**Tree Physiology** 14, 1163–1176


# $^{14}C$ Allocation in tree–soil systems

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Received March 10, 1993

**Summary**

We studied whole-tree C allocation with special emphasis on the quantification of C allocation to roots and root respiration. To document seasonal patterns of C allocation, 2-year-old hybrid poplar trees greater than 3 m tall were labeled with $^{14}CO_2$ in a large Plexiglas chamber in the field, in July and September. Climate and $CO_2$ concentration were controlled to track ambient conditions during labeling. Individual tree canopy $CO_2$ assimilation averaged 3.8 μmol $CO_2$ $m^{-2}$ $s^{-1}$ (12.9 g C $day^{-1}$ $tree^{-1}$) in July and 6.2 μmol $CO_2$ $m^{-2}$ $s^{-1}$ (9.8 g C $day^{-1}$ $tree^{-1}$) in September. Aboveground dark respiration was 12% of net daytime C fixation in July and 15% in September. Specific activity of root–soil respiration peaked 2 days after labeling and stabilized to less than 5% of maximum 2 weeks later. Low specific activity of root–soil respiration and a labeled pool of root C demonstrated that current photosynthate was the primary source of C for root growth and maintenance during the growing season. Root respiration averaged 20% of total soil respiration in both July and September based on the proportion of labeled C respired to labeled C fixed. In July, 80% of the recovered $^{14}C$ was found above ground and closely resembled the weight distribution of the growing shoot. By September, 51% of the recovered $^{14}C$ was in the root system and closely resembled the weight distribution of different size classes of roots. The finding that the distribution of biomass and $^{14}C$ were similar verified that the C introduced during labeling followed normal seasonal translocation pathways. Results are compared to smaller scale labeling studies and the suitability of the approach for studying long-term C fluxes is discussed.

*Keywords: biomass, growth, poplars, respiration, roots, whole-tree carbon allocation.*

## Introduction

The evaluation of gross C fixation, C allocation and respiratory C loss is important in interpreting the structure and function of managed and unmanaged ecosystems (Isebrands 1982). The potential flux of C below ground represents a considerable cost to trees (Dickson 1989) and belowground production is estimated to account for more than 50% of total production in forest ecosystems (Harris et al. 1975, Persson 1979, Keyes and Grier 1981, Fogel 1990, Hendrick and Pregitzer 1993). Despite the importance of C allocation to the plant–soil system, there have been few studies on belowground C allocation in forests (Tranquillini 1963, Kinerson et al. 1977, Ågren et al. 1980, Linder and Troeng 1981; Friend et al. 1991). The lack of research and the inherently difficult task of studying belowground processes continues to hinder our understanding of belowground production and its impact on tree productivity, nutrient cycling and soil organic matter dynamics (Santantonio 1989, Fogel 1990).

Detailed knowledge of belowground production is limited by the lack of suitable

methods to determine directly the simultaneous growth and turnover of roots, although some information can be obtained by means of $^{14}C$ pool dilution studies. Simple pulse-chase experiments with potted trees, in which a single leaf or branch is labeled with $^{14}CO_2$ in the field, have frequently been performed to determine C translocation pathways and allocation (e.g., Schier 1970, Isebrands and Nelson 1983). Studies using large quantities of high specific activity $CO_2$ often show high $^{14}C$ respiratory losses immediately following labeling (Isebrands and Nelson 1983, Kuhns and Gjerstad 1991). Relatively few field studies have determined $^{14}C$ allocation pathways by means of controlled-environment labeling techniques (Warembourg and Paul 1973, Webb 1977, Smith and Paul 1989, Gorissen et al. 1991).

The general objectives of our study were to label large field-grown trees with $^{14}C$ under controlled conditions to quantify whole-tree C allocation. We were specifically interested in determining seasonal differences in whole-tree respiration and C allocation, especially to roots. Additionally, we wanted to test the suitability of our C labeling system for long-term belowground C flux studies. Seasonal rhythms in growth and the allocation of carbohydrates and nitrogen reserves have been documented in *Populus* (Isebrands and Nelson 1983, Pregitzer et al. 1990, Nguyen et al. 1990), but budgets developed by labeling whole trees greater than 3 m tall have never been reported. We believe that C allocation patterns have ecological significance and that seasonal changes in source–sink relationships can greatly confound the interpretation of C cycling done at the ecosystem level. This paper represents our first attempt to study whole-tree gas exchange and C allocation in *Populus*–soil systems.

## Materials and methods

The study site was located at the Kellogg Biological Station, Michigan State University, Kalamazoo, Michigan (42°24′ N, 85°24′ W). The soils series is Kalamazoo (fine loamy, mixed, mesic, Typic Hapudalf). The site was prepared by moldboard plowing and disking. Cuttings of *Populus euramericana* cv. Eugenei, 10 cm in length, were planted on a 2 × 1 m spacing in the spring of 1989. Later in the first growing season, groups of eight trees were selected for uniform height and diameter. An undisturbed soil block (1 $m^3$) surrounding each tree was trenched with a narrow ditching machine (Ditchwitch, Howell, MI). Plywood dividers and vinyl sheeting (0.18 mm) were placed in the trenches before back-filling to form an airtight seal against the faces of each soil block.

The following summer, the vinyl sheeting of each soil block was sealed against the plywood dividers with foam sealant (Insta-Foam Products Inc., Marietta, GA). Polyvinyl chloride (PVC) sheets (3.2 mm thick) were sealed to the top of the plywood dividers with silicone caulking to create an enclosed headspace (15 cm tall × 1 m × 1 m) above the soil surface of each soil block. Tree stems were fixed with modeling clay, polyethylene plastic and duct tape before being sealed to the PVC plates with silicone caulking. The headspace atmosphere, containing $CO_2$ from root–soil respiration, was drawn via tygon tubing (1.3 cm I.D.) through an alkali trap

containing an aeration stone and 500 ml of 4.0 M NaOH (Figure 1). The belowground headspace atmosphere was displaced at a rate of 32.7 l $min^{-1}$ with a diaphragm pump (Cole Parmer Inst. Co., Chicago, IL). Thus, the headspace was displaced approximately twice per hour and provided sufficient exchange of oxygen.

A Plexiglas chamber (3.2 m tall × 3 m wide × 4 m long) was assembled over the trees and belowground partitions (Figure 1). The chamber was sealed to the plywood structure and PVC sheets with silicone caulking. A $^{14}CO_2$ generator was connected to a diaphragm pump (GAST, Benton Harbor, MI) to circulate $^{14}CO_2$ into the chamber. The $^{14}CO_2$ generator consisted of a vessel containing 1.0 l of concentrated $H_2SO_4$ and a reservoir containing 6.0 mol of $Na_2{}^{14}CO_3$ solution. The $Na_2{}^{14}CO_3$ solution was dispensed drop-wise into the acid through a solenoid valve (Skinner, New Brunswick, CT). The acid–carbonate mixture was continuously agitated with a magnetic stirrer to liberate $^{14}CO_2$. The $^{14}C$ was applied as a regulated one-day pulse.

Chamber $CO_2$ concentration was monitored with an infrared gas analyzer, IRGA (Analytical Development Co. Ltd., Hoddesdon, England). The IRGA was interfaced to a computer through a data acquisition and control device (Remote Measuring Systems Inc., Seattle, WA). Temperature sensors (Omega Engineering Inc., Stamford, CT) were installed to monitor the belowground headspace, chamber air and outside air. Software was written in Quick Basic (Microsoft Corp., Redmond, WA) to control the concentration of $CO_2$, monitor temperatures and collect data during the labeling procedure. Chamber photon flux density was measured with a radiometer/photometer (Li-Cor Inc., Lincoln, NE). The chamber temperature was controlled by forcing air through a water-cooled heat exchanger. The air turbulence created by the heat exchanger was sufficient to mix the $^{14}CO_2$ gas uniformly.

Belowground respiration traps were sampled at least twice daily to determine belowground $^{14}C$ and C flux from root–soil respiration. Total trap C was determined by titration. One-ml trap subsamples were titrated to a phenolphthalein end point with 0.1 M HCl after precipitation of carbonate species with excess $BaCl_2$. Labeled trap C was determined in 1-ml subsamples by liquid scintillation spectrometry

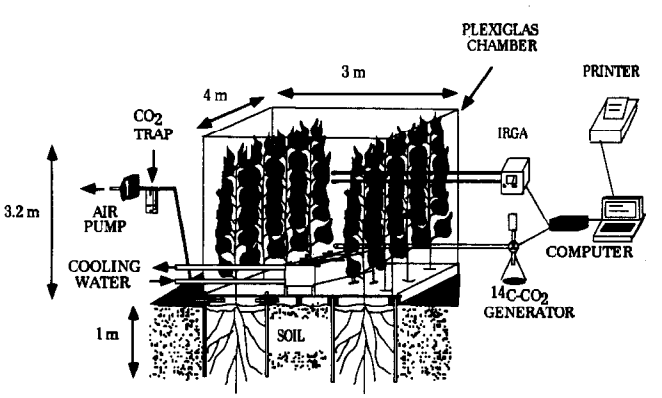


Figure 1. Schematic diagram of the $^{14}C$ exposure system used to label eight 2-year-old whole poplar trees. The trees were approximately 3 m tall when labeled.

(Packard Instrument Co., Downers Grove, IL) after adding 10 ml of scintillation cocktail (Safety Solve, Research Products International Corp., Mount Prospect, IL).

Eight trees were labeled on July 19, 1990 and eight more trees were labeled on September 5, 1990 for one day. A total of $3.04 \times 10^8$ Bq of $^{14}CO_2$ with a specific activity of 4215 Bq mg$^{-1}$ C was added to the labeling chamber in July. In September, a total of $2.85 \times 10^8$ Bq of $^{14}CO_2$ with a specific activity of 4300 Bq mg$^{-1}$ C was added. Each tree received approximately 1.0 mCi of $^{14}C$. Chamber $CO_2$ concentration was maintained at 290–350 ppm and closely tracked outside-air $CO_2$ concentrations. Aboveground nighttime respiration was monitored with the IRGA/computer control system. Carbon dioxide accumulated from aboveground night respiration was re-assimilated the following morning. The Plexiglas chamber was removed when the $CO_2$ concentration was depleted to 70 ppm, the approximate $CO_2$ compensation point for this genotype.

Three randomly selected trees were sampled 2 weeks after each labeling. The remaining trees were sampled the following year to determine the long-term disposition of labeled C and these data will be reported elsewhere. Shoot samples were separated into leaves, branches and stem. The soil block was excavated by depth (0–25, 25–60 and 60–100 cm), and the soil was weighed and sieved through an 8-mm screen to remove coarse roots. The sieved soil was then mixed in a cement mixer and a subsample taken. Plant material and soils were stored on ice in the field and then transferred to a 4 °C cold room.

Roots were sorted by hand into the following size classes: 0.5, 0.5–1, 1–3, > 3 and > 10 mm. Leaf area was measured with a Delta-T meter (Decagon Devices, Pullman, WA). Plant material was dried at 80 °C for 48–96 h and ground in a Wiley mill to pass a 60-mesh screen. The sieved soil samples were hydropneumatically elutriated (Smucker et al. 1982), dried at 70 °C and weighed to determine total fine root biomass of each soil block. Plant and soil samples were analyzed for C by gas chromatography–mass spectrometry (ANCA-MS, Europa Scientific, Crewe, U.K.). Whatman no. 1 filter paper was used as a reference standard. Labeled C from the gas chromatograph–mass spectrometer exhaust was collected in a $CO_2$ absorbing cocktail (J.R. Harvey Instrument Corp., Hillsdale, NJ) and analyzed for $^{14}C$ by liquid scintillation spectrometry (Harris and Paul 1989).

## Results and discussion

### *Tree growth and environmental variables*

Three weeks before the July labeling, the Eugenei clones were growing 35 mm day$^{-1}$ in height. By September, the trees had set bud and height growth had ceased. The eight trees labeled during July had an average diameter of 4.4 cm (at a height of 10 cm) and a height of 310 cm, whereas the eight trees labeled in September had an average diameter of 4.5 cm and were 337 cm tall. Total leaf area of individual trees averaged 6.1 m$^2$ in July and 3.1 m$^2$ in September. Decreasing leaf area late in the growing season is common for this genotype and is attributable to late-season

drought stress, *Melampsora* rust and *Marsonnina* leaf spot, all of which promote acropetal leaf loss. Decreasing leaf biomass and increasing root mass were characteristic of trees sampled in September. The increase in coarse root mass (> 3 mm) is associated with late-season translocation and storage of photosynthate (Pregitzer et al. 1990, Nguyen et al. 1990). The root/shoot ratio increased from 0.34 to 0.62 from July to September.

Chamber air temperature was maintained between 19 and 34 °C, which closely tracked ambient air temperature fluctuations of 18 to 36 °C during both labeling periods (Table 1). Belowground headspace temperature ranged from 20 to 24 °C for both labelings. To avoid water condensation and unnaturally dry air inside the chamber, the temperature of the heat exchanger was maintained at approximately the dew point of ambient air. Temperature control was important to maintain regulated conditions for realistic rates of C fixation and allocation in the field-grown poplars, because the $^{14}$C labeling method was intended to simulate C flow and stabilization for long-term studies.

*Carbon fixation and aboveground respiration*

Maximum chamber light intensity during labeling was 1250 μmol $m^{-2}$ $s^{-1}$ in July and 1060 μmol $m^{-2}$ $s^{-1}$ in September (Table 1). Net photosynthesis averaged for one day for an individual tree canopy was 3.8 μmol $CO_2$ $m^{-2}$ $s^{-1}$ in July and 6.2 μmol $CO_2$ $m^{-2}$ $s^{-1}$ in September. Chamber incident radiation reached saturating light intensities during midday. Saturating light intensities of 800–1000 μmol $m^{-2}$ $s^{-1}$ are typical for field-grown hybrid poplars (Ceulemans et al. 1980). Under saturating light conditions, net photosynthetic rates of 9.5–20.7 μmol $CO_2$ $m^{-2}$ $s^{-1}$ have been reported for individual leaves of hybrid poplars (Ceulemans et al. 1980, Nelson et al. 1982, Bassman and Zwier 1991). Average photosynthetic rates observed in our study were lower than other reported values, which is probably explained by a difference between whole-tree and single-leaf or branch determinations. In autumn, we observed an increase in $^{14}CO_2$ uptake (Table 1). This is typical of hybrid poplars (Nelson and Isebrands 1983, Friend et al. 1991) and may be attributable to acropetal

Table 1. Gas exchange characteristics during labeling periods in July and September.

| | July | September |
|---|---|---|
| Maximum photon flux density (μmol $m^{-2}$ $s^{-1}$) | 1250 | 1060 |
| Net assimilation (μmol $CO_2$ $m^{-2}$ $s^{-1}$)[1,2] | 3.8 | 6.2 |
| Aboveground dark respiration (μmol $CO_2$ $m^{-2}$ $s^{-1}$)[1,2] | 0.67 | 1.47 |
| Aboveground night respiration (% total assimilation during labeling) | 12 | 15 |
| Chamber air temperature (°C) | 19–33 | 19–34 |
| Ambient air temperature (°C) | 19–36 | 48–34 |
| Soil surface temperature (°C) | 18–24 | 18–24 |

[1] Represents the average of eight trees inside the chamber.

[2] Determination based on total leaf area in the chamber estimated from the average leaf area of sampled trees ($n$ = 3).

leaf loss in September, resulting in an increase in the proportion of young sun-adapted leaves.

Measurements of aboveground night respiration included leaves, branches and stem. Aboveground night respiration of individual trees, expressed on a leaf area basis, was 0.67 μmol $CO_2$ $m^{-2}$ $s^{-1}$ (1.5 g C $tree^{-1}$) in July and 0.47 μmol $CO_2$ $m^{-2}$ $s^{-1}$ (1.6 g C $tree^{-1}$) in September, or 12 to 15% of the previous day's net C assimilation (Table 1). Estimates of leaf dark respiration for hybrid poplars are between 1.1 and 1.6 μmol $CO_2$ $m^{-2}$ $s^{-1}$ on portions of intact leaves (Bassman and Zwier 1991). Our results emphasize the difficulty of extrapolating from single-leaf observations to whole-tree and stand-level C budgets.

There was excellent recovery of $^{14}C$ due, in part, to re-assimilation of nighttime aboveground $^{14}C$ respiration. We assumed that C assimilated early in the day was translocated in a manner similar to C assimilated later in the day. Photosynthate fixed during different parts of the day may be subject to different source–sink relations, i.e., C fixed early in the day may be transported and bypass leaf storage (Wardlaw 1990). Our data demonstrate that night respiration during warm periods can be significant and should be considered when calculating the C balance of trees.

*Root–soil respiration*

Belowground respiration measurements were determined on eight trees for 2 weeks following labeling (Figure 2). The soil respiration for each tree averaged 240 mg C $m^{-2}$ $h^{-1}$ in July and 218 mg C $m^{-2}$ $h^{-1}$ in September. On average, between 5.2 and 5.8 g C $m^{-2}$ $day^{-1}$ were respired from the soil surface of each soil block (1 $m^3$ of soil). The $CO_2$ emission values were similar to laboratory-incubated soils from the immediate area (Horwath 1993, Paul et al. 1994). The respiration measurement included roots, associated rhizosphere organisms and bulk soil organisms. The soil $CO_2$ emission was sufficient to provide approximately 50% of the C fixed by the trees in both July and September, during a 24-h period.

Respiration of labeled C from the root system occurred within 12 h of labeling (Figure 3). During both labeling periods, maximum specific activity (90–100 Bq $mg^{-1}$ C) of the belowground respiration occurred approximately 2 days after label-

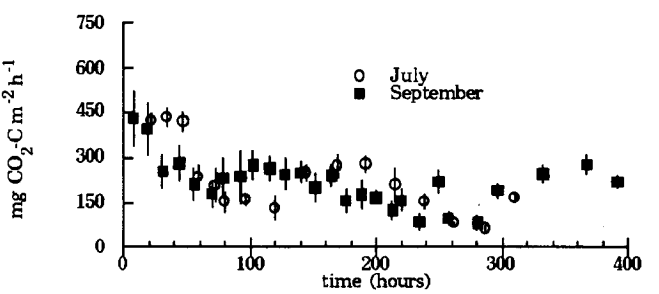


Figure 2. Root–soil respiration following labeling in July and September. Values are expressed as mg C $m^{-2}$ $h^{-1}$. Values are calculated from the average of the eight separate belowground headspace compartments. Standard error of the means are show as bars ($n$ = 8).

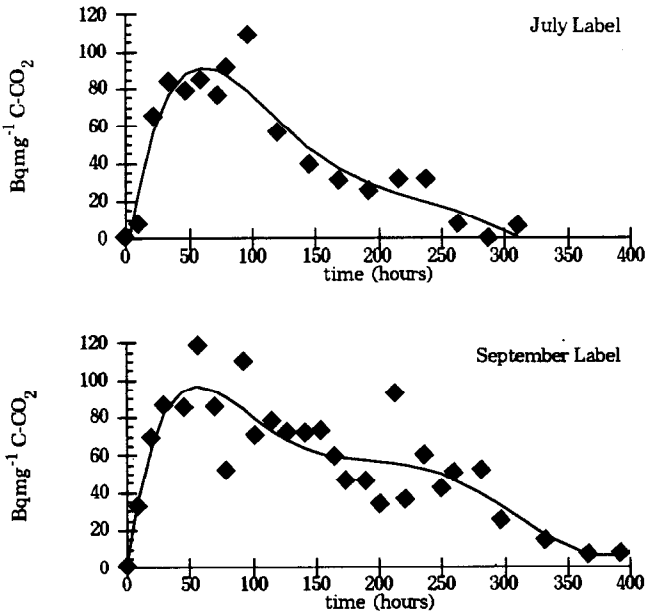


Figure 3. Specific activity (Bq mg C) of belowground respiration as measured by $CO_2$ (mg C) emission from the soil surface. Each symbol represents the mean of eight root–soil microcosms. Standard error of the measurement never exceeded 25% of the reported values.

ing. The time lag between labeling and belowground respiration of labeled C is the time required to translocate and utilize photosynthate in the root system. In this study, fixation and peak root respiration were separated by 2 days. This period is probably influenced by the length of the translocation pathway and anabolism of carbohydrates (Kozlowski et al. 1991). In both July and September, the rise in soil $^{14}$C emission 10 days after labeling indicates a second pulse of labeled C utilization. Secondary rises in belowground specific activity can be attributed to mycorrhizal symbionts sequestering remobilized root starch reserves, microbial utilization of root exudates, or root turnover (Harris et al. 1985, Harris and Paul 1991). Additional analyses are required to determine if any of the above scenarios apply to our results. In July and September, the specific activity of belowground respiration fell to less than 5% of maximum 2 weeks after labeling. It was assumed that $^{14}$C had by then stabilized in storage or structural forms in the tree, or was already in soil C pools (Harris and Paul 1991). Trees were harvested at this time to form a baseline for our long-term $^{14}$C studies.

The reduction in $^{14}$C emission 2 weeks after labeling occurred while the total soil respiration rate was constant (Figure 2 versus Figure 3). Dilution of $^{14}$C occurred because unlabeled current photosynthate increasingly dominated total soil respiration, indicating that these trees depend primarily on current photosynthate for root growth and maintenance during periods of active photosynthesis. Additionally, the results show that starch and other storage reserves formed during periods of photosynthesis are not utilized for current metabolic activities during the growing season

(Dickson 1989).

The $^{14}C$ recovered from belowground respiration expressed as a percent of that applied (Bq per Bq added) is an index of the quantity of photosynthate lost to root respiration. The amount of respired $^{14}C$ (of that applied) from roots and associated soil microorganisms was 8.1% in July and 9.8% in September (Figure 4). A single-component asymptotic exponential function described the respiration of $^{14}C$ from the root–soil system accurately. The exponential equation is

$$\frac{Bq_{root}}{Bq_{applied}} = Q(1 - e^{-kt}), \quad (1)$$

where $Bq_{root}$ is the amount of $^{14}C$ (Bq) in root–soil respiration, $Bq_{applied}$ is the total $^{14}C$ (Bq) added during labeling, $Q$ is the accumulation of $^{14}C$ (Bq) from root–soil respiration over time, $k$ is the rate constant for $^{14}C$ accumulation and $t$ is time. The calculated accumulation of $^{14}C$ in the respired root–soil C pool was 9.1% in July and 10.8% in September based on values extrapolated to 1000 h. In September, the accumulation of $^{14}C$ from belowground respiration was slower than in July. The additional time required to reach asymptotic values in September showed that the transport and utilization of labeled C in root–soil pools was slower at this time. The turnover rate of the $^{14}C$ respired in the root–soil system can be calculated from the rate constant for labeled C accumulation from Equation 1. The half-life of belowground $^{14}C$ respiration was 69 h in July and 99 h in September. The slower turnover rate in September reflects the fate of C during autumn. In September, the utilization of labeled C in the root–soil system may have been affected by the length of the translocation pathway (leaf drop was acropetal) and the cool nights following labeling.

Belowground $^{14}C$ respiration was similar during both labeling periods despite an increase in root $^{14}C$ allocation in September (Table 2). Carbon assimilated after bud set is primarily used to accumulate reserves (Dickson and Nelson 1982, Isebrands and Nelson 1983, Pregitzer et al. 1990). A similar amount of C was required to maintain metabolic activities of the root–soil system in both July and September.

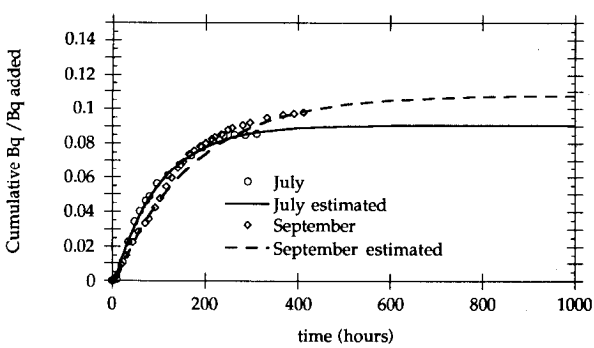


Figure 4. $^{14}C$ Respired from the root–soil system over time. Symbols represent real values and lines represent values calculated from Equation 1.

Table 2. Carbon concentration (%), specific activity (Bq mg$^{-1}$ C), % of $^{14}$C recovered of total applied (Bq/Bq added) and % $^{14}$C of the total amount of $^{14}$C recovered for the different tree components.[1] Standard error given in parenthesis ($n$ = 3).

| Tree component | % C | Bq (mg$^{-1}$ C) | Bq/Bq added[2] | % $^{14}$C of total $^{14}$C recovered |
|---|---|---|---|---|
| *July 15–August 1, 1990* | | | | |
| Stem | 43.0 (0.6) | 35.6 (2.5) | 31.3 (4.8) | 34.7 (2.0) |
| Branches | 42.5 (0.5) | 36.6 (4.8) | 16.8 (1.9) | 18.8 (0.5) |
| Leaves | 40.4 (0.3) | 42.8 (0.7) | 23.9 (1.7) | 26.9 (0.8) |
| Total aboveground | | | 72.0 (8.3) | 80.4 (1.8) |
| Roots | | | | |
| < 0.5 mm | 28.1 (2.4) | 12.9 (1.6) | 1.7 (0.0) | 2.0 (0.2) |
| 0.5–1 mm | 36.1 (2.0) | 13.3 (1.8) | 0.2 (0.0) | 0.2 (0.0) |
| 1–3 mm | 38.2 (1.7) | 15.9 (3.9) | 0.8 (0.1) | 0.9 (0.2) |
| 3–10 mm | 38.7 (1.0) | 21.3 (5.5) | 2.6 (0.3) | 3.2 (0.5) |
| > 10 mm | 40.7 (0.0) | 21.8 (5.4) | 2.1 (0.2) | 2.3 (0.2) |
| Cutting | 41.2 (1.2) | 23.0 (3.9) | 2.0 (0.1) | 2.3 (0.1) |
| Soil respiration | | 45.4 (1.8) | 7.7 (0.2) | 8.8 (0.9) |
| Total belowground | | | 17.1 (0.2) | 19.6 (1.8) |
| Total tree | | | 89.3 (8.1) | |
| *September 5–19, 1990* | | | | |
| Stem | 40.8 (1.0) | 17.3 (1.9) | 15.6 (3.7) | 19.2 (4.5) |
| Branches | 42.0 (2.0) | 12.4 (1.8) | 7.5 (1.0) | 9.3 (1.4) |
| Leaves | 39.5 (2.2) | 50.8 (4.6) | 17.0 (1.3) | 20.9 (1.3) |
| Total aboveground | | | 40.1 (3.9) | 49.4 (4.4) |
| Roots | | | | |
| < 0.5 mm | 28.7 (1.3) | 31.5 (6.3) | 5.5 (1.3) | 6.8 (1.8) |
| 0.5–1 mm | 31.9 (2.0) | 35.2 (4.4) | 0.8 (0.1) | 0.9 (0.2) |
| 1–3 mm | 34.0 (1.2) | 32.0 (5.7) | 1.6 (0.3) | 2.0 (0.3) |
| 3–10 mm | 36.6 (0.5) | 38.9 (7.3) | 8.8 (1.0) | 10.9 (1.4) |
| > 10 mm | 37.3 (1.2) | 38.1 (5.7) | 11.1 (3.2) | 13.5 (3.5) |
| Cutting | 43.0 (0.6) | 25.9 (9.2) | 3.4 (0.7) | 4.3 (1.0) |
| Soil respiration | | 39.0 (3.5) | 9.0 (1.2) | 12.2 (1.8) |
| Total belowground | | | 37.7 (4.2) | 50.6 (4.4) |
| Total tree | | | 81.1 (2.2) | |

[1] Belowground biomass determined from a 1 m$^3$ soil block. All roots were confined within these open-bottomed boxes surrounding an undisturbed cubic meter of soil (see Methods).

[2] Labeled C applied was 9 g tree$^{-1}$ (4215 Bq mg$^{-1}$ C) in July and 8.3 g tree$^{-1}$ (4300 Bq mg$^{-1}$ C) in September.

This is partially explained by the similar amounts of fine root mass (< 0.5 mm) in both July and September (Table 3). The root–soil system requires a certain amount of C for maintenance even though additional nonstructural reserves accumulated in the root system in autumn (Table 2).

Root–soil respiration, as measured, represents the fraction of $^{14}$C translocated below ground used to sustain the metabolic activity of roots and some associated soil organisms in the short-term. In both the July and September labelings, root–soil respiration accounted for 9 to 12% of the recovered $^{14}$C (Figure 4). The respired proportion of labeled C translocated to the root system was 45% in July and 24% in

Table 3. The dry weight of tree components in July and September. Values represent an average of three trees. Standard errors are shown in parenthesis.

| Tree component | July 17–August 1, 1990 | | September 5–19, 1990 | |
|---|---|---|---|---|
| | Weight (g) | % Weight distribution | Weight (g) | % Weight distribution |
| Stem | 781 (122) | 33 | 770 (125) | 30 |
| Branches | 432 (96) | 18 | 519 (28) | 20 |
| Leaves | 528 (52) | 23 | 303 (14) | 12 |
| Total aboveground | 1742 (270) | 74 | 1592 (158) | 62 |
| Roots | | | | |
| < 0.5 mm | 185 (15) | 8 | 213 (4) | 8 |
| 0.5–1 mm | 16 (2) | 1 | 24 (2) | 1 |
| 1–3 mm | 49 (2) | 2 | 53 (3) | 2 |
| 3–10 mm | 136 (36) | 6 | 230 (32) | 9 |
| > 10 mm | 116 (38) | 5 | 273 (53) | 11 |
| Cutting | 98 (18) | 4 | 191 (10) | 7 |
| Total belowground | 599 (104) | 26 | 984 (25) | 38 |
| Total tree | 2341 (370) | | 2576 (182) | |

September. Most of the respired $^{14}C$ was probably from root respiration, because the majority of the $^{14}C$ was evolved within 100 h of labeling (Figure 3). It was not possible to distinguish between root and microbial respiration. Van Veen et al. (1991) found that between 1 and 17% of the labeled C fixed by agronomic crops was due to microbial respiration. The $^{14}C$ found in microbial biomass is highly dependent on plant species, soil texture and soil fertility (Merckx et al. 1987, van Veen and Kuikman 1990).

As with our net photosynthesis measurements of the canopy, root–soil respiration represents the entire belowground system and must be considered in light of the 81 to 89% total recovery of $^{14}C$. We assumed that all $^{14}C$ added to the field chambers was fixed by the trees and that experimental error (incomplete recovery of $^{14}C$) was spread randomly throughout the labeling and sampling protocols. If the majority of the unrecovered $^{14}C$ left through the bottom of the unsealed soil block or by way of leaks in the belowground containment system, root respiration values could possibly double. However, it is more likely that the unrecovered $^{14}C$ was lost through aboveground respiration after the Plexiglas chamber was removed.

The proportion of $^{14}C$ respired from the roots was related to total labeled C fixation. If all of the $^{14}C$ respired over 500 h was due to root respiration, then the contribution of root respiration to total soil respiration was 19% in July and 21% in September. These values were calculated by comparing the proportion of labeled C lost in root–soil respiration to the total labeled C fixed during the labeling period. In contrast to our data, Cropper and Gholz (1991) estimated that two-thirds of soil respiration was from the fine root fraction in a mature slash pine stand. Edwards and Harris (1977) calculated that 35% of forest floor respiration in a deciduous forest evolved from the metabolic activity of roots. Root–soil $CO_2$ emission studies can be biased by root density in surface soils where root activity can produce proportion-

ately more $CO_2$ emission. It is likely that rooting density in our experiment was relatively low because the trees were young. Furthermore, the agricultural soil had a relatively high organic matter and labile C content, following several years of alfalfa cultivation, when we began the experiment. These facts may explain why fine root respiration represented a smaller proportion of total soil respiration compared with values in the literature. The high N status of the soil may also have influenced root–shoot C allocation. Nonetheless, our values for root respiration represent a direct field determination of the portion of the labeled C fixed used in root maintenance. Unlabeled $CO_2$ emission studies would be more meaningful if evaluated on a root density or root depth basis, and soil conditions should always be reported. The fate of C allocated to the root system is a key ecosystem process and its quantification is necessary to validate models of soil C balance used to simulate global climate change (van Veen et al. 1991).

*Seasonal carbon allocation*

Whole-tree $^{14}C$ allocation varied according to seasonal phenology. During active growth in July, 80% of the recovered $^{14}C$ was in aboveground components compared to 49% after bud set in September (Table 2). The $^{14}C$ recovered in July correlates strongly with the most actively growing portions of the tree. In September, more $^{14}C$ was allocated to roots and recovery closely resembled the biomass distribution in the different root size classes. The proportion of $^{14}C$ recovered in the belowground components, including root respiration, increased from 20% in July to 51% in September (Table 2). In July, the highest specific activity occurred in root–soil respiration. The high specific activity of root–soil respiration compared to root biomass indicates the high metabolic cost associated with root–soil maintenance during the growing season (Table 2). In September, the specific activity of the fine roots was similar to root–soil respiration, and between one and two times the activity of the growing shoot. Leaves were the exception; they had the highest specific activity of all tree–soil components during the autumn labeling period. The $^{14}C$ content of tree tissues for both labelings was between 1 and 2% of the total C content (Horwath 1993).

Carbon allocation in this clone is closely related to the termination of shoot growth (Michael et al. 1988). After bud set, source–sink relations change and roots become a major sink (Isebrands and Nelson 1983). Bud set and leaf senescence on branches occur acropetally. The onset of bud set in both the terminal and branch shoots shifts C allocation from the acropetal to basipetal direction (Isebrands 1982). Hybrid poplars gain root sink strength as autumn approaches through the loading of coarse roots (Pregitzer et al. 1990, Friend et al. 1991). Similar conclusions have been derived for apple trees (Quinlan 1969). During our labeling studies, translocation of labeled C to the root system increased 2.5 times between July and September.

The pronounced seasonal pattern of C allocation in *Populus* was first elucidated by Dickson and Nelson (1982) and Isebrands and Nelson (1983). Later, Pregitzer et al. (1990) and Nguyen et al. (1990) demonstrated that the root system of young Eugenei trees is a major site for the storage of nonstructural carbohydrates during the

dormant season, and that the clone exhibits a pronounced seasonal pattern of distribution and storage that reflects relative sink strength. This whole-tree tracer study confirms these earlier studies and demonstrates that whole-tree $^{14}C$ allocation patterns are similar to those of biomass. It is clear that this genotype allocates C to the different components of the tree preferentially, depending on the time of year.

*Comments on methodology*

Total recovery of $^{14}C$ ranged from 81% in September to 89% in July, which is comparable to rates observed in growth chamber labeling studies (Harris et al. 1985). This high recovery relative to that observed in other field studies must, in part, be related to the refixation of labeled aboveground night respiration. Another factor leading to good label recovery was complete C fixation under regulated labeling conditions. In contrast, pulse-chase labeling studies using large amounts of high specific activity $CO_2$ are prone to incomplete C fixation under unregulated conditions. Pulse-chase studies are also prone to unnaturally high respiratory losses of labeled C (Isebrands and Nelson 1983, Kuhns and Gjerstad 1991).

Regulated environmental conditions during photosynthesis assure realistic C flux rates and stabilization. The usefulness of our labeling technique was demonstrated by the finding that labeled and unlabeled C exhibited similar patterns of allocation. The results confirm both the magnitude and variation in seasonal C allocation documented by simple biomass studies (Pregitzer et al. 1990). The most interesting aspect of belowground respiration was its seasonal similarity despite large changes in C allocation to the root system. Additional research is required to determine if this observation holds true during the dormant and early growing seasons.

**Acknowledgments**

This research was supported by the National Science Foundation (grants BSR 8907565 and BSR 8702332) with additional support from the Michigan Agricultural Experiment Station. The NSF REU program provided critical additional support and we gratefully acknowledge this program and the students it supported. We also acknowledge Matthew Zwiernik for his contributions to this research.

**References**

Ågren, G.I., B. Axelsson, J.G.K. Flower-Ellis, S. Linder, H. Persson, H. Staaf and E. Troeng. 1980. Annual C budget for a young Scots pine. *In* Structure and Function of Northern Coniferous Forests—An Ecosystem Study. Ecol. Bull. (Stockholm) 32:307–313.

Bassman, J.H. and J.C. Zwier. 1991. Gas exchange characteristics of *Populus trichocarpa, Populus deltoides* and *Populus trichocarpa* × *Populus deltoides*. Tree Physiol. 8:145–159.

Ceulemans, R., I. Impens, F. Bebrant and R. Moermans. 1980. Evaluation of field productivity for several poplar clones based on their gas exchange variables determined under laboratory conditions. Photosynthetica 14:355–362.

Cropper, W.P. and H.L. Gholz. 1991. *In situ* needle and fine root respiration in mature slash pine (*Pinus elliottii*) trees. Can. J. For. Res. 21:1589–1595.

Dickson, R.E. and E.A. Nelson. 1982. Fixation and distribution of $^{14}C$ in *Populus deltoides* during dormancy induction. Physiol. Plant. 54:393–401.

Dickson, R.E. 1989. Carbon and nitrogen allocation in trees. Ann. Sci. For. 46(suppl.):631–647.

Edwards, N.T. and W.F. Harris. 1977. Carbon cycling in a mixed deciduous forest floor. Ecology 58:431–437.

Fogel, R. 1990. Root turnover and production in forest trees. HortScience 25:270–273.

Friend, A.L., G. Scarascia-Mugnozza, J.D. Isebrands and P.E. Hielman. 1991. Quantification of two-year-old hybrid poplar root systems: morphology, biomass and $^{14}$C distribution. Tree Physiol. 8:109–119.

Gorissen, A., G.C. Schelling and J.A. van Veen. 1991. Concentration-dependent effects of ozone on translocation of assimilates in Douglas fir. J. Environ. Qual. 20:169–173.

Harris, D., R.S. Pacovsky and E.A. Paul. 1985. Carbon economy of soybean, *Rhizobium, Glomus* associations. New Phytol. 101:427–440.

Harris, E. and E.A. Paul. 1989. Automated analysis of $^{15}$N and $^{14}$C in biological samples. Commun. Soil Sci. Plant Anal. 20:935–947.

Harris, D. and E.A. Paul. 1991. Techniques for examining the carbon relationships of plant–microbial symbioses. *In* Carbon Isotope Techniques. Eds. D.C. Coleman and B. Fry. Academic Press Inc., San Diego, pp 39–52.

Harris, W., F.P. Sollins, N.T. Edwards, B.E. Dinger and H.H. Shugart. 1975. Analysis of C flow and productivity in a temperate deciduous forest ecosystem. *In* Productivity of World Ecosystems. National Academy of Sciences, Washington, DC, pp 116–123.

Hendrick, R.L. and K.S. Pregitzer. 1993. Dynamics of fine root length, biomass and nitrogen content in two northern hardwood forest ecosystems. Can. J. For. Res. 23:2507–2520.

Horwath, W.R. 1993. The dynamics of carbon, nitrogen and soil organic matter in *Populus* plantations. Ph.D. Dissertation. Michigan State University, East Lansing, MI, 259 p.

Isebrands, J.G. and N.D. Nelson. 1983. Distribution of $^{14}$C-labeled photosynthate within intensively cultured *Populus* clones during the establishment year. Physiol. Plant. 59:9–18.

Keyes, M.R. and C.C. Grier. 1981. Above- and below-ground net production in 40-year-old Douglas-fir stands on low and high productivity sites. Can. J. For. Res. 11:599–605.

Kinerson, R.S., C.W. Ralston and C.G. Wells. 1977. Carbon cycling in a loblolly pine plantation. Oecologia 29:1–10.

Kozlowski, T.T., P.J. Krammer and S.G. Pallardy. 1991. The physiological ecology of woody plants. Academic Press Inc., San Diego, CA, 657 p.

Kuhns, M.R. and D.H. Gjerstad. 1991. Distribution of $^{14}$C-labeled photosynthate in loblolly pine (*Pinus taeda*) seedlings as affected by season and time of exposure. Tree Physiol. 8:259–271.

Linder, S. and E. Troeng. 1981. The seasonal variation in stem and coarse root respiration of a 20-year-old Scots pine (*Pinus sylvestris* L.). Mitt. Fordtl. Bunder Versuchtsanst 142:125–139.

Merckx, R., A. Dijkstra, A. den Hartog and J.A. van Veen. 1987. Production of root-derived material and associated microbial growth in soils at different nutrient levels. Biol. Fertil. Soils 5:126–132.

Michael, D.A., J.G. Isebrands, D.I. Dickmann and N.D. Nelson. 1988. Growth and development during the establishment year of two *Populus* clones with contrasting morphology and phenology. Tree Physiol. 4:139–152.

Nelson, N.D., D.I. Dickmann and K.W. Gottschalk. 1982. Autumnal photosynthesis in short-rotation intensively cultured *Populus* clones. Photosynthetica 16:321–333.

Nelson, N.D. and J.D. Isebrands. 1983. Late-season photosynthesis and photosynthate distribution in an intensively-cultured *Populus nigra* × *Laurifolia* clone. Photosynthetica 17:537–549.

Nguyen, P.V., D.I. Dickmann, K.S. Pregitzer and R.L. Hendrick. 1990. Late-season changes in allocation of starch and sugar to shoots, coarse roots and fine roots in two hybrid poplar clones. Tree Physiol. 7:95–105.

Paul, E.A., W.R. Horwath, D. Harris, R. Follett, S.W. Leavitt, B.A. Kimball and K.S. Pregitzer. 1994. Establishing the pool sizes and fluxes in $CO_2$ emissions from soil organic matter turnover. *In* Advances in Soil Science. Ed. B.A. Stewart, Lewis Publishers. In press.

Persson, H. 1979. Fine root production, mortality and decomposition in forest ecosystems. Vegetatio 41:101–109.

Pregitzer, K.S., D.I. Dickmann, R. Hendrick and P.V. Nguyen. 1990. Whole-tree carbon and nitrogen partitioning in young hybrid poplars. Tree Physiol. 7:79–93.

Quinlan, J.D. 1969. Mobilization of $^{14}$C in the spring following autumn assimilation of $^{14}CO_2$ by an apple rootstock. J. Hortic. Sci. 44:107–110.

Santantonio, D. 1989. Dry-matter partitioning and fine-root production in forests—new approaches to a difficult problem. *In* Biomass Production of Fast-growing Trees. Eds. J.S. Pereira and J.J. Landsberg. Kluwer Academic Publishers, Dordrecht, Netherlands, pp 57–72.

Schier, G.A. 1970. Seasonal pathway of $^{14}C$-photosynthate in red pine labeled in May, July and October. For. Sci. 16:1–13.

Smith, J.L. and E.A. Paul. 1989. Use of an *in situ* labeling technique for the determination of seasonal $^{14}C$ distribution in ponderosa pine. Plant Soil 106:221–229.

Smucker, A.J.M., S.L. McBurney and A.K. Srivastava. 1982. Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. Agron. J. 74:500–503.

Tranquillini, W. 1963. Die $CO_2$-jahresbilanz und stoffproduktion der zirbe (*Pinus cembra*). Mitt. Forstl. Bundes Versuchsanstalt Mariabrunn 60:535–546.

van Veen, J.A. and P. Kuikman. 1990. Soil structure aspects of decomposition of organic matter by microorganisms. Biogeochemistry 11:213–233.

van Veen, J.A., E. Lijeroth and L.J.A. Lekkererk. 1991. Carbon fluxes in plant–soil systems at elevated atmospheric $CO_2$ levels. Ecol. Appl. 1:175–181.

Wardlaw, I.F. 1990. The control of carbon partitioning in plants (Tansley Review No. 27). New Phytol. 116:341–381.

Warembourg, F.R. and E.A. Paul. 1973. The use of $^{14}CO_2$ canopy techniques for measuring carbon transfer through the plant–soil system. Plant Soil 38:331–345.

Webb, W.L. 1977. Seasonal allocation of photoassimilated carbon in Douglas fir seedlings. Plant Physiol. 60:320–322.