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▪ New Technology ▪

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

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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, $^{14}CO_2$ 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 $^{14}CO_2$ (e.g. Yamagata et al. 1987; Evdokimov et al. 2004). However, the extremely high cost of the ^{13}C-isotope

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source (approximately US$100 /L of 98% enriched $^{13}CO_2$; 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 CO_2, 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) C_3 plants grown in soils developed under C_4 plant-dominated vegetation (or C_4-soils); or (ii) C_4 plants grown in soils developed under C_3 plant-dominated vegetation (or C_3-soils). Therefore, this method cannot be applied to the plant-soil couplings of C_3 plants in "C_3 soils" or C_4 plants in "C_4-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 CO_2 has been used as a tracer for experiments under elevated CO_2 treatments (e.g. Andrews et al. 1999; Haile-Mariam et al. 2000). However, the ^{13}C-depleted tracers only occur in the elevated CO_2 treatment, not in the ambient CO_2 treatment. Therefore, the results only apply to systems under the elevated CO_2 condition.

In the present paper, we introduce a continuous labeling method using naturally ^{13}C-depleted CO_2 under any desirable CO_2 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 CO_2-free air injection rate of *Fi* (L/min), an ambient air injection rate of *Fa* (L/min) with a CO_2 concentration of *Ca* (μL/L) and a known $\delta^{13}C$ value of *δa* (‰), an air output rate of *Fo* (L/min) with a CO_2 concentration of *Co* (μL/L) and a $\delta^{13}C$ value of *δo* (‰); *Qi* (μL CO_2/min) is the rate of pure CO_2 injection from a high-pressure tank with a known $\delta^{13}C$ value of *δi*; *V* is the total effective volume of the growth chamber; *Cc* is the CO_2 concentration of the air inside the growth chamber; δ*c* is the $\delta^{13}C$ value of the CO_2-C inside the growth chamber; *Qn* (μL CO_2/min) is the net assimilation rate of all plants in the chamber with a $\delta^{13}C$ value of *δn*; *Rt* (μL CO_2/min) is the total respiration rate with a $\delta^{13}C$ value of *δr*; *Qa* (μL CO_2/min) equals *Fa* multipled by *Ca*; *Qo* (μL CO_2/min) equals *Fo* multiplied by *Co*; and *Qc* (μL CO_2) is the amount of CO_2 inside the growth chamber, which equals *V* multipled by *Cc*. The CO_2 concentration inside the chamber is controlled by automatic

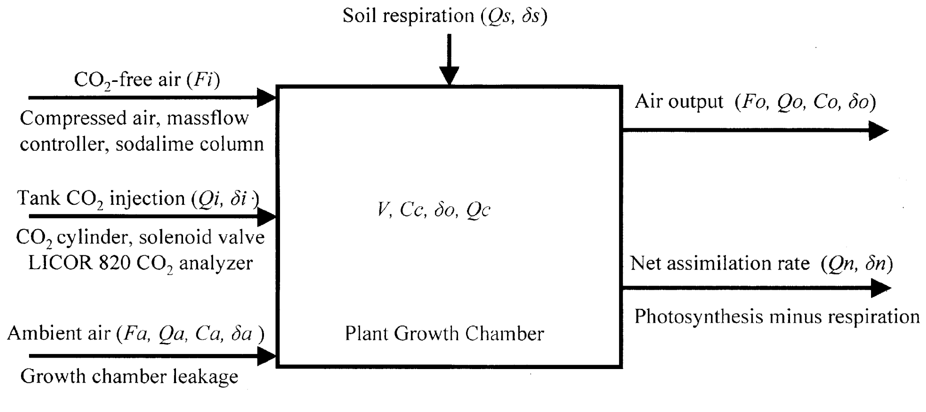

Figure 1. Designation and controls of parameters and variables in a theoretical plant labeling experiment.

Fi, airflow rate (L/min) of CO_2-free air injection; *Qi*, quantity of pure CO_2 injection from a high-pressure tank with a known $\delta^{13}C$ value of *δi*; *Fa*, airflow rate of ambient airflow with a CO_2 concentration of *Ca* and a known $\delta^{13}C$ value of *δa*; *Fo*, airflow rate of the total air output with a CO_2 concentration of *Co* and a $\delta^{13}C$ value of *δo*; *V*, total effective volume of the growth chamber; *Cc*, CO_2 concentration of the air in inside the growth chamber; *δc*, $\delta^{13}C$ value of the CO_2-C in side the growth chamber; *Qn*, net assimilation rate of all plants in the chamber with a $\delta^{13}C$ value of *δn*; *Qs*, soil respiration rate with a $\delta^{13}C$ value of *δs*. The CO_2 concentration inside the chamber is controlled by automatic injection of ^{13}C-depleted tank CO_2 using a solenoid valve activated or deactivated by a CO_2 analyzer.

injection of ^{13}C-depleted tank CO_2 using a solenoid valve activated or deactivated by a CO_2 analyzer. The $\delta^{13}C$ value inside the chamber is controlled indirectly by the rate of CO_2-free air in conjunction with injection of tank CO_2. In the following sections, we will show how we can logically control the $\delta^{13}C$ value inside the chamber by manipulating the rate of CO_2-free air injection.

Balancing the carbon inside the growth chamber, we have:

$$\mathrm{d}Qc/\mathrm{d}t = Qa+Qi+Qs-Qn-Qo \tag{1}$$

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:

$$\mathrm{d}Qc/\mathrm{d}t = Qa+Qi-Qn-Qo = 0 \tag{2}$$

$$Qo = Qa+Qi-Qn \tag{3}$$

and

$$Qi = Qo+Qn-Qa \tag{4}$$

Balancing the ^{13}C, we have:

$$Qc(\mathrm{d}\delta c/\mathrm{d}t) = \delta aQa+\delta iQi-\delta nQn-\delta oQo \tag{5}$$

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:

$$Qc(\mathrm{d}\delta c/\mathrm{d}t) = \delta aQa+\delta i(Qo+Qn-Qa)-\delta nQn-\delta oQo \tag{6}$$

After reconfiguring, eqn 6 becomes:

$$Qc(\mathrm{d}\delta c/\mathrm{d}t) = \delta aQa+\delta iQo-\delta iQa-\delta oQo+(\delta i-\delta n)Qn \tag{7}$$

Equation 7 shows that the necessary condition for $\mathrm{d}\delta c/\mathrm{d}t$ to be independent of Qn is $\delta n=\delta i$, so that the term $(\delta i-\delta n)Qn=0$. If $\delta n>\delta i$, δc decreases as Qn increases until δc approaches a level that makes $\delta n=\delta i$, because δn (the δ value of the net plant production) also decreases if δc (the δ value of CO_2 inside the chamber) decreases. Similarly, if $\delta n<\delta i$, δc increases as Qn increases until δc approaches a level that makes $\delta n=\delta i$. This is illustrated in Figure 2 using simulation results. This means that the system converges towards $\delta n=\delta i$. Therefore, we conclude that $\delta n=\delta i$ 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 CO_2.

Now we need to know how to reach the controlling goal of $\delta n=\delta i$. If we rearrange eqn 7, it becomes:

$$Qc(\mathrm{d}\delta c/\mathrm{d}t) = (\delta a-\delta i)Qa-(\delta o-\delta i)Qo+(\delta i-\delta n)Qn \tag{8}$$

When eqn 8 is at equilibrium, the condition for $\delta n=\delta i$ is:

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

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

$$(\delta a-\delta i)FaCa = (\delta o-\delta i)(FiCo+FaCo) \tag{10}$$

By definition (Farquhar et al. 1989),

$$\Delta = (\delta o-\delta n)/(1+\delta n/1\,000) \tag{11}$$

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:

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

Although the range for Δ values is known to be from 14.5‰ to 26.5‰ for C_3 plants and from 1.5‰ to 8.5‰ for C_4 plants, most measured values at the whole-plant level fall within the narrow range from 18‰ to 23‰ for C_3 plants and from 3‰ to 7‰ for C_4 plants (Vogel 1993). For plants grown under well-watered conditions, Δ tends to be a relatively stable approximately 20‰ for C_3 plants and 6‰ for C_4 plants. If we substitute δo-δi with Δ, eqn 10 becomes:

$$(\delta a-\delta i)FaCa = \Delta FiCo+\Delta FaCo \tag{13}$$

Dividing both sides of eqn 12 by Fa gives us:

$$(\delta a-\delta i)Ca = \Delta CoFi/Fa+\Delta Co \tag{14}$$

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:

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

Now eqn 15 finally gives us the needed condition for maintaining a constant δc value equal to $\delta i+\Delta$. We can maintain the $\delta^{13}C$ 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 CO_2 that has a $\delta^{13}C$ value of –40‰, let Δ be 20‰, let δa (the $\delta^{13}C$ vale of the ambient CO_2 source) be –9‰, and Cc is controlled to be at the ambient level of Ca (i.e. $Cc=Ca$), we can maintain the $\delta^{13}C$ value inside the chamber at a constant of –20‰ (because $\delta n = \delta i$, $\delta i = \delta c-\Delta$, $\delta c = -40+20 = -20$) by setting the $Fi : Fa$ ratio to 0.55. If C_4 plants are grown, the Δ value is approximately 6‰ and the $Fi : Fa$ ratio should be set to

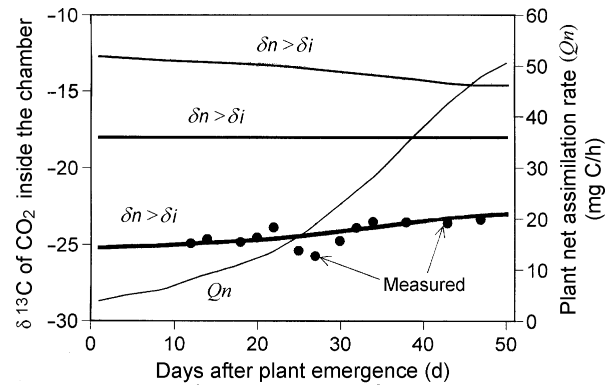

Figure 2. Changes in $\delta^{13}C$ values of CO_2 inside the labeling chamber as the theoretical plant net assimilation rate (Qn, the thin S-shaped curve) increases over time.

The lines represent results from simulations using the theoretical equations described in the text: a theoretical case when $\delta n > \delta i$, the case when $\delta n=\delta i$, and results when $\delta n<\delta i$, 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 $\delta n=\delta i$ as Qn and time increase.

4.17. Therefore, a much higher rate of CO_2-free air injection is required for C_4 plants than for C_3 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 CO_2-control module was added to the growth chamber. The CO_2-control module consisted of an infrared CO_2 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 CO_2 analyzer with built-in "high" and "low" alarm circuits. Both switching valves were connected with a pure CO_2 tank and controlled CO_2 injection (*Qi*) into the growth chamber. The CO_2 concentration inside the chamber (*Cc*) was set at 400 ppm (*v*) and controlled at an accuracy of ± 5 ppm (*v*).

A sodalime column was used for generating CO_2-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 (*Fi*) of the CO_2-free air injection into the chamber was controlled at 55 L/min using a mass-flow controller (Sierra Instruments, Monterey, California, USA). The $\delta^{13}C$ value of the tank CO_2 was –37.7‰. The flow rate of the ambient air into the growth chamber (*Fa*) was determined to be 48 L/min from chamber leakage. The CO_2 concentration of the ambient air (*Ca*) was (389±7) ppm (*v*). The $\delta^{13}C$ value of the ambient CO_2 (δa) 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 $\mu mol/m^2$ 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 CO_2 concentration and ^{13}C 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, CO_2-free air was fed into the growth chamber. The flow of CO_2-free air was discontinued when lights went off until 2 h before lights went on again. Air CO_2 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 $\delta^{13}C$ using the procedure described by Harris et al. (1997). Briefly, a 0.3 mol/L $SrCl_2$ solution was added to a subsample of the CO_2-trapping solution to form $SrCO_3$ precipitate. The $SrCO_3$ precipitate was repeatedly rinsed with deionized water until a solution pH of 7 was reached, then dried at 105 °C in an oven. The $SrCO_3$ precipitate samples were analyzed for $\delta^{13}C$ on a Hydra 20-20 continuous flow isotope mass spectrometer (PDZ Europa, Chesire, UK) using the isotope facility at University of California, Davis (CA, USA). The $\delta^{13}C$ values measured in the NaOH CO_2 traps were corrected for contamination from carbonate in the NaOH stock solution and from sample handling using the following equation (Cheng et al. 2003):

$$\delta^{13}C_j = (C_t\delta^{13}C_t - C_c\delta^{13}C_c)/(C_t - C_c) \qquad (16)$$

where $\delta^{13}C_j$ is the $\delta^{13}C$ value of a sample after correction, $\delta^{13}C_t$ is the $\delta^{13}C$ value of a sample before correction, $\delta^{13}C_c$ is the $\delta^{13}C$ value of the contaminant C (–6‰), C_t 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 $\delta^{13}C$ using the same facility as for the $SrCO_3$ samples.

The $\delta^{13}C$ value of the CO_2 inside the growth chamber was relatively constant and much more depleted in ^{13}C than the CO_2 in the ambient air, with an average $\delta^{13}C$ 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 $\delta^{13}C$ values of CO_2 inside the growth chamber, which changed little while plants were growing for the duration of the experiment. However, the $\delta^{13}C$ value of CO_2 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 $\delta^{13}C$ values for sunflower plant materials and soybean plants were approximately –45.5‰ and –43.5‰, respectively (Table 1). These $\delta^{13}C$ values were lower by approximately 16‰–23‰ than the $\delta^{13}C$ value of the soil organic carbon taken from C_3-plant dominated ecosystems, in which the $\delta^{13}C$ values often range from –22‰ to –27‰. Because of this difference in $\delta^{13}C$ values, the continuous labeling method allowed us to successfully separate new plant-derived CO_2 from original soil-derived CO_2 (Dijkstra et al. 2006) using the following equation:

$$C_s = C_t(\delta_p - \delta_t)/(\delta_p - \delta_s) \qquad (17)$$

Table 1. Mean (± *SEM*) δ^{13}C values for plant materials harvested at the end of the experiment, mean δ^{13}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 CO_2-C derived from soil organic matter, C_t is the total efflux of CO_2-C from both soil organic matter and plant-derived C, and δ_t, δ_s, and δ_p are the δ^{13}C values of the total efflux of CO_2-C from belowground, the efflux of soil-derived CO_2-C, and plant-derived CO_2-C, respectively. We used the δ^{13}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 δ^{13}C values between plant organs from the same plant species. However, the δ^{13}C values of the two plant species were significantly different from each other. Sunflower plants had a lower δ^{13}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 δ^{13}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 δ^{13}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 δ^{13}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 Schynder (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 *Fi* : *Fa* ratio, where *Fi* is the rate of CO_2-free air injection and *Fa* 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$^{14}CO_2$ or enriched $^{13}CO_2$, 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 δ^{13}C values of the labeled and unlabeled material, as well as by the associated measurement errors. The δ^{13}C value of the labeled material is largely determined by the δ^{13}C value of the tank CO_2, which may range from –35‰ to –50‰, whereas the δ^{13}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 δ^{13}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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