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Large-scale plant growth chamber design for elevated pCO_2 and $\delta^{13}C$ studies

William M. Hagopian[1]*, Brian A. Schubert[2] and A. Hope Jahren[1]

[1]Department of Geology and Geophysics, University of Hawaii, Honolulu, HI 96822, USA
[2]School of Geosciences, University of Louisiana at Lafayette, Lafayette, LA 70504, USA

RATIONALE: Throughout at least the next century, CO_2 fertilization and environmental stresses (e.g. nutrient, moisture, insect herbivory) are predicted to affect yields of economically important crop species. Stable isotopes of carbon are used to study plant stresses, which affect yields, but a growth chamber design that can be used to isolate the effects of environmental stresses on crop-sized species through precise maintenance of pCO_2 levels and the $\delta^{13}C$ values of atmospheric CO_2 ($\delta^{13}C_{CO2}$) is lacking.
METHODS: We designed and built low-cost plant growth chambers for growing staple crop species under precise pCO_2 and $\delta^{13}C_{CO2}$ conditions. Over the course of 14 hours, we assessed for pCO_2 stability at two targeted levels (ambient, ~400 ppm; and 2×, ~800 ppm) and measured the $\delta^{13}C_{CO2}$ value within the two chambers using a stable isotope ratio mass spectrometer. We also compared the temperature and relative humidity conditions within the two growth chambers, and in the ambient, outside air.
RESULTS: Across our experimental period, we achieved $\delta^{13}C_{CO2}$ stability (ambient: –8.05 ± 0.17‰; elevated: –12.99 ± 0.29‰) that showed nearly half the variability of any previously reported values for other chamber designs. The stability of the pCO_2 conditions (ambient: 406 ± 3 ppm; elevated: 793 ± 54 ppm) was comparable with that in previous studies, but our design provided ~8 times more growing space than previous chamber designs. We also measured nearly identical temperature and relative humidity conditions for the two chambers throughout the experiment.
CONCLUSIONS: Our growth chamber design marks a significant improvement in our ability to test for plant stress across a range of future pCO_2 scenarios. Through significant improvement in $\delta^{13}C_{CO2}$ stability and increased chamber size, small changes in carbon isotope fractionation can be used to assess stress in crop species under specific environmental (temperature, relative humidity, pCO_2) conditions. Copyright © 2015 John Wiley & Sons, Ltd.

Over the past 50+ years, hundreds of studies have documented the fertilization effect of elevated pCO_2 on plant growth (see, e.g.,[1–6]). The majority of these studies involved plant growth under pCO_2 levels projected for the next 100 years (i.e., ambient to 800 ppm[7,8]) using experimental designs ranging from miniature (e.g., 4 L size chambers for bryophytes[9]), to whole-forest scale open-air plots (e.g., Free Air CO_2 Enrichment (FACE) for mature trees).[10] The carbon isotope composition of plant tissue ($\delta^{13}C$ value) has been used as a proxy for environmental stress (e.g., heat stress,[11] water stress,[12] drought stress,[13] defoliation,[14] and insect herbivory[15]). As an extension of this technique, experiments could be performed to investigate the effect of environmental stress under changing pCO_2 levels. However, such experiments require precise control of the atmospheric conditions under which the plants are grown because both the pCO_2 level[16] and the carbon isotope composition of the atmospheric CO_2 ($\delta^{13}C_{CO2}$ value)[17] have been shown to affect the carbon isotope composition of plant tissue during photosynthesis.

Few studies have successfully varied the pCO_2 level while simultaneously monitoring the $\delta^{13}C_{CO2}$ value within plant growth chambers. Studies that reported sufficiently low $\delta^{13}C_{CO2}$ variability (i.e., <1‰) to assess differences in carbon isotope fractionation among treatments have been limited to small-scale (i.e. <0.8 m^3) chamber designs that can accommodate small-stature plants (e.g., herbs and grasses) only.[6,9,16,18,19] Large-scale experiments designed to accommodate shrubs and trees reported relatively large variability in $\delta^{13}C_{CO2}$ values (i.e., ± 0.9 to ± 1.4‰[20] and ± 1.4 to ± 3.3‰[21]). Recent work has intensified interest in the effects that elevated CO_2 has on crop species;[22] in order to pursue the importance of environmental stress within this context, the ability to control pCO_2 levels and maintain a constant $\delta^{13}C_{CO2}$ value within larger-sized growth chambers will be crucial. Towards this, we designed a growth chamber of suitable stature for staple crops (i.e., interior volume = 6.6 m^3). In order to evaluate the ability of the design to successfully control environmental conditions, we measured a suite of characteristics (i.e., pCO_2, $\delta^{13}C_{CO2}$, relative humidity, and temperature) over a 14-h period (06:00 and 20:00). Here we report on the environmental stability of the chambers at two levels of pCO_2 in order to assess their suitability for plant growth experiments that require simultaneous and precise control over both pCO_2 levels and $\delta^{13}C_{CO2}$ values.

* *Correspondence to:* W. M. Hagopian, 1680 East-West Road, 701 Post Building, University of Hawaii, Honolulu, HI 96822, USA. E-mail: william8@hawaii.edu

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EXPERIMENTAL

Plant growth chamber design

Two growth chambers (Fig. 1) were built within an outdoor greenhouse at the University of Hawaii Magoon Research Facility; the superstructure of this greenhouse was not glass enclosed, but instead covered with a shade cloth that provided 75% light transmission. The growth chambers were constructed using lengths of Douglas Fir 'two-by-four' wood and enclosed in 6 mil, UV-resistant Dura-Film Super 4 polyethylene greenhouse film (International Greenhouse Company, Danville, IL, USA) with a light transmission rating of 91%. The chambers had a footprint 2.4 m wide by 2.4 m long, and increased in height from 0.9 to 1.4 m (front to back) to allow for drainage of rainwater. The roof frames were constructed from Douglas Fir, with cross pieces for support, and positioned on top of the main frames with plastic spacers that provided a 1 cm ventilation gap along the perimeter of the roofline. The roof frames were covered with the same polyethylene greenhouse film as the main frame, with an overhang of 10 cm to provide a wind guard and drip edge. Two sides of the chamber were configured with removable doors to provide access to the interior. Foam weather stripping around the perimeter of the doors minimized atmospheric leakage. The chambers were placed on 0.6 m high nursery benches to provide a solid subflooring, easy access to the interior, and adequate airflow around the full exterior of the chamber.

The chambers featured a flow-through ventilation system where ambient air was supplemented with beverage-grade CO_2 cylinder gas (99.9%; Airgas-Gaspro, Honolulu, HI, USA) as it entered the intake system. Ambient air was drawn into the system through a 10.2 cm diameter PVC intake pipe using a 115-V fan (model #273-242; RadioShack, Fort Worth, TX, USA) and the amount of pure CO_2 gas that entered the air-intake system was controlled using an inline micro-control valve (model #1236012; SGE Analytical Science, Austin, TX, USA) (Figs. 1(A)–1(C)). Within the chamber, a series of parallel 5.1 cm diameter PVC manifolds with evenly spaced 1.9 cm diameter holes distributed the intake air throughout the footprint of the chamber (Fig. 1(D)). While we did not have the means to precisely measure air flow rates from the individual holes, we did verify that air was flowing from the holes along the full length of the distribution pipes. Air exited the chamber through the ventilation gap along the roofline. The chambers were designed to maximize the size of growing space, minimize CO_2 consumption, and optimize chamber air turnover rates. This design provided an interior volume of 6.6 m^3 and a footprint of 5.8 m^2. In order to elevate the chamber pCO_2 to our target level of ~800 ppm, we adjusted the fan air displacement rate to ~1.0 m^3 min^{-1}, and set the CO_2 bleed rate to ~400 cm^3 min^{-1}. This resulted in a mean residence time of air in the chamber of ~6 min and a CO_2 consumption rate of one 23 kg cylinder of beverage-grade CO_2 every 22 days. The 5.8 m^2 of floor space provides sufficient room to carry out growth experiments using sixteen 30 cm diameter (19 L volume) nursery pots. This allows for two treatments of eight plants to be conducted simultaneously within a chamber under uniform pCO_2 and $\delta^{13}C_{CO2}$ conditions; this number of plants (n = 8) corresponds to the median number of plants harvested in 350 previous elevated-CO_2 growth experiments.[4]

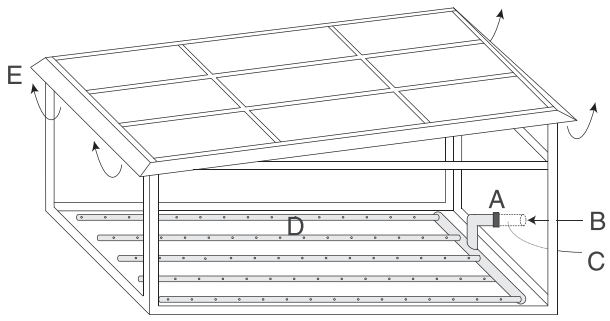

Figure 1. Controlled growth chamber design for maintenance of constant pCO_2 level, $\delta^{13}C_{CO2}$ value, relative humidity, and temperature. A fan located within a PVC intake pipe (A) draws ambient air (B) into the chamber. CO_2 from a gas cylinder bleeds into the intake pipe *via* a stainless steel capillary line (C) in order to elevate the chamber air to the desired pCO_2 level. The air is evenly distributed within the chamber *via* a perforated PVC manifold (D). Air is exhausted through the top of the chamber through a gap between the main structure and the roof. A 10 cm overhang of polyethylene greenhouse film provides a drip and wind guard (E). Access to the chamber is *via* removable side panels.

Monitoring pCO_2, temperature and relative humidity

Temperature and relative humidity were measured and logged using a HOBO U12-012 data logger (Onset Computer Corp., Bourne, MA, USA) and pCO_2 levels were measured using a WMA-4 CO_2 Analyzer (PP Systems, Amesbury, MA, USA). Data were downloaded from the data logger using the HOBOware Lite software, and then exported to Microsoft Excel for processing. We compared the environmental conditions within the chambers to that recorded by a weather station maintained just outside the greenhouse where the chambers were located. The weather station logged temperature, relative humidity, and solar radiation levels every 30 min.

Determination of $\delta^{13}C_{CO2}$ values

Between the hours of 06:00 (prior to sunrise) and 20:00 (post sunset), chamber air was sampled every half hour from inside the elevated chamber, and every hour from inside the ambient chamber. Samples were manually collected into 60 mL vials (catalog #26121; Restek Corporation, Bellefonte, PA, USA) and sealed with a Teflon-faced silicone septum. Access to the chamber for sampling was *via* an 18 cm long opening in the plastic siding of the chamber. The sampling location was 0.6 m from the wall of the chamber where the intake pipe was located. When not in use, the access hole was sealed with a piece of the polyethylene greenhouse film. The positive pressure generated inside the chamber did not allow any external air to enter the growth chamber during sampling. pCO_2 measurements during the experiment were taken at each sampling time at the same location as the $\delta^{13}C_{CO2}$ measurements. Prior to this experiment, we tested

whether the air was well mixed within the chambers by elevating the pCO_2 levels to ~800 ppm and measuring pCO_2 at eight different locations within the chamber. The readings were all within 5 ppm, which indicated that the air was well distributed within the chamber.

The $\delta^{13}C_{CO2}$ value of the chamber air was measured using the direct injection method described in Schubert and Jahren.[16] Briefly, sample aliquots were drawn from the sample vials using an SGE gas-tight syringe (model #008962; SGE Analytical Science) and injected into a modified Eurovector EA3000 automated combustion system (Eurovector SpA, Milan, Italy). Water was removed using a magnesium perchlorate trap, the CO_2 was frozen into a loop cooled with liquid nitrogen, and atmospheric N_2 and O_2 were sent to waste. Any nitrogen oxides in the sample were reduced to N_2 gas by passing the sample over a reduced copper column held at 650°C. The purified CO_2, within a flow of helium, then continued to an Isoprime stable isotope ratio mass spectrometer (Micromass UK Ltd, Manchester, UK) for $\delta^{13}C$ analysis. The $\delta^{13}C_{CO2}$ value of each sample was normalized to the Vienna Pee Dee Belemnite (VPDB) scale using two internal reference gases calibrated using CO_2 gas generated from NBS-19 calcium carbonate ($\delta^{13}C$ consensus value = 1.95‰) and LSVEC lithium carbonate ($\delta^{13}C$ consensus value = –46.6‰)[23] *via* reaction with 100% H_3PO_4.[24] The precision for reference injections and sample injections was better than 0.2‰ (1σ).

Cylinder CO_2 was sampled for $\delta^{13}C_{CO2}$ analysis using a 250-cm^3 metal vessel. The vessel was purged with the cylinder CO_2 for 1 min, and then both ends were closed. The gas was transferred to the dual micro-inlet of the Isoprime and measured against pure aliquots of CO_2 gas generated from NBS-19 calcium carbonate and LSVEC lithium carbonate. The precision for dual inlet analyses was better than 0.05‰.

RESULTS AND DISCUSSION

Environmental conditions

The temperature, relative humidity, and solar radiation levels within the two chambers are shown in Fig. 2 and Table 1. The differences between the average daytime (08:00 to 18:30) temperature and relative humidity between the two chambers were very small: 0.03°C and 0.78%, respectively, which fell within the precision specified for each gauge (0.36°C and 3.5%). The daytime temperature within the chambers averaged 3.9°C higher than open-air conditions reported by the greenhouse weather station. The largest difference occurred at midday when the chambers reached 8.4°C warmer than air outside the shaded greenhouse. Because the chambers were a semi-enclosed system, they were expected to operate at higher temperature than open-air experiments. Relative humidity is inversely proportional to temperature, which explains why the relative humidity was 7.9% lower, on average, within the chambers than that of the outside air where temperatures were lower. We calculated solar radiation levels within the chambers as being 68% of the open air values recorded at the greenhouse weather station by accounting for the amount of light transmission provided by the greenhouse shade cloth (75%) and the greenhouse film covering the chambers (91%) (Fig. 2).

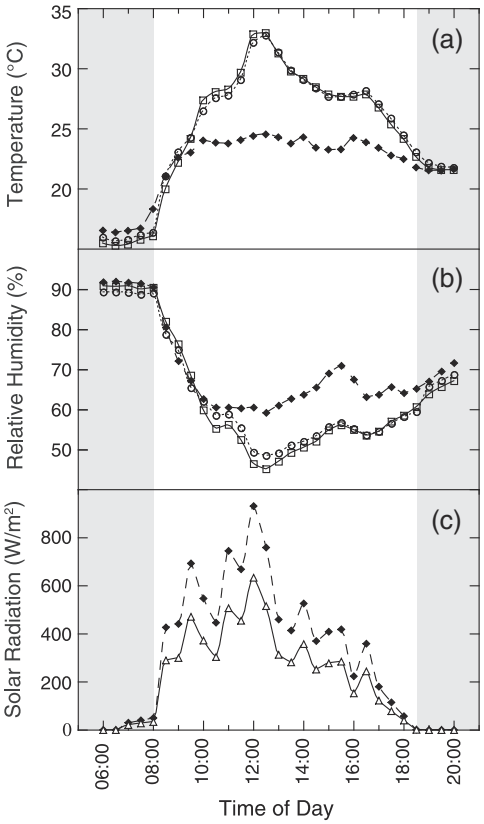

Figure 2. Temperature (a), relative humidity (b), and solar radiation (c) levels of the ambient (circles) and elevated (squares) chambers, and the open-air conditions outside the shaded greenhouse (diamonds). Shaded areas indicate nighttime, non-shaded areas indicate daytime. Uncertainty associated with the temperature and relative humidity gauge was ±0.36 °C and ±3.5%, respectively. Solar radiation was measured over a spectral range of 300 to 1100 nm with an uncertainty of ±10 W/m^2. Solar radiation levels inside the chambers (triangles) were calculated using the open-air measurements and transmission ratings of the shade cloth (75%) and greenhouse film (91%).

The design for a growth experiment can affect the environmental conditions greatly. FACE experiments were designed to most closely preserve natural environmental conditions, while closed environmental growth chambers have been designed to regulate each environmental parameter (i.e., temperature, light levels, relative humidity, and pCO_2 levels) precisely. Open top chambers are an intermediate design that mimics the natural environment more closely, although not exactly, with temperatures typically higher, relative humidity lower, and light intensity decreased compared with the surrounding environment.[25] The flow-through chambers described in this paper altered the temperature, relative humidity, and light levels in predictable ways, while preserving the diurnal patterns of the natural system (Fig. 2). The observation that the environmental conditions inside and outside the chambers differed is not problematic as long as: (1) the conditions within the chambers are conducive to growing the types of

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Table 1. Daytime environmental conditions (08:00 to 18:30)

Treatment	Temperature (°C)[a]	Relative humidity (%)[a]	pCO_2 (ppm)[b]	$\delta^{13}C_{CO2}$ value (‰)[b]
Ambient	27.4 (21.1–32.8)	57.6 (48.6–78.8)	406 ± 3	–8.05 ± 0.17
Elevated	27.4 (20.0–33.0)	56.8 (45.2–82.0)	793 ± 54	–12.99 ± 0.29
Open Air	23.5 (21.1–24.6)	65.1 (59.3–80.6)	N.M.	N.M.

N.M. = not measured.
[a]Average values; ranges in parentheses.
[b]Average values ± 1σ

species selected for an experiment, and (2) that the environmental parameters (e.g. light, relative humidity, temperature) are consistent between chambers. As with all chamber experiments, we suggest taking additional precautions to eliminate both inter- and intra-chamber effects by rotating the plants both within a chamber, and between chambers, on a regular basis.

pCO_2 and $\delta^{13}C_{CO2}$ values

The pCO_2 and $\delta^{13}C_{CO2}$ results are displayed in Fig. 3 and Table 1. The average pCO_2 level over the course of daylight hours was 406 ± 3 ppm (1σ) and 793 ± 54 ppm (1σ) for the ambient and elevated chambers, respectively. This compares favorably with environmental growth cabinets, greenhouse growth rooms that utilize an automatic CO_2 injection, and flow-through designs that were able to maintain stability of pCO_2 within ±8% of the average values (Table 2).[19,26–29] FACE studies defined an acceptable level of pCO_2 stability as maintaining pCO_2 levels within 10% of the mean for 90% of the time.[21,30,31] We maintained pCO_2 within 10% of the mean for 100% of the readings for the ambient chamber and for 91% of the readings for the elevated chamber.

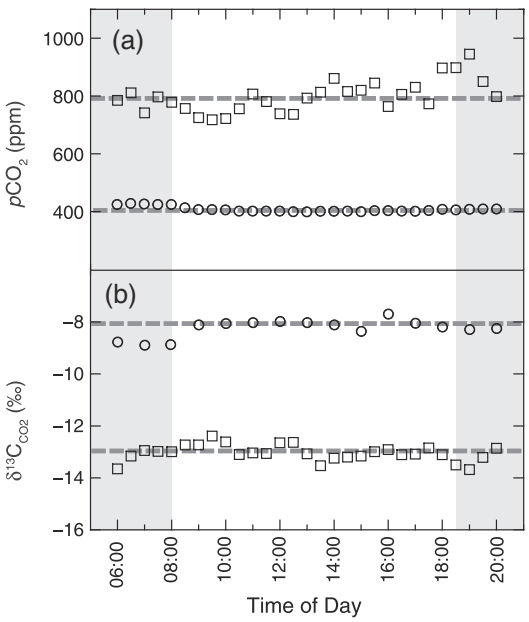

Figure 3. pCO_2 (a) and $\delta^{13}C_{CO2}$ (b) measurements of ambient (circles) and elevated (squares) chamber air throughout the 14-h experiment. Shaded areas indicate nighttime, non-shaded areas indicate daytime. Uncertainty associated with pCO_2 measurements was ± 10 ppm, and ± 0.2‰ for $\delta^{13}C_{CO2}$ measurements. Dashed lines indicate averages for the daylight hours (08:00 to 18:30).

The average daytime $\delta^{13}C_{CO2}$ value in the ambient chamber was –8.05‰ (1σ = 0.17‰; SE = 0.06‰; n = 10) and –12.99‰ (1σ = 0.29‰; SE = 0.05‰; n = 21) in the elevated chamber. While previous studies reported $\delta^{13}C_{CO2}$ values of environmental air for both ambient and elevated treatments, only half (n = 6) reported uncertainties for these values (Table 2). The standard deviations (and standard errors) of the average $\delta^{13}C_{CO2}$ values within our chambers were lower than those of all previous designs.

The higher variability in pCO_2 levels and $\delta^{13}C_{CO2}$ values for the elevated *versus* the ambient chamber can be attributed to several factors. Environmental temperature fluctuations can affect the flow characteristics of the CO_2 cylinder gas delivery system *via* thermal expansion and contraction of the gas regulator diaphragms, micro-valves, and metal transfer lines. A change in temperature can also affect the resistance and conductivity of the electrical components of the chamber intake fans, which can contribute to variations in intake flow. In addition, a change in temperature can change the number of moles of ambient air molecules flowing into the chamber (following the ideal gas law $PV = nRT$). Since the pCO_2 level of the elevated chamber is the result of two CO_2 sources mixing: (1) supplemental CO_2 from a cylinder and (2) ambient air, changes in amounts of either of these entering the chamber, due to one or more of the above causes, can affect the resulting pCO_2 level within the elevated chamber. The supplemental CO_2 has a different $\delta^{13}C_{CO2}$ value from the ambient air (–18.47‰ versus –8.05‰); therefore, changes in the ratio of CO_2 sources can also affect the $\delta^{13}C_{CO2}$ value of the elevated chamber air. As a result, we suggest shielding the CO_2 delivery system from direct sunlight and providing adequate airflow around the components to minimize temperature fluctuations within the system.

We observed that $\delta^{13}C_{CO2}$ and pCO_2 measurements made before sunrise and after sunset within the ambient chamber followed the same trends reported in the literature for ambient air that contained a source of nighttime respired CO_2 from roots and microbial decomposition.[25,32–35] We used the Keeling plot method to determine the $\delta^{13}C_{CO2}$ value of this contribution by plotting ambient chamber $\delta^{13}C_{CO2}$ values (06:00 to 20:00) against $1/pCO_2$. The least squares fit line through these points resulted in the equation: $\delta^{13}C_{CO2} = 5624/pCO_2 - 21.9$ ($R^2 = 0.78$), where the intercept represented the $\delta^{13}C_{CO2}$ value of mean local surface emissions.[35,36] This

Table 2. Comparison of published plant growth studies that vary pCO_2 levels and record $\delta^{13}C_{CO2}$ levels of the air used for photosynthesis

Type	Size	Plants studied	pCO_2 levels (ppm)[a]	$\delta^{13}C$ air values uncertainty[b]	References
Free Air CO_2 Enrichment (FACE), open air plots	25 m dia. plots	*Triticum aestivum*	360, 550 ±10%	±1.4‰ and ±3.3‰ (1σ)	Leavitt *et al.*[21]
Glass greenhouse room	not reported	*Sequoia sempervirens*, *Metasequoia glyptostroboides*, *Taxodium distichum*	400, 800 ±5%	not reported	Llorens *et al.*[29]
Growth room w/CO_2 injection	not reported	*Quercus petraea*	300,700	not reported	Tu *et al.*[40]
Growth Cabinet	not reported	*Aristida glabrata*, *Bouteloua curtipendula*, *Eragrostis lehmanniana*	370,690	±0.3‰ and ±0.6‰	Fravolini et al.[41]
Open top chambers	3 m dia.	*Sorghum bicolor*, *Glycine max*	358 to 732 ± <0.6%	±0.9‰ and ±1.4 ‰	Torbert *et al.*[20]
Closed chamber w/CO_2 injection	0.72 m^3	*Sorghum bicolor*	350, 700 ±7%	±0.22‰ and ±0.24‰ (stand error)	Watling *et al.*[27]
Closed chamber w/CO_2 injection	0.72 m^3	*Arabidopsis thaliana*	380 to 3000	not reported	Lomax *et al.*[42]
Flow through	0.004 m^3	*Lunularia cruciata*, *Marchantia polymorpha*. *Funaria hygrometrica*, *Leptobryum pyriforme*	375 to 6000	not reported	Fletcher *et al.*[9]
Flow through	0.004 m^3	*Phaseolus vulgaris* and *Sinapis alba*	300, 360, 450	~ ±1‰	Beerling *et al.*[43]
Flow through	0.125 m^3	*Puccinellia nuttalliana*	350, 1300	not reported	Guy and Reid[18]
Flow through	0.50 m^3	*Arabidopsis thaliana*, *Raphanus sativus*	370-4200 ±3-8%	±0.54‰ (1σ)	Jahren *et al.*;[19] Schubert and Jahren[6,16]
Flow through	2.43 m^3	17 herb and grass species	350, 525, 700 ±3%	not reported	Beerling and Woodward[26]

[a] pCO_2 uncertainty included for studies that reported it.
[b] $\delta^{13}C$ uncertainty is included for studies that reported it. Uncertainty was reported as 1σ, standard error, or not indicated.

value was within the range of nighttime respired $\delta^{13}C_{CO2}$ values reported by other researchers (–21.1 to −26.35‰).[34,37] During daylight hours only (the time when plants fix carbon), we did not observe the effects of nighttime respiration on chamber air $\delta^{13}C_{CO2}$ values and pCO_2 measurements. This can be explained by the short mean residence time of 6 min for air within the chamber, with the nighttime respired CO_2 contribution being completely flushed out of the chamber soon after sunrise.

We tested whether the elevated chamber pCO_2 was the result of a two end-member mixing system (ambient air CO_2 and CO_2 cylinder gas) to verify that no other sources of CO_2 contributed to the elevated chamber $\delta^{13}C_{CO2}$ values. We used the following isotope mass balance equation:

$$d/dt\left(\delta^{13}C_e\right)(M) = \left(\delta^{13}C_a\right)(F_a) + \left(\delta^{13}C_s\right)(F_s) - \left(\delta^{13}C_{out}\right)(F_{out}) \quad (1)$$

where F_a is the flux of CO_2 from ambient air, F_s is the flux of supplemental CO_2 from the cylinder, and F_{out} is the flux of CO_2 out of the chamber. M represents the number of moles within the chamber. $\delta^{13}C$ represents the carbon isotope ratio of each CO_2 component (subscripts *e*, *a*, *s*, and *out* indicate elevated, ambient, supplemental, and chamber exhaust, respectively). We assumed that:

$$\delta^{13}C_{out} = \delta^{13}C_e \quad (2)$$

and that,

$$dM/dt = F_a + F_s - F_{out} \quad (3)$$

We combined Eqns. (1), (2), and (3) and, when $d/dt(\delta^{13}C_e) = 0$:

$$\delta^{13}C_e = \left[\left(\delta^{13}C_a\right)(F_a)/(F_a + F_s)\right] + \left[\left(\delta^{13}C_s\right)(F_s)/(F_a + F_s)\right] \quad (4)$$

The $\delta^{13}C_a$ value was measured at each sampling time and $\delta^{13}C_s$ was the value of the cylinder gas (–18.47‰). F_a was calculated for each sampling time using the pCO_2 measurement for the ambient chamber, the intake airflow rate, and the diameter of the intake pipe. F_s was calculated using the pCO_2 measurements for the ambient and elevated chambers, and assuming that any rise in CO_2 above ambient was derived from the supplemental CO_2 bleed line (the CO_2 bleed rate

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could not be directly measured during the experiment without affecting the chamber pCO_2).

Using Eqn. (4), we calculated the average daytime $\delta^{13}C_e$ = –13.14‰ (1σ = 0.44‰; n = 10), which was not statistically different (p = 0.33) from the average $\delta^{13}C_{CO2}$ value measured *via* the direct injection method (–12.99‰; 1σ = 0.29‰; n = 21). This verified that the composition of the elevated chamber air was a simple two end-member mixing system that consisted of ambient CO_2 and supplemental cylinder CO_2.

These chambers, along with the majority of controlled pCO_2 experiments, utilized local ambient air supplemented with CO_2 cylinder gas to achieve elevated pCO_2 levels. In urban and industrial settings, anthropogenic CO_2 emissions can alter localized/daytime ambient pCO_2 levels and $\delta^{13}C_{CO2}$ values by as much as 550 ppm and 11‰, respectively.[33,37–39] It is therefore important to choose a location for the chambers where local ambient conditions are stable for the length of the growth experiment.

We expect plant stress growth experiments to last longer than 14 h (of the order of weeks to months), and the data presented here suggests that the chamber conditions will remain sufficiently stable over these longer time periods. We believe that temperature changes are of most concern to the stability of the pCO_2 levels and $\delta^{13}C_{CO2}$ values within the elevated chamber, and we have shown that across a wide range of temperatures (i.e., 20 to 33°C), stability was maintained. For changes in large-scale external environmental conditions (e.g., atmospheric pressure), maintenance of the elevated pCO_2 level would involve a simple adjustment of the supplemental CO_2 flow rate.

CONCLUSIONS

We have described low-cost growth chambers capable of maintaining nearly identical environmental conditions and precise $\delta^{13}C_{CO2}$ control under both ambient and elevated pCO_2 levels. Previous chamber designs aimed at using the $\delta^{13}C$ value of plant tissue as an investigative tool reported greater $\delta^{13}C_{CO2}$ variability and were limited in interior chamber volume (maximum of 0.72 m^3). The chamber design that we tested here provided at least 8 times more interior volume (6.6 m^3) while limiting $\delta^{13}C_{CO2}$ variability to nearly half that of all previous designs. The large interior volume allows for growth experiments of economically important crop-sized species, such as sweet potato, soybean, and tomato. Because the $\delta^{13}C_{CO2}$ value[17] and pCO_2 level[16] both affect the measured carbon isotope fractionation in plant tissues, the unmatched stability in atmospheric conditions (i.e., $\delta^{13}C_{CO2}$ and pCO_2) of this design will enable researchers to detect differences in isotope discrimination from environmental stresses that would not otherwise be possible.

Acknowledgements

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