Design and Operation of a Continuous $^{13}$C and $^{15}$N Labeling Chamber for Uniform or Differential, Metabolic and Structural, Plant Isotope Labeling

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Abstract

Tracing rare stable isotopes from plant material through the ecosystem provides the most sensitive information about ecosystem processes; from CO$_2$ fluxes and soil organic matter formation to small-scale stable-isotope biomarker probing. Coupling multiple stable isotopes such as $^{13}$C with $^{15}$N, $^{18}$O or $^2$H has the potential to reveal even more information about complex stoichiometric relationships during biogeochemical transformations. Isotope labeled plant material has been used in various studies of litter decomposition and soil organic matter formation. From these and other studies, however, it has become apparent that structural components of plant material behave differently than metabolic components (i.e. leachable low molecular weight compounds) in terms of microbial utilization and long-term carbon storage. The ability to study structural and metabolic components separately provides a powerful new tool for advancing the forefront of ecosystem biogeochemical studies. Here we describe a method for producing $^{13}$C and $^{15}$N labeled plant material that is either uniformly labeled throughout the plant or differentially labeled in structural and metabolic plant components.

Here, we present the construction and operation of a continuous $^{13}$C and $^{15}$N labeling chamber that can be modified to meet various research needs. Uniformly labeled plant material is produced by continuous labeling from seedling to harvest, while differential labeling is achieved by removing the growing plants from the chamber weeks prior to harvest. Representative results from growing Andropogon gerardii Kaw demonstrate the system's ability to efficiently label plant material at the targeted levels. Through this method we have produced plant material with a 4.4 atom% $^{13}$C and 6.7 atom% $^{15}$N uniform plant label, or material that is differentially labeled by up to 1.29 atom% $^{13}$C and 0.56 atom% $^{15}$N in its metabolic and structural components (hot water extractable and hot water residual components, respectively). Challenges lie in maintaining proper temperature, humidity, CO$_2$ concentration, and light levels in an airtight $^{13}$C-CO$_2$ atmosphere for successful plant production. This chamber description represents a useful research tool to effectively produce uniformly or differentially multi-isotope labeled plant material for use in experiments on ecosystem biogeochemical cycling.

Video Link

The video component of this article can be found at http://www.jove.com/video/51117/

Introduction

Understanding the dynamics of plant-soil-atmosphere processes is critical for accurately predicting how the global carbon (C) and nitrogen (N) cycles function under current and future environmental conditions. Stable isotopes are powerful tools in quantitative studies of plant-soil-atmosphere C and N cycling. Tracing rare stable isotopes from plant material through the ecosystem provides sensitive information in studies of biogeochemical cycling, from CO$_2$ fluxes and soil organic matter formation to small-scale stable-isotope biomarker probing. Combining $^{13}$C labeling with $^{15}$N labeling, or other stable isotopes such as $^2$H or $^{18}$O in plant tissue provides a high-detection, traceable, yet complex substrate for use in coupled studies of plant and soil biochemistry. The ability to uniformly or differentially label structural and metabolic plant material adds further ability to address complex questions about C and N cycling through ecosystems. The benefit of using isotope labeled plant material in quantitative studies of C and N accounting, however, depends on the ability to produce $^{13}$C and $^{15}$N labeled material that is either uniformly or differentially labeled.

Isotope labeling has been used in studies addressing plant C and N assimilation, allocation and rhizodeposition. Uniformly $^{13}$C and $^{15}$N labeled plant material provides a complex labeled substrate for studies of litter decomposition, soil organic matter formation, soil CO$_2$ emissions, soil food web studies, and studies of soil C residence times. Studies utilizing $^{13}$C labeled biochar from labeled plant material
are also beginning to reveal new information about formerly overlooked soil char pools\(^\text{15}\). While \(^{15}\text{N}\), \(^2\text{H}\), and \(^{18}\text{O}\) labeling are relatively easy to achieve through water and fertilizer treatment, the challenge exists in producing uniformly \(^{13}\text{C}\) labeled plant material through \(^{13}\text{C}-\text{CO}_2\) fixation.

Continuous isotope labeling from seedling to maturity in a sealed chamber produces uniform isotope labeling throughout the plant. Other methods such as repeated pulse labeling\(^\text{16}\) and foliar application or wicking\(^\text{17,18}\) do not produce uniformly isotope labeled plant material, nor clear differential labeling of specific C-compounds (e.g. metabolic vs. structural)\(^\text{19}\). An important consideration in isotope labeling is labeling efficiency, due to the high cost of rare isotope enriched compounds used in labeling. Although continuous \(^{13}\text{C}\) labeling has been used in the past\(^\text{2,4,20}\), there is not to our knowledge a published detailed technical description of a continuous labeling chamber with evidence of high labeling efficiency and accurate control of the amount and uniformity of isotope labeling.

On the forefront of litter decomposition and soil organic matter formation research is the concept that metabolic plant material (i.e. leachable, labile, low molecular weight compounds) and structural plant material (i.e. lignin, cellulose, hemicellulose) are processed differently in terms of microbial use efficiency, soil organic matter formation, and long term soil C storage\(^\text{5-7}\). Plant material that is differentially labeled in its structural and metabolic components, therefore, is a useful tool in advancing litter decomposition and soil organic matter formation research. Differential labeling with dual isotopes allows for the tracing of structural and metabolic components separately through the ecosystem using a multiple-pool isotope technique\(^\text{21}\).

Continuous isotope labeling with \(^{13}\text{C}\) and other isotopes in a sealed chamber requires careful attention to plant physiological conditions to maximize plant productivity and isotope labeling efficiency. Daytime temperature spikes must be controlled to prevent plant damage when growing in an airtight chamber. An optimal range of humidity and temperature are required to maintain open plant stomata and \(^{13}\text{C}\) uptake\(^\text{15}\). High levels of humidity cause fogging of the chamber walls, which minimizes light availability and may damage the chamber structure. Careful consideration to isotope labeling efficiency by eliminating natural abundance isotopes from the chamber (e.g. coming from potting with soil organic matter) and preventing exposure to external air is important when working with expensive heavy-isotope labeled compounds.

Here, we present a method for building and operating a continuous dual \(^{13}\text{C}\) and \(^{15}\text{N}\) isotope labeling chamber for the production of plant material that is either uniformly labeled or has its structural and metabolic components labeled at distinct levels. \(^{13}\text{C}\) labeling is controlled at the chamber level, while fertilization and \(^{15}\text{N}\) labeling is controlled at the individual pot level. Representative results are shown to demonstrate the ability of this method to control temperature, humidity, and \(^{13}\text{C}\) concentration throughout the growing season. Results from growing *Andropogon gerardii*, Kaw also demonstrate this method’s ability to produce uniformly or differentially labeled plant material. The specific chamber design and operation scheme described can be modified to grow different plant species, as well as to accommodate \(^2\text{H}\) or \(^{18}\text{O}\) labeling.

### Protocol

#### 1. Chamber Construction

1. Construct the labeling chamber in a greenhouse to allow for maximum natural light potential for plant growth. Make sure that adequate power supply is available to power all chamber components.

2. Construct the labeling chamber by mounting 3.175 mm thick transparent acrylic walls (polycarbonate would also be suitable) and a 6.35 mm thick transparent acrylic ceiling on an aluminum frame with a white-painted steel floor to maximize solar reflectance. The dimensions of the chamber can be tailored to suit individual research needs.

3. Mount the chamber on \(\frac{3}{4}\) in (19 mm) plywood on cinder blocks.

4. Drill holes in the acrylic glass, aluminum frame and steel floor and use screws to fasten all components together.

5. Seal all seams with silicone caulk to insure an airtight seal.

6. Construct a door by mounting one section of the acrylic paneling on long screws, which can be screwed down using removable wing nuts.

7. Seal the door with weather stripping to prevent air leakage.

8. Select an area directly adjacent to the chamber as the control center to mount all temperature, humidity, \(^{13}\text{C}\) controls, and monitoring equipment.

9. Carefully drill small holes in the chamber wall adjacent to the control center for all electrical wires and gas tubing. Use silicone caulk to seal the holes around all wires and tubing to prevent air leakage.

10. Test the chamber for air leakage by filling it with a high level of \(^{13}\text{C}\) (e.g. 800 ppm) and letting it sit overnight. If the \(^{13}\text{C}\) concentration in the chamber is maintained at its original level then it is airtight. If the concentration drops overnight then all seams should be examined and resealed with silicone caulk until an airtight seal is achieved.

11. For some plants adapted to high light conditions, add lights connected to a timer to the immediate exterior of the chamber to increase light penetration and plant productivity.

#### 2. Temperature and Humidity Controls

1. Regulate chamber temperature by installing a commercial split type air conditioner with the cooling (evaporator) coils located inside the chamber and the compressor and condenser coils located outside the greenhouse to dissipate the heat. Set the air conditioner to maintain the desired temperature.

2. Use a small room dehumidifier to control humidity in the chamber.
   1. Drill a drainage hole through the floor of the chamber adjacent to the dehumidifier.
   2. Remove the condensate collector from the dehumidifier, and connect a drainage tube from the dehumidifier through the drainage hole in the floor of the chamber.
   3. Underneath the chamber, place an open jar filled with water for the dehumidifier to drain directly into. This creates an airtight seal but also allows for pressure equilibration.

3. Install a controller, such as the Omega iSeries controller, in the control center with a humidity sensor inside the chamber.
1. Connect the controller to the dehumidifying system with a solid-state relay and set the humidity controller with a high alarm and swing to maintain optimal growing conditions in the chamber. Optimal temperature and humidity conditions will differ for different plant species.

3. CO₂ Controls

1. The 13C-CO₂ enrichment is achieved using two pure CO₂ gas tanks, one of 10 atom% 13C-CO₂ or higher and one of 1.1 atom% 13C-CO₂ (natural abundance).
2. Monitor CO₂ concentration by having a diaphragm pump continuously draw chamber air through an Infrared Gas Analyzer (IRGA) and then pump the air back into the chamber, thus maintaining a closed system (Figure 1).
3. Set a low alarm and dead band on the IRGA software to maintain CO₂ concentrations within a desired range. Here, we use a low alarm of 360 ppm with a 40 ppm dead band to maintain CO₂ concentrations between 360-400 ppm.
4. Connect a metering valve to each tank and carefully adjust them to achieve the target 13C enrichment level. Set the tank regulators to 20 psi.
5. Insert a solenoid valve between the metering valve and the regulator of each tank. Join the outlets of the two metering valves together and pipe them into the center of the chamber. Wire a solid-state relay to the IRGA output to control the solenoid valves (Figure 1).

4. Web-based Remote Monitoring System

1. Monitor CO₂ concentrations from the IRGA software by logging it to a local file once every 30 sec.
2. Create a custom utility (e.g. in perl or another programming language) to pick up entries from the local CO₂ logging file, along with the current laptop timestamp, and upload them to a back-end web application.
3. Set the web application to query the temperature and humidity sensor data.
4. Use a monitoring system to check the status of the temperature in the chamber every five minutes to prevent potential temperature spikes that would destroy the plants if the air conditioning system failed.
5. Monitor the CO₂, temperature and humidity data on any standard web browser so that any unexpected temperature spikes or CO₂ drops can be immediately attended to.

5. Irrigation System

1. Drill one small hole in the wall of the acrylic glass chamber per pot.
2. Use irrigation tubing to create one drip irrigation ring per pot and feed the irrigation tubing through the chamber wall to the exterior.
3. Seal the holes around the irrigation tubing with silicone caulk to prevent air leakage.
4. On the exterior of the chamber, connect the irrigation tubing to the tubing for a peristaltic pump.
5. Use a small hose clamp to close off all irrigation tubing to prevent air leakage between watering.

6. Potting Plants

1. Select a pot size appropriate for the plants being grown. Here, 40, 15 L pots are used.
2. Create a soil-free potting mix by mixing sand, vermiculite, and profile porous ceramic.
3. Test the water holding capacity of the potting mix by weighing a filled pot dry, soaking the pot with water and allowing it to drain completely, and weighing the pot wet. Use this maximum water holding capacity to ensure that excess labeled fertilizer and water does not leak from the pots during watering.
4. Germinate seeds in potting soil prior to planting them in the pots. This ensures that only successfully germinated seeds are started in the labeling chamber.
5. Inoculate the seedlings with fresh soil slurry to introduce beneficial microbes.
6. Once the seeds have germinated, carefully transplant seedlings to the pots in the desired number.
7. Once potted, move the pots into the chamber and assemble each pot with an individual irrigation hose.

7. Sealing the Chamber

1. When first sealing the door to the chamber a large mass of external air fills the chamber space. Scrub out this external CO₂ by connecting a soda lime scrubber to the air pump to scrub the CO₂ concentration down to at least 200-250 ppm before filling the chamber back up to 400 ppm using the 13C-CO₂ tank mixture.
2. Try to keep the chamber closed through the duration of the growing season to minimize natural abundance CO₂ contamination.
3. Monitor plant growth visually and adjust fertilization and irrigation according to demand.

8. Fertilization and Irrigation

1. Use a fertilizer solution, such as a modified Hoagland's solution, to fertilize the plants through the irrigation system.
2. Label the fertilizer with 15N by using a 15N-KNO₃ subsolution at the targeted atom% 15N level by mixing 98 atom% 15N-KNO₃ with natural abundance 15N-KNO₃ (0.37 atom% 15N).
3. Mix up enough of the fertilizer solution upon each fertilization event for the entire chamber, based on the water holding capacity of the potting mix. Place the proper amount of fertilizer for one pot in a glass jar, and prepare as many jars there are pots.
4. Unclamp the irrigation hoses and place each of them in a jar with the fertilizer solution, and then connect them to the peristaltic pump.
5. Water plants by pumping water through the peristaltic pump through the individual drip irrigation hoses regularly as the plants need it.
6. Fertilization with Hoagland's solution should follow the plant demand or experimental design, with increasing nutrient demand as the plant productivity increases to maximize productivity.
7. First, pump the Hoagland's solution through the irrigation hose, then pump a water rinse through to minimize algal and bacterial growth in the tubes.
8. Reclamp all hoses after fertilization and irrigation to eliminate chamber air leakage.

9. Uniform and Differential Labeling

1. For differential labeling of structural and metabolic components, remove plants from labeling chamber 1-3 weeks prior to harvest. Plants that are to be uniformly labeled can remain in the sealed labeling chamber continuously until harvest and irrigated with $^{15}$N-Hoagland's solution.
2. Keep the removed plants in the greenhouse during this time so that they receive adequate light and CO$_2$ at natural abundance $^{13}$C.
3. For differential $^{15}$N labeling, continue to fertilize and irrigate the plants as usual, but use natural abundance $^{15}$N-KNO$_3$ in the Hoagland's fertilizer solution.

10. Harvest

1. Stop watering plants 1 week prior to harvest so plants begin to senesce and potting medium dries out.
2. Open the chamber and move the pots out for immediate clipping and harvest of the aboveground biomass.
3. Pour out the potting mix and roots over a coarse screen.
4. Use the screen to separate out the roots from the potting mix and shake the roots free of potting mix.
5. Place the roots on a 2 mm sieve and rinse them with water to remove any remaining potting material. Use tweezers to remove any vermiculite that may cling to the roots.
6. Allow roots to air-dry in preparation for future experiments.

11. Litter Chemistry

1. Weigh air-dry plant material to determine labeling chamber biomass.
2. Grind a subsample for chemical analysis.
3. Place 2.0 g of oven dried (60 °C) litter in a 125 ml acid washed flask and add 50 ml of deionized water.
4. Place the sample on a preheated (60 °C) stirrer plate and place a stirrer bar in the flask. Set the stirring for 200 rpm and allow the sample to heat for 30 min.
5. After 30 min, filter the litter solution through a 20 μm Nylon mesh on a vacuum filtration system.
6. Transfer the extract to a preweighed acid washed tube and freeze.
7. Transfer the solid litter residue to a preweighed aluminum pan and dry at 60 °C. Weigh pan and litter after drying to determine hot water residue mass.
8. Freeze dry the hot water extract and weigh to determine the hot-water extract mass.
9. Analyze oven-dried (60 °C) litter, freeze-dried hot water extract, and oven-dried hot water residue in an Elemental Analyzer - Isotope Ratio Mass Spectrometer (EA-IRMS).

**Representative Results**

Our labeling chamber is 1.2 m x 2.4 m x 3.6 m in size and holds 40,15 L pots (Figure 1). The computerized IRGA control system maintained CO$_2$ concentrations between our set values of 360 and 400 ppm during the photosynthetically active period of the day (Figure 2a). The low CO$_2$ alarm feature on the IRGA triggered solenoid valves to allow CO$_2$ from the $^{13}$C enriched and natural abundance tanks into the chamber when the concentration dropped below the minimum threshold (e.g. 360 ppm). The dead band feature stopped the flow when the concentration reached the upper set point (e.g. 400 ppm). The iSeries temperature and humidity monitoring system connected to the air conditioner and dehumidifier held climate conditions within the set parameters throughout the growing season (Figure 2b). We used a one-ton (3.5 kW) air conditioning unit to keep the chamber cool.

The remote monitoring system allowed the logged data to be viewed at any time by a standard web browser. The CO$_2$ concentrations, temperature and humidity values were down sampled by the web application to display graphs over the past 24-240 hr, in 24 hr increments. This created a quick visual to confirm that the daily fluctuations were within the expected limits. Viewing the web interface also showed the current chamber status, as well as provided alerts to potential problems such as not receiving recent data. At any time the complete dataset could also be downloaded from the web interface.

We measured photosynthetically active radiation (PAR) in the immediate interior and exterior of the chamber at four points with and without the lights on in the middle of the summer and the middle of the day using a quantum sensor. The PAR in the chamber was 31.5% lower than the exterior when the chamber lights were on in the middle of the summer and the middle of the day using a quantum sensor. The PAR in the chamber was 31.5% lower than the exterior when the chamber lights were off and 22% lower than the exterior when the lights were on. Thus, the chamber lights help to significantly increase PAR penetration within the chamber by 9.5% ($P<0.05$).

Our continuous labeling system was able to produce 2759 g of A. gerardi biomass, 37% of which was aboveground biomass and 63% of which was belowground biomass. We achieved a 4.4 atom%$^{13}$C whole plant label in our uniform plant material by setting the solenoid valves on the two CO$_2$ tanks accordingly (Figure 1, Table 1). We achieved a 6.7 atom%$^{15}$N whole plant label in our uniform plant material by mixing 98 atom %$^{15}$N-KNO$_3$ with 0.37 atom%$^{15}$N-KNO$_3$ in the KNO$_3$ subslution of a modified Hoagland's solution$^{22}$ (Table 1). We watered the A. gerardi weekly with 750 ml total fluid (water plus Hoagland's solution) throughout the growing season. We fertilized with 200-500 ml of $^{15}$N labeled Hoagland's solution per week depending on plant productivity.
We utilized the hot water extraction method to determine if there were isotopic differences between the uniform and differentially labeled plant material. For the differentially labeled plants, upon harvest we removed any leaves that were completely dead and handled these separately as they were likely not differentially labeled. When looking at $^{13}\text{C}$ content, all four incorporation days were significantly different from each other for the whole plant and the hot water extract, but for the hot water residue day 14 and 22 were not significantly different from each other (Table 1). When comparing the plant tissue fractions within each day, the hot water extract and residue were significantly different from each other for all four days and by day 22 the whole plant, extract, and residue were all significantly different from each other (Table 1). For the $^{15}\text{N}$ incorporation into plant components, there were differences between days of incorporation and plant tissue fractions. For the hot water extract all four of the incorporation days were significantly different from each other for $^{15}\text{N}$, and for the whole plant and the hot water residue the shorter days of incorporation were significantly different than the longer days of incorporation (Table 1). The plant tissue fractions in the uniform plants were not significantly different from each other in $^{15}\text{N}$, but the hot water extract and residue were significantly different from each other in $^{15}\text{N}$ for the differentially labeled litter.

All isotopic values are reported using the atom percent (atom %) notation (Equation 1), which is a more accurate notation than % to use at high levels of heavy isotope enrichment\textsuperscript{21}. For example:

\begin{equation}
\text{Atom \%}^{13}\text{C} = \frac{^{13}\text{C}}{^{13}\text{C} + ^{12}\text{C}} \times 100
\end{equation}

For this study, we ran statistical analyses using SAS version 9.2. We tested differences between the chamber interior and exterior light levels using a paired t-test. We tested differences between $^{13}\text{C}$ and $^{15}\text{N}$ labeling of hot water extracts and hot water residues using one-way analysis of variance (ANOVA) in PROC ANOVA. We used Duncan's multiple range test for multiple comparisons analysis. Significance was accepted at a P-level of 0.05. We used a Wilcoxon rank sum test to test that the data met the assumptions of the analysis.

Figure 1. Schematic diagram of the 40 pot capacity continuous multi-isotope labeling chamber from a bird's eye view. Dotted lines represent electrical wiring, while solid lines represent gas or water tubing. Click here to view larger image.
Figure 2. A) Average CO\(_2\) concentration (ppm) (+/− SE) over a twenty-four hour period for an entire growing season. B) Average temperature (ºC), open circles, and humidity (%), closed circles (+/− SE) over a twenty-four hour period for an entire growing season. Click here to view larger image.

Table 1. Isotopic composition and litter chemistry for uniform and differentially labeled litter. Days of differential labeling outside the chamber are in parentheses. Comparisons between days of incorporation are in capital letters (across rows) and between litter fractions are in lower case letters (down columns) for each variable.

|                        | Uniform (0) | Differential (7) | Differential (14) | Differential (22) |
|------------------------|-------------|------------------|-------------------|-------------------|
| **Whole Litter**       |             |                  |                   |                   |
| \(^{13}\)C Atom %      | 4.46±0.02 Aab | 3.93±0.05 Ba     | 3.64±0.03 Ca      | 3.35±0.06 Db      |
| \(^{15}\)N Atom %      | 6.69±0.07 Aa | 6.72±0.01 Aa     | 6.33±0.06 Ba      | 6.41±0.07 Ba      |
| **Hot Water Extract**  |             |                  |                   |                   |
| \(^{13}\)C Atom %      | 4.59±0.04 Aa | 3.35±0.06 Bb     | 2.79±0.06 Cb      | 2.37±0.03 Dc      |
| \(^{15}\)N Atom %      | 6.69±0.03 Aa | 6.43±0.01 Bb     | 5.89±0.07 Db      | 6.16±0.05 Cb      |
| **Hot Water Residue**  |             |                  |                   |                   |
| \(^{13}\)C Atom %      | 4.37±0.06 Ab | 4.1±0.03 Ba      | 3.79±0.10 Ca      | 3.66±0.05 Ca      |
| \(^{15}\)N Atom %      | 6.57±0.04 Ba | 6.71±0.02 Aa     | 6.45±0.02 Ca      | 6.44±0.03 Ca      |

Discussion

This design for a continuous isotope labeling chamber was used to produce uniformly and differentially \(^{13}\)C and \(^{15}\)N labeled *A. gerardii* for subsequent field and laboratory experiments. During three growing seasons of operation, the chamber has successfully maintained temperature, humidity, and CO\(_2\) concentrations within the set parameters (Figure 2). The reliability of the temperature control system is critical during the peak...
of the summer when high solar radiation can cause overheating in the air-tight chamber. Eliminating excess humidity caused by transpiration from the growing plants ensures that plant stomata remain open for photosynthetic uptake and that water condensation does not inhibit light penetration or damage the structure of the chamber.

The near continuous monitoring of CO₂ concentration by the IRGA software maintained continuous ¹³C labeling of the plants while they were growing in the chamber. Due to the high photosynthetic activity of A. gerardii growing in this chamber, CO₂ was injected into the system frequently during daylight hours of the peak growing season when photosynthetic activity drew CO₂ concentrations down to 360 ppm, approximately every 15-20 min. The metering of the enriched and natural abundance ¹³C-¹²C CO₂ tanks allowed for a controlled 4.4 atom% ¹³C atmosphere through the growing season for uniform plant tissue labeling. ¹³C-¹²C CO₂ production can also be achieved by mixing ¹³C-sodium bicarbonate or ¹³C-sodium carbonate and hydrochloric acid, however this type of system is more complicated and requires more monitoring and maintenance, so we recommend using ¹³C-¹²C CO₂ gas. An important consideration for monitoring CO₂ concentrations using an IRGA is that infrared analyzers lose two thirds of their sensitivity when measuring ¹³C-¹²C CO₂. This underestimation of approximately 2.9% ppm for our 4.4 atom% ¹³C-¹²C CO₂ mixture was not of great concern to us, but could become a more significant issue when labeling at higher ¹³C levels.

A. gerardii is a warm season perennial tallgrass prairie graminoid species. The design of this chamber was optimized for A. gerardii production (Figure 1). The size and height of the chamber were chosen in consideration for the maximum height productivity of A. gerardii in the field, as well as for the desired plant biomass production for future experiments. A. gerardii is known to be light limited in the field. PAR within a greenhouse can be diminished by 30-47% as compared to exterior levels. Since our plants were grown in an acrylic glass chamber inside a greenhouse, PAR limitation was a concern. When turned on, the fluorescent lights increased PAR within the chamber by 9.5%, which may have helped to increase productivity in this light sensitive species. When using this chamber design to grow other types of plants specific physiological needs such as size, lighting, nutrient demands, temperature sensitivity, and soil moisture should be carefully tailored to maintain optimal growing conditions for the plants.

When working with expensive isotopically labeled compounds, such as 10 atom% ¹³C-¹²C CO₂ and 98 atom% ¹⁵N-KNO₃, efficiency of labeling is an important consideration. This chamber design optimizes ¹³C labeling by making all efforts to seal the chamber and minimize air leakage into and out of the chamber. If this chamber is never opened during the growing season, then none of the ¹³C labeled CO₂ from the chamber is leaked out to the atmosphere. The CO₂ build up during nighttime respiration does not appear to damage the growing plants and is quickly taken up after sunrise (Figure 2). During differential labeling, the chamber was briefly opened to remove the differentially labeled pots but this did not appear to dilute the targeted 4.4 atom% ¹³C labeling of the continuously labeled plants (Table 1). ¹³C labeling was also optimized by scrubbing out the initial atmospheric air trapped in the chamber upon sealing. During preliminary tests on the chamber without an initial scrub of atmospheric CO₂, plants were measured to have a diluted ¹³C level in the first leaves produced than in the later leaves produced. The initial scrub of atmospheric CO₂ upon chamber closure appears to eliminate this issue by allowing for continuous ¹³C labeling from seedling to maturity. Maintaining a soil-free potting mixture of sand, vermiculite, and clay also eliminates natural abundance CO₂ contamination from soil respiration. The elimination of soil from the system does require careful fertilization and inoculation considerations, which may be unique to different plant species. ¹⁵N labeling through the targeted 7 atom% ¹⁵N Hoegland's solution produced highly labeled plant material at 6.7 atom% ¹⁵N (Table 1). A slight dilution from the targeted ¹⁵N label may be caused by some natural abundance N in the potting mix or from the native soil inoculation.

During biosynthesis of compounds, natural discrimination of ¹³C (or ¹⁵N) occurs as a result of kinetic fractionation and nonstatistical isotope distribution in the synthesized compounds. Thus, in the case of C, secondary products (e.g. lipids, phenol compounds) are generally depleted in ¹³C as compared to primary products (carbohydrates). This natural ¹³C discrimination appears to persist also when plants are grown in an enriched ¹³C atmosphere, as can be seen in the slight difference in atom% ¹³C of the hot water extracts and hot water residues of the uniformly labeled plants (Table 1). This natural kinetic fractionation is very small compared to the enrichment and does not compromise the uniformity of the labeling.

Differential labeling of structural and metabolic plant tissues is a novel technique with potential for advanced studies in litter decomposition, microbial ecology and soil organic matter formation. The difference in ¹³C and ¹⁵N of the hot water extracts and hot water residues indicates a significant dilution of ¹³C and ¹⁵N in the leachable, low molecular weight compounds (hot water extracts) from the structural plant material (hot water residues) after 7, 14, and 22 days of differential labeling (P<0.005). This differential labeling of plant tissues can be used to track the fate of structural and metabolic components separately through an ecosystem. The ¹³C differential labeling was more extreme than ¹⁵N differential labeling. This may be due to the immediacy of ¹³C dilution when removing the plants from the ¹³C-¹²C CO₂ labeled atmosphere, while the ¹⁵N label still remains in the potting mix for some time and ¹⁵N dilution occurs more slowly. For more drastic differential labeling of ¹⁵N, one may consider flushing the pots with water prior to natural abundance fertilization during the final weeks of growth outside the chamber.

The design and operation of this continuous ¹³C and ¹⁵N labeling system for uniform or differential, metabolic and structural, plant tissue labeling provides a novel method for producing isotopically labeled plant material for advanced research. The design and operational details of this chamber have been chosen for 4.4 atom% ¹³C and 7 atom% ¹⁵N labeling of A. gerardii, but can be tailored to other plant types and isotope labeling levels. The growing conditions described here should be tailored to suit the size, temperature, humidity, light, water and nutrient demands of the particular plant species of interest. Labeling with ¹⁸O or ²H can also be achieved by labeling the water used in the irrigation system. The system described here addresses many of the challenges of uniform and differential ¹³C labeling of plant material. This basic chamber design can be used by other research groups to produce highly labeled plant material for advanced studies in ecosystem biogeochemistry.

**Disclosures**

The authors have nothing to disclose.
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