

Methods

Measuring tree root respiration using ^{13}C natural abundance: rooting medium matters

Weixin Cheng¹, Shenglei Fu^{1,3}, Richard B. Susfalk^{1,4} and Robert J. Mitchell²

¹University of California, Santa Cruz, CA 95064, USA; ²Jones Ecological Research Center, Newton, GA 39870, USA; ³Present address: Ecological Research Center, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, 510650 China; ⁴Present address: Desert Research Institute, Reno, NV 89512, USA

Summary

Author for correspondence:

Weixin Cheng

Tel: +1 831 4595317

Fax: +1 831 4594015

Email: wxcheng@ucsc.edu

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- Tree root respiration utilizes a major portion of the primary production in forests and is an important process in the global carbon cycle. Because of the lack of ecologically relevant methods, tree root respiration *in situ* is much less studied compared with above-ground processes such as photosynthesis and leaf respiration.
- This study introduces a new ^{13}C natural tracer method for measuring tree root respiration *in situ*. The method partitions tree root respiration from soil respiration in buried root chambers.
- Rooting media substantially influenced root respiration rates. Measured in three media, the fine root respiration rates of longleaf pine were 0.78, 0.27 and 0.18 mg CO_2 carbon mg^{-1} root nitrogen d^{-1} at 25°C in the native soil, tallgrass prairie soil, and sand-vermiculite mixture, respectively. Compared with the root excision method, the root respiration rate of longleaf pine measured by the field chamber method was 18% higher when using the native soil as rooting medium, was similar in the prairie soil, but was 42% lower if in the sand-vermiculite medium.
- This natural tracer method allows the use of an appropriate rooting medium and is capable of measuring root respiration nondestructively in natural forest conditions.

Key words: C-13 tracer, *Pinus palustris*, *Pinus ponderosa*, *Populus fremontii*, *Pseudotsuga menziesii*, root chamber, root excision.

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Introduction

The global carbon cycle has been a focal area in global change research. The processes controlling carbon fluxes between the atmosphere and terrestrial ecosystems are critical components of the global carbon cycle (Fan *et al.*, 1995; Phillips *et al.*, 1998). To predict the course and the consequences of climate change, it is essential to understand and quantify these processes because the primary 'greenhouse' gases (i.e. CO_2 and CH_4) are taken up and released in large quantities by terrestrial ecosystems at the same time (Schlesinger, 1991). Forests have been identified as important processors of carbon (Tans *et al.*, 1990; Vogt *et al.*, 1991; Gregor, 1992; Houghton, 1993).

However, our lack of understanding of below-ground carbon fluxes in forest systems and specifically the role of roots is the greatest limitation in our ability to assess the contribution of forests as global carbon processors (Schimel, 1995; Jackson *et al.*, 1997).

Total rhizosphere respiration has been defined as CO_2 respired by a system of living roots and their closely associated rhizosphere microbes that utilize organic substrates from live roots (Cheng *et al.*, 1993; Cheng, 1996). However, total rhizosphere respiration has commonly been regarded as 'root respiration' in most ecological studies because the two components are intimately associated and rarely considered separately (Andrews *et al.*, 1999). 'Root respiration' in this study

is defined similarly, including both components, and will be used as such in this study. According to current rough estimates, root respiration may range from 30% to 80% of total below-ground CO₂ efflux (Hanson *et al.*, 2000) in various forest ecosystems. Therefore, root respiration is one of the most important below-ground processes responsible for carbon release.

Most direct measurements of tree root respiration are made on roots excised from trees (Coleman, 1973; Edwards & Sollins, 1973; Winship & Tjepkema, 1985; Burton *et al.*, 1996; Zogg *et al.*, 1996). Some reports have suggested that excision does not affect respiration over short intervals (Lambers *et al.*, 1981; Marshall & Perry, 1987; Lee *et al.*, 2003) whereas other studies have shown that excision significantly decreases root respiration (Mindermann & Vulto, 1973; Bloom & Caldwell, 1988). In addition to a possible physiological response to the wound, root excision introduces a very high degree of disturbance and a highly artificial physical and chemical environment; and excludes most of the rhizosphere microbial components. The relevance of the root excision method to the processes in forest ecosystems is often questionable and has rarely been tested. However, root excision remains the method of choice because of the lack of a better alternative. Tree root respiration has rarely been measured *in situ*, mostly because of difficulties involved in separating root respiration from soil microbial respiration in field conditions. Separating root respiration from soil microbial respiration has been accomplished by using isotope-labeling techniques under laboratory conditions. Most reported data on root respiration have been obtained by either continuous ¹⁴C labeling (Barber & Martin, 1976; Whipps & Lynch, 1983; Whipps, 1984; Merckx *et al.*, 1987; Kuikman *et al.*, 1991; Liljeroth *et al.*, 1991) or pulse labeling (Cheng *et al.*, 1993, 1994; Horwath *et al.*, 1994; Swinnen *et al.*, 1994) of small plants. However, both of these ¹⁴C-labeling methods have limited utility in forest ecosystems. Isotope labeling of large trees in a forest is too difficult because of the high cost, long time required, and other logistical considerations. Thus, these labeling approaches have never been used in forest conditions.

Root respiration in the field has also been estimated by indirect means including: (1) budgeting inputs of detritus to the soil and subtracting from total soil respiration (Phillipson *et al.*, 1975; Ewel & Cropper, 1987; Bowden *et al.*, 1993) and (2) measuring respiration of root-free soil and subtracting from total soil respiration (Edwards & Ross-Todd, 1983; Nakane *et al.*, 1983; Lamade *et al.*, 1996). The method of budgeting inputs of detrital carbon available for heterotrophic decomposition and subtracting it from total soil CO₂ efflux as an estimate of root respiration is both difficult and costly. Errors in any of the separate input estimates will aggregate in the estimate of root respiration. Estimating input from root turnover is particularly difficult because the periodicity of fine root turnover may range from weeks to years (Cheng *et al.*, 1990; Hendrick & Pregitzer, 1992). Measuring root respiration

by subtracting respiration of root-free soils from total below-ground respiration usually introduces complications that are difficult to resolve. Creation of root-free soils causes disturbance to the soil profile, either physically through sieving or chemically by killing the roots and increasing fresh detritus (Gadgil & Gadgil, 1975; Fisher & Gosz, 1986; Faber & Verhoefer, 1991; Ehrenfeld *et al.*, 1997). Both of these types of disturbances typically enhance heterotrophic respiration. The budgeting method is based on the assumption that interactions between the roots and soil are minimal (e.g. changes in soil moisture, priming effect of exudates, inputs of sloughed fine roots or the presence of rhizosphere organisms do not appreciably affect heterotrophic respiration). This assumption is often invalid, as indicated by recent studies using a ¹³C natural tracer method (Cheng, 1996; Fu & Cheng, 2002; Cheng *et al.*, 2003). Budgeting methods also often require an accurate assessment of the net change in total soil carbon stock during the measurement period (Giardina & Ryan, 2002). Because of the inherent high degree of temporal and spatial variability often associated with measurements of soil carbon stocks, accurate detection of a normally small net change compared with its large stock size is often unapproachable (Smith, 2004). However, the net change in soil carbon stock is a crucial component for estimating root respiration when budgeting methods are used. Other methods, if available, must be used to overcome these difficulties.

Recent developments of the ¹³C natural tracer method (Cheng, 1996; Qian *et al.*, 1997; Rochette & Flanagan, 1997) have significantly increased our ability to investigate rhizosphere C fluxes under more realistic conditions. However, these approaches have been limited to highly altered agricultural ecosystems after a change of rotation from a C₃ or a C₄ crop (Rochette & Flanagan, 1997) or exporting soil from C₄-dominated prairies to forests (Susfalk *et al.*, 2002), which introduces unavoidable complications associated with the mixing of ¹³C signals from the native soil source. Seeking solutions to these problems, we introduce a new root chamber method that employs natural ¹³C isotope tracers, and portable CO₂ trapping.

The primary goals of this study were to (1) investigate the potentials and the limitations of this new approach of coupling root chambers with natural ¹³C tracers; (2) assess the effects of different rooting media on the measured respiration rates of roots attached to mature trees; and (3) compare tree root respiration rates measured using the chamber method with the rates produced from an root excision method.

Materials and Methods

The ¹³C natural tracer method

The principle of this ¹³C natural tracer method (Cheng, 1996; Qian *et al.*, 1997; Rochette & Flanagan, 1997) is based on the difference in ¹³C : ¹²C ratio (often reported in δ¹³C

value) between plants with the C_3 photosynthetic pathway whose mean $\delta^{13}C$ is -27‰ , and plants with the C_4 pathway whose mean $\delta^{13}C$ is -12‰ (Smith & Epstein, 1971), and on the subsequent $\delta^{13}C$ difference in soil C derived from the two types of plants. Soil C derived from C_4 plant-dominated vegetation (C_4 soil), such as tallgrass prairie and tropical grasslands, has $\delta^{13}C$ values ranging from -12 to -20‰ , whereas $\delta^{13}C$ values of soil C derived from cold and temperate forest (C_3 soil) range from -24 to -29‰ . By growing tree roots in a chamber filled with a C_4 -derived soil, the amount of CO_2 produced from tree root respiration can be partitioned from soil respiration because of their different $\delta^{13}C$ values. Based on the logic of two-end member separation (Cheng, 1996), the following equation can be used to calculate tree root-derived CO_2 and soil-derived CO_2 :

$$C_{tr} = C_t(\delta_t - \delta_s)/(\delta_{tr} - \delta_s) \quad \text{Eqn 1}$$

($C_t = C_{tr} + C_s$ and is the total amount of CO_2 -C trapped; C_{tr} is the amount of CO_2 -C derived from tree root respiration; C_s is the amount of CO_2 -C derived from soil microbial respiration; δ_t is the $\delta^{13}C$ value of the C_t ; δ_{tr} is the $\delta^{13}C$ value of the root-derived CO_2 (end member #1); δ_s is the $\delta^{13}C$ value of the soil-derived CO_2 (end member #2)). In this study, the $\delta^{13}C$ value of the root-derived CO_2 is assumed to be the same as the $\delta^{13}C$ value of the roots used in the experiment because previous studies have indicated that there is no detectable isotope fractionation during plant/rhizosphere respiration (Cheng, 1996; Lin & Ehleringer, 1997). The $\delta^{13}C$ value of the soil-derived CO_2 is measured from the soil-only treatment. This method has several advantages compared with ^{14}C -labeling of either the plants or the soil: (1) uniform and natural labeling of plants and soils; (2) no radiation safety problems; and (3) it can be used in natural forests where radioisotope labeling is virtually impossible. The major limitation of this method is that it involves soil substitution that may influence tree root respiration because of the creation of different soil environments.

Sites, tree species, and rooting media

One experiment was done using roots of 21 yr-old longleaf pines in a plantation. The longleaf pine (*Pinus palustris* L.) plantation was located at the Joseph W. Jones Ecological Research Center in Newton, GA, USA. The plantation was established in 1980. The soil used in the root chambers was taken from under long-term wiregrass (*Aristida stricta* L.) patches. Wiregrass is a common plant in the understory of this ecosystem. Wiregrass is a C_4 plant, therefore the soil organic carbon under a long-term wiregrass patch had a ^{13}C abundance similar to that of a C_4 plant origin ($\delta^{13}C = -17\text{‰}$). The difference in $\delta^{13}C$ values between the soil under wiregrass and roots of longleaf pine trees was sufficiently high that the natural ^{13}C tracer method could be used to separate root respiration from

soil heterotrophic respiration if used in a root chamber together. Another experiment was carried out using roots attached to Douglas fir (*Pseudotsuga menziesii* Mib. Franco) trees of approx. 25 yr old growing in the Campus Reserve of the University of California, Santa Cruz, CA, USA. The Douglas fir trees were at the center of a pure stand of approx. 10×10 m size. For comparison, the respiration rates of whole-root systems of ponderosa pine (*Pinus ponderosa* Laws., 2 yr old) and cottonwood (*Populus fremontii* Watson, 1.5 year old) were also measured in a pot experiment using the natural ^{13}C tracer method.

Three kinds of media were used in these experiments. A surface soil (0–15 cm) under long-term wiregrass patches (C_4 soils) near to the longleaf pine plantation was collected, sieved to pass 2 mm screen, removed of roots by hand-picking, air-dried, and homogenized for later use in longleaf pine root chambers. This wiregrass soil was significantly enriched with ^{13}C ($\delta^{13}C$ - CO_2 was -17.83‰ from wiregrass soil and -27.56‰ for pine roots, i.e. approx. 10‰ higher). The soil under these wiregrass patches was composed of sand and a low level of organic matter, lacking silt and clay fractions, and had C and N contents of 8.0 g kg^{-1} and 0.3 g kg^{-1} , and a pH of 4.79. Similar pH value, C content, and N content were found in the soil taken from the top layer of the longleaf pine plantation. Wiregrass is the most common understory species of this pineland ecosystem. Roots of longleaf pine naturally colonize the soil under wiregrass patches (Jones *et al.*, 2003). Another soil was obtained from Konza Prairie, KS, USA, where C_4 grasses dominated. It had a $\delta^{13}C$ of -14.6‰ , C and N contents of 20 g kg^{-1} and 1.9 g kg^{-1} , respectively, and a pH of 7.05. The tallgrass prairie soil was sieved with a 2-mm mesh sieve air-dried, and inoculated with the native soil (1% v : v) before use. The pH of this tallgrass prairie soil was much higher than the soil pH values either from the wiregrass–longleaf pine ecosystem (pH = 4.8) or from the Douglas fir stands (pH = 5.3). The surface (0–20 cm depth) mineral soil at the Douglas fir stand had a C and N contents of 18 g kg^{-1} and 1.6 g kg^{-1} , similar to the C and N contents of the tallgrass prairie soil. The third medium was a mixture of acid-washed sand and burnt vermiculite in which half-strength Hoagland's nutrient solution (Hoagland & Arnon, 1950) was added. The sand–vermiculite mixture was inoculated with the native soil (1% v : v) before use. This mixture was used as a reference medium because it was free of carbon.

Root chamber design and operation

Root chambers were built using PVC pipes (7.6 cm diameter, 0.5 cm wall thickness, and 30 cm long) (Fig. 1). A PVC cap was glued onto one end of each chamber, and a hole 3.2 cm in diameter was drilled, allowing an intact live root system to be inserted. Plastic tubing was connected to each chamber for air circulation and CO_2 trapping. A temperature probe (calibrated thermistor, TMC6-HB, Onset Computer Corp, Bourne, MA, USA) was installed inside each chamber and connected to a data logger (HOBO H8 outdoor/industrial

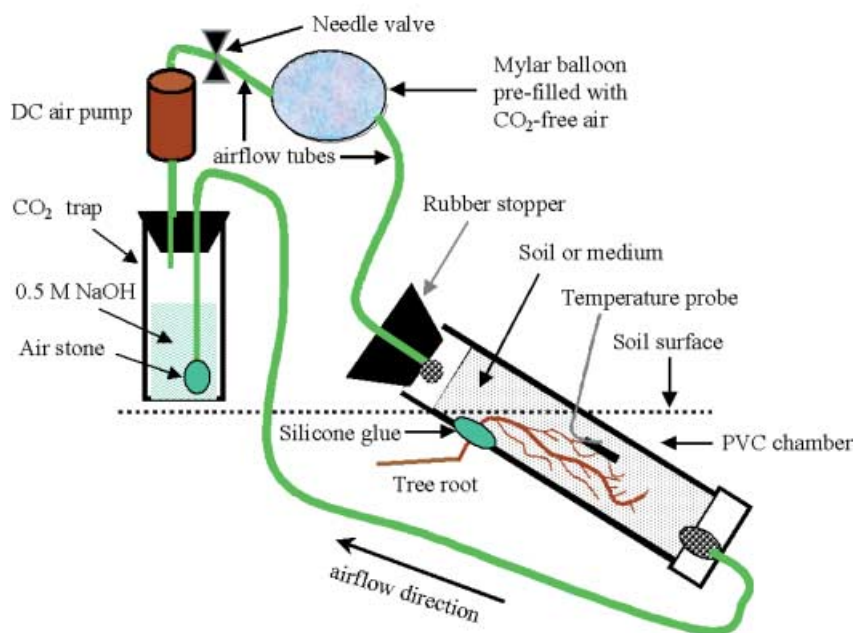


Fig. 1 Root chamber and closed-circulation CO_2 -trapping system. Temperature probe is connected to a data logger.

four-channel logger Part# H08-008-04). In each experiment, five replicate chambers were used for each treatment with roots, and five replicate chambers were used for soil-only control (for measuring the $\delta^{13}\text{C}$ value of the soil-derived CO_2).

Intact live root systems were carefully isolated by hand and then washed clean with deionized (DI) water. Extreme care was taken to minimize physical stress to the roots. A typical isolated root system consisted of four to five branching orders starting at the root tip. Each isolated root system was threaded into a root chamber. A known amount of C_4 -derived soil (soil from under wiregrass for longleaf pine, and soil from under a Kansas tallgrass prairie for Douglas fir) was added through the open-top end of the root chamber. The root-chamber junction was sealed using a two-part silicone glue (Kuzyakov & Cheng, 2001). The integrity of the seal was verified by submerging the air outlet tube in water and checking for air leaks. DI water (or half-strength Hoagland nutrient solution in the case of sand-vermiculite mix) was added to each chamber to bring the initial soil water content to 80% of field capacity. Root chambers were then buried at an approx. 45° angle to the vertical with their original topsoil and litter around the chamber, and positioned to insure the open-top end stayed 5 cm above the soil surface. Before any measurement took place, an acclimatization period of 20 d was allowed for all root chambers in the longleaf pine experiment; 34 d of acclimatization period was allowed for the root chambers in the Douglas fir experiment. Before trapping CO_2 , the headspace of each target chamber was flushed with CO_2 -free air for 5 min every 2 h during a period of at least 24 h by connecting a timer-controlled direct current (DC) air pump to the airflow tubes, but without NaOH trapping solution connected to the system. At the end of the conditioning period, a CO_2 -

trapping period was begun by adding 50 ml of 0.5 M NaOH solution to the CO_2 trap, and connecting a Mylar balloon pre-filled with CO_2 -free air to the closed circulating system. The balloon provided air pressure relief and adequate oxygen supply that could last well beyond the duration of the experiment. The use of the CO_2 -free air in the circulation should not significantly affect root respiration because tree root respiration is insensitive to CO_2 concentration during measurements (Burton & Pregitzer, 2002). Using this closed circulation system, CO_2 from each root chamber was trapped in an alkaline solution for 5 min at a 2-h interval for 21 d (Cheng *et al.*, 2000, modified to run on 12 V batteries, see Fig. 1). The CO_2 trapping efficiency with this system was greater than 99.9% (Cheng & Coleman, 1989), eliminating preferential absorption of $^{12}\text{CO}_2$ vs $^{13}\text{CO}_2$. Blanks were included to correct for handling errors. An aliquot of the NaOH solution was analysed for total inorganic C using a TOC analyser (5050A; Shimadzu, Columbia, MD, USA). The remainder of the trapping solution was mixed with excess SrCl_2 and the $\delta^{13}\text{C}$ of the precipitate (SrCO_3) was analysed by mass spectrometry (Harris *et al.*, 1997). Immediately after the CO_2 trapping period, the root system in each chamber was cut at the root-chamber junction, washed with tap water, and dried in an oven at 70°C in the laboratory. The respiration rates of excised roots from longleaf pine were measured before oven drying. Oven-dried roots were ground in a ball mill before analyses for ^{13}C , total C and total N. Analysis of ^{13}C abundance of all samples were carried out using a continuous flow isotope ratio mass spectrometer coupled with an automated C and N combustion analyser at the Stable Isotope Facility of the University of California, Davis, CA, USA. Rhizosphere respiration and decomposition of original soil organic matter was partitioned

using Eqn 1, where soil-derived CO₂ was a measure of original soil organic matter decomposition and root-derived CO₂ was a measure of rhizosphere respiration.

The whole-plant experiment

The respiration rates of the whole root systems of cottonwoods and ponderosa pine were measured in a pot experiment at the University of California, Santa Cruz, CA, USA. The natural ¹³C tracer method (Cheng, 1996; Fu & Cheng, 2002) was used. Cottonwoods (1.5 yr old, a C₃ plant) and ponderosa pine (2 yr old, a C₃ plant) were grown in either the prairie soil ('C₄' soil) taken from Kansas, USA, or in the carbon-free sand-vermiculite mixture. No-plant controls were included for both the soil treatment and the sand-vermiculite treatment. Five replicates were used. For the soil treatment, 6.8 kg of dry soil was added to a PVC pot (13.8 cm inner diameter, 43 cm tall) and subsequently brought to 31% volumetric water content with DI water. For the sand-vermiculite treatment, each pot was filled with 0.6 kg of vermiculite and 3.4 kg of sand admixed with 1.6 l of half-strength Hoagland's solution (Hoagland & Arnon, 1950). Both treatments were inoculated with 1.0% of field-moist soil from under native cottonwood or ponderosa pine. The experiment was conducted outdoors at the University of California, Santa Cruz, CA, USA. Additional DI water was added as necessary to maintain 34% volumetric water content. The sand-vermiculite treatments was fertilized with 100 ml of 200% strength Hoagland's solution on day 23, with additional fertilization of both treatments on day 32 using 200 ml of 200% strength Hoagland's solution.

Below-ground CO₂ was trapped 100 d after planting, using the closed-circulation method of Cheng *et al.* (2003). Briefly, the pot was sealed by covering the soil surface with plastic boards and low-temperature melting-point wax. Air-flow entered the pot through an inlet tube at the top of the pot and exited through an outlet at the bottom of the pot. A vacuum pump pulled air through a sand column filled with 4 N NaOH to trap CO₂. Carbon dioxide was trapped for 30 min every 4 h over a 2-d period. The flow rate was adjusted to 0.30 l min⁻¹ with a needle valve. An aliquot of NaOH solution from each column was analysed for inorganic C using a Shimadzu 5050A TOC analyser. Another aliquot of NaOH solution was mixed with SrCl₂ to generate the SrCO₃ precipitate that was analysed for δ¹³C by a ratio mass spectrometer (Harris *et al.*, 1997) at the University of California, Davis. Tree root-derived CO₂ and soil-derived CO₂ were partitioned using Eqn 1 and used as a measure of root respiration and soil respiration, respectively.

Plants were harvested immediately after CO₂ trapping. Root systems were removed from the pot, separated from the soil by hand, and briefly stored at 4°C until they could be washed free of rooting medium with DI water. Plant tissues were dried at 65°C for 2 d. Individual samples were

ground and analysed for δ¹³C at the University of California, Davis.

Respiration of excised roots

Only respiration rates of excised roots from the longleaf pine experiment were measured. At the end of the CO₂ trapping for the field measurements described earlier, roots were cut at the chamber-root juncture, and chambers were destructively sampled and immediately transported to a nearby laboratory. All roots inside each sampled chamber were quickly washed clean. The roots from each root chamber were then placed in a flask that was sealed using a rubber stopper connected with plastic tubing for air circulation. Excised root respiration was measured using an open-flow gas exchange method modified from Cheng and Virginia (1993). An infrared gas analyser (6262; Li-Cor, Lincoln, NE, USA) was set to a differential mode using a standard gas (2500 p.p.m., v : v, CO₂ in air) for both the carrier gas and the reference gas. The 2500 p.p.m. (v : v) CO₂ gas was chosen because it was similar to the soil CO₂ concentration in the field and because measured root respiration rates could be sensitive to the CO₂ concentration that the root is exposed to during the measurement (Qi *et al.*, 1994; Burton *et al.*, 1997). However, this control on the measurement CO₂ concentration might not be necessary, as indicated by a recent study (Burton & Pregitzer, 2002). The air flow rate through both the sample cell and the reference cell was set at 35 ml min⁻¹ and measured using a digital mass flow meter. The temperature of the carrier gas and the incubating flask was controlled using a water bath at a preset temperature based on the overall mean soil temperature data from data loggers connected to the root chambers at a particular site.

Results and Discussion

The average dry weight of the root systems in five replicated root chambers under each treatment combination ranged from 0.42 g to 0.55 g (Table 1). The sizes of root systems used in root chambers were reasonably similar. No statistically significant differences were found between these biomass values. However, the root biomass of cottonwoods was much higher than that of ponderosa pine in the greenhouse experiment. The N contents of the root systems ranged from 0.44% to 0.83%. Douglas fir roots had significantly higher N content than other species. In general, medium types did not significantly affect the root N content except for the longleaf pine roots in sand-vermiculite mixture, which had a significantly lower N content than the rest.

The detection limit of this natural ¹³C method was largely determined by the absolute difference between the mean δ¹³C values of the two end members and the associated measurement errors. The difference between the mean δ¹³C values of the two end members ranged from 10.3‰ (longleaf pine

Table 1 Mean dry weights, carbon contents, and nitrogen contents of the roots enclosed in root chambers of four tree species (longleaf pine (*Pinus palustris*), Douglas fir (*Pseudotsuga menziesii*), ponderosa pine (*Pinus ponderosa*) and cottonwood (*Populus fremontii*) and three different growth media

Tree species	Media ¹	Dry weight (g)	%C (wt : wt)	%N (wt : wt) ²
Longleaf pine	WG	0.44 (0.11)	43.1 (0.5)	0.60 (0.06)a
Longleaf pine	KS	0.40 (0.15)	44.5 (0.7)	0.63 (0.02)a
Longleaf pine	SV	0.54 (0.14)	44.2 (0.7)	0.44 (0.02)b
Douglas fir	KS	0.42 (0.20)	42.9 (1.3)	0.83 (0.03)c
Douglas fir	SV	0.45 (0.23)	43.0 (1.4)	0.81 (0.05)c
Ponderosa pine	KS	11.28 (1.32)	46.5 (0.5)	0.63 (0.04)a
Ponderosa pine	SV	12.15 (0.83)	46.5 (0.5)	0.73 (0.05)a
Cottonwood	KS	70.06 (8.19)	45.8 (0.7)	0.71 (0.04)a
Cottonwood	SV	67.62 (7.15)	46.4 (0.5)	0.64 (0.05)a

Each value is the mean of five replicates with SE in parentheses.

¹Media used: WG, soil under wiregrass patches; KS, soil taken from a tallgrass prairie; SV, carbon-free sand–vermiculite mixture.

²Values with the same letter are not significantly different as indicated by *t*-tests ($P < 0.05$).

Table 2 Mean $\delta^{13}\text{C}$ values (‰PDB) and associated measurement errors ($n = 5$) of two end members used in rhizosphere respiration measurements for longleaf pine (*Pinus palustris*), Douglas fir (*Pseudotsuga menziesii*), ponderosa pine (*Pinus ponderosa*) and cottonwood (*Populus fremontii*)

Plant species	Medium	Method	Roots	SE ³ (roots)	Soil CO ₂	SE (soil CO ₂)	Difference	%Error ⁵
Longleaf pine	Wiregrass soil ¹	Root chamber	−28.1	0.18	−17.78	0.36	10.32	3.5
Douglas fir	C ₄ prairie soil ²	Root chamber	−27.34	0.21	−15.19	0.32	12.15	2.6
Ponderosa pine	C ₄ prairie soil	Whole plant ⁴	−29.32	0.52	−14.8	0.08	14.52	3.6
Cottonwood	C ₄ prairie soil	Whole plant	−26.53	0.29	−14.8	0.08	11.73	2.5

¹Soil from long-term wiregrass patches.

²Soil from tallgrass prairie in Kansas.

³Standard error calculated using five replicates.

⁴The method described in Cheng *et al.* (2003). Roots of the whole plant were measured.

⁵Error is calculated using the higher SE of the two end members divided by the difference between the means of the two and multiplied by 100, indicating the detection limit of this method.

roots in wiregrass soil) to 14.5‰ (Douglas fir roots in prairie soil) (Table 2). The measurement errors, as expressed in coefficient of variation ranged from 2.5% to 3.6%. A treatment difference of approx. 10% of the mean would be detected as statistically significant. Therefore, the detection power of this method would be reduced if the difference between the $\delta^{13}\text{C}$ values of the two end members was smaller or the measurement errors were larger. Application of this method would require prior knowledge of the relevant information in order to avoid unexpected low detection limits.

To contrast root respiration rates measured in all three experiments, a Q_{10} value of 2 was used to convert respiration rates measured at different temperatures to rates at the same temperature of 25°C. In addition to temperature, root nitrogen content tended to be another important determinant of root respiration rate (Burton *et al.*, 2002). Root respiration rates were expressed as mg of CO₂-C per mg of root N per day at a standard temperature of 25°C, so that rates from different root systems with different N contents could also be compared (Table 3, Figs 2 and 3).

Root respiration rates differed significantly among different tree species in the same medium. The respiration rate of cottonwood roots growing in the prairie soil more than doubled the root respiration rates of coniferous trees (Table 3, Fig. 2). However, when measured in sand–vermiculite mixture, the root respiration rate of cottonwoods was similar to the rate of ponderosa pine roots, but significantly higher than the rates of longleaf pine roots and Douglas fir roots. Each root–soil coupling gave different respiration rate. This result further indicated that appropriate root–soil coupling is an essential requirement for any realistic assessment of root respiration.

Respiration rates of longleaf pine roots were measured using both the chamber plus isotope method in the forest and the root excision method under laboratory conditions. Three media were used in the chambers: C₄ soil under long-term wiregrass patch, C₄ from tallgrass prairie, and sand–vermiculite mixture (carbon-free). The overall mean of hourly field temperature measurements in the chambers was 24.5°C during the whole measurement period, with low diurnal and daily variation, as indicated by the standard deviation of 1.56. The

Table 3 Root respiration rates of four tree species (longleaf pine (*Pinus palustris*), Douglas fir (*Pseudotsuga menziesii*), ponderosa pine (*Pinus ponderosa*) and cottonwood (*Populus fremontii*) as measured using two different methods and three different growth media

Plant species	Medium	Temperature (°C)	Method	Respiration rate	SE
Longleaf pine	Wiregrass soil ¹	25	Root chamber	0.782	0.062
Longleaf pine	C ₄ prairie soil ²	25	Root chamber	0.269	0.113
Longleaf pine	Sand + vermiculite ³	25	Root chamber	0.179	0.048
Douglas fir	C ₄ prairie soil	25	Root chamber	0.249	0.028
Douglas fir	Sand + vermiculite	25	Root chamber	0.327	0.016
Ponderosa pine	C ₄ prairie soil	25	Whole plant	0.337	0.019
Ponderosa pine	Sand + vermiculite	25	Whole plant	0.565	0.077
Cottonwood	C ₄ prairie soil	25	Whole plant	0.833	0.108
Cottonwood	Sand + vermiculite	25	Whole plant	0.617	0.035

Each respiration rate ($\text{mg CO}_2\text{-C mg}^{-1}$ root N d^{-1} at 25°C) value is a mean of five replicates.

¹Soil from long-term wiregrass patches.

²Soil from tallgrass prairie in Kansas.

³Acid-washed sand plus burned vermiculite with half-strength Hoagland nutrient solution.

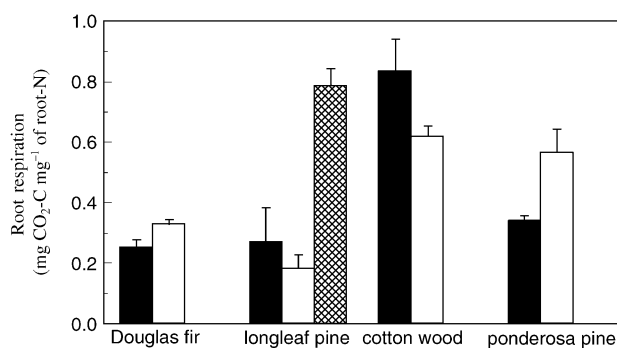


Fig. 2 Root respiration rates of four tree species, as measured in three kinds of rooting media: C₄ prairie soil (closed bars), sand and vermiculite mixture (open bars) and wiregrass soil (crossed bars). All rates were adjusted to a standard rate at 25°C assuming $Q_{10} = 2$. All error bars represent ± 1 SE ($n = 5$).

same temperature (24.5°C) was also maintained in the respiration measurements using excised roots. Compared with the chamber method using different rooting media, the root excision method gave a significantly lower respiration rate for the roots grown in the wiregrass soil, a significantly higher respiration rate for roots grown in the sand–vermiculite mixture and a similar rate for roots grown in the tallgrass prairie soil (Fig. 3). Other studies (Dwivedi, 2000; Cabrera & Saltveit, 2003) suggested that physiological responses to injury from excision may increase the respiration rate, such as observed for roots grown in the sand–vermiculite mixture relative to the chamber method. These differences may have resulted from the termination of the carbohydrate supply and other disturbances from root excision. The lower rates measured by the root excision method may have also been caused by the exclusion of mycorrhizal associations and a reduction of root exudation after excision. Both components are closely coupled with carbohydrate supply and the above-ground photosynthetic activity (Rygielwicz & Andersen, 1994; Rygielwicz

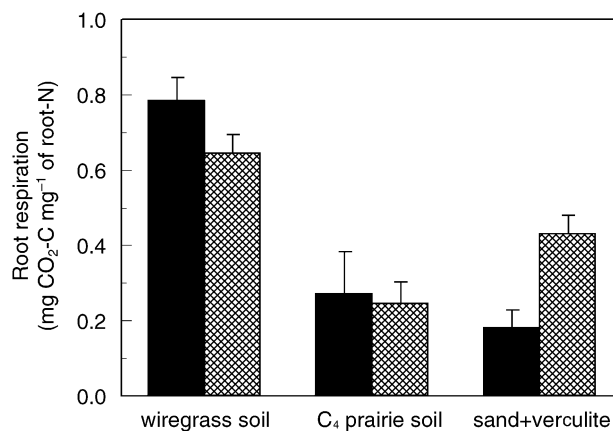


Fig. 3 Root respiration rates of longleaf pine (*Pinus palustris*) trees as measured by the natural tracer methods (closed bars) and the excision method (hatched bars) using three kinds of media. All rates were adjusted to a standard rate at 25°C assuming $Q_{10} = 2$. All error bars represent ± 1 SE ($n = 5$).

et al., 1997; Högberg *et al.*, 2001; Kuzyakov & Cheng, 2001). However, some laboratory studies show that root respiration rates measured after excision remain similar to that observed before excision (Lambers *et al.*, 1981; Lee *et al.*, 2003; Lipp & Andersen, 2003).

The effect of rooting media on the measured respiration rates of longleaf pine was consistently higher than the difference caused by the two methods (Fig. 3). Both methods produced a significantly higher respiration rate for roots grown in the 'native' soil from under wiregrass patches than roots grown either in the tallgrass prairie soil or the sand–vermiculite mixture. In the chamber measurements, root respiration rate with the prairie soil and with the sand–vermiculite mixture was only 34% and 12%, respectively, of that measured in the native wiregrass soil. These percentages increased to 41% and

51%, respectively, when the root excision method was used. In another field measurement with Douglas fir trees, the rate of root respiration in the prairie soil medium was 9.3% lower than in the sand-vermiculite mixture (Fig. 2). In the greenhouse experiment, 2-yr-old ponderosa pine trees and 1-yr-old cottonwood trees were grown in both tallgrass prairie soil and sand-vermiculite mixture. The respiration rate of the whole root system of ponderosa pine trees grown in the tallgrass prairie soil was significantly lower than those grown in the sand-vermiculite mixture. By contrast, the respiration rate of cottonwood roots was significantly higher when grown in the tallgrass prairie soil than in the sand-vermiculite mixture. These results indicated that the rooting media played a critical yet inconsistent role in determining the measured tree root respiration rates. Root respiration rates could not be assessed reliably and realistically using methods without appropriate soils as rooting media. In addition to setting temperature and moisture conditions, soils were known to have crucial controls on many important processes such as rhizosphere microbial associations, nutrient availability, and physical and chemical matrix. As stated in the Introduction section, tree root respiration was defined as the total rhizosphere respiration including physiological root respiration and rhizosphere microbial respiration that originated from the microbial utilization of substrates from roots. Because rhizosphere microbial associations, especially mycorrhizas, could contribute as much as 60% of the total rhizosphere respiration (Cheng *et al.*, 1993; Rygiel & Andersen, 1994; Högberg *et al.*, 2001), different rooting soils would affect root respiration rates by changing the microbial components as well as root physiology. Soil conditions would impose equal, if not more, control on rhizosphere microbial associations (Bachmann & Kinzel, 1992). Better characterization of rhizosphere microbial associations, especially mycorrhizal fungi, in future studies would help to explain the real causes of these differences in measured root respiration rates.

The ranges of respiration rates were similar between the field experiments and the greenhouse experiment (0.2–0.78 mg CO₂ C mg⁻¹ root N d⁻¹ at 25°C for chamber, 0.3–0.83 mg CO₂ C mg⁻¹ root N d⁻¹ for whole plant). This seemed to indicate that the disturbance associated with root isolation and chamber installation did not seriously affect root physiology after the period of acclimatization.

The rates of fine root respiration determined in these three experiments were well within the ranges of reported respiration rates of excised tree roots (Burton *et al.*, 1997; Pregitzer *et al.*, 1998; Widén & Majdi, 2001), of whole-root systems of seedlings grown in liquid media (Qi *et al.*, 1994) and of washed root systems attached to mature trees with or without artificial media (George *et al.*, 2003). The range of published fine tree root respiration rates in the literature might have been unrealistically wide because drastically different methods were used in different studies. For example, in the mini-review by George *et al.* (2003), the range spanned three to four orders

of magnitude from the lowest value of 0.04 nmol CO₂ g⁻¹ root s⁻¹ at 15°C for Douglas fir roots (McCreary & Zaerr, 1987) to the highest value of 35.61 nmol CO₂ g⁻¹ root s⁻¹ at 15°C for *Picea engelmannii* (Sowell & Spomer, 1986). The wide spread of reported fine root respiration rates alone is an indication of the urgent need for better methods for assessing tree root respiration.

All existing approaches for assessing tree root respiration are plagued with methodological problems. The root excision approach is the simplest and easiest to use, thereby producing the majority of root respiration data in the literature. This approach has been used to demonstrate that root N content is an important determinant of root respiration rate in addition to temperature during the measurement (Burton *et al.*, 2002). However, the ecological relevancy of this method is questionable because of the inherent high degree of disturbance and the associated artifacts, especially the possible wound response, the exclusion of rhizosphere microbial associations and the absence of realistic soil conditions. In order to avoid some of these problems, root chambers or cuvettes have been applied to roots that remain attached to the tree after being carefully evacuated and cleaned (often by rinsing) (George *et al.*, 2003; Lipp & Andersen, 2003). By using attached roots, this approach may prevent the alteration of respiration resulting from a wound response. However, the other problems associated with disturbances during root evacuation and measurements remain, even though the environment in the chamber can be controlled according to the predominant field conditions. Another common concern with these approaches is the absence of an appropriate medium for maintaining a realistic rhizosphere environment and therefore a realistic respiration value.

Another commonly used method to measure root respiration is soil trenching. This approach assumes that root respiration is absent in the trenched plot (or 'root-free' plot) and the CO₂ efflux from the trenched plot realistically represents the respiration rate of soil microbial communities when roots are present and remain intact. If this assumption is valid, the difference in total CO₂ efflux rates between the trenched plot and the intact control plot is a measure of root respiration. However, this assumption has often been shown to be invalid (Tate *et al.*, 1993; Uchida *et al.*, 1998; Lee *et al.*, 2003), because soil trenching introduces a series of disturbances, including root excision, subsequent root decomposition, and an often increased soil moisture owing to the absence of water removal by transpiration. Given all these concerns, soil trenching does offer an estimate of root respiration at the field plot scale, and therefore a closer link to ecosystem-level C flows.

Compared with existing methods, the root chamber method coupled with natural ¹³C tracers has several advantages. By using natural isotope tracers, real soils can be used as rooting media, instead of commonly used nutrient solution or artificial media. Clean sampling of CO₂ (i.e. free of contamination from atmospheric CO₂), is assured by using a closed system during

sampling. Both the chamber and the CO₂-trapping system are portable and suited for any field conditions. After chamber installation, rhizosphere respiration in a root chamber can be measured nondestructively at daily to annual time scales with little additional disturbance. The amount of roots in each chamber can be quantified initially and can be measured by sequential harvests afterwards. Integration of this ¹³C tracer method with other chamber methods (Espeleta *et al.*, 1999), such as the sand chamber method used in quantifying biochemical markers of external mycelium of ectomycorrhizal fungi in the field (Wallander *et al.*, 2001), may offer new opportunities for further deciphering the complex interactions in the rhizosphere under more realistic conditions.

This new approach also has several limitations. First, this method needs compatible rooting medium in the chamber. This requirement is made clear by the results of this study. Because of this requirement, the general applicability of this method is limited to situations that have large enough differences in the δ¹³C values between the two end members (see Table 2). This method is most suitable for ecosystems that have C₃ and C₄ plants coexisting naturally or by design, such as the longleaf pine–wiregrass ecosystem used in this study, a tropical savanna which has C₃ trees and C₄ grasses coexisting and woody plants recently invaded into C₄-dominated grasslands. Second, the disturbances during initial chamber installation are inevitable, which can negatively impact the ecosystem and necessitate an adequate acclimation period to recover. Third, a long-lasting leak-proof seal at the root–chamber junction is a critical part of the operation, which demands a suitable live root system that has a flexible suberized base for such a seal to work. This means that the respiration measurement in the chamber has to be for roots of mixed branching orders. This difficulty can potentially be overcome through miniaturizing the current design. Fourth, the respiration rate of live roots can be in error if there is a large enough input from dead roots after chamber installation. This may be a serious problem particularly for trees that have faster root turnover rates or experiments that require a long period of operation. Quantifying total rhizodeposits along with the respiration measurement may be used to partly correct for this error. Fifth, like other root chamber methods, this method is difficult to use with roots located in deeper soil horizons because of the high degree of associated soil disturbance that may require much longer acclimatization period to recover than the method allows. Finally, isotope analysis involves more sample preparation and greater cost.

Given the advantages and the limitations discussed above, this method moves a step forward towards ecological reality in rhizosphere research, and offers some opportunities for comparisons with existing methods. This method can also be used to test some of the critical assumptions, either explicit or implicit, with the existing methods briefly mentioned earlier in this paper. The potential and the problems inherited with this method await more tests in future utilization of it.

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