

RESEARCH ARTICLE

Plant growth chamber design for subambient $p\text{CO}_2$ and $\delta^{13}\text{C}$ studiesWilliam M. Hagopian¹  | Brian A. Schubert² | Robert A. Graper³ | A. Hope Jahren¹ ¹Centre for Earth Evolution and Dynamics, University of Oslo, N-0315 Oslo, Norway²School of Geosciences, University of Louisiana at Lafayette, Lafayette, LA 70504, USA³School of Ocean and Earth Science and Technology, University of Hawaii at Manoa, Honolulu, HI 96822, USA**Correspondence**W. M. Hagopian, Postbox 1028 Blindern, 0316 Oslo, Norway.
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Rationale: Subambient $p\text{CO}_2$ has persisted across the major Phanerozoic ice ages, including the entire late Cenozoic (*ca* 30 Ma to present). Stable isotope analysis of plant-derived organic matter is used to infer changes in $p\text{CO}_2$ and climate in the geologic past, but a growth chamber that can precisely control environmental conditions, including $p\text{CO}_2$ and $\delta^{13}\text{C}$ value of CO_2 ($\delta^{13}\text{C}_{\text{CO}_2}$) at subambient $p\text{CO}_2$, is lacking.

Methods: We designed and built five identical chambers specifically for plant growth under stable subambient $p\text{CO}_2$ (*ca* 100 to 400 ppm) and $\delta^{13}\text{C}_{\text{CO}_2}$ conditions. We tested the $p\text{CO}_2$ and $\delta^{13}\text{C}_{\text{CO}_2}$ stability of the chambers both with and without plants, across two 12-hour daytime experiments and two extended 9-day experiments. We also compared the temperature and relative humidity conditions among the chambers.

Results: The average $\delta^{13}\text{C}_{\text{CO}_2}$ value within the five chambers ranged from -18.76 to -19.10‰ ; the standard deviation never exceeded 0.14‰ across any experiment. This represents better $\delta^{13}\text{C}_{\text{CO}_2}$ stability than that achieved by all previous chamber designs, including superambient $p\text{CO}_2$ chambers. Every $p\text{CO}_2$ measurement ($n = 1225$) was within 5% of mean chamber values. The temperature and relative humidity conditions differed by no more than 0.4°C and 1.6%, respectively, across all chambers within each growth experiment.

Conclusions: This growth chamber design extends the range of $p\text{CO}_2$ conditions for which plants can be grown for $\delta^{13}\text{C}$ analysis of their tissues at subambient levels. This new capability allows for careful isolation of environmental effects on plant ^{13}C discrimination across the entire range of $p\text{CO}_2$ experienced by terrestrial land plants.

1 | INTRODUCTION

Plant growth chamber designs that are capable of maintaining precise control of both the concentration of atmospheric carbon dioxide ($p\text{CO}_2$) and the carbon isotope composition of atmospheric CO_2 ($\delta^{13}\text{C}_{\text{CO}_2}$ values) are needed in order to quantify the effect of $p\text{CO}_2$ on ^{13}C discrimination during photosynthesis. Our previous work showed that under elevated $p\text{CO}_2$, ^{13}C discrimination by C_3 land plants increases with increasing $p\text{CO}_2$,¹ and led to the conclusion that interpretations of environmental change based on the $\delta^{13}\text{C}$ value of terrestrial plant material must account for changes in $p\text{CO}_2$.² Consequently, this work has been applied to multiple intervals of Earth history with elevated $p\text{CO}_2$.³⁻⁵

The last 20 million years of Earth history may have included $p\text{CO}_2$ levels lower than today (i.e. 170–400 ppm) as indicated by both proxies⁶ and the CO_2 concentration in air bubbles trapped in ice.⁷ Data on plant ^{13}C discrimination across subambient $p\text{CO}_2$ are generally limited to measurements of the $\delta^{13}\text{C}$ values of preserved plant material coupled with historical and ice core $\delta^{13}\text{C}_{\text{CO}_2}$ data,⁸⁻¹² and the discrimination has not been systematically quantified across multiple levels of $p\text{CO}_2$.

Previous studies of plant growth under subambient $p\text{CO}_2$ generally relied on closed or semi-closed systems where $p\text{CO}_2$ was regulated via removal of atmospheric CO_2 either by photosynthetic fixation or by chemical scrubbing, and then supplemented with cylinder CO_2 or ambient air. Most of these studies focused on maintenance

of $p\text{CO}_2$ levels, but did not measure or maintain the $\delta^{13}\text{C}_{\text{CO}_2}$ value during experimentation.

The goal of the work reported here was to create a growth chamber design capable of providing stable subambient $p\text{CO}_2$ levels and $\delta^{13}\text{C}_{\text{CO}_2}$ values. Here we describe our subambient chamber design and test the stability of $p\text{CO}_2$ levels and $\delta^{13}\text{C}_{\text{CO}_2}$ values within four experiments designed to assess both intraday and day-to-day variation. We compare our results with those from other published designs, and evaluate the potential of these chambers for quantifying changes in ^{13}C discrimination across subambient $p\text{CO}_2$.

2 | EXPERIMENTAL

2.1 | Plant growth chamber design

Five plexiglass boxes each capable of supporting twelve small plants for several weeks of growth at subambient levels of $p\text{CO}_2$ were constructed from readily available hardware store materials (Figure 1). Chambers (122 cm \times 91 cm \times 46 cm) were constructed out of 6.4 mm thick plexiglass sheets; the 122 cm \times 46 cm sides of the chambers were removable for access to the interiors in order to perform daily maintenance of plants. Weather stripping was used to create seals between the door panels and chamber frames to prevent ambient air from leaking into the growing space.

Each chamber featured a flow-through ventilation system where compressed air flowed through a CO_2 scrubbing canister filled with Sofnolime 812 mesh, 797 grade CO_2 absorbent (Molecular Products Group Ltd, Harlow, UK) in order to completely remove ambient CO_2 . The CO_2 scrubbing canister consisted of a 50 cm section of 10.2 cm diameter PVC pipe with a screen mesh at one end designed to hold the Sofnolime granules in place. Fernco flexible couplings (Fernco Inc., Davison, MI, USA) on each end connected the canister to the compressed air inlet and to the PVC pipe (10.2 cm diameter) leading to the chamber. This provided an easily removable canister for replenishing the depleted Sofnolime. In order to raise $p\text{CO}_2$ to the desired subambient level within the chamber, 99.9% pure CO_2 gas (Airgas-Gaspro, Honolulu, HI, USA) was introduced to the chamber intake via stainless steel tubing. An inline SGE model 1236012

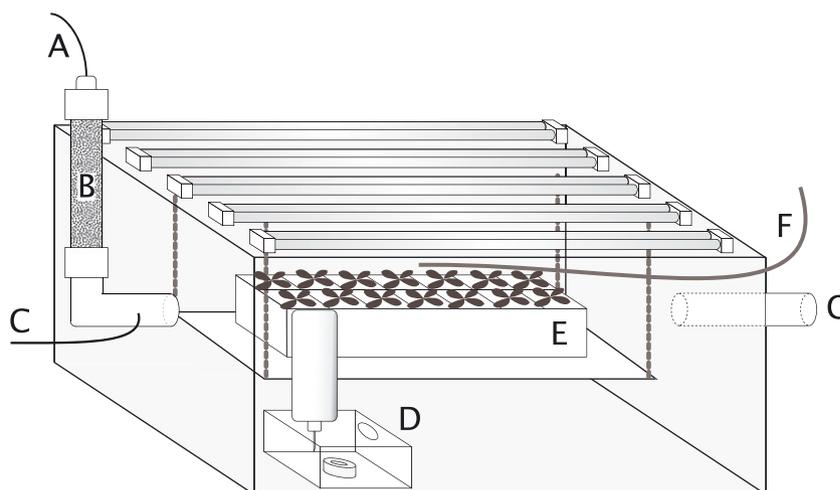
micro-control valve (SGE Analytical Science, Austin, TX, USA) enabled precise control of the CO_2 flow rate, adjusted to between 1.5 and 11.0 mL/minute depending on the target $p\text{CO}_2$ level.

Carbon dioxide levels were measured for each chamber using an LI-840A CO_2 gas analyzer (LI-COR Inc., Lincoln, NE, USA), which has a manufacturer-reported accuracy of better than 1.5% of reading. The gas analyzer was zeroed daily by diverting the intake flow through a Sofnolime scrubber and manually zeroing via the software interface. A 10 mm diameter Tygon tube allowed chamber air to be sampled for $p\text{CO}_2$ measurements directly from the growing area without needing to remove the plexiglass chamber sides.

Chamber air was collected for stable isotope analysis by diverting the $p\text{CO}_2$ sampling line for the LI-COR CO_2 gas analyzer through a line containing a septum sampling port where air could be drawn into an SGE gas-tight syringe, model 008962 (SGE Analytical Science). The $\delta^{13}\text{C}_{\text{CO}_2}$ value of chamber air was then measured using the direct injection method described in Schubert and Jahren.¹ Briefly, sample aliquots were injected from the syringe 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. 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 $\delta^{13}\text{C}$ analysis. The $\delta^{13}\text{C}_{\text{CO}_2}$ value of each sample was normalized to the Vienna Pee Dee Belemnite (VPDB) scale using two internal reference gases (-10.27 and -25.32‰) calibrated using CO_2 gas generated from NBS-19 calcium carbonate ($\delta^{13}\text{C}$ consensus value = 1.95‰) and LSVEC lithium carbonate ($\delta^{13}\text{C}$ consensus value = -46.6‰)¹³ via reaction with 100% H_3PO_4 .¹⁴ A third calibrated gas (-19.73‰) was used as a quality control sample to ensure accuracy of measurements. The precision for reference injections and quality assurance injections was better than 0.2‰ (1σ).

The temperature and relative humidity were measured and logged using a HOBO U12-012 data logger (Onset Computer Corp., Bourne, MA, USA). The measurement precision specified by the manufacturer was 0.36°C and 3.5% for temperature and relative humidity, respectively.

FIGURE 1 Controlled growth chamber design for maintenance of stable subambient $p\text{CO}_2$, $\delta^{13}\text{C}_{\text{CO}_2}$ value, relative humidity, and temperature. Compressed ambient air (A) flows through a Sofnolime CO_2 scrubber (B) where CO_2 -free air is then supplemented with cylinder CO_2 via a stainless steel capillary (C) to elevate $p\text{CO}_2$ of the chamber air to the desired level. A humidifier (D) maintains stable relative humidity within the chamber. A hanging shelf (E) provides an adjustable platform for plant trays. Air is sampled for $p\text{CO}_2$ and $\delta^{13}\text{C}_{\text{CO}_2}$ measurements via a tube (F) leading to the plant growth area. Chamber exhaust is vented (G) to a fume hood [Color figure can be viewed at wileyonlinelibrary.com]



For chamber humidity control, a custom humidifier was constructed using a Mist Maker element (AGPtek, Brooklyn, NY, USA) placed within a 15 cm × 15 cm × 8 cm plastic container (Glad Products Company, Oakland, CA, USA) and a modified 0.5 L Nalgene squirt bottle provided an extended water reservoir. A simple closed-loop control program running on an Arduino Mega 2560 R3 microcontroller (Adafruit.com, New York, NY, USA) configured with an SHT15 humidity and temperature sensor (Sensirion AG, Stäfa, Switzerland) maintained relative humidity via user-configurable upper and lower set points.

The lighting system consisted of five rows of two-element T8 lighting units located on the outside of the chamber in order to reduce heat buildup in the chamber. An adjustable shelf hanging by chains from the ceiling of the chamber provided a platform for plant pots where the shelf distance from the lights could be adjusted as the plants grew in height, therefore maintaining a constant light intensity at canopy level. In order to provide uniform $p\text{CO}_2$, temperature, and relative humidity within the chamber, a 12 V fan was used to circulate air.

2.2 | Environmental stability experiments

The intraday and day-to-day environmental variability of the subambient chamber design was quantified in four separate experiments: (1) chambers with no plants operating over a 12-hour daytime period; (2) chambers with no plants over the course of 9 days; (3) chambers with plants over a 12-hour daytime period; and (4) chambers with plants over the course of 9 days.

Experiment 1 consisted of three empty chambers with $p\text{CO}_2$ maintained at *ca* 100, 250, and 400 ppm over the course of a typical 12-hour chamber day: chamber $p\text{CO}_2$ and $\delta^{13}\text{C}_{\text{CO}_2}$ measurements were taken approximately every 60 minutes. This experiment was designed to show the diurnal variability of the chamber environment under ideal conditions where no carbon fixation by plants could affect the chamber $p\text{CO}_2$ or $\delta^{13}\text{C}_{\text{CO}_2}$ value.

Experiment 2 consisted of three empty chambers with $p\text{CO}_2$ maintained at *ca* 100, 250, and 400 ppm over the course of 9 days: chamber $p\text{CO}_2$ was recorded approximately every 30 minutes, and $\delta^{13}\text{C}_{\text{CO}_2}$ measured four times daily at *ca* 1, 2, 9 and 10 hours after lights on. This experiment was designed to show the variability of the chamber environment over multiple days, under ideal conditions where no carbon fixation by plants could affect the chamber $p\text{CO}_2$ or $\delta^{13}\text{C}_{\text{CO}_2}$ value.

Experiment 3 consisted of two chambers, each containing 12 *Arabidopsis thaliana* plants, with $p\text{CO}_2$ maintained at *ca* 100 and 400 ppm over the course of a typical 12-hour chamber day: chamber $p\text{CO}_2$ and $\delta^{13}\text{C}_{\text{CO}_2}$ measurements were taken approximately every 30 minutes. This experiment was designed to show the diurnal variability of the chamber environment under typical plant growth conditions where carbon fixation could potentially alter the chamber $p\text{CO}_2$ or $\delta^{13}\text{C}_{\text{CO}_2}$ value.

Experiment 4 consisted of five chambers, each containing 12 *Arabidopsis thaliana* plants, with $p\text{CO}_2$ maintained at *ca* 100, 175, 250, 325, and 400 ppm over the course of 9 days: the chamber $p\text{CO}_2$ was recorded approximately every 30 minutes, and the $\delta^{13}\text{C}_{\text{CO}_2}$ value was measured once daily at *ca* 4 hours after lights on. This experiment was designed to show the variability of the chamber environment under plant growth conditions over multiple days where

carbon fixation could potentially alter the chamber $p\text{CO}_2$ or $\delta^{13}\text{C}_{\text{CO}_2}$ value. The nine days of close monitoring was chosen to encompass the period when *Arabidopsis* were growing most rapidly, during weeks 3 and 4 after germination, just prior to flowering. This provided an opportunity to assess for the maximum potential effect of photosynthesis on $p\text{CO}_2$ and the $\delta^{13}\text{C}_{\text{CO}_2}$ value.

For experiments 3 and 4, *A. thaliana* plants were grown in small plastic pots (7.6 cm × 7.6 cm × 10.2 cm) containing Miracle-Gro Potting Mix (ScottsMiracle-Gro Co., Marysville, OH, USA) and the gravimetric soil moisture was measured and adjusted in order to maintain moisture at 1.9 g of water per gram of soil.

The following conditions and protocols were the same for all chambers in the four experiments: compressed air flow rates were set to 28 ± 0.5 L/min; supplemental CO_2 flow rates were set at the beginning of the experiments to maintain $p\text{CO}_2$ levels, with no further adjustment; the lighting cycles were set to 12 hours on, 12 hours off, using Philips bulbs (model F32 T8/TL850/ALTO II; Philips Corp., Andover, MA, USA) with an intensity of $180 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$ (400–700 nm) at canopy height; the relative humidity control was set to maintain a value between 63 and 68%. Two hours prior to each start of the “day” (lights on), the chambers were opened for maintenance and the humidifier water reservoirs were replenished, the plants were watered, and the Sofnolime scrubber granules were replaced with 1000 ± 50 g of fresh granules. The chambers were then closed and allowed to stabilize to the set $p\text{CO}_2$ levels prior to lights on. All the chambers of the four experiments used the same supplemental cylinder CO_2 with $\delta^{13}\text{C}$ value of -19.09% . The relative humidity and temperature were recorded every 10 minutes throughout all experiments.

3 | RESULTS AND DISCUSSION

Results for the four experiments are displayed in Table 1 and Figures 2 and 3. The standard deviations of the $p\text{CO}_2$ measurements for every chamber, across all four experiments were less than 4.2 ppm, and 100% of the $p\text{CO}_2$ values were within 5% of their mean chamber values. This compared favorably with previous subambient growth chamber studies that reported standard deviations between 1 and 40 ppm,^{15–28} and was more stable than observed in superambient $p\text{CO}_2$ chamber studies (refer to Table 2 in Hagopian et al²⁹).

The mean $\delta^{13}\text{C}_{\text{CO}_2}$ chamber values ranged from -18.76 to -19.10% across all experiments (both intraday and long-term monitoring, with and without plants), and the standard deviation of the $\delta^{13}\text{C}_{\text{CO}_2}$ values for each chamber never exceeded 0.14‰ in any experiment. Every $\delta^{13}\text{C}_{\text{CO}_2}$ measurement was within 0.36‰ of the chamber mean value with 98% of all $\delta^{13}\text{C}_{\text{CO}_2}$ measurements within 0.2‰ (the analytical uncertainty of the measurement) of their chamber mean value.

To assess the effects of plant CO_2 fixation on the $\delta^{13}\text{C}_{\text{CO}_2}$ values within our chambers, we compared the mean $\delta^{13}\text{C}_{\text{CO}_2}$ values of chambers with and without plants at the same $p\text{CO}_2$ levels and found the differences to be $\leq 0.22\%$, indicating that there was no significant $\delta^{13}\text{C}_{\text{CO}_2}$ value increase from CO_2 fixation effects. We conclude that the ventilation rate of our chambers was sufficient to offset any carbon fixation effects.

TABLE 1 Subambient chamber $p\text{CO}_2$, $\delta^{13}\text{C}_{\text{CO}_2}$ values, relative humidity (RH) and temperature results^a

| Experiment 1: no plants (intraday) | | | |
|--------------------------------------|---|-------------------|-------------------|
| $p\text{CO}_2$ (ppm) | $\delta^{13}\text{C}_{\text{CO}_2}$ (‰) | RH (%) | Temperature (°C) |
| 98 ± 1.3 (15) | -18.81 ± 0.07 (15) | 64.0 ± 1.9 (144) | 23.8 ± 3.3 (144) |
| 244 ± 1.5 (15) | -18.91 ± 0.07 (15) | 64.8 ± 2.1 (144) | 23.7 ± 3.2 (144) |
| 387 ± 0.9 (15) | -19.02 ± 0.06 (15) | 64.3 ± 1.6 (144) | 24.1 ± 3.1 (144) |
| Experiment 2: no plants (9 days) | | | |
| $p\text{CO}_2$ (ppm) | $\delta^{13}\text{C}_{\text{CO}_2}$ (‰) | RH (%) | Temperature (°C) |
| 98 ± 1.5 (211) | -18.86 ± 0.12 (36) | 63.6 ± 1.9 (1084) | 24.2 ± 3.3 (1084) |
| 253 ± 4.2 (203) | -18.90 ± 0.14 (36) | 64.6 ± 2.1 (1084) | 24.0 ± 3.1 (1084) |
| 391 ± 2.7 (203) | -19.02 ± 0.09 (36) | 64.1 ± 1.6 (1084) | 24.4 ± 3.1 (1084) |
| Experiment 3: with plants (intraday) | | | |
| $p\text{CO}_2$ (ppm) | $\delta^{13}\text{C}_{\text{CO}_2}$ (‰) | RH (%) | Temperature (°C) |
| 93 ± 0.9 (25) | -18.89 ± 0.09 (25) | 65.0 ± 2.1 (144) | 20.7 ± 2.0 (144) |
| 386 ± 1.4 (25) | -18.80 ± 0.10 (25) | 64.7 ± 2.1 (144) | 20.9 ± 1.9 (144) |
| Experiment 4: with plants (9 days) | | | |
| $p\text{CO}_2$ (ppm) | $\delta^{13}\text{C}_{\text{CO}_2}$ (‰) | RH (%) | Temperature (°C) |
| 96 ± 1.5 (175) | -18.89 ± 0.10 (9) | 65.4 ± 2.7 (1513) | 22.5 ± 2.0 (1225) |
| 167 ± 1.8 (181) | -18.84 ± 0.09 (9) | 64.8 ± 2.6 (1513) | 22.2 ± 1.9 (1225) |
| 243 ± 1.7 (184) | -18.76 ± 0.09 (9) | 66.4 ± 2.4 (1513) | 22.3 ± 2.1 (1225) |
| 322 ± 4.0 (180) | -19.10 ± 0.04 (9) | 64.8 ± 2.3 (1513) | 22.5 ± 1.9 (1225) |
| 390 ± 2.2 (179) | -18.97 ± 0.11 (9) | 65.2 ± 2.2 (1513) | 22.3 ± 1.9 (1225) |

^aMean values ±1 standard deviation. Number of measurements reported in parentheses.

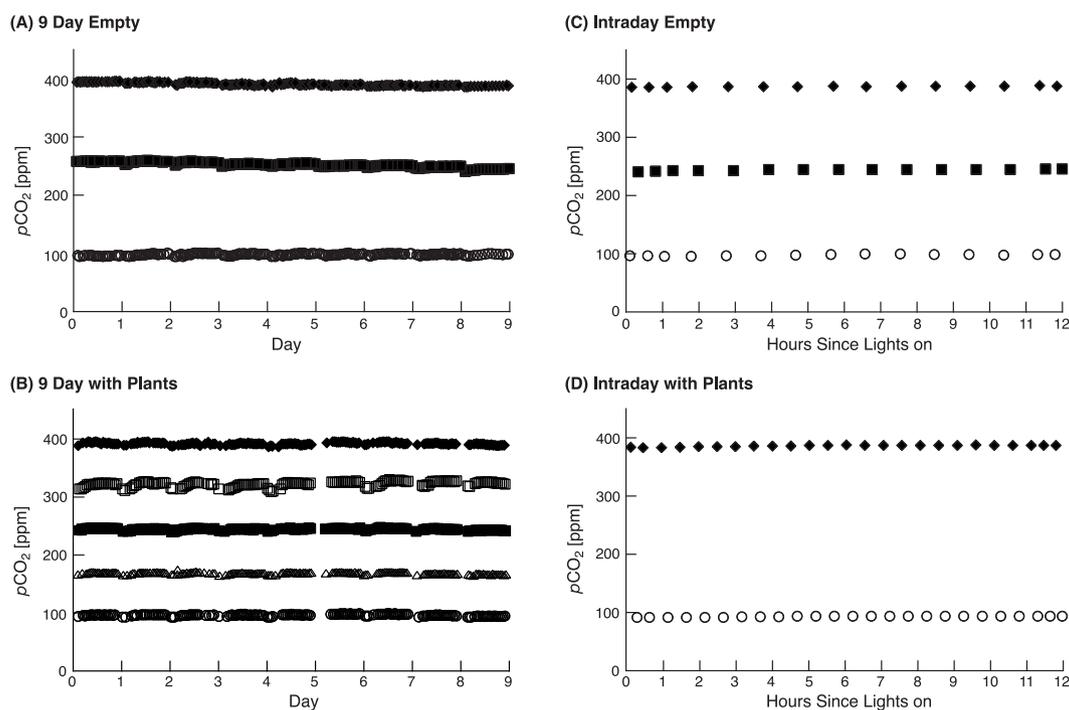


FIGURE 2 Values of $p\text{CO}_2$ for (A) a 9-day period with no plants in the chambers; (B) an 11-day period with *Arabidopsis thaliana* actively growing in the chambers; (C) intraday levels for empty chambers; and (D) intraday levels for chambers with *A. thaliana*. Solid triangles represent ca 400 ppm chambers, open squares ca 325 ppm, closed squares ca 250 ppm, open triangles ca 175 ppm, and open circles ca 100 ppm. Uncertainty for $p\text{CO}_2$ measurements was 1.5% of the reading. Data for (A) and (B) include only the time period of 12 hours while lights were on

For all the chambers in this study, the $\delta^{13}\text{C}_{\text{CO}_2}$ mean values were within 0.33‰ of the cylinder $\delta^{13}\text{C}_{\text{CO}_2}$ value (-19.09‰). The small differences can be explained by small amounts of ambient air (2 to 5 ppm of the total $p\text{CO}_2$) leaking into the chambers. Because calculation of ^{13}C plant discrimination accounts for absolute differences in

atmospheric $\delta^{13}\text{C}_{\text{CO}_2}$ values among treatments,^{1,2,30} maintaining a specific atmospheric $\delta^{13}\text{C}_{\text{CO}_2}$ value is not required. Therefore, these small differences in the chamber $\delta^{13}\text{C}_{\text{CO}_2}$ values are not a concern. However, accurate interpretation of small changes in ^{13}C discrimination in response to small changes in environmental conditions (e.g. $p\text{CO}_2$) does

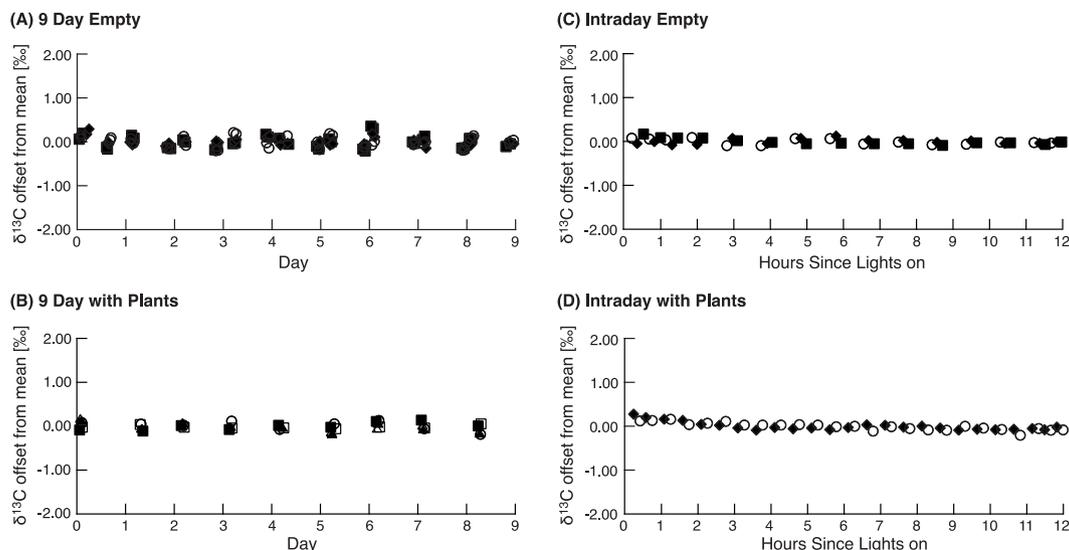


FIGURE 3 Deviation of single $\delta^{13}\text{C}_{\text{CO}_2}$ measurements from mean chamber values for (A) 9-day period with no plants in the chambers; (B) 9-day period with *Arabidopsis thaliana* actively growing in chambers; (C) intraday levels for empty chambers; and (D) intraday levels for chambers with *A. thaliana*. Solid triangles represent ca 400 ppm chambers, open squares ca 325 ppm, closed squares ca 250 ppm, open triangles ca 175 ppm, and open circles ca 100 ppm. Uncertainty for $\delta^{13}\text{C}_{\text{CO}_2}$ measurements was 0.2%. Data for (A) and (B) include only the time period of 12 hours while lights were on

require a high degree of stability in the chamber $\delta^{13}\text{C}_{\text{CO}_2}$ value and the chamber design that we have described here satisfies this requirement.

Isolation of the effect of $p\text{CO}_2$ on plant discrimination also requires consistent environmental conditions among chambers. The computer-controlled humidifiers used in each chamber were able to maintain a similar relative humidity between chambers within each experiment (differing by $\leq 1.6\%$). The chamber temperatures were also well constrained, differing by $\leq 0.4^\circ\text{C}$ within each experiment.^{29,31-35}

Two methods are reported in the literature for reducing $p\text{CO}_2$ to subambient levels for growth chambers: scrubbing CO_2 with a chemical absorbent^{19-22,24,28}; and utilizing the natural process of carbon fixation by the plants to decrease the chamber $p\text{CO}_2$.¹⁵⁻¹⁸ Previous studies that utilized chemical scrubbing were designed to investigate the effects of $p\text{CO}_2$ on plant growth and reproduction and did not study carbon isotope discrimination; hence there is no chamber $\delta^{13}\text{C}_{\text{CO}_2}$ data reported in these papers with which we can compare our $\delta^{13}\text{C}_{\text{CO}_2}$ results.

Of the studies that utilized the natural CO_2 fixation by plants within the chamber environment to create subambient CO_2 conditions, Beerling et al¹⁸ and Polley et al²⁷ were the only ones to report $\delta^{13}\text{C}_{\text{CO}_2}$ data. The experimental approach described within Polley et al²⁷ relied on CO_2 fixation along a 38 m gradient in order to maintain $p\text{CO}_2$ between 200 and 365 ppm. Variability in $\delta^{13}\text{C}_{\text{CO}_2}$ value was not quantified within this experiment because this value was only measured two times throughout a two-year period, with air values at other times determined indirectly via a C_4 plant proxy.

Beerling et al¹⁸ used the natural CO_2 drawdown by plants within individual chambers to create subambient CO_2 conditions down to 300 ppm with standard deviations for 14 treatments that ranged from 0.4 to 3.6‰ (standard deviations calculated from reported standard errors). In comparison, the standard deviations for 13 treatments using our chamber design ranged from 0.05 to 0.14‰. The reasons for the large variation in the study by Beerling et al were not discussed, but

may be related to the reliance on the plants to reduce the CO_2 levels, which enriches the remaining CO_2 with ^{13}C .³⁶ When CO_2 fixation decreased the $p\text{CO}_2$ below the target value, supplemental cylinder CO_2 , which is typically depleted in ^{13}C relative to ambient air (with values of -18 to -55% ^{29,37-39}), was introduced to the system. Based on the wide range of chamber $\delta^{13}\text{C}_{\text{CO}_2}$ values that they reported (-3.2 to -14.2%), it appears that both ^{13}C enrichment of ambient CO_2 ($\delta^{13}\text{C}_{\text{CO}_2} \sim 8\%$) from photosynthesis, and ^{13}C depletion from supplemental cylinder CO_2 injection, were significant and probably contributed to the day-to-day variability.

Every subambient chamber in this study showed less $\delta^{13}\text{C}_{\text{CO}_2}$ variability ($1\sigma \leq 0.14\%$) than reported in the superambient $p\text{CO}_2$ (>400 ppm) literature (best $1\sigma = 0.17\%$).²⁹ This improved stability for both $\delta^{13}\text{C}_{\text{CO}_2}$ values and $p\text{CO}_2$ over superambient chambers can be explained by the differences in source CO_2 for each chamber type – the subambient chambers reported here utilized ambient air that had been completely scrubbed of CO_2 , then supplemented with a stable source of cylinder CO_2 , whereas the superambient chambers utilized a mixture of air containing ambient CO_2 and supplemental cylinder CO_2 . Both the $p\text{CO}_2$ and the $\delta^{13}\text{C}_{\text{CO}_2}$ value of ambient air can vary due to external contributions of CO_2 (e.g. human breath, ecosystem level respiration, industrial sources),³¹⁻³⁵ which in turn add instability to the final superambient air mixtures. Superambient chamber studies could possibly benefit from our new chamber design, providing a more stable $\delta^{13}\text{C}_{\text{CO}_2}$ and $p\text{CO}_2$ environment; however, this would need to be verified with further tests operating the chambers at $p\text{CO}_2$ levels >400 ppm.

4 | CONCLUSIONS

The development of a subambient plant growth chamber capable of providing stable $\delta^{13}\text{C}_{\text{CO}_2}$ and $p\text{CO}_2$ values is critical for constraining the effect of $p\text{CO}_2$ on ^{13}C discrimination across subambient $p\text{CO}_2$.

Previous subambient growth chamber designs failed to assess $\delta^{13}\text{C}_{\text{CO}_2}$ variability, suffered wide $\delta^{13}\text{C}_{\text{CO}_2}$ variations within chamber treatments, or encompassed a limited range in subambient $p\text{CO}_2$ levels. The chamber design reported here overcomes these limitations and provides an affordable option for carrying out controlled carbon isotope discrimination experiments through precise $\delta^{13}\text{C}_{\text{CO}_2}$ control under both ambient and subambient $p\text{CO}_2$ (ca 100 to 400 ppm). The highest variability in the $\delta^{13}\text{C}_{\text{CO}_2}$ value determined here across all chambers and experiments ($1\sigma = 0.14$) is approximately three times better than any previously reported chamber data in the subambient literature. This increased stability now makes it possible to carry out subambient growth experiments under $p\text{CO}_2$ characteristic of widespread Phanerozoic glaciations (e.g. Neogene through today, and Permo-Carboniferous) where ^{13}C discrimination is predicted to be most sensitive to changing $p\text{CO}_2$ (i.e. $>1\%$ per 100 ppm change in $p\text{CO}_2$).¹

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REFERENCES

- Schubert BA, Jahren AH. The effect of atmospheric CO_2 concentration on carbon isotope fractionation in C_3 land plants. *Geochim Cosmochim Acta*. 2012;96:29-43.
- Schubert BA, Jahren AH. Global increase in plant carbon isotope fractionation following the Last Glacial Maximum caused by increase in atmospheric $p\text{CO}_2$. *Geology*. 2015;43(5):435-438.
- Schubert BA, Jahren AH. Reconciliation of marine and terrestrial carbon isotope excursions based on changing atmospheric CO_2 levels. *Nat Commun*. 2013;4:1653. <https://doi.org/10.1038/ncomms2659>
- Cui Y, Schubert BA. Quantifying uncertainty of past $p\text{CO}_2$ determined from changes in C_3 plant carbon isotope fractionation. *Geochim Cosmochim Acta*. 2016;172:127-138.
- Cui Y, Schubert BA. Atmospheric $p\text{CO}_2$ reconstructed across five early Eocene global warming events. *Earth Planet Sci Lett*. 2017;478:225-233.
- Foster GL, Royer DL, Lunt DJ. Future climate forcing potentially without precedent in the last 420 million years. *Nat Commun*. 2017;8:14845. <https://doi.org/10.1038/ncomms14845>
- Bereiter B, Eggleston S, Schmitt J, et al. Revision of the EPICA Dome C CO_2 record from 800 to 600 kyr before present. *Geophys Res Lett*. 2015;42(2):542-549.
- Berninger F, Sonninen E, Aalto T, Fu J. Modeling ^{13}C discrimination in tree rings. *Global Biogeochem Cycles*. 2000;14:213-223.
- Van de Water PK, Leavitt SW, Betancourt JL. Trends in stomatal density and $^{13}\text{C}/^{12}\text{C}$. *Science*. 1994;264(5156):239-242.
- Feng X, Epstein S. Carbon isotopes of trees from arid environments and implications for reconstructing atmospheric CO_2 concentration. *Geochim Cosmochim Acta*. 1995;59:2599-2608.
- Peñuelas J, Estiarte M. Trends in plant concentration and plant demand for N throughout this century. *Oecologia*. 1997;109:69-73.
- Krishnamurthy RV, Epstein S. Glacial-interglacial excursion in the concentration of atmospheric CO_2 : effect in the $^{13}\text{C}/^{12}\text{C}$ ratio in wood cellulose. *Tellus B*. 1990;42(5):423-434.
- Coplen TB, Brand WA, Gehre M, et al. After two decades a second anchor for the VPDB delta C-13 scale. *Rapid Commun Mass Spectrom*. 2006;20(21):3165-3166.
- Brand WA, Huang L, Mukai H, Chivulescu A, Richter JM, Rothe M. How well do we know VPDB? Variability of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in CO_2 generated from NBS19-calcite. *Rapid Commun Mass Spectrom*. 2009;23(6):915-926.
- Anderson LJ, Derner JD, Polley HW, Gordon WS, Eissenstat DM, Jackson RB. Root responses along a subambient to elevated CO_2 gradient in a C_3 - C_4 grassland. *Glob Chang Biol*. 2010;16(1):454-468.
- Anderson LJ, Maherali H, Johnson HB, Polley HW, Jackson RB. Gas exchange and photosynthetic acclimation over subambient to elevated CO_2 in a C_3 - C_4 grassland. *Glob Chang Biol*. 2001;7:693-707.
- Baker JT, Allen LH Jr, Boote KJ, Jones P, Jones JW. Rice photosynthesis and evapotranspiration in subambient, ambient, and superambient carbon dioxide concentrations. *Agron J*. 1990;82:834-840.
- Beerling DJ, Lake JA, Berner RA, Hickey LJ, Taylor DW, Royer DL. Carbon isotope evidence implying high O_2/CO_2 ratios in the Permo-Carboniferous atmosphere. *Geochim Cosmochim Acta*. 2002;66(21):3757-3767.
- Cowling SA, Sage RF. Interactive effects of low atmospheric CO_2 and elevated temperature on growth, photosynthesis and respiration in *Phaseolus vulgaris*. *Plant Cell Environ*. 1998;21:427-435.
- Cunniff J, Charles M, Jones G, Osborne CP. Reduced plant water status under sub-ambient $p\text{CO}_2$ limits plant productivity in the wild progenitors of C_3 and C_4 cereals. *Ann Bot*. 2016;118:1163-1173.
- Cunniff J, Osborne CP, Ripley BS, Charles M, Jones G. Response of wild C_4 crop progenitors to subambient CO_2 highlights a possible role in the origin of agriculture. *Glob Chang Biol*. 2008;14:576-587.
- Dippery JK, Tissue DT, Thomas RB, Strain BR. Effects of low and elevated CO_2 on C_3 and C_4 annuals I. Growth and biomass allocation. *Oecologia*. 1995;101:13-22.
- Mayeux HS, Johnson HB, Polley HW, Malone SR. Yield of wheat across a subambient carbon dioxide gradient. *Glob Chang Biol*. 1997;3:269-278.
- Mohan JE, Clark JS, Schlesinger WH. Genetic variation in germination, growth, and survivorship of red maple in response to subambient through elevated atmospheric CO_2 . *Glob Chang Biol*. 2004;10:233-247.
- Polley HW, Johnson HB, Marino BD, Mayeux HS. Increase in C_3 plant water-use efficiency and biomass over Glacial to present CO_2 concentrations. *Nature*. 1993;361:61-63.
- Polley HW, Johnson HB, Mayeux HS. Nitrogen and water requirements of C_3 plants grown at glacial to present carbon dioxide concentrations. *Funct Ecol*. 1995;9(1):86-96.
- Polley HW, Johnson HB, Derner JD. Soil- and plant-water dynamics in a C_3/C_4 grassland exposed to a subambient to superambient CO_2 gradient. *Glob Chang Biol*. 2002;8:1118-1129.
- Ward JK, Strain BR. Effects of low and elevated CO_2 partial pressure on growth and reproduction of *Arabidopsis thaliana* from different elevations. *Plant Cell Environ*. 1997;20:254-260.
- Hagopian WM, Schubert BA, Jahren AH. Large-scale plant growth chamber design for elevated $p\text{CO}_2$ and $\delta^{13}\text{C}$ studies. *Rapid Commun Mass Spectrom*. 2015;29:440-446.
- Farquhar GD, Ehleringer JR, Hubick KT. Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol*. 1989;40:503-537.
- Szaran J, Dudziak A, Trembacowski A, Niezgodna H, Halas S. Diurnal variations and vertical distribution of delta C-13, and concentration of atmospheric and soil CO_2 in a meadow site, SE Poland. *Geol Quarterly*. 2005;49(2):135-144.

32. Demeny A, Haszpra L. Stable isotope compositions of CO₂ in background air and at polluted sites in Hungary. *Rapid Commun Mass Spectrom*. 2002;16(8):797-804.
33. Yanes Y, Yapp CJ. Indoor and outdoor urban atmospheric CO₂: stable carbon isotope constraints on mixing and mass balance. *Appl Geochem*. 2010;25(9):1339-1349.
34. Longinelli A, Selmo E. Seasonal and diurnal variations of delta C-13 and concentration of atmospheric CO₂ at Parma, Italy. *Geol Quarterly*. 2005;49(2):127-134.
35. Widory D, Javoy M. The carbon isotope composition of atmospheric CO₂ in Paris. *Earth Planet Sci Lett*. 2003;215(1-2):289-298.
36. Edwards CE, Haselhorst MSH, McKnite AM, Ewers BE, Williams DG, Weinig C. Genotypes of *Brassica rapa* respond differently to plant-induced variation in air CO₂ concentration in growth chambers with standard and enhanced venting. *Theor Appl Genet*. 2009;119(6):991-1004.
37. Fletcher BJ, Beerling DJ, Brentnall SJ, Royer DL. Fossil bryophytes as recorders of ancient CO₂ levels: experimental evidence and a Cretaceous case study. *Global Biogeochem Cycles*. 2005;19(3):GB3012. <https://doi.org/10.1029/2005GB002495>
38. Lomax BH, Knight CA, Lake JA. An experimental evaluation of the use of C₃ δ¹³C plant tissue as a proxy for the paleoatmospheric δ¹³CO₂ signature of air. *Geochem Geophys Geosyst*. 2012;13(1):1-12.
39. Leavitt SW, Pendall E, Paul EA, et al. Stable-carbon isotopes and soil organic carbon in wheat under CO₂ enrichment. *New Phytol*. 2001;150(2):305-314.

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