

Theoretical Proof and Empirical Confirmation of a Continuous Labeling Method Using Naturally ^{13}C -Depleted Carbon Dioxide

Weixin Cheng* and Feike A. Dijkstra

(Environmental Studies, University of California, Santa Cruz, CA 95064, USA)

Abstract

Continuous isotope labeling and tracing is often needed to study the transformation, movement, and allocation of carbon in plant-soil systems. However, existing labeling methods have numerous limitations. The present study introduces a new continuous labeling method using naturally ^{13}C -depleted CO_2 . We theoretically proved that a stable level of ^{13}C - CO_2 abundance in a labeling chamber can be maintained by controlling the rate of CO_2 -free air injection and the rate of ambient airflow with coupling of automatic control of CO_2 concentration using a CO_2 analyzer. The theoretical results were tested and confirmed in a 54 day experiment in a plant growth chamber. This new continuous labeling method avoids the use of radioactive ^{14}C or expensive ^{13}C -enriched CO_2 required by existing methods and therefore eliminates issues of radiation safety or unaffordable isotope cost, as well as creating new opportunities for short- or long-term labeling experiments under a controlled environment.

Key words: carbon flux; CO_2 ; isotope; rhizosphere; tracers.

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Carbon, as the central element in all life forms, has been studied across a range of scales from simple molecules to global biogeochemical cycles. In order to understand the transformation and movement of carbon or carbon compounds through various stages of life in terrestrial ecosystems, many carbon isotope labeling methods have been developed and applied (Coleman and Fry 1991). Through pulse labeling of plants using ^{14}C , a radioactive isotope, many intricate processes of

carbon transformation in plant-soil systems have been deciphered. For example, $^{14}\text{CO}_2$ pulse labeling has been used widely to study the short-term transfer of plant photo-assimilates from leaves to other parts of the plants and into the rhizosphere and surrounding soils (e.g. Kuzyakov et al. 1999; Warembourg and Estelrich 2000). For long-term and quantitative investigation of such transfer, continuous ^{14}C -labeling has been used (e.g. Barber and Martin 1976; Whipps and Lynch 1983). However, both of these ^{14}C -labeling methods have critical limitations. Pulse ^{14}C -labeling is only suitable for short-term investigation of mostly non-quantitative measures. Continuous ^{14}C -labeling requires special facilities that are limited to a few places in the world. It often requires transplanting of seedlings, which may have considerable unlabeled food reserves, and it may take some time for all plant parts to become evenly labeled (Lynch and Whipps 1990). Because of safety concerns due to the use of radioactive materials, accessibility to ^{14}C continuous labeling experiments is often limited and therefore this method is mostly applied to experiments of short duration, 1 or 2 months at most.

To avoid radioactivity from ^{14}C , ^{13}C -enriched CO_2 has been used to replace $^{14}\text{CO}_2$ (e.g. Yamagata et al. 1987; Evdokimov et al. 2004). However, the extremely high cost of the ^{13}C -isotope

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*Author for correspondence.

Tel: +1 831 459 5317;

Fax: +1 831 459 4015;

E-mail: <wxcheng@ucsc.edu>.

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source (approximately US\$100/L of 98% enriched ^{13}C CO₂; Cambridge Isotope Laboratories) often confines the size of the experiment and the labeling duration. Because of the cost issue associated with the use of ^{13}C -enriched CO₂, this approach has not been used widely.

Without actual labeling, a ^{13}C natural tracer method has been used in recent studies for tracing carbon of current plant photosynthesis separately from carbon derived from the soil (Cheng 1996; Qian et al. 1997; Rochette and Flanagan 1997). This natural tracer method eliminates some of the major limitations of earlier labeling methods. Although progress has been made by using the ^{13}C natural tracer method (Cheng and Kuzyakov 2005), this method also has some limitations. One major limitation is the required differential in ^{13}C natural abundance between the SOM-derived C and the plant-derived C, which means that this method can only be used in two types of plant-soil couplings: (i) C₃ plants grown in soils developed under C₄ plant-dominated vegetation (or C₄-soils); or (ii) C₄ plants grown in soils developed under C₃ plant-dominated vegetation (or C₃-soils). Therefore, this method cannot be applied to the plant-soil couplings of C₃ plants in "C₃ soils" or C₄ plants in "C₄-soils". The natural ^{13}C tracer method relies on an assumption that the switched plant-soil couplings do not significantly alter the measured results. This assumption is not always valid because different soil types significantly affect root respiration of the same plant species (Cheng et al. 2005).

Naturally ^{13}C -depleted CO₂ has been used as a tracer for experiments under elevated CO₂ treatments (e.g. Andrews et al. 1999; Haile-Mariam et al. 2000). However, the ^{13}C -depleted tracers only occur in the elevated CO₂ treatment, not in the

ambient CO₂ treatment. Therefore, the results only apply to systems under the elevated CO₂ condition.

In the present paper, we introduce a continuous labeling method using naturally ^{13}C -depleted CO₂ under any desirable CO₂ concentrations in a growth chamber. In the following sections we will deliberate the theoretical foundation of the new methods, show experimental results that validate the calculated outcomes, and discuss the potentials and limitations of the method.

Theoretical Foundation

As shown in Figure 1, a plant growth chamber has a certain effective air volume of V (Liters), a CO₂-free air injection rate of F_i (L/min), an ambient air injection rate of F_a (L/min) with a CO₂ concentration of C_a ($\mu\text{L/L}$) and a known $\delta^{13}\text{C}$ value of δa (‰), an air output rate of F_o (L/min) with a CO₂ concentration of C_o ($\mu\text{L/L}$) and a $\delta^{13}\text{C}$ value of δo (‰); Q_i ($\mu\text{L CO}_2/\text{min}$) is the rate of pure CO₂ injection from a high-pressure tank with a known $\delta^{13}\text{C}$ value of δi ; V is the total effective volume of the growth chamber; C_c is the CO₂ concentration of the air inside the growth chamber; δc is the $\delta^{13}\text{C}$ value of the CO₂-C inside the growth chamber; Q_n ($\mu\text{L CO}_2/\text{min}$) is the net assimilation rate of all plants in the chamber with a $\delta^{13}\text{C}$ value of δn ; R_t ($\mu\text{L CO}_2/\text{min}$) is the total respiration rate with a $\delta^{13}\text{C}$ value of δr ; Q_a ($\mu\text{L CO}_2/\text{min}$) equals F_a multiplied by C_a ; Q_o ($\mu\text{L CO}_2/\text{min}$) equals F_o multiplied by C_o ; and Q_c ($\mu\text{L CO}_2$) is the amount of CO₂ inside the growth chamber, which equals V multiplied by C_c . The CO₂ concentration inside the chamber is controlled by automatic

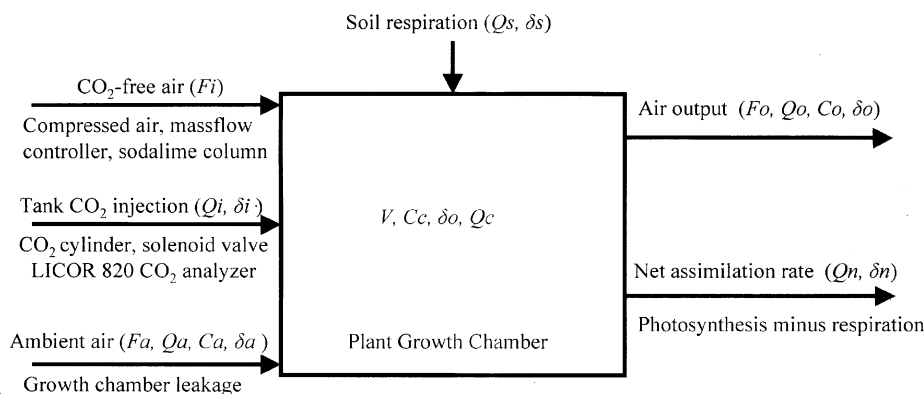


Figure 1. Designation and controls of parameters and variables in a theoretical plant labeling experiment.

F_i , airflow rate (L/min) of CO₂-free air injection; Q_i , quantity of pure CO₂ injection from a high-pressure tank with a known $\delta^{13}\text{C}$ value of δi ; F_a , airflow rate of ambient airflow with a CO₂ concentration of C_a and a known $\delta^{13}\text{C}$ value of δa ; F_o , airflow rate of the total air output with a CO₂ concentration of C_o and a $\delta^{13}\text{C}$ value of δo ; V , total effective volume of the growth chamber; C_c , CO₂ concentration of the air in inside the growth chamber; δc , $\delta^{13}\text{C}$ value of the CO₂-C in side the growth chamber; Q_n , net assimilation rate of all plants in the chamber with a $\delta^{13}\text{C}$ value of δn ; Q_s , soil respiration rate with a $\delta^{13}\text{C}$ value of δs . The CO₂ concentration inside the chamber is controlled by automatic injection of ^{13}C -depleted tank CO₂ using a solenoid valve activated or deactivated by a CO₂ analyzer.

injection of ¹³C-depleted tank CO₂ using a solenoid valve activated or deactivated by a CO₂ analyzer. The δ¹³C value inside the chamber is controlled indirectly by the rate of CO₂-free air in conjunction with injection of tank CO₂. In the following sections, we will show how we can logically control the δ¹³C value inside the chamber by manipulating the rate of CO₂-free air injection.

Balancing the carbon inside the growth chamber, we have:

$$dQc/dt = Qa + Qi + Qs - Qn - Qo \quad (1)$$

If we ignore soil respiration because soil respiration rate is often very low compared with other rates (see Discussion) and set adequate airflow rates so that the system can reach its equilibrium within a desirable period (for the implications of this setting, please refer to the Discussion), at equilibrium, eqn 1 becomes:

$$dQc/dt = Qa + Qi - Qn - Qo = 0 \quad (2)$$

$$Qo = Qa + Qi - Qn \quad (3)$$

and

$$Qi = Qo + Qn - Qa \quad (4)$$

Balancing the ¹³C, we have:

$$Qc(d\delta c/dt) = \delta a Qa + \delta i Qi - \delta n Qn - \delta o Qo \quad (5)$$

where δa, δi, δn, and δo are δ values for Qa, Qi, Qn, and Qo, respectively. Substituting Qi in eqn 5 with Qi = Qo + Qn - Qa, gives:

$$Qc(d\delta c/dt) = \delta a Qa + \delta i (Qo + Qn - Qa) - \delta n Qn - \delta o Qo \quad (6)$$

After reconfiguring, eqn 6 becomes:

$$Qc(d\delta c/dt) = \delta a Qa + \delta i Qo - \delta i Qa - \delta o Qo + (\delta i - \delta n) Qn \quad (7)$$

Equation 7 shows that the necessary condition for dδc/dt to be independent of Qn is δn = δi, so that the term (δi - δn)Qn = 0. If δn > δi, δc decreases as Qn increases until δc approaches a level that makes δn = δi, because δn (the δ value of the net plant production) also decreases if δc (the δ value of CO₂ inside the chamber) decreases. Similarly, if δn < δi, δc increases as Qn increases until δc approaches a level that makes δn = δi. This is illustrated in Figure 2 using simulation results. This means that the system converges towards δn = δi. Therefore, we conclude that δn = δi is our primary controlling goal; that is, we want to control the system so that the δ value of the net plant production equals the δ value of the tank CO₂.

Now we need to know how to reach the controlling goal of δn = δi. If we rearrange eqn 7, it becomes:

$$Qc(d\delta c/dt) = (\delta a - \delta i) Qa - (\delta o - \delta i) Qo + (\delta i - \delta n) Qn \quad (8)$$

When eqn 8 is at equilibrium, the condition for δn = δi is:

$$(\delta a - \delta i) Qa - (\delta o - \delta i) Qo = 0 \text{ or } (\delta a - \delta i) Qa = (\delta o - \delta i) Qo \quad (9)$$

Because Qo = FoCo, Fo = Fi + Fa and Qa = FaCa, eqn 9 becomes:

$$(\delta a - \delta i) Fa Ca = (\delta o - \delta i) (Fi Co + Fa Co) \quad (10)$$

By definition (Farquhar et al. 1989),

$$\Delta = (\delta o - \delta n) / (1 + \delta n / 1000) \quad (11)$$

where Δ is the so-called isotope discrimination factor (unit: ‰). The second term in the denominator of eqn 11 is quite small and is often neglected. So, eqn 11 can be approximated by:

$$\Delta = \delta o - \delta n; \text{ or } \Delta = \delta o - \delta i \text{ because } \delta n = \delta i \quad (12)$$

Although the range for Δ values is known to be from 14.5‰ to 26.5‰ for C₃ plants and from 1.5‰ to 8.5‰ for C₄ plants, most measured values at the whole-plant level fall within the narrow range from 18‰ to 23‰ for C₃ plants and from 3‰ to 7‰ for C₄ plants (Vogel 1993). For plants grown under well-watered conditions, Δ tends to be a relatively stable approximately 20‰ for C₃ plants and 6‰ for C₄ plants. If we substitute δo - δi with Δ, eqn 10 becomes:

$$(\delta a - \delta i) Fa Ca = \Delta Fi Co + \Delta Fa Co \quad (13)$$

Dividing both sides of eqn 12 by Fa gives us:

$$(\delta a - \delta i) Ca = \Delta Co Fi / Fa + \Delta Co \quad (14)$$

After rearrangement and substituting Co with Cc because Co = Cc when the air inside the growth chamber is well mixed, eqn 13 takes the following form:

$$Fi / Fa = (\delta a - \delta i) / \Delta (Ca / Cc) - 1 \quad (15)$$

Now eqn 15 finally gives us the needed condition for maintaining a constant δc value equal to δi + Δ. We can maintain the δ¹³C inside the chamber (δc) at a constant value by setting the ratio of the two flow rates at a certain fixed value. For example, if we use a tank of CO₂ that has a δ¹³C value of -40‰, let Δ be 20‰, let δa (the δ¹³C value of the ambient CO₂ source) be -9‰, and Cc is controlled to be at the ambient level of Ca (i.e. Cc = Ca), we can maintain the δ¹³C value inside the chamber at a constant of -20‰ (because δn = δi, δi = δc - Δ, δc = -40 + 20 = -20) by setting the Fi : Fa ratio to 0.55. If C₄ plants are grown, the Δ value is approximately 6‰ and the Fi : Fa ratio should be set to

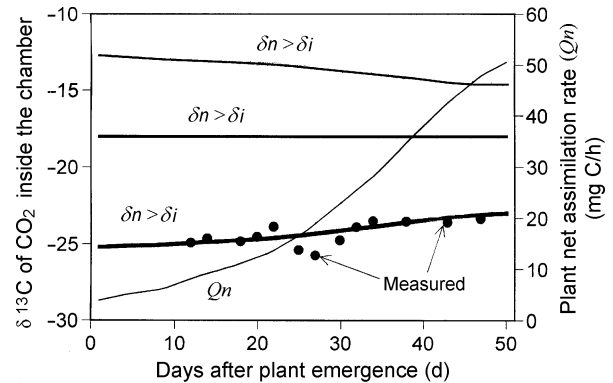


Figure 2. Changes in δ¹³C values of CO₂ inside the labeling chamber as the theoretical plant net assimilation rate (Qn, the thin S-shaped curve) increases over time.

The lines represent results from simulations using the theoretical equations described in the text: a theoretical case when δn > δi, the case when δn = δi, and results when δn < δi, as in the case of the empirical experiment. Filled circles are measured results from the empirical experiment. Overall, this figure shows that the system converges towards δn = δi as Qn and time increase.

4.17. Therefore, a much higher rate of CO₂-free air injection is required for C₄ plants than for C₃ plants.

Empirical Validation

An experiment was performed in order to validate the theoretical conclusion given above. A plant growth chamber (Model EGC-W15; Environmental Growth Chambers, Chagrin Falls, Ohio, USA) was used for this purpose. A CO₂-control module was added to the growth chamber. The CO₂-control module consisted of an infrared CO₂ Analyzer (Model LICOR-820; LICOR, Lincoln, Nebraska, USA) and two automatic switching valves that were controlled by two solid-state relays. The two relays were activated or deactivated by the signals generated from the CO₂ analyzer with built-in "high" and "low" alarm circuits. Both switching valves were connected with a pure CO₂ tank and controlled CO₂ injection (Q_i) into the growth chamber. The CO₂ concentration inside the chamber (C_c) was set at 400 ppm (v) and controlled at an accuracy of ± 5 ppm (v).

A sodalime column was used for generating CO₂-free air. The column was constructed using PVC pipe (20 cm diameter, 200 cm length). The column was filled with approximately 40 kg fresh sodalime and fed with compressed air. The flow rate (F_i) of the CO₂-free air injection into the chamber was controlled at 55 L/min using a mass-flow controller (Sierra Instruments, Monterey, California, USA). The $\delta^{13}\text{C}$ value of the tank CO₂ was -37.7‰ . The flow rate of the ambient air into the growth chamber (F_a) was determined to be 48 L/min from chamber leakage. The CO₂ concentration of the ambient air (C_a) was (389 ± 7) ppm (v). The $\delta^{13}\text{C}$ value of the ambient CO₂ (δ_a) averaged -9‰ . The total effective volume of the growth chamber (V) was 3.1 m^3 .

In order to test the theoretical results described above, 16 plants were grown in the plant growth chamber for 54 days in PVC plastic pots (15 cm diameter, height 40 cm, closed at the bottom except for an air outlet for air circulation). Eight pots were planted with soybean (*Glycine max*) and another eight pots were planted with sunflower (*Helianthus annuus*). Each pot was filled with 7 500 g air-dried soil before planting. Four plants of each species were grown either in soil taken from an organic farm or in soil taken from an annual grassland on the campus reserves of the University of California, Santa Cruz (CA, USA). Plants were grown from seeds in the growth chamber with 12 h light (approximately $800\ \mu\text{mol}/\text{m}^2$ per s), at 25 °C when lights were on and at 20 °C when lights were off, and 40% relative humidity. Pots were watered daily with deionized water and maintained at 80% water holding capacity by weighing the pots before watering. Disturbances in CO₂ concentration and ¹³C signature inside the growth chamber during light hours were kept at a minimum by watering during the dark period.

Two hours before the start of each daylight period, CO₂-free air was fed into the growth chamber. The flow of CO₂-free air was discontinued when lights went off until 2 h before lights

went on again. Air CO₂ inside the growth chamber was sampled every other day when growth chamber lights were on by pumping air through a glass airstone immersed in 4 mol/L NaOH solution (from 30 min after lights were on until 30 min before lights went off; total 11 h). Samples were analyzed for $\delta^{13}\text{C}$ using the procedure described by Harris et al. (1997). Briefly, a 0.3 mol/L SrCl₂ solution was added to a subsample of the CO₂-trapping solution to form SrCO₃ precipitate. The SrCO₃ precipitate was repeatedly rinsed with deionized water until a solution pH of 7 was reached, then dried at 105 °C in an oven. The SrCO₃ precipitate samples were analyzed for $\delta^{13}\text{C}$ on a Hydra 20-20 continuous flow isotope mass spectrometer (PDZ Europa, Cheshire, UK) using the isotope facility at University of California, Davis (CA, USA). The $\delta^{13}\text{C}$ values measured in the NaOH CO₂ traps were corrected for contamination from carbonate in the NaOH stock solution and from sample handling using the following equation (Cheng et al. 2003):

$$\delta^{13}\text{C}_j = (C\delta^{13}\text{C}_r - C_c\delta^{13}\text{C}_c) / (C_r - C_c) \quad (16)$$

where $\delta^{13}\text{C}_j$ is the $\delta^{13}\text{C}$ value of a sample after correction, $\delta^{13}\text{C}_r$ is the $\delta^{13}\text{C}$ value of a sample before correction, $\delta^{13}\text{C}_c$ is the $\delta^{13}\text{C}$ value of the contaminant C (-6‰), C_r is the total amount of C in the sample solution including contaminant C, and C_c is the amount of C in blank control solutions.

All plants were harvested after 54 d of growth in the chamber. Plants were separated into stems, leaves, reproductive organs, and roots, were then dried (65 °C), weighed, and ground. Plant stem and leaf materials were combined before isotope analysis. Samples of ground plant materials were analyzed for $\delta^{13}\text{C}$ using the same facility as for the SrCO₃ samples.

The $\delta^{13}\text{C}$ value of the CO₂ inside the growth chamber was relatively constant and much more depleted in ¹³C than the CO₂ in the ambient air, with an average $\delta^{13}\text{C}$ of -24.4‰ , a minimum of -24.9 , and a maximum of -23.4‰ (Figure 2). These values confirmed our results produced from a simulation with a computer model. The model was built using the eqn 7 given above. The model calculated similar $\delta^{13}\text{C}$ values of CO₂ inside the growth chamber, which changed little while plants were growing for the duration of the experiment. However, the $\delta^{13}\text{C}$ value of CO₂ inside the growth chamber would slowly approach -18‰ , which was the value when $\delta n = \delta c - \Delta$ or $\delta n = \delta i$, because net primary productivity increased through time if the experiment lasted much longer.

The $\delta^{13}\text{C}$ values for sunflower plant materials and soybean plants were approximately -45.5‰ and -43.5‰ , respectively (Table 1). These $\delta^{13}\text{C}$ values were lower by approximately 16‰ – 23‰ than the $\delta^{13}\text{C}$ value of the soil organic carbon taken from C₃-plant dominated ecosystems, in which the $\delta^{13}\text{C}$ values often range from -22‰ to -27‰ . Because of this difference in $\delta^{13}\text{C}$ values, the continuous labeling method allowed us to successfully separate new plant-derived CO₂ from original soil-derived CO₂ (Dijkstra et al. 2006) using the following equation:

$$C_s = C_i(\delta_p - \delta_i) / (\delta_p - \delta_s) \quad (17)$$

Table 1. Mean (\pm SEM) $\delta^{13}\text{C}$ values for plant materials harvested at the end of the experiment, mean $\delta^{13}\text{C}$ values of the CO_2 inside the chamber, and the resulting discrimination factor, Δ

Plant type	Stem+leaf	Reproductive organs	Roots	Whole plant	Air	Δ
Soybean	-43.9 ± 0.24	-43.5 ± 0.52	-43.5 ± 0.29	-43.8 ± 0.29	-24.4	19.4
Sunflower	-46.0 ± 0.12	-45.0 ± 0.13	-45.4 ± 0.28	-45.6 ± 0.13	-24.4	21.2

where C_s is the efflux of $\text{CO}_2\text{-C}$ derived from soil organic matter, C_t is the total efflux of $\text{CO}_2\text{-C}$ from both soil organic matter and plant-derived C, and δ_t , δ_s , and δ_p are the $\delta^{13}\text{C}$ values of the total efflux of $\text{CO}_2\text{-C}$ from belowground, the efflux of soil-derived $\text{CO}_2\text{-C}$, and plant-derived $\text{CO}_2\text{-C}$, respectively. We used the $\delta^{13}\text{C}$ value measured from soil respiration in control pots (no plants, averaged by soil type) for δ_s and from plant biomass in each pot for δ_p .

There was no significant difference in $\delta^{13}\text{C}$ values between plant organs from the same plant species. However, the $\delta^{13}\text{C}$ values of the two plant species were significantly different from each other. Sunflower plants had a lower $\delta^{13}\text{C}$ value than soybean plants, indicating a higher level of ^{13}C discrimination for sunflower plants than soybean plants (Table 1). The average ^{13}C discrimination factor was 19.4‰ and 21.2‰ for soybean and sunflower plants, respectively. These values were expected for the well-watered condition.

Discussion

There are several important variables that may influence the stability and controllability of the system. First, we have assumed that the airflow and CO_2 concentration inside the chamber are at equilibrium. In reality, this assumption has to be carefully considered because it is unavoidable that the chamber system is frequently disturbed during the experiment, such as door openings, watering activities, or any other physical access to the plants inside the chamber. If the system departs from the equilibrium, the above theoretical deliberation and the final eqn 15 would not be usable. In practice, maintaining a reasonable air turnover rate inside the chamber is a simple approach to ensure that this assumption is valid throughout the experiment. A related issue of setting the air turnover rate is the cost of generating CO_2 -free air, which can be a critical factor of financial limitation. Because the ratio of CO_2 -free air to ambient air flow rates is the necessary control parameter for maintaining a constant $\delta^{13}\text{C}$ value in the chamber air, setting a higher air turnover rate requires a proportional increase in the flow rate of CO_2 -free air, thereby increasing the amount of sodalime needed.

Another relevant issue is the choice of using net CO_2 uptake rate as a variable. By using this approach, we have assumed that the CO_2 assimilation rate is larger than or equal to the rate

of total respiration inside the chamber during the active photosynthetic period. However, there may be situations in which the respiration rate is higher than the assimilation rate. For example, at dawn or dusk, low photosynthetic active radiation may result in a much lower assimilation rate, but the respiration rate may not be much different than during other hours. The total system respiration rate may also be higher than the assimilation rate during the initial period of the experiment when plants are small. Fortunately, the ^{13}C abundance of the respired CO_2 from the soil normally ranges from -24‰ to -26‰ , except for soils from C_4 plant-dominated ecosystems, and are not too different from set values in the chamber air. In addition, the CO_2 efflux rate from soil respiration is often a fraction of the rate of CO_2 injection for balancing the CO_2 -free air injection. Therefore, the deviation of ^{13}C abundance in the air caused by soil respiration is often small and negligible.

The results given above show that the effect of fast-growing plants on the $\delta^{13}\text{C}$ value of the CO_2 inside the growth chamber when δn (-5‰) does not equal δi (-37.7‰) is relatively small for the short-term labeling experiment, approximately 1.5‰ in 50 d. This indicates that the labeling system can maintain a relatively stable ^{13}C abundance inside the chamber for a short period of time even if it is not at the ideal setting. This is probably why the effect of plant growth on the $\delta^{13}\text{C}$ value of the CO_2 inside the growth chamber over time can sometimes be ignored in some experiments (Dyckmans et al. 2000; Dyckmans and Flessa 2001). However, this systematic effect is definitely significant if the labeling experiment lasts longer or the assimilation rate is changing fast. Another way to reduce this plant growth effect to an insignificant level is to substantially increase the turnover rate of the chamber air. This approach has been chosen in some studies (Schnyder 1992; Schnyder et al. 2003) using a flow-through system design. The main drawback of the flow-through system is the extremely high demand for CO_2 -free air, which is costly to accommodate. Our system only requires less than 10% of the CO_2 -free air used by the flow-through system of Schnyder (1992).

Both theoretically and empirically, we have shown that inexpensive ^{13}C -depleted CO_2 produced from natural gas can be used in continuous labeling by controlling the $F_i : F_a$ ratio, where F_i is the rate of CO_2 -free air injection and F_a is the rate of ambient air injection. We have used this continuous labeling method for the purpose of partitioning total belowground CO_2 efflux into root-derived and soil-derived components (Dijkstra

et al. 2006). For this type of application, the continuous labeling method works roughly in a way similar to the natural tracer method (Cheng 1996), but eliminates the issue of unnatural plant-soil switches that the natural tracer method requires.

The continuous labeling method can be used for producing uniformly labeled (or uniformly ^{13}C -depleted) litter for decomposition studies. This application has the similar principle as applying C_3 plant litter to a C_4 plant-dominated ecosystem, or vice versa, using C_4 plant litter in a C_3 plant-dominated ecosystem, except that no such switches are needed if the continuous labeling method is used to produce the litter.

In principle, the continuous labeling method has the same potential applications as other labeling methods that use either $^{14}\text{CO}_2$ or enriched $^{13}\text{CO}_2$, except that the ^{13}C -depletion method has a much lower isotopic differential between the labeled and unlabeled materials. The low isotopic differential of this ^{13}C -depletion method inevitably reduces the detection limit compared with enrichment labeling methods. The detection capability of the ^{13}C -depletion method is largely determined by the absolute difference between the mean $\delta^{13}\text{C}$ values of the labeled and unlabeled material, as well as by the associated measurement errors. The $\delta^{13}\text{C}$ value of the labeled material is largely determined by the $\delta^{13}\text{C}$ value of the tank CO_2 , which may range from -35‰ to -50‰ , whereas the $\delta^{13}\text{C}$ value of the unlabeled material may range from -22‰ to -29‰ for C_3 plants and from -10‰ to -15‰ for C_4 plants. A reasonable difference between the $\delta^{13}\text{C}$ values of the labeled and unlabeled materials for C_3 plants may be in the range of 13‰ – 21‰ . Measurement errors may range from 0.1‰ – 2.0‰ depending on the actual variability of the replicated samples. Therefore, measurement errors may account for 0.5% – 10% of the isotopic difference between the labeled and unlabeled material, which indicates that the detection capability of the ^{13}C -depletion method is quite low compared with enrichment labeling methods. Aside from this low detection power, the new continuous labeling method described herein avoids the use of radioactive ^{14}C or expensive ^{13}C -enriched CO_2 required by existing methods and therefore eliminates issues of radiation safety or unaffordable isotope cost, as well as creating new opportunities for short- or long-term labeling experiments under a controlled environment.

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