hardware

Article

Design of a Low-Cost Open-Top Chamber Facility for the Investigation of the Effects of Elevated Carbon Dioxide Levels on Plant Growth

Nicola Novello [1,*], Mani Naiker [1], Haydee Laza [2], Kerry B. Walsh [1] and Sabine Tausz-Posch [3]

[1] School of Health, Medical and Applied Sciences, Central Queensland University, Bruce Hwy, Rockhampton, QLD 4702, Australia; m.naiker@cqu.edu.au (M.N.); k.walsh@cqu.edu.au (K.B.W.)
[2] Department of Plant and Soil Science, Texas Tech University, Lubbock, TX 79409, USA; haydee.laza@ttu.edu
[3] Faculty of Science, The University of Melbourne, Dookie College, VIC 3647, Australia; sposch@unimelb.edu.au
* Correspondence: n.novello@cqu.edu.au

Abstract: Open-top chambers (OTCs) consist of semi-open enclosures used to investigate the impact of elevated carbon dioxide [CO_2] on crops and larger plant communities. OTCs have lower operational costs than alternatives such as controlled environment cabinets and Free Air Carbon Dioxide Enrichment (FACE). A low-cost design is presented for an OTC with a surface area of 1.2 m^2 and a target elevated CO_2 concentration [CO_2] of 650 μmol mol^{-1} adequate for trials involving cereals or grain legumes. The elevated CO_2 chambers maintained an average concentration $\pm$ standard deviation of 652 $\pm$ 37 μmol mol^{-1} despite wind and air turbulences, in comparison to 407 $\pm$ 10 μmol mol^{-1} for non-enriched chambers. Relative to ambient (non-chamber) conditions, plants in the chambers were exposed to slightly warmer conditions (2.3 °C in daylight hours; 0.6 °C during night environment). The materials' cost for constructing the chambers was USD 560 per chamber, while the CO_2 control system for four chambers dedicated to CO_2-enriched conditions cost USD 5388. To maintain the concentration of 650 μmol mol^{-1} during daylight hours, each chamber consumed 1.38 L min^{-1} of CO_2. This means that a size G CO_2 cylinder was consumed in 8–9 days in the operation of two chambers (at USD 40).

Keywords: modified environment; greenhouse effect; CO_2 enrichment

Citation: Novello, N.; Naiker, M.; Laza, H.; Walsh, K.B.; Tausz-Posch, S. Design of a Low-Cost Open-Top Chamber Facility for the Investigation of the Effects of Elevated Carbon Dioxide Levels on Plant Growth. *Hardware* **2024**, *2*, 138–153. https://doi.org/10.3390/hardware2020007

Academic Editor: Peter C. Hauser

Received: 9 April 2024
Revised: 9 May 2024
Accepted: 13 May 2024
Published: 15 May 2024

1. Introduction

The study of plant responses to changed atmospheric composition can be conducted using leaf cuvettes for the consideration of short-term effects on leaf physiology prolonged for minutes or a few hours. For the study of a whole plant and plant community (crop) responses observed in the medium-term, including weeks to several months, larger cuvettes are required. This can be accomplished using controlled environment cabinets, also known as phytotrons, which involve an artificial environment with a high level of control of the temperature, humidity, light levels and gas levels, but these involve high capital and maintenance costs [1].

An alternative is the Free Air CO_2 Enrichment (FACE) design [2]. This design could be used for any gas, but it has achieved some popularity in CO_2 enhancement research. The FACE design involves modification of the atmosphere of an open (wall-less) area of a crop or native ecosystem providing insights on plants' phycological responses that are noticeable in the long term, typically from one to more years. A pipe system is used for distribution within a typically circular area, with a control system adjusting gas (CO_2) release in response to wind speed and direction and consequent ambient CO_2 levels. The wall-less nature of these systems is attractive in terms of maintaining ambient conditions other than the gas of interest; however, their CO_2 use is high. Also, the control of CO_2 levels could be compromised in windy conditions. For instance, Allen et al. [3] reported that fluctuations in FACE experiments can be >200 ± 50 µmol mol^{-1} above the target [CO_2], although some designs showed that concentrations can be maintained at or higher than 90% of the target for 93–98% of the time [4].

OTCs are semi-open framed structures designed to maintain the desired concentration of gaseous pollutants and represent a compromise between cost and environmental modification [5,6]. An area of ground is enclosed by greenhouse film or transparent sheeting, with the top left open to the atmosphere. A concentrated gas source is ducted into a mixing chamber, mixed with ambient air, and the mixed gas is then ducted into the base of the OTC. The gas concentration inside the open cuvette is usually monitored to control how much gas is delivered. For elevated CO_2 studies, the source of CO_2 is typically a cylinder of compressed pure CO_2, which is diluted to the desired concentration with ambient air before introduction to the cuvette.

Early examples of studies employing OTCs include investigations on the impact of air pollution on plants, i.e., the effect of ozone (O_3) on tobacco [7] and the effect of elevated CO_2 [8–10]. OTC designs generally comprise a framework support for a transparent cover, e.g., polyvinyl chloride, plexiglass, or LDPE glasshouse film, forming an open-topped chamber. The chambers are typically cylindrical [11–13], although square or rectangular [14], hexagonal [15,16], and octagonal [17,18] shapes have been used.

The chamber size is varied, from small conical units enclosing single plants to multiple plants and larger plant communities enclosed in structures having >10 m diameter [5]. In the context of climate change, OTCs represent a valuable tool for studying the effects of elevated CO_2 on plants, but assessing their long-term sustainability and scalability is crucial. The advantage of using OTCs relies on their sustainability due to both low environmental impact in terms of carbon footprint and costs, including reasonable initial investments and ongoing operational expenses.

Moreover, OTCs can be effectively expanded from small-scale experiments to larger ecosystems or broader research applications involving growth environment manipulation, such as, for instance, nitrogen sink and source experiments [19]. However, while on one hand, larger structures with wider openings allow for studying the long-term impacts of elevated CO_2 on trees and forest ecosystems, on the other hand, they are more exposed to wind interference, which consequently makes it difficult to maintain the gas concentration close to the target involving higher capital and operational costs [20,21].

The chamber can also cause a heating effect, mitigated by the high volume of air passing through the ventilation system. However, it has been noted that the temperature inside OTCs dedicated to temperature-manipulation experiments is uniform across vertical and horizontal space [22].

With CO_2 levels currently rising at ~2.17 µmol mol^{-1} per annum [23], there is a wide interest in undertaking studies to anticipate the impact of this change on crop performance. Such studies need to be undertaken at the whole plant/crop level to evaluate not only the effect of elevated CO_2 on leaf-level photosynthetic rates but also on plant functions such as assimilate partitioning between organs. In our case, interest in the effect of elevated CO_2 on peanut growth in the context of a 'graze and grain' strategy, i.e., off-take of a hay crop before flowering and pod set, initiated our interest in the use of an OTC design.

The present study outlines the design, specifications, and performance of an open-top chamber (OTC) of 1.2 m^2 area tailored specifically for investigating the impact of elevated CO_2 levels on smaller broadacre crops. This study builds upon the designs implemented in previous research, refining and adapting these earlier models to better suit the specific requirements of assessing CO_2 effects under controlled environmental conditions, particularly Messerli et al. [15].

The key point of our approach lies in presenting a cost-effective open-top chamber (OTC) design. These modifications not only aim to enhance the accuracy and reliability of the results but also provide critical insights into the effects of increased CO_2 concentrations on crop growth and development in smaller agricultural settings. This practical, economical solution allows for detailed observation and analysis, ensuring that our findings are both accessible and applicable to a wide range of agricultural research scenarios.

Although this facility was specifically designed to conduct CO_2 enrichment experiments, the design provides substantial versatility to accommodate studies involving a range of gaseous pollutants. For instance, the chambers can be suited for investigating the impacts of nitrogenous compounds such as ammonia (NH^{4+}), commonly emitted from agricultural practices and livestock operations, as well as sodium fluoride, which often originates from industrial processes including aluminium production and phosphate ore processing.

Additionally, the facility could be adapted for experiments with ozone, a pollutant produced when nitrogen oxides (NOx) from vehicle exhaust and industrial emissions reacting with sunlight. To facilitate ozone research, significant modifications would be necessary, such as the application of PTFE coatings to protect the structural integrity of the chamber from ozone's highly reactive nature.

2. Design

2.1. OTC Design

The design goals included the following:

(i) a chamber large enough to produce a crop of peanuts, allowing sampling of 'non-edge' plants.
(ii) a chamber that is sufficiently low-cost and operationally easy to build to allow multiple units to be built and replicated in experimental designs.
(iii) imposition of a 650 μmol mol^{-1} elevated [CO_2] treatment.

The chambers were designed to enclose a ground area of 1.25 m^2 (Figure 1a). A raised garden bed of 1.25 m diameter was used to facilitate the use of farm soil and ease of operator access to the crop (Figure 1b)

The enclosing octagonal chamber was 1.2 m high, with an open conical top (frustrum) to reduce the amount of wind-induced turbulence within the chamber, extending the suggestion of Drake et al. [9].

CO_2 sensors were installed within the chambers, with feedback to a CO_2 control system operating with a set point of 650 $\pm$ 50 μmol mol^{-1}, with a final control element of a valve on the CO_2 input line. The CO_2 source was bottled pressurized pure CO_2 (BOC, NSW, Sydney, Australia).

A VKM 100 Centrifugal Inline blower, (Fanco, Melbourne, VIC, Australia) was used to deliver a relatively high flow rate into the chamber, causing an even CO_2 concentration distribution within the chamber compared to the injection of pure CO_2 and to mitigate temperature increase inside the OTC due to a chamber greenhouse effect [24].

Air intake to the blower consisted of the regulated pure CO_2 feed from the bottled pressurized pure CO_2, ambient air, and recycled chamber air from pick-up bottoms around the base of the frustrum. The opening at the bottom of the mixing box allowed the supply of fresh ambient air at the flow rate of 0.047 m^3 s^{-1}, while the backflow pipe connected to the box allowed the recycling of CO_2 air mix at the rate of 0.028 m^3 s^{-1} (as measured using a thermo-anemometer Protech, model QM1646, Electus Distribution, Sydney, Australia). Hence, approximately 35% of the CO_2 air mix was recycled into the system through the backflow piping.

2.2. Replication

Replication is required in any experimental evaluation. In the case of OTC work, the replication of chambers is required at the chamber level. However, the chamber itself may impact the plant growing environment, and so, a treatment involving plants grown in the raised bed without a chamber is recommended. At least three replicate units are recommended, giving a minimum build of nine units, i.e., three beds without chambers, three beds with chambers and ventilation operating at ambient CO_2, and three beds with chambers and chambers operating at elevated CO_2, placed in a random arrangement. In our build, four replicate units were constructed for each of the three treatments (Figure 2).

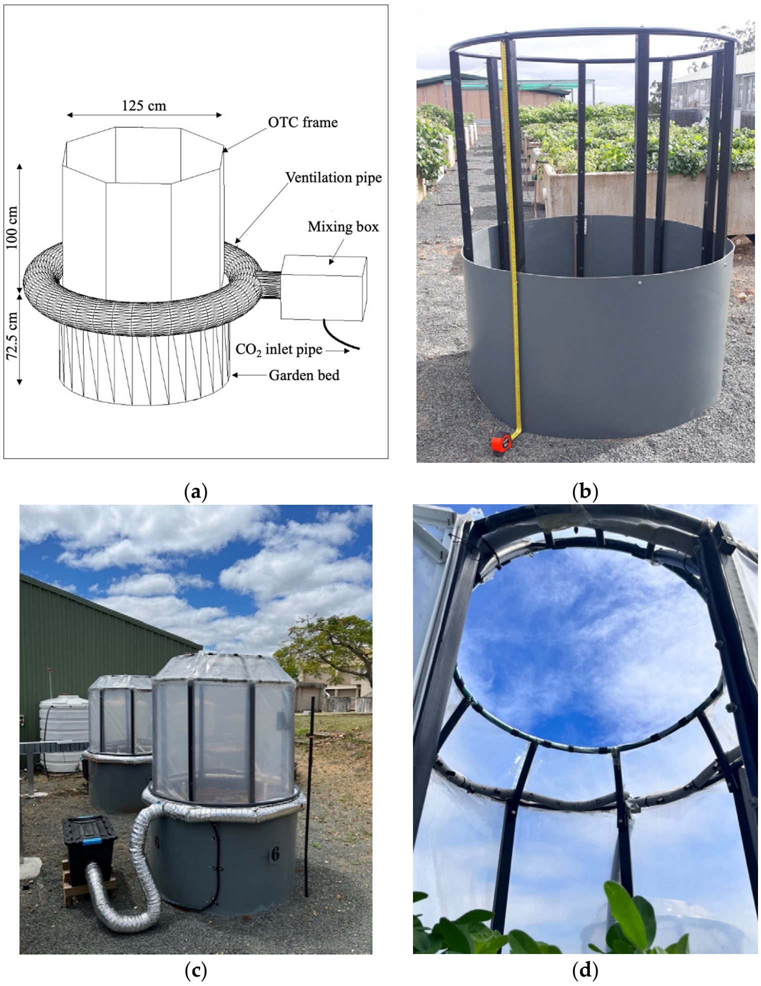


Figure 1. Open-top chamber (OTC) design, with octagonal framing mounted on a raised garden bed (**a**). Pure CO_2 is delivered at a controlled rate into a mixing box equipped with an industrial blower, which delivers the mixed gas to a perforated duct installed around the OTC circumference. (**b**) An OTC under fabrication where the framing and the garden bed are visible. (**c**) OTC showing connection to ventilation system. (**d**) Perforated back flow pipe inside the OTC. The original design document is in the Supplementary Materials.

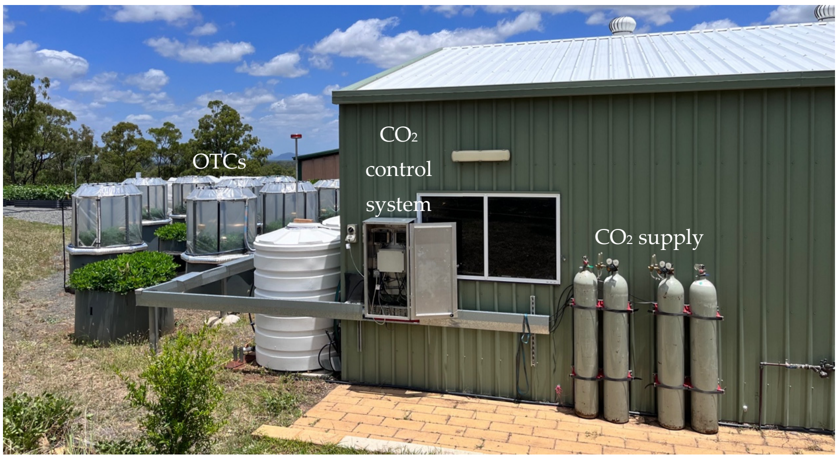


Figure 2. Overview of the experimental site, featuring the main components of the facility. The gas cylinders supplying carbon dioxide stand alongside the CO_2 control system, with distinct octagonal-shaped OTCs and control plots without chambers.

3. Build Instructions

3.1. Bill of Materials

A Bill of Materials is presented in Table 1, describing the costs for the OTC framing, ventilation, and CO_2 regulation systems and irrigation.

Table 1. Items used for the fabrication of one OTC with cost description for installing a CO_2 system for four elevated CO_2 OTCs, four ambient CO_2 OTCs, and four control plots. Costs are reported in USD as per 2021.

Item	Brand/Model	Specifications/Description	Cost	Units	Total
		OTC Framing and Ventilation System			
Garden beds	Plastic Forests	Diameter: 125 cm; height 72.5 cm; thickness: 5 mm; length 400 cm	118.80	1	118.80
Plastic posts	Plastic Forests	Section: 3.8 × 3.8 cm; length; 180 cm	7.20	8	57.60
Poly pipe	Holman	Length: 500 cm per OTC; diameter: 2.5 cm	7.20	1	7.20
Greenhouse LDPE film	Redpath, Hortiplus180	Light transmission >91%; thickness: 180 µm	36.00	1	36.00
Mounting tape	Permastik	Length used: 800 cm; width: 2.4 cm	7.20	2	14.40
Mixing box	Inabox	Size: 60 × 39 × 39 cm; capacity: 55 L	10.18	1	10.18
Box sealant	Moroday	Length used: 200 cm	5.03	1	5.03
Blower	Fanco VKM 100	Voltage 220/240, capacity 270 $m^3\ h^{-1}$, 2830 r min^{-1}	151.20	1	151.20
Ducting	Fanco	Length: 4 m; diameter: 10 cm	18.72	1	18.72
Plugs (nozzles)	K-Rain	Internal diameter: 1.9 cm	0.27	40	10.66
T-joint drain coil	Vinidex	Diameter: 10 cm	6.31	1	6.31
PVC connector	Vinidex	Diameter: 10 cm	2.87	2	5.75
Duct clamps	Pacific Air	Diameter: 12.5 cm	2.95	2	5.90
Screws	Buildex	Length: 3–5 cm	NA	50	15.84
Angle molding	Brutus	Size: 25 × 25; thickness: 2.5 mm	4.64	2	9.29
Backflow pipe	Vinidex	Length used: 600 cm; diameter: 5 cm	15.53	1	15.53
Consumables	NA	Cable ties, wire, tape, silicone sealant, etc.	NA	NA	72.00
			Subtotal		**560.41**
		Irrigation for an OTC (Inside OTC only)			
Poly pipe	Holman	Length used: 300 cm	1.94	1	1.94
Barbed elbow	Pope	Diameter: 1.9 cm	0.92	2	1.84
T-joint	Pope	Diameter: 1.9 cm	0.78	1	0.78
Inline tap	Pope	Diameter: 1.9 cm	5.58	1	5.58
End plug	Pope	Diameter: 1.9 cm	0.57	3	1.71
Drippers	Netafim	Flow: 0.5 L h^{-1}	0.35	30	10.58
			Subtotal		**22.43**
		CO_2 regulation for four units			
CO_2 probe	GMP252 CO_2 Vaisala	Operating temperature: −40/+60 °C	607.00	4	2428.00
CO_2 transmitter *	Indigo 520 Vaisala	M12 5-pin cable; RJ45-ethernet cable; built-in web server	1179.00	2	2358.00
Solenoid	Bürkert 6013	Voltage: 24 V; 2 ports; 1/4 in G; flow factor: 0.23 $m^3\ h^{-1}$	92.16	1	92.16
Gas regulator *	BOC CO_2 Regulator	Inlet: type 30; outlet: Side 5/8″ UNF RH; outlet pressure 400 kPa	164.95	2	330.00
Other/Not Classified	NA	Fittings, connectors, tubing, clamps, etc.	NA	NA	180.00
			Subtotal		**5388.16**
		Cost for 4 elevated CO_2 units.			USD 7719
		Cost for 4 ambient CO_2 units.			USD 2331
		Cost for 4 control CO_2 units.			USD 565
Total OTC facility cost					**USD 10,615**

Note. * Served two OTCs. NA: not applicable

3.2. Garden Beds

The garden beds (125 cm diameter × 72.5 cm height; from Plastic Forests, North Albury, New South Wales, Australia) were manufactured from a stabilized UV-resistant recycled plastic material (Simply Cups recycling program). Each garden bed was divided into two halves by inserting a plastic board, to allow for sub-plot treatments, e.g., of water or fertilization. Each bed was filled with 1.0 m^3 of agronomically relevant soil, in this case, a ferrosol (according to the Australian soil classification), acquired from a farm located in Rossmoya, Queensland. The soil was excavated from the horizons between A1 and B2 (0–600 mm).

3.3. Chamber

The construction of each chamber was based on eight 3.6 cm square, 180 cm long recycled-plastic pickets (Plastic Forests, North Albury, NSW, Australia). These uprights were placed in an octagonal pattern against the inside wall of the garden bed ring, driven through the garden bed soil to ground level, and fixed to the bed using 3 cm hex-head screws (Figure 1b). The posts were connected at their tops with a polyethylene (PE) pipe ring (392 cm circumference, 3 cm diam), providing more rigidity to the structure, and a truncated conical frame was then mounted onto this structure.

A glasshouse low-density polyethylene (LPDE) film of 180 μm thickness and >91% light transmission (Hortiplus180, from Redpath, Bendigo, VIC, Australia) was placed around the frame and fixed with double-sided clear mounting tape. As specified by the manufacturer, the plastic film contains surface tension modifiers to prevent condensation and dirt accumulation that could degrade light transmission and is UV-stabilized, with a 30-month warranty provided. An opening allowed operator access into the OTC. Initially, 'door' closing was facilitated by adhesive "hook and loop" strips with double-sided tape, applied on an opening flap of plastic film and on an upright frame. However, this arrangement was not robust. A simple hinged door frame (approx. 100 × 45 cm) was constructed of PVC angle building molding (2.5 × 2.5 cm).

3.4. CO_2 Injection System

Bottled CO_2 (size G, cylinder water capacity 50 L containing 31 kg CO_2, or 18 m^3 at 25 °C) and 1 atm Industrial Grade (gas code 081, >99.9% CO_2, initial pressure 20 MPa, 2.0 Sm^3 at 15 °C and 101.3 kPa) was used. Outlet pressure was set at 90–100 KPa (12–14 psi) using a CO_2 regulator (BOC 801325, NSW, Sydney, Australia). The regulator outlet was fitted with a dual split connector on which two flowmeters (BOC 10521, NSW, Sydney, Australia) set at 1 L min^{-1} were installed. Plastic tubing (65 mm diameter) was used in the connection of the flowmeter output and the solenoid inlet and in the connection of the solenoid outlet to the mixing box of the respective OTC chamber.

The gas mixing box consisted of a plastic container (60 × 40 × 35 cm) with an opening of 5 × 5 cm width cut into the base of the container to allow air entry. A neoprene adhesive foam tape was placed along the top edges of the box to affect a seal with the lid. An electric blower (73 W, 270 m^3 h^{-1}, 2830 r min^{-1}, Figure 3) (VKM 100, Fanco) was mounted inside the container with the intake facing the internal area of the mixing box which was employed to propel CO_2 through a lay-flat aluminium/plastic flexible ventilation ducting (~50 cm in length; 10 cm diam) connecting the blower to the chamber with a PVC dual split coil (10 cm diam), and finally, a ducting made of the same material (400 cm long; 10 cm diam) was connected to both ends and placed along the OTC circumference to serve as a plenum.

The plenum was perforated at regular intervals (~5 perforations of 22 mm diameter per linear meter), and a plastic irrigation barbed plug having 19 mm internal diameter was placed in each hole as a nozzle and fixed with reinforced insulation tape. The decision to add a nozzle instead of just leaving an open perforation in the ducting was justified by several considerations. Having outlets in each perforation ensures a steady distribution of the air mix, allowing for consistent levels of gas injection. Additionally, the angle of each nozzle can be adjusted as needed, which allows for a more targeted dispersal of CO_2 in the OTC environment. Lastly, this setup prevents the perforation from enlarging with time and prevents water entering the plenum and, consequently, into the mixing box.

To improve CO_2 level stability during windy periods, a corrugated PVC pipe (50 mm diameter) (Vinidex, Sydney, NSW, Australia) was installed around the chamber's top opening, with a connection to the gas mixing box. This allowed a partial capture of exiting gases, recycling into the air mix cycle. This backflow pipe was perforated at 10 cm intervals with 19 mm diameter holes and equipped with nozzles for the plenum.

(**a**) (**b**)

Figure 3. Representation of the Open-top chamber ventilation system which included (**a**) an industrial blower, (**b**) mixing box with inside fan used to propel the CO_2/ambient air mix into the chamber.

The CO_2 sensors (GMP252, Vaisala, Helsinki, Finland) were mounted inside the four elevated CO_2 chambers. The sensors (Figure 4) were connected to two control systems (Indigo520, Vaisala, Helsinki, Finland). The CO_2 controllers were programmed to log the CO_2 concentration data at two-minute intervals, with data downloaded through a LAN linked to the Vaisala web interface and saved as a .csv file. The transmitters also activated solenoid valves (Bürkert 6013 G1/4 24V) to regulate CO_2 release on a set point of 650 µmol mol^{-1}, with the hysteresis value set at 150 µmol mol^{-1}. The value of 150 µmol mol^{-1} was chosen with the purpose of avoiding short cycling of the system which can cause wear and tear of the solenoid components and over-heating. Therefore, the solenoids were switched off automatically when the internal [CO_2] exceeded 800 µmol mol^{-1} and switched on when dropping below 500 µmol mol^{-1}.

(**a**) (**b**)

Figure 4. The internal CO_2 concentration was monitored using two CO_2 system controllers Indigo 520 Vaisala (**a**); a CO_2 probe protected by a weather shield was installed inside each open-top chamber dedicated to the CO_2-enriched conditions (**b**).

4. Operating Instructions

4.1. Set up and Run

In our experience, the operation of the CO_2 dosing chain to the OTCs can be trouble-free, although maintenance of and access to a stock of spare components (blower, sensors, valves) is recommended, as for any equipment. The primary operational issue is allowance for purchasing and delivery time of CO_2 cylinders. Longer-term (over several years) maintenance includes the replacement of the plastic sheeting, yearly calibration of sensors, and checking of electrical components, e.g., blower.

The gas supply to the four OTCs was carried out using two 31 kg gas cylinders which were monitored daily for timely replacement, as needed, to avoid CO_2 enrichment gaps. The size of the cylinder was relatively easy to handle; however, gas supply involved manual replacement of cylinders, which is time-consuming and labour-intensive. Such limitations could be overcome by using an automatic electronic switching system able to open the valves of another couple of cylinders, by using high-capacity tanks or by producing CO_2 on-site.

Also, it is recommended to progressively raise the CO_2 sensors within each chamber to a constant distance above crop canopy height throughout the growing season, to ensure that the foliage does not cover the sensor.

The use of soil moisture probes, not discussed in this report, is recommended for agronomic studies, given the biomass and water use efficiency (WUE) differences, and thus water use, of plants between treatments.

4.2. Maintenance

The ducting used for the plenum and the clear plastic sheeting are affected by the UV component of solar radiation, with degradation obvious after 12 months in the harsh tropical location of the current experiment. Metal ducting would be resistant to UV and extreme weather conditions but would result in higher cost. Annual replacement is the alternative. The chamber plastic film required cleaning with a soapy solution to remove soil dust, insects, and bird droppings. However, the light transmissibility of the clean plastic decreased to 71% after 30 months, presumably due to UV damage, and replacement (nominally at 2 years) is recommended.

4.3. Troubleshooting

During severe precipitation events, the CO_2 control system showed low input signal from the CO_2 probes, Vaisala GMP 252, due to condensation on the optical surface of the sensor. The issue was solved by letting the sensor dry for some hours. In some situations, it was required to remove the filter for drying (using a compressed air instrument followed by placement in a sealed container with dry silica for 12 h).

During the trials conducted on peanuts from 2021 to 2024, it was observed that crops inside OTCs were slightly more prone to pests and diseases with mild infestations of Cowpea aphids (*Aphis craccivora*) and peanut mites (*Paraplonobia* spp.)

Finally, in our experimental trials, we observed that crops cultivated in garden beds without OTC tended to grow beyond the bed edges, benefiting from unrestricted space. To mitigate the edge effect and maintain standardized growth conditions, we suggest using guard plants planted in the outer areas of the beds and regularly clipping overgrown branches during the season. These guard plants must not be considered during in-season measurements and must be discarded at harvest. Alternatively, we suggest installing short barriers, e.g., plastic or metal mesh, around the garden beds to contain the plants, especially if using crops with a decumbent or prostrate habitus like peanuts.

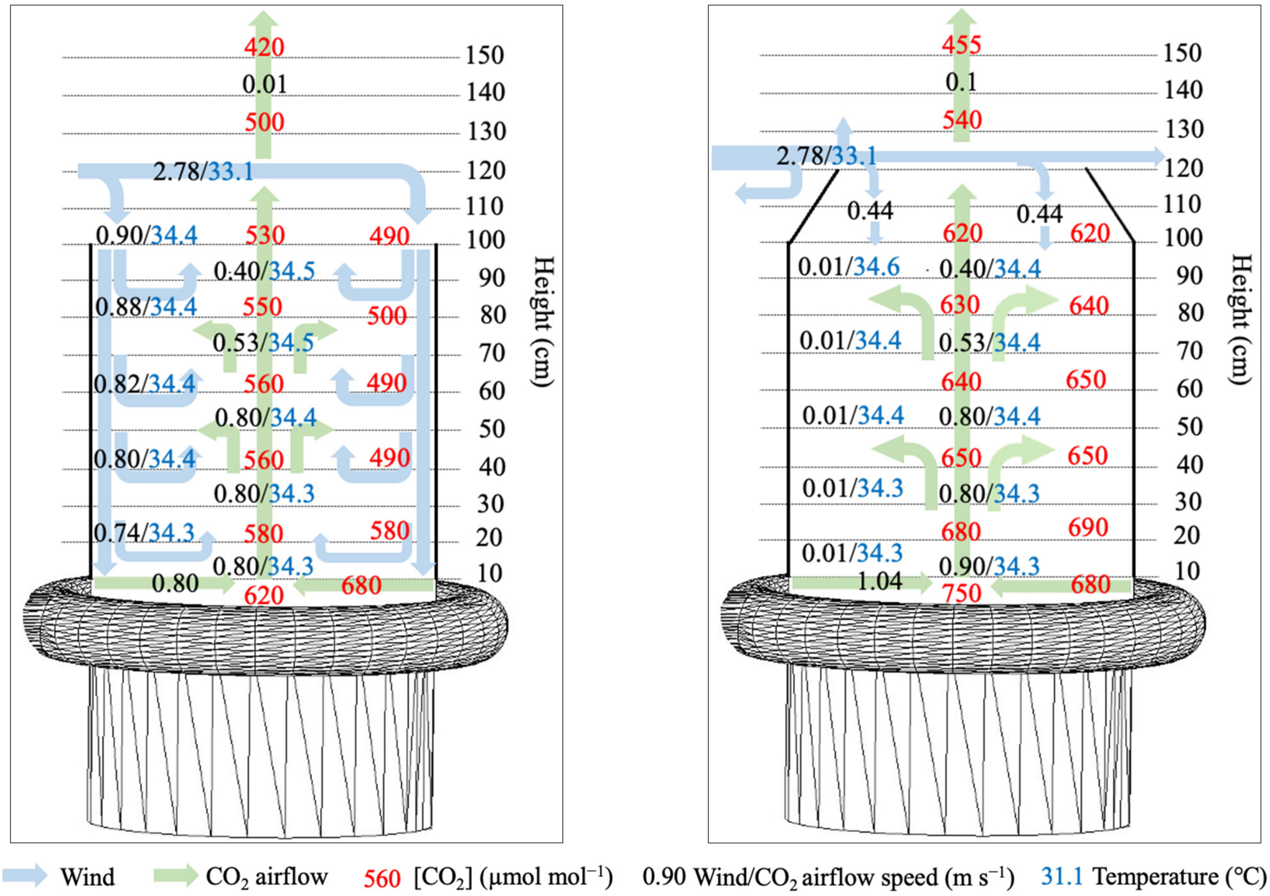
5. Validation

5.1. Airflow

Airflow direction was visualized using a non-toxic smoke-emitting tool (Smoke-Pen, Bjornax, Sweden), which is used for testing airflow in industrial ventilation systems.

Airflow speed and temperature were recorded for each point with a handheld thermo-anemometer (Protech, model QM1646, Electus distribution, Sydney, Australia). CO_2 concentration was measured using a CO_2 probe, Vaisala GMP 252. Measurements were taken at regular intervals (20 cm) from ground level to above the open chamber top, both along the side wall and in the centre of the OTC. Measurements were conducted during a calm period and a period with a constant (2.78 m s^{-1} at time of measurement) wind. Measurements were also made of a chamber without the open conical collar top on the day of constant wind.

Chamber CO_2 concentrations were not different ($p \leq 0.05$) in the calm and windy period. CO_2 concentration profiles and airflow patterns were quite different for an OTC without the conical collar, with much less uniformity in CO_2 concentration within the chamber (Figure 5). Air from the ventilation ducting flowed in horizontally at the base of the chamber, then tended to flow upwards through the centre part of the chamber and out into the general atmosphere. Without the collar, a downward draught along the inner wall of the chamber brought atmospheric air into the OTC, lowering CO_2 concentration (Figure 5a). This did not occur with the collar installed, providing more consistent elevated CO_2 levels within the OTC (Figure 5b).



(**a**) (**b**)

Figure 5. Airflow direction and speed, CO_2 concentration, and temperature within (**a**) OTC without a conical collar and (**b**) with a collar. Measurements were conducted with a crop (canopy height = ~35 cm) planted in the beds and placing a control sensor at 45 cm from ground level. The original design document is in the Supplementary Materials.

The position of the CO_2 control system sensor was considered. The target concentration of 650 µmol mol^{-1} was maintained at positions up to 60 cm from ground level, which exceeds the canopy height expected for this crop. A sensor height of between 30 and 45 cm was adopted, a choice that was driven by the optimal performance of the system to maintain the target concentration enhancing the accuracy in monitoring the CO_2 levels (Figure 6).

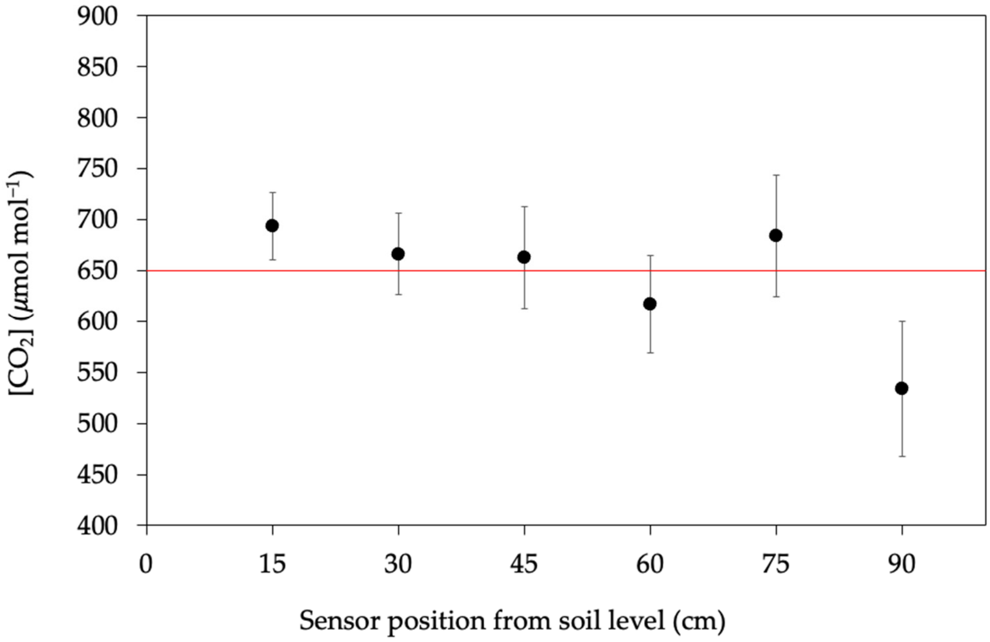


Figure 6. Average [CO_2] as measured on blank soil with no crop planted in the beds when the sensor was positioned at different heights from the soil level in an OTC with conical collar. Bars represent and standard deviation on measurements made over 3 h at 2 min intervals, i.e., n = 90. The red line represents the target concentration.

The CO_2 target was not maintained when the control sensor was positioned at 90 cm above ground level, due to the dilution of CO_2 in the chamber air with ambient air (Figure 7). In this circumstance, the control system attempts to maintain the set point, resulting in excess CO_2 levels within the crop.

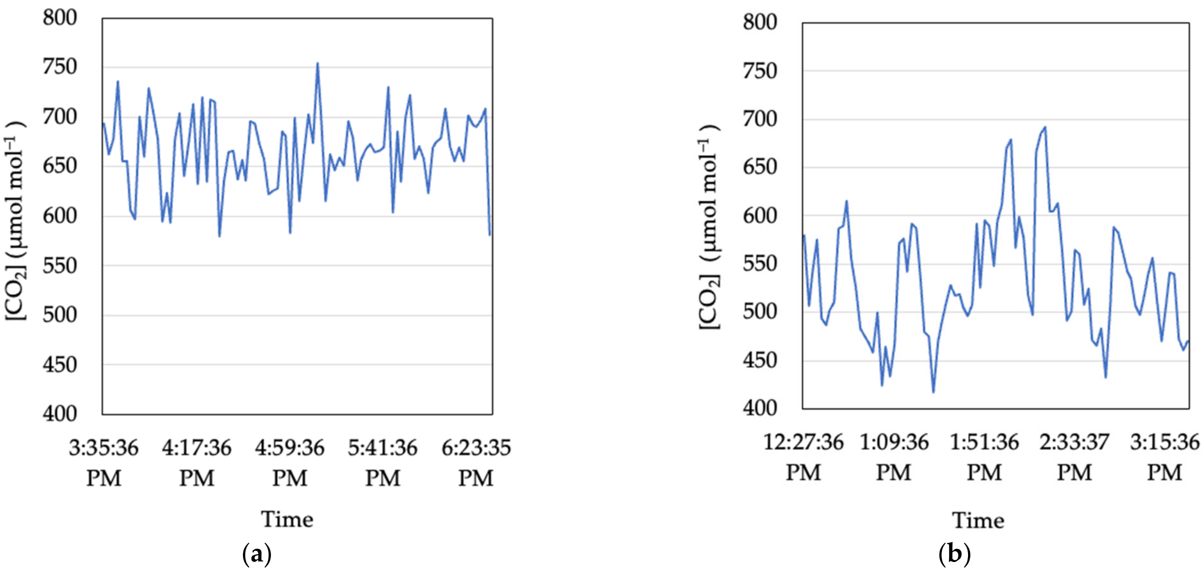


Figure 7. Comparison of system's performance when CO_2 sensor was installed at 30 cm (**a**) and 90 cm (**b**) from the ground level.

The suction in the return line situated on the inside of the base of the plenum was visually determined using a smoke pen (Figure 8). The suction strength was greatest in the inlets located in the proximity (30° to either side) of the junction with the main pipe, medium at 30°–120° either side of the junction, and low in the range 120°–180° on either side of the junction.

(**a**) (**b**) (**c**) (**d**)

Figure 8. Smoke test conducted on the backflow pipe inlets installed around the OTC circumference in the upper area. (**a**) The negative pressure formed in the mixing box generated a suction force which was maximum near the inlets located in proximal distance to the main backflow pipe; (**b**) and (**c**) The air suction reduced its effectiveness for the inlets located between 30°–120° and 240°–300° from the main pipe (0°). (**d**) The suction strength was minimal in the furthermost inlets to the main pipe (120°–240°).

5.2. CO_2 Concentration

CO_2 measurements were acquired at 2 min intervals over a 12 h period. The concentration inside the chamber was within 50 μmol mol^{-1} (±8%) of the target 650 μmol mol^{-1} in 43% of observations, within 100 μmol mol^{-1} (±15%) of the target in 79% of observations, and within 150 μmol mol^{-1} (±23%) of the target in 92% of observations (Table 2).

Table 2. Percentage of observations in each of three CO_2 concentration ranges. The set point was 650 μmol mol^{-1}.

CO_2 Concentration Range (μmol mol^{-1})	**Range as % of Set Point**	**% of Observations**
±50	±8	43
±100	±15	79
±150	±23	92

5.3. Air Temperature

OTC internal temperature was recorded at hourly intervals with temperature dataloggers positioned above canopy height (45 cm) next to the CO_2 probes. There was no difference in day time (05:00–18:00) temperature averages for OTC operated at ambient and elevated CO_2 levels. However, these chambers were warmer (with approximately 2.3 °C higher temperature) than the no-chamber control plots (Figure 9). The within-OTC and control temperatures gap was narrower in cloudy or rainy days. The night air temperature (19:00–4:30) was increased by 0.6 °C inside the OTCs (23.99 °C), compared to unchambered (control) plots' temperature (control), with an average value of 23.40 °C. These temperature variations could alter biological processes such as plant growth, metabolic rates, and phenological events. OTCs also modify other aspects of the microclimate, including humidity, light exposure, and wind flow. Such differences, particularly between day and night, introduce additional variability that may not accurately reflect natural conditions, thereby challenging the ecological validity and reproducibility of the results across different settings. To mitigate these effects, it is recommended to standardize OTC design, accurately monitor environmental conditions, and employ ambient OTC for direct comparison, while unchambered plots can serve as a reference for current environmental conditions. This "chamber effect" can be easily detected by investigating changes in morphological traits on plants such as, for instance, flower count, fresh weight measurements, and physiological parameters such as photosynthetic rates and stomatal conductance [25].

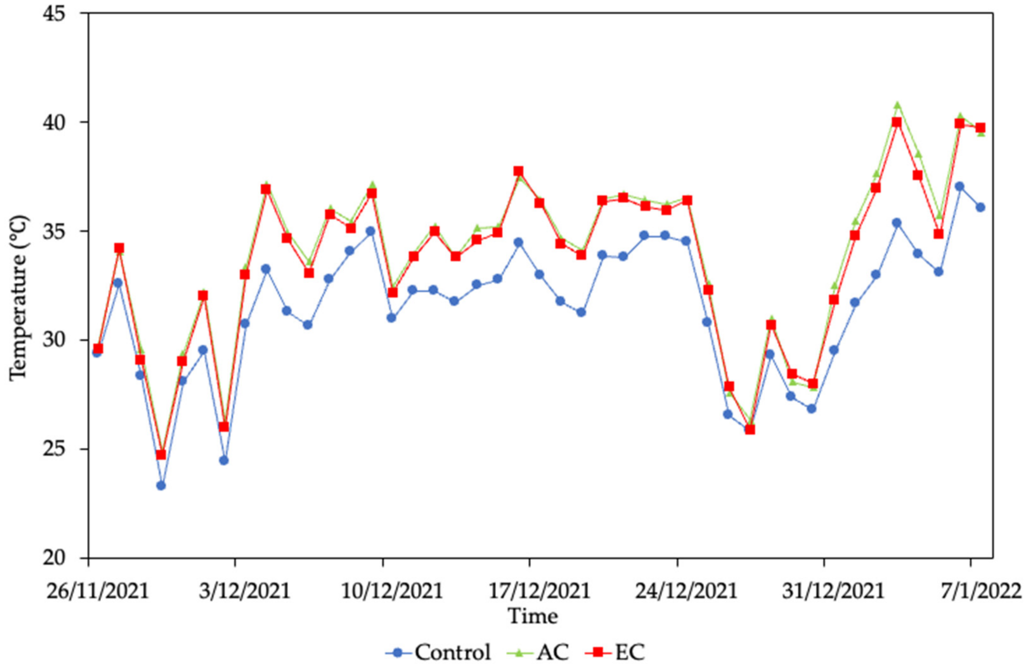


Figure 9. Average daily day-time air temperature in OTCs with ambient (AC) and elevated (EC) CO_2 levels, and for the control plots without chambers.

When the ventilation system of an OTC was turned off at the start of a day, the average air temperature through the monitored day was higher by 0.7 °C within the chamber without ventilation than the chamber with ventilation (Figure 10).

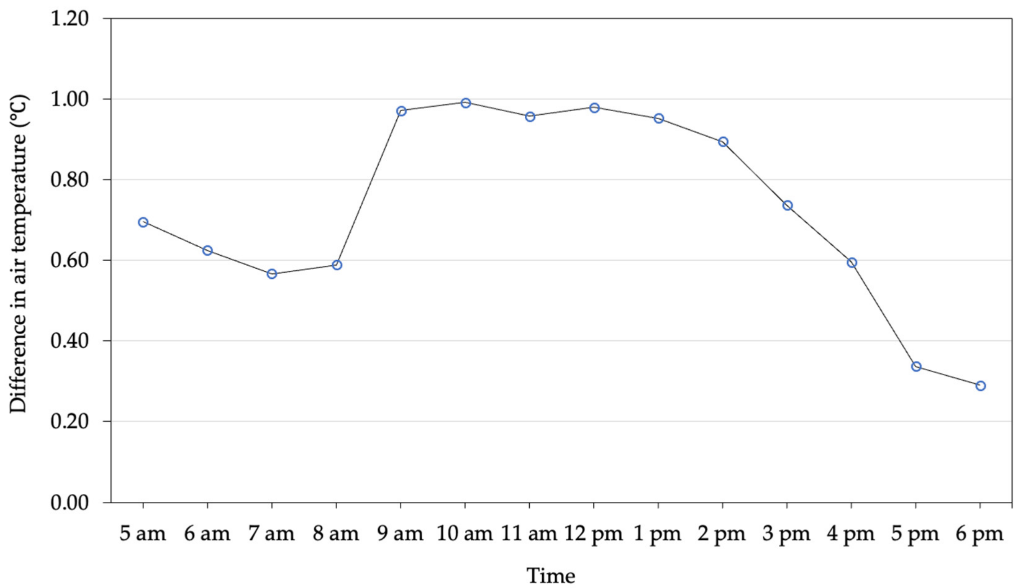


Figure 10. OTC differential air temperature through a day calculated as the difference between temperatures recorded with blower on and off.

5.4. System CO_2 Consumption

With CO_2 injected to maintain a concentration of 650 µmol mol^{-1}, CO_2 consumption was, on average, 3.15 L min^{-1} (0.22 m^3 h^{-1}) per chamber for the non-collared OTC design, operated 24 h per day. Consumption was decreased to 2.67 L min^{-1} (0.16 m^3 h^{-1}) when the system was operated during daylight hours (0530–1800) only. With the installation of the conical collar and recirculation of a portion of the airflow, consumption was decreased to 1.38 L min^{-1} (0.09 m^3 h^{-1}) per chamber, requiring the replacement of a 31 kg CO_2 cylinder replacement every 8–9 days for the operation of four chambers.

A single size G compressed CO_2 bottle contains 31 kg CO_2, or 704 moles CO_2, which, at 25.4 L mole^{-1}, represents 17,881 L at 1 atm. This source can be diluted with ambient air to produce approximately 17,881/0.00065 = 27.5 M L of mixture, or 27,509 m^3 (×2 = approx. 60 ML or 60,000 m^3 of gas for 650 µmol m^{-2} s^{-1}). With the blower operating at 270 m^3 h^{-1}, one bottle should, therefore, support operation for 60,000/270 = 300 h for a single chamber or 75 h for four chambers. Allowing for 35% recirculation of chamber air, the operation should be extended to 105 h for four chambers. With operation at 12 h a day, it should operate for approximately 9 days, consistent with observed usage.

The capital costs, OTC specifications, CO_2 consumption rates, temperature, and overall performance results are reposted in Table 3. The OTCs performance is compared to the findings presented by Messerli at al. [15], providing a comprehensive analysis of how our current results align or differed with the previous studies.

Table 3. Summary of the OTC specifications and comparison with Messerli et al. [15] OTC design.

Attribute	This Study	Messerli et al. [15]
Capital cost (USD per 12 units)	USD 10,615 for 12 units.	USD 14,000 for eight units.
Ground area per chamber	1.25 m^2	1.2 m^2
Chamber volume	1.5 m^3	1.2 m^3
CO_2 usage	×2 31 kg CO_2 cylinders every 8–9 days (daylight hours injection) to maintain 650 µmol mol^{-1} target in 4 OTC. Average daily consumption of 0.99 kg/day/chamber at the rate of 1.38 L min^{-1}	22.68 kg CO_2 every 4–7 days to maintain 600 µmol mol^{-1} 4 OTCs, 5.5 kg/5 day = 1.1 kg/day/chamber Average daily CO_2 consumption of 3.0 kg m^{-2} of elevated CO_2 area (design to mix fresh air and recirculated air in 5:1 ratio)
Air flow into chamber	Rate of the fresh ambient air taken inside the mixing box: 0.047 m^3 s^{-1}; backflow: CO_2/air mix recycling rate: 0.028 m^3 s^{-1}.	5 air changes per minute 5.66 m^3/min^{-1}
CO_2 control	79% of observations within 100 µmol mol^{-1} ±15% to target 650 µmol mol^{-1}	93% of the time within ±20% (i.e., 120 µmol mol^{-1}) of the targeted 600 µmol mol^{-1} CO_2, based on 10 min averages
Temperature	OTCs warmer than unchambered plots (control) by 2.3 °C (30-day measurements) during daylight h. Night temperatures were 0.6 °C in OTC over control plots. OTCs warmer when ventilation system was turned off by 0.7 °C	OTCs 0.7 °C warmer than control plot similar to climate change prediction (seasonal measurements). Midday temperature increase 1.03 °C; night temperature increases 0.43 °C

5.5. Plant Performance

Commercial peanut plants (*Arachis hypogaea* L., cultivar Sutherland) were planted in December 2021 into the 12 beds, including four OTC with 650 µmol mol^{-1} CO_2 (EC), four OTC with ambient CO_2 levels (AC), and four control beds. The injection of CO_2 to the four chambers (EC) started when the first leaves were fully developed (V-3) [26], and injection was terminated at harvest, 30 days after planting, when the peanuts reached the flowering stage (R1). The chamber's internal temperature was monitored with temperature probes (HOBO dataloggers, Bourne, MA, USA). Leaf gas exchange parameters were measured on one per plot of the last fully expanded young leaflet at the canopy top, one day before harvest, using a portable photosynthesis system (LI-COR 6800, LI-COR Inc., Lincoln, NE, USA). Measurements were conducted with a solar photosynthetic photon flux density (PPFD) manually set at 2000 μmol mol^{-1}. Humidity reference was set at 60%, fan speed at 8000 rpm, air flow at 500 mmol^{-1}, and cuvette temperature at 30 °C. The cuvette [CO_2] was adjusted based on the growth condition: 400 μmol mol^{-1} for AC OTC and 650 μmol mol^{-1} for EC OTC. Measurements were taken between 9:00–11:00 a.m. and 14:00–16:00 p.m. The intrinsic water use efficiency (WUE) was calculated as a ratio between net CO_2 assimilation rates and the transpiration. The crop performance results are reported in Table 4.

Table 4. Peanut crop parameters after 30 days of growth in (**a**) no chamber, (**b**) OTC with ambient CO_2 levels; (**c**) OTC with 650 μmol m^{-2} s^{-1} CO_2. Means ± SD, n = 4 chambers per each treatment.

Attribute	No Chamber (Control)	OTC with Ambient (~400 μmol mol^{-1}) CO_2 Levels (AC)	OTC with 650 μmol mol^{-1} CO_2 (EC)	*p* Value
Above ground dry biomass (g) per chamber (of 10 plants)	61.4 ± 8.9 ^{b}	60.9 ± 12.1 ^{b}	87.8 ± 24.8 ^{a}	0.0060
Net CO_2 assimilation rate (μmol m^{-2} s^{-1})	32.2 ± 3.9 ^{a}	29.1 ± 3.5 ^{a}	35.1 ± 8.2 ^{a}	ns
Intrinsic WUE (μmol CO_2 mol^{-1} H_2O)	3.2 ± 0.1 ^{b}	3.1 ± 0.1 ^{b}	4.4 ± 0.3 ^{a}	0.0024
PAR at ground level (% of Control)	-	76	76	-

Note. Same superscript letters within the row indicate no statistical difference between groups according to Tukey's post hoc test.

Over the 30-day growth period, the accumulated above-ground biomass, measured as the dry weight of 10 plants per chamber, was similar between the control (no chamber) and the OTC with ambient CO_2 levels (AC), with values of 61.4 g (no chamber) compared to 60.9 g for AC. In contrast, the accumulated above-ground biomass was ~40% greater in the OTC with elevated CO_2 (EC) compared to the chambers with ambient CO_2) (p = 0.0060) (Table 4). Similarly, net CO_2 assimilation rates were greater for EC-grown peanuts compared to peanuts grown under ambient control and AC. This difference, however, was not significant. In contrast, intrinsic WUE was greater for EC-grown peanuts compared to control and AC-grown peanuts, and this difference was significant (p = 0.0024). These results are in close agreement with previous studies reporting the stimulation of biomass, photosynthesis, and water use efficiency of crops when grown under elevated CO_2 [27].

6. Conclusions

The design of an open-top chamber facility described in this research effectively showcases the impact of elevated CO_2 levels on crops, combining precision with cost effectiveness for a sustainable approach to agricultural research. Details of its construction and the results provided for the facility evaluation show that it represents a viable and practicable alternative to costly methods of ascertaining the effects of CO_2 on plants.

The test performed on the OTCs and CO_2 concentration control equipment highlighted the system's efficacy in maintaining the internal target concentration throughout the trial, which, for this research, was set to 650 ± 50 μmol mol^{-1}. Additionally, recycled chamber air was used, reducing overall CO_2 usage from 2.68 min^{-1} to 1.38 L min^{-1}.

The daily average CO_2 consumption observed during daylight hours injection in this study was 1.56 kg m^{-2}.

Some adjustments and system fine-tuning were required to improve CO_2 consumption rates and limit wind interference. Simple modifications implemented to the OTC structure, such as partially modifying the opening with a conical top opening (frustum), resulted in effective measures for mitigating the issues caused by wind and improving the system's functioning. However, the use of a conical frame for the frustrum could lead to changes in the indoor–outdoor continuum as a trade-off, modifying the amount of received rainfall. However, such an issue can be overcome with automated irrigation systems set to release the amount of water according to the crop need, geographical location, and soil characteristics. A potential limitation that could affect the interpretation of the effects of elevated CO_2 is the increased temperature inside the chambers, which could confound the effects attributed solely to CO_2. As a mitigation measure, each chamber ventilation system was equipped with a blower which effectively contributed to reduce temperature through the high-volume air produced. Furthermore, the facility included OTCs with ambient CO_2 levels for direct comparison and "control plots" without chambers which are recommended to reflect current CO_2 and environmental conditions accurately.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/hardware2020007/s1.

Author Contributions: Conceptualization, N.N. and S.T.-P.; methodology, N.N and S.T.-P.; software, N.N.; validation, N.N., M.N., K.B.W., H.L. and S.T.-P. formal analysis, N.N.; investigation, N.N.; resources, M.N. and S.T.-P.; data curation, N.N.; writing—original draft preparation, N.N.; writing—review and editing, N.N., M.N., K.B.W., H.L. and S.T.-P.; visualization, N.N.; supervision, M.N., K.B.W., H.L. and S.T.-P.; project administration, N.N.; funding acquisition, N.N. and S.T.-P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by in part by the Elevate Scholarship (2021–2024) from Central Queensland University and the Cooperative Research Centre for Developing Northern Australia (CRCNA) awarded to one of the authors (N.N.) and by a CQ University Merit Grant granted to S.T.-P.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the finding of this research are available upon request from the corresponding author.

Acknowledgments: We would like to express our gratitude to Ruan Nortje for his invaluable contribution to the realization of the electrical plant of the CO_2 control systems, an aspect that played a pivotal role in ensuring the overall success of our experiments. Additionally, we extend our sincere thanks to CQU Technical Officer Andrew Bryant for the support in coordinating the CO_2 supply during the experiments. Finally, we gratefully acknowledge the contribution of the Peanut Company of Australia (Bega group), Kingaroy, Queensland, Australia in providing us the seed material used in the experiments.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Allen, J.L.; Drake, B.; Rogers, H.; Shinn, J. Field techniques for exposure of plants and ecosystems to elevated CO_2 and other trace gases. *Crit. Rev. Plant Sci.* **1992**, *11*, 85–119.
2. Norby, R.J.; Zak, D.R. Ecological Lessons from Free-Air CO_2 Enrichment (FACE) experiments. *Annu. Rev. Ecol. Evol. Syst.* **2011**, *42*, 181–203. [CrossRef]
3. Allen, L.H.; Kimball, B.A.; Bunce, J.A.; Yoshimoto, M.; Harazono, Y.; Baker, J.T.; Boote, K.J.; White, J.W. Fluctuations of CO_2 in Free-Air CO_2 Enrichment (FACE) depress plant photosynthesis, growth, and yield. *Agric. For. Meteorol.* **2020**, *284*, 107899. [CrossRef]
4. Mollah, M.; Norton, R.; Huzzey, J. Australian grains free-air carbon dioxide enrichment (AGFACE) facility: Design and performance. *Crop Pasture Sci.* **2009**, *60*, 697–707. [CrossRef]
5. D'andrea, L.; Rinaldi, M. Systems to evaluate the effects of atmospheric CO_2 concentration on field crops: A review of open top chambers. *Ital. J. Agrometeorol.-Riv. Ital. Agrometeorol.* **2010**, *15*, 23–34.
6. Machacova, K. Open top chamber and free air CO_2 enrichment-approaches to investigate tree responses to elevated CO_2. *Iforest-Biogeosci. For.* **2010**, *3*, 102–105. [CrossRef]
7. Heagle, A.S.; Body, D.E.; Heck, W.W. An open-top field chamber to assess the impact of air pollution on plants. *J. Environ. Qual.* **1973**, *2*, 365–368. [CrossRef]
8. Hardy, R.W.F.; Havelka, U.D. Photosynthate as a major factor limiting nitrogen fixation by field grown legumes with emphasis on soybeans. In *Symbiotic Nitrogen Fixation in Plants*; Cambridge University Press: London, UK, 1976; pp. 421–429.
9. Drake, B.G.; Leadley, P.W.; Arp, W.J.; Nassiry, D.; Curtis, P.S. An Open Top Chamber for field studies of elevated atmospheric CO_2 concentration on saltmarsh vegetation. *Funct. Ecol.* **1989**, *3*, 363–371. [CrossRef]

10. Leadley, P.W.; Drake, B.G. Open top chambers for exposing plant canopies to elevated CO_2 concentration and for measuring net gas exchange. *Vegetatio* **1993**, *104*, 3–15. [CrossRef]
11. Norby, R.; Edwards, N.; Riggs, J.; Abner, C.; Wullschleger, S.; Gunderson, C. Temperature-controlled open-top chambers for global change research. *Glob. Chang. Biol.* **1997**, *3*, 259–267. [CrossRef]
12. Mandl, R.H.; Weinstein, L.H.; McCune, D.C.; Keveny, M. A Cylindrical, Open-Top Chamber for the exposure of plants to air pollutants in the field. *J. Environ. Qual.* **1973**, *2*, 371–376. [CrossRef]
13. Khan, M.R.; Rizvi, T.F. Effect of elevated levels of CO_2 on powdery mildew development in five cucurbit species. *Sci. Rep.* **2020**, *10*, 4986. [CrossRef] [PubMed]
14. Silva, J.B.L.d.; Ferreira, P.A.; Pereira, E.G.; Costa, L.C.; Miranda, G.V. Development of experimental structure and influence of high CO_2 concentration in maize cro. *Eng. Agrícola* **2012**, *32*, 306–314. [CrossRef]
15. Messerli, J.; Bertrand, A.; Bourassa, J.; Belanger, G.; Castonguay, Y.; Tremblay, G.; Baron, V.; Seguin, P. Performance of low-cost open-top chambers to study long-term effects of carbon dioxide and climate under field conditions. *Agron. J.* **2015**, *107*, 916–920. [CrossRef]
16. Wang, B.; Li, R.; Wan, Y.; Li, Y.E.; Cai, W.; Guo, C.; Qin, X.; Song, C.; Wilkes, A. Air warming and CO_2 enrichment cause more ammonia volatilization from rice paddies: An OTC field study. *Sci. Total Environ.* **2021**, *752*, 142071. [CrossRef] [PubMed]
17. Meng, F.; Zhang, J.; Yao, F.; Hao, C. Interactive effects of elevated CO_2 concentration and irrigation on photosynthetic parameters and yield of maize in Northeast China. *PLoS ONE* **2014**, *9*, e98318. [CrossRef]
18. Qiao, Y.F.; Miao, S.J.; Li, Q.; Jin, J.; Luo, X.S.; Tang, C.X. Elevated CO_2 and temperature increase grain oil concentration but their impacts on grain yield differ between soybean and maize grown in a temperate region. *Sci. Total Environ.* **2019**, *666*, 405–413. [CrossRef] [PubMed]
19. Ofori-Amanfo, K.K.; Klem, K.; Veselá, B.; Holub, P.; Agyei, T.; Juráň, S.; Grace, J.; Marek, M.V.; Urban, O. The effect of elevated CO_2 on photosynthesis is modulated by nitrogen supply and reduced water availability in Picea abies. *Tree Physiol.* **2023**, *43*, 925–937. [CrossRef] [PubMed]
20. Whitehead, D.; Hogan, K.P.; Rogers, G.N.D.; Byers, J.N.; Hunt, J.E.; McSeveny, T.M.; Hollinger, D.Y.; Dungan, R.J.; Earl, W.B.; Bourke, M.P. Performance of large open-top chambers for long-term field investigations of tree response to elevated carbon dioxide concentration. *J. Biogeogr.* **1995**, *22*, 307–313. [CrossRef]
21. Juráň, S.; Grace, J.; Urban, O. Temporal changes in ozone concentrations and their impact on vegetation. *Atmosphere* **2021**, *12*, 82. [CrossRef]
22. Frei, E.R.; Schnell, L.; Vitasse, Y.; Wohlgemuth, T.; Moser, B. Assessing the effectiveness of in-situ active warming combined with open top chambers to study plant responses to climate change. *Front. Plant Sci.* **2020**, *11*, 539584. [CrossRef] [PubMed]
23. NOAA. Trends in Atmospheric Carbon Dioxide. 2024. Available online: https://gml.noaa.gov/ccgg/trends/gl_gr.html (accessed on 29 January 2024).
24. Van Oijen, M.; Schapendonk, A.; Jansen, M.; Pot, C.; Maciorowski, R. Do open-top chambers overestimate the effects of rising CO_2 on plants? An analysis using spring wheat. *Glob. Chang. Biol.* **1999**, *5*, 411–421. [CrossRef]
25. Porter, A.S.; Evans-Fitz. Gerald, C.; McElwain, J.C.; Yiotis, C.; Elliott-Kingston, C. How well do you know your growth chambers? Testing for chamber effect using plant traits. *Plant Methods* **2015**, *11*, 44. [CrossRef] [PubMed]
26. Boote, K. Growth stages of peanut (*Arachis hypogaea* L.). *Peanut Sci.* **1982**, *9*, 35–40. [CrossRef]
27. Tausz-Posch, S.; Tausz, M.; Bourgault, M. Elevated [CO_2] effects on crops: Advances in understanding acclimation, nitrogen dynamics and interactions with drought and other organisms. *Plant Biol.* **2020**, *22* (Suppl. S1), 38–51. [CrossRef]