Summary of the
Enriched Background Isotope Study (EBIS) Workshop
Irvine, California January 27 and 28, 2003

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

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Project Summary and EBIS 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 for the first full year of the experiment. The following bullets provide highlights from the workshop derived from the task-specific progress descriptions provided later in this report.

Operational bullets:

- PDF versions of the power point presentations given at the workshop can be downloaded at http://www.esd.ornl.gov/programs/WBW/EBISwkshp2003.htm.

- 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, therefore, should not confound analysis of carbon cycling processes in cross-site comparisons.

- The Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory processed more than 1100 samples in support of the EBIS research project during the first year of the EBIS study.

- Subcontracted funds for the research task on macrobiotic vertical transfer of carbon (earthworm movement) have been put in place. This task's field activity will begin this summer.

Scientific Findings:

- Air monitoring to track new $^{14}CO_2$ additions to the experimental forests under study have shown two additional ^{14}C-spikes since 2000. However, because they both occurred outside of the period of active canopy photosynthesis, they did not confound the EBIS experimental assumptions. Contrasting levels of ^{14}C in surface air at western-enriched vs. eastern-background sites suggests transient surface accumulation of soil derived CO_2.

- Analysis of organic and mineral soil carbon and nitrogen showed similar stocks at all four experimental sites. These absolute values will be revised with the collection of additional estimates of soil reference densities.

- Analysis of the ^{14}C-signature of bulk organic and mineral soil carbon after 1-year of litter-manipulation, indicated little vertical movement of carbon from the surface litter pools to the mineral soils.

- Lysimeter dissolved-organic-carbon measurements, however, do provide evidence for rapid movement of at least small quantities of carbon deep within the soil profile after only 1 or 2 years. Laboratory analyses documented differential carbon absorbing capacities for the B horizon of the Ultisol and Inceptisol soils being studied.

- Using enrichment or dilution of the ^{14}C-signature, an analysis of the turnover of carbon within the surface organic layer showed that litter pools were not homogeneous, and must be represented by a layered sequence of litter cohorts to capture the true nature of carbon turnover.

- The ^{14}C-signature of CO_2 evolved from the forest floor has allowed us to distinguish both litter vs. mineral soil components of soil respiration, and autotrophic vs. heterotrophic sources within the mineral soil profile. Moisture has a strong impact on the contribution of the litter layer decomposition to total soil respiration.

- Fine root biomass distributions are comparable at all four EBIS sites with the exception of greater organic layer root density at the Haw Ridge site (explanation pending). The ^{14}C-signatures for west-end enriched and east-end background roots are well defined. These time-zero data have established an excellent baseline for the evaluation of fine root turnover rates as 1- and 2-year data on the root ^{14}C-signatures become available.

- Time-zero analysis of physically fractionated carbon pools in the surface mineral horizons (0-15 cm) of enriched vs. background sites suggests substantial incorporation of new carbon derived from fine root sources into particulate organic matter pools in both soil types, and lower but significant incorporation into mineral-associated carbon pools in the Ultisol. (Note: roots were enriched for two growing seasons – 1999 and 2000 – before time-zero sampling).

- Evaluation of ^{14}C-signatures of mycorrhizal fungi confirm their autotrophic source of carbon.

- The Rothamsted soil-carbon-turnover model's carbon pools were successfully redistributed to coincide with organic matter pools sampled as a part of the EBIS project. Initial simulations based on previous data and time-zero assumptions show reasonable agreement with the 1-year patterns of ^{14}C.

Action Items

1. Funding for the University of California-Irvine's portion of the EBIS project was initially provided in a two-year lump sum payment to cover expenditures in FY2002 and FY2003. Plans need to be made to ensure FY 2004 funding of **~$123K** for Susan Trumbore's group at the University of California-Irvine consistent with the revised budget submitted in August of 2001.

2. Workshop participants concluded that it would be important to continue the EBIS measurements on an annual basis through at least FY2007. This would allow us to track the ^{14}C pulse (added over the 2000 to 2003 period) while it migrates deep within the soil profile and dissipates from decomposition processes. Additional justification for extended operation is found on pages 33 to 34 of this report.

3. EBIS operations beyond FY2004 will extend beyond the original EBIS proposal/workplan submitted in October 2000. We need guidance on how to proceed with an extended/revised workplan for FY 2005 through FY 2007. Should it be in the form of a workplan for peer review? What deadline would you like us to work towards to allow sufficient time for internal and/or peer review?

EBIS Publications and Presentations

Complete Publication:

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

Complete presentation:

Gaudinski J, Trumbore S, Hanson PJ, Southon JR (2000) A Whole Ecosystem Radiocarbon Label In A Southeastern Hardwood Forest Near Oak Ridge, TN, USA, Eos Trans. AGU, 81 (48), Fall Meet. Suppl., Abstract U21A-01.

Cisneros-Dozal M, 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, Eos Trans. AGU, 83(47), Fall Meet. Suppl., Abstract B11C-0766, 2002

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.

Planned manuscripts and presentations over the next 12 months:

Manuscripts:

Cisneros-Dozal M, Trumbore S, Hanson PJ. Partitioning sources of soil respired CO2 using radiocarbonî

Gaudinski JB, Joslin JD, Torn MS. Assessing turnover times for various fractions of fine root pools in a temperate forest using a whole ecosystem radiocarbon label.

Hanson PJ, Swanston C, et al. Reconciling Litter Decomposition with Isotopic Changes in the Oi Horizon. Soil Sci Soc Amer J

Jardine PM, Todd DE, Palmer JA, Swanston C, Hanson PJ. Hydrogeochemical controls on the fate and transport of dissolved organic C in subsurface environments. Vadose Zone J.

Jardine PM, Palmer JA, Todd DE, Nesbit RM, Hanson PJ. Physical and geochemical processes controlling organic-C sequestration in highly weathered subsurface soils. Soil Sci Soc Am. J

Treseder K, Torn MA, et al. Carbon substrates for mycorrhizal fungi in a temperate forest.

Presentations:

Gaudinski JB, Joslin JD, Torn MS. Determination of fine root dynamics in a temperate forest using a whole ecosystem radiocarbon label: diameter size class versus root architecture. American Geophysical Union, Fall Meeting 2003

Jardine PM, Palmer JA, Todd DE, Nesbit RM, Hanson PJ. Physical and geochemical processes controlling organic-C sequestration in highly weathered subsurface soils. Second Annual Conference on Carbon Sequestration: Developing and Validating the Technology Base to Reduce Carbon Intensity. May 5-8, Alexandria, VA.

Jastrow JD, O'Brien SL, Swanston C, et al. Incorporation of a whole ecosystem radiocarbon label into unprotected and protected soil carbon pools. Ninth International Conference of the Soil Ecology Society, Palm Springs, CA. 11-14 May 2003.

Joslin JD, Gaudinski JB, Torn MS, Swanston C, Jastrow J. Assessing turnover times and the short-term fate of fine root carbon in a temperate hardwood forest with a whole ecosystem radiocarbon label. Soil Science Society of America Annual Meeting, November 2003

Todd DE, Palmer JA, Swanston C, Jardine PM, Hanson PJ. Hydrogeochemical controls on the fate and transport of dissolved organic C in subsurface environments. Soil Society of America National Meetings. Nov. 2-6. Denver, CO.

Torn MS, Treseder K, et al., Microbial carbon and fungal dynamics in a temperate forest for EBIS (Enriched Background Isotope Study). 18th International Radiocarbon Conference. September 2003.

EBIS Project Information

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 organic matter pools that reside in soils for many years to decades will be 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 will enable 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.

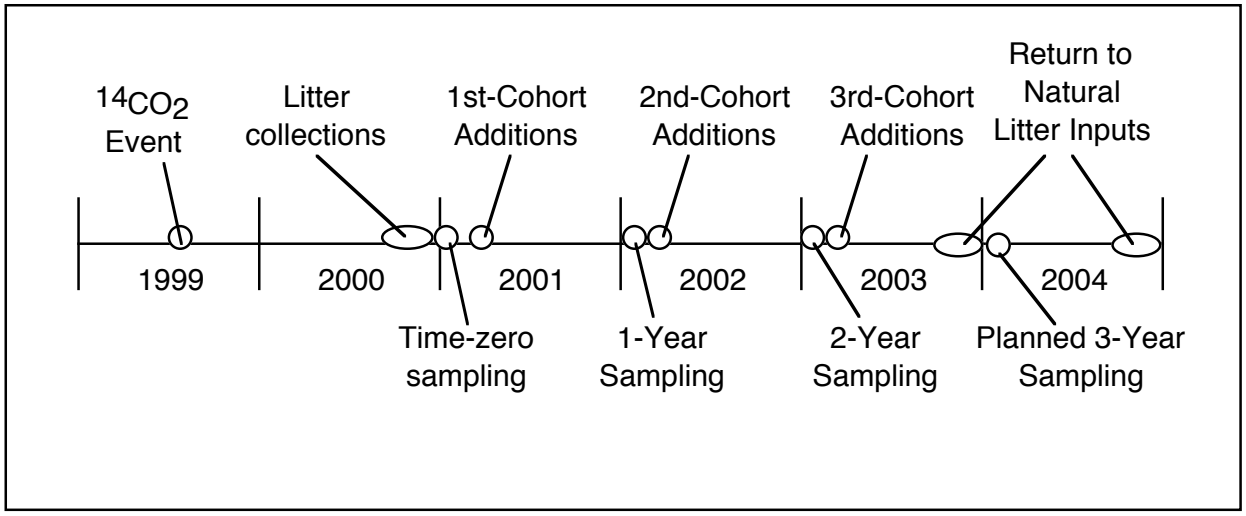
The fifth issue, longevity and turnover of fine roots, will be addressed by tracing the radiocarbon label through the fine root pool over time. Doing so will allow us to determine the dynamics of different constituents of the fine root population, as well as their specific contributions (relative to 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 By Task and Investigators

Experimental Manipulations (Task 1)

Paul Hanson and Donald E. Todd

A timeline of experimental activities accomplished since the ^{14}C-pulse of 1999 is provided below. As of mid-February 2003 all annual cohorts of either enriched or background litter (collected in the fall of 2000) were applied to the four EBIS experimental plots. Landscape cloth was added to all experimental plots from mid-October through mid-December in 2000, 2001, and 2002 to exclude ambient leaf litter inputs.



Time-zero and 1st-year litter layer and soil core samples from March of 2001 and January 2002, respectively, were processed and distributed to individual EBIS researchers in FY2002. The 2nd-year litter layer and soil core samples, collected in January 2003, are frozen pending processing and distribution to various EBIS research groups in FY2003.

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

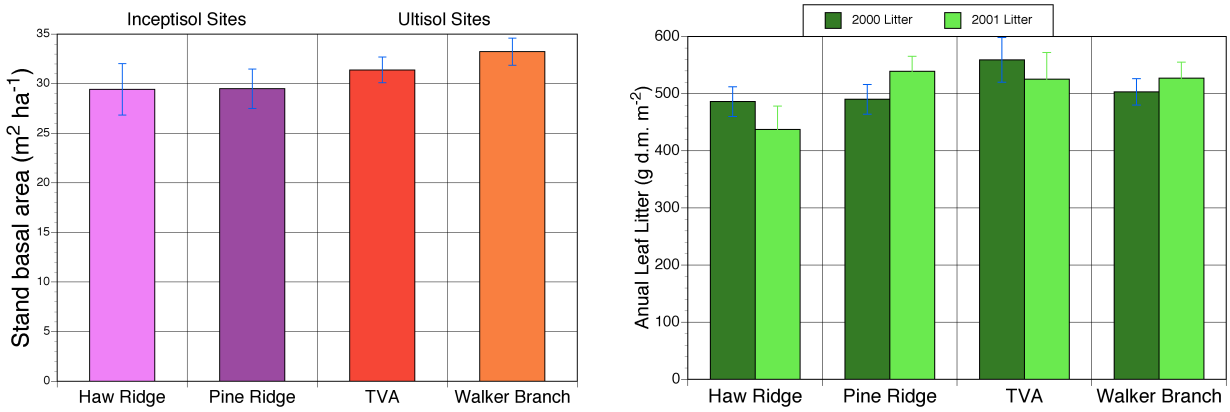
Chris Swanston and Tom Guilderson

Approximately 1100 AMS radiocarbon measurements have been carried out on soil, soil fractions, roots, ambient and soil atmosphere, and aboveground biomass in support of all EBIS research tasks. Precision of AMS measurements of graphite from these samples has been high (~5‰). However, the variation of radiocarbon within the bulk soil matrix is much higher than the measurement precision, and may necessitate a more extensive set of radiocarbon analyses than originally anticipated.

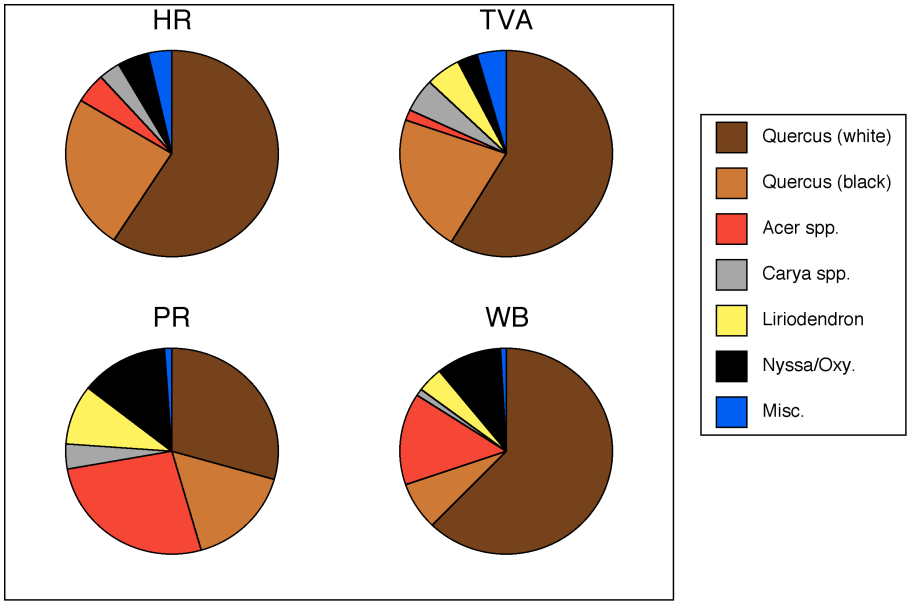
Site Characterization (Task 1)

Paul J. Hanson and Donald E. Todd

Automated measurements of environmental variables (not shown) and periodic measures of vegetation growth (dendrometer bands), 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, but this will not play a role until natural litter is allowed to accumulate on the plots in the fall of 2003 and beyond. 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.



Stand basal area and annual litter production for the four EBIS sites.



Species contribution to the basal area at each of the EBIS sites.

Bulk Soil Carbon Pools: Concentrations and Stocks By Site (Task 1)

Charles T. Garten, Donald E. Todd and Paul J. Hanson

Soil carbon and nitrogen stocks were determined based on initial soil sampling (February-March 2001) at the four EBIS study sites on the Oak Ridge Reservation. 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 ($P > 0.05$). 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) (Figure G1). 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.

There are presently uncertainties in the measurement of reference soil densities at the various sites (especially for the deeper soil depths). Multiple regression analysis indicated that, across all mineral soil samples from the four study sites, variation in soil C concentrations had at least a 2-fold greater effect on soil C stocks than reference density estimates. Soil C stocks calculated using the individual reference density data from each core and the mean reference density data at each site were in good agreement. The difference between the two estimates of soil C stocks was usually less than 200 g C m-2.

SITE DIFFERENCES (C STOCKS)

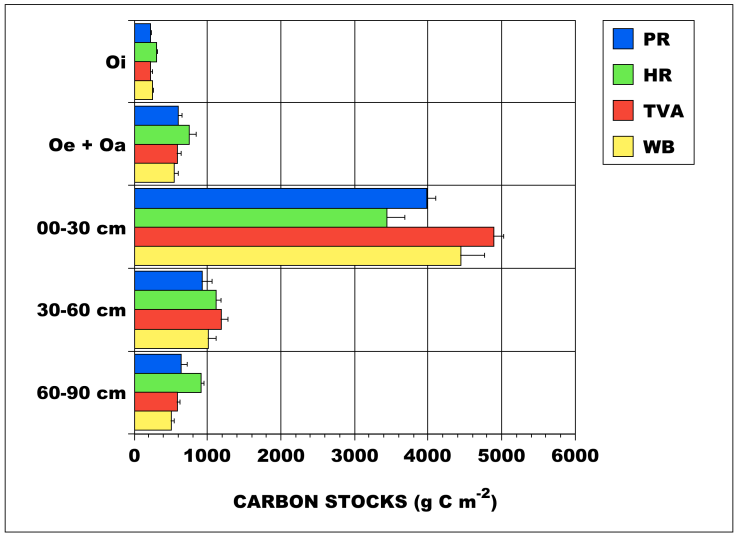


Figure G1. Carbon stocks for each EBIS research site: Pine Ridge (PR), Haw Ridge (HR), TVA, and Walker Branch (WB).

Air Monitoring of ^{14}C and ^{13}C (Task 1)

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

We have monitored ^{14}C and ^{13}C in sub-canopy air during the growing season at Walker Branch and Pine Ridge sites since September 2000. Air is collected by slowly filling an evacuated 32-liter canister over a two-week period through a capillary restrictor. Figure C1 shows the ^{14}C data since September 2000. Very high ^{14}C levels at both sites, indicative of new ^{14}C releases, were observed in early November, 2000 and mid-April, 2002 . The ^{14}C values were higher at Pine Ridge than at Walker Branch (+1438 ‰ vs. +867 ‰ in November 2000, and +2504 ‰ vs. +1683 ‰ in April 2002). 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‰.

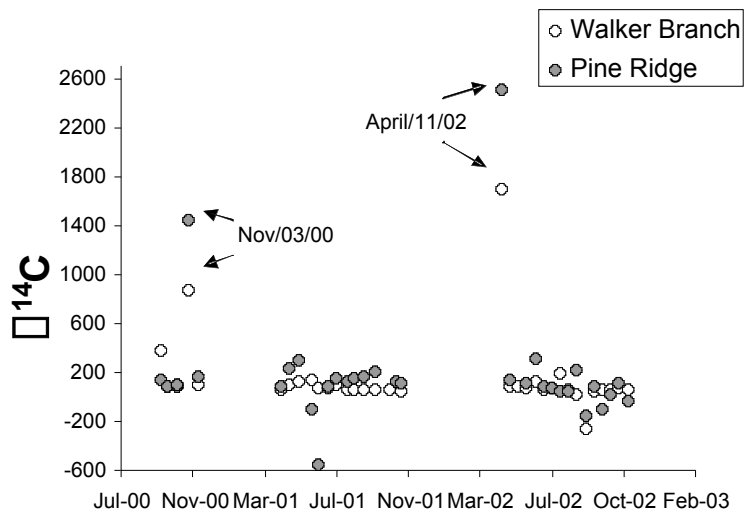


Figure C1. ^{14}C in ambient air CO_2

In the growing seasons of both 2000 and 2001, levels of ^{14}C in air were usually higher at Pine Ridge than at Walker Branch (Figure C1). This was attributed in 2000 (our last report) to the influence of re-respired C on our air sample, with much higher ^{14}C values observed for soil respired C at Pine Ridge/TVA than at Walker Branch. In 2002, this difference is less pronounced, as were differences in the ^{14}C of soil-respired C between eastern and western portions of the ORR (see also discussion of soil respiration).

The $\Delta^{14}C$ values in air sampled at Walker Branch were generally lower than expected for background air, and the opposite was true at Pine Ridge (Figure C2). We also observed negative values of $\Delta^{14}C$ mostly at Pine Ridge (Figure C1). We believe that these variations in isotopic signatures reflect the combined effect of two sources: 1) burning of fossil fuels and 2) soil respiration fluxes. The signature of fossil fuel burning would deplete the isotopic signature of the air sample while the signature from soil respiration would have the opposite effect. The incorporation of soil respiratory fluxes into the air sample is inferred from the ^{13}C signature of the sample. The ^{13}C signature in air is always more depleted than the atmospheric level of -8 ‰ (according to NOAA as of 1999) at both sites (Figure C3). This we believe reflects the more

depleted signature of soil respiration (usually about -26 ‰ at both sites) that could be present in the air sample. The topography and frequent nocturnal inversions that trap air in the surface boundary layer on the ORR combine with our 24-hour sampling close to the soil surface so that the incorporation of respired or locally produced fossil fuel CO_2 may be more pronounced in our air monitoring samples than experienced by photosynthesizing plants. Next summer we will attempt to have an automated sampler in the field that takes samples only during the daytime hours for comparison with the 24-hour integrated samples.

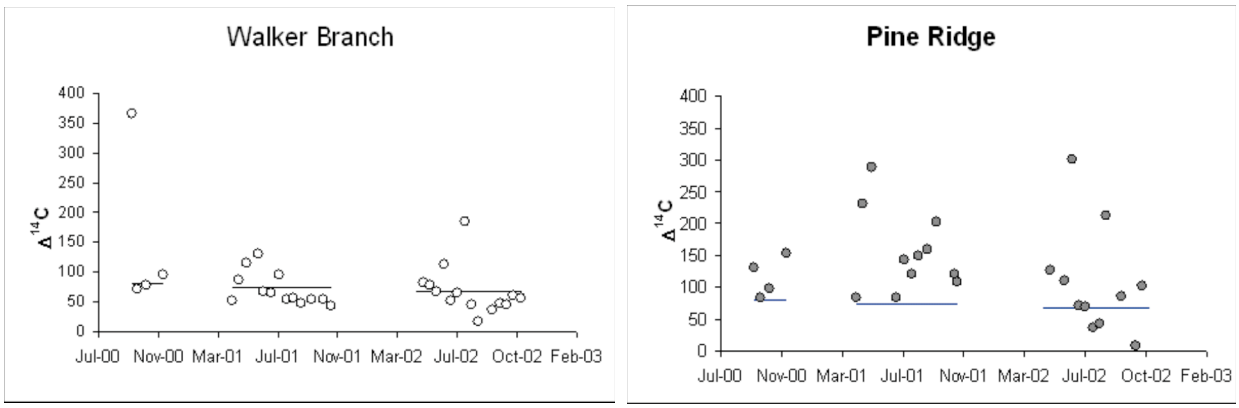


Figure C2. ^{14}C in ambient air at the sites (positive values only) and expected ^{14}C levels in the atmosphere [solid line from Levin I, Hesshaimer V (2000) A unique tracer of global carbon cycle dynamics. *Radiocarbon* 42,69-80, 2000].

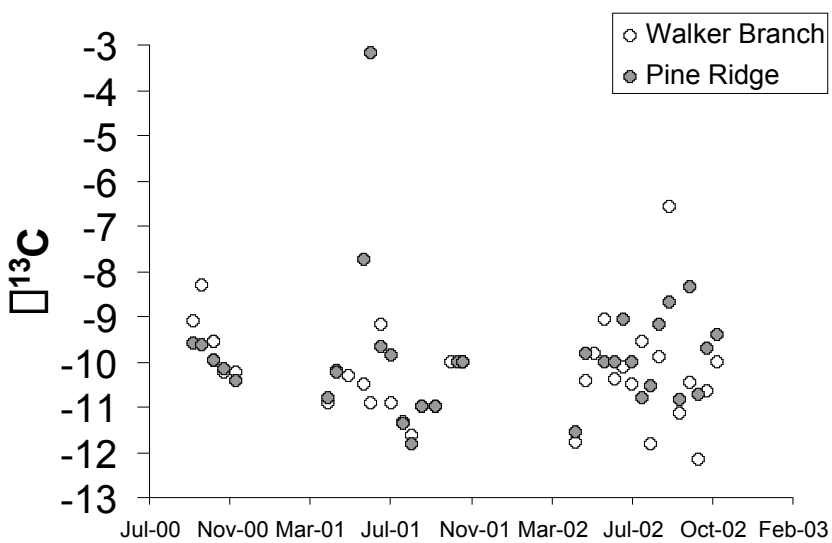


Figure C3. □^{13}C in ambient air CO_2 at the two sites

Bulk-Soil ^{14}C Signatures (Task 1)

Paul J. Hanson, Donald E. Todd and Chris Swanston

Following one year of decomposition and incorporation of the initial litter cohort on the research sites, the trends in the surface organic layer ^{14}C were as expected. 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. After just one year, little or no ^{14}C label was detected in the bulk analysis of the mineral soil horizons of any of the sites. Root additions (current root exudates and post-2000 senescent roots) to the Oe/Oa and mineral soil horizons may have diluted the signal from the enriched litter. 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 initial years' ^{14}C signal.

With only two points in time, there is still a paucity of data for determining trends in ^{14}C movement through the soil profile. The 2nd and 3rd cohort additions of litter in 2002 and 2003 will be necessary to reveal trends of C cycling through the bulk soil. All three cohorts from the 2000 collections have been added to the experimental plots (as of January 2003), and sustained operation of the experimental sites and annual sampling through at least the 2005 growing season will be required to track the movement of ^{14}C from litter sources to soil carbon sinks within the mineral soil along several parallel pathways.

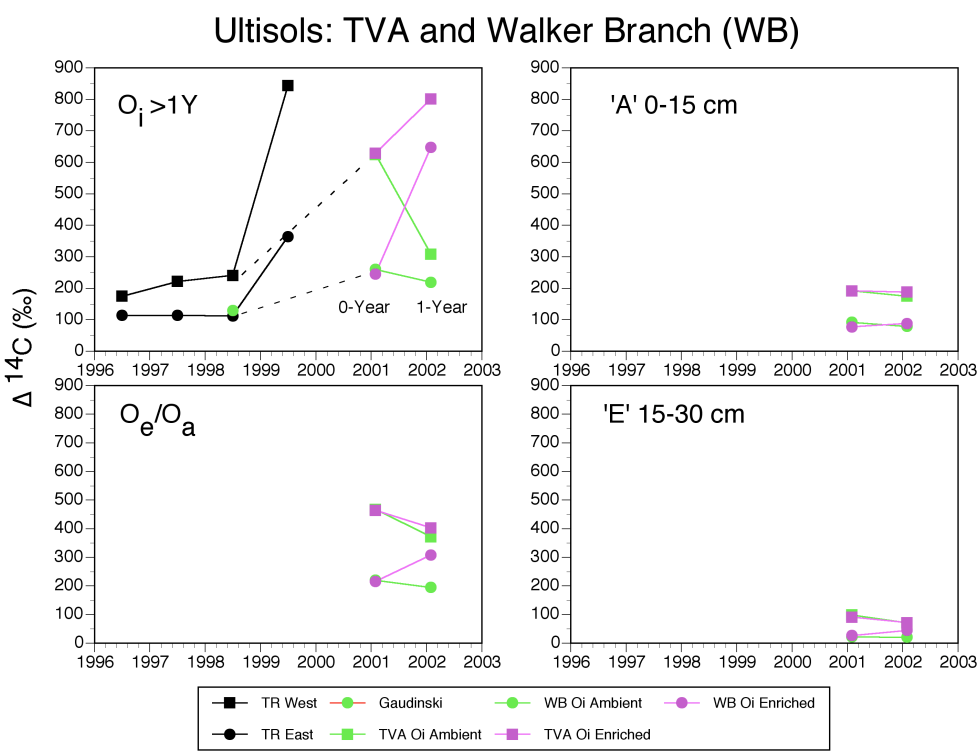
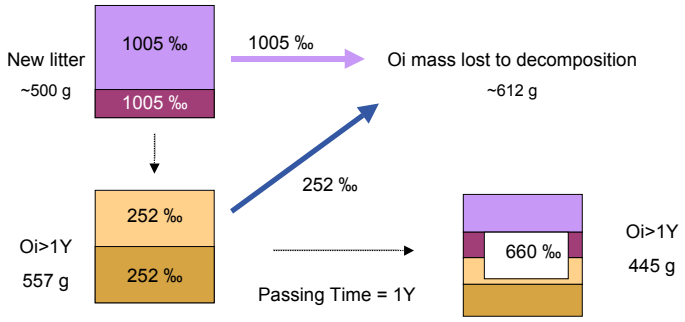


Figure H1. Bulk ^{14}C-analysis by site and horizon for the Ultisol systems. Black symbols are 14C in tree ring cellulose (TR) for historical comparison. Similar data are available for the Inceptisol sites.

Reconciling Litter Decomposition with Isotopic Changes in the Oi Horizon (Task 1)
Paul J. Hanson and Chris Swanston

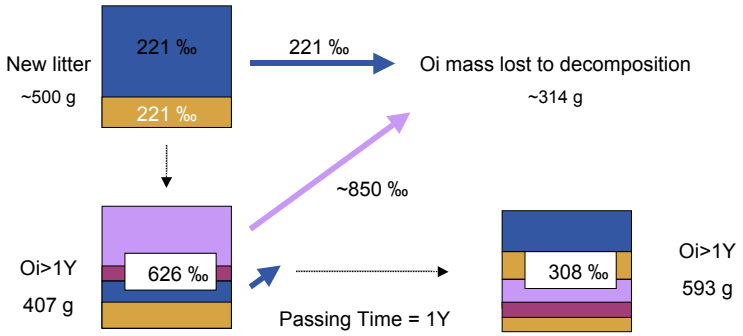
Using enrichment or dilution of the ^{14}C-signature, an analysis of the turnover of carbon within the surface organic layer showed ~50% turnover for enrichment of background litter (as expected), but nearly 75% turnover for dilution of enriched litter at TVA or Pine Ridge. This unacceptable discrepancy suggested that the Oi litter pools were not necessarily homogeneous, and therefore, should be represented by a layered sequence of litter cohorts to capture the true nature of carbon turnover. The graphic below suggest that preferential loss of an enriched cohort in the Oi layer at 'west-end' sites is responsible for the discrepancy.

The 'Well-behaved' Case
(Enrichment of WB Oi-layer)



Homogeneous pools mix to yield 50% change in Oi horizon

The 'Problem' Case
(Dilution of TVA Oi-layer)



Non-homogeneous pools mix to yield an apparent ~75% change in the Oi horizon because of preferential loss of material having higher than bulk ^{14}C-signature.

Soil Respiration and C-Source Quantification (Task 2a)

Susan Trumbore, Malu Cisneros-Dozal, 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 May, July and September 2002 at Walker Branch (WB) and TVA sites (east and western sides of the ORR respectively). At each site, we measure 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 infra-red 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 air-tight 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.

To measure the ^{14}C signature of CO_2 in soil gas at depth, stainless steel tubes were installed in the soil at different depths in two treatments (one enriched and one background leaf litter) at each site. An automated system was deployed at Walker Branch to collect CO_2 from the stainless steel tubes. The system takes a time-integrated sample into a molecular sieve trap. Traps are then taken to the laboratory for ^{14}C and ^{13}C analysis.

The results shown here include: 1) rates of total soil respiration 2) ^{14}C signature of total fluxes (from chamber measurements), 3) contribution from leaf litter decomposition and root respiration to total soil respiration and 4) ^{14}C signatures of soil CO_2 at depth at the Walker Branch site.

Results

Total C fluxes in mg C/m^2-hr are shown in Figure T1 and Figure T2. No significant difference is observed between treatments or across sites except in July, when fluxes in TVA were lower than in Walker Branch. This we believe may be the result of the lower soil moisture observed in TVA during that period. Figure T3 shows the average soil moisture content in the upper 10 cm at the time of sampling. July at the TVA site was the driest period of the 3 sampling dates with only 11% volumetric water content.

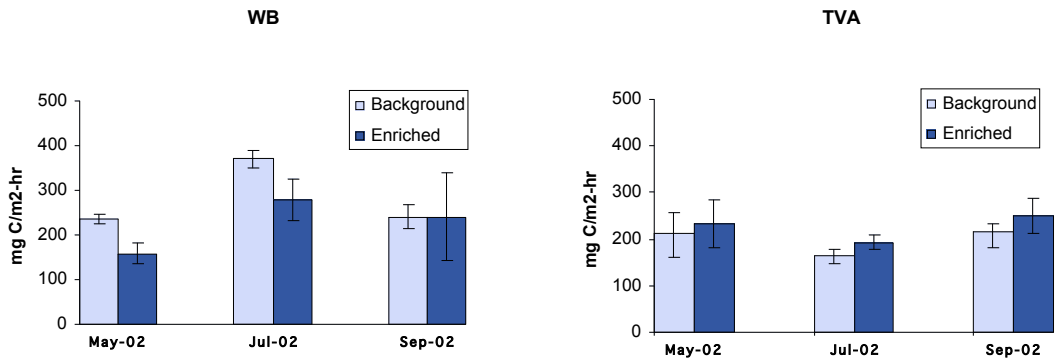


Figure T1. Total surface fluxes per treatment (mean value of 3 chamber measurements) for Walker Branch (left) and TVA (right)

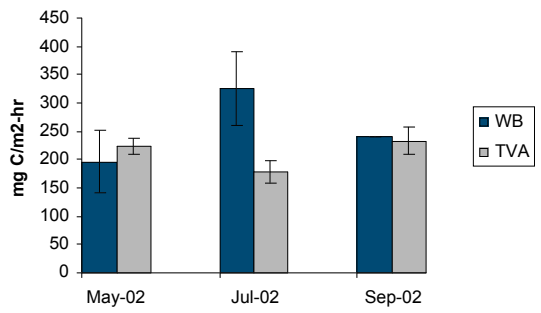


Figure T2. Total surface fluxes per site (mean value of 6 chamber measurements)

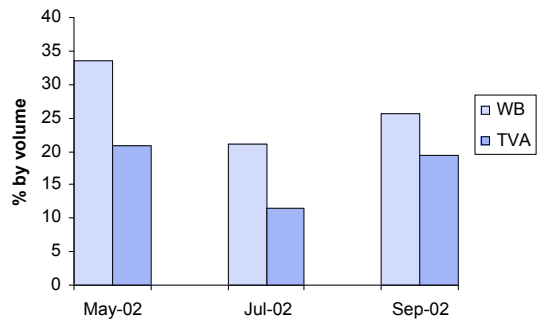


Figure T3. Soil moisture content in the upper 10 cm (mean value of hourly data recorded during the time of sampling)

Figure 4 shows the ^{14}C signature of total soil respiration. As expected, the ^{14}C signature was more enriched from plots with enriched leaf litter than from those with background levels at both sites. One exception was observed again in July at the TVA site, when there was no difference in the isotopic signature between treatments.

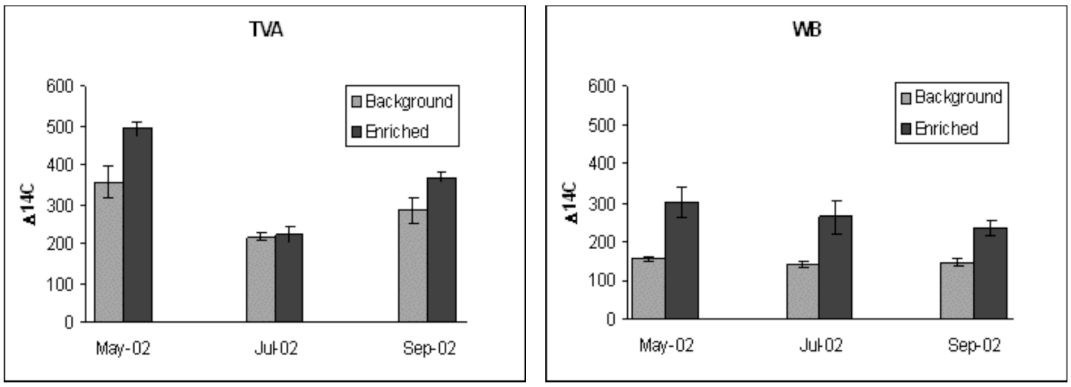


Figure T4. $\Delta^{14}C$ of total soil respiration (mean value of 3 chamber measurements) for TVA (left) and Walker Branch (right)

As noted earlier, soil moisture was markedly lower at the TVA site during July (Figure T3). The same effect was observed in August 2001, when surface leaf litter was completely dry.

Fraction from leaf litter decomposition (FLD)

Within a site, the difference in the ^{14}C of total soil respiration between enriched and background plots is proportional to the contribution of leaf litter decomposition, since the radiocarbon content of CO_2 derived from root and soil decomposition and root respiration should be the same. We therefore can use the observed differences in ^{14}C in total soil respiration between elevated and background plots to calculate the fraction of total soil respiration coming from leaf litter decomposition (FLD; Figure T5). FLD decreased from 0.18 in May to 0.11 in September, reflecting the depletion of substrate with time. As expected, leaf litter decomposition depended strongly on moisture content, accounting for only 1% to total soil respiration in July at the TVA site.

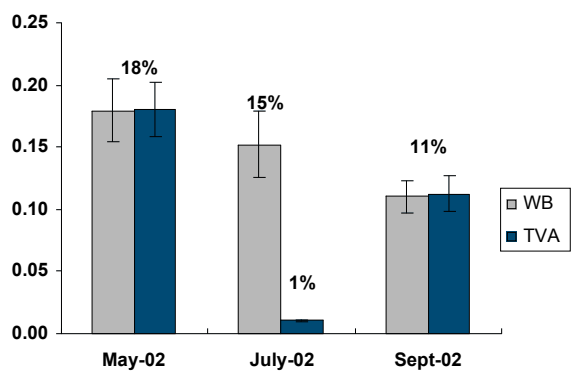


Figure T5. Fraction of total soil respiration coming from leaf litter decomposition (FLD). The same contribution was found at both sites in May and September.

Fraction from root respiration (FRR)

We calculated the fraction of total soil respiration coming from root respiration (FRR) by applying a mass balance approach for ^{14}C at each site and treatment:

$$\Delta^{14}C_{\text{total respiration}} = \Delta^{14}C_{\text{autotrophic}} * FRR + \Delta^{14}C_{\text{heterotrophic}} * (1-FRR)$$

$\Delta^{14}C_{\text{total respiration}}$ is obtained from chamber measurements, $\Delta^{14}C_{\text{autotrophic}}$ from root sampling and $\Delta^{14}C_{\text{heterotrophic}}$ from incubations of soil and leaf litter. In background plots we found that the differences in isotopic signature between sources and total respired carbon were too small to resolve the mass balance. In contrast, the differences between sources in the enriched plots are significant and the results are more consistent. The value of FRR was calculated to vary from 0.43 to 0.80 across sites, with the highest value corresponding to the very dry soil at TVA in July (Figure T6).

Figure T7 shows the absolute contributions from FRR and FLD to total soil respiration at each site. At Walker Branch, total respiration fluxes reached a maximum value in July with all of the sources increasing with respect to May. At the same site, the contribution from root

respiration (FRR) increased from May to September. At TVA, total respiration was highly suppressed in July with a much lower contribution from heterotrophic sources. The fraction from root respiration shows the same increase as in Walker Branch from May to September but it was the highest in the driest period.

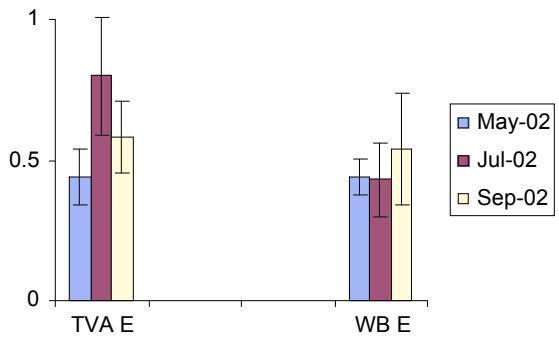


Figure T6. Fraction of total soil respiration coming from root respiration (FRR) at TVA (left) and Walker Branch (right) for the three sampling dates.

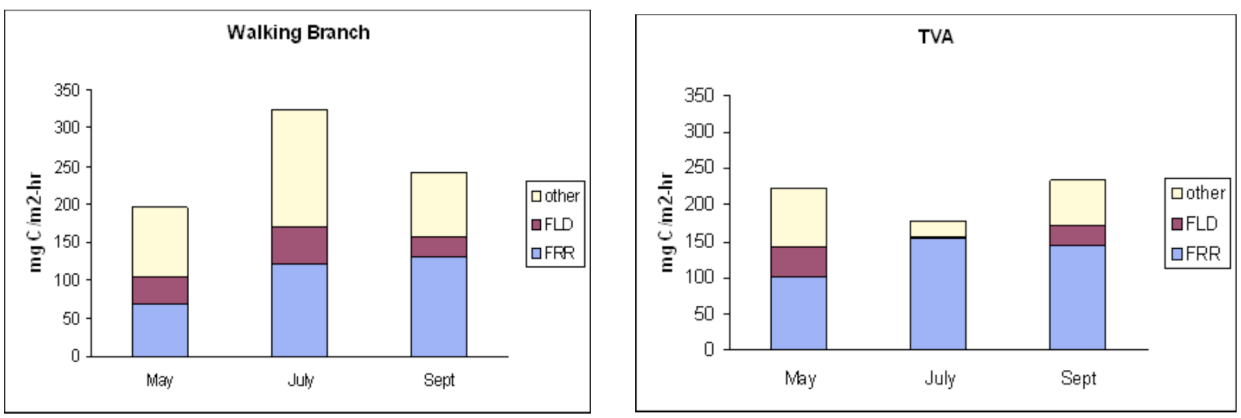


Figure T7. Absolute contributions from leaf litter decomposition (FLD) and root respiration (FRR) to total soil respiration at each of the sites.

In 2003, we will continue to measure the components of soil respiration as in previous sampling efforts. We will increase the sampling frequency under different moisture conditions in leaf litter to better establish the pattern of response of FLD to moisture content. To better constrain the seasonal variations in root respiration, we will monitor the ^{14}C signature of carbohydrates in roots and in ambient air on the same day of root sampling.

<u>^{14}C in soil CO_2 at depth</u>

Figure T8 shows the ^{14}C of CO_2 at the depth profile from samples taken with the automated system over a one-week period. We observe very little change below 20 cm soil depth and a clear contribution from leaf litter C to the soil CO_2 at 10 cm (noticeable in both

treatments). This suggests that leaf litter C may be transported into the A horizon in dissolved form and then decomposed.

In 2003 we will continue to monitor these plots with several samplings and we will also deploy this system at the TVA site.

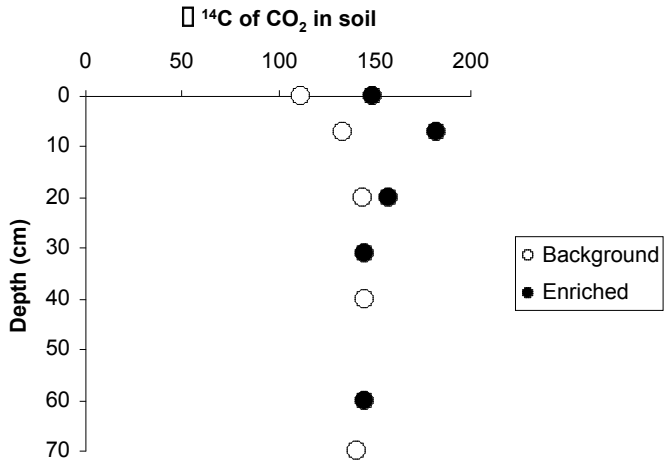


Figure T8. $\Delta^{14}C$ of CO_2 in soil gas samples taken at Walker Branch in May 2002

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 (Task 2b)

Julia B. Gaudinski and J. Dev Joslin

Accurate measurements of dead and live fine root biomass by depth and their ^{14}C signature through time are essential for several components of the EBIS project. For example, for partitioning soil respiration into autotrophic and various heterotrophic components; estimating the turnover time and stabilization of SOM; and modeling ecosystem carbon dynamics. Fine root samples are sorted into two size class categories (<0.5 mm and 0.5-2 mm in diameter) and by live and dead categories. Year 0 samples show that fine root biomass averages 2130 ± 182 g m^{-2} for all four sites. The greatest proportions of roots are found in the 0-15 cm depth interval and in the 0.5-2 mm diameter size class (Figure G1) and 52% of all roots sampled were live. Distribution of fine roots by depth indicate that the two dolomitic sites are quite similar (Figure G2), however Haw Ridge is unique among the four sites in having four times as much fine root material in the Oa horizon and 50% more fine roots below 30 cm and more coarse root biomass (data not shown).

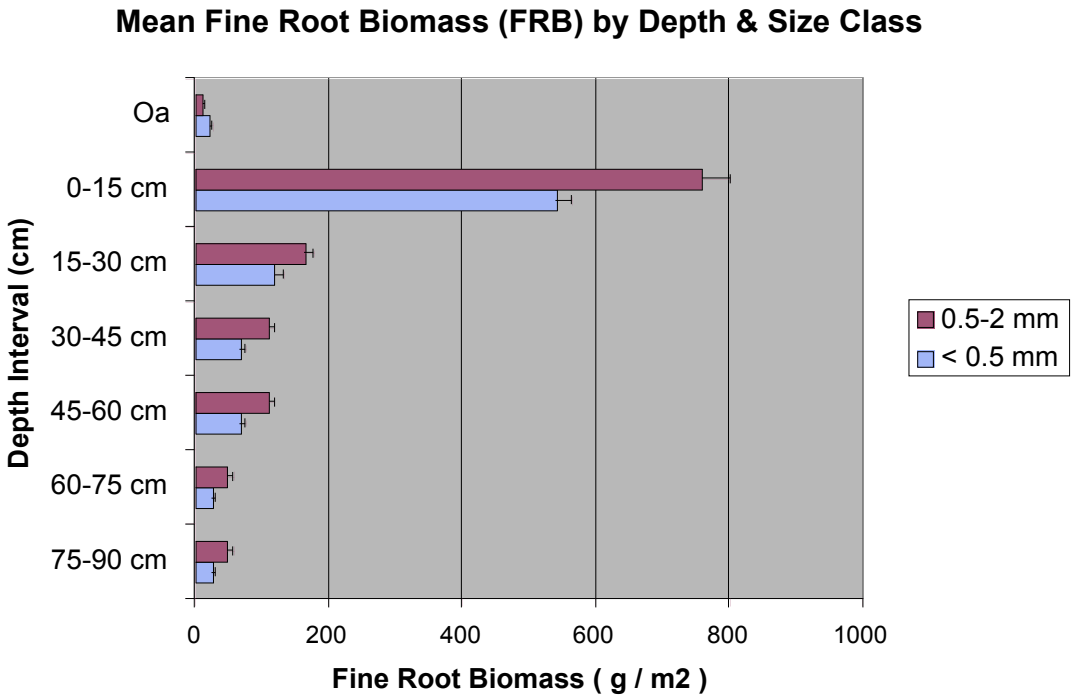


Figure G1. Fine root biomass as a function of depth and diameter size class averaged over all four sites.

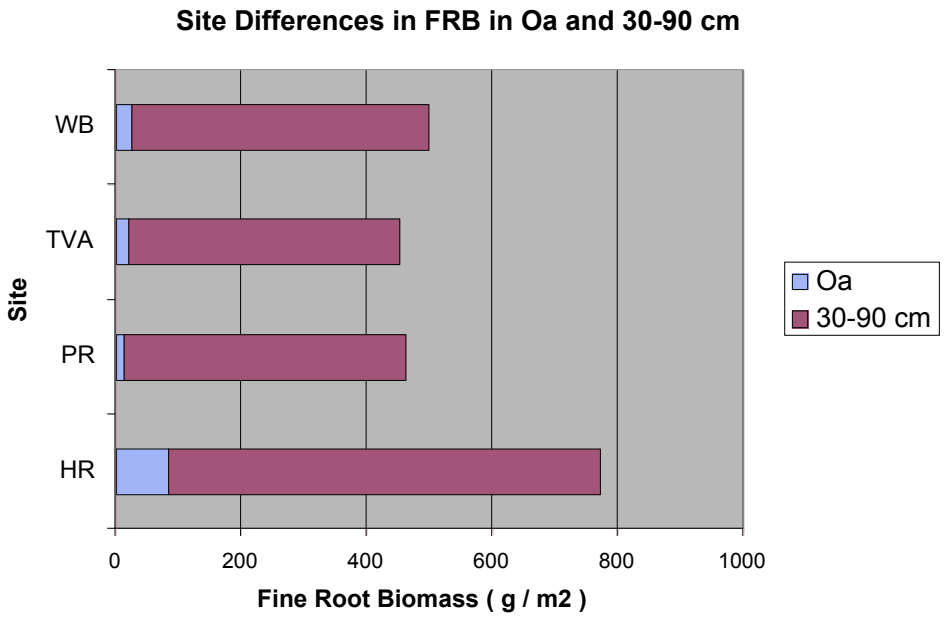


Figure G2. Fine root biomass by site in the Oa and 30-90 cm intervals. Note that Haw Ridge has larger biomass in both Oa and the 30 – 90 cm intervals.

Several published studies of fine root dynamics show a correlation between fine root lifetimes and diameter size class, and that the < 0.5 mm diameter size class is the most dynamic portion of the fine (< 2mm) root pool. Our year 0 ^{14}C data, however, do not show any consistent trend in $\Delta^{14}C$ signatures between our two size class groupings (Figure G3). Based on our recent findings in other forests, we are currently looking at $\Delta^{14}C$ signature as function of position on the root branch system at two of the four EBIS sites.

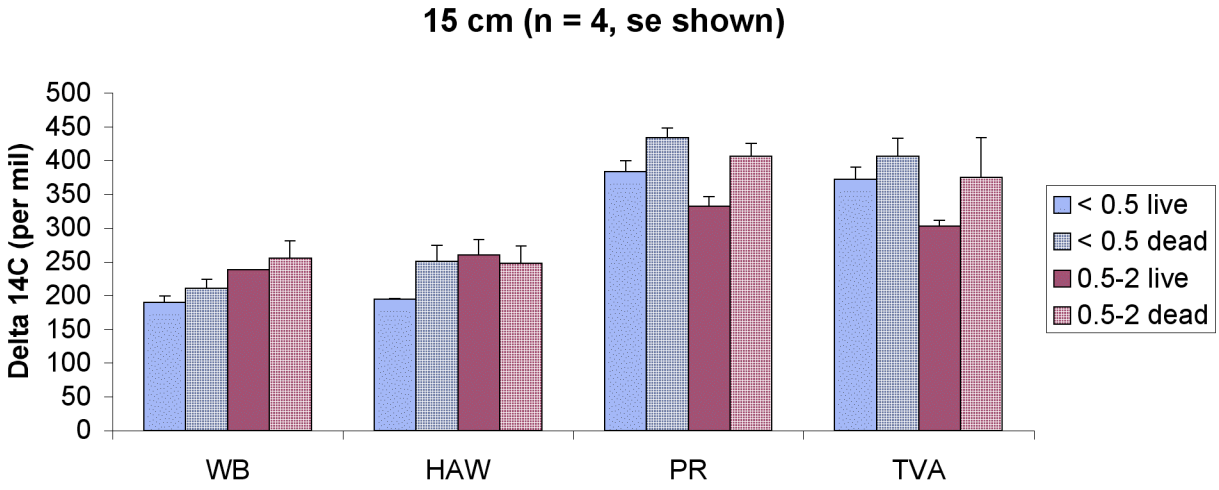


Figure G3. $\Delta^{14}C$ of fine roots as a function of live and dead and diameter size class for the 0-15 cm soil interval. While data shown only for the 0-15 cm interval, both the $\Delta^{14}C$ magnitudes and trends as a function of live/dead category and diameter are similar for the root mat (Oa horizon) and the 30-60 cm interval.

Dead roots tend to have the same or higher $\Delta^{14}C$ values relative to live roots (Figure G3). There is not a significant change in $\Delta^{14}C$ as a function of depth in the soil profile of live or dead roots (Figure G4). The $\Delta^{14}C$ of both live and dead roots from the east end of the reservation average 230 ± 6‰ and 234 ± 13‰ respectively, while west end roots have significantly higher values at 351 ± 12‰ and 403 ± 13‰, for live and dead respectively. The $\Delta^{14}C$ of new root growth appears to retain 10-20% of the $\Delta^{14}C$ signature of the previous years growth (Figure G5). This is an important result that will help characterize inputs to root lifetime models and estimates of the rates of fine root turnover based on the changing atmospheric record of $\Delta^{14}CO_2$.

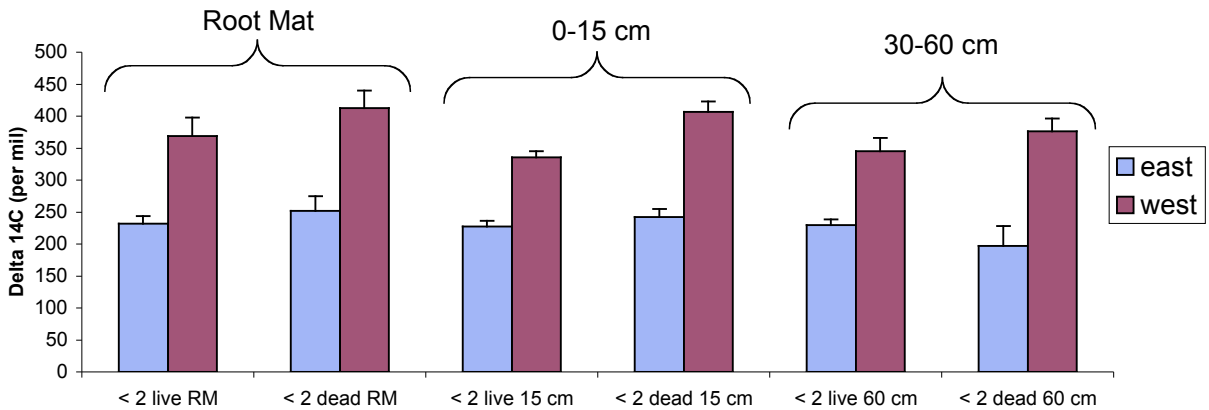


Figure G4. $\Delta^{14}C$ values in the root mat, 0-15 cm and 30-60 cm intervals, for all roots < 2 mm ($\Delta^{14}C$ represents a mass weighted composite for the <0.5 and 0.5 to 2 mm diameter size classes) grouped by location on the reservation (east or west). Root mat samples are representative of the Oa horizon.

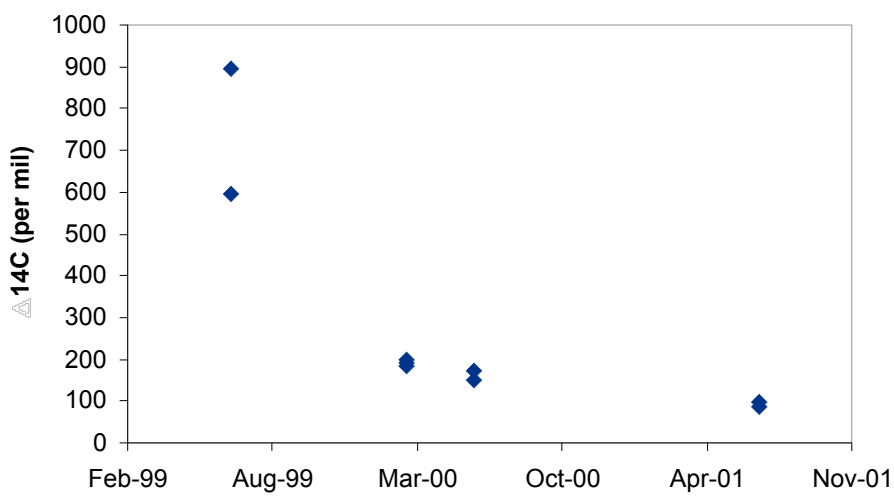


Figure G5. The change in $\Delta^{14}C$ of new root growth (sampled from root screens) over time. At each sampling time, the screens are completely cleaned of all roots, so each successive sampling represents roots grown from a distinct and known time interval. The time point shown corresponds to the midpoint of the sampling period.

DOC Transport (Task 3a)

P. Jardine, Todd, J.A. Palmer, C. Swanston, and R.M. Nesbit

The objectives of this task are to (1) quantify the impact of coupled hydrological and geochemical processes on the fate and transport of dissolved organic C through the EBIS soil profiles, and (2) quantify the mechanisms that control enhanced carbon accumulation within deep subsoils of forested Ultisols and Inceptisols. Our approach involved a multi-porosity sampling scheme coupled with a non-reactive 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 plots) were 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. 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 application of ^{14}C labeled litter, a nonreactive Br tracer was evenly applied over each of the instrumented areas in an effort to quantify the hydrodynamics of each site. Solution samplers were monitored during all storm events over the next year and analyzed for Br, DOC, and inorganic anions. Select samples were analyzed for ^{14}C as well. Bulk soil samples from each plot were also characterized for select physical and chemical properties and organic C sorption isotherms were quantified for each subsoil. These investigations were designed to quantify the geochemical processes controlling the movement of organic C through the soil profiles.

Results to date suggested that DOC fluxes were higher for A-horizons relative to B-horizons. Haw Ridge (sandy Inceptisol) had the highest C flux which was consistent with its more rapid flow and transport characteristics and lower organic C retention capacity. Contrasting DOC sampled under experimental plots with enriched vs. background litter additions, clearly shows that ^{14}C-enriched DOC from newly applied litter is present at both shallow and deep lysimeters within one-year of application. Additional analysis of the magnitude of the DOC carbon flux is being done to assess the significance of DOC transport as a means for delivering significant C to deep soil storage pools.

Clayey soils (PR, TVA, WB) had lower organic C fluxes yet greater flux decreases during movement from A- to B-horizons. This was related to their higher organic C retention capacity. Pore water $\Delta^{14}C$ signatures were higher on enriched plots relative to background plots, and the data was consistent with site hydrological and geochemical characteristics. DOC sorption was significantly higher in soils rich with Fe-oxides and clay. The Fe-oxide coatings on mineral surfaces strongly sequester pore water organic C which limits its bioavailability and transport to groundwater. In summary, organic C fluxes at each site were consistent with the hydrodynamics and geochemical retention capacities of the soils. The data will be useful for prediction strategies concerned with the hydrogeochemical controls on organic C fate and transport in subsurface environments.

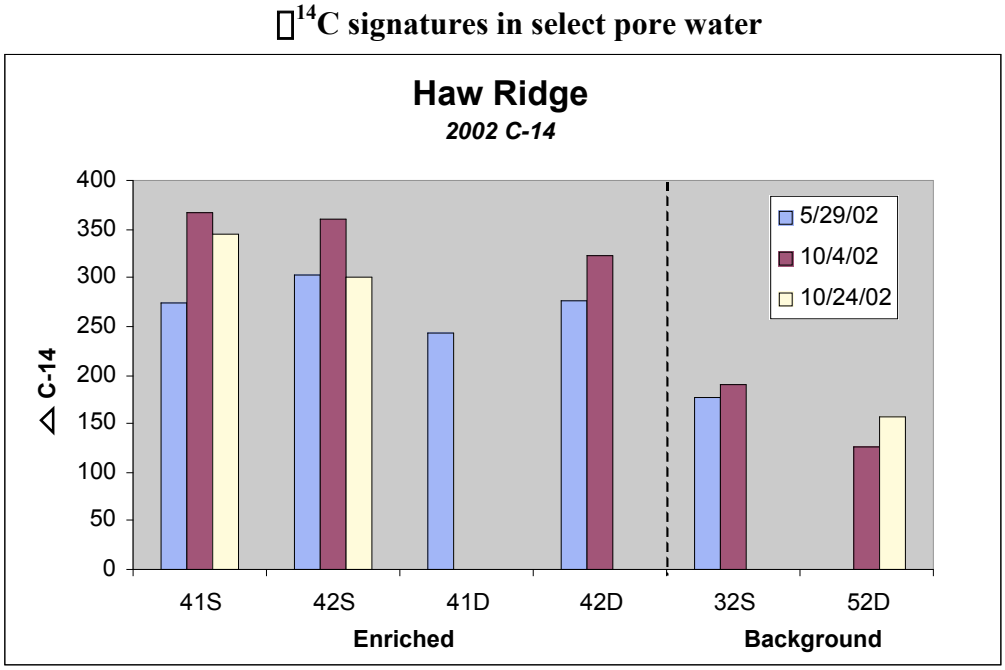


Figure D1. Contrasting 14C-signatures of DOC collected in shallow (S) and deep (D) lysimeters in plots with either enriched or background litter additions.

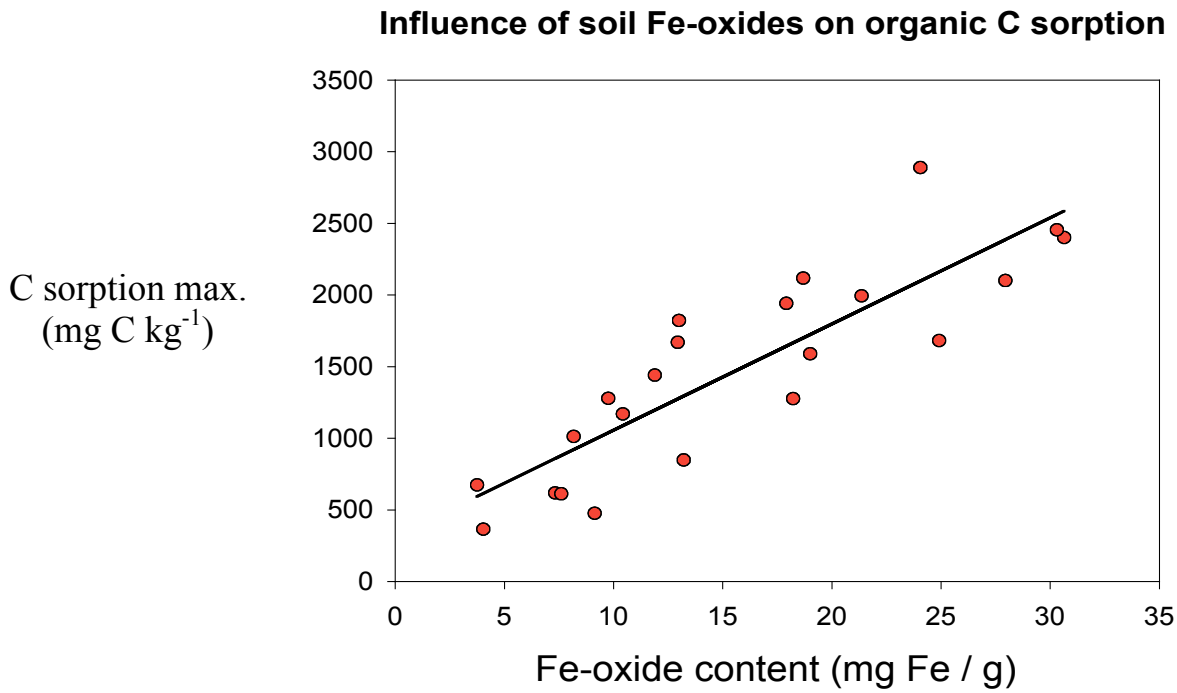


Figure D2. Relationship between carbon absorption capacity of soils and their iron(Fe) ozide content which explains differential carbon adsorption capacities of the Ultisol and Inceptisol soils.

Soil C Dynamics in Unprotected and Protected Pools:
Process/mechanistic-based fractionations (Task 4a)

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

Mineral soil samples (Year 0; 0-15 cm) were fractionated into unprotected and microaggregate-protected particulate organic matter (POM and mPOM), microaggregated silt and clay (mSILT and mCLAY), and silt and clay located outside microaggregates (SILT and CLAY). For the purpose of tracking the movement of input sources into stabilized C pools, it is only necessary to fractionate samples from the treatments with enriched litter/background roots, background litter/enriched roots, and background litter/background roots. The sum of C amounts in the six fractions was found to be in remarkable agreement with independent analyses of whole soil C concentrations, indicating excellent fractionation recoveries. The $\Delta^{14}C$ signatures of the fractions also correlated well with each other and with whole-soil signatures. Comparing west side (root enriched) to east side (root background) soils (Figure J1), the greatest ^{14}C enrichment occurred in unprotected POM for PR and in both POM and mPOM for TVA. The negative $\Delta^{14}C$ signatures for mPOM in WB soil appear to be associated with the presence of significant amounts of charcoal in this fraction. Similarly, charcoal may account for the lower POM signatures in WB compared to HR. Higher $\Delta^{14}C$ signatures were also found for all mineral fractions, but only TVA mineral fractions were significantly enriched.

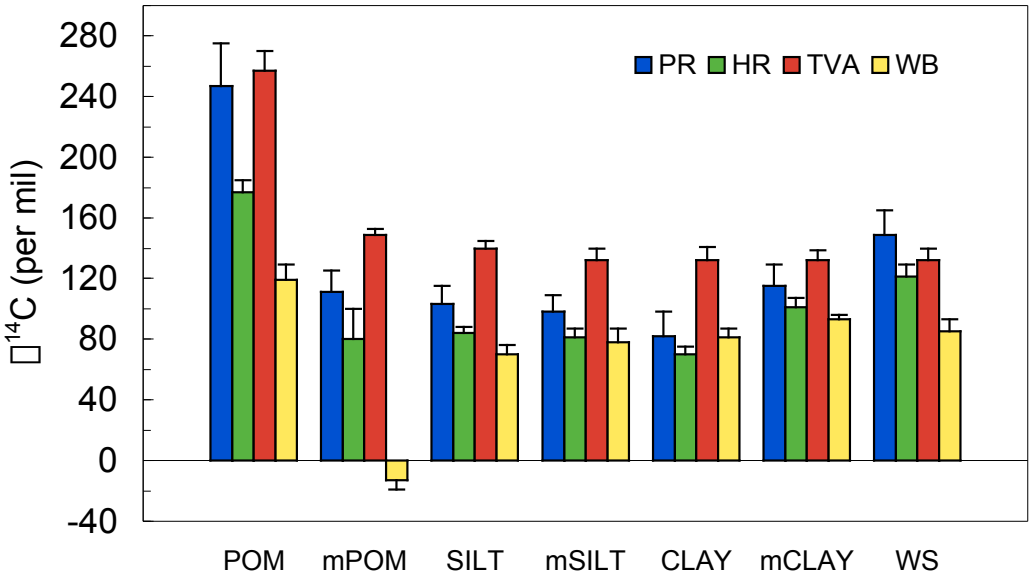


Figure J1. $\Delta^{14}C$ signatures in microaggregated soil fractions (mPOM, mSILT, mCLAY) and non-microaggregated fractions (POM, SILT, CLAY) at Year 0.

At this time (two years of significantly enriched root inputs), mineral fraction enrichment probably results mostly from exudation and microbial activities. If true, after acid hydrolysis of the mineral fractions, we expect most of the labeled C to be hydrolyzable. The amount of new C in each fraction was estimated (Figure J2) by mass balance, assuming two years of inputs with $\delta^{14}C$ signatures equal to that of PR and TVA dead roots in 2000. Although the amounts of new C in PR seem possible, the TVA amounts are higher than might be expected, suggesting (1) that ^{14}C inputs, especially in 1999, may have been higher than estimated by the 2000 dead roots or (2) that ^{14}C inputs from before 1999, albeit at a lower level, may be contributing to the soil signatures. Better estimates of C dynamics and residence times will be obtained by following changes in the signatures of each fraction over time. This information will also be required to compare the fates of enriched inputs from litter and roots. To accomplish these goals, it will be necessary to track fraction signatures every year through Year 3 or 4 and then again after waiting a few years. It may be possible to prepare a manuscript within the next 12 months, depending on the signatures of the hydrolyzed mineral fractions or on data obtained from Year 1 samples (which should be fractionated by the end of April).

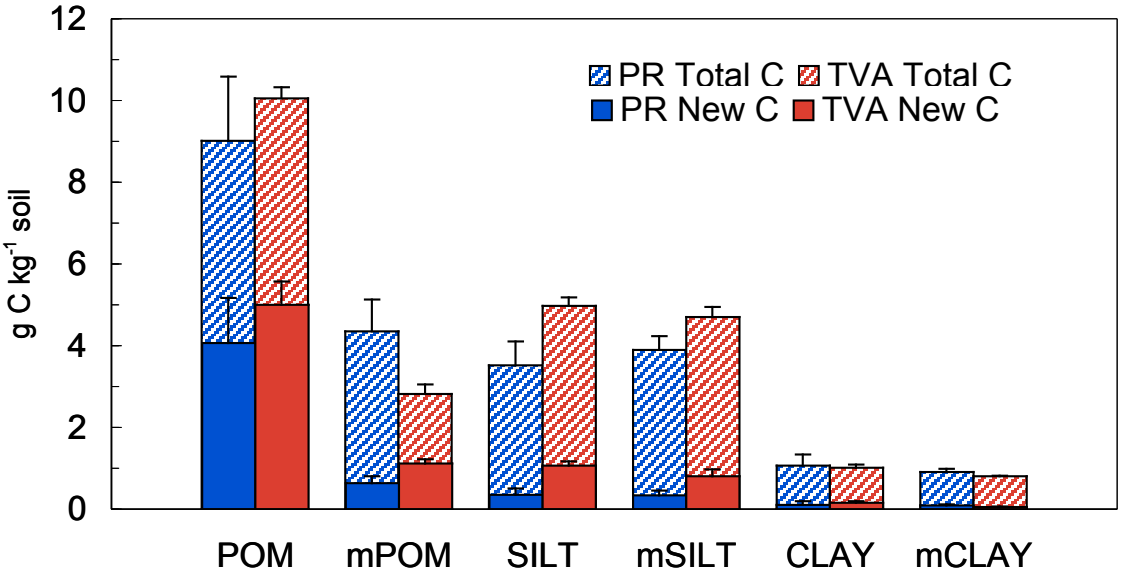


Figure J2. Estimated new C concentrations in PR and TVA (enriched) soils relative to total soil organic C at Year 0.

Soil Organic Matter Fractions (Task 4b)

Margaret Torn

We have developed a method that will compliment observations underway in Task 4a but emphasize the influence of soil mineralogy and depth on carbon stabilization. The laboratory has been set up and initial investigation of the soil has begun.

Microbial Carbon Pools (Task 4c)

Margaret Torn

Soil Fungi

Two recent papers (Chapela et al 2001, Hobbie et al 2002) have cited radio- and stable isotopic data as indirect evidence for the uptake of soil organic carbon by ectomycorrhizal fungi, and have even suggested that this flux could contribute to plant carbon budgets. We sampled roots from the reciprocal transplant experiment in September 2001, and hand-picked ectomycorrhizal tips for ^{14}C analysis. Our results indicated that the ectomycorrhizal fungi received no carbon from decomposition of the litter. Only ectomycorrhizal fungi growing on trees on the west end of the reservation (more ^{14}C enriched) were themselves enriched. On the east end, ectomycorrhizal fungi were not enriched even for those from plots with isotopically enriched litter (Figure M1).

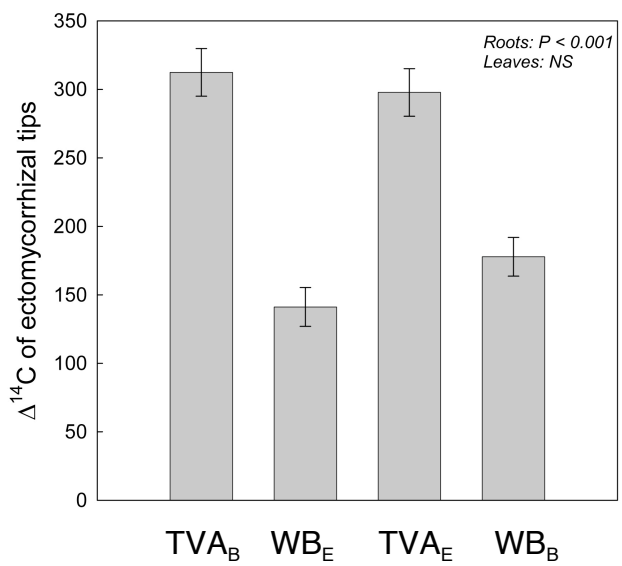


Figure M1. Radiocarbon content of ectomycorrhizal fungal tips from the TVA and WB sites of EBIS, collected September 2001.

We are excited about these results because they indicate that ectomycorrhizal fungi in this system had not switched between mutualistic and saprotrophic functions at any point over the course of one year. Ectomycorrhizal fungi produce enzymes that break down organic material, and can survive in sterile culture when supplied with only organic compounds. It is possible that they could switch to a saprotrophic lifestyle if carbon supplies from plants decline due to changes in allocation or photosynthetic rate. Yet, they apparently only acquired mineral nutrients from the soil. This evidence also supports assumptions that fungi release extracellular enzymes to break down organic material externally, and then take up the products. The distinction is important when measuring 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 or ectomycorrhizal lifespan.

We also measured the influx of carbon into pools of glomalin in the soil. Glomalin is produced only by arbuscular mycorrhizal fungi, and it can account for ~5% of soil carbon pools. Natural abundance ^{14}C signatures indicate that glomalin can be decades old in Hawaii (Rillig et al, 2001), but the rate of glomalin production is not known.. Our preliminary data at WB and TVA show an influx of the radiocarbon pulse into sites that were directly affected by the pulse (data not shown), and we can now begin to model the flux of carbon into this microbial pool.

Microbial Biomass

To look at movement of radiocarbon into soil organic matter, we measured the radiocarbon content of bulk microbial biomass and extractable soil carbon from soils collected in July 2002. Extracts from the standard chloroform fumigation-extraction method (CFEM) were freeze-dried and combusted for graphite preparation. We verified that we were able to achieve complete combustion by comparing carbon density from elemental analysis and combustion independently. In preliminary results (10 out of 32 pairs of samples completed), the amount of carbon (extractable and microbial carbon) and the radiocarbon enrichment were greater in the top 5 cm of soil compared to 5-15 cm depth. In terms of carbon sources, the radiocarbon content of extractable carbon was most enriched in plots with enriched roots (Figure M2). In contrast, the microbial biomass showed incorporation of the radiocarbon pulse in all treatments, but was most enriched in the plots receiving enriched litter (Figure M3). The rapid enrichment of the microbial carbon is consistent with conceptual models of the microbial biomass as a highly active carbon pool that decomposes root, litter, and dissolved organic material. The relatively high enrichment of the microbial biomass (~500 ‰) compared well with values of heterotrophic respiration measured in 2002. Over the next year, we can evaluate the efficacy of using the ^{14}C signature of the microbial biomass pool as an integrated signature of soil respiration $^{14}CO_2$ over a season.

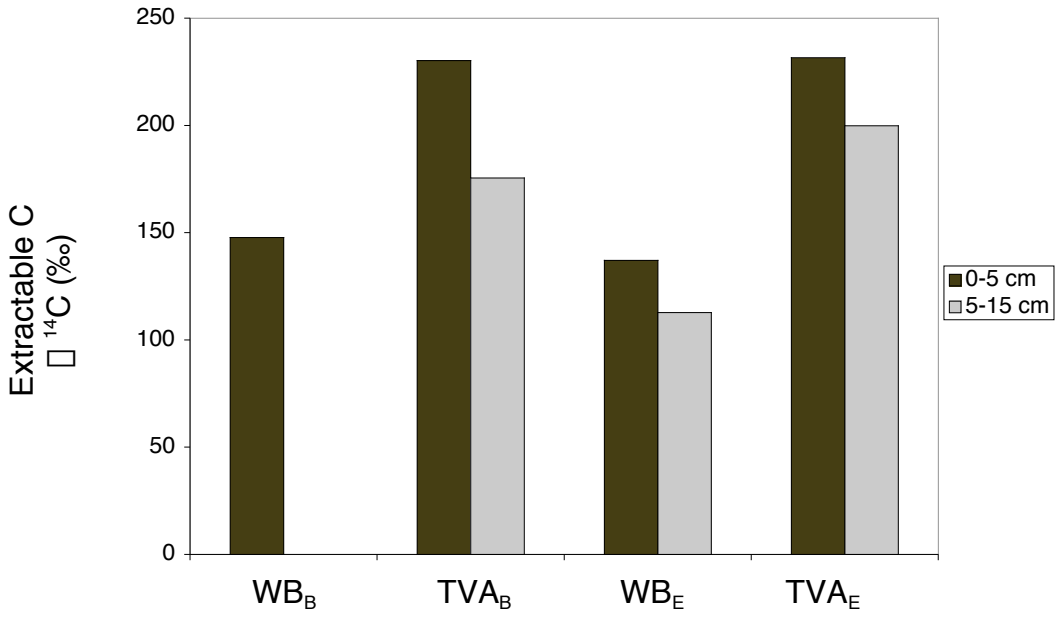


Figure 4c-2a. Extractable-carbon ^{14}C for a preliminary set of 10 samples collected in July 2002. Samples are extracted with 0.5 M K_2SO_4.

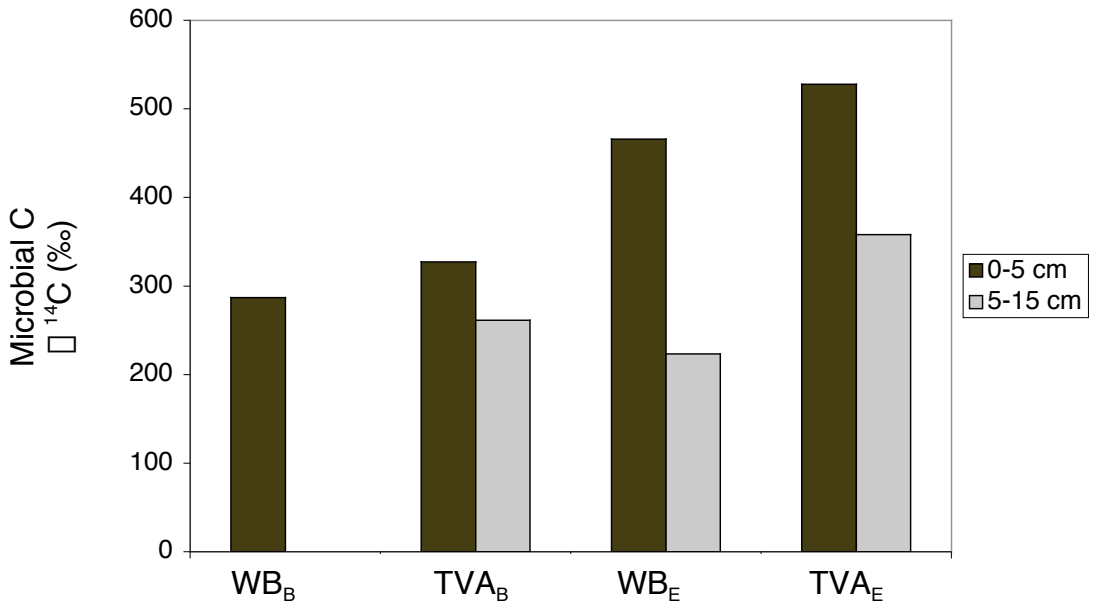


Figure 4c-2b. Microbial biomass ^{14}C for a preliminary set of 10 samples collected in July 2002. The microbial biomass values are calculated from the mass balance of the extractable and fumigated samples' carbon and ^{14}C content.

Soil C Modeling (Task 6)
W. Mac Post et al.

The Rothamsted soil carbon turnover model was chosen for initial simulations because of its simplicity and it's history of dealing with ^{14}C data. It's pool structure is hueristic and conceptually process based. It is largely the same pool structure that is adopted by the widely used CENTURY model. With the shift in recognition that biological and physical processes dominate over organic chemical properties in determining the dynamics of soil carbon it has been shown that the conceptual pools of the Rothamsted model largely align with physically separated fractions of soil organic carbon. It is possible, therefore, to redistribute the organic matter pools of the Rothamsted model into organic matter pools measured directly by investigators in the EBIS project. While the correspondence is not exact, initial simulations show it is close enough to capture the mass and radiocarbon data of the EBIS plots. The following table shows the simulated pool sizes (re-categorized) compared to the measured pools for Walker Branch Watershed.

Table P1 Modeled vs. observed carbon pools for Walker Branch Watershed.

Equilibrium Pools

Compartment	Model	WBW
DPM + associated RPM (Oi)	2.0	2.8
RPM + BIO (Oe + Oa)	9.8	9.4
HUM + IOM (A)	50.7	44.2

Figure P1 shows the radiocarbon signal of the various litter and soil carbon pools for the bomb period and the first year of EBIS additions for Walker Branch Watershed. The only pool that was adjusted was the inert organic matter (IOM) pool in order to get the correct total soil organic matter ^{14}C content. Figure P2 shows the litter and surface soil radiocarbon measurements plotted on top of the simulation results.

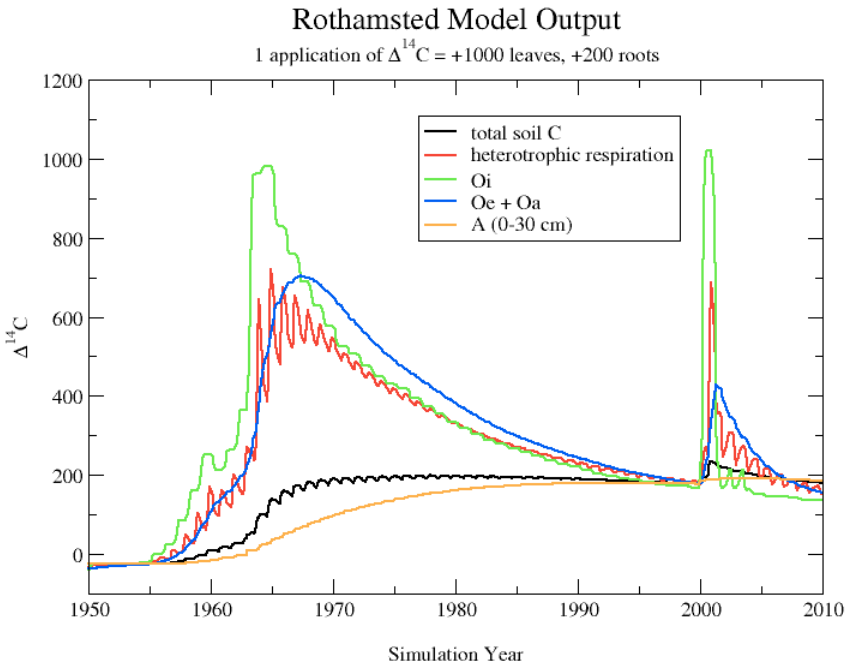
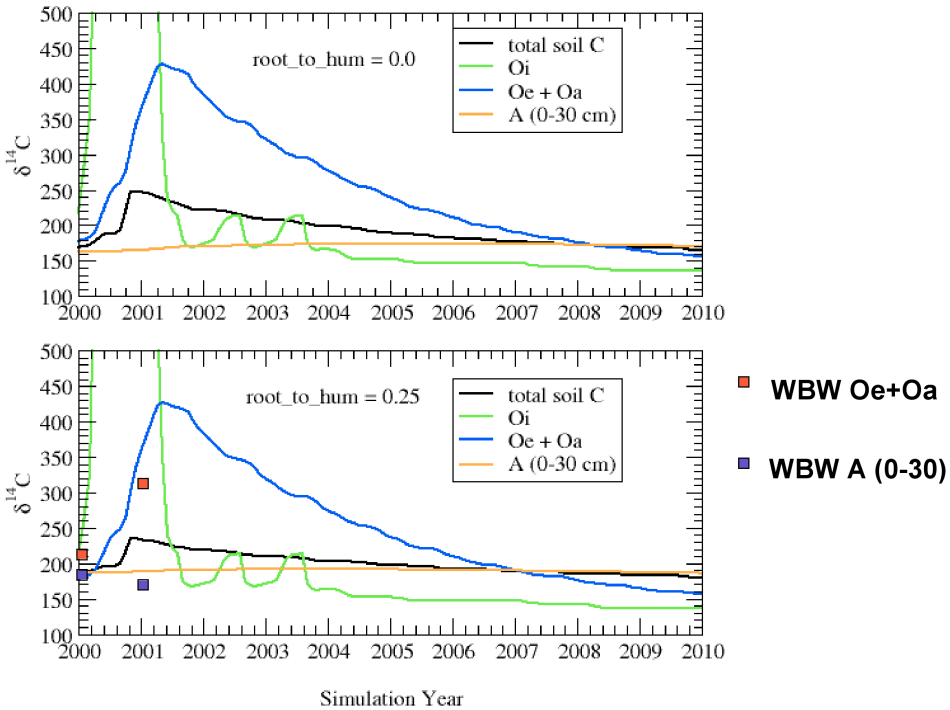


Figure P1 Simulated ^{14}C-signatures of adjusted Rothamsted carbon pools for a single cohort of enriched litter and roots added to the ecosystem.



Figuer P2 Simulated and observed (lower panel) ^{14}C signatures of soil carbon pools.

The success of a well tested, standard soil organic matter model to capture the main features of the carbon pool sizes and radiocarbon dynamics is encouraging, both for the usefulness of the measurements and for more detailed modeling that the new tracer data will now allow. The Rothamsted organic matter pools, especially the HUM (35-50 yr turnover time) is a hueristic pool that capture the dynamics of several processes related to aggregate dynamics and the physical protection of particulate organic matter, adsorption/desorption of DOC and colloidal sized organic matter molecules, chemical transformation into resistant material.

Measurements of movement of organic matter as DOC, particulate material, or translocation by decomposer organisms will allow us to develop a multilayer model of organic matter dynamics. While layered models have been developed, they are largely empirical models calibrated to bulk measurements. EBIS project data will allow us to develop a layered model based on the physical and biological processes that have been used in the widely used single layer agricultural based models. As the number of layers increases, however, the number of compartments and flows that must be estimated increases rapidly. While it appears that the number of measurements that are currently being collected will be sufficient for model enhancements, development and application of a layered model must proceed quickly so that the unexpected measurement requirements might be addressed (for example, do we need measurements of the microbial biomass in deeper layers).

Justification for EBIS Measurements Beyond FY 2004

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The October 2000 EBIS proposal requested funding for 3 years to cover the establishment and initial measurements associated with a multi-year study of soil-carbon-cycling processes. With initial data and analyses completed (as described above), we are in a position to evaluate the appropriate time frame for continued operation and completion of EBIS field measurements. The hypothetical figure below is an extension of the graph of bulk ^{14}C-signature of the organic horizon previously described (Figure H1), but includes hypothesized changes into the future. The observed pattern is a crude representation of changing ^{14}C signatures in the surface organic horizon in response to manipulated litter additions in 2001, 2002, and 2003 followed by a return to background litter additions from canopy leaf fall in October-December 2003. We have currently collected all samples through 2003 and analyzed samples through 2002 as shown in Figure F1 below, but can anticipate substantial changes in organic and mineral horizon (not shown) ^{14}C-signatures in the years to come. Understanding the migration of C from litter to mineral horizons, and turnover in mineral soil C pools (not shown in Figure F1 below) will also require monitoring over several years to capture the dynamics and deep migration of the ^{14}C inputs.

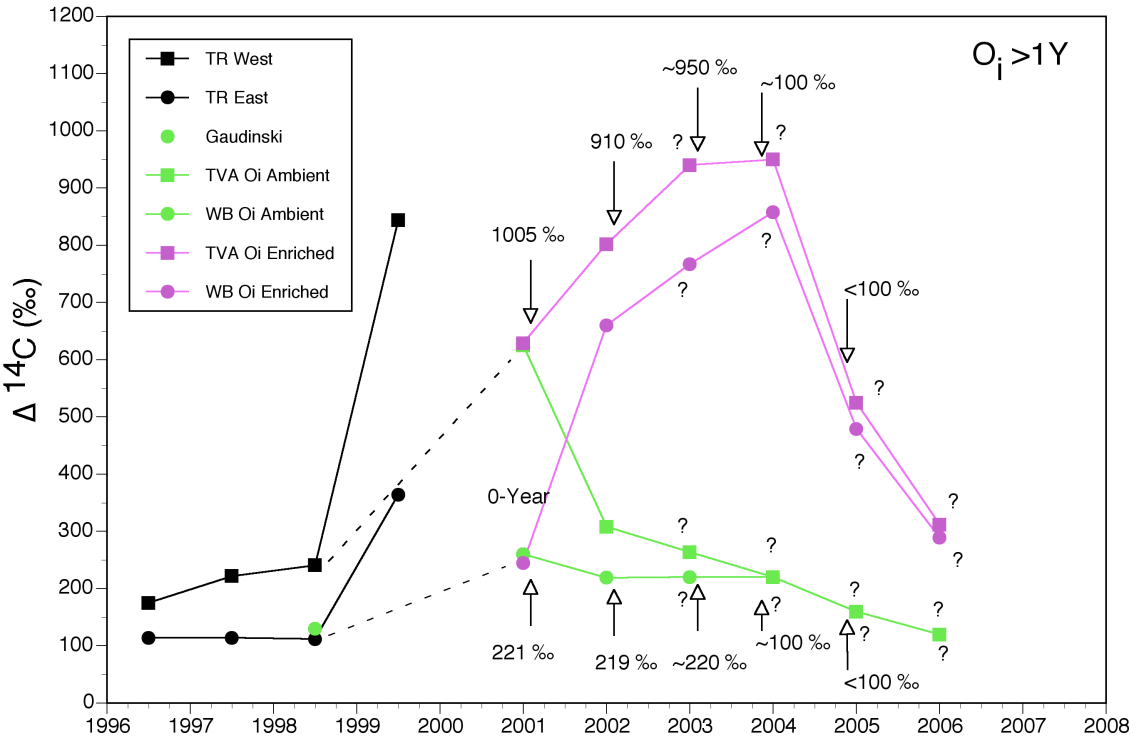

Figure F1. Measured or hypothesized (?) changes in the ^{14}C-signature of the surface organic horizon (Oi layer) from late 2000 through 2006, and the ^{14}C-signature of historical tree ring cellulose (TR). The tree ring ^{14}C data are provided as a reference to historical ^{14}C signatures of the Oi horizon, but in 1999 the Oi layer would not have reached the ^{14}C-signature of wood cellulose because it is the mean of 1-3 years of carbon inputs. The dashed line represents the transition for this time period. Arrows represent manipulated litter additions (2001, 2002, and 2003) and natural litterfall beyond 2003.

The 7x7 meter plots have already been destructively sampled as shown in below (only 2% of the area; Figure F2), but can easily handle replicate (x3) organic and mineral soil sampling for three additional annual samples (October 2004, October 2005, and October 2006) for a total of only 3.1 % of the treated area sampled. These permanent plots will also be maintained for potential sampling at a 2- or 3-year intervals beyond annual sampling to follow the ^{14}C label from the 2001-2003 additions as it cycles down and out of the soil profile.

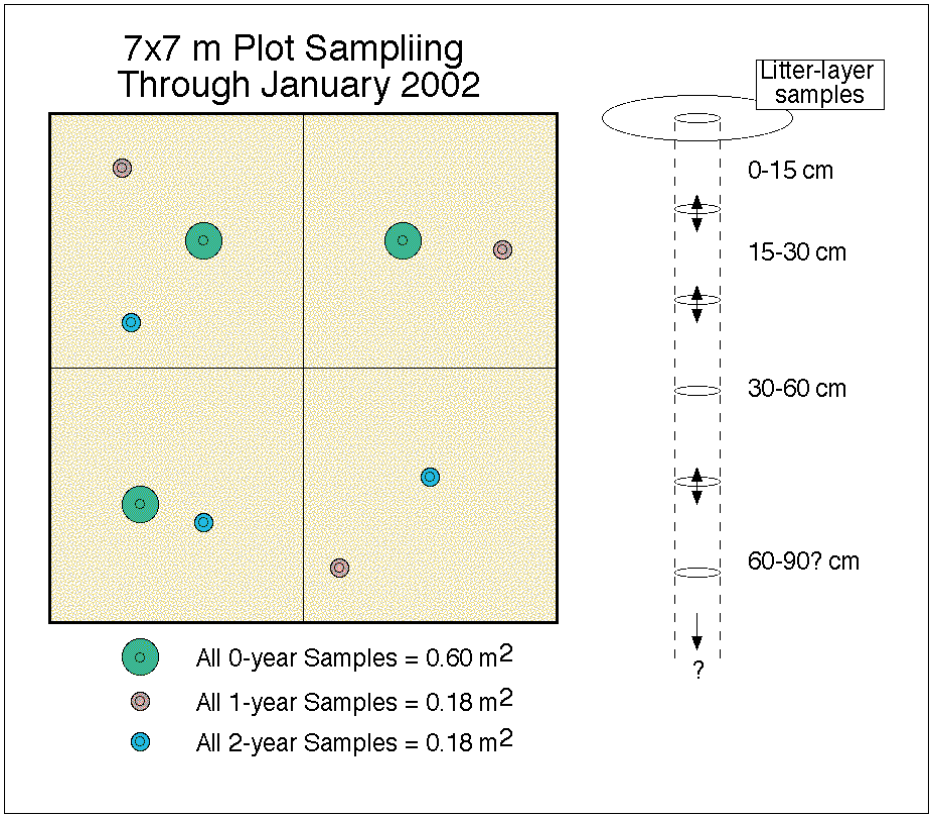

Figure F2. Example diagram of the destructive annual sampling of the organic and mineral soils within each of the eight 7x7 m plots located at the four EBIS treatment sites. Time-zero sampling of the organic horizons (0.2 m^2) was scaled back in the 2nd and 3rd year to multi-year observations without impacting a significant area or volume of the experimental plot.

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