO_2, and with fluctuating or constant elevated CO_2 concentration. Values are averaged over four measurement dates, and leaves were measured at the mean daytime elevated CO_2 concentration of 550 μmol mol^{-1}. Values within columns followed by *different letters* differed between CO_2 concentration treatments at P=0.05, using *ANOVA*.

Treatment	P_N [μmol m^{-2} s^{-1}]	g_s [mmol m^{-2} s^{-1}]	C_i [μmol mol^{-1}]
None	39.4^A	1338^A	472^A
Constant	37.1^B	1180^B	477^A
Fluctuating	34.4^C	1037^C	467^A

Wheat: The leaf gas-exchange data as a whole showed significant effects of treatment, date, and treatment by date interactions for both P_N and g_s. However, restricting the statistical analysis to measurements on flag leaves (four dates) the treatment by date interaction term became nonsignificant for both gas-exchange parameters. For flag leaves, P_N and g_s measured at the mean elevated CO_2 concentration was highest in leaves from chambers without CO_2 addition, intermediate in leaves from chambers with constant elevated CO_2 concentration, and lowest in leaves from the fluctuating elevated CO_2 concentration chambers (Table 3). Similar patterns and magnitudes of treatment effects also occurred on some other measurement dates, but there were also measurement dates when no significant treatment effects

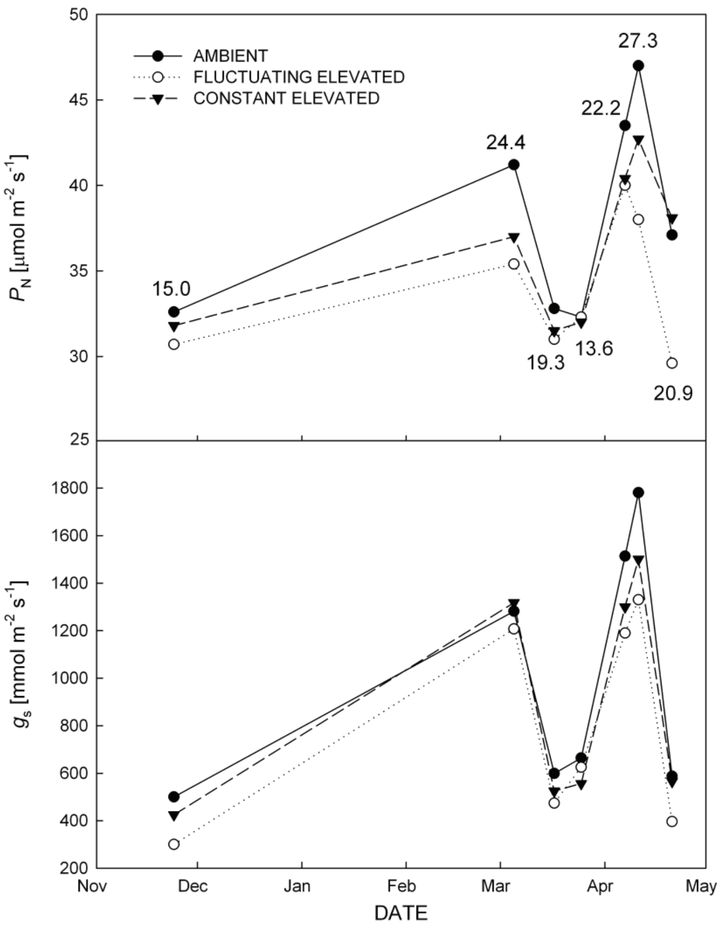

Fig. 3. Mean values of leaf net photosynthetic rate (P_N) and stomatal conductance (g_s) in leaves of vegetative wheat plants on different measurement dates, for three CO_2 treatments. Measurements were made at 370 μmol mol^{-1} for the "ambient" CO_2 concentration treatment and at 550 μmol mol^{-1} for the "elevated" CO_2 concentration treatments. Numbers indicate mean leaf temperatures during the measurements on each date. The treatment, date, and treatment by date interaction terms were all significant at P=0.05 for both P_N and g_s, using repeated measures *ANOVA*.

Table 4. Harvest data for winter wheat crops grown in open-top chambers without added CO_2, with constant elevated CO_2 concentration, and with fluctuating elevated CO_2 concentration. Values are means for four chambers per treatment. Values within columns followed by *different letters* differed between growth CO_2 concentration treatments at P=0.05, using *ANOVA*. DM – dry mass.

Treatment	At day 44 Total shoot [g(DM) plant^{-1}]	At crop maturity Total shoot [g(DM) m^{-2}]	Seed [g(DM) m^{-2}]
None	0.98^C	373^B	125^B
Constant	1.43^A	445^A	149^A
Fluctuating	1.20^B	395^B	131^B

occurred, without any obvious relationship with growth stage or measurement temperature (Fig. 3).

The dry mass of shoots of wheat plants at an early vegetative stage of development (44 days after sowing) was highest in the constant elevated CO_2 concentration treatment and lowest in the treatment without added CO_2 (Table 4). At maturity, total shoot mass and seed mass per ground area were significantly higher for the constant elevated CO_2 concentration treatment than either the fluctuating elevated CO_2 concentration treatment or the treatment without CO_2 addition, which did not differ significantly from each other (Table 4).

Discussion

The results of this study indicated that the cyclically varying elevated CO_2 concentration treatment reduced the long-term growth of both species compared with a more constant CO_2 concentration treatment with the same mean CO_2 concentration. Lower P_N also occurred, and could have been responsible for the slower dry matter production. As pointed out by Hendrey *et al.* (1997) and Holtum and Winter (2003), because P_N has a saturating response to CO_2 concentration, mean P_N would always be less for leaves exposed only to the maximum and minimum CO_2 concentration than for those exposed only to the mean concentration. In both our and the Holtum and Winter (2003) fluctuating CO_2 concentration treatments, plants were exposed to the whole range of concentrations (Fig. 2), not just to the minimum and maximum, as in the Cardon *et al.* (1994) and the Hendrey *et al.* (1997) treatments, so the anticipated effect on mean P_N would be smaller. From the frequency distribution of exposure to different CO_2 concentration, in combination with the observed CO_2 concentration response curves, assuming an instantaneous response of P_N to CO_2 concentration leads to an estimated reduction in mean P_N of only 3 ± 1% for both cotton and wheat due to this direct effect of variation in CO_2 concentration. In FACE systems which expose plants intermittently to much higher CO_2 concentrations than used here, this effect would be larger, because very high CO_2 concentrations would increase mean CO_2 concentration but have little additional effect on P_N. In these experiments in open-top chambers, the observed down-regulation of P_N was far more important in reducing P_N in the fluctuating CO_2 concentration treatments, especially in cotton, than was this effect due to the curvilinear photosynthetic response.

This is the first report to document down-regulation of P_N in response to long-term exposure to fluctuating CO_2 concentrations. Both Holtum and Winter (2003) and Hendrey *et al.* (1997) exposed plants to fluctuating CO_2 concentrations only for several minutes. The larger down-regulation of P_N at lower measurement CO_2 concentration than at high CO_2 concentration documented here in cotton suggests a larger reduction in carboxylation capacity than in RuBP regeneration capacity, because carboxylation capacity is generally limiting at low measurement CO_2 concentrations, and regeneration capacity becomes limiting at high CO_2 concentrations. Lack of change in C_i with growth CO_2 concentration treatment suggests that the treatment effects on g_s were a response to changes in P_N, rather than the reverse (Bounoua *et al.* 1999). Cardon *et al.* (1994) reported that fluctuations in CO_2 concentration disrupted g_s, but did not report responses to fluctuations with periods as short as the 1-min cycle used in these experiments.

Although the period of the cyclic variation in CO_2 concentration used here was based on periods observed in a FACE system, FACE systems also expose plants to a much wider range of CO_2 concentration and to more abrupt changes in CO_2 concentration than used here (Hendrey *et al.* 1999, Bunce 2011). The large effects of this limited magnitude, cyclic variation in CO_2 concentration observed here on plant biomass production in both cotton and wheat could conceivably be larger or smaller than possible effects of the more variable fluctuations in CO_2 concentration occurring in FACE systems. Season-long, side-by-side comparisons of plant growth responses to elevated CO_2 concentration in FACE and open-top chambers, like the shorter study by Kimball *et al.* (1997), would be worthwhile, as well as other efforts to evaluate and understand the impacts of rapid fluctuations in CO_2 concentration on plants.

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