A laboratory-scale greenhouse for spectroscopic monitoring of plants and associated gas-phase isotopic fractionation

OPEN ACCESS
IOP Publishing
Measurement Science and Technology
Meas. Sci. Technol. **34** (2023) 085902 (8pp)
https://doi.org/10.1088/1361-6501/accbd9

A laboratory-scale greenhouse for spectroscopic monitoring of plants and associated gas-phase isotopic fractionation

Samuel M Greer, Istvan Robel, Andrew C Beveridge, Aaron S Anderson, Ronald K Martinez, Robert P Currier, George B Perkins, Leslie Thalmann and Kristy L Nowak-Lovato*

Los Alamos National Laboratory, Los Alamos, NM 87545, United States of America

E-mail: knowak@lanl.gov

Received 10 March 2023
Accepted for publication 11 April 2023
Published 28 April 2023

Abstract

The ability to monitor isotopic fractionation in terrestrial ecosystems is a challenge due to the presence of interacting variables. A laboratory-scale apparatus for controlled experiments could serve as a useful platform to deconvolute the variables that affect isotopic fractionation. Such a device could offer a powerful means to understand fractionation of carbon stocks in terrestrial ecosystems and to probe the effects of photosynthesis or interactions between the soil and plants on carbon fractionation. To this end, an enclosed and artificially-lit benchtop soil and plant chamber was constructed and equipped to monitor atmospheric isotopic composition. Fourier-transform infrared (FTIR) spectroscopy was employed for isotopic sensing since it enables *in-situ* measurements. The validity of FTIR for isotopic ratio determination was confirmed by comparing FTIR and isotope ratio mass spectrometry data for a series of CO_2 gas samples with known quantities of ^{13}C and ^{12}C. The greenhouse chamber was also equipped with an optically-based trace gas analyzer capable of continuously tracking CO_2, CH_4, and H_2O concentrations and a residual gas analyzer mass spectrometer. Reflectance spectroscopy was also incorporated by way of sealed fiber optic feed-throughs coupled to a spectro-radiometer, for quantifying changes in leaf spectra induced by various environmental stressors. The resulting greenhouse chamber can be a useful tool for determining the effects of atmospheric trace gases on plant morphology and physiology as a function of concentration and isotopic composition. Microecosystems can be examined under controlled laboratory conditions and a wide variety of plant species can be accommodated. The bench-scale greenhouse should prove useful in assessing the impact of environmental variables and in guiding the design of field experiments.

Keywords: isotope uptake, ecosystems, environmental fate

(Some figures may appear in colour only in the online journal)

* Author to whom any correspondence should be addressed.

1. Introduction

It was established decades ago within the realm of plant sciences that greenhouses enable year-round research under conditions wherein the effects of external environmental variations can be controlled. Guidelines for the design and operation of research greenhouses that promote accurate and reproducible results have been formulated and critically evaluated [1–3]. Using such guidelines, laboratory-scale greenhouses (also referred to as phytotrons) have become useful tools in assessing environmental effects on the growth of a wide range of plant species. In addition to carbon dioxide (CO_2) and moisture levels, other growth parameters include the light source (spectrum and intensity), temperature, atmospheric gas composition, and nutrients. The physical configuration of the greenhouse, growing protocols, and adequate environmental control are additional operational parameters.

Quantifying the response of terrestrial carbon and water cycles to changes in the climate is of considerable value in formulating and tuning predictive models. These cycles are coupled through photosynthesis and evapotranspiration but the fluxes occurring at multiple spatial and temporal scales require better understanding. For example, predictions are complicated by the uncertainties associated with scaling the processes involving movement or retention of carbon. The greenhouse-/phytotron provides an experimental platform in which the interaction of plants with the environment can be studied under a range of controlled conditions. Specifically, it is of practical interest to know both the effects of atmospheric gases on plants and the fate of specific chemical constituents within ecosystems. In addition to variations in CO_2 levels, the effects of other potent greenhouse gases such as methane (CH_4) can be studied within closed laboratory greenhouses. The effects of environmental pollutants can be interrogated as well. This may include nitrogen oxides and sulphur- and iodine-containing species.

As a result of advances in instrumentation, stable isotopes have become an increasingly important tool in determining chemical reaction pathways, in probing material provenance, and in establishing the ultimate fate of specific molecules. Thus, isotopic analysis is proving to be a useful technique for probing interactions between plants and their environment.

A change in isotopic composition within a molecule gives rise to distinct chemical species, which are often referred to as isotopologues. Simply put, isotopologues are variants of a molecule that differ in the isotopic identity of one or more of the constituent atoms. The change in molecular mass resulting from the change in isotopic composition affects the zero-point vibrational energy [4–7]. Consequently, the chemical bonds involving heavier stable isotopes are usually stronger than the bonds between lighter atoms. This in turn alters the rate of chemical reactions, with the heavier isotopologues typically reacting slightly slower than lighter isotopologues. This is referred to as the kinetic isotope effect. A change in isotopic composition also has subtle effects on chemical reactions and thermodynamics. At equilibrium, a chemical reaction may attain a state where isotopic fractionation exists between the pool of reactants and the pool of products. This is referred to as an equilibrium isotope effect. In addition, intermolecular interactions with other molecular species differ for different isotopologues [8]. This can result in differences in adsorption and transport of the various isotopologues and also in fractionation between phases that are in equilibrium [9].

Tracking isotopic composition within a greenhouse must reconcile all the important fractionation mechanisms. These include kinetic isotope effects associated with photosynthesis and respiration, preferential adsorption of one or more of the isotopologues onto exposed surfaces (e.g. walls, tubing, soil, pores, etc.), and any significant fractionation associated with the molecular gases dissolving into, and equilibrating with, liquid water. In addition, with some chemical pollutants, the rates of photon-induced chemistry may depend on the isotopic composition. In a natural ecosystem, one isotope fractionation mechanism may serve to enrich the atmosphere with specific isotopologues while other mechanism may act to deplete the atmosphere of those same isotopologues. These effects create non-trivial challenges for controlling the greenhouse and for the unambiguous interpretation of results.

In this communication, the assembly, instrumenting, and testing of a bench-scale greenhouse designed for tracking isotopic compositions is described. In validation experiments using CO_2, ^{12}C and ^{13}C isotopes were measured in the presence of live plants to illustrate the capabilities and potential of the system, as well as, to identify some of the limitations.

2. Phytotron/greenhouse apparatus

A bench-scale enclosed plant growth chamber was built to measure isotopic changes in the enclosed atmosphere resulting from interactions of atmospheric components with soil and plants. A schematic of the system is shown in figure 1 and a photograph appears in figure 2. The basic description of the apparatus is as follows. Live plants were housed within a 30 cm $\times$ 30 cm $\times$ 45 cm clear acrylic chamber (40 l volume; Fisher Scientific 08-642-23C). The chamber was equipped with an access door sealed by rubber gaskets. Light-emitting diode (LED) grow lights were positioned above the chamber. Their spectral range was roughly 400–800 nm. This spectral window covers the range absorbed by chlorophyl (roughly 400–900 nm). However, the use of LEDs does provide less spectral range than a quartz-tungsten-halogen lamp. Therefore, a 70-watt quartz-tungsten-halogen light source with a wider spectral range (350–2500 nm) was also installed and used for recording reflectance spectra (see below). However, it resulted in considerably more long-term heating within the chamber. Two gas feedthrough ports on the chamber walls enabled continuous closed-loop flow of the greenhouse atmosphere and provided access to multiple analytical instruments, as described below.

A Fourier-transform infrared (FTIR) spectrometer was connected to the system (Thermo Scientific, Nicolet iS50). FTIR provides a means of identifying a wide variety of gaseous species and quantifying individual molecular isotopologues. The

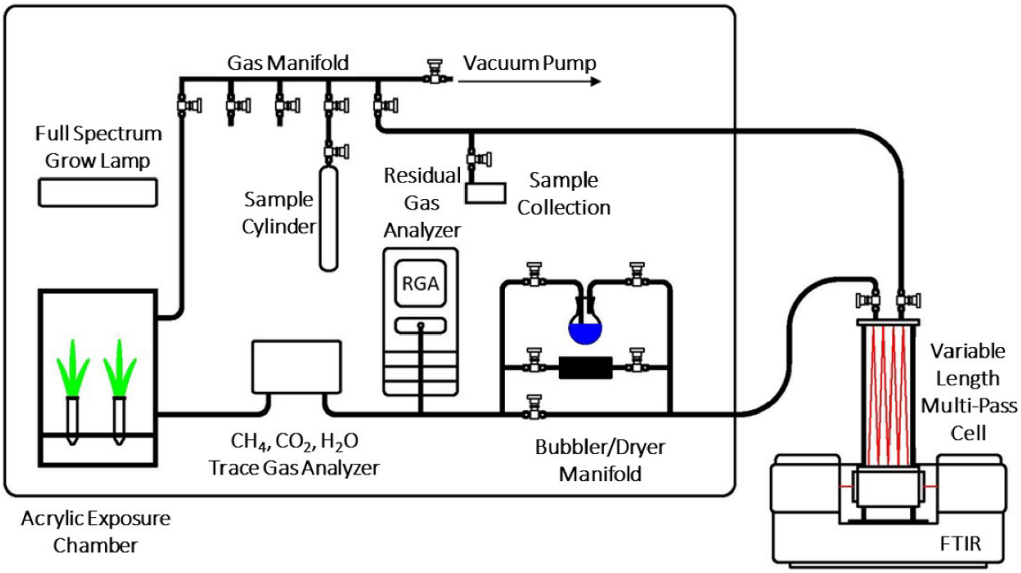

Figure 1. Schematic highlighting the key components of the laboratory-scale greenhouse system. While not shown in the schematic, gases discharged from the RGA were recycled back to the chamber housing the plants.

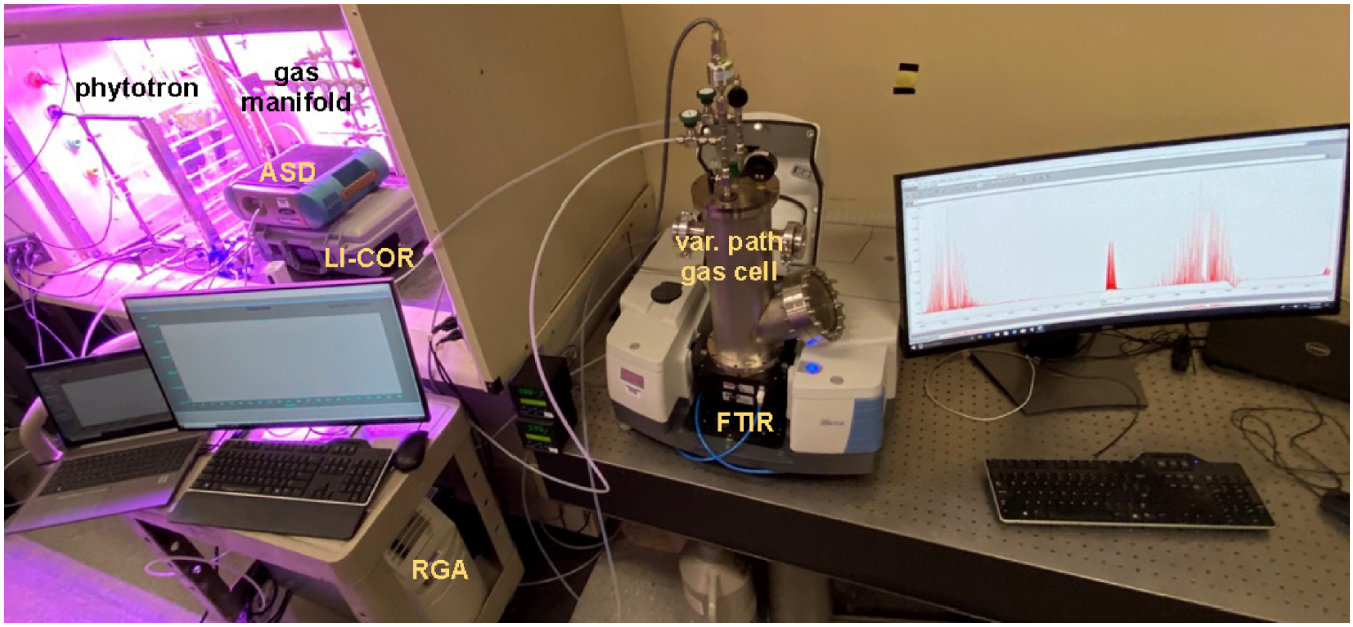

Figure 2. Phytotron chamber with a gas handling manifold connected in a closed loop to analytical instrumentation that includes a FTIR, trace gas analyzer, residual gas analyzer, and spectroradiometer.

installed FTIR could be used either as part of the continuously operating closed loop or opened to sample gas within the loop, as needed. The FTIR spectrometer was equipped with a multipass variable path length attachment that enabled measurement of both high concentration components and trace species from the same gas sample, using a maximum path length of 16 m. The volume of the FTIR gas cell was approximately 4 l. Analysis of the FTIR spectra enabled determination of both chemical species and isotopic content.

A quadrupole mass spectrometer residual gas analyzer (RGA) designed for atmospheric pressure gases was also incorporated into to the system (Stanford Research Systems QMS300). It was equipped with a 1–300 amu atmospheric sampling system. A capillary tube intake ensured suitably low flow rates (milliliters per minute). The RGA could be used for continuous monitoring over periods of hours or days with little effect on the pressure in the closed loop system. The RGA exhaust could also be fed back into the closed loop to avoid loss of pressure, if necessary.

An optical trace gas analyzer (LI-COR, model LI-7810) was connected to the closed gas loop for monitoring the concentrations of CO_2, H_2O, and CH_4. Two gas-handling and

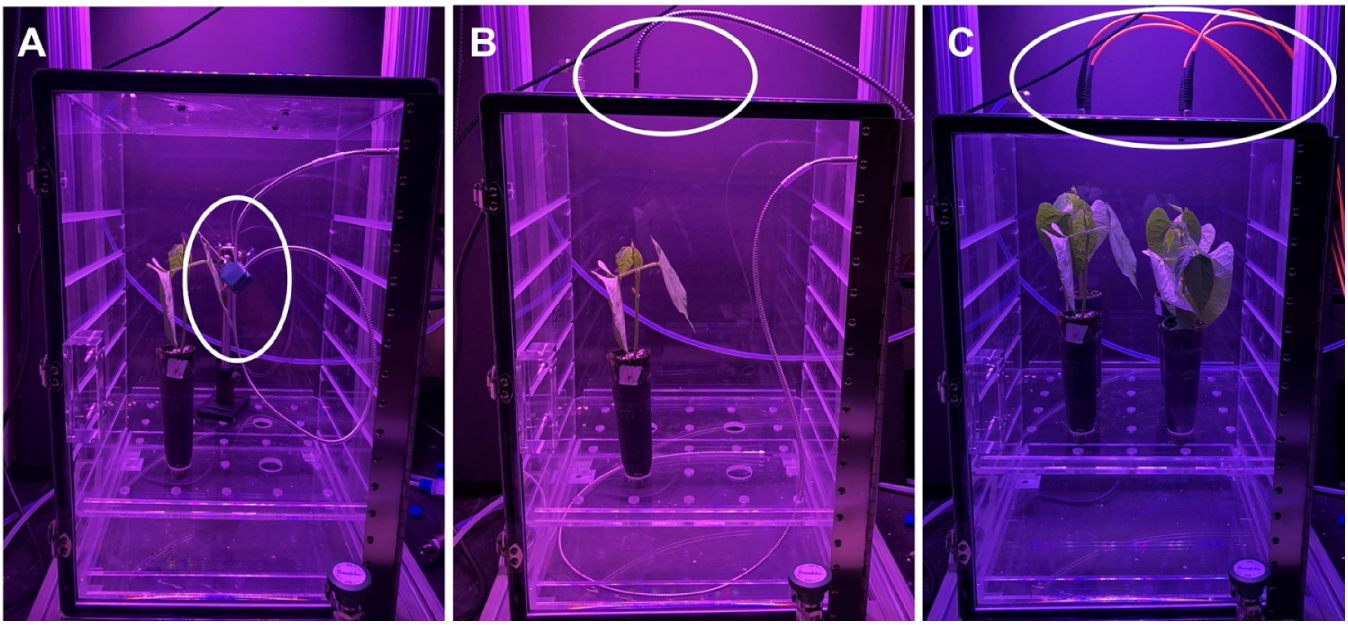

Figure 3. Three photographs showing the different modes of coupling a fiber into the growth chamber. (A) Fiber coming in from the side and monitoring a single leaf on a single plant. (B) A single overhead fiber is used to monitor a single plant. (C) Four fibers monitor all for plants at the same time. The purple hue is due to the grow lights.

sampling manifolds were also interconnected to the exposure chamber flow loop. They allow for the controlled introduction, or extraction, of gases and control of pressure and relative humidity through water bubbler and dryer lines, respectively.

Leaf reflectance spectra [10, 11] were obtained using a spectroradiometer (analytical spectral devices (ASD) Inc., FieldSpec 4). We employed several methods of coupling the ASD fibers into the plant chamber. The first method employs an optical fiber that was fed through the side of the chamber. It monitors a single leaf by using a small clamp to hold the collection fiber in place. This method produces the highest quality spectra but is limited to monitoring a single leaf on a single plant (figure 3(A)). The second method involved positioning the fiber directly over one of the plants. This overhead monitoring records reflection spectra of the entire region below the fiber. This fiber could be placed above any one or all of the four plants in the chamber (figure 3(B)). The third method involved a fiber splitter and combiner to position an optical fiber above each plant in the chamber. The reflected light from these fibers was combined into a single fiber that was connected to the ASD (figure 3(C)). This configuration allows for the simultaneous monitoring of all four plants. All of these options allow for *in-situ* observation and analysis of spectral changes as a result of stress factors incurred by the plants upon exposure to gases.

3. Experimental

To establish a baseline, the ability of FTIR spectroscopy to quantify isotopic content was verified first, without the added complexity of the plants, and soil. A range of gaseous CO_2 mixtures, with varying $^{12}C/^{13}C$ ratios, was prepared and analyzed using the variable path length FTIR cell. Following validation, the effects of plants and soil on CO_2 fractionation under 24 h of illumination were measured. The associated experimental protocols are described in this section. This includes preparation of gas mixtures having the desired $^{12}C/^{13}C$ isotopic composition, preparation and monitoring of live plant samples, and a description of the spectroscopic data analysis techniques that were used.

3.1. Preparation of isotopic gas mixtures for FTIR and IRMS measurements

Commercially-obtained lecture bottles containing gases of known isotopic composition were purchased from Sigma-Aldrich/Isotec ($^{12}CO_2$ with 99 atom % ^{12}C and $^{13}CO_2$ with 10 atom % ^{13}C). These gases were used to create stock mixtures containing 1%, 2%, and 5% $^{13}CO_2$. The 1%–2% mixtures were subsequently diluted with $^{12}CO_2$ to create a range of CO_2 mixtures with 0.1%–2% ^{13}C. To prepare stock gases of known isotopic composition, the following dilutions were performed. 500 cm^3 cylinders (Swagelok, 304 l Stainless, fitted with a Swagelok needle valve) were evacuated to a base pressure of $<$0.02 Torr. The source bottles were fitted with regulators and connected to a gas handling manifold. The tubing between the manifold and the lecture bottle (or gas cylinder) was evacuated to $<$0.1 Torr and refilled to 600 Torr three times to remove residual air. The appropriate amount of the lower percentage component (e.g. ^{13}C) was introduced, followed by the higher percentage component (^{12}C or gas of natural abundance). The total pressure was brought to $\sim$1000 torr. These mixtures were then used to create a dilution series with small inter-sample percentage difference. The cylinders were closed and capped until needed. FTIR and Isotope ratio mass spectrometry (IRMS) gas samples were prepared as follows. Dilutions

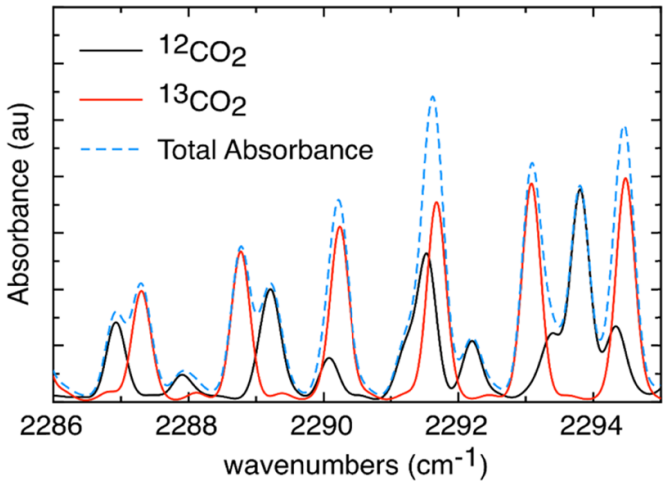

Figure 4. Simulated absorption spectrum of a mixture of 99% $^{12}CO_2$ and 1% $^{13}CO_2$.

were carried out as above, but using 25 or 50 cm^3 stainless steel cylinders (Swagelok 316 l fitted with Swagelok needle valves). After preparing the desired isotopic mixture, the gas bottles were pressurized with nitrogen (to ∼1000 Torr) to facilitate gas transfer into the IRMS cell.

3.2. Plant sample germination and growth protocols

The initial measurements used immature pinto bean plants (*Phaseolus vulgaris*). Pinto beans were chosen as they are a common agricultural product, are readily available, and are easy to cultivate. The beans were planted, germinated, and grown in cone-shaped containers housed in either a Percival or Thermo Scientific Precision growth chamber. The growth chambers were stand-alone units programmed to maintain a temperature of 20 °C and a diurnal cycle of 12 h, i.e. 12 h with the growth light on and 12 h of darkness. The plants were typically used in an experiment within 1–2 months of planting to minimize root growth restrictions imposed by the containers. As described below in the results section, watering protocols varied with the experiment.

3.3. Spectroscopic data analysis techniques

FTIR spectroscopy is an established means of quantifying carbon isotopes [12–14]. In this study, FTIR measurements were converted from transmission to absorbance and then processed for analysis. The only processing step was a simple baseline removal, using the Estimated Background function in Mathematica™. Baseline removal effectively serves as a high pass, low cutoff filter that removes any offsets from zero while preserving the spectral peaks. The open-source code PGopher [15] was used for fitting the data. PGopher simulates and fits electronic, rotational, and vibrational spectra. To support fitting of the raw data, known molecular state transitions for the molecular isotopologues were imported from the HITRAN database. Line transitions and partition function data for each species was also imported into PGopher. For accurate determination of relative concentrations, inclusion of partition function data proved to be essential. Partition function data was obtained from the HITRAN database.

Using both the HITRAN and experimental data, PGopher was used to convolute the HITRAN line data with both Gaussian and Lorentzian functions to create a Voigt line-shape. Voigt parameters were optimized using multiple non-linear fits. Parameters determined via least-squares regression were assumed to provide the best fit. Relative concentrations were then inferred using the fitted amplitudes. Using known line strengths and path lengths for gases at known composition (calibration data), quantitative results were obtained. Two key aspects employed during fitting included: (1) ensuring the fitted peak amplitudes were low enough to not be affected by the FTIR baseline adjustment (typically an absorbance of 0.1 or less was sufficient); and (2) identification of a region where the peaks for distinct isotopologues were commensurate with each other. The region 2286–2295 cm^{-1} was determined to be ideal to fit small concentrations of $^{13}CO_2$. In this region, peaks of a relatively small quantity of $^{13}CO_2$ are commensurate with the peaks from $^{12}CO_2$ (see figure 4). In addition, this region was sensitive to small changes in $^{13}CO_2$ concentration. Typical error for the fitted amplitude for each component was small (on the order of less than 1%). Using standard methods of error propagation, the fitting error for a 1% ratio of $^{13}CO_2$ to $^{12}CO_2$ was approximately 1.00 ± 0.05; thus small changes in composition were detectable using this protocol (provided the signal to noise ratio was adequate).

4. Results

A series of measurements on prepared mixtures of known concentration was used to validate the FTIR technique for measuring isotope ratios. Isotopic ratios determined by FTIR analysis were then verified using IRMS. For these measurements, collected samples were back-filled with nitrogen to ambient pressure. The $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios in CO_2 were

Table 1. Comparison of isotopic ratios determined by FTIR and IRMS.

Sample number	% ^{13}C (nominal)	% ^{13}C (FTIR)	% ^{13}C (IRMS)
1	0.1000	0.0993	0.203
2	0.5000	0.4641	0.624
3	0.9500	0.8986	1.026
4	1.4700	1.4470	1.446
5	1.5400	1.4808	1.660
6	2.0000	1.8793	2.02

then measured in continuous flow mode using the GV Instruments Isoprime IRMS coupled to a TraceGas unit (GV Instruments, Manchester, UK). The automated TraceGas instrument preconcentrated CO_2 cryogenically (via liquid nitrogen, −196 °C), chemically (via magnesium perchlorate), and by gas chromatography (using a 25 m PoraPLOT Q; 0.32) mm i.d.). The concentrated CO_2 was carried in a helium stream (99.999% purity) into the mass spectrometer, which was tuned specifically for isotopic analysis at *m/z* values of 44, 45, and 46. IRMS measurements were made relative to a reference gas CO_2 cylinder that was in turn calibrated to a known 750 ppm CO_2 -in-air standard (Scott-Marrin Inc., USA) with certified δ^{13}C and δ^{18}O isotope contents of −20.882‰ and −17.50‰ respectively. Here, per mil values (‰) are relative to the Vienna Pee Dee Belemnite standard typically used in environmental and geochemical analyses [16].

As summarized in table 1, the FTIR results are in good agreement with the nominal concentrations in the prepared mixtures, with the values differing by less than 10%. For each sample, the composition of gas with known isotopic composition is compared to the composition determined by FTIR and by IRMS.

Comparison indicates that with adequate care, FTIR can serve as a useful tool for monitoring ^{12}C/^{13}C ratios, even at very low levels of ^{13}C. At ^{13}C concentrations at and above natural abundance, the FTIR values are consistent with isotopic compositions determined by IRMS, with differences appearing at lower concentrations (the IRMS calibration was performed at natural abundance composition thus the highest IRMS accuracy can be expected near that composition).

A series of experiments to monitor ^{13}C/^{12}C isotope ratios was then performed. In these experiments, dry sterilized soil, wet sterilized soil, wet soil, and wet soil with healthy pinto beans were examined. This set of experiments was designed to explore the effects of microbes and moisture on isotopic distribution. Samples were sterilized using a high temperature pressure vessel (i.e. autoclave) that exposed the sample to a temperature >120 °C for at least 30 min. For each sample type, four samples in containers were sealed within the chamber. Three experimental trials were performed with each FTIR spectra measurement repeated thrice. The FTIR spectra were recorded ∼24 h after the samples were sealed in the chamber using the variable path length chamber at a pressure of 240 Torr. From these spectra, the simulation procedure described above was used to extract the isotopic composition of the CO_2. The LI-COR system was also employed to monitor the total concentration of CO_2 in the chamber. The results of these experiments are summarized in table 2 and figure 5.

Table 2. Initial and final CO_2 concentrations.

Sample	Trial	Initial (ppm)		Final (ppm)	
Dry auto-claved	1	378	Mean	863	Mean
	2	430	410	1132	1598
	3	421	±28	2800	±1049
Wet auto-claved	1	577	Mean	1059	Mean
	2	422	495	1408	1343
	3	486	±78	1562	±258
Live soil	1	481	Mean	825	Mean
	2	406	433	866	980
	3	411	±42	1250	±234
Live soil with plants	1	398	Mean	52	Mean
	2	542	460	227	137
	3	441	±74	132	±88

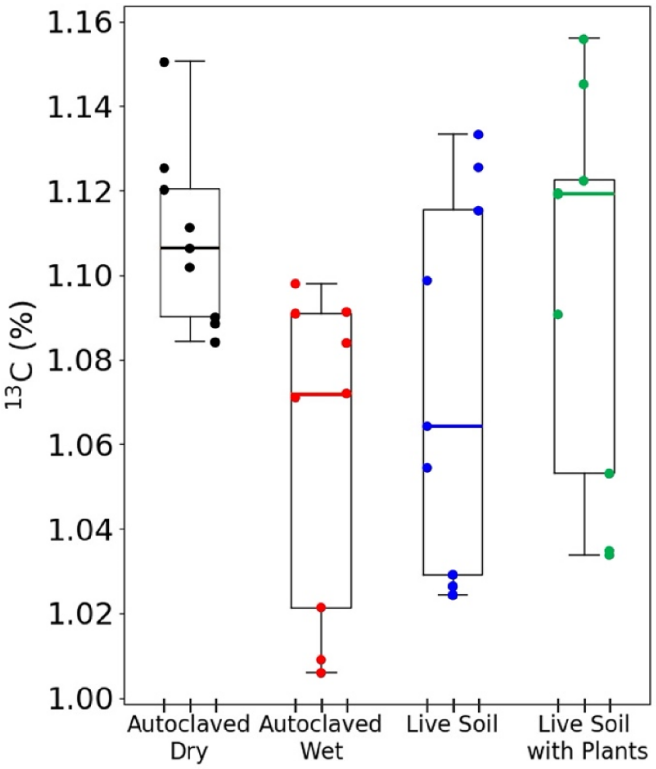

Figure 5. Results of experiments using the FTIR as incorporated into the phytotron system. The individual points and accompanying vertical lines are the mean and standard deviation respectively of each individual trial. This box-and-whisker plot includes accumulated data for all three measurements of all three trials for each sample.

The apparatus can be used to monitor changes in isotopic composition induced by the respiration of plants, or other organisms. Monitoring both the environment and isotopic fractionation can be used to interrogate ecosystem dynamics under well-controlled conditions. For example, figure 6 provides an example of monitoring the temporal evolution of atmospheric composition.

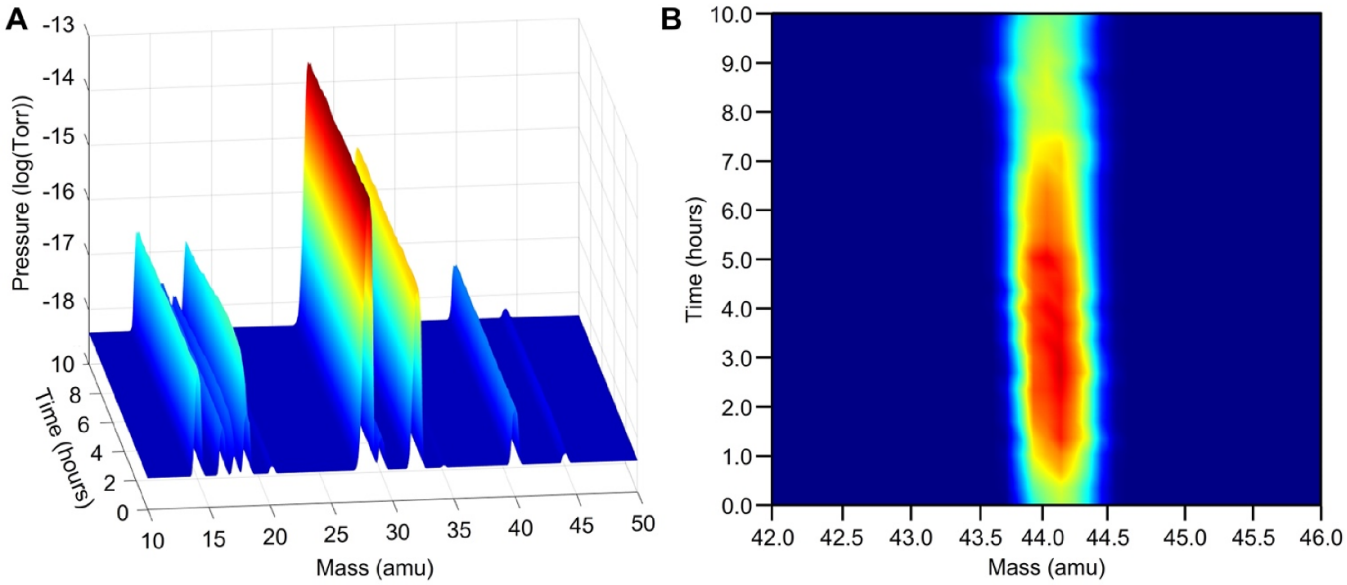

Figure 6. (A) 3D plot showing the evolution of gaseous composition as a function of time. (B) 2D false color plot of the mass region corresponding to CO_2 at 44 amu. Red represents highest pressures and blue the lowest.

5. Discussion

These preliminary results demonstrate the possibilities of the laboratory-scale phytotron. Specifically, the results show that autoclaved soil under illumination, and its associated heating, affects the outgassing of CO_2 over the course of 24 h experiments. Plants under illumination are able to consume CO_2 faster than the outgassing rate from soil. Use of the device for carbon isotope fractionation studies showed that variations within trials of each sample were small in absolute value, but significant. One can simply compare the average fractionation between the four samples. The observation is that the $^{13}CO_2$ average is higher when dry autoclaved soil is present, compared to the wet autoclaved and live soil samples, which are roughly equal in water content. The general trend aligns with CO_2 fractionation into an aqueous phase [17–19]. Of all the samples studied here, the live soil with plants had the highest average $^{13}CO_2$ concentration, which suggests that the plants preferentially uptake $^{12}CO_2$ while soil preferentially accumulates $^{13}CO_2$. This observation is consistent with previous studies [20] showing that live plants discriminate against $^{13}CO_2$. Improvements to the phytotron apparatus and the simulation procedure (some of which are discussed below) should quantitatively improve these results.

Quantitative measurements with live plants and specific pollutants should include replication and randomization experiments to account for plant-to-plant variability. Future studies should also include quantifying the effects of spatial variations within the phytotron itself, which can be an important variable [3]. Plants subject to various environmental stressors (e.g. drought conditions, nutrient deprivation) should also be examined.

While the system intentionally employed an inexpensive and easily replaceable acrylic exposure chamber, it was not perfectly air-tight. Future iterations may opt for an air-tight chamber to better maintain the desired atmosphere within the phytotron. Such a modification comes with larger expense. However, depending on the purpose of the experiments, this trade-off may be justified. If hazardous or highly reactive contaminant gases are to be used, a chemically compatible exposure chamber and adequate seals are recommended.

As configured, the phytotron is suitable for the study of plant response to acute exposures involving isotopically labeled environmental stressors. A enhancement of the system would involve long-term tests in an open system configuration involving continuous flow-through of atmospheric gases and contaminants. This requires the use of labeled gas sources of considerably larger volume as well as long-term humidity control. With the addition of the necessary flow meters and controlled air supply, the system could then be used to monitor the ecosystem response to long-term exposures. Also, instead of using conventional FTIR spectroscopy, cavity ring-down spectroscopy [21] could be employed. It is also capable of measuring the isotopic composition of trace atmospheric gases [22], CH_4 [23], and even radiocarbon emissions [24].

The natural carbon and water cycles are coupled through photosynthesis and evapotranspiration, but in many situations the individual steps and fluxes are not well understood. Use of a laboratory phytoron may provide an opportunity to identify and quantify specific processes. However, since container-grown plants often differ from field-grown plants in size and morphology, considerable care must be exercised in direct extrapolation of greenhouse results to field-grown plants. Never-the-less, a bench-scale chamber can provide useful estimates of the expected changes in isotopic composition within specific ecosystems. This information can be used to guide design and execution of subsequent field experiments,

which are typically more difficult to control and considerably more expensive to conduct.

Data availability statement

The data cannot be made publicly available upon publication because they are owned by a third party and the terms of use prevent public distribution. The data that support the findings of this study are available upon reasonable request from the authors.

Acknowledgments

The authors gratefully acknowledge support from the National Nuclear Security Administration's Office of Defense Nuclear Nonproliferation Research and Development. This work was performed under U.S. Department of Energy contract DE-AC52-06NA25396.

ORCID iD

Kristy L Nowak-Lovato https://orcid.org/0000-0002-1676-4094

References

[1] Both A J, Benjamin L, Franklin J, Holroyd G, Incoll L D, Lefsrud M G and Pitkin G 2015 Guidelines for measuring and reporting environmental parameters for experiments in greenhouses *Plant Methods* **11** 43

[2] Both A J and Wheeler E F 2002 *Rutgers Cooperative Extension, New Jersey Agricultural Experiment Station* vol E276 (New Brunswick: The State University of New Jersey) (available at: http://horteng.envsci.rutgers.edu/factsheets/e276.pdf)

[3] Lee C and Rawlings J O 1982 Design of experiments in growth chambers—uniformity trials in the North Carolina state university phytotron 1 *Crop Sci.* **22** 551

[4] Urey H C 1947 The thermodynamic properties of isotopic substances *J. Chem. Soc.* 562–81 (available at: http://pubs.rsc.org/en/Content/ArticleLanding/1947/JR/jr9470000562)

[5] Bigeleisen J and Mayer M G 1947 Calculation of equilibrium constants for isotopic exchange reactions *J. Chem. Phys.* **15** 261

[6] Wolfsberg M 1969 Isotope effects *Annu. Rev. Phys. Chem.* **20** 449

[7] Jancso G, Rebelo L P N and Van Hook W A 1993 Isotope effects in solution thermodynamics: excess properties in solutions of isotopomers *Chem. Rev.* **93** 2645

[8] Herman M F, Currier R P and Clegg S M 2015 An isotopic mass effect on the intermolecular potential *Chem. Phys. Lett.* **639** 266

[9] Eiler J M *et al* 2014 Frontiers of stable isotope geoscience *Chem. Geol.* **372** 119

[10] Stasinski L, White D M, Nelson P R, Ree R H and Meireles J E 2021 Reading light: leaf spectra capture fine-scale diversity of closely related, hybridizing arctic shrubs *New Phytol.* **232** 2283–94

[11] Zea M, Souza A, Yang Y, Lee L, Nemali K and Hoagland L 2022 Leveraging high-throughput hyperspectral imaging technology to detect cadmium stress in two leafy green crops and accelerate soil remediation efforts *Environ. Pollut.* **292** 118405

[12] Esler M B, Griffith D W T, Wilson S R and Steele L P 2000 Precision trace gas analysis by FT-IR spectroscopy. 2. The $^{13}C/^{12}C$ isotope ratio of CO_2 *Anal. Chem.* **72** 216

[13] Zanasi R, Alfano D, Scarabino C, Motta O, Viglione R G and Proto A 2006 Determination of $^{13}C/^{12}C$ carbon isotope ratio *Anal. Chem.* **78** 3080

[14] Mohn J, Werner R A, Buchmann B and Emmenegger L 2007 High-precision $\delta^{13}CO_2$ analysis by FTIR spectroscopy using a novel calibration strategy *J. Mol. Struct.* **834–836** 95

[15] Western C M and Billinghurst B E 2017 Automatic assignment and fitting of spectra with pgopher *Phys. Chem. Chem. Phys.* **19** 10222

[16] Weiser M E and Brand W A 2000 A 2000 Isotope studies using mass spectrometry *Encyclopedia of Spectroscopy and Spectromentry* (Amsterdam: Elsevier) 1072

[17] Mook W G, Bommerson J C and Staverman W H 1974 Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide *Earth Planet. Sci. Lett.* **22** 169

[18] Vogel J C, Grootes P M and Mook W C 1970 Isotopic fractionation between gaseous and dissolved carbon dioxide *Z. Phys.* **230** 225

[19] Szaran J 1998 Carbon isotope fractionation between dissolved and gaseous carbon dioxide *Chem. Geol.* **150** 331

[20] O'Leary M H 1981 Carbon isotope fractionation in plants *Phytochem.* **20** 553

[21] Crosson E R, Ricci K N, Richman B A, Chilese F C, Owano T G, Provencal R A, Todd M W, Glasser J, Kachanov A A and Paldus B A 2002 Stable isotope ratios using cavity ring-down spectroscopy: determination of $^{13}C/^{12}C$ for carbon dioxide in human breath *Anal. Chem.* **74** 2003

[22] Bitter M, Ball S M, Povey I M and Jones R L 2005 A broadband cavity ringdown spectrometer for *in-situ* measurements of atmospheric trace gases *Atmos. Chem. Phys.* **5** 2547

[23] Bicer A, Bounds J, Zhu F, Kolomenskii A A, Tzortzakis S and Schuessler H A Cavity ring-down spectroscopy for the isotope ratio measurement of methane in ambient air with DFB diode laser near 1.65 um *2017 European Conf. on Lasers and Electro-Optics and European Quantum Electronics Conf.* paper ch, p 39 (Optica Publishing Group)

[24] Lehmuskoski J, Vasama H, Hämäläinen J, Hokkinen J, Kärkelä T, Heiskanen K, Reinikainen M, Rautio S, Hirvelä M and Genoud G 2021 On-line monitoring of radiocarbon emissions in a nuclear facility with cavity ring-down spectroscopy *Anal. Chem.* **93** 16096