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ORIGINAL ARTICLES

Synthesis of a ^{13}C-Labeled Tracer for Stream DOC: Labeling Tulip Poplar Carbon with $^{13}CO_2$

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ABSTRACT

Ecosystem tracer-level additions would benefit from a stable isotope-labeled source of complex organic molecules. We tested a method to label tree C with ^{13}C and create a stable isotope tracer for stream dissolved organic carbon (DOC) using tulip poplar (*Liriodendron tulipifera L.*) seedlings. In 2000, seedlings were grown with 0.82 moles of $^{13}CO_2$ to assess the distribution and level of ^{13}C enrichment in the tree tissues. In 2001, seedlings were grown with 25 times more $^{13}CO_2$ to generate tissues with a ^{13}C signal strong enough for a ^{13}C-DOC stream tracer addition. ^{13}C enrichment in the trees varied in each year and by tissue age and type. Tissues formed during labeling (new) were more enriched in ^{13}C than tissues established prior to the $^{13}CO_2$ injection (old). Stems were most enriched in ^{13}C in both new and old tissues. A higher percentage of $^{13}CO_2$ was incorporated into seedlings in 2000 (59% ±1) than 2001 (43% ±0). Percent ^{13}C incorporation among tree tissue types paralleled biomass distributions. Although tree C and ^{13}C were equally soluble in both years, a greater percentage of tree C went into solution in 2001 (30%) than 2000 (20%). The water-soluble tree C accounted for approximately 12% of the injected $^{13}CO_2$ and had both humic and polysaccharide components. Results from a whole-stream ^{13}C-DOC tracer addition demonstrated that tree C could be sufficiently labeled with $^{13}CO_2$ to create a stream DOC isotope tracer with some polymeric constituents.

Key words: dissolved organic carbon; streams; carbon 13; stable isotope tracers; trees; leachate; tree carbon allocation.

INTRODUCTION

Stable and radioactive isotopes provide useful tracers in aquatic ecology and biogeochemistry to elucidate flow paths, transformations, and sources of dissolved and particulate materials, as well as to measure rates of biological and chemical processes (Newbold and others 1983; Bower and others 1987; Michener and Schell 1994; Hall 1995; Peterson and others 1997). Whole ecosystem tracer-level additions are a relatively new approach employing isotopes to examine elemental cycles and food webs in lakes, streams, and estuaries (for example, Newbold and others 1983; Hall 1995; Peterson and others 1997; Hughes and others 2000; Tank and others 2000; Cole and others 2002). This approach has focused primarily on nitrogen ($^{15}NH_4^+$, $^{15}NO_3^-$) and phosphorus ($^{32}PO_4^{-3}$) dynamics in streams (for example, Newbold and others 1983; Peterson and others 1997; Tank and others 2000; Peterson and others 2001) and estuaries (Holmes and others 2000; Hughes and

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others 2000); C cycling has been examined to a lesser degree (Hall 1995; Hall and Meyer 1998; Cole and others 2002). Complex mixtures of ^{14}C-labeled compounds from algal and wetland plant leachates have been used to examine C dynamics in laboratory experiments (for example, Wetzel and others 1995; Tranvik and Kokalj 1998), however, radiolabeled mixtures like these are not typically released into the environment. To date, the flow of organic C through lotic and lacustrine food webs has been tracked with stable isotopes using either an inorganic substrate for photosynthesis ($H^{13}CO_3^-$) or a monomeric labile organic molecule (^{13}C-acetate) (Hall 1995; Hall and Meyer 1998; Cole and others 2002). The intricacies of the C cycle, however, cannot be completely elucidated with either bicarbonate or acetate as tracers because stream dissolved organic carbon (DOC) comprises a mixture of compounds primarily of terrestrial origin that vary in their biological availability (Thurman 1985; Kaplan and Newbold 1993; Meyer and others 1998; Palmer and others 2001; Kaplan and Newbold 2003). Thus, to examine stream organic matter dynamics, we labeled deciduous trees with $^{13}CO_2$ to create a stable isotope tracer more representative of stream DOC. Deciduous leaf litter leachate like stream DOC is a complex mixture of organic compounds with substantial polymeric character and various biodegradable fractions (Cummins and others 1972; McClaugherty 1983; Thurman 1985; Qualls and others 1991; Hongve 1999). We recognize that leaf litter leachate is not a perfect model for stream DOC; it has not been extensively processed in the soil like most DOC entering streams. Nevertheless, we feel that it is a good tool to begin measuring *in situ* stream dynamics given that DOC leached from leaf litter can account for 30% of the daily DOC export from small forested streams on an annual scale and can comprise 42% of the DOC inputs in the fall (McDowell and Fisher 1976; Meyer and others 1998).

Numerous studies have isotopically labeled tree C, focusing on the physiology of photosynthate allocation (Schier 1970; Webb 1977; Isebrands and Nelson 1983; Smith and Paul 1988; Horwath and others 1994). To our knowledge, trees have not been isotopically labeled to generate a product for an environmental tracer experiment. Most tree C labeling studies have used ^{14}C (that is, Schier 1970; Webb 1977; Isebrands and Nelson 1983; Smith and Paul 1988; Horwath and others 1994) rather than ^{13}C (Maillard and others 1994; Vivin and others 1996; Simard and others 1997) because of its lower cost and widely available sensitive detection methods; ^{14}C has a six-orders of magnitude lower detection limit than ^{13}C. For health, safety, and environmental reasons, radiolabeled C is generally not used for field releases (Hesslein and others 1980; Bower and others 1987). However, with new methodology and instrumentation advances, the environmentally safe ^{13}C is now being used as a tracer.

Table 1. Environmental Conditions in Growth Chambers for Years 1 and 2 Tree C Labeling Experiments

Conditions	Years 1 & 2
Photoperiod (h)	13
Irradiance (μmol photons m^{-2}/s^{-1} PAR)	1250
Day/Night Temp. (°C)	27/20
Relative Humidity (%)	70
CO_2 Conc. (ppm)	360

The overall goal of our study was to generate a mixture of terrestrially derived, ^{13}C-labeled DOC molecules that could be used to examine *in situ* stream DOC dynamics associated with fresh tree litter inputs. Here, we present a method to label tree C with $^{13}CO_2$ and test the efficacy of using labeled leachate from the trees in a whole-stream DOC tracer addition.

Methods

Trees were labeled with ^{13}C in two different years (2000, 2001) at the National Phytotron located at Duke University, Durham, North Carolina. During 2000 (Year 1), tree seedlings were grown with 0.82 moles of $^{13}CO_2$ to assess the distribution and level of ^{13}C enrichment. During 2001 (Year 2), tree seedlings were grown with 25-fold more $^{13}CO_2$ to generate tree tissues with a strong enough ^{13}C signal for a ^{13}C-DOC stream tracer addition. In 2002, we used leachate generated from Year 2 ^{13}C-labeled tree tissues in a whole-stream isotope tracer addition.

Tree Cultivation

Thirty-two one-year-old bare root tulip poplar (*Liriodendron tulipifera L.*) seedlings were planted prior to leaf out in 10 L (16-cm diameter, 51-cm depth) PVC containers filled with a 3:1 (v/v) mixture of sterilized gravel and vermiculite. The seedlings were placed in a 1.2 m × 2.4 m × 2.1 m walk-in plant growth chamber. The trees were watered to saturation twice daily, using one-half strength Hoagland's solution in the morning and distilled water in the afternoon (Downs and Hellmers 1975). The photoperiod, irradiance, temperature, relative humidity, and atmospheric CO_2 concentration in the chamber were the same during Years 1 and 2 (Table 1). As

Table 2. $^{13}CO_2$ Injection Rates for Years 1 and 2 Tree C Labeling Experiments

Year	DAP	Daily Length of Injection (h)	Injection Rate (L $^{13}CO_2$ d^{-1})	Moles of 99 atom % ^{13}C-CO_2 Injected	$\delta^{13}C$ Bkg. CO_2
Year 1	0–52	0	0	0	−8
	53–66	8	0.48	0.27	1.8
	67–71	8	0.72	0.15	1.8
	72–78	8	0.72	0.20	4.66
	79–84	8	0.96	0.19	4.66
	Total			0.82	
Year 2	0–50	0	0	0	−8
	51	13	5.1	0.21	−44
	52–64	13	6.2	3.27	−44
	65–69	13	7.8	1.58	−44
	70–76	13	8.3	2.36	−44
	77–86	13	10.1	4.1	−44
	87–98	13	12.5	6.09	−44
	99–107	13	11	3.57	−44
	Total			21.2	

(DAP = days after planting). $\delta^{13}C$ values for the background (Bkg.) CO_2 in the growth chambers for Years 1 and 2 are shown.

soon as the seedlings were planted, CO_2 was injected into the growth chamber to maintain the CO_2 concentration set point (Tables 1 and 2). This CO_2 provided the background CO_2 in the growth chamber (Table 2; Bkg. CO_2); $^{13}CO_2$ was later added to the chamber air to label the trees with ^{13}C.

Year 1

At 52 days after planting (DAP), the locations of the youngest fully expanded leaf on each plant's main stem and branches were marked to delineate aboveground plant tissues that formed prior to the $^{13}CO_2$ injection (old tissues) from those that formed during the injection (new tissues). Immediately before the injection, an automatic watering system was installed and the chamber was sealed to minimize $^{13}CO_2$ loss. $^{13}CO_2$ was injected into the chamber from a lecture bottle of 99 atom % ^{13}C-CO_2 (Cambridge Scientific Laboratories) for 8 h during daylight starting on 53 DAP. A mass flow valve (0–10 sccm) was used to meter the $^{13}CO_2$ injection rate into the chamber and help mix the $^{13}CO_2$ with the chamber air (Table 2). Eighty-four DAP, the seedlings were harvested and their leaf, stem, and root tissues were separated into old and new tissues. Old and new leaf and stem tissues were separated using the marks placed on the seedlings prior to the $^{13}CO_2$ injection. Root tissue was separated into two portions following washing; the upper four-fifths and the lower fifth of the root ball were considered old and new tissues, respectively. All tissues were dried to a constant weight at 60°C.

Year 2

At 24 DAP, seven seedlings were performing poorly and replaced with pot-grown seedlings of the same age. At 42 and 50 DAP, the trees were sprayed with Safer's® sulfur spray to control powdery mildew. Fifty DAP, the trees were marked to distinguish old and new tissues using the same protocol as in Year 1. The $^{13}CO_2$ injection began 51 DAP following the protocol from Year 1 with minor modifications to the daily injection period and rate (Table 2). One hundred and seven DAP, the seedlings were harvested and their leaf, stem, and root tissues were separated into old and new tissues. Old and new leaf, stem, and root tissues were separated and dried according to the protocol from Year 1.

Tree Tissue Preparation

Dried tissues were ground in a Wiley Mill through a 250-μm mesh sieve prior to the cold water extraction and isotope analysis. Tree tissues were ground from least to most enriched (old to new) and the mill was cleaned between samples. Composite samples (30 to 100 mg) of each tissue type were prepared for isotope analysis from equal aliquots of ground tissues from all 32 seedlings.

Cold Water Extraction of New Tree Tissues

In Year 1, approximately 3 g of both ground new leaves and stems were added to 1 L of 0.2-μm filtered (polyethersulfone membrane filter, Gelman Su-

por®-200) de-ionized water (DIW). The water and plant material were gently stirred with a Teflon-coated stir bar in a 2-L glass container in the dark at 4°C for 24 h. After 24 h, the water and plant material were centrifuged at 17,931 × g for 0.5 h. The supernatant liquid was sequentially filtered through DIW-rinsed paper (Whatman 4) and GF/F (Whatman) filters. Sub-samples of the leachate were taken for DOC concentration (total DOC = ^{12}C-DOC + ^{13}C-DOC), dissolved total saccharides (DTS) and free monosaccharides (DFMS) (0.2-μm filtered; HT Tuffryn® membrane Acrodisc®), humic substances, and δ^{13}C-DOC isotope analyses. DOC and humic substance samples were analyzed immediately following collection and isotope and DFMS samples were stored frozen until analysis. DTS samples were acidified to pH 1 with 6 N HCl and stored at 4°C until analysis. All glassware and GF/F filters were baked at 500°C for 6 h prior to use.

In Year 2, approximately 1 g of ground new tree tissue (60.9% leaves, 24.6% stems, 14.5% roots; % weight of new tissues) was added to 1 L of 0.2-μm filtered DIW. The procedure for cold water extraction was similar to that used in Year 1 with the exception that the leachate was only filtered through a GF/F filter following centrifugation. Samples of the tree tissue before leaching, as well as the leachate were taken for C, DOC, and ^{13}C isotope analyses. Samples of the leachate were also taken for humic substance and carbohydrate analyses. DOC, humic substance, carbohydrate, and isotope samples were stored following Year 1 procedures.

^{13}C-DOC Whole-Stream Tracer Addition

In October 2002, 120 L of ^{13}C-labeled tree tissue leachate (120 mg C L^{-1}; δ^{13}C 6160) were released into White Clay Creek, a third-order forested piedmont stream located in southeastern Pennsylvania (39°53′N, 75°47′W), and its concentration was measured along a 1265-m reach at eight stations. Tree tissue leachate was prepared using Year 2 new tree tissues; approximately 4 g of tissue (60.9% leaves, 24.6% stems, 14.5% roots; % weight of new tissues) were added to 4 L of 0.2-μm (polyethersulfone membrane, Gelman Supor®-200) filtered DIW. The procedure for cold water extraction was similar to that used in Year 2 with the exception that the leachate was Tyndallized for 0.5 h in a 70°C water bath following GF/F filtration. Twenty-four hours after Tyndallization, the process was repeated to ensure the biological stability of the leachate. The leachate was stored in 2-L sterile plastic containers in the dark at 4°C until the stream injection. The ^{13}C-DOC was injected into the stream over the course of 115 min using a peristaltic pump at the rate of 1062 ml min^{-1}. After the ^{13}C-DOC was thoroughly mixed in the stream water (~60 min after the pump started) and its concentration stabilized (plateau period), water samples for DOC and ^{13}C-DOC were collected at the eight stations and filtered through GF/F filters (Stream Solute Workshop 1990). DOC samples were immediately analyzed following collection and ^{13}C-DOC samples were stored frozen until analysis. A further description of data analysis to estimate ^{12}C-DOC uptake from the ^{13}C-DOC release is beyond the scope of this manuscript (Wiegner and others unpublished data).

Analytical Measurements

DOC was measured by Pt-catalyzed persulfate oxidation using either an OI 700 or an OI 1010 (O.I. Corp.) carbon analyzer (Kaplan 1992). Humic substances were measured using XAD-8 resin chromatography (Stanley and Kaplan 1998). Humic carbon was defined as the difference between DOC concentrations of the sample and XAD-8 resin effluent. Dissolved carbohydrates were assayed using high-performance liquid chromatography and pulsed amperometric detection (Cheng and Kaplan 2001, 2003). DFMS were measured on samples without pretreatment and DTS were measured following acid hydrolysis. Dissolved polysaccharide concentrations were calculated as the difference between the DTS and DFMS concentrations.

Tree tissue samples were prepared for isotope analysis and subsequently combusted by a modified Dumas technique (Macko and others 1987). Preparation of DOC for isotope analysis followed the method of Feuerstein and others (1997), except that the samples were not dialyzed. The gases resulting from the Dumas combustion of tree tissues and DOC were purified by cryogenic gas separation and subsequent isotope determinations were performed using either a dual inlet on a PRISM (Micromass) stable isotope ratio mass spectrometer or an Eurovector elemental analyzer interfaced to a ISOPRIME (Micromass) stable isotope mass spectrometer (Wong and others 1992). The elemental analyzer method yielded results (average ±S.D.) that were within 0.14‰ (±0.15) of the modified dual inlet method (Feuerstein and others 1997) at natural abundance levels and 0.024 atom % (±0.037) at enriched levels (Gandhi and others 2004). Stable carbon isotope ratios were expressed as:

$$\delta^{13}\mathrm{C} = [(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1] \times 1000 \qquad (1)$$

where R is ^{13}C/^{12}C in the samples and standard. The

Table 3. Dry Weight and Average (±S.D.) Carbon Content, δ^{13}C, Atom %, and Moles of ^{13}C from the $^{13}CO_2$ Tank (n^{13}C) in the Old and New Tree Tissues from Years 1 (n = 2) and 2 (n = 3) Tree C Labeling Experiments

Year	Tree Tissue	Dry Wt. (g)	%C	δ^{13}C	Atom %	n ^{13}C
Year 1	Old Leaves	364	48 (±3)	81 (±0)	1.20 (±0.00) c[a]	0.02 (±0.00)
	Old Stems	651	49 (±4)	217 (±5)	1.35 (±0.00) a	0.07 (±0.00)
	Old Roots	1436	39 (±0)	114 (±5)	1.24 (±0.01) b	0.07 (±0.00)
	New Leaves	1065	53 (±6)	317 (±16)	1.46 (±0.02) b	0.18 (±0.01)
	New Stems	461	51 (±1)	471 (±16)	1.63 (±0.02) a	0.11 (±0.00)
	New Roots	333	35 (±3)	197 (±0)	1.33 (±0.00) c	0.03 (±0.00)
	Total	4310				0.48
Year 2	Old Leaves	249	37 (±1)	3757 (±7)	5.07 (±0.01) c	0.31 (±0.00)
	Old Stems	777	42 (±1)	4888 (±19)	6.21 (±0.02) b	1.40 (±0.01)
	Old Roots	2058	40 (±8)	5088 (±3)	6.40 (±0.00) a	3.74 (±0.00)
	New Leaves	1072	43 (±1)	5986 (±29)	7.28 (±0.03) c	2.42 (±0.01)
	New Stems	436	43 (±0)	6143 (±20)	7.43 (±0.02) a	1.01 (±0.00)
	New Roots	256	38 (±1)	6042 (±14)	7.33 (±0.01) b	0.51 (±0.00)
	Total	4848				9.39

[a]*Parameter means with different letters within a year's age group (old and new tissues) are significantly different at* $\alpha = 0.05$ *using Tukey's HSD mean separation procedure.*

standard used was V-PDB and the reproducibility of these measurements at natural abundance levels was not more than 0.2‰ and less than 0.03 atom % for enriched values (Table 3). The concentration of organic carbon in tree tissue samples was estimated using either a calibrated baratron capacitance manometer (MKS Instruments) during gas separation or the Eurovector elemental analyzer.

Calculations

^{13}C enrichment of the tree tissues (fractional abundance, F) was calculated from measured δ^{13}C values and is reported as atom % (atom % = $F \times 100$), where:

$$F = R/(R+1) = {}^{13}\mathrm{C}/({}^{12}\mathrm{C} + {}^{13}\mathrm{C}) \qquad (2)$$

These carbon isotope data were used in a two-source mixing model to provide estimates of the fraction of ^{13}C from the enriched CO_2 tank incorporated by the trees. The equation derived is similar to that used by Johnston and others (1997):

$$TDC = [(F_T - F_{CT})/(F_{CO2} - \Delta - F_{CT})] \qquad (3)$$

where TDC (tank derived carbon) is the fraction of carbon from the ^{13}C-enriched CO_2 tank in a given sample of the enriched tree tissue, F_T is the measured fractional abundance of ^{13}C in the enriched tree tissue (Table 3), F_{CT} is the fractional abundance of ^{13}C in the control tree tissue end member (0.010815, the average F for deciduous trees grown with atmospheric CO_2; Broadmeadow and Griffiths 1993), F_{CO2} is the fractional abundance of ^{13}C in the $^{13}CO_2$ tank (0.99 in Year 1; 0.9994 in Year 2), and Δ is a photosynthesis fraction factor expressed in ^{13}C fractional abundance terms (0.011332; Fogel and Cifuentes 1993). The moles of C in the tree tissues derived from the $^{13}CO_2$ tank (n^{13}C) were calculated as:

$$n^{13}\mathrm{C} = TDC \times M_T \qquad (4)$$

where M_T is the total moles of C in the enriched tree tissues (Table 3). M_T was calculated for each tree tissue type using their respective δ^{13}C value, carbon content, and dry weight (Table 3). To calculate the ^{13}C labeling efficacy of the tulip poplar C, we first assumed that all the C in the tree tissues derived from the $^{13}CO_2$ tank was ^{13}C and then applied the following equation:

$$\%^{13}\mathrm{C}\text{ - Labeling Efficacy } = (\mathrm{n}^{13}\mathrm{C}_T/\mathrm{n}^{13}\mathrm{CO}_2) \times 100 \qquad (5)$$

where $n^{13}C_T$ is the sum of all the moles of C in the trees derived from the $^{13}CO_2$ tank (old and new) and $n^{13}CO_2$ is the number of ^{13}C moles injected from $^{13}CO_2$ tank into the growth chamber (Table 2).

Statistics

Atom % and % ^{13}C labeling efficacy data for Years 1 and 2 tree tissues were analyzed by analysis of variance (ANOVA) using SAS (version 8.1; GLM). Post hoc analyses were performed using Tukey's Studentized Range test ($\alpha = 0.05$).

Results

^{13}C enrichment in the tulip poplar trees varied in each year ($P < 0.0001$) and by tissue age ($P <$

0.0001) and type ($P \leq 0.0006$). Year 2 tree tissues were five times more enriched with ^{13}C than Year 1 tissues; this enrichment corresponded to a 20-fold increase in the number of moles of ^{13}C in the tree tissue derived from the $^{13}CO_2$ tank and a 25-fold increase in the number of $^{13}CO_2$ moles injected into the growth chamber during Year 2 (Table 2 and 3). New tissues were more enriched in ^{13}C than old tissues ($P \leq 0.003$) and stems were generally the most enriched tissue type (Table 3). In Year 1, ^{13}C comprised 1.26 atom % (±0.07) and 1.47 atom % (±0.13) of the C within the old and new tree tissues, respectively. In Year 2, ^{13}C comprised 5.89 atom % (±0.72) and 7.06 atom % (±0.57) of the C in the old and new tissues, respectively.

Overall, a higher percentage of the injected $^{13}CO_2$ was incorporated into the trees in Year 1 (59% ±1) than in Year 2 (43% ±0) ($P < 0.0001$; Figure 1A, B). At the whole tree scale (old + new tissues) each year, a similar percentage of $^{13}CO_2$ was found in the leaves and stems, which differed from that found in the roots ($P \leq 0.0008$). The roots had a smaller percentage of the $^{13}CO_2$ than the leaves or stems in Year 1 (Figure 1A) and a higher percentage of the $^{13}CO_2$ in Year 2 (Figure 1B). We observed distinct patterns of preferential plant tissue labeling in both years when considering old and new tissues separately ($P < 0.0001$). Old root tissues incorporated the greatest percentage of ^{13}C followed by stems and leaves ($\alpha = 0.05$) whereas new leaf tissues incorporated the most ^{13}C followed by stems and roots ($\alpha = 0.05$). Percent ^{13}C incorporation parallels the biomass distribution among the different tree tissues (Table 3; Figure 1A, B).

Tree C and ^{13}C were equally water-soluble in Years 1 and 2 (Table 4). Overall, a higher percentage of tree C was water-soluble in Year 2 than in Year 1 (Table 4). The percent of ^{13}C that was water-soluble in Year 1 and 2 represents 12% and 13% of the $^{13}CO_2$ injected into the growth chamber, respectively. Humic substances comprised a similar percent of the water-soluble tree C in Years 1 (24% ±2) and 2 (26% ±2). Likewise, a similar percent of the water-soluble tree C was carbohydrate-C in Years 1 (14% ±0.5) and 2 (13% ±0.2), with monosaccharides and polysaccharides representing 5% (±2) and 8% (±1) of this C (average of both years), respectively (Table 4). The dominant carbohydrates in the tree tissue leachates, expressed as % of DTS C-concentration (average of both years), were glucose (63% ±1), rhamnose (14% ±0), galactose (11% ±1), fructose (9% ±1), and mannose (2% ±1). All of the rhamnose, galactose, and mannose were polysaccharide-derived whereas all of the fructose was monosaccharide. Glucose was found in both the mono- (23% ±8) and polysaccharide fractions (40% ±8).

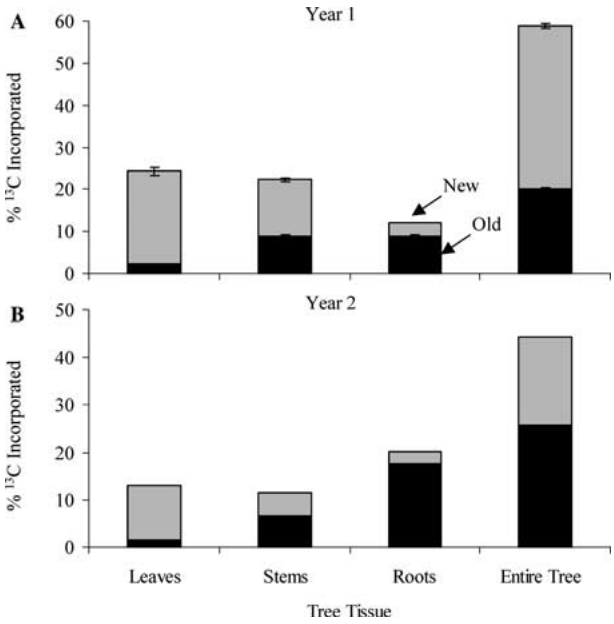

Figure 1. Average (±S.D.) percent ^{13}C incorporation into old and new tree tissues for (A) Year 1 and (B) Year 2. Percent ^{13}C incorporation is calculated as the percent of the $^{13}CO_2$ released into the growth chambers that was incorporated into the tree tissues.

The ^{13}C-DOC whole-stream tracer addition sufficiently enriched the DOC pool in White Clay Creek. The average background $\delta^{13}C$-DOC value for the stream prior to the injection was −25.8 (±0.4); the tracer addition elevated it up to 337 (±35; average of stations 1 and 2) and only increased the stream DOC concentration by less than 5% (±4), from a background of 1.48 (±0.06) mg C L^{-1} to a maximum of 1.55 (±0.03) mg C L^{-1} following the addition of the ^{13}C-DOC. The ^{13}C enrichment of the tree tissue leachate was strong enough for its signal to be easily detected 1265-m downstream from the injection site when the average stream discharge and width were 15.1 L s^{-1} and 3.3 (±1.4) m, respectively (Figure 2). The correction of these data for background ^{13}C-DOC concentration and stream water dilution, and the ultimate calculation of uptake length, mass transfer coefficient, and total DOC supply to the benthos are topics for a different manuscript.

Discussion

^{13}C Enrichment in Tree Tissues

The ^{13}C enrichment patterns we observed in the tulip poplar tissues were consistent with previous

observations of photosynthate allocation and natural abundance signals. Photosynthate generated during plant growth is allocated for growth and development of new tissues (Quinlan 1965), and this should explain the preferential enrichment of newly formed tissues compared to older tissues. Natural abundance studies have shown that stem/trunk tissues are more enriched in ^{13}C than leaf tissues (Leavitt and Long 1982, 1986; Schleser 1992; Terwilliger and Huang 1996; Arndt and Wanek 2002). For example, the trunks of juniper and beech trees were 1.2 to 5‰ heavier than the leaves (Leavitt and Long 1982; Schleser 1992). Placing our data in similar terms, old stems were 136‰ and 1131‰ heavier than old leaves and new stems were 154‰ and 157‰ heavier than new leaves in Years 1 and 2, respectively. Diffusion processes cannot explain this fractionation pattern because diffusion away from the leaves would enrich them in ^{13}C (Leavitt and Long 1982). The variant δ^{13}C signals among major biochemical components of plant tissues, which are a function of biosynthetic fractionation factors, may explain differences in the ^{13}C enrichment among the tree tissues examined here (Abelson and Hoering 1961; Benner and others 1987; Ghashghaie and others 2001; Fernandez and others 2003). Additionally, the role of newly developed phloem cells in the translocation of ^{13}C-enriched photosynthate from the canopy to leaves lower on the tree and roots may cause the trunk tissue to become more enriched in ^{13}C relative to the leaves adjacent to the trunk (Schleser 1992). In our study, stem tissues were comprised primarily of developing trunk.

Allocation of ^{13}C in Trees

During the growing season, leaves incorporate most of the new photosynthate while allocation to the stems and roots varies with tree type (Schier 1970; Webb 1977; Isebrands and Nelson 1983; Smith and Paul 1988; Horwath and others 1994). In our study, the new leaves had the highest percent of ^{13}C followed by the stems and roots, respectively (Figures 1A, B); this allocation pattern parallels the biomass distribution among the different tree tissues (Table 3). We expected this ^{13}C distribution pattern within the tulip poplars given that active regions of growth act as sinks for photosynthate (Hansen 1967).

C turnover in the new tissues resulted in a high percentage of ^{13}C in the old tree tissues. Overall, 20% and 26% of the $^{13}CO_2$ taken up by the tulip poplars was found in the old tissues for Years 1 and 2, respectively. In addition, the percent ^{13}C distribution in the old tissues was different from the pattern observed for the new tissues (Figures 1A, B); the roots had the greatest percentage of ^{13}C in them followed by the stems and then leaves. This percent ^{13}C incorporation pattern parallels the biomass distribution among the different tree tissues (Table 3) and is similar to C allocation patterns reported for deciduous trees during the fall using ^{14}C (Horwath and others 1994).

^{13}C Recovery

Controlled environment and pulse-chase experiments are commonly used to isotopically label tree C. In controlled environment experiments like ours, an entire tree is exposed to isotopically labeled CO_2 while its concentration is monitored and maintained (Webb 1977; Smith and Paul 1988; Horwath and others 1994). In contrast, during a pulse-chase experiment, a segment of a tree (that is, leaf, branch) is exposed to the C label until approximately 99% of it has been taken up (for example see, Balatinecz and others 1966; Dickson and Nelson 1982; Kuhns and Gjerstad 1991; Simard and others 1997; Mikan and others 2000). In studies employing both labeling techniques, young (seedlings to 2-years-old) trees are exposed to CO_2 for 30 min to 1 d, and harvested 8 h to 2 weeks after exposure (Balatinecz and others 1966; Dickson and Nelson 1982; Kuhns and Gjerstad 1991; Horwath and others 1994; Simard and others 1997; Mikan and others 2000). High (77%) C recoveries have been reported for controlled environment labeling experiments and are thought to result from complete C fixation under regulated labeling conditions and re-fixation of the labeled aboveground night respiration (Horwath and others 1994). C recoveries for pulse-chase experiments (24 to 75%) are more variable than controlled environment experiments, which may result from incomplete C fixation and high respiratory losses of the labeled C (Horwath and others 1994).

The ^{13}C recoveries observed for our experiments (43% and 59%) are within the range, albeit on the low side, reported for tree C labeling experiments employing both controlled environment and pulse-chase designs (Balatinecz and others 1966; Dickson and Nelson 1982; Kuhns and Gjerstad 1991; Horwath and others 1994; Simard and others 1997; Mikan and others 2000). Discrepancies in the C isotope incorporation between our study and other C labeling experiments may be due to differences in the C isotopes used, experimental designs (as discussed above), tree types, CO_2 exposure times, and tree tissue harvest times or may have resulted from

leaky chambers and/or unused $^{13}CO_2$ in the chamber atmosphere at the end of labeling. Photosynthetic and metabolic discrimination against ^{13}C is much less than that against ^{14}C (Van Norman and Brown 1952); therefore, it is unlikely that discrimination differences between ^{13}C and ^{14}C were responsible for our low label recovery. Loss of the ^{13}C to aboveground respiration, the soil atmosphere, and/or root exudation may have contributed to our low recoveries; however, the ^{13}C lost to the aforementioned processes should have been re-fixed by the trees given our controlled environment design. Similarly, the tulip poplars were harvested immediately following labeling to maximize the ^{13}C recovery and minimize the amount of ^{13}C that could have been lost to respiration. To rule out the remaining factors that may have contributed to our low ^{13}C recoveries, we calculated the remaining amount of injected $^{13}CO_2$ in the chamber after labeling using a two-source mixing model, similar to the one in the calculations section. Parameters used in this calculation were the fractional abundance of ^{13}C in the chamber air at the end of labeling (0.0196758), background CO_2 tank (0.011164), and $^{13}CO_2$ tank (0.99), as well as the number of CO_2 moles in the chamber given the CO_2 concentration point (Table 2) and chamber volume (5728 L). This calculation could only be done for Year 1 where the $\delta^{13}C$ of the chamber air had been measured during labeling. Our calculation suggests that approximately 49% of the $^{13}CO_2$ injected into the chamber was still there at the end of labeling and that the chambers were relatively leak-free. Assuming the conditions were similar for Year 2, our low ^{13}C recoveries were the result of unused injected $^{13}CO_2$ in the growth chambers. A more even $^{13}CO_2$ injection rate over the course of labeling or a more intense injection rate at the beginning could have resulted in higher ^{13}C recoveries in the trees.

In addition, a higher percentage of the injected $^{13}CO_2$ was incorporated into the trees in Year 1 than in Year 2 (Figures 1A, B). Given that Year 2 trees were exposed to 25 times more $^{13}CO_2$ for a longer time (23-d more) under the same environmental conditions as Year 1 trees, they should have incorporated at least as much, if not more, $^{13}CO_2$. The only difference in the growth conditions between Years 1 and 2 is that Year 2 trees were sprayed with Safer's ® sulfur spray to control powdery mildew which may have lowered the trees' carbon fixation rate, resulted in a lower $^{13}CO_2$ recovery, and a higher percentage of $^{13}CO_2$ in the chamber air at the end of the experiment. However, we cannot verify that the mildew and/or the anti-fungal agent affected the trees' carbon fixation rate because, as mentioned above, no $\delta^{13}C$ measurements for the chamber air were made in Year 2.

^{13}C-DOC Tracer

Tree tissue leaching studies have shown that 6 to 47% percent of leaf C goes into solution during cold-water extractions (for example, Nykvist 1962, 1963; Cummins and others 1972; Petersen and Cummins 1974; Gessner and Schwoerbel 1989; Magill and Aber 2000). In our study, a similar percentage of tree C and ^{13}C went into solution during the cold-water extractions, suggesting that the ^{13}C label was evenly distributed among the water-soluble components of the new tree tissues (Table 4). In addition, our results indicate that most ($\sim$70 and 80%) of the tree C, as well as ^{13}C was allocated into non-soluble, structural components. The water-soluble tree C had both humic and polysaccharide components, confirming that our ^{13}C-DOC tracer, like stream water, possesses polymeric constituents. Though clearly, the structure of the constituents isolated from our ^{13}C-DOC tracer may differ from those found in stream water.

Enriching plant material in ^{13}C is a costly endeavor. CO_2 highly enriched in ^{13}C is expensive and large quantities are needed to sufficiently label material for an isotope tracer experiment, placing a premium on maximizing recovery. For a stream injection experiment, the $\delta^{13}C$ value of the DOC injectate needs to be sufficiently high to overwhelm the background fluctuations in the $\delta^{13}C$ of the stream DOC. In addition, the DOC injectate needs to be adequately labeled so that its addition to the stream does not increase the DOC concentration and can be followed for a considerable distance downstream. It is important that the tracer addition does not increase the stream DOC concentration so that rates of *in situ* processes can be measured. Preliminary results from the whole-stream ^{13}C-DOC tracer addition demonstrate that the tulip poplar tissues were sufficiently labeled with ^{13}C to meet the criteria for a whole-ecosystem isotope tracer addition (Figure 2).

Results from our study illustrate that large quantities of CO_2 highly enriched with ^{13}C are needed to generate a stream DOC tracer for a whole-stream isotope addition. Calculations suggest that the contribution of the ^{13}C-enriched CO_2 (99 atom % ^{13}C) to the ^{13}C enrichment of the trees was not affected by the $\delta^{13}C$ value of the background CO_2; it was the volume (amount) of $^{13}CO_2$ added relative to the growth chamber volume that influenced the ^{13}C tree tissue enrichment. Thus,

Table 4. Average (±S.D.) Percent Total C (C_T) and ^{13}C Recovery for the Cold Water Extractions of the New Tree Tissues from Years 1 and 2 Tree C Labeling Experiments

	Year 1	Year 2
$\delta^{13}C_{Tree}$	393[a]	5941 (±29)
$\delta^{13}C_{Leachate}$	460 (±8)	6095 (±92)
DOC Conc. (mg C L^{-1})	705 (±3)	130 (±1)
% C_T Recovery[b]	20	30
% ^{13}C Recovery[b]	21	30
% Humic-C[c]	24 (±2)	26 (±2)
% Carbohydrate-C	14 (±0.5)	13 (±0.2)
% Monosaccharide-C	6 (±0.6)	4 (±0.2)
% Polysaccharide-C[d]	8 (±0.8)	9 (±0.3)

[a]*Calculated from the $\delta^{13}C$ values and percent weight distribution of the new leaves and stems used to make the leachate.*
[b]*%C_T and ^{13}C were calculated as the percent of the tree C_T or ^{13}C recovered in the dissolved form.*
[c]*Calculated as the difference in DOC concentrations between the sample and XAD-8 effluent.*
[d]*Calculated as the difference between the dissolved total saccharide- and monosaccharide-carbon concentrations.*
Average (±S.D.) humic- and carbohydrate-carbon content for the cold water extracts are also shown.

the greater the amount of $^{13}CO_2$ used to label the trees, the greater the ^{13}C enrichment in the tree tissues (Table 2 and 3). Additionally, if we had used CO_2 with a $\delta^{13}C$ value similar to the background CO_2 used in Year 1 (Table 2), a CO_2 source more enriched with ^{13}C than the atmosphere and several orders of magnitude less expensive than the 99% atom ^{13}C-CO_2 used to label the trees, the ^{13}C signal of our tracer would not have been strong enough for a whole-stream isotope addition.

Conclusions

Tree C can be successfully labeled with $^{13}CO_2$ and used to create a DOC tracer with humic and polysaccharide constituents that is more representative of stream/riverine DOC than previously used carbon isotope tracers (Hall 1995; Hall and Meyer 1998). Although our tracer is not a perfect model for stream DOC, we feel that it is a good tool for starting to measure *in situ* stream DOC dynamics given the fact that DOC leached from leaf litter accounts for 30% of daily DOC export from small forested streams on an annual scale and can comprise 42% of the DOC inputs in the fall (McDowell and Fisher 1976; Meyer and others 1998). Specifically, our tracer will allow for the direct measurement of stream DOC uptake lengths and rates, transformations, turnover rates, consumer-food linkages, interactions with mineral surfaces, and metabolism, and may even provide some insight into why there is so little terrestrial C in the oceans (Bader 1956; Meyers-Schulte and Hedges 1986; Hedges and others 1997; Opsahl and Benner 1997). The utility of our ^{13}C-DOC tracer could potentially be enhanced by aging it in the soil to generate a tracer that is chemically and biologically more similar to stream DOC. Additionally, a tracer like ours can be used to examine soil processes like organic matter degradation, DOC production, humic substance formation, and DOC sorption (that is, Bottner and others 2000; Coûteaux and others 2001). Ultimately, having a carbon stable isotope tracer more representative of natural organic matter will allow ecosystem scientists to examine carbon dynamics across the terrestrial-aquatic gradient.

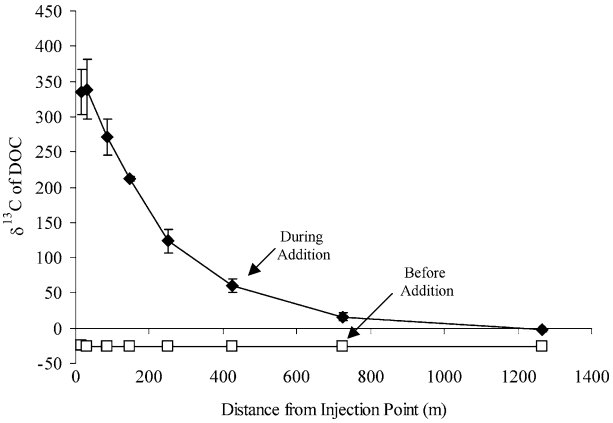

Figure 2. Decline of the δ^{13}C-DOC along a 1265-m reach in White Clay Creek, PA, during a 115 min whole-stream ^{13}C-DOC tracer addition. Average (±S.D.) δ^{13}C-DOC values before and during the whole-stream isotope addition are shown. Note δ^{13}C-DOC values during the stream injection are not corrected for stream water dilution and background ^{13}C-DOC concentration.

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