Summary of the 2005
Enriched Background Isotope Study (EBIS) Workshop
Livermore, California January 20 and 21, 2005

A progress report to DOE's Office of Science, Biological and Environmental Research (BER), Terrestrial Carbon Processes (TCP) Program

Submitted by the EBIS Working Group
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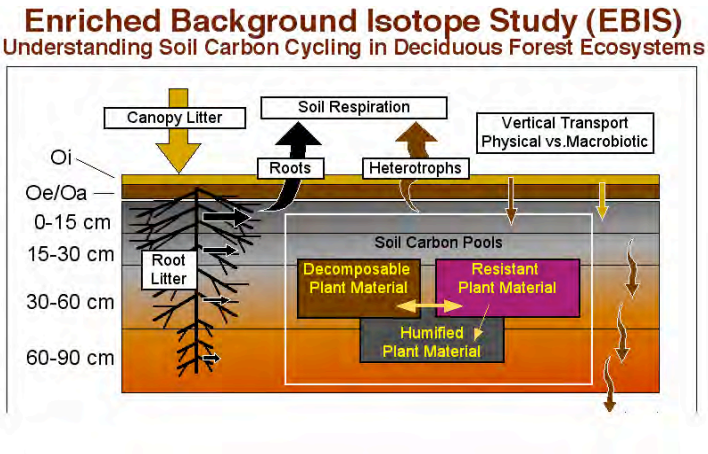

EBIS Project Web Site:
http://ebis.ornl.gov/

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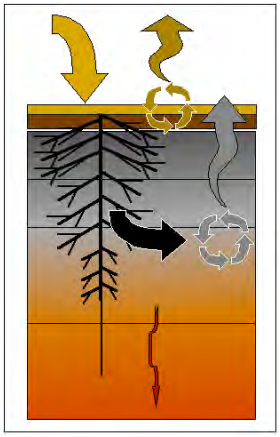
PROJECT SUMMARY AND WORKSHOP HIGHLIGHTS

Elevated levels of ^{14}C enriched CO_2 in the air and soil atmosphere as well as leaf, stem, and root tissues were observed on the Oak Ridge Reservation (ORR) during the summer of 1999, and were attributed to local incinerator activities on and/or near the ORR. The isolated enrichment of ^{14}C in local forest ecosystems represented a unique opportunity to study unresolved carbon cycling processes such as the contribution of leaf versus root litter to soil carbon accumulation, the rate of vertical transport of carbon into deep soil storage pools, and the differential contribution of physicochemical versus faunal driven processes to soil carbon cycling and sequestration. We are conducting a cooperative, multi-institutional study, centered on a field manipulation experiment, which takes advantage of the whole-ecosystem isotopic label generated by the 1999 ^{14}C-release. Experimental results from this study are being used to parameterize and refine existing carbon dynamics models for the quantification of the long-term fate of ecosystem carbon inputs and the potential for ecosystem carbon sequestration.

This report summarizes experimental progress by all participants through the first four years of the experiment. The following bullets provide highlights from the workshop derived from the task-specific progress descriptions provided in this report.

Key Scientific Findings to Date:

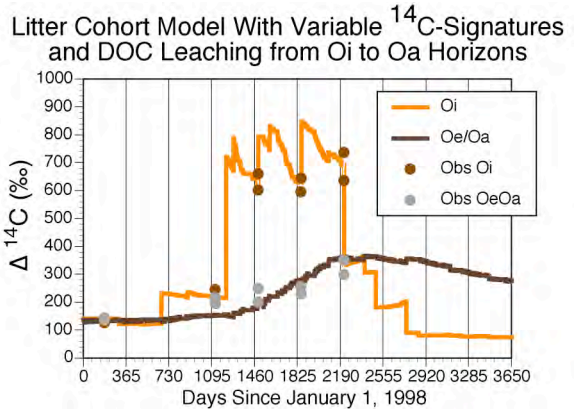
- EBIS observations from all experimental tasks support the conclusion that intra- and inter-annual soil carbon cycling in eastern deciduous hardwood forest soils should be characterized as a two-compartment system with surface litter inputs representing the primary carbon source for an organic-layer carbon cycle, and root litter inputs the primary carbon source for a mineral-soil carbon cycle. Only over long time periods (e.g., decades) will litter-layer carbon represent a significant source of mineral soil C in these forests.



- Analysis of the ^{14}C-signature of bulk organic horizons have only just begun to indicate the arrival of enriched litter into the humus (Oe/Oa) layer 4-years after the initiation of litter manipulations. This step-by-step downward migration of litter cohorts over time is consistent with a humus layer turnover time on the order of ~9 years similar to values suggest by bomb-^{14}C analysis at Walker Branch. Bulk-soil ^{14}C data have yet to demonstrate significant accumulation of litter layer carbon into mineral soil C horizons.

- Dissolved-organic-carbon (DOC) measurements from subsurface lysimeters do show evidence of rapid movement of ^{14}C-labeled-litter-C into deep soils, but the quantity of carbon moved by DOC transport into deep soils is small (<10 gC m^{-2} y^{-1}). Carbon accumulation associated with soil Fe-oxide content in Ultisols at depth has been demonstrated as a viable mechanism for C immobilization, but EBIS observations suggest the annual net rate of such accumulation would be small.

- Second- and third-year analyses of the bulk ^{14}C-signature of the recognizable litter layer (Oi) did not show hypothesized continuous increases in Δ^{14}C anticipated from fixed turnover-time mixing models. However, multiple-cohort organic-layer models with realistic representations of the short-term dynamics of decomposition and DOC leaching do explain observed patterns of Δ^{14}C. Such models will be needed to successfully capture organic layer C cycling and C transfer to the mineral soil.



- The EBIS experimental design has allowed the use of combinations of enriched radiocarbon litter and soils to partition soil respiration among three components: autotrophic (root) respiration, leaf litter decomposition, and decomposition of dead roots and other organic matter substrates. Leaf litter decomposition (derived from the difference between elevated and background plots) contributes ~10-40% of the total soil respiration flux, with variations tied to leaf litter moisture conditions and season. Root respiration contributes 40-80% of total soil respiration, with highest relative contributions before leaf-out and after senescence. Overall, variability in total soil respiration flux is more closely linked to differences in heterotrophic respiration sources than to autotrophic sources.

- Observation of the Δ^{14}C signatures of root tissues over time has provided considerable insight into root turnover processes. These measurements are unique in the history of research on the processes of fine root production, mortality, and decomposition. Measured changes in root Δ^{14}C were used to develop a root turnover model that accounts for all transfers of ^{14}C over time. There is evidence that in the surface horizons the very fine roots (< 0.5 mm) turn over slightly more rapidly than the medium fine roots (0.5 to 2.0 mm), but support for this size class difference appears to decline with depth.

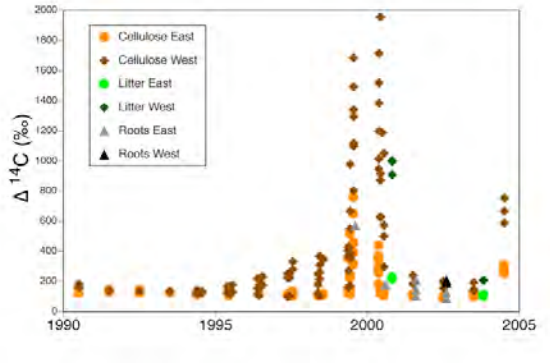
- Fractionation of soil C by particle size and secondary organization into microaggregates is supports our original hypothesis that relatively greater proportions of labeled root inputs will be physically protected by incorporation into microaggregates. Enriched root C is steadily accumulating in microaggregated silt- and clay-sized particles in addition to microaggregated particulate organic matter. In non-microaggregated soil, however, the level of enrichment in silt- and clay-sized particles has changed little since the experiment started. This 'aggregate-based' fractionation approach provides the sensitivity needed to observe increasing ^{14}C-signatures of several fractions from the multi-year enriched litter treatment. Differences between soil types in the fractions that are being

enriched are hypothesized to be related to differences in sites available for sorption of dissolved organic C within specific soils (Ultisols vs. Inceptisols).

- Density-based methods for isolating functionally different soil organic matter pools have successfully traced enriched ^{14}C from litter and roots into unprotected, protected, and mineral-associated pools. The Ultisol sites, initially been dominated by root inputs, showed no appreciable contribution from surface litter. The Inceptisol mineral soils also received the majority of their C inputs from root inputs, but additionally appear to have begun incorporating enriched litter ^{14}C. Both soil types reveal a general path of rapid particulate input into the unprotected particulate pool (free light fraction), followed by a much slower incorporation from the free light fraction into the protected particulate pool (occluded light fraction). The Ultisols appear to more effectively stabilize non-particulate SOM, but the mineral fractions of both soil types clearly include a fast- and slow-cycling pool.

- A generic carbon cycling model based on fixed turnover-time assumptions for litter and soil C pools (i.e., Rothamsted) was not adequately capturing the dynamics of organic layer C-cycling indicated by the measured changes in ^{14}C. Modifications to this and similar models and the application of multi-compartment litter and root decomposition models are being considered to better align model predictions of C pool structure and decomposition dynamics with field observations.

- A new mid-season pulse of ^{14}C was observed in the summer of 2004 of sufficient magnitude to increase the ^{14}C signature of new root growth at western EBIS sites (TVA and Pine Ridge). This pulse represents a new opportunity to address root turnover and decomposition with a defined initialization period that was not available in the first grant period.



- A new model of root growth, turnover, and senescence was developed to capture observed ^{14}C dynamics measured over the first 3 years of EBIS measurements. A key feature of the root model is the inclusion of two live root pools: one that turns over rapidly (on the order of months) and one that consists of longer-lived roots (>5 years in age). The delineation of these two pools explains the relatively rapid enrichment of live roots only one year after the release (caused by turnover of the short-lived roots), and the relatively slow decline of the enrichment in the subsequent years (caused by persistence of the long-lived roots). To represent the complexity in root dynamics while minimizing the number of unknown parameters, the model is also designed with the following attributes: root ages that are non-normally distributed, representation of fine roots pools, with both structural and non-structural C, variable plant storage of $C/^{14}C$, seasonal growth and respiration patterns, Monte Carlo analysis of uncertainty, and a X^2 approach to find best-fit parameters to match root C and $\Delta^{14}C$ data.

- Evaluation of the C mass balance for the bulk forest soils provides a reasonable accounting of C sources, sinks and losses, but a key uncertainties remains associated with the rate of root litter production. Organic- to mineral-layer vertical transfers of particulate and dissolved organic carbon also still need to be resolved to explain C mass balance for the Oe/Oa (humus layer) and A horizons of the soil.

Operational-progress:

- All cohorts of enriched or background litter, collected in the fall of 2000, have been added to the EBIS treatment plots representing three full years of litter manipulations.
- Vegetation cover, litter production, species composition, and climate are comparable across the four spatially distributed EBIS research sites and will allow similar analysis of carbon cycling processes in all cross-site comparisons.
- The Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory processed more than 3400 ^{14}C-samples in support of the EBIS research project during the first 3-years of the EBIS study.
- New atmospheric releases of ^{14}C have taken place since the large pulse in 1999. However, measured releases in 2000 and 2002 did not have significant overlap with the growing season and are of no concern to the interpretation of the EBIS study. Documented growing-season ^{14}C-releases in August of 2003 and in May, June, and July of 2004 were largely restricted to the west-end of the Oak Ridge Reservation. Litterfall and new root growth samples have been collected for those time periods. The ^{14}C-signature data from those samples will be used as needed to adjust our analyses of treatment effects and model runs to account for new ^{14}C inputs in future years.
- PDF versions of the power point presentations given at the Gaithersburg (2004) and Livermore (2005) workshops are available for downloading at http://ebis.ornl.gov.

Post-workshop deliverables:

1. Posting of Livermore workshop presentations on the project web site – Completed.
2. Submission of the Livermore EBIS workshop report to DOE – This report.
3. Prepare a renewal proposal for work in FY2006, 2007 and 2008 by March 1, 2005.
4. Develop a subcontract between ORNL and Bill Parton to being initial application of the *Daycent* model to the EBIS data from FY2005 funds.
5. Completion of a multi-authored, initial EBIS synthesis manuscript for submission to a high profile journal detailing the nature of soil carbon cycling under eastern deciduous hardwood forests and key hypotheses remaining to be tested – delayed deliverable from October-November 2004 now planned for early summer 2005.
6. Complete the task-specific manuscripts described below in 2005.

EBIS PUBLICATIONS AND PRESENTATIONS

Completed Publications:

Trumbore S, Gaudinski JB, Hanson PJ, Southon JR (2002) A whole-ecosystem carbon-14 label in a temperate forest. *EOS* 83:265,267-268.

Swanston CW, Torn MS, Hanson PJ, Southon JR, Garten CT, Hanlon EM, Ganio L (2005) Initial characterization of processes of soil carbon stabilization using forest stand-level radiocarbon enrichment. *Geoderma* (in press; available online January 8, 2005).

Cisneros-Dozal LM, Trumbore SE, Hanson PJ (2005) Estimating sources of soil respired CO_2 and their seasonal variation using a unique radiocarbon tracer. *Global Change Biology* (in press).

Hanson PJ, Swanston CW, Garten CT Jr., Todd DE, Trumbore SE (2005) Reconciling Change in Oi-Horizon ^{14}C With Mass Loss for an Oak Forest. *Soil Science Society of America Journal* (in press).

Joslin JD, Gaudinski JB, Torn MS, Riley WJ, Hanson PJ (2005) Unearthing live fine root turnover times in a hardwood forest: the roles of root diameter, soil depth, and root branching order. *Biogeochemistry* (accepted pending revisions).

Submitted manuscripts:

Callaham MA Jr., González G, Hale C, Heneghan L, Lachnicht S, Zou X (In Review) Policy and management responses to earthworm invasions. *Biological Invasions*.

Manuscripts in preparation for anticipated submission in 2005:

Cisneros Dozal LM. Using a ^{14}C release to partition soil respiration sources in a southeastern hardwood forest. PhD thesis, University of California, Irvine [Expected 9/2005].

Cisneros Dozal, LM, Trumbore SE, Winston G, Hanson PJ. Importance of leaf litter in soil respiration. For submission to *Journal of Geophysical Research-Biogeoscience*.

Cisneros Dozal, LM, Trumbore SE, Xu X, Zheng S, Seasonally changing sources of carbon for root respiration. For submission to *New Phytologist*.

Garten CT Jr. et al. Applications of natural abundance 13C measurements for interpreting soil C dynamics in undisturbed forest ecosystems. For submission to *Biogeochemistry*.

Gaudinski JB, Torn MS, Riley WJ, Dawson TE, Joslin JD, Trumbore SE, Majdi H, Hanson PJ. Growth of new roots and leaves from stored carbon reserves in temperate forest ecosystems estimated with a numerical model and radiocarbon data. For submission to *Ecological Applications*.

Hanson PJ, Garten CT, Post WM, Swanston CW, and Trumbore SE. Multi-year fate and transport of carbon through the organic horizons of a deciduous forest: facilitated observations with ^{14}C-enriched litter cohorts. For submission to *Soil Science Society of America Journal*.

Riley WJ, Gaudinski JB, Torn MS, Swanston C, Hanson PJ. Root lifespan and growth in a forested ecosystem using a numerical model tested with locally released radiocarbon. For submission to *Ecological Applications*.

Jardine PM, Todd DE, Palmer JA, Swanston C, Hanson PJ. Hydrogeochemical controls on the fate and transport of dissolved organic C in subsurface environments. For submission to *Soil Science Society of America Journal*.

Treseder KK, Torn MS, Masiello CA. Do ectomycorrhizal fungi use soil carbon? A field test with large-scale radiocarbon enrichment. For submission to *Oecologia*.

Published abstracts:

Cisneros-Dozal L, Trumbore S, Hanson P, & Xu X (2002) Partitioning of Soil Respiration Sources Using ^{14}C-Enriched Leaf Litter and Roots in a Temperate Forest, Oak Ridge, TN. San Francisco, California, 6-10 December 2002, Eos Trans. AGU, 83(47), Fall Meet. Suppl., Abstract B11C-0766, 2002.

Cisneros-Dozal LM, Trumbore S, Hanson PJ and Winston G (2004) Quantifying Leaf Litter Decomposition and the Response to Moisture Changes Using ^{14}C. American Geophysical Union, Joint Assembly, 17-21 May 2004, Montreal, Québec, Canada. Paper number B42A-03, Abstract 903.

Cisneros-Dozal LM, Trumbore S and Zheng S (2004) The Source of C for Root/Rhizosphere Respiration Using ^{14}C Enriched Roots in a Temperate Forest. American Geophysical Union, Fall Meeting 13-17 December, 2004, San Francisco, CA, Abstract B23A-0945.

Cisneros Dozal LM, Trumbore S, Hanson PJ (2004), Partitioning Sources of Soil Respired CO_2 and Their Response to Moisture and Temperature Changes Using a Whole-Ecosystem Radiocarbon Tracer, *Eos Trans. AGU, 85*(17), Jt. Assem. Suppl., Abstract B42A-03.

Hanson PJ, Trumbore S, Gaudinski J, Swanston C, Torn M, Jastrow J, Joslin JD, & Jardine P (2002) Enriched background isotope study (EBIS): application of an ecosystem-scale ^{14}C tracer to soil-carbon-cycle studies. Annual meeting of the Soil Science Society of America, November 10-14, 2002, Indianapolis, Indiana. Annual Meetings Abstracts 2002, S07-hanson095634.

Hanson PJ, Trumbore SE, Swanston CW, et al. (2004) Distinct organic and mineral soil carbon cycles in an upland oak forest: evidence from multi-year ^{14}C enriched litter additions. Annual Meeting Abstracts Soil Science Society of America, October 31 to November 4, 2004, Seattle, Washington. S07-Hanson 6065.

Jastrow JD, O'Brien SL, Van Til BE, Matamala R, Swanston CW, & Hanson. 2003. Incorporation of a whole ecosystem radiocarbon label into unprotected and protected soil carbon pools, p. 47. In Book of Abstracts, Soil Ecology Society Ninth Biannual International Conference, Palm Springs, California. 11-14 May 2003.

Joslin JD, Gaudinski JB, Torn MS, Swanston C, Hanson PJ & Todd DE (2003) Turnover of fine root carbon in a ^{14}C-labeled forest ecosystem. In the 95th Annual Meeting of the American Society of Agronomy, Denver, CO, November 2-6, 2003. Agronomy Abstracts.

Todd DE, Tarver JA, Mehlhorn TL, Swanston C, Hanson PJ and Jardine PM (2003) Transport and sequestration of organic C in contrasting soils amended with C-14 enriched leaf litter. In the 95th Annual Meeting of the American Society of Agronomy, Denver, CO, November 2-6, 2003. Agronomy Abstracts.

Treseder KK, Torn MS, Masiello CA (2003) Do ectomycorrhizal fungi use soil carbon? A field test with large-scale radiocarbon enrichment. Abstracts the Ecological Society of America 88th Annual Meeting, 3-8 August 2003, Savannah, Georgia, p. 337.

Popular-Press EBIS Summary

Cabage B (2002) Carbon-14 levels discovered on ORR provide a unique scientific opportunity. ORNL Reporter 44:1,4.

Other presentations:

Cisneros Dozal LM, Trumbore S, Hanson PJ, Xu X (2003) Quantifying Sources of Soil Respiration Using ^{14}C-Enriched Leaf Litter and Roots in a Temperate Forest. National Science Foundation North American Carbon Program Joint PI Meeting (NACP '03), Arlington, Virginia, 12-14 May 2003.

Gaudinski J, Riley W, Torn M, and Joslin J (2003) Using a Locally Released Radiocarbon Label to Constrain Fine Root Stored Carbon Inputs to Roots and Refinement of Isotopically Derived Fine Root Lifespans Using A Locally Released Radiocarbon Label in Oak Ridge, TN. AGU Fall Meeting, 8-12 December, 2003, San Francisco, California.

Gaudinski JB, Torn M, Dawson T, Hooshang M & Joslin D (2003) Quantifying fine root carbon inputs to soil: improving estimates of BNPP by combining results from radiocarbon and traditional methodologies. Conference on Fine Root Turnover Workshop in forested ecosystems. Swedish University of Agricultural Sciences, Uppsala, Sweden, September 8-10, 2003.

Hanson PJ, Trumbore S, Swanston C, Torn M, Jastrow J, Gaudinski J, Jardine P, Post WM, Cisneros-Dozal M, Garten CT Jr., Guilderson T, Joslin JD, Winston G, & Callaham M (2003) Enriched Background Isotope Study (EBIS). DOE/BER Carbon Cycle Investigators Meeting, Boulder, Colorado. 16-17 October 2003.

Hanson PJ, Trumbore SE, Swanston CW, et al. (2004) Distinct organic and mineral soil carbon cycles in an upland oak forest: evidence from multi-year ^{14}C enriched litter additions. Annual Meeting Abstracts Soil Science Society of America, October 31 to November 4, 2004, Seattle, Washington.

Jastrow JD, O'Brien SL, Van Til BE, Matamala R, Swanston CW & Hanson PJ (2003) Incorporation of a whole ecosystem radiocarbon label into unprotected and protected soil carbon pools. North American Carbon Program Joint PI Meeting 2003, Arlington, Virginia. 12-14 May 2003.

Jastrow JD, O'Brien SL, Van Til BE, Matamala R, Swanston CW & Hanson PJ (2003) Incorporation of a whole ecosystem radiocarbon label into unprotected and protected soil carbon pools. DOE/BER Carbon Cycle Investigators Meeting, Boulder, Colorado. 16-17 October 2003.

Swanston C, Torn M, Hanson P, and Gaudinski J (2003) Radiocarbon flux into labile and stable soil organic matter pools in the Enriched Background Isotope Study (EBIS) 18th International Radiocarbon Conference, Wellington, New Zealand, from 1–5 of September 2003.

Swanston C, Torn M, Hanson P, and Gaudinski J (2003) Tracing radiocarbon from forest litter and roots into labile and stable carbon pools. International Conference on Mechanisms and Regulation of Organic Matter Stabilization in Soils, October, 5th - 8th, 2003, Munich, Germany.

Torn MS (2004) Soil Carbon Dynamics in Two Novel Cases: The Historic Russian Archives and the Tennessee Burp. Informal Seminar. University of Zurich, Switzerland.

Torn M, Gaudinski J, Treseder K, Westbrook J, Joslin D, Swanston C, Hanson P (2003) A Forest ^{14}C Pulse-Label Study of Root Turnover and Microbial Dynamics (EBIS). 18th International Radiocarbon Conference, Wellington, New Zealand, from 1–5 September 2003.

Torn MS, Treseder K, Westbrook J, Swanston C, and Hanson P (2003) A whole-forest ^{14}C pulse-label study of microbial dynamics and soil organic matter (EBIS). International Conference on Mechanisms and Regulation of Organic Matter Stabilization in Soils, October, 5th - 8th, 2003, Munich, Germany.

EBIS BACKGROUND AND APPROACH

Purpose of the EBIS Project

A unique, large release of $^{14}CO_2$ occurred near the Oak Ridge Reservation (ORR), Oak Ridge, TN in July/August 1999. At a local level, this pulse label is similar to or larger in magnitude than the pulse of ^{14}C produced by atmospheric weapons testing. Measurements of ^{14}C in tree ring cellulose throughout the ORR area demonstrated that at all sites, the 1999 release was unprecedented in its uptake by vegetation. We are taking advantage of the whole-ecosystem isotopic label generated by this release to address several outstanding issues in the terrestrial carbon cycle:

(1) the partitioning of soil respiration between autotrophic and heterotrophic sources, and quantification of that partitioning seasonally and inter-annually,
(2) the partitioning of heterotrophic respiration sources between aboveground litter decomposition and belowground root detritus decomposition,
(3) the pathways leading from leaf and root detritus to long-term stabilization of soil organic matter, including the role of soil fauna,
(4) the role of dissolved organic carbon (DOC) transport in distributing carbon within the soil profile, and
(5) the longevity and turnover time of fine roots.

Furthermore, we are using the results of our work to parameterize and refine existing carbon dynamics models. Such models will then be used to quantitatively address the long-term fate of ecosystem carbon inputs and the potential for ecosystem carbon sequestration.

Experimental Approach

The first four issues listed above are being addressed through a reciprocal litter transplant experiment. At four sites on the ORR encompassing two soil types and two levels of ^{14}C exposure in 1999, we established replicated permanent plots for the manipulation of forest litter through reciprocal transplants of enriched versus near back ground litter among sites. With a combination of incubations, soil surface chamber flux measures and soil CO_2 profiles, and continuous measurements of soil temperature and moisture controls, we are tracking the changes in soil respiration partitioning over several years of climate variations. The nature and source of long-lived soil organic matter pools are being tracked with differently labeled root and surface litter sources. Experiments to exclude soil fauna will allow understanding of their importance in facilitating those transformation processes. Finally, sampling of soils and soil solutions and the use of inert tracers are enabling us to investigate the chemical nature and form of DOC and its transport in surface soil horizons. By replicating the litter transplant study on two soil types we can address the influence of soil chemical and physical properties on all these issues.

The fifth issue, longevity and turnover of fine roots, was addressed by tracing the radiocarbon label through the fine root pool over time. Doing so will allow us to determine the dynamics of different constituents of the fine root population, as well as their specific contributions (relative to leaf litter) to heterotrophic respiration and soil organic matter formation. Intensive root longevity studies will be conducted at a single site where root samples are available from previous years.

PROGRESS TO DATE

Experimental Litter Manipulations (Task 1)

Paul Hanson and Don Todd

A timeline of experimental activities accomplished since the ^{14}C-pulse of 1999 is provided below (Figure 1-1). As of January 2005 three annual cohorts of either enriched or background litter (collected in the fall of 2000) have been applied to the four EBIS experimental plots. Time-zero, 1st-year, 2nd-year, and 3rd-year litter layer and soil core samples from March of 2001 January 2002, January 2003, and January 2004, respectively, were processed and distributed to individual EBIS researchers.

Landscape cloth was applied to all experimental plots from mid-October through mid-December in 2000, 2001, and 2002 to exclude ambient leaf litter inputs in those years. In the fall of 2003 and 2004 triplicate 50x50 cm squares of landscape cloth were added to each plots at the locations for 3rd and 4th-year sampling in January of 2004 and 2005, respectively. Fifth-year sampling of the organic horizons is underway as of the date of this report in late January and early February of 2005.

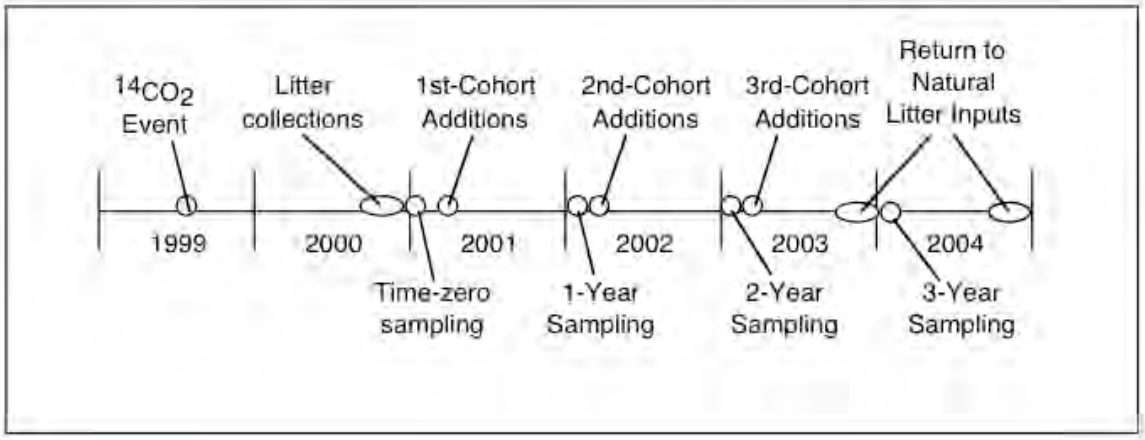


Figure 1-1. EBIS experimental timeline.

Site Vegetation and Litter Production (Task 1)

Paul J. Hanson and Donald E. Todd

Automated measurements of environmental variables (not shown) and periodic measures of vegetation growth (dbh tape measurements), canopy litter production, and species composition are being conducted in conjunction with the EBIS litter manipulations to evaluate the potential influence of site-specific conditions on soil carbon cycling processes. Environmental drivers, such as temperature and soil water status, show no significant differences across sites outside of occasional localized summer precipitation events. There is some potential for species differences (and corresponding changes in litter quality) to impact soil carbon cycling processes across sites (Figure 1-2), but this will not impact the analysis for years 1 to 3 when only the litter from the 2000 cohort will be added back to the plots The Pine Ridge site has more red maple litter that may decompose at a faster rate than the dominant oak litter at the other three sites.

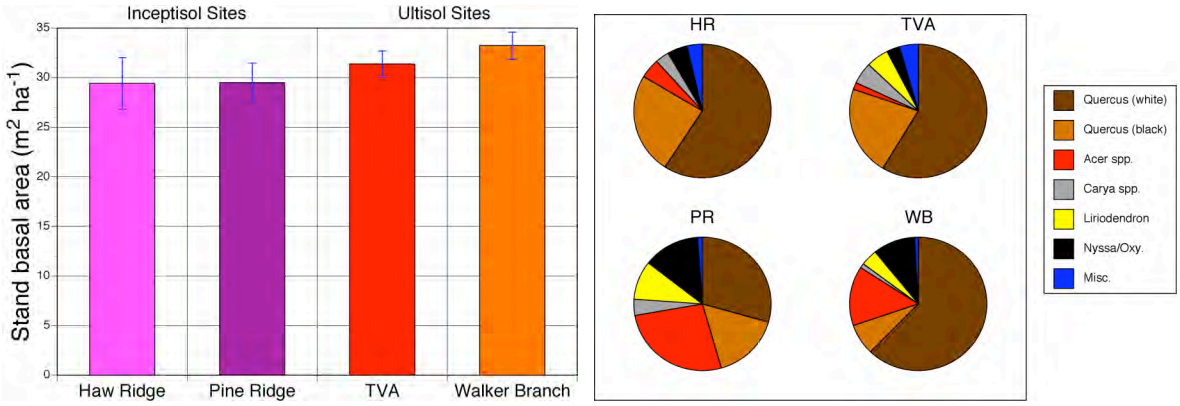


Figure 1-2 Stand basal area for the four EBIS sites, and the species contribution to the basal area at each of the EBIS sites.

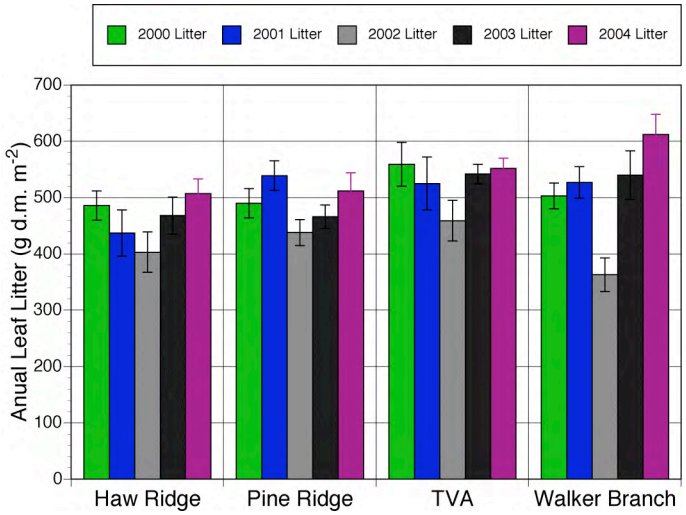


Figure 1-3. Mean annual ambient litterfall mass from 2000 to 2004 for each EBIS site.

From 2000 to 2004 annual litter production averaged 518 and 475 g dry matter m^{-2} for those sites with Ultisol and Inceptisol soils, respectively (Figure 1-3). These levels closely approximate the experimental additions of 500 g dry matter m^{-2} y^{-1} added to all treatment plots in May 2001, February 2002, and February 2003.

Air and soil temperatures at the four EBIS sites are not statistically different. Although some variation in mineral soil water status is expected from differences in soil texture at each site. The seasonal patterns of soil water availability and hydrologic flux through all sites is very similar. Litter water content, a highly dynamic but important driver for organic matter decomposition, is also similar at all sites, but isolated summer thunderstorms can generate occasional wet/dry cycles at a single site that may not be represented at all four study sites.

Analysis of Soil C and N at the EBIS Study Sites (Task 1)

Chuck T. Garten, Paul J. Hanson, Don E. Todd, and Bonnie Lu

Litter additions, O-horizons (i.e., Oi > 1 year and Oe/Oa), and four mineral soil depth increments (0-15, 15-30, 30-60, and 60-90 cm) from the EBIS study sties have been analyzed for total C and N concentrations. Samples were analyzed on a LECO CN-2000 using calibration standards traceable to NIST reference materials. Soil C and N concentrations (gC g-1) in combination with average reference densities (g soil < 2 mm cm-3) were used to calculate C and N stocks (g m-2) in the 0-15, 15-30, 30-60, and 60-90 cm soil layers. Similar calculations were performed for the forest floor horizons (Oi> 1 yr and Oe/Oa) using concentration data in combination with measurements of forest floor dry mass (g m-2). A two-way analysis of variance including effects of study site, litter addition (background vs. enriched), and site x litter addition was used to analyze the data. The effects of litter addition and the site x litter addition interaction were generally not statistically significant ($P > 0.05$).

Mineral soil C stocks (0-90 cm) ranged from 4084 to 4716 g C m^{-2} across the four sites with the highest inventories at Walker Branch Watershed (Figure 1-4). Revised mineral soil N stocks (0-90 cm) range from 104 to 222 g N m^{-2} with the highest inventories at the TVA site (data not shown). Both N and C concentrations decline sharply with increasing soil depth, and most of the C inventory at the EBIS sites resides in the surface 30 cm of mineral soil. Soil C stocks in the surface mineral soil (0-30 cm) were approximately twice greater than those measured in the deeper soil layers (30-60 and 60-90 cm). There were also statistically significant differences in soil C stocks and soil C:N ratios among the four sites. Haw Ridge had the lowest surface mineral soil C stocks and the highest C:N ratios. The TVA site had the highest soil C stocks and the lowest C:N ratios. The remaining two sites (Pine Ridge and Walker Branch) were intermediate in their soil C stocks and soil C:N ratios. Soil N stocks followed a similar pattern, but the differences between Haw Ridge and the TVA site were even greater than those for soil C stocks.

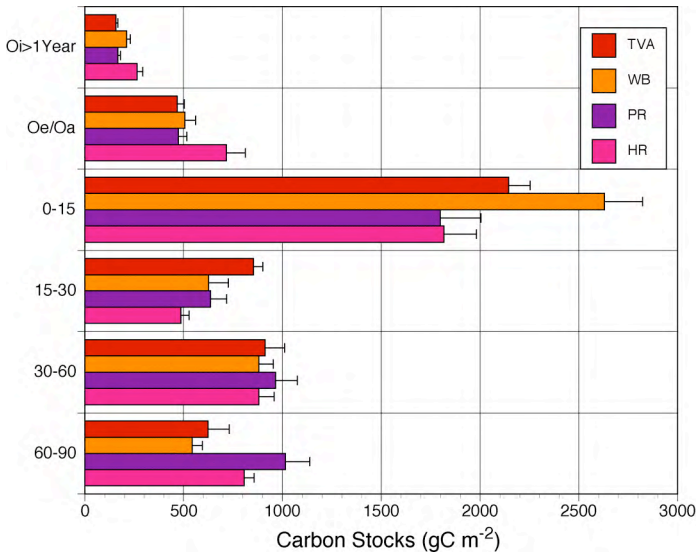


Figure 1-4. Revised estimates of organic-layers and mineral soil carbon stocks for each EBIS research site: Pine Ridge (PR), Haw Ridge (HR), TVA, and Walker Branch (WB).

Mean (±SE) N concentrations in litter additions were 0.63 ±0.02 % in 2001 (year 1) and ranged from 0.56 ±0.03 to 0.61 ±0.02 % in 2002 (year 2). Measured mean (±SE) C

concentrations in litter additions at the four study sites ranged from 45.5 ±0.16 to 45.9 ±0.08 % in 2002 and were less than measured litter C concentrations in 2001 (50.1 ±0.20 %). The C:N ratio of litter additions ranged from 75.1 to 82.5 in 2002; comparable to the C:N ratio of litter additions in 2001 (79.5).

On average, N concentrations in the O-horizons at the EBIS study sites range from 1.0 to 1.4 %. For Oi litter greater than 1 year old, N concentrations in 2002 tend to be greater than those measured in 2001 at all four sites. There is also a trend toward lower O-horizon C concentrations in 2002 (year 2) than in 2001 (year 1). Carbon concentrations in the Oi>1-year layer (43 to 49%) are greater than those measured in the Oe/Oa layer (28 to 36%) at most sites. Across all sites, measured mean C:N ratios in the Oi>1 year layer range from 31 to 42 and those in the Oe/Oa layer range from 23 to 29. There is a clear trend toward decreasing C:N ratios in the Oe/Oa layer from 2000 (year 0) to 2002 (year 2). There are generally greater C stocks in the Oe/Oa horizon than in Oi litter greater than 1 year old.

Across the four sites, mean surface soil (0-15 cm) N concentrations range from 0.057 to 0.118% and mean surface soil C concentrations range from 1.86 to 2.49%. The lowest surface soil N and C concentrations were measured at Haw Ridge. Soil C:N ratios at the Pine Ridge (23 to 30) and TVA (18 to 24) study sites are generally less than those measured at Haw Ridge (32 to 54) and at Walker Branch Watershed (25 to 39).

Soil Chemical Characterization

Four mineral soil samples (0-15 cm) from each site were chemically separated into humic acid and humin fractions using traditional methods of soil extraction with sodium hydroxide followed by precipitation of humic acids by the addition of hydrochloric acid. Carbon in the fulvic acid fraction was not measured directly but estimated by mass balance. Carbon concentrations in the humic acid fraction (15 to 18 %) were much greater than those in the humin (0.6 to 1.0 %). There were no significant differences among the four EBIS sites in the partitioning of soil C among the humin, humic acid, and fulvic acid fractions (Figure 1-10). Approximately 35 to 40 % of the soil C was found in the fulvic acid fraction, which is highly soluble and may contribute to the downward movement of ^{14}C in soils. Approximately 36 to 39% of the soil C resides in the humin fraction and approximately 21 to 26 % was found to be associated with humic acids. The latter two fractions are more refractory than fulvic acids. Considering all three fractions, C in humin is considered to be the most resistant to decomposition.

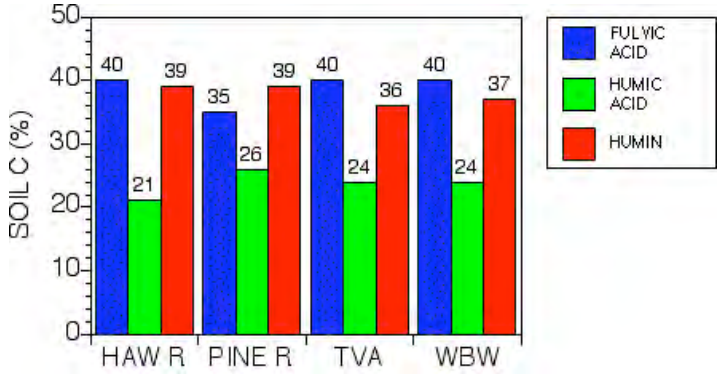


Figure 1-5. Distribution of C among fulvic acid, humic acid, and humin fractions in surface (0-15 cm) soil samples from the four EBIS study sites.

^{14}C Analyses and Bulk-Carbon-Pool ^{14}C Signatures (Task 5)

Chris Swanston and Tom Guilderson

Approximately 3400 AMS radiocarbon measurements have been carried out in the first three years of the EBIS project on bulk soil, soil fractions, microbial biomass, dissolved organic carbon, roots, ambient and soil atmosphere, and aboveground biomass in support of all EBIS research tasks. By February of the fourth year, approximately 300 samples have been analyzed. Precision of AMS measurements of graphite from these samples has been high (~5 ‰). This large and expensive number of ^{14}C analyses is unprecedented within a single study, but it is necessary as we attempt to appropriately interpret the fate of the 1999 ^{14}C-pulse using a replicated study design and appropriate statistical methods.

Bulk-Soil ^{14}C Signatures and a Model of Oi-Layer C and ^{14}C cycling (Task 1)

Paul J. Hanson, Chris Swanston, Charles T. Garten Jr., and W. Mac Post

Sampling of bulk organic (Oi>1Y and Oe/Oa) and mineral soil (0-15, 15-30, 30-60, and 60-90 cm) 'horizons' was conducted in January 2001, 2002, 2003, and 2004 representing 0-Year, 1-Year, 2-Year, and 3-Year experimental samples, respectively. Year-zero organic samples were 0.75 m^2 per 49-m2 plot, but that was reduced to 0.18 m^2 for all subsequent annual measurements. Mineral soil sampling to a target depth of 90 cm (seldom achieved due to rock and high soil density constraints) were collected over 0.02 m^2 per 49 m^2 plot in all years. Through four years of sampling, <3.6% of the organic layer and <1% of the mineral soil available sampling surface (36 m^2) has been sampled to date.

After the first year of enriched (1005‰) or background (221‰) litter additions (2002 data points in Figure 1-6), recognizable litter greater than 1-year of age (Oi>1Y) showed the expected patterns of enrichment or dilution, respectively. In the western sites (Pine Ridge and TVA) the addition of enriched litter led to a moderate spike in ^{14}C-signature of the surface organic layer, and the background litter led to a significant dilution in ^{14}C. In the eastern sites (Walker Branch /Haw Ridge), the addition of enriched litter led to a large spike in ^{14}C, whereas no change was detectable with the addition of background litter. Little or no ^{14}C was detected in the bulk mineral soil horizons of any of the sites. There was little evidence of new C movement below the Oi horizon after one year of treatment.

After the second and third year of experimental litter additions, we were surprised to see that ^{14}C-signatures of the Oi>1Y layer did not continue to rise in the enriched plots or fall in the near-background litter treated plots (2003 and 2004 data points in Figure 1-6). Lack of continued increases in the Oi>1Y ^{14}C-signature towards the 1000 ‰ bulk signature of the added litter cohorts following three years of treatment was hypothesized to result from: (1) differential decomposition of individual litter cohorts resulting from inter-annual variations in decomposition driven by variable weather patterns, (2) variable losses of ^{14}C from the metabolism of fresh litter, (3) variable losses of ^{14}C from leaching of dissolved organic carbon (DOC) from fresh litter, and (4) preferential removal of ^{14}C in fresh litter via macrobiotic transport. The presence of litter fences, lack of observed mass movement of fresh litter, and consistent plot-to-plot error bars for Oi-layer mass and ^{14}C-signatures led us to conclude that disturbance was not a contributing

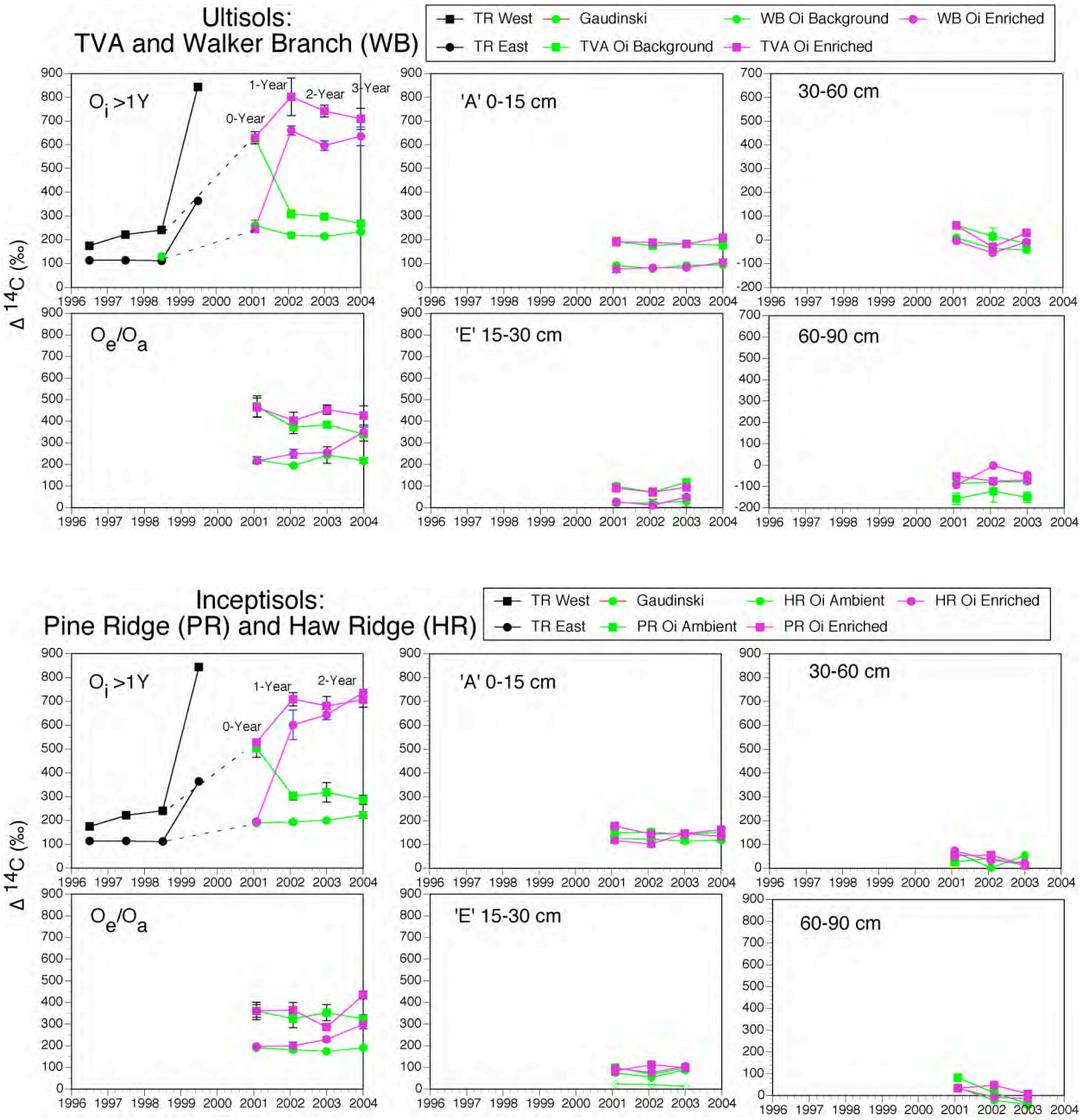


Figure 1-6. Bulk ^{14}C-analysis by site and horizon for the Ultisol (upper graph) and Inceptisol (lower graph) sites showing the following layers: recognizable litter (Oi>1Y), humus layer including some root material (Oe/Oa), 0 to 15 cm mineral soil (0-15), 15 to 30 cm mineral soil (15-30), 30 to 60 cm mineral soil (30-60), and 60 to 90 cm mineral soil (60-90). Black symbols are the ^{14}C in tree ring cellulose (TR) for years prior to time-zero.

factor. Within the Oe/Oa and mineral soil horizons root-carbon sources from exudates and root turnover for may have been responsible for some dilution of the enriched litter.

Following three years of litter manipulation there remains little evidence of new litter-C movement below the Oe/Oa horizon, but enriched litter material has impacted the Oe/Oa layer by 2004 suggesting a gradual and organized year-by-year migration of litter cohorts into the

humus layer. The combination of large stocks of soil C, high variability of the soil matrix, and a low rate of vertical transport of litter-layer carbon may be responsible for obscuring the downward movement of ^{14}C signal into the large carbon stocks of the 0-15 cm soil layers.

Further discussion of these ^{14}C patterns and the model complexity required to reproduce them is included under Task 6 below. Briefly, cohort based models of litter migration and a knowledge of DOC leaching and transport will be required to reconcile explain the ^{14}C signatures in our data.

^{14}C Content of the 2000-Litter-Cohort Carbon Pools (new effort)

Laura J. Hainsworth

Analysis of the ^{14}C content of various carbon pools in the 2000 leaf-litter-cohort was initiated to gain a better understanding of the nature of the pulse-labeling event and the distribution of the radiocarbon label within the foliar litter. Variations in the ^{14}C-content of carbon pools within the leaf litter might account for observed ^{14}C-signatures in the Oi horizon. An initial laboratory experiment was conducted on enriched and near-background *Quercus prinus* L. litter collected in 2000 to determine if leaching of fresh litter would yield DOC with a different ^{14}C-signature from the original bulk material (Figure 1-7).

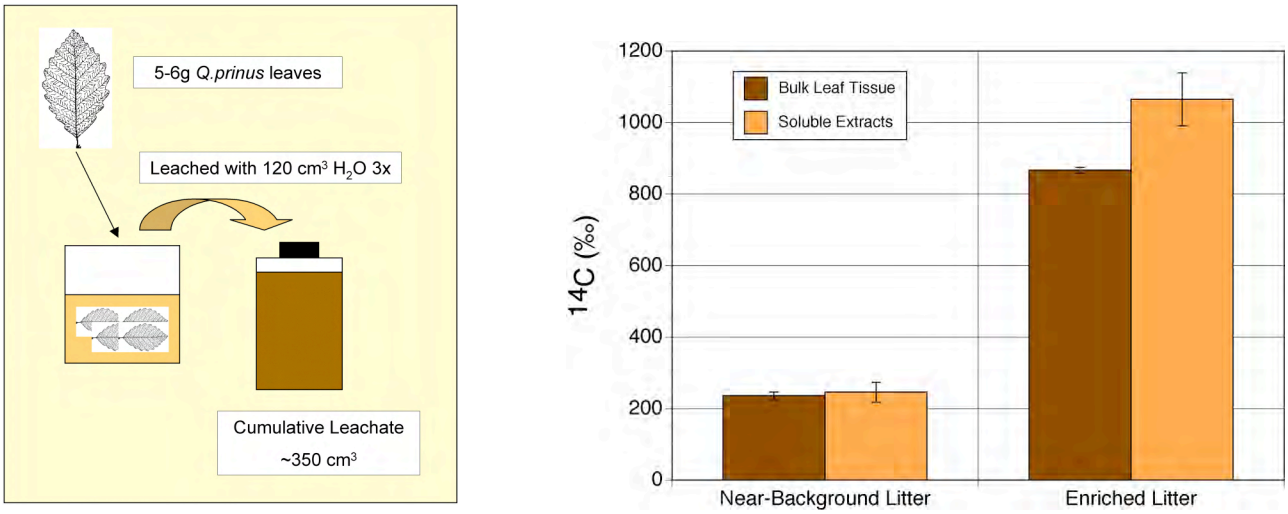


Figure 1-7. Diagram of the experimental litter leaching study conducted on *Quercus prinus* L. (left graph) and the results (right graph).

The mean leachate [C] was 378 µg cm^{-3} and did not differ by source of the litter source (near-background vs. enriched). Near-background tissues showed uniform ^{14}C-signatures for leached and bulk tissues, but the soluble materials leaching from enriched litter had a significantly higher^{14}C-signatures ($p = 0.02$; Figure 1-7). This previously unrecognized phenomenon is critical to modeling ^{14}C movement through the organic and mineral soil horizons because these data suggest that the ^{14}C-signature was not uniformly distributed among the soluble and insoluble fractions of *Quercus prinus* L. litter collected at the highly enriched western end of the study area. Leachable carbon was found to be significantly enriched in ^{14}C relative to the bulk leaf tissue.

In an effort to better characterize the allocation of the radiocarbon label in the litter, a series of chemical fractionations are being analyzed for litter from three dominant species: *Q. prinus*, *Q. alba L.*, and *Acer rubrum L.* Samples were collected in the fall of 2000 at sites having near-background (Walker Branch) vs. enriched (Pine Ridge) ^{14}C-signatures. Isolation of soluble components (sugars, amino acids and soluble phenolic compounds) and insoluble residue (cellulose and lignin) from ground leaf tissue has been completed (Figure 1-8).

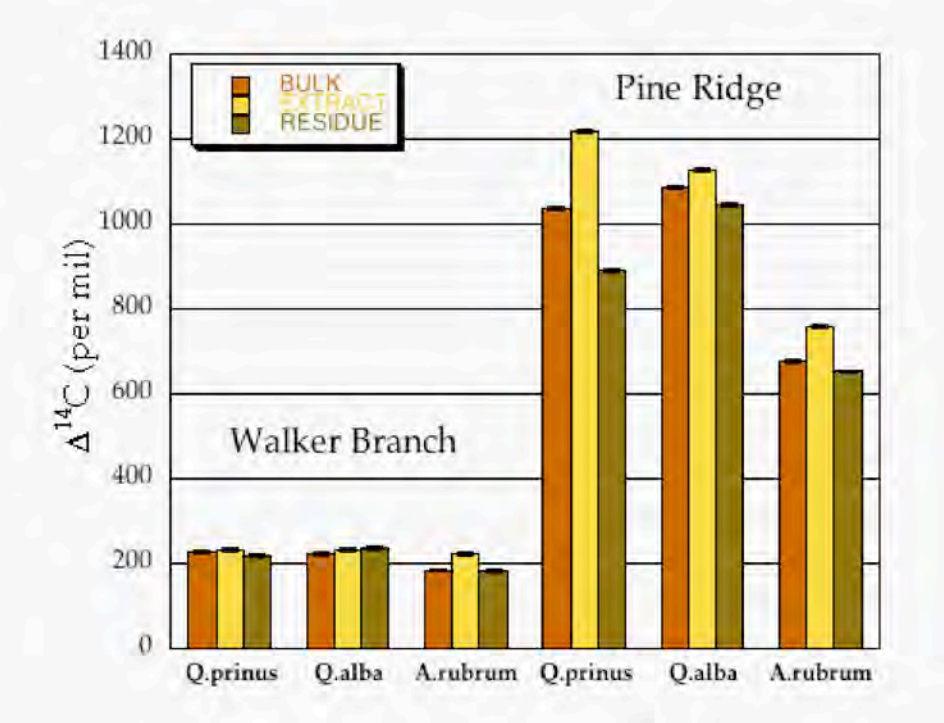


Figure 1-8. ^{14}C content of bulk, soluble and insoluble carbon pools in 2000 litter.

These preliminary results confirm the observations made in the initial leaching experiment, with soluble compounds showing significant ^{14}C enrichment over the insoluble, structural compounds in the highly labeled Pine Ridge litter. An apparent species dependency is revealed as well, with *Q. prinus* exhibiting the greatest enrichment and most significant variation. Preferential loss of this relatively enriched, soluble material from leaching and/or microbial decomposition would result in a lower than expected ^{14}C-signature in the residual organic matter of the Oi>1Y horizon. More detailed compound class isolation and analysis of neutral sugars, phenolic acids, cellulose and lignin is currently underway. Mass balances will be completed for the sample fractions, and these data will be used to improve our simulations of C cycling via the processes of leaching and annual, downward vertical migration of litter cohort residue.

Air Monitoring of ^{14}C (Task 1)

Malu Cisneros-Dozal, Paul J. Hanson, and Susan E. Trumbore

The ^{14}C in sub-canopy air has been monitored during the growing season at Walker Branch and Pine Ridge sites since September 2000. Air is collected approximately 1 m above ground by slowly filling an evacuated 32-liter canister over a two-week period through a capillary restrictor. Figure 1-9 shows the ^{14}C data since September 2000. The data are 2-week averages, likely biased towards nighttime CO_2 values when local inversions are associated with higher CO_2 concentrations at the ground.

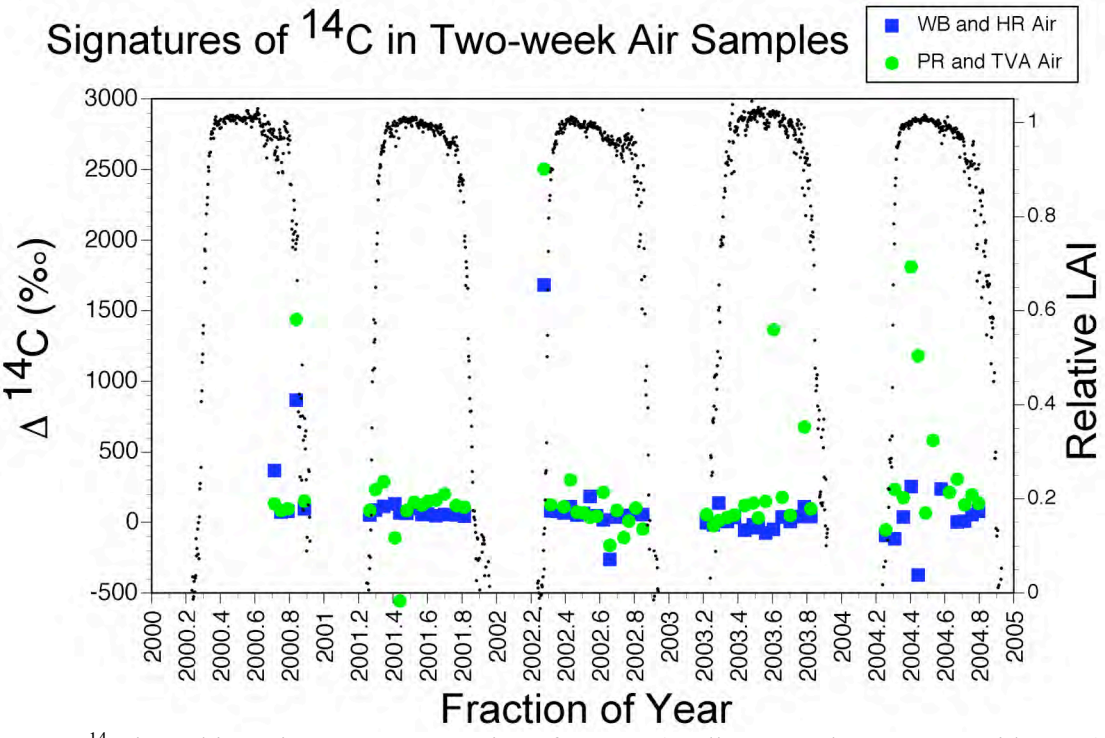


Figure 1-9. ^{14}C in ambient air CO_2 representative of eastern (Walker Branch WB; Haw Ridge HR) and western (Pine Ridge PR; TVA) on the Oak Ridge Reservation.

High ^{14}C levels at both sites, indicative of new ^{14}C releases, were observed in early November, 2000, mid-April, 2002, August of 2003 and during several events in 2004. The air ^{14}C-values are typically higher at Pine Ridge than at Walker Branch. The ^{14}C release in November 2000 took place after leave senescence, and thus was likely not incorporated into plants. The April 2002 release occurred at the beginning of canopy leaf development, but samples of oak leaves and squaw root plants in early May (approximately 15 days after the event) showed no evidence of incorporation of the elevated ^{14}C from this atmospheric peak having ^{14}C values ranging from +98 to +125‰. The August 2003 release in the vicinity of Pine Ridge is likely to have been incorporated by photosynthesis of the 'west-end' forest canopy, and 2003 canopy litter fall showed west-end TVA and PR litterfall to be enriched with respect to litterfall at WB and HR (~208 vs. 107 ‰, respectively). That enrichment may, however, also be the result of multi-year exposures to ^{14}C since 1995.

Summertime releases of ^{14}C in May, June, and July of 2004 were responsible for enriching current photosynthate in 2004, and were incorporated into new root growth at both

western and (to a lesser extend) eastern sites. Carbon dioxide at the Walker Branch measurement site is periodically impacted by a local fossil fuel source leading to substantially negative ^{14}C numbers. Root respiration ^{14}C shows small increases following the August 2003 release at both sites (Figure 1-10), and large increases at both sites in early 2004. Interestingly, the root respired CO_2 values returned to earlier levels in August 2004, which may be an indication of either a switch in the pools used to fuel root respiration or the time it took for the new release to move through respirable C pools.

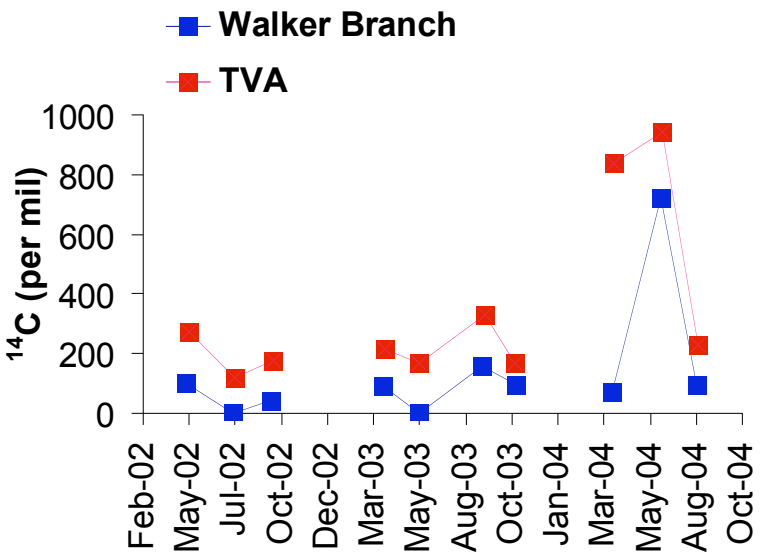


Figure 1-10. Root-respiration-derived ^{14}C-signatures for roots sampled at the west-end TVA and the eastern Walker Branch study sites.

Notwithstanding the pulses of ^{14}C received by the forest ecosystems in late 2003, the air monitoring data shows that our analysis and modeling of the fate of enriched vs. near-background litter additions from 2001 through the third year of stable 2000-cohort additions in 2003 will be largely unaffected. The extent and importance of the summer ^{14}C-pulse in 2004 will be quantified through measured changes in root and litterfall ^{14}C.

^{14}C in Tree Rings (New effort)

Chris Swanston et al.

Assessments of ^{14}C within tree ring wood were conducted in preparation for the EBIS project. However, the need for comprehensive site-by-site information on the ^{14}C history of all four EBIS sites on the Oak Ridge Reservation caused us to make additional observations in 2004. Duplicate increment cores 5mm in diameter for *Q. alba* and *Q. prinus* trees were collected in July of 2004. Rings were removed in 1-year or 1/3 year increments. Rings representing the growing season of calendar years 2003 to 1999 were each separated into thirds. Years 2004, 1998 – 1990, 1985, and 1980 were collected as complete years. Soxhlet extractions were carried out on a subset of about 100 'thirds' and rings, sufficient to characterize the major atmospheric fluctuations at the EBIS sites. The method, adapted from that used by Julia Gaudinski on root samples, extracted holocellulose by boiling in a 2:1 mixture of toluene and ethyl alcohol for 24 hr, followed by another 24 hr in ethyl alcohol, before the samples were bleached in a sonic bath with acetic acid and sodium chlorite. The extracted cellulose was then converted to graphite using the standard hydrogen-reduction method.

Figure 1-11 shows the reconstructed ^{14}C signatures for western (TVA and Pine Ridge) and eastern (Walker Branch and Haw Ridge) sites on the Oak Ridge Reservation. The data appropriately demonstrate the presence of ^{14}C exposures in 1999 and perhaps early 2000, and show that TVA and PR sites began to experience elevated ^{14}C as early as 1995. These data are in agreement with the ^{14}C-air measurements for 2000 to 2003 and show the importance of the 2004 mid-summer ^{14}C pulse to biological processes.

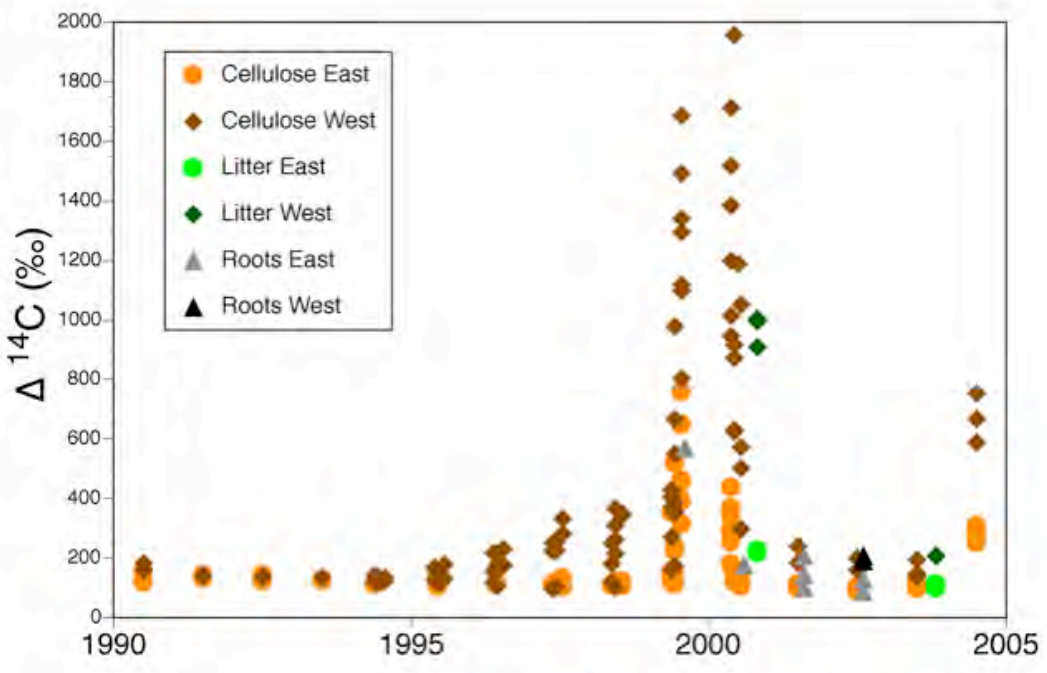


Figure 1-11. The ^{14}C-signatures for *Quercus alba* wood holocellulose and selected leaf litter and fine root samples from 1990 through 2004 demonstrating the importance of the 1999 ^{14}C pulse and a gradient of higher ^{14}C on the western portions of the Oak Ridge Reservation since 1995.

Partitioning Soil Respiration and C-Source Quantification (Task 2A)

L. M. Cisneros Dozal, Susan Trumbore, and Greg Winston

Soil respiration is made up of CO_2 respired from root metabolism (autotrophic respiration) as well as CO_2 derived from decomposition of dead organic matter (heterotrophic respiration). In the EBIS studies, we use the different amounts of ^{14}C respired by each of these pools to determine their relative contribution to total soil respired C. The experimental plot design also allowed us to determine the fraction of total soil respiration derived from litter decomposition.

Measurements of soil respiration were carried out in 2002, 2003 and 2004 at Walker Branch (WB) and TVA sites (east and western sides of the ORR respectively). At each site, we measured 6 plots in total (3 plots with enriched and 3 with background leaf litter). Rates of total soil respiration were measured using a closed dynamic chamber and an infrared gas analyzer. CO_2 was collected for determination of ^{13}C and ^{14}C using molecular sieve traps (Gaudinski et al., 2000). To determine the isotopic signature of root and heterotrophic respiration, we collected the CO_2 evolved during incubations. At each site, living roots were excavated from the surface soil, shaken and washed free of soil, and placed immediately in an airtight container attached to a molecular sieve trap to collect CO_2 for ^{14}C and ^{13}C analysis. For heterotrophic respiration, we collected soil cores (representing the top 5 cm of mineral soil) and leaf litter. These were stored refrigerated for ~1 week, then placed in 1 L jars flushed with CO_2-free air. We allowed the CO_2 to accumulate for 1 week to measure respiration rates and then collected CO_2 for ^{14}C and ^{13}C analysis.

Microbially respired (heterotrophic CO_2 sources

The radiocarbon content of CO_2 evolved in incubations of O horizon and 0-5 cm mineral soil is used to determine the carbon sources of microbial (heterotrophic) respiration. CO_2 evolved from O horizon organic matter clearly shows the influence of the litter label, though ^{14}C values decline or stay constant from May 2002 – August 2003 (Figure 2A-1 upper graph). A dramatic decrease in the ^{14}C of CO_2 evolved from plots with enriched litter additions is observed beginning in 2004, when local leaf litter was permitted to fall on all sites. Sites with near-background litter additions do not change dramatically during the course of the experiment. The TVA site, which had higher root ^{14}C values (and likely also higher pre-experiment ^{14}C values in the Oe and Oa horizons) have consistently higher ^{14}C values than the Walker Branch site. Radiocarbon signatures of CO_2 derived from incubation of the 0-5cm of mineral soil show increases, especially during the growing season of 2002-2003 (Figure 2A-2).

Again, differences between elevated and near background plots are smaller than differences between eastern and western sites, though there are small increases in the ^{14}C of CO_2 respired from the elevated litter plots, especially in TVA. Hence, some of the decomposition of DOC carrying enriched litter material gets decomposed in the upper part of the mineral soil; this makes up < 5-10 % of the microbially respired carbon from this horizon. Increases in the radiocarbon signature of CO_2 evolved in incubations from 2004 likely are showing the influence of the new radiocarbon label, which was present in newly grown roots.

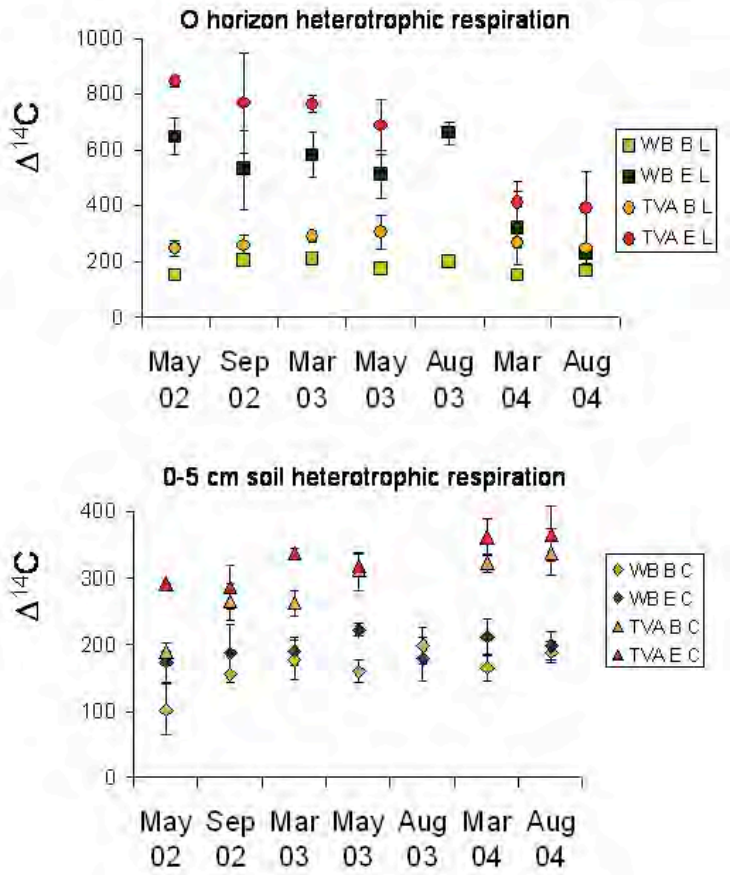


Figure 2A-1. Radiocarbon signature of CO_2 evolved during incubation of O horizon (top graph) and 0-5cm of mineral soil (bottom graph) for Walker Branch (WB; low root ^{14}C label) and TVA (high root and pre-experiment O horizon ^{14}C label). E = Enriched litter addition plot; B = near-background litter plot.

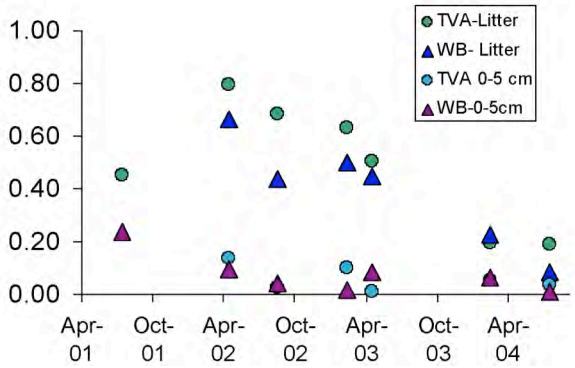


Figure 2A-2. Fraction of respired CO_2 in incubations derived from the leaf litter label. Since the entire O horizon was incubated, including presumably litter material present prior to the litter additions, these are expected to vary through the experiment. Non-zero values in the 0-5 cm core incubations indicate that a small fraction of labeled litter C is being decomposed in the mineral soil.

Absolute contributions of leaf litter (LD), root respiration (RR) and mineral soil decomposition (other) to total soil respiration at Walker Branch (from Cisneros Dozal et al., in press) at various periods from 2002 through 2004 are shown in Figure 2A-3. Heterotrophic respiration sources are highly variable in space and time, especially leaf litter contributions, which are clearly linked to litter moisture conditions. Decomposition in the O horizon contributes up to 40% of the total soil respiration flux under optimal moisture conditions. Autotrophic respiration accounts for ~20 to 60% of soil respiration, though absolute rates of root respiration remain nearly constant for most time periods. Overall, the variability in total soil respiration flux is more linked to differences in heterotrophic respiration sources than to autotrophic sources.

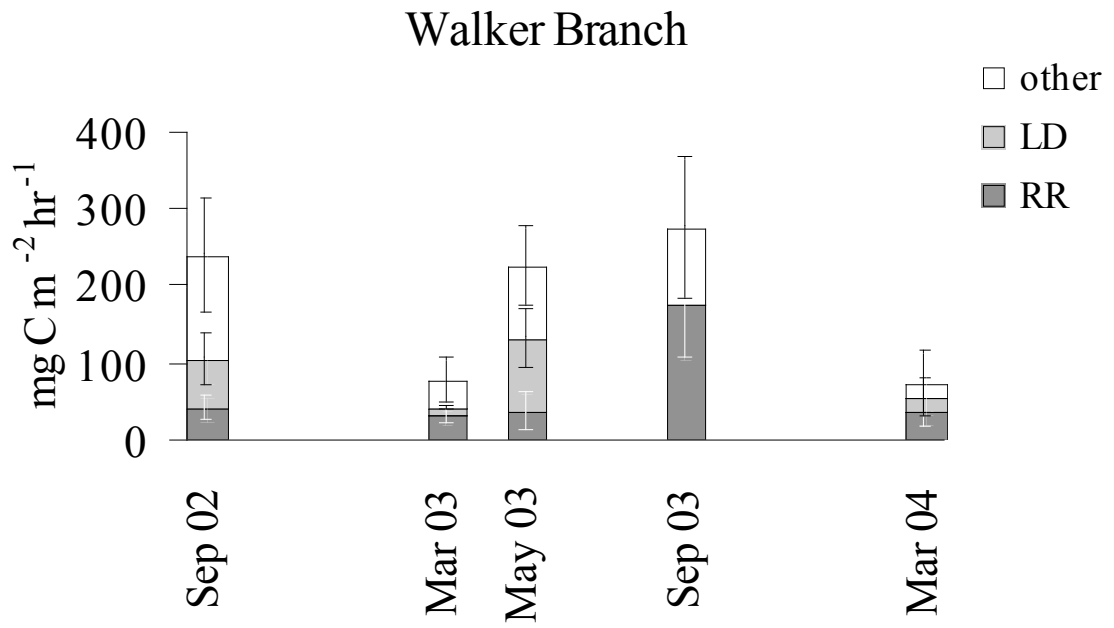


Figure 2A-3. Cumulative contribution of litter-layer decomposition (LD), root respiration (RR) and other heterotrophic forms of respiration to total CO_2 efflux from the forest floor.

Root (autotrophic) respiration shows significant variation in radiocarbon with time (Figure 2A-4). Values for root respiration are highest in spring (March-April), and then drop during the growing season consistent with the idea that the source of root respired C shifts from nonstructural carbohydrate stores to more recently photosynthetic products from spring to summer. The exception to this pattern occurs after the ^{14}C release seen in Pine Ridge in August 2003; however, this release did not appear to affect air at the WB sites, which also showed increased ^{14}C in root respiration. Consistently high ^{14}C values for root respiration at TVA cannot be explained by Pine Ridge air samples being higher in ^{14}C (Figure 1-9), and may alternatively be the result of residual labeled nonstructural carbohydrate pools from prior ^{14}C pulses. Future research on root carbon pools will focus on the measurement of carbohydrate and sugar pools in roots to see if they match the patterns seen in root respiration, from archived samples from the 2003 sampling periods.

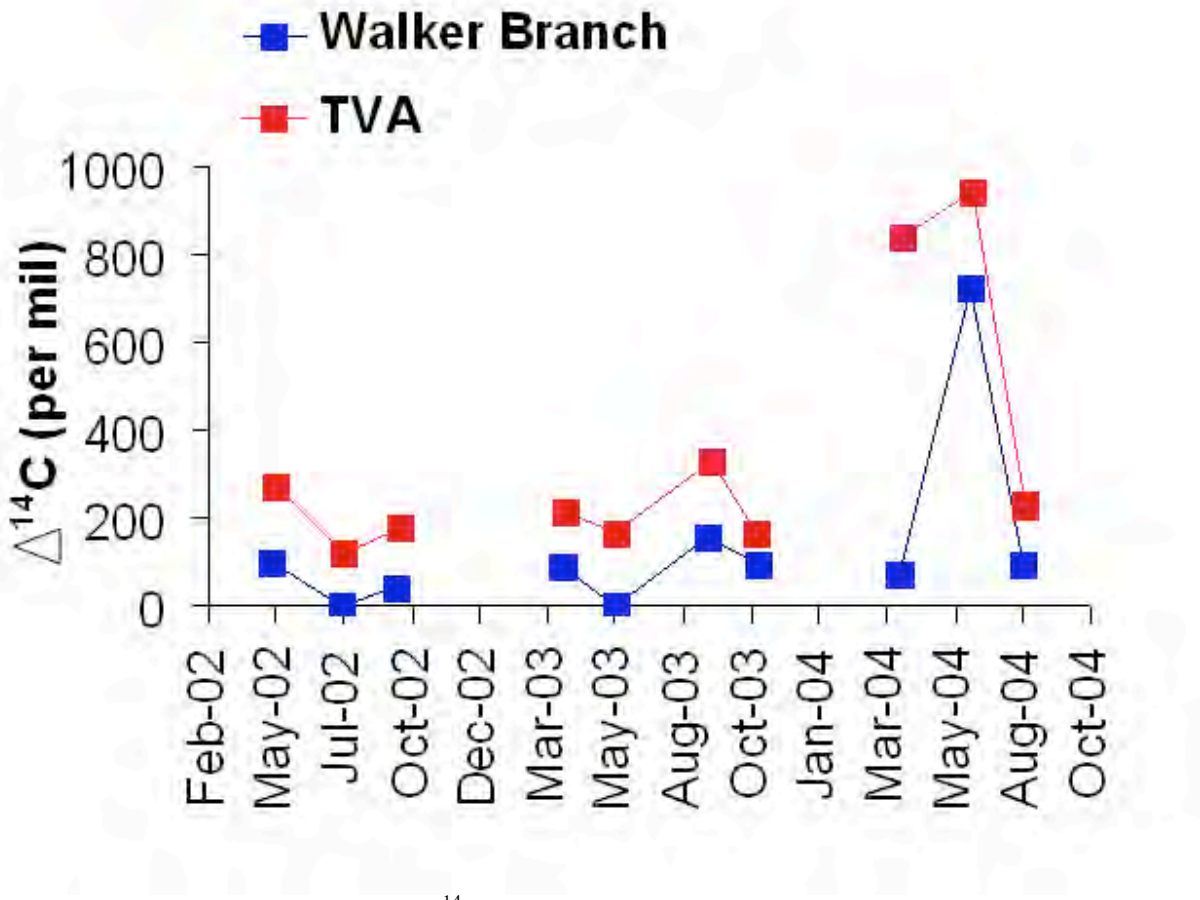


Figure 2A-4 Pattern of ^{14}C in roots collected on east (Walker Branch) and west (TVA) ends of the Oak Ridge Reservation.

Early gas-well data contrasting $\Delta^{14}C$-signatures beneath background and enriched litter plots also suggests rapid downward vertical transport of ^{14}C into the upper mineral horizons, but insignificant changes in the bulk ^{14}C levels of the surface mineral soils suggests that much of the transported carbon may be subsequently lost to microbial decomposition.

Reference:
Gaudinski, J.B., S.E. Trumbore, E.A. Davidson and S. Zheng. Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes, *Biogeochemistry*, 51:33-69, 2000.

Fine Root Turnover: Measurements (Task 2B)

Julia B. Gaudinski, J. Dev Joslin, and Margaret S. Torn

The distribution of fine root C and fine root C mortality turnover times are central components for characterizing C cycling and sequestration in mature forests. Essential to this characterization is quantifying the C transfers involved in root growth, root death, and the subsequent transformation of root C to soil organic matter and CO_2. To address these issues we have performed a series of measurements and developed a numerical model to better quantify the pathways and turnover times of C in fine roots. We measured fine root biomass and ^{14}C content by soil depth, size class, and live/dead for time-zero, year-1, and year-2 (Joslin et al., *in review*). We subdivided roots into very fine (<0.5 mm diameter) live roots, very fine dead, medium fine (0.5–2.0 mm) live roots, and medium fine dead roots. There was no trend in biomass among sites, so sites are grouped for discussion of C content.

In each depth and size class, the mass of dead roots was approximately the same as the mass of live roots. Live root biomass and dead root biomass were roughly in steady state, with no significant trends over the three years (Fig 2B-1). Taken together, the observations of equal and steady masses suggests that that the decay rate of dead roots was roughly equal to the turnover time of live roots in these forests. Turnover-time is also quantified below using the ^{14}C time series and a new model of ^{14}C in the root pool. In terms of depth distribution, almost 50% of the total pedon root mass was concentrated into the A horizon between 0-15 cm (even including the O horizon; Figure 2B-1).

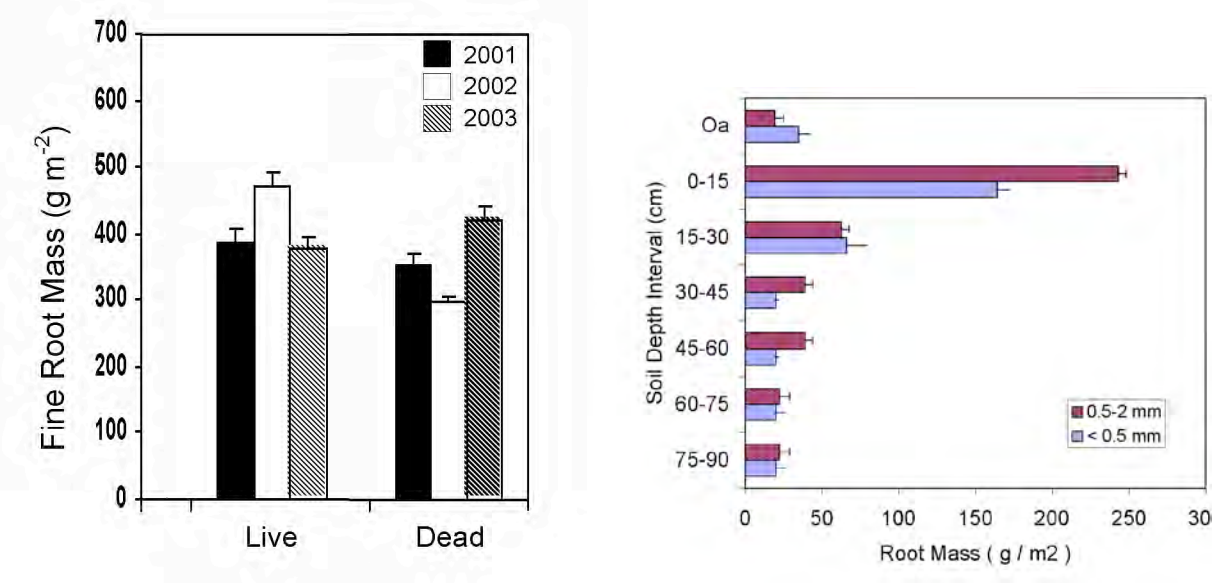


Figure 2B-1. *Left graph*: Live root and dead root biomass by year. Error bars depict standard error of the mean of four sites. *Right graph*: Fine root mass (live and dead) distribution by depth and diameter-size class, mean over three years and four sites.

As expected, the $\Delta^{14}C$ values of live and dead roots were much higher at the two sites at the West End of the reservation (TVA and PR) compared to the East End (WB and HR) (Figure 2B-2). All live root pools retained a significant amount of the pulse enrichment through the latest harvest, indicating that mass-weighted turnover time for the live fine root population is on

the order of years. However, dead root pools had elevated $\Delta^{14}C$ values within 6-18 months of the labeling, pointing to a component of live roots with short turnover times (months).

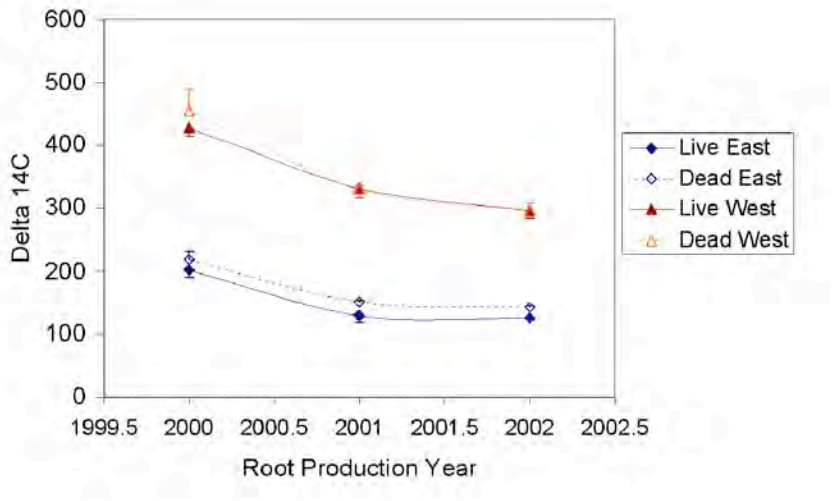


Figure 2B-2. The $\Delta^{14}C$ value of very fine (< 0.5 mm) roots of the O horizon for live and dead root pools separately, showing change over three years. The two west-end sites and two east-end sites have been pooled. Unidirectional error bars indicate standard error of the mean.

Another area of interest—and of controversy in belowground forest research—concerns the utility of root diameter or branching order as predictors of root turnover rate. It has been assumed that the narrower the root, the shorter its life span. We have not found consistent support for this hypothesis. Root $\Delta^{14}C$ declined faster in < 0.5 mm live roots than in 0.5–2 mm roots, and faster in surface roots than in deeper roots. Based on these results, very fine roots do turn over slightly more rapidly than medium fine roots in the O horizon, but turnover times between size classes converge with depth. The convergence occurs because turnover of medium fine roots does not vary with depth, whereas very fine roots turnover more rapidly close to the surface. In addition to tracking roots by size class, we also investigated turnover time by branching order, where branch order increases from most distal to closer to tree. At WB, there was an increase in ^{14}C with branch order, consistent with higher order roots being older and thus containing more of the pulse ^{14}C than do younger roots that grew after the pulse. Branch order data from TVA were confounded by the more complex time-course of elevated ^{14}C at that site.

Transport and Sequestration of Dissolved Organic Carbon in Contrasting Soils Amended with ^{14}C-enriched Leaf Litter (Task 3A)

Philip Jardine, Donald Todd, Paul J. Hanson, Jana Tarver, and Chris Swanston

The objectives of this task are to (1) use ^{14}C enriched litter as a well defined source to quantify dissolved organic C flux through soil profiles as a function of storm events, (2) quantify the impact of coupled hydrological and geochemical processes on the fate and transport of dissolved organic C through contrasting soil profiles being used in the Enriched Background Isotope Study at ORNL, and (3) quantify the mechanisms that control enhanced carbon accumulation within deep subsoils of forested Ultisols and Inceptisols. Our approach involved a multi-porosity sampling coupled with a nonreactive tracer to quantify the movement of indigenous and ^{14}C labeled dissolved organic C through the various soil profiles. Two background and two enriched plots from each of the four EBIS sites (16 total plots) were each instrumented with four tension lysimeters and four tension-free lysimeters. Two of each type were placed within the A- and B-horizons of the soil profiles (8 samplers per plot). Tension-free lysimeters provided a measure of solute fluxes through macro-and mesopores, while tension lysimeters provided a measure of solute fluxes through the microporosity of the media. Just prior to the addition of enriched and background litter, a nonreactive Br tracer was evenly applied over each of the instrumented areas using a backpack sprayer (i.e. distributed initially to the soil matrix porosity) in an effort to quantify the hydrodynamics of each site during storm events. Solution samplers were monitored during all storm events and analyzed for Br, TOC, inorganic anions, and pH. Numerous select samples were analyzed for ^{14}C. Bulk soil samples from each plot were also characterized for select physical and chemical properties. Organic C sorption isotherms were also quantified for each subsoil. These laboratory investigations were designed to quantify the geochemical processes controlling the movement of organic C through the soil profiles.

Storm driven breakthrough curves of nonreactive Br provided useful data for quantifying the flow and transport processes at the various sites. Haw Ridge exhibited the most rapid infiltration characteristics which is consistent with its more highly structured media and lower microporosity relative to the more clayey Pine Ridge and Ultisol soils. Likewise, Haw Ridge consistently had the highest pore water organic C concentration in both the A- and B-horizons relative to the other soils which is consistent with its more labile organic C source in the upper A and O horizons. Using storm driven percolation data from WBW and assuming it is serves as a conservative estimate of the infiltration rate on Haw Ridge, Figure 3A-1 shows that Haw Ridge exhibited the highest C flux relative to the other soils which is consistent with its larger Oe/Oa carbon pool and higher pore water DOC concentrations. The flux of organic C through Haw Ridge was 2 to 3 times greater than for WBW. Results from 2003 (not shown) were consistent with those obtained in 2002 (Figure 3A-1). Estimates of organic C inputs in the WBW Ultisol and Haw Ridge Inceptisol B-horizons showed that these horizons typically received more C than they lost which is consistent with laboratory DOC isotherms showing that these soils can sorb significant quantities of organic C.

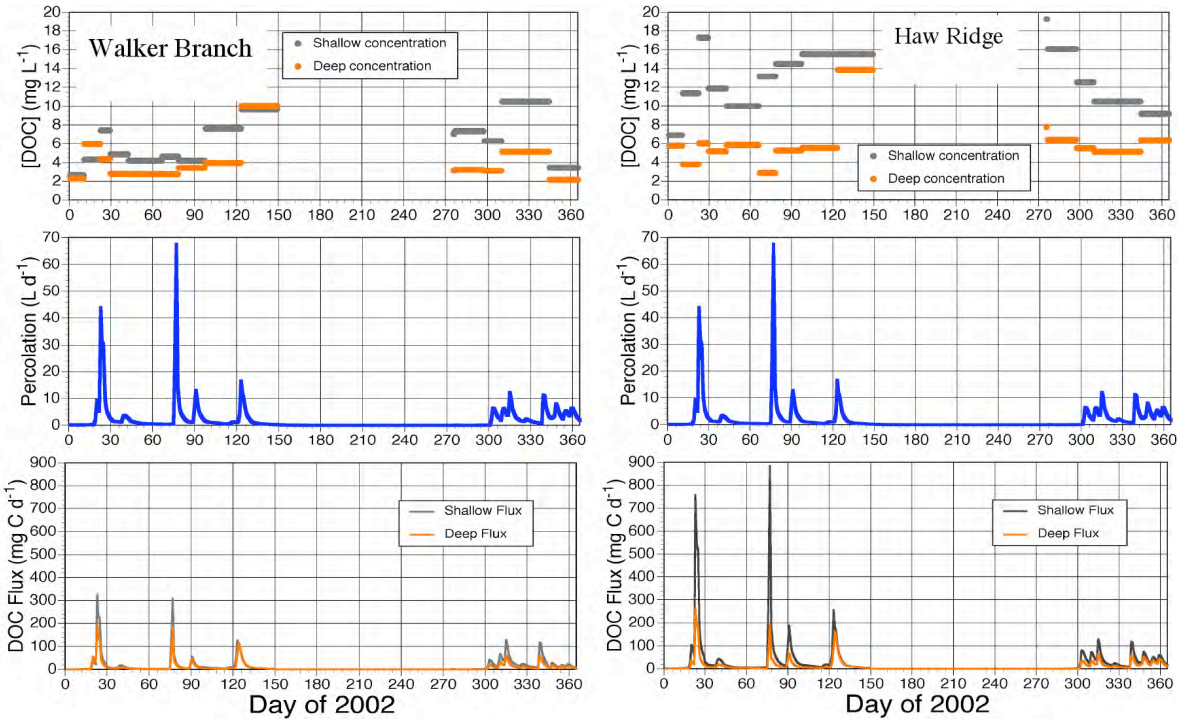


Figure 3A-1. Estimated organic C flux through the WBW and Haw Ridge soil profiles

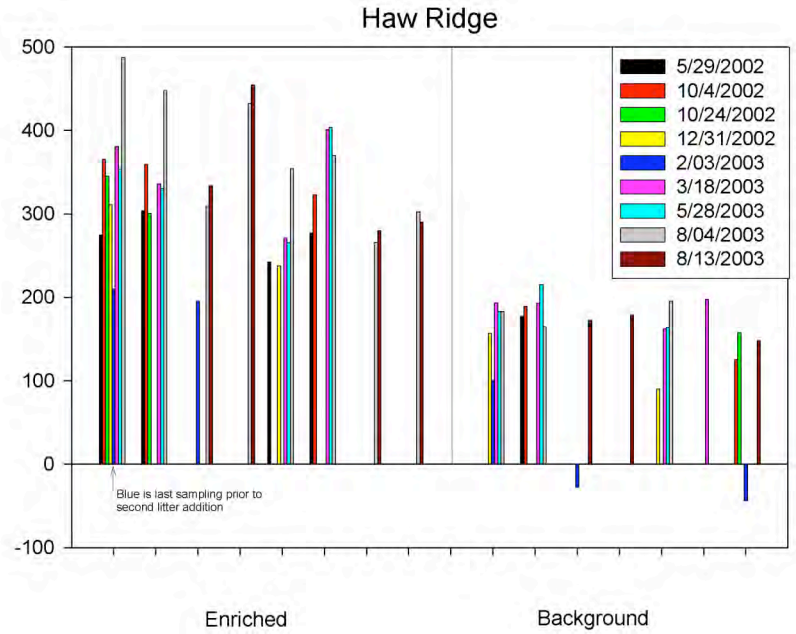


Figure 3A-2. $\Delta^{14}C$ signatures in select pore water from the Walker Branch Ultisol soil profile.

Enriched plots for all soils had higher $\Delta^{14}C$ signatures in pore water relative to background plots (e.g. Figure 3A-2). Pore water from Haw Ridge had higher $\Delta^{14}C$ signatures relative to Walker Branch, which is consistent with the more rapid flow and transport characteristics and lower organic C retention capacity of HR. Trends for Pine Ridge and TVA are complicated by $\Delta^{14}C$ enrichments that occurred prior to time-zero sampling of the EBIS project (March 2001). However, experimental results clearly show that Enriched A- and B-

horizons have consistently higher $\Delta^{14}C$ pore water than background A- and B-horizons (Figure 3A-3). It was also noted that enriched pore water moves deeper in the Inceptisols (Haw and Pine Ridge) vs. the Ultisols (WBW and TVA). On enriched plots, pore water $\Delta^{14}C$ signatures increased with time and were quite responsive to the yearly addition of enriched litter whereas background $\Delta^{14}C$ pore water was essentially flat at -200 (Figure 3A-3). These data combined with DOC concentration data will enable quantitative modeling of the fate and transport of organic C through the various soil profiles (Michalzik et al., 2003).

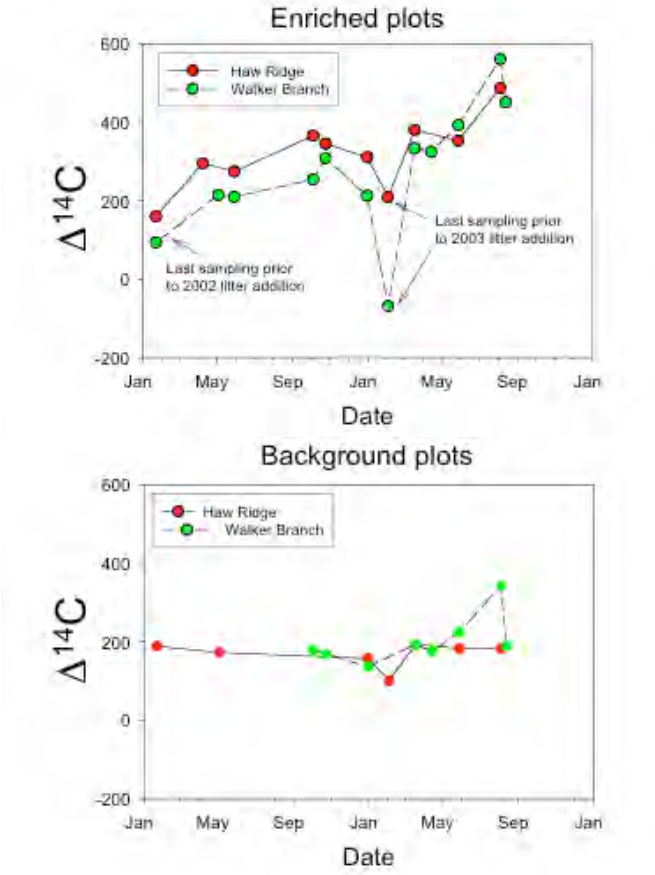


Figure 3A-3 Movement of the pore water $\Delta^{14}C$ signature through the A-horizons of enriched and background plots on Haw Ridge and Walker Branch.

In summary, non-reactive Br tracer provided useful data for quantifying flow and transport processes at the various sites and dissolved organic C fluxes at each site were consistent with the soil hydrodynamics and labile nature of the A-horizon organic matter. Net organic C accumulations were observed in the B-horizon of all soils where organic C sorption was strongly correlated with the soil Fe-oxide content. Pore water $\Delta^{14}C$ signatures look promising for enhancing our knowledge and predictive capability concerning organic C cycling. Enriched plots clearly showed higher values than background plots, and the data was consistent with site hydrological and geochemical characteristics.

Reference:

Michalzik, B., E. Tipping, J. Mulder, JFG Lancho, E. Matzner, C.L. Bryant, N. Clarke, S. Lofts, and MAV Esteban. 2003. Modeling the production and transport of dissolved organic carbon in forest soils. Biogeochemistry. 66:241-264.

Macrobiotic-Facilitated Transport of Carbon to the Mineral Soil: Earthworms (Task 3B)

Mac Callaham, Paul J. Hanson, and Donald E. Todd

Although earthworms are not a dominant biota in the ridge-top sites representing the focus of the EBIS study, they are a large component of most temperate systems and are known to have a large effect on soil carbon cycling processes. Epigeic earthworms feed on fresh litter but rarely enter mineral soil horizons, and anecic earthworms build deep permanent burrows in the mineral soil and drag fresh surface litter below ground where they feed on the 'fermented' litter material. Using the ^{14}C-enriched litter available from the EBIS 2000 collections, we developed a parallel study to distinguish the net effect of earthworm activity on vertical transport of carbon between surface litter layers and the mineral soil horizon. The study design will also allow us to distinguish rates of transport associated with the distinct anecic vs. epigeic earthworm feeding habits.

Replicated experimental plots (Figure 3B-1) were established on the Walker Branch Watershed during 2003 to which ^{14}C-enriched litter supplies were added to track the vertical transport of litter-layer carbon. These plots were arranged in three blocks of four treatments: 1) unmanipulated control with ambient/ native earthworms; 2) native removal plus anecic (*Lumbricus terrestris*) earthworm additions; 3) native removal plus epigeic (*Lumbricus rubellus*) earthworm additions; and 4) native population removed with no additions. At the chosen study location the ambient earthworm populations are predominantly native species (*Diplocardia* spp.). Individual plots measured 2 x 2 meters. EBIS ^{14}C labeled litter was added (500 g dry matter m^{-2}) shortly after the earthworm manipulations were completed. Soils were sampled from the A and B horizons of each plot on a quarterly basis for one year, and a second cohort of EBIS litter was applied in January 2005. Soil samples from the first year have been processed and submitted for ^{14}C analysis. When those data are available, changing ^{14}C-levels in the surface soils will be evaluated over time, and the incorporation of ^{14}C into the earthworm population will be calculated. Soil sampling is planned for a second year, followed by destructive sampling of the plots for microbial biomass, aggregate fractionation, and earthworm sampling.

Figure 3B-1. November 2003 photographs of one block of the EBIS earthworm carbon transport study. The photo on the right shows the octet-shocking device installed and operating for the extraction (and or counting) of earthworm populations from a defined soil volume.

Soil C Dynamics in Unprotected and Protected Pools:
Aggregated-based fractionations (Task 4A)

Julie Jastrow, Sarah O'Brien, Brent Van Til, Chris Swanston

For the purpose of tracking the movement of input sources into stabilized C pools, we fractionated mineral soil (A horizon; 0-15 cm) from three of the four treatments: CONTROL (background litter/background roots), LITTER (enriched litter/background roots), and ROOTS (background litter/enriched roots) in both Inceptisols (HR & PR) and Ultisols (WB & TVA). Samples collected in winter 2001, 2002, and 2003 (i.e., Year-0, Year-1, and Year-2) were physically fractionated into unprotected and microaggregate-protected particulate organic matter (POM and mPOM), microaggregated silt and clay (mSILT and mCLAY), and silt and clay not associated with microaggregates (SILT and CLAY). In addition, all silt and clay fractions from Year-0 were chemically fractionated by acid hydrolysis. At least initially, we expected that treatment induced changes in the ^{14}C label of the mineral fractions (especially clay) would be hydrolyzable (likely derived from exudation, dissolved organic C and microbial activities). Acid-resistant fractions were expected to be largely composed of recalcitrant, plant-derived compounds (e.g., lignin and lignin derivatives) and other degraded materials. Because the ^{14}C label in the acid-resistant fractions was not expected to change appreciably during the course of the study, only a few samples from Year-1 were hydrolyzed to verify this assumption.

The sum of the C contents in the six physically isolated fractions was found to be in remarkable agreement with independent analyses of whole-soil C concentrations, indicating excellent fractionation recoveries. The $\Delta^{14}C$ of the fractions also generally correlated well with each other and with whole-soil signatures.

The POM fractions contained about 50-60% of the total soil organic C in both soil types; whereas, the silt and clay fractions comprised 30-40% and ~10% of total soil organic C, respectively. Interestingly, 38-52% of the C in silt-sized fractions was hydrolyzable with a consistent tendency at all sites for more hydrolyzable C in SILT than mSILT. Overall, a higher proportion (46-63%) of the C in the clay-sized fractions was hydrolysable. But, in contrast to the silts, significantly higher percentages occurred in the mCLAY than in CLAY, suggesting greater protection of clay-associated hydrolyzable C within microaggregates. Fraction C:N ratios decreased with decreasing particle size (Fig 4A-1), which is consistent with the inverse relationship between particle size and degree of rendering observed in many soils. The C:N ratios of the hydrolyzable fractions ranged from 8 to 11, suggesting this fraction could include microbial biomass and byproducts as well as amino compounds and other N-rich materials. The C:N ratios of the acid-resistant fractions, however, were all much higher than even the POM and differed for microaggregated and non-microaggregated fractions. Consistent with conceptual models of root-derived organic matter cycling through microaggregates, the highest acid-resistant C:N ratios were found in mSILT suggesting relatively unprocessed plant-derived materials accumulate in microaggregates due to physical protection. In contrast, for the acid resistant clay fractions, the lowest C:N ratios occurred in mCLAY, which could be related to protection of more microbial residues and/or more degraded materials in microaggregated clays compared to non-microaggregated clays.

As expected, the highest ^{14}C enrichment occurred in the non-microaggregated POM in all treatments for both soil types (Fig. 4A-2). This fraction is the least degraded and freshest of all fractions and is composed largely of root fragments and other pieces of litter. Although LITTER

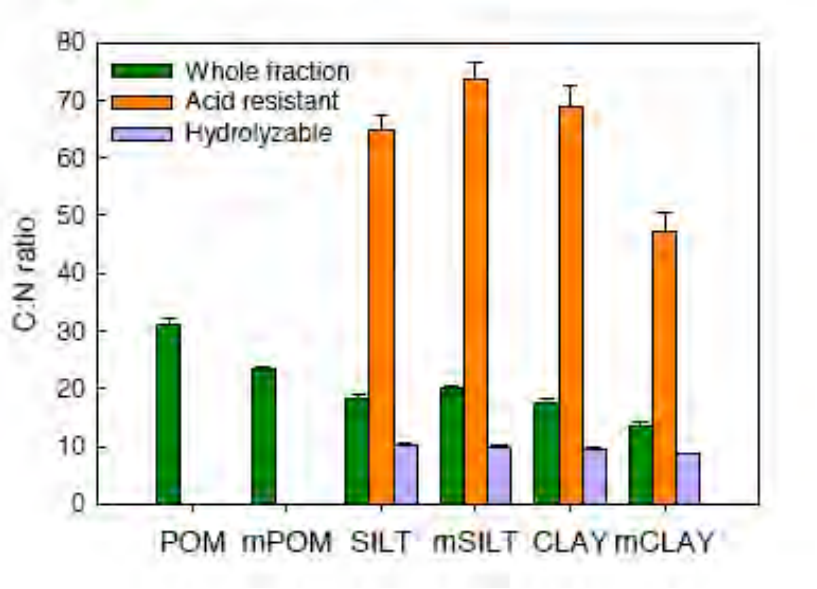


Figure 4A-1. C:N ratios of physically and chemically isolated soil fractions in Year-0, averaged over both soil types (error bars indicate SE; n = 24).

had little effect on POM $\Delta^{14}C$, ROOTS significantly increased the ^{14}C label in POM. Negative ^{14}C signatures for mPOM in WB (Ultisol) were associated with visible amounts of black carbon (charcoal or soot), which appears to be limited to the WB site. The slightly lower POM $\Delta^{14}C$ in the CONTROL and LITTER plots of the Ultisols compared to the Inceptisols can also be explained by the presence of relatively smaller amounts of black carbon. Thus, apparent magnitude of ROOTS enrichment of POM and, especially, mPOM relative to the CONTROL is probably overestimated for the Ultisols because of the black carbon. The ^{14}C signatures of all silt- and clay-sized fractions in CONTROL plots (Fig. 4A-2, panels A and B), showed very little difference between soil types and remarkably similar temporal patterns in both soils. ROOT enrichment of ^{14}C was considerably higher in the Ultisols compared to the Inceptisols (Fig. 4A-2, panels E and F) for all fractions except mCLAY. This difference was present even in Year-0, and the higher label in the mineral fractions as well as POM and mPOM suggests that plants at TVA were exposed to higher atmospheric ^{14}C and/or for longer time periods than at PR. Although it is possible that less C is retained by the mineral fractions in the Inceptisols, this hypothesis is not supported by the fact that ^{14}C signatures were so similar between WB and HR.

Because of the temporal patterns in different fractions that seem to be occurring across sites and treatments, it is difficult to discern from Fig. 4A-2 whether labeled C is being transferred in or out of the various fractions. However, when the differences between the LITTER or ROOTS treatments and the CONTROL are graphed over time (Fig. 4A-3), some trends are becoming apparent. The differences in Fig. 4A-3 are all illustrated at the same scale to facilitate comparisons across treatments and soils.

In the Ultisol LITTER treatment, there appears to be relatively large and steady increases in the mSILT and CLAY fractions relative to the CONTROL with a smaller trend in mCLAY. The POM and SILT fractions also increased over time but not at the steady rate observed in the other fractions. In contrast, for the Inceptisols, there appeared to be trend for loss from mSILT and CLAY during Year 0 and Year 1 followed by a large increase in Year 2. Steadier increases occurred in POM and mPOM for this soil. If these trends continue in Year 4, then they may be

indicative of soil differences in available exchange sites for sorption of DOC derived from the labeled litter.

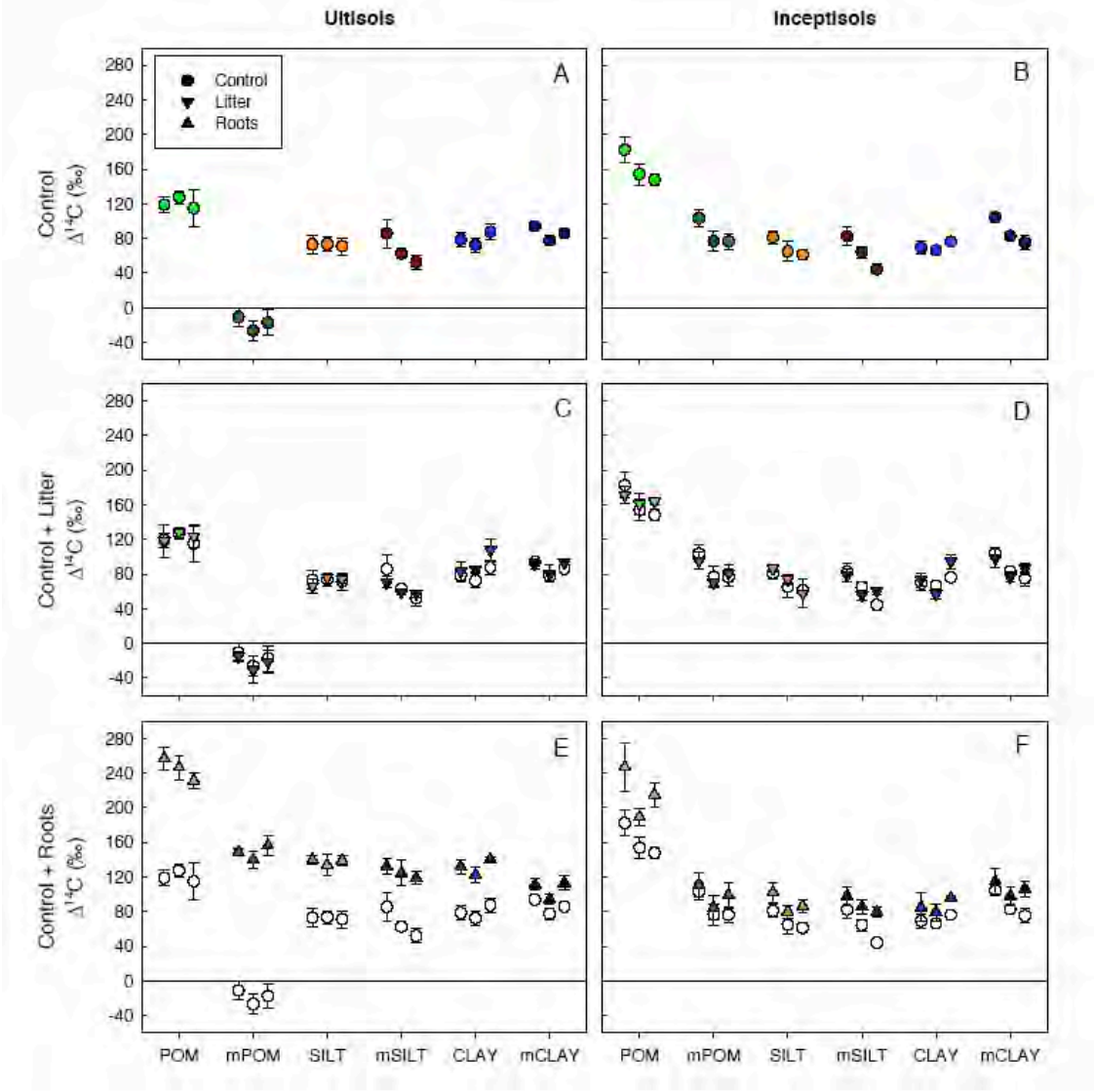


Figure 4A-2. Mean (± SE) $\Delta^{14}C$ for physically isolated fractions in (A & B) CONTROL, (C & D) LITTER and (E & F) ROOTS treatments for Ultisols and Inceptisols. For LITTER and ROOTS, the CONTROL values are also shown as open circles for comparison.

In the ROOTS plots, both soils followed similar patterns that are consistent with our conceptual model of root detritus cycling through microaggregates. There was consistent ^{14}C enrichment of all three microaggregated fractions in both soils. Radiocarbon in Ultisol POM is declining as expected, but it is not yet clear what is happening to this fraction in the Inceptisol. Non-microaggregated SILT and CLAY do not appear to be changing even though these fractions achieved some level of ^{14}C enrichment before the experiment started. This finding supports the expectation that the mineral fractions consist of more than one pool of C and that these pools have varying residence times.

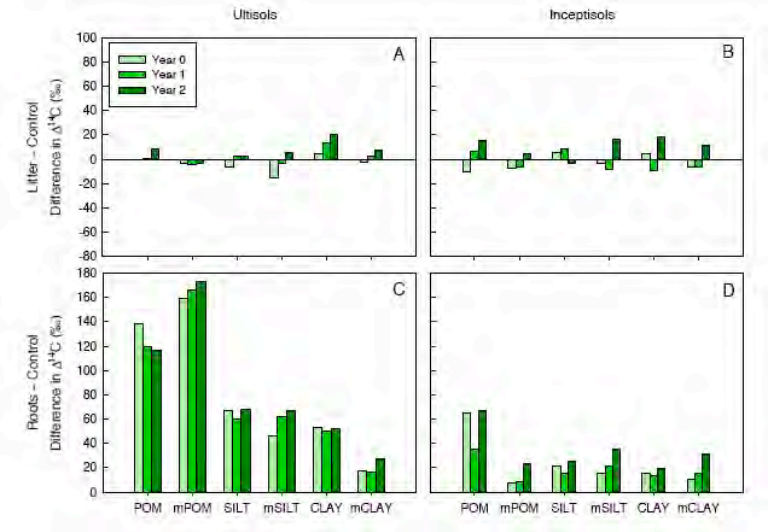


Figure 4A-3. Differences relative to the CONTROL for the $\Delta^{14}C$ of physically isolated fractions in the LITTER (A & B) and ROOTS (C & D) treatments over time for each soil type.

We used acid hydrolysis in an attempt to separate labile and more recalcitrant pools in each of the mineral fractions. Results from Year-0 samples indicate that acid hydrolysis clearly separates two fractions with different ^{14}C signatures and mean residence times (Fig. 4A-4). In addition, the acid resistant $\Delta^{14}C$ was not consistent across mineral fractions or treatments. In both near background soils, the acid resistant SILT fraction was the oldest. In the near background Ultisols (WB) the CLAY fraction was the youngest, but the mSILT fraction was the youngest in the near background Inceptisols (HR). In the Ultisols, the increase in $\Delta^{14}C$ observed for the ROOT mineral fractions (Fig. 4A-2 & 4A-3) was clearly due to an increase in acid resistant C for both silt-sized fractions and an increase in hydrolyzable C for the CLAY fraction. Except for mSILT, a similar but not as clear-cut pattern existed for the Inceptisols. The mSILT fraction was the most variable in both soils. The generally lower $\Delta^{14}C$ of acid resistant mSILT in the Inceptisol ROOTS treatment suggests that there are likely inherent differences between PR and HR in this fraction.

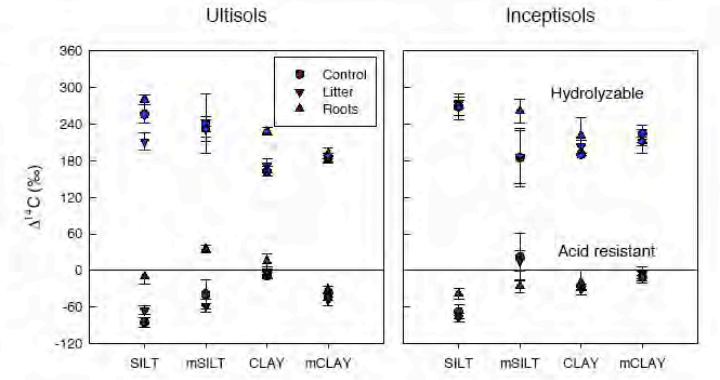


Figure 4A-4. Mean (± SE) $\Delta^{14}C$ of the acid resistant and hydrolyzable components of the mineral fractions in Year 0 for each treatment and soil type.

When the study was initiated, we assumed that changes to the acid resistant fraction would be slow enough that values from Year-0 could be used together with the intact mineral fractions from each succeeding year to determine changes in the hydrolyzable component. However, the apparent enrichment due to the ROOTS treatment and results from a few Year-1 check samples suggest that the acid resistant pool of at least some fractions has a dynamic component in addition to the older highly resistant and more stable component, which may require a change in the approach to the chemical separation of labile and resistant pools.

Forms and fate of carbon in soils: density-based method (Task 4B)

Chris Swanston, Margaret Torn and Paul Hanson

In the first grant period, we determined that the density-based soil fractionation scheme was effective in describing carbon dynamics (Swanston et al. 2005). We have completed analysis of Y0, Y1, and Y2 at 0–15 and 15–30 cm, and for Y1 at 30–60 cm. Our observations are summarized here, and lead to the hypotheses about soil carbon cycling and mechanisms of stabilization that we will test in the next phase of the proposal.

- Enriched root litter has been incorporated into SOM pools but almost no enriched leaf litter has.
- There is less enrichment from the 1999-pulse with depth for all density fractions.
- Differences in background ^{14}C and treatment effects between soil types suggest that soil mineralogy and texture influence the transport and stabilization of plant inputs.
- Although dominated by stable SOM, the dense fraction shows some enrichment, depending on soil mineralogy, indicating that it comprises fast- and slow-cycling C.
- Although its molecular chemistry (C/N, %C, ^{13}C) is more similar to that of the free light fraction, the residence time of the occluded C is similar to that of the dense (presumably mineral-associated) fraction.
- There appears to be almost no time lag between the ^{14}C enrichment of plant biomass and incorporation of the ^{14}C into the free light fraction, but a significant lag for incorporation into the occluded light fraction.

These results have been presented at the 18th International Radiocarbon Conference (2003), the International Conference on Mechanisms and Regulation of Organic Matter Stabilization in Soils (2003), and as an invited presentation at the Commonwealth Scientific and Industrial Research Organization (CSIRO, Land and Water, Australia, 2004). In the remaining period of the current grant we will (1) present current results at the 2nd International Conference on Mechanisms and Regulation of Organic Matter Stabilization in Soils and the 22nd International Meeting on Organic Geochemistry, (2) submit a manuscript of the results of the first three years of EBIS density-based fractionation to *Biogeochemistry*, and (3) begin work on a methods comparison paper with Julie Jastrow, who is using a aggregate-based separation method (Task 4A).

Results

For the control treatment (WB or HR, near-background litter, Figure 4B-1a) here were no clear trends through time in any of the density fractions. Free and occluded light fraction (LF) were more alike in %C, C/N and morphology than the dense fraction (DF), but at time-zero the apparent mean residence time (MRT) of the occluded LF was either intermediate to the free LF and DF, or more like the DF. The exception was the free LF in the Inceptisol, which decreased through time, possibly indicating a more rapid incorporation of the 1999 ^{14}C-pulse into SOM. This is supported by the DF being more elevated in the Inceptisol initially (but possibly decreasing through time), and by greater DOC flux through the Inceptisol (Jardine et al., this report).

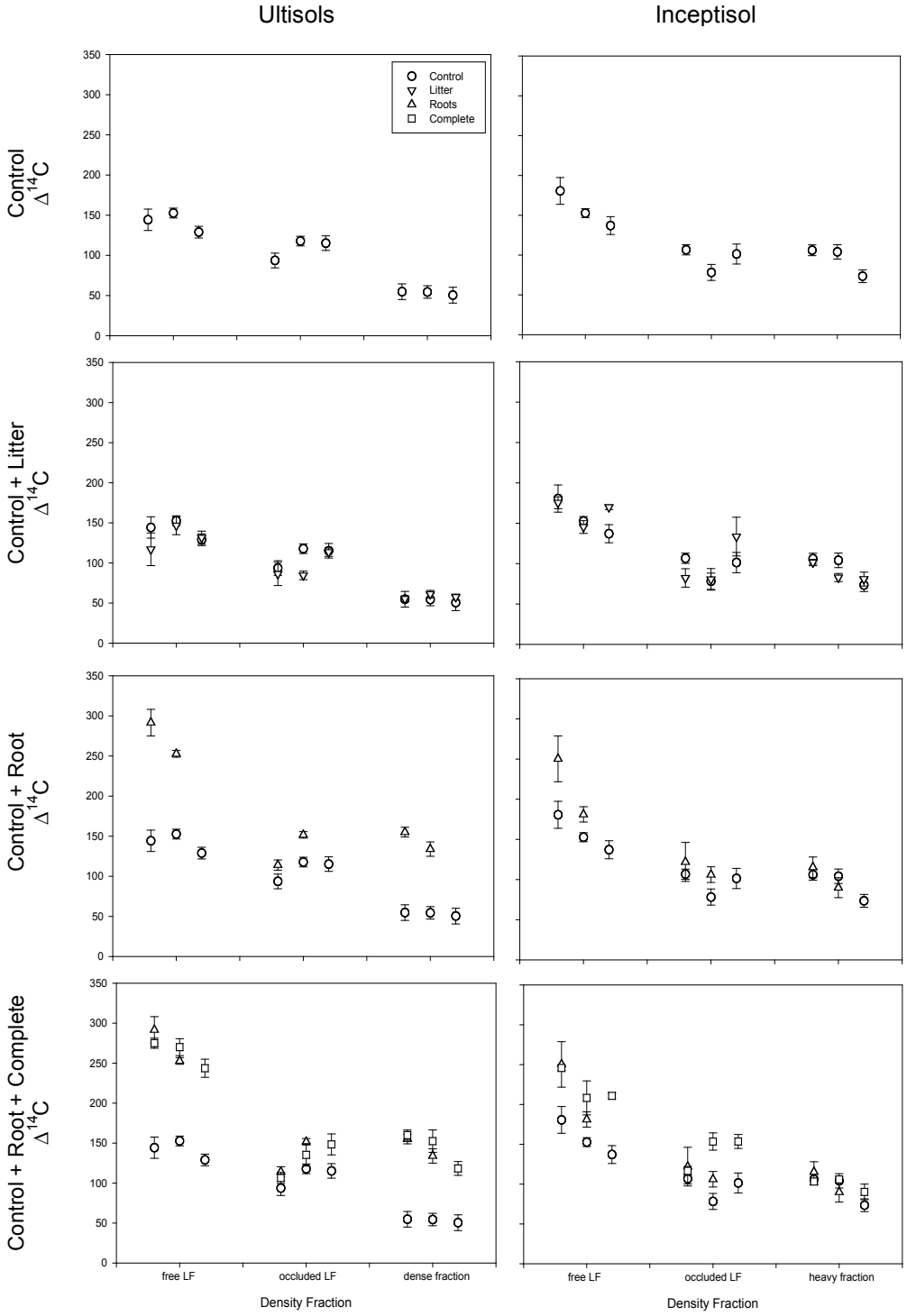


Figure 4B-1. Radiocarbon values for density fractions of Ultisols and Inceptisols. Data are presented in $\Delta\ ^{14}C$ with error bars (1SE) of four plots. The three data points given for a specific fraction correspond (moving left to right) to EBIS 0-year (2001), 1-year (2002), and 2-year (2003). Considering the source of enriched ^{14}C, fractions for each mineralogy are shown for the (a) Control treatments, (b) Control and Litter treatments, (c) Control and Roots treatments, and (d) Control, Roots, and Complete treatments.

The litter treatment (WB or HR, enriched litter, Figure 4B-1b) showed no evidence at any time of a substantial contribution of enriched ^{14}C from the litter treatment in the Ultisol, or by 1-year in the Inceptisol. After the second year of litter addition, however, there was some evidence of enriched ^{14}C input into the free LF and the occluded LF of the Inceptisol. It is possible that the coarser-textured soils more easily transfer particulates from litter horizons, and more rapidly cycle particulate SOM.

With respect to the roots treatment (TVA or PR, near-background litter, Figure 4B-1c) the Ultisol and Inceptisol each showed ^{14}C-enrichment of the free LF at 0-year, likely reflecting multiple pre-1999 small ^{14}C pulses in addition to the large 1999 ^{14}C pulse. Furthermore, each showed dilution of the enriched free LF through time as fresh, ambient-^{14}C root-derived organic matter was added to the soil. The $\Delta^{14}C$ of the free LF in the Inceptisol appeared to decrease more, consistent with the idea of more rapid SOM cycling in the Inceptisol. The free LF and the DF were each initially more ^{14}C-elevated in the Ultisol than the Inceptisol, probably reflecting higher pre-1999 atmospheric $\Delta^{14}C$ in the Ultisol (TVA). The depletion of ^{14}C in the DF of both soils indicates a rapid-cycling pool of C within the DF. The occluded LF showed the opposite trend of the free LF and DF in the Ultisol; as both of the other fractions decreased in $\Delta^{14}C$, the occluded fraction increased. This indicates a contribution to the occluded LF from the other fractions after a lag time.

Finally, the complete treatment (TVA or PR, enriched litter, Figure 4B-1d) did not appear to have any additional effect in the Ultisol over the roots treatment, supporting the idea that root-dominate C inputs to that soil. The Inceptisol, however, did show some evidence of increased $\Delta^{14}C$ in the complete treatments, consistent with the findings from the litter treatment in that soil.

Microbial Biomass and Soil Fungi (Task 4c)

Margaret Torn, Kathleen Treseder, Jessica Westbrook

Microbial Biomass

Soil bacteria and fungi are the main agents of decomposition, and nearly all heterotrophic respiration and humification in soils results from their activity. As a result, the isotopic composition of microbial biomass provides an estimate of the isotopic composition of the substrate that is being decomposed. In EBIS, where the treatments are root and litter inputs of widely different ^{14}C content, we are using microbial biomass ^{14}C to test the hypothesis that root inputs—and not leaf litter—form the basis of most heterotrophic respiration and OM formation in the mineral soil. We are also comparing biomass ^{14}C with incubation-based estimates of heterotrophic respiration ^{14}C (Task 2) and providing our results as direct input to the "Bio" component of the ecosystem models in Task 6. Under the first grant, we developed and tested the method for ^{14}C determination of microbial biomass, and completed analysis of samples collected in March 2002, 2003, and 2004 at TVA (west) and WB (east).

To isolate microbial biomass carbon from soil, we used the chloroform fumigation-extraction method, freeze-dried the extracts, and combusted with extra silver. Graphite was analyzed at CAMS. We verified complete combustion by comparing carbon density from elemental analysis and combustion independently. Microbial biomass C is expressed as the difference between fumigated and control extract values ($C_m = C_f - C_c$). We did not use a conversion factor for extraction efficiency. Microbial biomass ^{14}C is estimated by mass balance using the C and ^{14}C content of the control and fumigated extracts ($C_f D_f = C_m D_m + C_c D_c$), where C = carbon, D = ^{14}C ratio, m=microbial, f=fumigated, c=controls. There was no significant difference in total microbial biomass between sites or treatments.

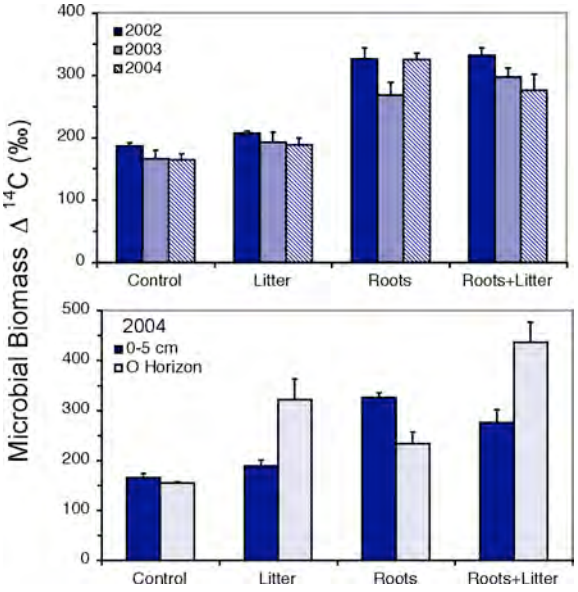


Figure 4C-1. Upper panel: Microbial biomass ^{14}C of A horizon in 2002, 2003, and 2004. Samples collected at 0-10 cm in 2002 and 0-5 cm thereafter. Lower panel: Microbial biomass ^{14}C of A horizon and O horizon (Oi, Oe/a) in 2004. The x-axis shows the source of radiocarbon enrichment at the site (corresponds to WBB, WBE, TVAB, TVAE where subscript = background or enriched litter treatment.

The ^{14}C enrichment treatments have had interesting effects on the isotopic signature of the microbial biomass (Figure 4C-1b). The most dramatic difference was between the sites: microbial biomass ^{14}C was much higher at TVA than at WB in all three years, and there was only a small effect of litter treatment within sites (Figure 4C-1). These observations support the hypothesis that root inputs are a quantitatively more important source of soil organic matter carbon than are leaf litter inputs in these temperate forest soils. The O horizon microbial biomass was enriched by the litter treatment (Fig 4C-1), showing that the lack of a large effect in mineral soil was not due to time lags or other artifacts, but rather to a profound disconnect in the C cycling between litter and soil in this upland forest.

Soil fungi

Ectomycorrhizal (ECM) fungi grow on plant roots but extend from the rhizosphere to the bulk soil. They can utilize C directly from roots, but they also produce enzymes that decompose organic matter. Recent work has cited indirect, isotopic evidence that ECM fungi take up soil organic carbon, and has suggested that this C could contribute to plant carbon budgets. We are investigating whether the litter treatments had a significant effect on ^{14}C enrichment of ECM fungi, to test if ECM fungi at Oak Ridge grow exclusively from root C or also decompose and incorporate litter-derived C. We sampled roots from the reciprocal transplant experiment in late summer 2001 and 2003. Ectomycorrhizal tips were handpicked, converted to graphite, and analyzed for ^{14}C at CAMS.

Ectomycorrhizal fungi were significantly ^{14}C-enriched at TVA, where the trees had ^{14}C-enriched roots (Figure 4C-2). In contrast, there was no effect of litter ^{14}C treatment. Thus, we found no evidence of incorporation of ^{14}C tracer from litter or soil organic matter during the first growing season of treatments, or, more surprisingly, after two full winters of treatments. Instead, over these three years, the ECM fungi acquired all C from live roots, rather than from

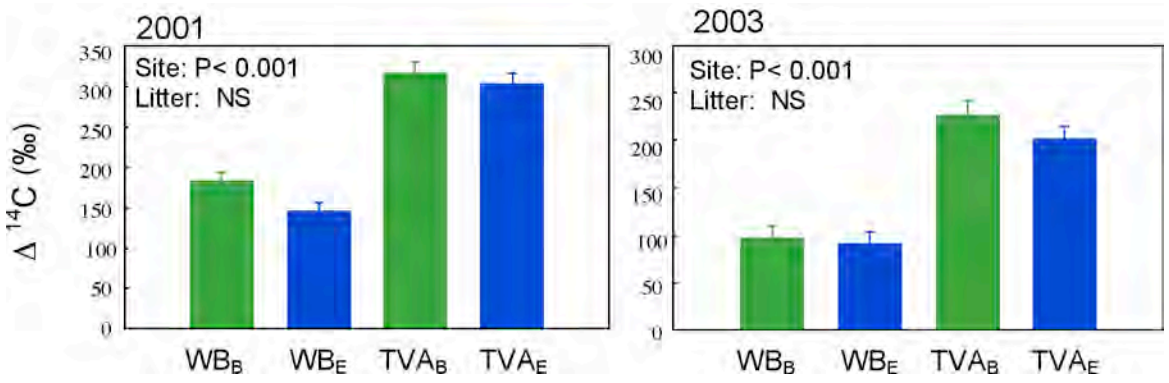


Figure 4C-2. The radiocarbon content of Ectomycorrhizal fungi from TVA and WB, in September 2001 and August 2003. Site, as a proxy for tree root enrichment, had a significant effect on fungal ^{14}C, but litter treatment within a site did not. The x-axis is the site name with litter treatment as a subscript (B=near-background; E=enriched). TVA is an enriched site and WB is a near-background site.

decomposition of soil organic matter. If the fungi did switch from mycorrhizal to saprophytic function (i.e., did decompose dead organic matter), it may have been to acquire nutrients rather than carbon substrate. In that case, the extracellular enzymes produced by ECM fungi were used to mineralize N and P rather than to acquire organic C. This mineralization by fungi can increase

nutrient availability to plant roots. The distinction between mycorrhizal and saprophytic activity is important when measuring N or C mineralization rates in the soil, and when modeling nutrient cycling in ecosystems where ectomycorrhizal fungi dominate. In addition, our results indicate that incorporation of soil C is not a likely confounding factor in radiocarbon-based estimates of root lifespan (e.g., Task 2B).

Supplemental Estimates of Fine-root and Soil C Dynamics Using Stable C and N Isotope Measurements (new effort related to Task 2B and 3A)

Charles T. Garten Jr.

The purpose of this task is to provide supplemental data on fine root dynamics and dynamics of soil organic matter decomposition at the four EBIS sites. These supplemental data are derived based field studies using stable N and C isotopes, and will be used to help interpret temporal patterns and between site differences in the behavior of ^{14}C in the main EBIS study.

A. Root dynamics

Successful quantification of fine root dynamics appears to be a critical element in understanding and interpreting ^{14}C behavior at the EBIS research sites. Results indicate that the production of new roots is diluting the ^{14}C signal in forest litter layers and some mineral soil fractions. Past studies of fine roots have yielded qualitative estimates of root turnover times at the study sites (Joslin and Wolfe 2003), and live root dynamics appear to be described by a combination of fast (monthly) and slow (yearly) cycling components as described above. Although microbes in the O-horizon utilize both root and litter substrates, nearly all of the ^{14}C-labeled organic matter in the surface mineral soil is derived from root inputs. Because root dynamics are an important element of EBIS, a combination of approaches are being utilized to quantify fine root dynamics at the forest sites, including ^{15}N tracer techniques for quantification of root production and mortality (Hendricks et al., 1997).

B. Verification of roots as the source of soil C

Movement of dissolved organic C through the soil profile is one mechanism for moving C to long-term storage pools in deeper mineral soil horizons. Carbon leaching from litter material is enriched in ^{14}C, and light fraction organic matter at depth (30-60 cm) also has a ^{14}C-signature suggesting downward movement of relatively recent C inputs at the EBIS sites. Results indicate a greater flux of dissolved organic C (DOC) on Inceptisols (HR and PR) than Ultisols (WB and TVA). Although the annual C flux to deeper soil horizons is small (≤ 5 g C m^{-2}), this could be a significant mechanism for soil C accumulation at depth over decades to centuries that is difficult to detect in the short-term because of the presence of a large soil C pool. Because accumulation in deep soils is a potential mechanism for protecting new C inputs from decomposition processes, we undertook a comparison of vertical ^{13}C profiles in soils from the EBIS study sites.

Methods

Nitrogen-15 tracer methods (Hendricks et al., 1997) are being used to quantify fine root production and mortality at the four EBIS sites. Eight 1-m^2 plots were labeled during the first half of 2004 by applying ^{15}N-enriched ammonium nitrate or ammonium chloride at a rate that approximately doubled wet-only atmospheric N deposition. Measurements through time

(August 2004, November 2004, and April 2005) of ^{15}N abundance in the fine root structural pool from the O-horizon and the surface mineral soil and ^{15}N abundance in available soil N will be used to calculate fine root production and mortality by ^{15}N mass balance at the end and beginning of the growing season. The rationale and equations developed for this method are presented elsewhere (Hendricks et al., 1997). Buried ion exchange resins were used to determine the ^{15}N content of available soil N.

Natural abundance measurements of ^{13}C at different soil depths and in different soil fractions (particulate organic matter and mineral-associated organic matter) were undertaken at the four EBIS sites. Samples were analyzed in the Environmental Sciences Division, ORNL, using continuous-flow, isotope ratio, mass-spectrometry (PDZ Europa Integra CN). Prior studies (Garten et al., 2000) indicate that ^{13}C abundance increases in forest soils along a continuum from fresh C inputs to old, highly stable soil C pools. The relationship along the continuum of decomposition is described by a nonlinear regression unique to each study site. Regressions of ^{13}C abundance versus C concentration were developed and used to define the isotopic signature of "old" and "new" soil C at each EBIS site. A mixing model was then used to calculate the mean (±SE) fraction of "new" soil C remaining at different soil depths and in different soil fractions.

Progress to date:

In FY 04, we completed labeling of eight 1-m^2 plots at the EBIS study sites and completed initial sampling of the O-horizons and surface (16 cm deep) mineral soils. Root biomass in the O-horizon ranged from a low of 29 ±9 g m^{-2} at WB to a high of 63 ±18 g m^{-2} at TVA. Root biomass in the surface mineral soils was much greater and ranged from 407 ±41 to 584 ±66 g m^{-2} across the four sites. All of the root biomass in the O-horizon was classified as fine roots (<2 mm diameter). Fine root biomass in the mineral soil was 40 to 60% of the total root biomass. Initial fine root N pools (O-horizon + mineral soil) ranged from 3.5 to 5.0 g N m^{-2} with no differences among sites. Samples of fine roots contained significantly elevated ^{15}N concentrations at the initial root sampling (Figure CG-1). Roots in the O-horizon were more enriched than those in the mineral soil, but ^{15}N abundance in all roots was elevated ≈10 to 100 times over natural abundance levels.

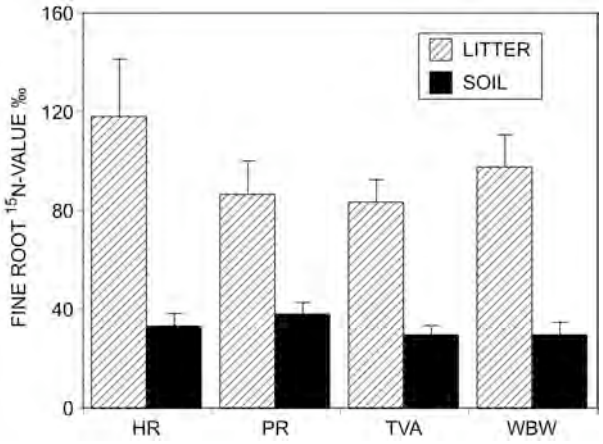


Figure CG-1. Nitrogen-15 abundance in fine roots from labeled study plots at four EBIS sites.

In early FY 05, we completed the first round of root and soil sampling from ^{15}N-labeled plots at each study site. We are in the process of sorting roots from O-horizon and soil samples

collected in November 2004. Analysis of ion exchange resins from the study plots indicated a variable signature for available soil N (ranging from 26 ±9 ‰ at WBW to 95 ±16 ‰ at PR). Available soil N in the study plots appeared to be enriched in ^{15}N by 5 to 20 times background in November 2004. Cation resins contained more ^{15}N than anion resins and isotopic signatures on the anion resins were consistent with expected low rates of net soil nitrification and low rates of nitrate leaching.

Carbon-13 analysis of soils and soil fractions at the EBIS sites were completed in early FY 05. Logarithmic regressions of natural ^{13}C abundance against C concentration were statistically significant at all four locations (r^2 from 0.90 to 0.96). There were significant between site differences in the slope of the regression indicating more recent C inputs remaining at the terminus of decomposition at sites with higher soil C:N ratios. Mixing model calculations were used to estimate the fraction of new soil C inputs remaining at different soil depths (Figure CG-2). In deep soils (60-90 cm), ^{13}C data indicated there is a greater fraction of new C remaining in Inceptisols (HR and PR) than in Ultisols (WBW and TVA). The occurrence of more new C in deep soil horizons is consistent with an observed greater downward leaching of ^{14}C in Inceptisols than in Ultisols. However, the presence of more new C in deep Inceptisols may also be due to less OM processing by decomposition (^{13}C abundance tends to increase from newer to older soil constituents) or site differences in patterns of deep rooting. We are not yet able to distinguish between the latter two mechanisms because of nondetectable ^{14}C concentrations in deep bulk-soil samples . These studies of natural abundance ^{13}C provide information on the distribution and fate of C as a result of long-term forest soil C dynamics and will be valuable in interpreting the short-term behavior of ^{14}C inputs.

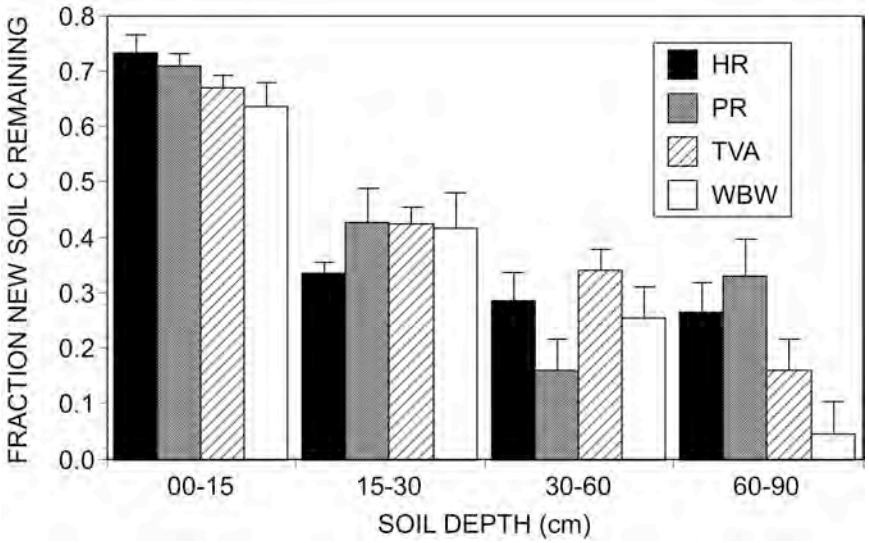


Figure CG-2. Mean (±SE) fraction of fresh soil C inputs remaining at different soil depths.

References:

Garten CT Jr., Cooper LW, Post WM III, Hanson PJ (2000) Climate controls on forest soil C isotope ratios in the southern Appalachian mountains. *Ecology* 81: 1108-1119.

Hendricks JJ, Nadelhoffer KJ, Aber JD (1997) A ^{15}N tracer technique for assessing fine root production and mortality. *Oecologia* 112:300-304.

Carbon-Cycle Modeling (Task 6A)

Rothamsted Model

W. Mac Post

Modeling in EBIS has two main purposes. One is tactical and targeted to achieve proximate objectives. These include organizing data into logical constructs consistent with measurements observations. Such data analysis models are being developed within tasks of the EBIS project to investigate specific components of ^{14}C data such as: What is the potential impact of differential leaching or decomposition of materials on ^{14}C distribution in litter cohorts? How can root carbon pools and turnover rates be reconciled with observed ^{14}C data? Can the carbon budget be closed with current measurements? In addition to these tactical modeling objectives, a second goal of EBIS Task 6 is to apply what is learned about carbon cycling, particularly decomposition dynamics, to a more general description of ecosystem C dynamics. With such a description the experimental results from the EBIS project can be generalize for other ecosystems.

Modeling Ecosystem C Dynamics

A widely used soil organic matter turnover model that is often used with ^{14}C data (RothC, Jenkinson 1990) was used to generate hypotheses about the distribution of ^{14}C among various decomposition pools prior to the experimental applications of labeled litter (Figure 6A-1).

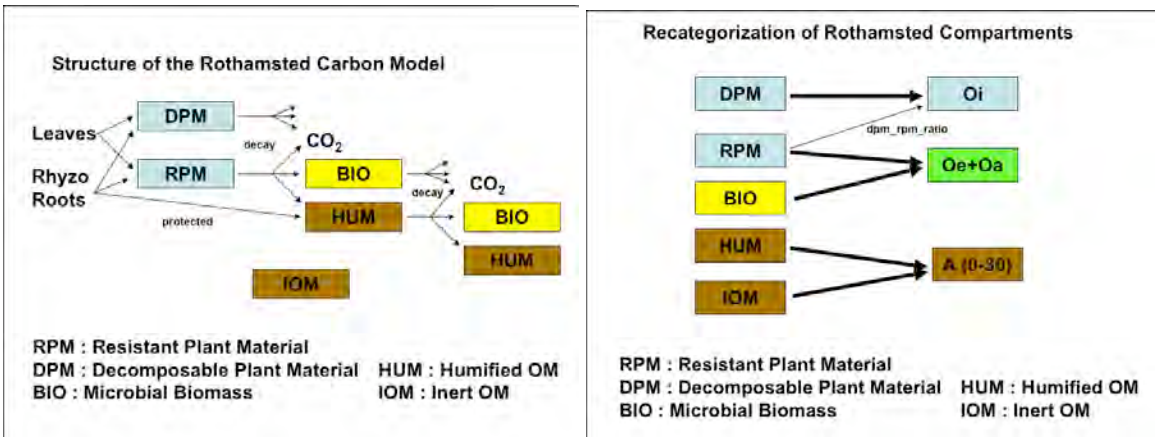


Figure 6A-1. Original structure of the RothC model (left) and the reassignment of the structural alignment for application to the measured EBIS carbon pools.

Simulations were again performed with the RothC model after 4 years of measurements to determine the adequacy of the model for describing the experimental data. Estimates of aboveground and belowground inputs combined with a reassignment of carbon pools in the RothC model to align with those measured in the experiment it was found that total carbon of each of the pools was estimated accurately by the model.

The amount of radiocarbon in the litter pools, however, was overestimated by 15% in year 1 and 30% in years 2 and 3 (Figure 6A-2). The discrepancy may be caused by a more complete separation of litter dynamics from mineral soil decomposition dynamics than is modeled. The RothC model, as well as all other process oriented soil C models were developed

for agricultural or grassland ecosystem application. As a result, discrete litter layers are not well handled. For the EBIS experiment, a more detailed litter decomposition model is required.

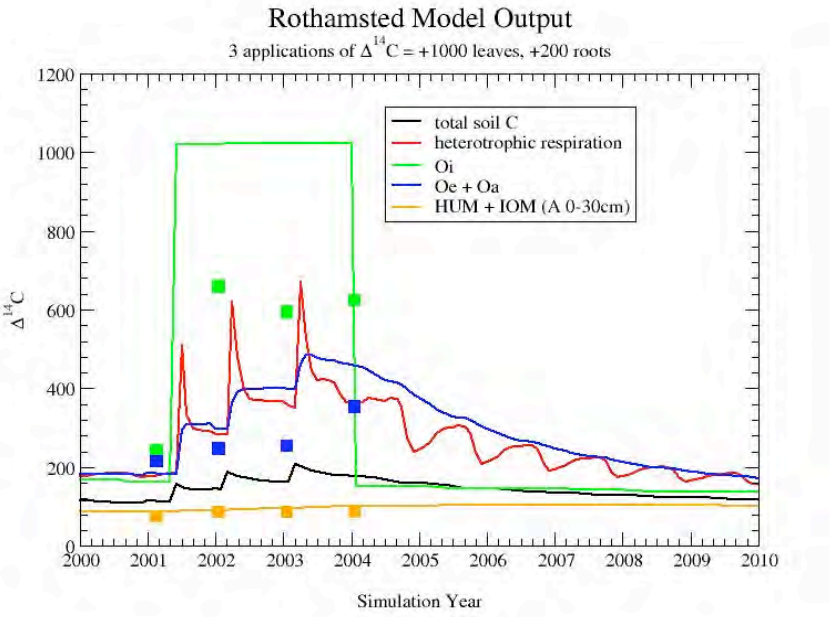


Figure 6A-2. RothC simulations for the first three-years of the EBIS experiment (2000 to 2004) and a hypothetical progression for years beyond 2004.

Litter decomposition models that follow the change in amount and quality of annual additions of organic matter have been developed for forest ecosystems (Pastor and Post 1986). A general theoretical formulation of such models has been developed by Ågren and Bosatta (1996) called the Q-model or Quality model. A version of the Q-model is being developed to apply to the labeled litter cohorts of the EBIS experiment. Several challenges need to be overcome in applying the fairly theoretical model to the data at hand. First, there a description of how the cohorts change in quality through time needs to be developed. A litter decompositions model developed previously (LINKAGES, Pastor and Post 1986) uses the empirical description of litter quality change developed by Aber and Melillo (1982). This is approach links quality to lignin and nitrogen dynamics of the decomposing litter. Appropriate parameters for the Q-model partial differential equations can be estimated for the EBIS plots by comparison to the LINKAGES litter decomposition model which may be though of as a discrete approximation to the Q-model. Utilization of the Q-model will allow model components to be better aligned with pool structure and decomposition dynamics in a layered forest floor, allow inter-annual variation in decomposition, and account for preferential decomposition of more labile components of litter have greater influence on the model dynamics.

References:

Ågren GI, Bosatta E (1996) *Theoretical Ecosystem Ecology: Understanding Element Cycles*. Cambridge University Press, Cambridge.

Pastor J, Post WM (1986) Influence of climate, soil moisture, and succession on forest carbon and nitrogen cycles. *Biogeochemistry* 2:3-27.

Jenkinson DS (1990) The turnover of organic carbon and nitrogen in soil. Philosophical Transactions of the Royal Society of London Series B - Biological Sciences, 329:361-368.

Modeling Bulk Organic Layer ^{14}C (Task 6B)

Paul J. Hanson, Charles T. Garten, W. Mac Post, Chris Swanston and Sue Trumbore

The unexplained patterns of Oi-layer ^{14}C accumulation over time and the presence of differential ^{14}C-signatures between decomposing litter cohorts and DOC leaching suggested that a cohort specific model of Oi-layer C and ^{14}C cycling was needed that could capture cohort specific carbon movements and intra-annual decomposition patterns driven by weather variation from year-to-year (see also the discussion of Task 6). An initial form of such a model was developed using STELLA® Version 8.1 ((Figure 6B-1). The model allows for multiple cohorts of Oi (i.e., recognizable litter) and a single layer of Oe/Oa (i.e., humus). Litter decomposition was simulated using either fixed litter turnover times, or variable rates driven by daily temperature and litter or soil water potential values derived from laboratory-based relationships reported by Hanson et al. (2003). Litter leaching from all Oi-layer cohorts was allowed to take place when throughfall events exceeded 15 mm -- a level of precipitation input needed to force vertical hydrologic movement that would not transfer more than 60 gC m-2 y-1 from all Oi-layer cohorts (the threshold was set based on observations of Anderson 1973).

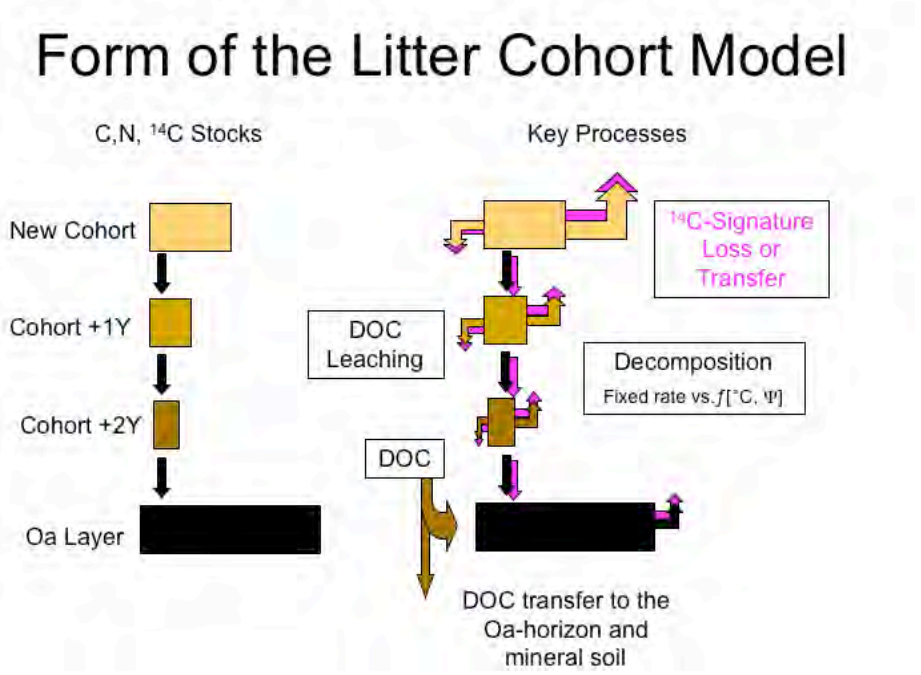


Figure 6B-1. Component of a multi-cohort litter decomposition model structured to handle C and ^{14}C cycling.

The model was capable of handling multi-year decomposition of litter layer cohorts consistent with observed litter mass loss for Walker Branch Watershed (Hanson et al. 2003; Figure 6B-2). When the model was run with fixed litter cohort turnover times and no DOC leaching of C or ^{14}C it failed to capture the observed patterns of ^{14}C in the Oi>1Y-layer shown in Figure 6B-3 (left). Substantial improvements in capturing the observed ^{14}C patterns in the Oi>1Y-layer were obtained by including the following in the model: (1) soil temperature and

water dependent decomposition functions, (2) DOC leaching of C, and (3) the observed differential patterns of ^{14}C-signatures between leachable C and bulk litter (Figure 1-6).

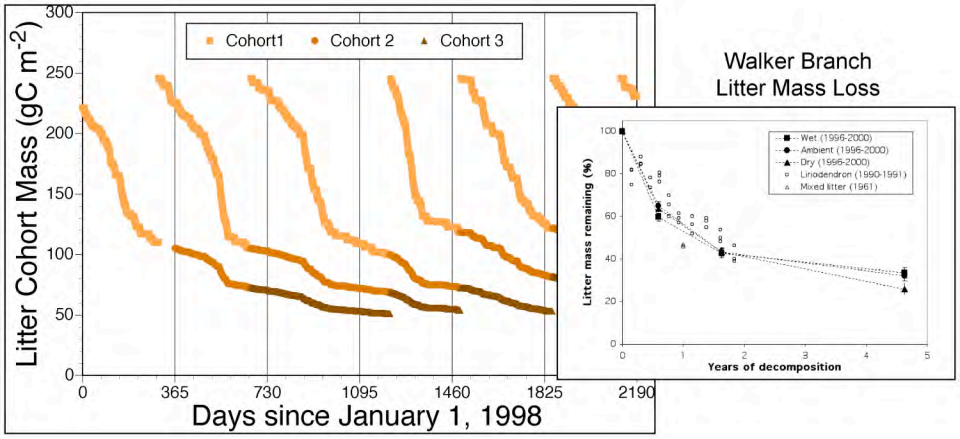


Figure 6B-2. Modeled litter mass loss and observed litter mass loss for Walker Branch (inset).

Although these model runs could still be improved it is clear that multiple cohort models with realistic representations of decomposition and leaching dependent on intra-annual temperature and soil water conditions will be needed to successfully capture organic layer C cycling and C transfer to the mineral soil. Movement of C from fresh litter to underlying mineral soil horizons by bioturbation (e.g., earthworms) is also one mechanism that remains to be tested.

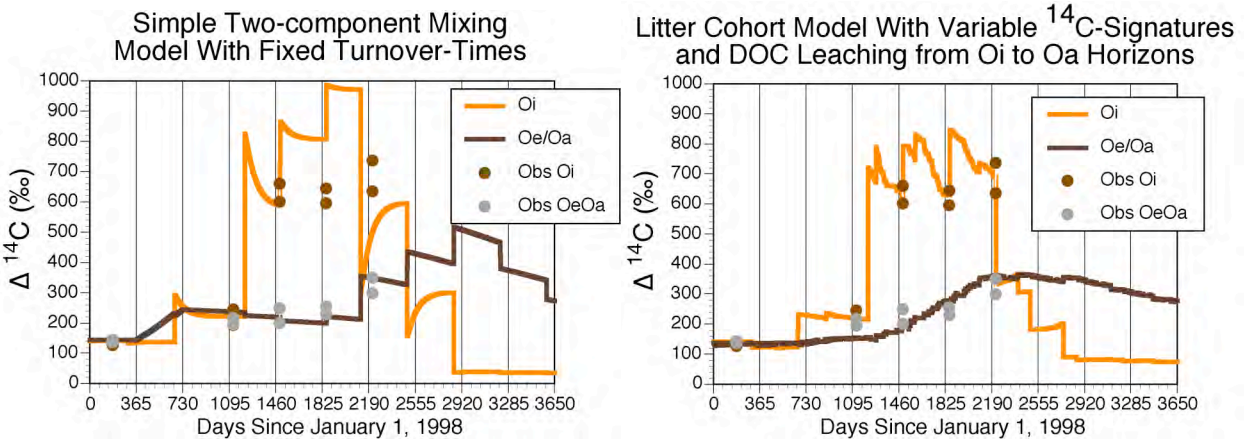


Figure 6B-3. Modeled litter $\Delta^{14}C$ using a fixed turnover time model dependent on tissue C:N ratios (left graph) contrasted with a complete litter turnover mode as described in the text (right graph). The brown and grey circles represent measured Oi>1Y and OeOa horizon litter samples for the litter enrichment treatment on Walker Branch.

References:

Hanson PJ, O'Neill EG, Chambers MLS, Riggs JS, Joslin JD, Wolfe MH (2003) Soil respiration and litter decomposition. In: Hanson PJ, Wullschleger SD, Eds, North American Temperate Deciduous Forest Responses to Changing Precipitation Regimes. Springer, New York, pp. 163-189.

Andersen JM (1973) The breakdown and decomposition of sweet chestnut (Castanea sativa Mill.) and beech (Fagus sylvatica L.) leaf litter in two deciduous woodland soils. I. Breakdown, leaching and decomposition. Oecologia 12:251-274.

Fine Root Turnover Modeling (Task 6C)

William Riley, Margaret S. Torn, Julia B. Gaudinski, and J. Dev Joslin

Root mortality turnover times in temperate forests have been estimated by several methods, including mini-rhizotron observations, sequential coring, and isotopic analysis. These methods yield very different estimates of mortality turnover times. Some of the differences undoubtedly reflect the fact that different methods tend to preferentially observe some root pool dynamics better than others. Nevertheless, a significant amount of the discrepancy stems from limitations in the models used to translate the empirical observations to root mortality turnover times. The EBIS data sets provide an opportunity to develop and test a more general model of root population dynamics. For example, root screens installed in the soil for fixed periods of time can be used to characterize the ^{14}C content of new root growth, and thus constrain estimates of the use of stored C reserves in root growth.

With the unique isotopic labeling of an intact, mature forest, the EBIS experiment has provided new and valuable insights into the processes of fine root production, mortality, and decomposition. We have used the EBIS observations of root ^{14}C content over time to develop and parameterize a dynamic root model (Figure 6C-1) that can quantify the (a) use of stored C reserves for new root growth; (b) mortality turnover times of multiple live root pools and decomposition turnover times of multiple dead root pools; (c) C fluxes from living to dead root pools; and (d) C fluxes out of the dead pool via decomposition.

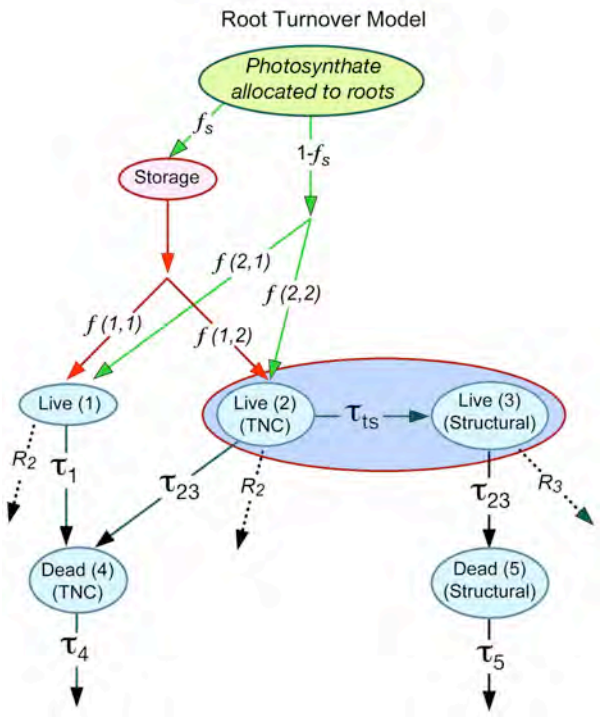


Figure 6C-1. Schematic of Root Model. TNC = total non-structural carbon.

A central feature of the root model is the inclusion of two live root pools; one that turns over rapidly (on the order of months) and one that consists of longer-lived roots (>5 years in age). The delineation of these two pools explains the relatively rapid enrichment of live roots

only one year after the release (caused by turnover of the short-lived roots), and the relatively slow decline of the enrichment in the subsequent years (caused by persistence of the long-lived roots). To represent the complexity in root dynamics while minimizing the number of unknown parameters, the model is designed with the following attributes:

- Root ages that are non-normally distributed,
- Representation of fine roots as two pools, each with structural and non-structural C,
- Stored C/^{14}C,
- Seasonal growth and respiration patterns,
- Monte Carlo analysis of uncertainty, and
- A X^2 approach to find best-fit parameters to match root C and Δ^{14}C data.

To apply the model we also had to develop an estimate of atmospheric Δ^{14}C values over time. Unfortunately, direct measurements of atmospheric Δ^{14}C were unavailable during the 1999 pulse (and possible West ORR pulse in 2000), so we used a combination of Δ^{14}C measurements in tree cores, soil gas, leaves, and soil respiration to construct a consistent atmospheric history that will also be valuable to other investigators in the EBIS project (Figure 6C-2).

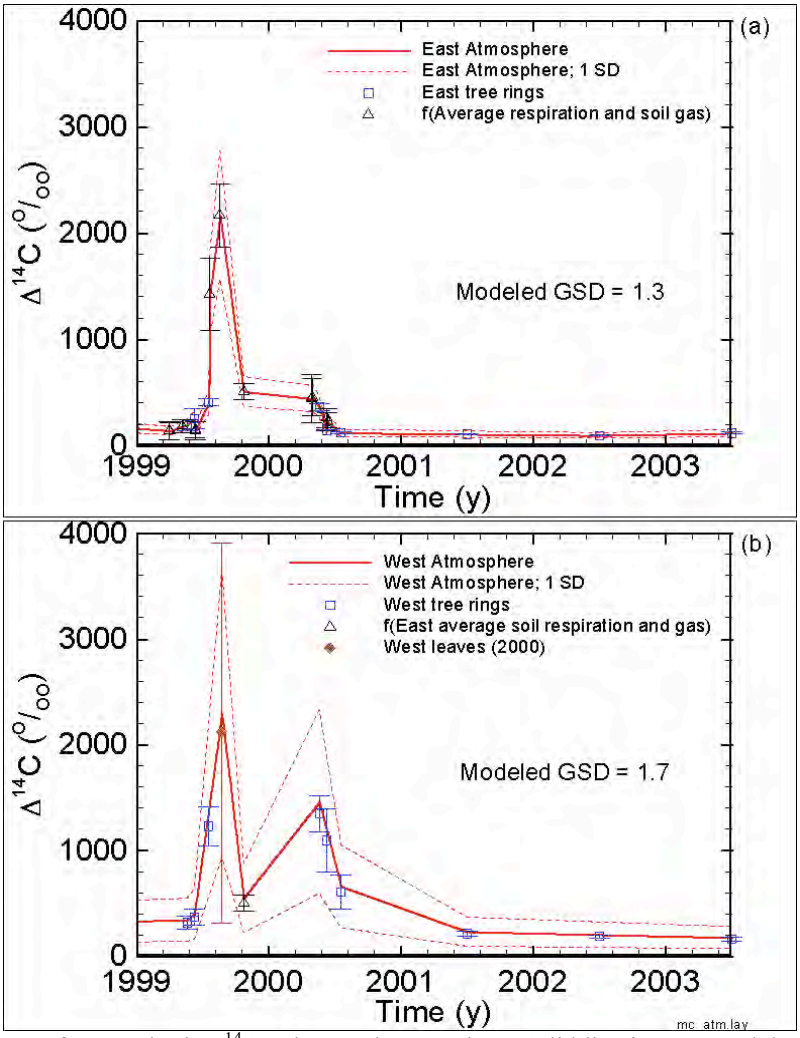


Figure 6C-2. Estimates of atmospheric Δ^{14}C values and uncertainty. Solid line is mean and dashed lines are ±1 SD for a Monte Carlo analysis of estimated variability.

To date, we have applied the model to (1) estimate the mortality turnover time in the fast live root pool and fraction of BGPP being stored and (2) estimate mortality turnover times for the longer-lived fine roots and decomposition turnover times in the dead root pools. First, using Monte Carlo error propagation, the best-fit X^2 approach, and the root screen data, we estimate that 5% of BGPP is being stored over the course of the year and that the flux-weighted C turnover time in the storage pool is about 0.7 y (Figure 6C-3). Notably, this turnover time is about half what would be calculated from a simple exponential curve fit to the root screen measurements.

Second we have used the model and root core data to improve estimates of the slower fine root pool mortality turnover times in the 0-15 cm depth interval and <0.5 mm root class (Figure 6C-4). In these simulations, the predicted best-fit turnover time of the live root pools was 6 months and 5 years; and of dead root pools was 1.9 and 5.4 years. The best-fit turnover times were estimated from a combination of east and west side ^{14}C and biomass data.

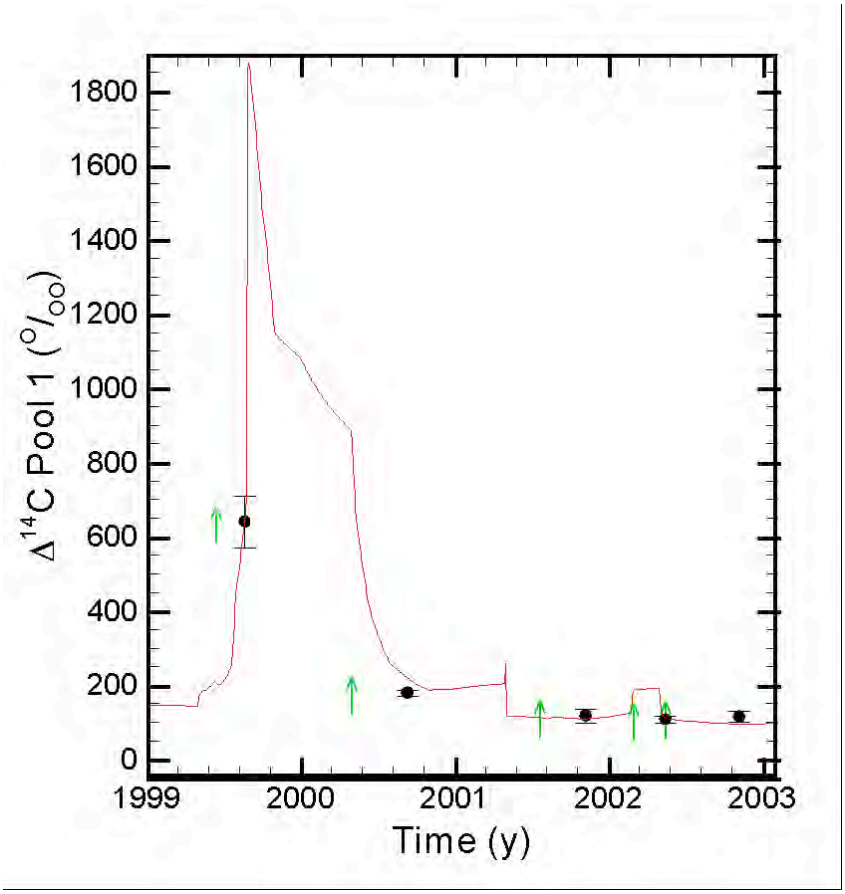


Figure 6C-3. Best-fit model results of root screens. The green arrows indicate implantation of the root screens and the data points indicate when each core was removed. The best-fit parameters for this simulation are $f_s = 0.05$ and $\tau_{sn} = 0.7$ y.

One of the key goals of the EBIS experimental design is to compare the fate of plant C that is input to the soil as leaf litter versus root tissues. Preliminary model predictions of the C fluxes out of the fast cycling and long-lived non-structural pools and out of the dead root pools are shown in Table 6C-1.

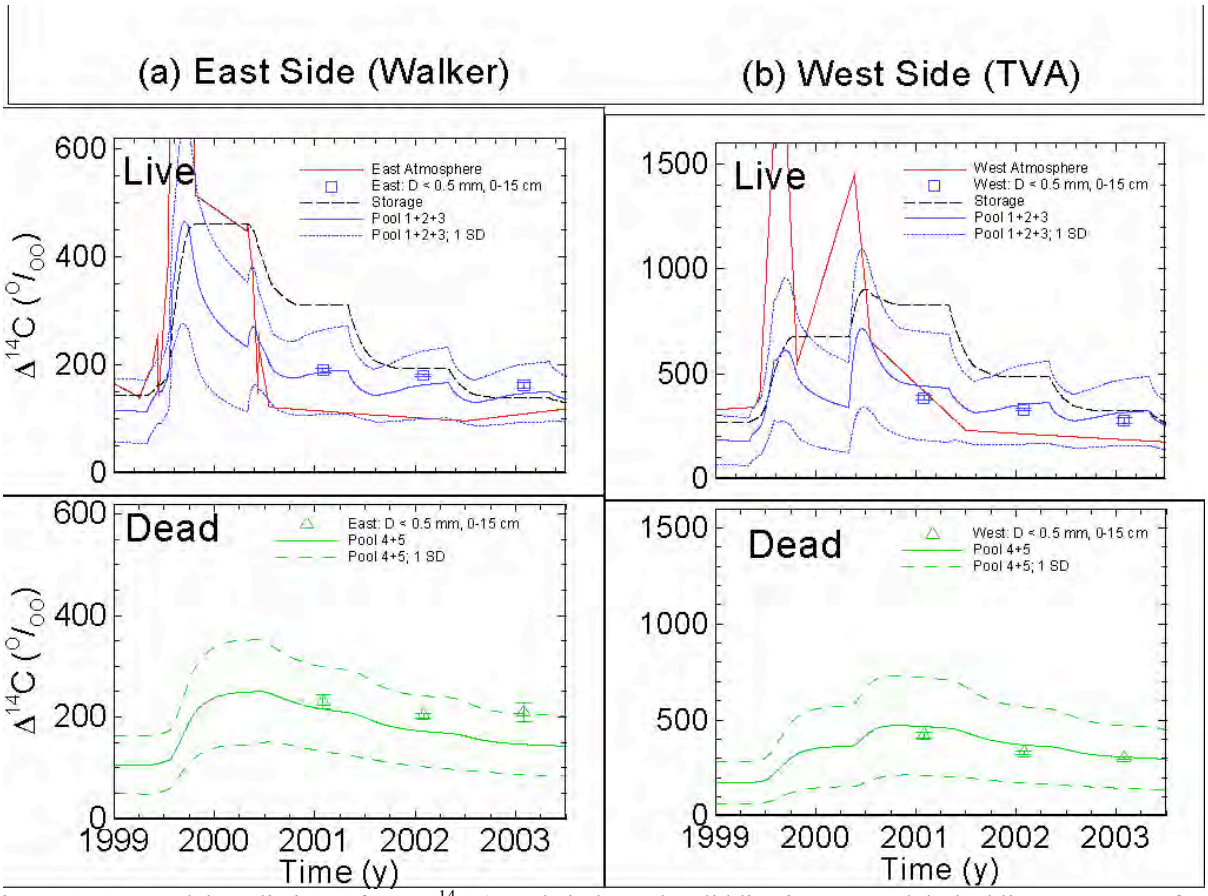


Figure 6C-4. Model predictions of root $\Delta^{14}C$ ("pool" in legend; solid line is mean and dashed lines are ±1 SD for a Monte Carlo analysis of estimated variability in parameters) and empirical root core ^{14}C data, for <0.5 mm root class, 0-15 cm depth. The upper-panel shows live roots. The lower-panel shows dead roots.

Table 6C-1. Preliminary prediction of $\Delta^{14}C$ flux to soil from root death or decay, for very fine roots (≤ 0.5 mm) in the A horizon (0-15 cm). Because that depth/size category represents about one-quarter of total fine roots (Fig 2B-1), we estimate a flux from dead roots of roughly 100 g m^2 y^{-1} for the total profile. The C fluxes are yearly integrals of daily predictions. Non-structural C is the net C flux from the live non-structural pool to the labile dead root pool. The dead roots column shows the net C leaving the dead root pool and entering SOM.

Year	Non-Structural C *<0.5 mm, A horizon*		Dead Roots <0.5 mm, A horizon	
	$\Delta^{14}C$ (‰)	Flux (g m^{-2} y^{-1})	$\Delta^{14}C$ (‰)	Flux (g m^{-2} y^{-1})
1999	464		395	
2000	329		416	
2001	145		314	
2002	117		269	
2003	125		247	
Average		21.2		25.2

Soil C and ^{14}C Mass Balance

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An evaluation of the carbon mass balance for our site using annual total CO_2 efflux from the forest floor as an integrative reference point shows general agreement between inputs and outputs (Figure S-1). While the approximate 100 gC m^{-2} y^{-1} difference is not trivial, such a difference might easily be accounted for by a larger estimate of annual root production and/or a change in the fractional contribution of autotrophic and heterotrophic respiration to total flux from the forest floor (see also Task 2A). Leaching losses from the root/soil complex are small and not responsible for the observed imbalances.

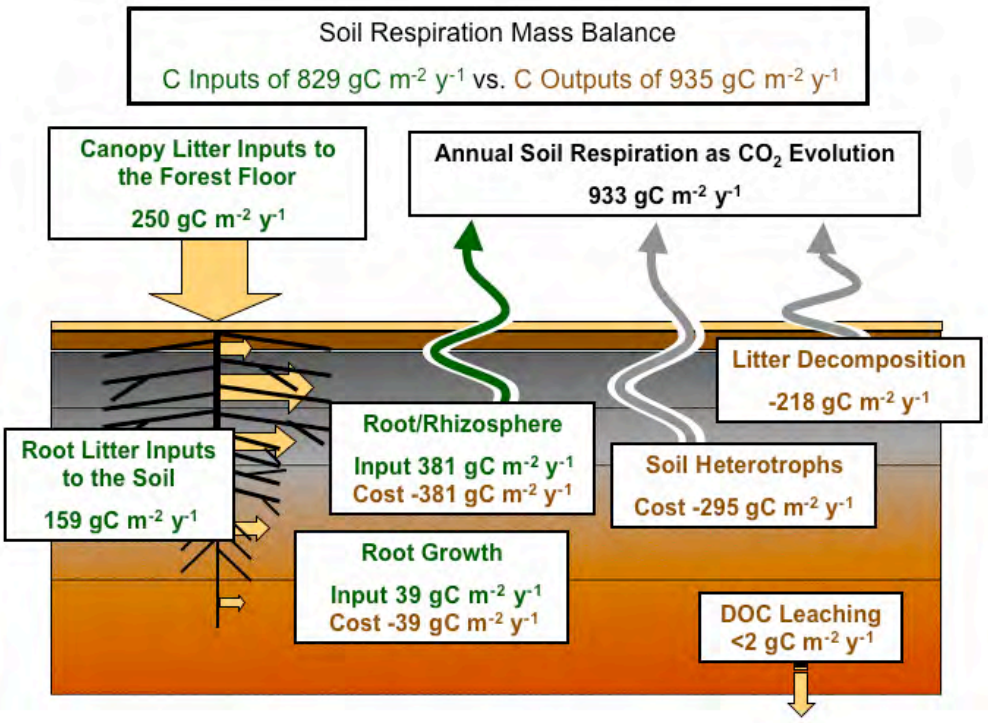

Figure S-1. Measured and approximated values for carbon inputs and outputs associated with the annual soil C cycle on the Oak Ridge Reservation. Values in green and brown letters represent C inputs and outputs, respectively. Annual soil respiration is an integrated value for recent years on Walker Branch watershed.

The annual root production in Figure S-1 was derived from a root ingrowth core and minirhizotron observations for Walker Branch (Joslin and Wolfe 2003) and is considered highly variable. Because Task 2B observations demonstrated that the root standing crop is near equilibrium for the EBIS sites, it follows that root production should be nearly equivalent to heterotrophic losses from soils horizons occupied by roots minus the carbon inputs from canopy litter. Furthermore, because we have demonstrated that net DOC leaching inputs are small and that vertical movement of particulate carbon into the mineral soil is slow for these soils we might anticipate agreement between the level of soil heterotrophic losses from the mineral soil and annual root litter inputs, but that is not the case (159 vs. 295 gC m^{-2} y^{-1}). The remaining difference (136 gC m^{-2} y^{-1}) could represent an underestimate of annual root litter production that if corrected for would go a long way towards resolving the C imbalance in Figure S-1. A key objective of the sustained EBIS measurement and modeling tasks will be to resolve this issue.

The previous sections and discussion has shown how we are using detailed process-based models to reconcile and understand measured and modeled ^{14}C flux patterns for the EBIS study sites, and underscore the inability of coarse resolution soil C cycling models to capture our data without substantial improvement. Table S-1 lists key process for which we have C and ^{14}C pool and/or flux data within the EBIS project and areas for which data are still lacking. Under sampled processes will an added focus in the renewal proposal for future EBIS work.

Table S-1. Key soil carbon cycling process and the status of data collected for them in the first three years of the EBIS project.

Critical Process	Related C measurement	Related ^{14}C measurement
Total CO_2 Efflux	Flux chambers	Flux chambers
Canopy Litter inputs	Litter baskets	Litter baskets
Root litter production	Subject to large error	^{14}C dilution
Litter decomposition	Not attempted	Incubation of isolated tissue and ^{14}C mixing calculations
Mineral soil heterotrophic respiration	Incubation of isolated soil minus roots	Incubation of isolated tissue and ^{14}C mixing calculations
Root respiration	Subject to large error	From ^{14}C evolution
DOC transport from Oi to Oe/Oa horizons	No	No
DOC transport from Oe/Oa to A horizon	No	No
DOC leaching from deep soils	Lysimeter data X water flux	Analysis of ^{14}C transport
Macrobiotic transport potential	Subject to large error	^{14}C tracer approach in progress

Reference

Joslin JD, Wolfe MH (2003) Fine root growth response. In: Hanson PJ, Wullschleger SD, Eds, North American Temperate Deciduous Forest Responses to Changing Precipitation Regimes. Ecological Studies, Vol. 166, Springer, New York, pp. 274-302.

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