Enriched Background Isotope Study (EBIS)
Litter reciprocal transplant studies to understand sources, transport and fate of carbon in soils and watersheds

An unsolicited, multi-laboratory proposal to study terrestrial carbon cycling processes using a regional terrestrial pulse label of ^{14}C-CO_2 on the Oak Ridge National Environmental Research Park

Coordinating Investigator:
Paul J. Hanson
Environmental Sciences Division
Oak Ridge National Laboratory
P.O. Box 2008, Building 1059
Oak Ridge, TN 37831-6422
Phone: 865/574-5361
Fax: 865/576-9939
E-mail: hansonpj@ornl.gov

Co-Principal Investigators:
Susan Trumbore (UC-Irvine))
Julia Gaudinski (UC-Berkeley)
John Southon (CAMS-LLNL)
Margaret Torn (LBNL)
Julie Jastrow (ANL)
(Additional investigators listed on Page 3)

Requested Funding by Institution and Year

Institution	Year 1	Year 2	Year 3	3-Year Total
ORNL	$ 348,000	$ 408,000	$ 412,000	$1,168,000
LLNL-CAMS	398,899	386,226	397,727	1,182,852
LBNL	194,525	193,043	201,261	588,829
ANL	137,294	138,374	144,395	420,063
UC-Irvine	155,923	117,504	122,756	396,183
Totals	$1,234,641	$1,243,147	$1,278,139	$3,755,927

[Comment; Funded at a lower level ~$1,000,000 per year]

Use of human subjects in proposed project: No
Use of vertebrate animals in proposed project: No

Official Signing for ORNL:
Stephen G. Hildebrand, Division Director
Environmental Sciences Division
Oak Ridge National Laboratory
P.O. Box 2008
Oak Ridge, TN 37831-6037
Phone: 865/574-7374
Fax: 865/574-7287
E-mail: sgh@ornl.gov

_____
Principal Investigator Date

_____
Laboratory Official Date

_____
T.S. Reagan, ESD Finance Officer/Date

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[Budget pages, vitae, and statements of support were removed from this archived version.]

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P.J. Hanson[1], Susan Trumbore[5], Julia Gaudinski[6], John Southon[2], Margaret Torn[3], Julie Jastrow[4], Philip Jardine[1], Wilfred M. Post[1], Patrick Mulholland[1], Charles T. Garten Jr.[1], J. Devereux Joslin[7], Mac Callaham[1], and Caroline A. Masiello[2]

[1]ORNL = Oak Ridge National Laboratory, Oak Ridge, TN 37831
[2]LLNL-CAMS = Lawrence Livermore National Laboratory, Livermore, CA 94550
[3]LBNL = Lawrence Berkeley National Laboratory, Berkeley, CA, 97420
[4]ANL = Argonne National Laboratory, Argonne, IL 60439
[5]UC-Irvine = University of California, Irvine, CA 92697
[6]UC-Berkeley = University of California, Berkeley, CA 94720
[7]Belowground Forestry Research, Oak Ridge, TN 37830

ABSTRACT

A unique, large release of radiocarbon 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 demonstrate that at all sites, the 1999 release was unprecedented in its uptake by vegetation. We propose to take advantage of the whole-ecosystem isotopic label generated by this release to address six 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 above-ground litter decomposition and below-ground 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,
(5) the longevity and turnover time of fine roots, and
(6) the sources of DOC in streams in relation to stream hydrology.

Furthermore, we will use our field findings 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.

The first four issues will be 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 will establish 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 incubation, soil surface chamber and soil CO_2 profiles, and continuous measurements of soil temperature and moisture controls, we will be able to track 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.

The sixth issue, the origin and fate fluvial organic carbon at the watershed scale, will be addressed by measuring ^{14}C labeled dissolved and particulate organic carbon in stream water across a range of watersheds having contrasting ^{14}C exposures and soil types. The long-term hydrologic studies at the Walker Branch Watershed provide a unique reference for this study of the contribution of recently fixed carbon to stream particulate and dissolved organic carbon pools.

While the cost of ^{14}C measurements needed to support this work is large, they are not inordinate considering (1) the cost of doing a whole ecosystem labeling experiment of this magnitude de novo, and (2) the cost of efforts of similar magnitude to study effects of elevated CO_2 or temperature on forest ecosystems. We cannot currently guarantee that there will be no future release of ^{14}C in or around the ORR, however, our experimental design will control the ^{14}C of future leaf litter inputs and monitor the signature of future root litter inputs with a combination of direct root measurements and air and soil CO_2 monitoring. Using the extremely unique opportunity of a whole-ecosystem label provided by the enriched isotopic release to the Oak Ridge atmosphere we will make significant inroads into understanding key issues in the terrestrial C cycle.

PROJECT NARRATIVE

1.0 Introduction

Soil organic matter is the largest terrestrial pool of carbon globally (Post et al. 1990; Schimel 1995), but it also represents a preferred site for mitigative carbon storage. Enhanced carbon sequestration in both above- and below-ground terrestrial ecosystem C pools would help to mitigate greenhouse gas accumulation in the atmosphere (IPCC 1996; IGBP 1998; Johnson 1995; Johnson and Curtis 2000). Soil carbon sequestration is preferential to above ground carbon accumulation because it is better protected from periodic disturbance (i.e., wind throw, fire, pests) and has the potential for much longer retention (McFee and Kelly 1995). Before engineering technologies for the sequestration of C in terrestrial systems can be appropriately tested or put in practice, a fundamental understanding of the processes driving C sequestration and accurate methods for determining rates of change in both above and below ground components are needed.

Soil respiration is a large terrestrial flux of carbon, and variability in soil respiration rates dominates year-to-year variations in net ecosystem production. However, fundamental questions remain about the factors that influence soil respiration. These include determining what fraction of soil respiration comes from autotrophic (below-ground plant metabolism) versus heterotrophic (decomposition of litter residues and humus) sources, and how climate factors that change soil temperature and moisture conditions influence that partitioning. Another key question is how much of the respired C is derived from decomposition of leaf versus root detritus versus the larger pools of humus material. Since CO_2 enrichment studies show increases in below-ground carbon allocation (Zak et al. 2000), understanding the residence time and fate of that material is key to determining how and over what time frame ecosystems can store carbon.

This proposal seeks funding to enhance our understanding of processes responsible for short- and long term changes in soil C cycling processes. Documented changes in soil carbon stocks over decadal time steps are almost exclusively based on empirical field observations (Trettin et al. 1999; Johnson and Todd 1998; Richter et al. 1994; Post and Kwon 2000) without regard to the processes that drove the direction of change. However, the processes responsible for soil carbon sequestration are most often characterized and manipulated at short time steps associated with complex interactions between biomass production and soil respiration (Davidson et al. 1998; Edwards 1975; Edwards and Sollins 1973; Edwards et al. 1977; Hanson et al. 1993; Paul et al. 1999). Linking records of soil C change over time with a fundamental understanding of the processes responsible for observed patterns of soil carbon sequestration will allow estimates of potential rates of future soil carbon accumulation. Independent of the potential for future soil carbon sequestration, the development of a fundamental understanding of the underlying processes will yield a decision making framework that can be used to judge the efficacy of future proposed mitigative actions.

1.1 The ^{14}C opportunity

Fundamental knowledge of soil organic matter (SOM) dynamics is critical to understanding carbon sequestration, carbon cycling, and ecosystem nutrient cycling processes and ultimately growth. Despite years of research by the soil science community, we still lack critical information regarding how soil organic matter is formed and the mechanisms whereby it is distributed within the soil profile. Advances in understanding SOM dynamics will require that we reach beyond traditional approaches and explore new measurement methods and

experimental opportunities. One such opportunity is represented by the summer 1999 ^{14}C pulse-label of a significant fraction of the forest ecosystems on the Oak Ridge Reservation (Gaudinski et al. 2000a).

As part of a DOE/NIGEC study of soil carbon cycling along a latitudinal gradient in eastern forests, Gaudinski et al. (2000a) measured ^{14}C in soil respiration, roots, leaves and soil organic matter at the Walker Branch watershed on the Oak Ridge National Environmental Research Park in 1998 and 1999. Elevated levels of ^{14}C in air, soil air, and soil respiration were observed in July and August 1999 (Figure 1). The rapid rise in the ^{14}C signature of soil derived CO_2 could only be explained by a local release of ^{14}C in the form of $^{14}CO_2$ gas which was assimilated by photosynthesis and translocated to roots. Subsequent root respiration or decomposition of root exudates or fine roots would account for a near-instantaneous increase in the ^{14}C signature of soil CO_2. A number of local waste incinerator companies located on or around the ORR are candidate sources of the $^{14}CO_2$, but we have not yet identified the exact source or timing of the release(s). Subsequent measurement of ^{14}C in the cellulose from tree rings formed in 1999 showed that this growing season release was unique in its magnitude (Figure 2). Additional surveys of 1999 tree wood and 2000 leaves showed that the release encompassed a large area (at least the area of the ORR). The highest amounts of the ^{14}C label were found in vegetation in the western portion of the ORR (Figure 3).

This inadvertent enrichment of ^{14}C in vegetation over 10 to 100 km^2 provides the research community with a unique tool with which to observe and manipulate carbon cycling processes within eastern deciduous hardwood forests. It is an especially good research opportunity because the timing of the pulse-label was such that we can separate root and leaf inputs to the soil. Because the $^{14}CO_2$ release occurred following canopy leaf production in 1999, foliar litterfall from 1999 was not strongly labeled. However, the 2000 canopy is well labeled since stored carbohydrate levels from 1999 contributed to the production of canopy tissues (leaves, twigs, flowers, etc.). Therefore, the year 2000 litterfall represents a unique and large source of ^{14}C enriched foliar tissues from a broad range of species for application to a number of edaphic, physiological and ecosystem-scale research questions. This proposal describes experimental plans to enhance the ^{14}C enrichment even further. We propose in situ studies of soil carbon cycling and transport processes that will be facilitated by the reciprocal transport of litter between two areas of the ORR: those that have ^{14}C enriched leaf litter, roots, and soil and those with near background levels of ^{14}C. The manipulation we propose will provide a unique opportunity to separate root and leaf litter contributions to the production of soil organic matter and to soil respiration. Testing our experimental design with commonly used model of C dynamics (the Rothamsted model) we demonstrate that the response of C pools is likely to be large and will differ significantly between treatments (Section 3.6),. The model calculations indicate that the 1999 local enrichment of the ORR is equivalent in magnitude to the bomb-^{14}C enrichment of the global atmosphere in the early 1960's, but is better defined due to its short duration (See Figure 6 in Section 3.6). Ultimately, our results will enable us to test some of the underlying assumptions in these models, thereby improving our predictions of below-ground response to global change.

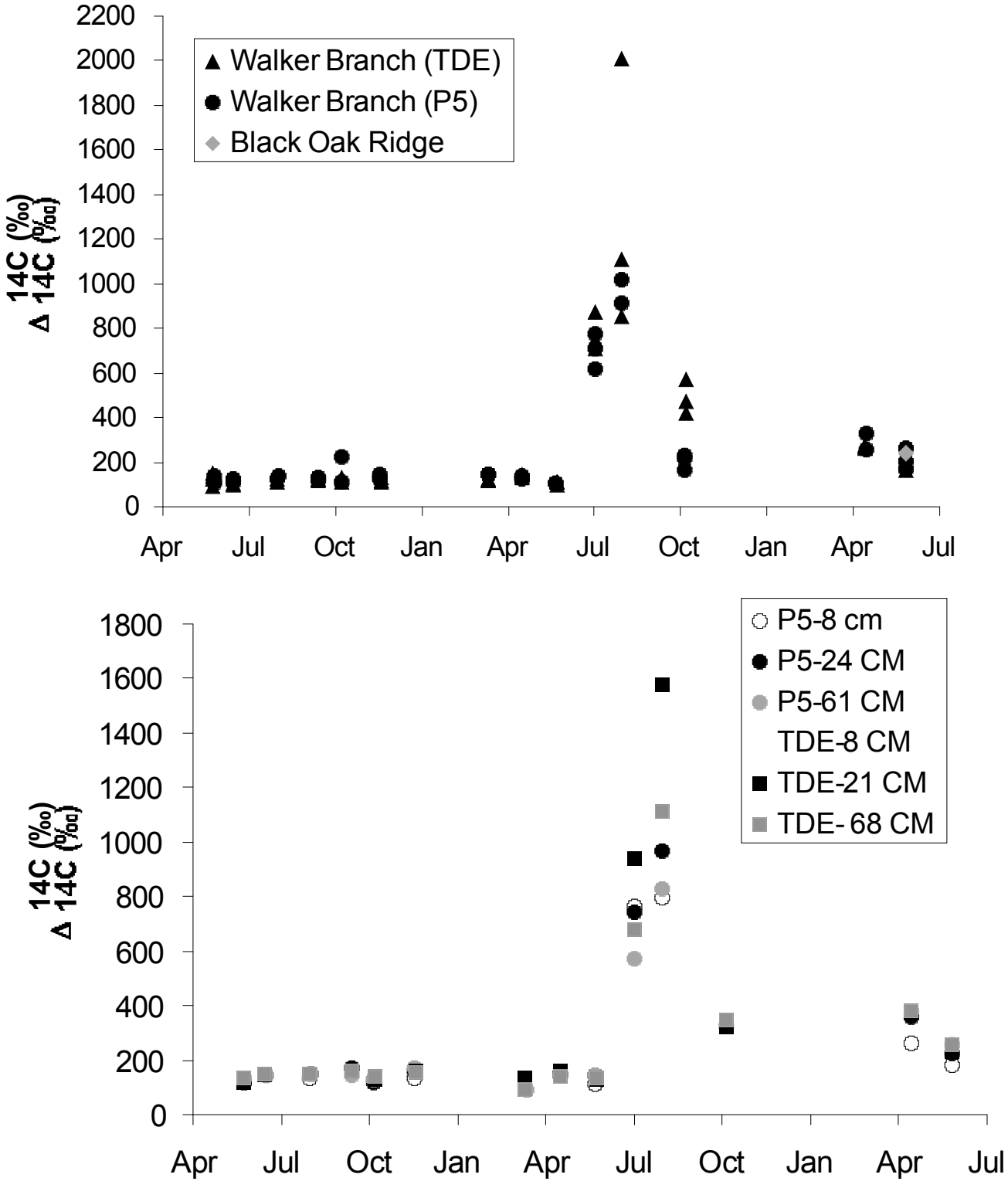

Figure 1. Radiocarbon in CO_2 of total soil respiration (top) and soil gas (bottom) for two sites on the Walker Branch Watershed.

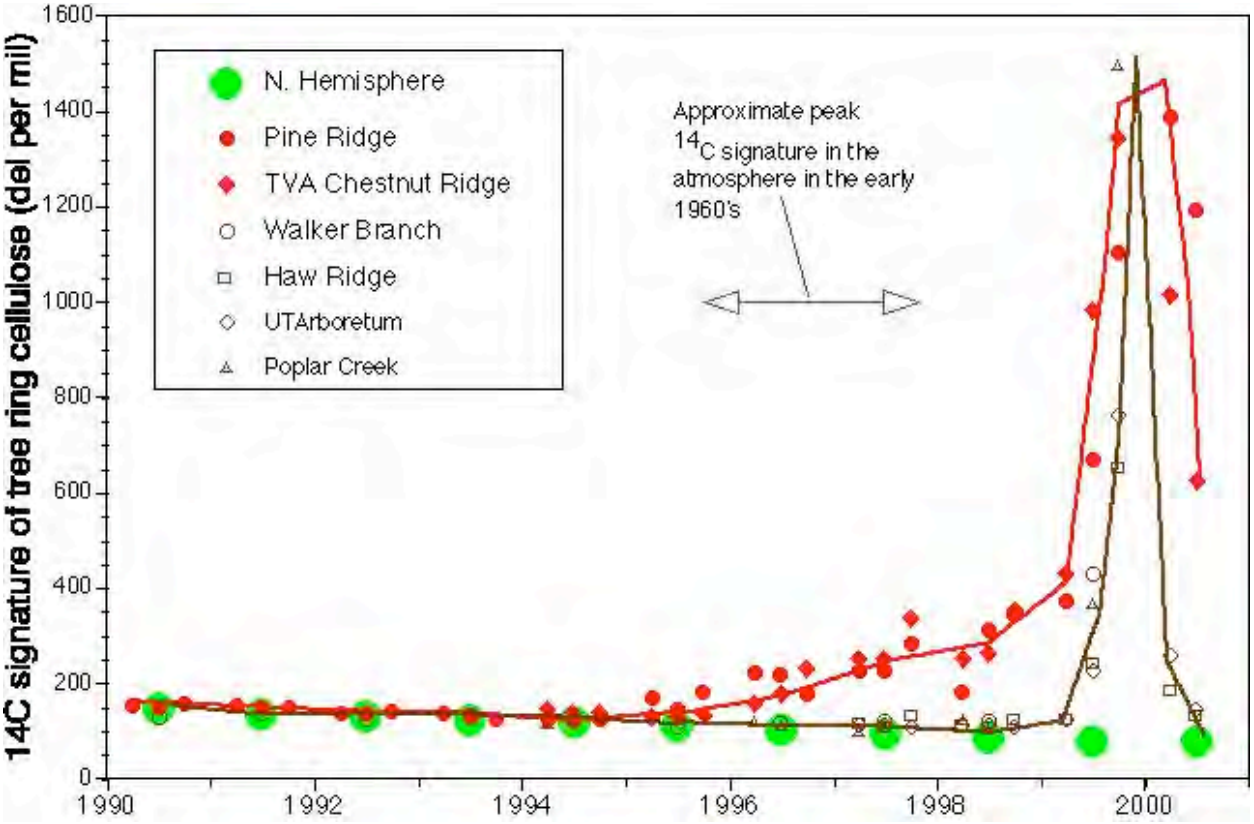

Figure 2. Pattern of tree ring and northern hemisphere ^{14}C signatures in *Quercus* tree wood cellulose over time. Trees on the west edge of the ORR in the vicinity of two local waste incinerators (closed symbols) show gradual increases in ^{14}C starting around 1996 and the entire reservation becomes enriched in 1999 event.

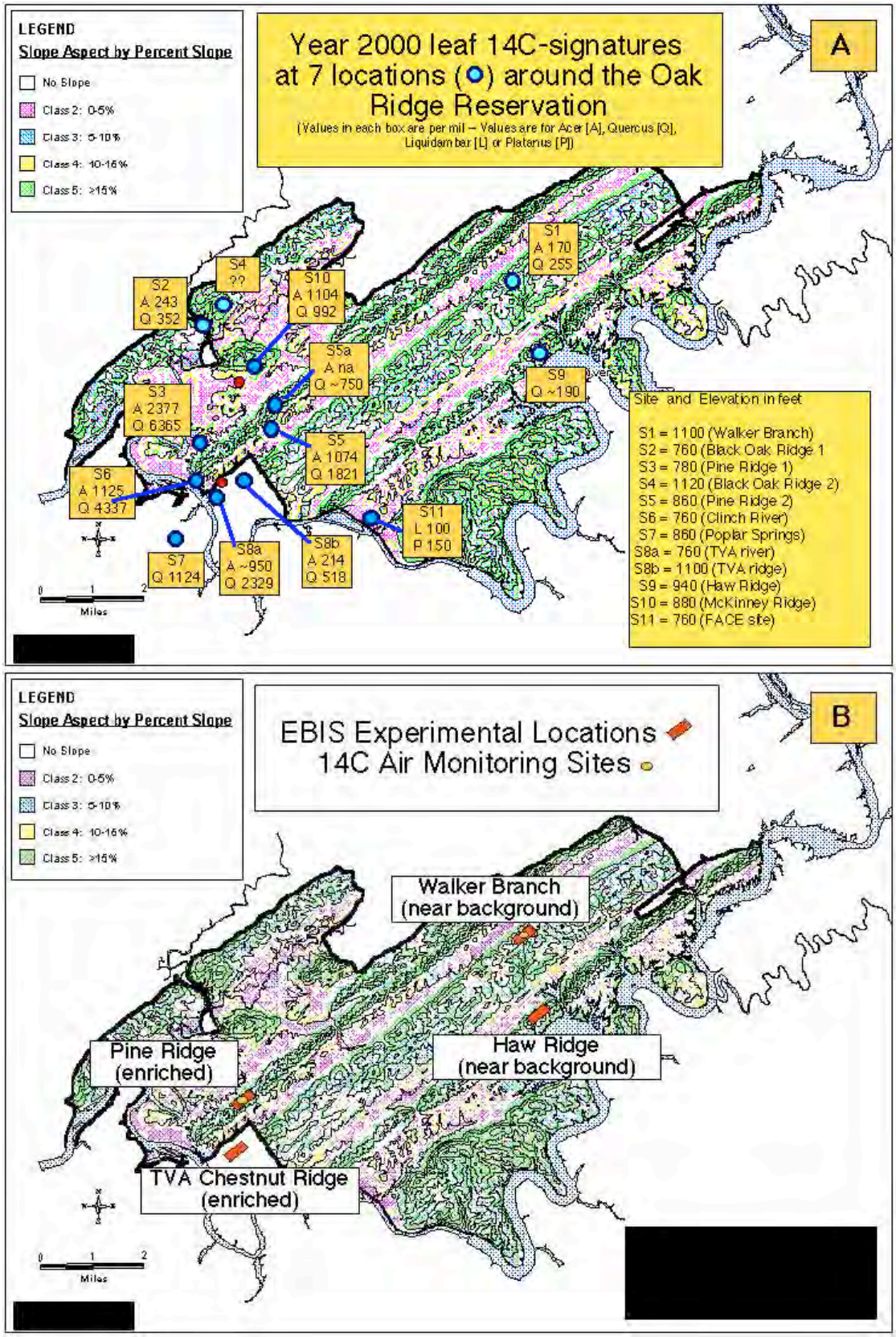

Figure 3. Map of the Oak Ridge Reservation showing the distribution of year 2000 growing season leaf ^{14}C signatures (A), and the location of the enriched (Pine Ridge, TVA Chestnut Ridge) and near background (Walker Branch, Haw Ridge) research sites proposed below (B).

1.2 Research topics

Origin of soil organic matter (SOM) -- Soil organic carbon is derived from plant tissues, either from above ground leaf litter or from root products. Its vertical distribution is affected by four processes: (1) transport within root phloem to different soil depths with subsequent transformation and deposition of fine root litter, (2) transport from one depth to another (either above or below) by animals, and (3) mass flow with percolating water as dissolved organic or inorganic carbon (DOC or DIC), and (4) stabilization of soil organic matter by incorporation into mineral inter-layers or aggregates. This experiment will address three main aspects of soil organic matter storage: rates of vertical transport; rates of movement of soil C into and out of stabilized forms (rates of stabilization); and the differential fate (location or timing) of leaf versus root inputs to the soil.

Components of soil respiration -- Soil respiration is a combination of root respiration, above ground litter decomposition, below ground litter decomposition (fine root turnover and associated rhizodeposition), and decomposition of soil humus. The amount each source contributes to soil respiration varies among the soil layers. The enhanced ^{14}C tracer along with manipulation of the aboveground litter inputs will allow partitioning of soil respiration among these sources by layer. Seasonal variation in soil respiration is influenced by the interaction between phenology, weather, and microclimate. Partitioning the components of soil respiration during the year will allow evaluation of the influence of different controls on the magnitude of soil respiration.

Residence and turnover time of roots -- Enhanced ^{14}C tracer will allow determination of the longevity of fine roots, which influence the amount and turnover of root derived soil carbon. Previous results using the bomb ^{14}C label have shown that a significant portion of fine (<1mm in diameter) root biomass lives for several years and up to decades (Gaudinski et al., in review). These results differ from many previous estimates of fine root longevity using mass balance approaches and root-viewing cameras which generally report lifetimes on the order of a few months to one or two years. The discrepancies are likely the result of the different techniques used, each of which has biases that tend to emphasize one end of the lifetime spectrum within the population.Fine root lifetimes tend to increase with both size class, and depth and decrease with branching order (Gaudinski et al. 2000b and Gaudinski et al. in review). Thus the ^{14}C technique can be applied so as to measure the entire spectrum by measuring different size classes and branching orders.

We know from ^{14}C testing of roots grown only during the 1999 growing season on Walker Branch that there was significant label incorporation (two samples were 600 and 900 ‰). Thus we can determine the spectrum of ages and average lifespan of fine roots within populations by monitoring the ^{14}C concentration of the whole fine root population (in-situ soil cores) and the ^{14}C of newly growing fine roots (collected from in-growth root cores or root screens). Radiocarbon analyses of fine roots will not only provide new information on the dynamics of fine root populations, but is an essential component of the quantification of inputs to SOM and soil respiration.

Residence and turnover time of unprotected and protected SOM pools -- The ^{14}C tracer addition will allow us to investigate critical components of carbon dynamics and stabilization in soils.

The pathways by which leaf litter- and root-derived inputs are incorporated into mineral soil organic matter are quite different. Yet little is known about the relative contributions of leaf and root sources to protected and unprotected forms of forest soil organic matter. Soil organic matter is stabilized through physicochemical associations with minerals, incorporation into aggregates, and microbial processing into biochemically recalcitrant compounds. We propose to use physical, density, and chemical fractionation techniques to follow the movement and fate of ^{14}C-labeled litter and root sources into protected and unprotected soil carbon pools. By studying sites with different parent materials, we will also be able to compare the effectiveness of two contrasting types of clay minerals in stabilizing recent plant inputs.

Models of organic matter movement and turnover will be refined and constrained by empirical results, including the amount and ^{14}C content of isolated organic matter pools and fluxes over time (Trumbore and Zheng 1996). See also Section 3.6.

Role of soil macrofauna in carbon transport and sequestration -- Soil organisms influence the rate and magnitude of allocation to different SOM components and different soil layers through the processes of comminution (i.e., fragmentation), vertical/lateral transport, and bioturbation (soil turnover and carbon incorporation). Enhanced ^{14}C tracer combined with manipulations to distinguish aboveground from belowground inputs with manipulations of soil organisms (electroshocking of earthworms, exclosures, enhancements) will allow quantification of the influence of organisms on amount and distribution of SOM components.

Watershed-scale transport of organic carbon -- The enhanced ^{14}C tracer will allow determination of whether recently-fixed carbon in terrestrial portions of the watershed is a significant source of dissolved organic carbon (DOC) and particulate organic carbon (POC) exported from the watershed in stream water. Observations of ^{14}C in soil SOM together with observations of ^{14}C of streamwater DOC and POC under different hydrologic conditions and during different seasons will be used to identify the contribution of recently-fixed carbon versus that of older and presumably more recalcitrant organic carbon to stream DOC and POC export.

2.0 Preparations for EBIS

In order for the work proposed in this document to proceed significant work has already been completed towards the archiving and collection of year 2000 ^{14}C –enriched litterfall to ensure that the unique research opportunities afforded by this serendipitous event are not lost. Plot locations and the current isotopic signatures of canopy leaves across the ORR are shown in Figure 3. Approximately 150 15'x30' plastic tarps were laid out on the forest floor along Pine Ridge for the collection of all forest leaf litter. A similar number and distribution of tarps was laid out at the east end of Walker Branch Watershed. Tarps were deployed starting in September of 2000 and will be left in place through mid-December. To avoid litter degradation, litter will be collected weekly during the fall from tarps laid out in late summer 2000. The leaf litter will be transferred to ORNL greenhouses for air drying and subsequent storage in large (1 x 1.5 m) vacuum bags. The litter vacuum bags will be stored in rodent free trailers until needed. These litter collections will be of sufficient magnitude to support the study proposed in this document, and future laboratory or chamber-based studies.

Based on our knowledge of the ^{14}C signatures of canopy leaves collected during June and July of 2000, we expect a strong spatial gradient in ^{14}C signatures for the Pine Ridge enriched litter collections. Therefore, the Pine Ridge collections are being harvested along an east-west

gradient with appropriate labeling and identification to track this phenomenon. Following the completion of litter collections in December, samples of ^{14}C enriched and background litter will be obtained for analysis of their bulk ^{14}C isotopic signatures before being used in the litter transplant operations for this proposal.

3.0 Research design and task descriptions

3.1 Site description and design, environmental data, and annual sampling of C compoents

3.1A Research sites

The proposed research plots are all located on the U.S. Department of Energy's National Environmental Research Park near Oak Ridge, Tennessee. Mean annual precipitation is 1358 mm and mean temperature is 14.1°C (Johnson and Van Hook 1989). Forest vegetation is chiefly upland oak (*Quercus* spp.; *Acer* spp.; *Carya* spp.) with scattered pine (*Pinus* echinata and P. virginiana) and mesophytic hardwoods (*Liriodendron tulipifera, Fagus grandifolia*). All research plots will be located in the upland oak forest type on ridge and upper slope positions. The ages of the overstory trees covers a broad range from about 40 to over 150 years, and the maximum canopy height was approximately 26 meters above the surface. Maximum leaf area index is typically about 6 m^2 m^{-2}.

We have located research sites on or near the west end of the ORR that received sufficiently high amounts of ^{14}C label in the 1999 pulse to allow the signature to be followed for long periods of time (5 years or more). Near background sites with minimum label are also available on the eastern end of the ORR. Two enriched sites will be established on Pine and Chestnut ridges on the west end of the ORR and two near background sites will be established on Chestnut and Haw ridges further east. Pine and Haw ridges have soils derived from shale and the Chestnut ridge soils are derived from dolomitic parent materials. We anticipate that soil depth, structure, and chemistry may play an important role in the transport and cycling of soil C and replicate study sites are therefore located on each formation. Aerial photographs of the four research sites and other supporting information can be found at the following web address: http://www.esd.ornl.gov/programs/WBW/C14.htm.

The Ultisols to be used in this proposal are deep, highly weathered soils derived from dolomitic parent material. In general the soils have a 10 cm thick A horizon which resides above a well-developed EB horizon and multiple Bt subsoil horizons. The soils have acidic, well structured B horizons whose clay fraction is dominated by kaolinite and lesser quantities of hydroxy-Al interlayered vermiculite. They have typical cation exchange capacities between 4 and 6 cmol / kg. Clay material is often coated with 2-4% Fe-oxides, primarily as hematite and maghemite. The abundance of clay in the subsoil results in impeded vertical drainage near 1.5 m and the creation of transient perched water tables during sizable storm events. Storm water often flows laterally during these conditions and follows topography. Significant vertical transport below 1.5 m occurs in cracks within the blocky structure of the media. Since the matrix porosity constitutes nearly 95% of the total water content (40-50% v/v), large hydraulic and physical gradients develop during solute transport resulting in nonequilibrium conditions during storm events.

The Inceptisols to be used in this proposal are shallow, less weathered soils derived from limy shale formations. The soils have been weathered from interbedded shale-limestone sequences, where the limestone has been weathered to massive clay lenses devoid of carbonate and the more resistant shale has been weathered to an extensively fractured saprolite. The soils

are acidic with thin A-horizons (0-3 cm) and weak B-horizons ranging from 0.5 to 3 m in depth. Typical cation exchange capacities are 15-20 cmol / kg, with the < 2 um clay fraction dominated by illite and lesser quantities of 2:1 interstratified material and vermiculite. The solid phase is coated with 2-4 % Fe-oxides and up to 1 % Mn-oxides. Within the weathered shales, fractures are highly interconnected with densities in the range of 200 fractures/m. Fracture orientation and connectivity give rise to extensive preferential flow within the media. Fractures surround low permeability, high porosity matrix blocks that have water contents ranging from 30 to 50%. Such a condition results in large hydraulic and physical gradients during storm drainage thus creating nonequilibrium flow and transport processes during solute migration. Transient perched water tables develop within the subsoil during storm events resulting in lateral flow that preferentially follows bedding plane dip that often times is counter to the direction of topography. A detailed description hydrologic, physical, geochemical, and mineralogical properties of these soils can be found in previous studies (Wilson and Luxmoore, 1988; Arnseth and Turner, 1988; Jardine et al., 1988, 1989a,b, 1990a,b, 1993a,b, 1998; Wilson et al., 1989, 1990, 1991a,b, 1993, 1998; Reedy et al., 1996)

3.1B Experimental design

At each research site eight 7x7 m permanent plots will be established for the manipulation of forest litter with known levels of ^{14}C and monitoring of the fate of ^{14}C within the soil profile over a 5-year study period. All of the plots at each research site will have their normal litterfall excluded, and half of the plots will receive litter enriched in ^{14}C and the other half background litter. The permanent plots will be installed for studies of the contribution of labeled leaf versus root litter to (a) soil respiration (b) production and downward transport of DOC in soils and to streams, and (c) transformations of detrital organic matter into more stable forms. The following combination of replicated research plots will be generated:

(1) plots with enriched leaf litter, enriched root litter, and enriched soil C (west ORR)
(2) plots with unlabeled leaf litter, enriched root litter, and enriched soil C (west ORR),
(3) plots with enriched leaf litter, and low enrichment roots and soil C (east ORR), and
(4) plots with unlabeled leaf litter and low enrichment roots and soil C (east ORR)

Although not manipulated, reference plots will be established away from the ORR on each soil type to provide relevant information on the levels of litter, root, and soil C and ^{14}C signatures to be expected in native soils from natural cycling with normal background levels of atmospheric ^{14}C.

At each of the four research sites on the ORR, eight square 7x7 m plots will be delineated with metal fence posts (hammered ~12 inches deep) and plastic fencing (~61 cm tall). From late September through early December of each growing season, the forest floor within each plot will be covered with landscape cloth to allow the native litter to be removed and replaced with ^{14}C enriched or background litter of known composition and ^{14}C signatures (i.e., the year 2000 litter collections). Treatments will be randomly applied to the eight plots at each site. A single soil pit (maximum depth 1 m) will be dug on one side of six of the eight plots for the installation of soil temperature and soil water probes. Miniature soil gas sampling devices will also be installed into the face of each soil pit. The soil pits will be backfilled immediately following the installation of instruments.

Within each plot two shallow (~20 cm) and two deep (~80 cm) soil tension samplers will be installed from the surface into augered holes for sampling dissolved organic C levels and ^{14}C signatures and the calculation of vertical ^{14}C transport rates within the soil horizon. Annual 10 cm diameter cores of soil from the surface to a depth of not greater than 1 m will be collected from each plot for the evaluation of root mass, soil C content, and root and soil ^{14}C signatures. Permanent rings to be used for periodic measurements of soil respiration and surface soil air samples will be centered in each of the eight plots.

A single weather station and data logger will be located at each site for hourly measurements of air temperature, relative humidity, precipitation reaching the litter layer (i.e., throughfall), and litter layer water content. Biweekly integrated samples of air at one east (Walker Branch) and one west (Pine Ridge) site will be collected as a direct measurement of future, growing season inputs of atmospheric ^{14}C into each site. Where possible, solar panels and associated batteries will be installed in nearby clearings to provide power to the instruments at these remote sites.

Ten litter collections baskets (i.e., plastic laundry baskets of known dimensions) will be installed in each research area to determine the quantity of litter fall (dry mass) to be added the plots.

3.1C Environmental monitoring at the long-term reference plots

Hourly air (1.5 m), organic horizon (below the Oi), and surface soil (-0.1 m) temperatures will be logged hourly at all long-term reference plots throughout 2001 and 2002 using sealed thermistor probes (Stowaway Tidbit, ONSET Corporation, Bourne, MA). In addition, three buried time domain reflectometer waveguides (TDR; Soil Moisture Equipment Corp., Santa Barbara, California) will be installed at -15 cm on each site to be monitored monthly from April through November. Soil water contents are at or near saturation during the December through March period.

A number of environmental variables will be logged hourly in a central location near the eddy covariance towers (Section 4.6A). We will measure photosynthetically active radiation at 1.5 meters (PAR in μmol m-2 s-1; Quantum sensor, LiCor Inc.), air temperature and relative humidity (combined sensor; HMP35C Vaisala, Inc.), and soil temperature (thermistors). Soil temperature will be measured throughout a single depth profile at -0.15, -0.3, -0.5, -0.7 and -1.0 m in the side wall of a back filled soil pit. Instrumentation for monitoring soil water to a depth of 1 m will be added to the soil pits so that we can estimate stand water leaving the rooted soil profile. The majority of tree roots (>90%) are to be found in the upper 60 cm of soil in most Walker Branch soils (Harris et al. 1973; Joslin and Wolfe, 1998). Soil water content will be measured using water content reflectometers (Model CS615, Campbell Scientific, Inc., Logan, Utah). Time domain reflectometer waveguides (TDR; Soil Moisture Equipment Corp., Santa Barbara, California) will be co-located with the soil water reflectometers as an in situ check against factory supplied calibrations. All climate data will be measured every minute and the hourly means or sums will be stored on a Campbell Scientific CR10 data logger. A cellular phone will installed at each site to allow for weekly remote downloading of data from the CR10. Phone access to each data logger allows for efficient maintenance of the automated equipment and better quality assurance of the data sets.

Continuous direct measurements of forest litter water content based on the electrical resistance characteristics of wet vs. dry litter (Hanson et al. 1996; Wilson et al. 2000) will also be conducted for the Oi, and Oe/Oa litter layers. These data are essential for the estimation of

organic layer contributions to total soil respiration. Litter water content observations will be multiplied by the litter specific respiration rates adjusted for water content (Section 3.2) and the residual standing mass of litter in each layer (an estimated value).

Reference data for above canopy conditions including: total irradiance (W m-2; pyranometer sensor, LiCor Inc., Lincoln, NE)), PAR, air temperature, relative humidity, and rainfall (0.25 mm resolution tipping bucket rain gauge) will be obtained from equipment operated as a part of the Throughfall Displacement Experiment (Hanson et al. 1995; 1998).

The ^{14}C content of atmospheric CO_2 will be continuously monitored throughout the experiment using a time integrated sampling procedure. This will enable us to identify whether or not further $^{14}CO_2$ releases of significance have occurred and to take advantage of them to look at short-term responses. Sampling stations have been set up at Walker Branch and on Pine Ridge and monitoring was started in September 2000.

3.1D Annual sampling of carbon components critical to soil carbon cycling **(Task 1 - Hanson)**

During the late September and early October of 2000 initial 'time zero' samples of the litter layer, roots and soil mineral matter will be collected from all 32 plots for the evaluation of the standing stock of C of each component and its ^{14}C signature. These data will be the basis for calculation of C flux described in each task below.

Vegetation compositon -- A single vegetation inventory will be conducted for each site to record the species composition of each site. Vegetation inventories will be conducted using nested circular plots as described by Grigal and Goldstein (1971). At each site three locations will be evaluated. Each location will consist of three concentric circles marked by a single central stake making three plots of 810, 405, and 40.5 m2 (i.e., 1/5, 1/10, and 1/100 acre plots). The dbh of trees > 24, 9 or 1.5 cm will be measured in the 810, 405, and 40.5 m2 circles, respectively. All of the selected trees will be identified as to species.

Above-ground litter production -- Because soil carbon accumulation in soils is the net effect of carbon accumulation from leaf, branch, flower , seed and root inputs minus the loss of carbon through heterotrophic decomposition processes, data for above-ground litter inputs are needed. A seven-year record of litter inputs to the Throughfall Displacement Experiment (Todd and Hanson 1999; Joslin et al. 2000) based on 147 litter collection baskets demonstrates that inter-annual variation in above-ground litter production is low (maximum range was 480 to 520 g m-2 from 1994 through 1998) indicating that stable inter-annual production may be assumed in our calculations. Fallen leaves and other materials (i.e., twigs, flowers, seeds, etc.) will be collected from eight 0.2 m2 litter collection baskets placed at random locations within the 810 m2 circle of each forested plot. The baskets will be installed prior to canopy leaf senescence in the autumn of 2000 and fresh litter will be collected biweekly during fall senescence in 2000, 2001, and 2002 for deciduous plots. Litter samples will be dried (24-48 hours at 70°C) and subsequently separated by leaf, twig and seed components for mass determinations.

Below ground litter inputs -- Measurements of root growth activity and root turnover indicative of belowground litter inputs have been studied extensively in recent years as a part of the Walker Branch Throughfall Displacement Experiment (Joslin and Wolfe 1998; Joslin et al. 2000a, 2000b). TDE data suggest that quantitative estimates of root litter inputs would be difficult and expensive to obtain for all of the experimental plots. However, estimates can be obtained from

calculations based upon the root turnover time and the size of the root biomass pool. Root turnover rate information will be available for each site based upon dilution of the radiocarbon signal in the fine root pool over time and ^{14}C inputs from new root growth. More precise information on turnover will be available from detailed work on fine root turnover at the Walker Branch site (see section 3.2). Estimates of the size of fine root biomass pool will be available from the annual root coring conducted in each plot. Belowground litter input estimates will be compared to a long-term data set of annual fine root production estimates at the TDE site on Walker Branch Watershed.

An independent estimate of belowground litter inputs will be based upon the allometric relationship between annual tree ring growth and annual root production. Root growth in a given year does appear to correlate with levels of above ground production. Hence, we propose to monitor selected aboveground tree growth as a surrogate for belowground root litter production during 2001 and 2002 and use historical tree ring data to develop root litter input estimates for previous study years (i.e., the period from ~1960 through current time).

Circumferential tree growth will be monitored on the six most dominant trees of representative north aspect, south aspect, ridge, and riparian plots (3 plots each). Dendrometer bands following the design of McLaughlin and Downing (1996) will be used to provide the necessary resolution for single year growth observations. All dendrometer bands will be installed during the dormant season, ahead of the initial growth measurements, to eliminate potential first year bias in the dendrometer band measurements (Keeland and Sharitz 1993). Measurements will be initiated in late March and sustained at biweekly intervals throughout the period of annual growth.

Radiocarbon inputs by roots -- Ingrowth root cores or root screens (8) will be installed outside of the manipulation plots at each site at the beginning of the growing season. Ingrowth cores will be installed to a depth of 30 cm using 10-cm diameter soil cores prepared using soil collected from pits in the vicinity of the plots. Cores will be repacked to original soil bulk densities, enclosed in mesh bags, and inserted into identically sized core holes within the plots, according to the method of Joslin and Henderson (1982). Root screens will be installed by inserting fine mesh (1 mm) screens into narrow slots in the soil/root matrix created by driving a sharpened metal plate to a depth of 30 cm. After allowing a growing season for regrowth of roots into the cores or screens, roots will be extracted, composited, and analyzed to determine the input ^{14}C signature to all four sites for new roots grown that particular year.

Annual litter layer(s), mineral horizons, and roots -- To sample litter, mineral soil, roots, and soil organic matter, three cores to a depth of 90 cm will be taken from each plot (96 total cores), using a 10-cm diameter coring tool. Cores will be separated into the following 2 organic and 4 mineral increments: (1) Oi, (2) Oe + Oa, (3) A horizon (based on average depth for each site), (4) Mineral soil from bottom of A horizon (average depth) to 30 cm, (5) Mineral soil 30-60 cm, (6) Mineral soil 60-90 cm. Samples will be stored at refrigerator temperatures until further processing can be completed.

After sieving to collect large roots and rocks (8-mm sieve) for later weighing, each split core depth-increment will be subdivided into two samples and the volume of each "half" determined. After compositing each set of three halves per plot, samples will be stored at refrigerator temperatures. One set of samples will be further processed to obtain samples for fine root analysis and the other processed for collection of samples of various soil organic matter

fractions (See section 3.4). Fine roots will be separated from soil both to determine root biomass and to collect representative root samples for ^{14}C analyses. Fine roots will be subdivided into two size classes and into live and dead pools.

Sample processing C/N analysis, and laboratory incubations -- O horizon litter samples, roots and mineral soils from which roots have been extracted will be oven dried to a constant mass at 100°C. Soils will be separated from the coarse fraction (sand/gravel > 2mm) and live root components with a 2 mm sieve prior to chemical analyses. Mass of roots retained in the sieve will be recorded as an estimate of root density by horizon. Organic horizon samples will be ground in a Tecator Cyclotec sample mill at 20 mesh (Tecator, Herndon, VA). A portion all homogenized wood and organic layer soils will be heated at 500 °C to obtain ash content. Total dry mass per unit ground area (m^2) for each organic horizon will be corrected for ash content so that the inclusion of variable mineral matter contents in the samples will not bias the results. All processed soils and organic horizons samples will be stored in archival containers.

Dry samples of aboveground litter inputs, organic horizons, and soil will be analyzed for total carbon (and nitrogen) using a Perkin Elmer 2400 Series II CNHS Analyzer.

3.2 Components of soil respiration

Soil respiration rates are indicative of the net effect of a host of autotrophic and heterotrophic process responsible for soil carbon cycling (Hanson et al. 2000). We will measure the radiocarbon in total soil respiration, CO_2 in soil air pore space as function of depth, and CO_2 released during short-term soil incubations to separate these components using an isotope mass-balance approach (Gaudinski et al., 2000b). In addition, a significant portion of soil respiration comes from decomposition of fine roots (20-32% for a mixed deciduous forest in central Massachusetts [Gaudinski et al., 2000b]). As such, accurate knowledge of fine root lifetimes and their isotopic composition is critical in order to be able to partition soil respiration using isotopic mass balance. We will utilize the enriched labeling event to evaluate the longevity of fine root pools which will also allow for more accurate quantification of their total C and ^{14}C input to soil respiration and soil organic matter.

3.2A Evaluation of C sources for total soil respiration **(Task 2a - Trumbore)**

At Walker Branch Watershed we have two years of data including data pre-release for soil organic matter stocks, soil respiration and soil CO_2. The ^{14}C signature in soil respiration and soil gas declined from August, 1999 to October, 1999 However we expect we may see secondary pulses as this year's leaf litter, and root litter grown last year (both of which have significant label incorporation) decompose. We will continue monitoring at these two existing sites on the Walker Branch Watershed, in addition to measuring the manipulated litter sites.

H1: *The overall decline in ^{14}C in soil respiration observed since August 1999 will be temporarily reversed in spring 2001 as leaf litter fractions with higher amounts of label begin to decompose.*

In conjunction with the factorial manipulations we will measure the ^{14}C of total soil respiration and soil gas as a function of depth at two of the four sites in order to separate the components of total soil respiration (T) coming from; 1) autotrophic respiration by plant roots (A), 2) decomposition of leaf litter (L) 3) decomposition of roots (R) and 4) decomposition of humified soil material (H). We also plan to perform soil organic matter incubations as an additional way to

separate autotrophic and heterotrophic contributions to soil respiration. The manipulations will allow us for the first time to separate litter and root decomposition contributions to soil respiration. The depth-dependence of ^{14}C sources will allow us to determine whether downward-leaching of DOC with the elevated ^{14}C signature is a significant source of soil respiration at depth, or whether root decomposition dominates. An experimental design that also provides for continuous monitoring of litter and soil moisture and temperature will allow us to test the following hypotheses:

H2: *During the growing season, variation in the magnitude of soil respiration will be caused more by fluctuations in the overall magnitude of heterotrophic respiration relative to autotrophic respiration and moisture conditions in the upper soil horizons will be a significant determiner of the ratio between the two sources.*

H3: *Leaf litter decomposition dominates the heterotrophic component of decomposition in the early spring and mid-summer, while fine root decomposition dominates in the late summer and fall.*

H4: *Leaf litter decomposition is more responsive to precipitation events than root decomposition.*

H5: *Root decomposition is a greater source of CO_2 in the A and B horizons than is DOC leached from the litter layer and decomposed at greater depth in the soil.*

In order to partition the four components described above we utilize a series of equations based on total C and ^{14}C mass balance. ΔT, ΔR, ΔL, ΔH and ΔA represent the ^{14}C signature of T, R, L, H and A respectively. We will measure T, ΔT, ΔR, ΔL, ΔH, and ΔA. The isotopic signature of root respiration (ΔA) is assumed to equal the $\Delta^{14}C$ of CO_2 in the current atmosphere. This assumption is supported by data presented in Gaudinski et al. (2000b) and also by one direct measure of root respiration performed August 12, 1999 on Walker Branch. The ^{14}C signature of root respiration trapped onto molecular sieve from a closed chamber was 84 ± 6‰, which compares well to an air sample of 71 ±7 ‰ taken the same day. The remaining four unknowns (R, L, H, and A) can be solved for using the four equations below.

Mass balance of soil respiration

$$T = R + L + H + A \quad (1)$$

Mass balance of radiocarbon

$$T\Delta T = R * \Delta R + L * \Delta L + H * \Delta H + A * \Delta A \quad (2)$$

In the leaf litter manipulation experiments, we assume the relative contributions of R, L, H, and A at a given site are the same and the only differences between the plots with (1) labeled leaf litter and (2) near background leaf litter are in the $\Delta^{14}C$ of total respiration (ΔT) and the $\Delta^{14}C$ of litter (ΔL).

Therefore equation 2 (with subscripts 1 and 2 representing labeled vs. near background litter treatments) can be expressed as:

$$T(\Delta T_1 - \Delta T_2) = L(\Delta L_1 - \Delta L_2) \quad \text{and} \quad \frac{L}{T} = \frac{(\Delta T_1 - \Delta T_2)}{(\Delta L_1 - \Delta L_2)} \tag{3}$$

Comparison **across sites** that have different amounts of ^{14}C label in the roots, but which are amended with leaf litter that has the same $\Delta^{14}C$ would lead to a similar result (and our fourth equation). Here the only reason that the total soil respiration would have a different isotopic signature is because of the differences in the isotopic signature of the decomposing roots at the different sites with different amount of label in the roots. The proportions of R, L, A, and H in total soil respiration are again assumed to be equal across sites.

Therefore (with subscripts a and b representing two sites with high vs. low amounts respectively, of ^{14}C label in the roots):

$$T(\Delta T_a - \Delta T_b) = R(\Delta R_a - \Delta R_b) \quad \text{and} \quad \frac{R}{T} = \frac{(\Delta T_a - \Delta T_b)}{(\Delta R_a - \Delta R_b)} \tag{4}$$

Incubations of soil organic matter produce the isotopic signature of decomposition from L, R and H sources combined (ΔInc) without root respiration (A). Combing incubations with the ^{14}C signature of soil respiration (ΔT) allows for another way to determine the amount of soil respiration coming from A.

The equations used here are:

$$\Delta Inc = (R\,\Delta R + L\,\Delta L + H\,\Delta H)/(R+L+H) \tag{5}$$

The total soil respiration isotope value is then a linear combination of

$$\Delta T = (1-A/T)(\Delta Inc) + (A/T)\,\Delta A \quad \text{and} \quad \frac{A}{T} = \frac{(\Delta T - \Delta Inc)}{(\Delta A - \Delta Inc)} \tag{6}$$

We can use the approach of Davidson and Trumbore (1995) and Gaudinski et al (2000b) to use CO_2 and $^{14}CO_2$ profiles together with radon-based estimates of effective diffusivity to partition respiration sources as a function of depth in the soil. In August, 2000, we made a preliminary assessment of this approach to partitioning soil respiration using the methods described above. Comparison of the TDE/Haw Ridge (low root label) and Pine Ridge (high root label) sites yielded soil respiration $\Delta^{14}C$ signatures of 150 ‰ (± 20; n=5), and 370 ‰ (± 28; n=3), respectively. A one-week incubation of minimally disturbed soils from O and A horizons for these two sites yielded CO_2 with $\Delta^{14}C$ values of 185 ‰ and 580 ‰ for the two sites.

Using eq (6), above, and ΔA of 84‰ (directly measured August 12, 2000) the fraction of total soil respiration that is autotrophic (A/T) can be calculated for both sites, and is 35% for Haw Ridge/TDE (150-185)/(84-185) and 42% for Pine Ridge (370-580)/(72-580). Assuming the contribution of the litter layer to the ^{14}C flux is identical for the two sites that have different root labels, and using proxy samples of parasitic plants (squaw root, *Canopholus americana*) to estimate the isotopic composition of root decomposition (ΔR), we can use equation (4) to estimate R, the fraction of respiration derived from the labeled root pool: R/T = (370-150)/(780

(acorn) – 320(squaw root)), or 48%. Summing the 48% from (R) and the 35-42% from (A) mean leaf litter decomposition is contributing 10-17% of the total flux in August 2000. Equation four requires a robust number for ΔR and this is just an example calculation using acorns and parasitic squaw root to estimate the value of ΔR. However this value will be known much more confidently after the ^{14}C analysis of fine roots.

The preliminary results from August 12, 2000 indicate little difference in the soil respiration at the two low label sites Haw Ridge (shale parent material) and the Walker Branch (dolomite parent material). Soil respiration was 153 ± 30‰ (n=2) and 154 ± 24‰ (n=3) for both sites respectively. We therefore conclude that in the short-term plant effects will dominate soil respiration and NOT the influence of parent material derived mineral-C interactions. Thus, in an effort to decrease ^{14}C costs, we will measure soil respiration, soil gas and perform incubations at only the Walker Branch (low label) and TVA (high label) sites with dolomite parent material.

Soil gas samples -- In order to determine changes in SOM sources over time as a function of depth we will monitor the ^{14}C concentration at 3 depths at the two dolomite sites (i.e., Walker Branch and TVA Chestnut Ridge). We will use estimates of soil gas diffusivity and soil CO_2 and $^{14}CO_2$ profiles to calculate the production in each horizon and its $\Delta^{14}C$ signature (Davidson and Trumbore, 1995). These results will be compared with incubations to determine the importance of autotrophic respiration with depth in the soil profile. A time series of $^{14}CO_2$ production with depth will be useful confirmation of vertical movement (as DOC) of labile carbon through the soil profile.

Methods for Task 2a

The methods for sampling ^{14}C in soil respiration and soil gas are described in detail in Gaudinski et al. (2000b). Briefly, closed dynamic chambers will be used to sample ^{14}C in soil respiration. The chamber headspace will be cleared of atmospheric CO_2 by circulating three chamber volumes through a soda lime trap prior to trapping the CO_2 on molecular sieve (13x). Trapping continues until approximately 1.5 mg of C are sorbed onto the trap. Molecular sieve 13X traps CO_2 quantitatively at room temperatures and then releases it when baked at 475 °C (Bauer et al. 1992). To measure CO_2 and its ^{14}C signature in the soil atmosphere we will collect soil gas samples from stainless steel tubes (3 mm OD) inserted horizontally into soil pit walls (the soils pits will be back-filled). Evacuated stainless steel containers (0.5 - 2.0 L volume) are then attached to the tubing and filled slowly using a capillary to restrict flow and thus minimize disturbance of the concentration gradient. Incubation samples will be taken by coring, chilled in coolers with blue ice and immediately shipped to UCI where incubations will begin upon arrival. Soil will be placed in 1-2 liter mason jars with air tight lids containing stopcocks. Atmospheric CO_2 is removed at the beginning of the incubation by circulating 3 volumes of jar air through a soda lime trap. After approximately 10 days of incubation, at temperatures near field conditions, total CO_2 concentration and ^{14}C and ^{13}C will be measured. The carbon from all samples will be analyzed for both ^{13}C and ^{14}C. The ^{13}C is used to correct for any mass-dependent isotopic fractionation to –25‰ in $\delta^{13}C$.

Measurements of ^{14}C in total soil respiration and soil gas will be taken five times a year at two of the factorial sites (Walker Branch and TVA) and at the existing Walker Branch sites. Timing of sampling will be once prior to leaf out, three times during the growing season and once after leaf fall. Respiration measurements will be taken at three collars, from three plots at each treatment. Soil gas samples will be taken from three depths at three plots within each

treatment. Incubations will be performed three times throughout the year at the two factorial sites (Walker Branch and TVA). Four incubations will be performed from four samples taken at three depth intervals (O and A horizons and mineral soil from bottom of A horizon to 30 cm) from samples taken by coring.

3.2B Fine root lifetimes **(Task 2b – Gaudinski/Joslin)**

The decomposition of fine roots is a major component of soil respiration, and a major source of SOM. Therefore a detailed analysis of fine root lifetimes in in-situ root populations will lead to new knowledge about the lifetime of fine roots. It will also contribute critical information needed to achieve two of the goals in this proposal—(1) partitioning sources of soil respiration and (2) partitioning sources of protected soil organic matter. We have chosen to focus on Walker Branch to address the question of fine root longevity because it is the only site where we have archived samples of new roots grown only during the 1999 and 2000 growing seasons respectively (collected from root screens implanted in the top 0-10 cm). Also archived are representative samples of the total fine root population collected in July 1999 and in April 2000. Using our measurements of the ^{14}C inputs from the 1999 pulse in both 1999 and 2000 roots, we can assess the contribution of roots grown in 1999 and 2000 to the entire population. Figure 4 shows the difference in ^{14}C between new roots that grew in 1999 (sampled in late August) relative to roots sampled from a soil pit (sampled July 23, 1999). These data imply that newly grown fine roots are incorporating significant amounts of newly fixed photosynthate.

The ^{14}C signature for the soil pit roots from July 23, 1999 are also elevated by 70 to 215‰ relative to the pre-pulse atmosphere on Walker Branch. Since these soil pit roots were sampled on July 23, 1999, one day after the atmosphere was measured to be 435‰, one would not expect C from the pulse to be incorporated into these samples. The fact that the ^{14}C signatures are so significantly elevated in these July 23 roots relative to the pre-pulse atmosphere simply indicates that these roots were made from carbon fixed several years previously when the atmospheric CO_2 contained more ^{14}C from thermonuclear weapons testing in early 1960's (the rate of decrease has been roughly 8 ‰ per year from 1990-1996 and is now roughly 4 ‰ per year). Since the roots that are newly formed after the July pulse will have incorporated considerable C with the very strong signal from that pulse, we expect to see large differences between the standing stock measured in 2000 and subsequent years relative to the inputs from the 1999 pulse.

Previous work at three other eastern forests shows that root lifetimes are significantly heterogeneous within the commonly defined size class inclusive of fine (< 2 mm in diameter) roots (Gaudinski et al. in review). A small population of the standing stock have ^{14}C signatures consistent with one to two year lifetimes while the bulk of fine root stocks have ^{14}C signatures consistent with 5-10+ year lifetimes. Fine root lifetimes tend to increase with both size class, and depth and decrease with branching order (Gaudinski et al. 2000b and Gaudinski et al. in review). It is important to realize that the ^{14}C technique is in general most representative of the largest mass of the sample. Thus a bulk ^{14}C measurement of fine roots is going to be biased toward the ^{14}C signature of the slowest turnover component (biggest mass) while the fastest turnover component (smallest mass), which is actually contributing to the measured isotopic signature of soil respiration and to SOM, will be effectively masked by the ^{14}C signature of the larger mass.

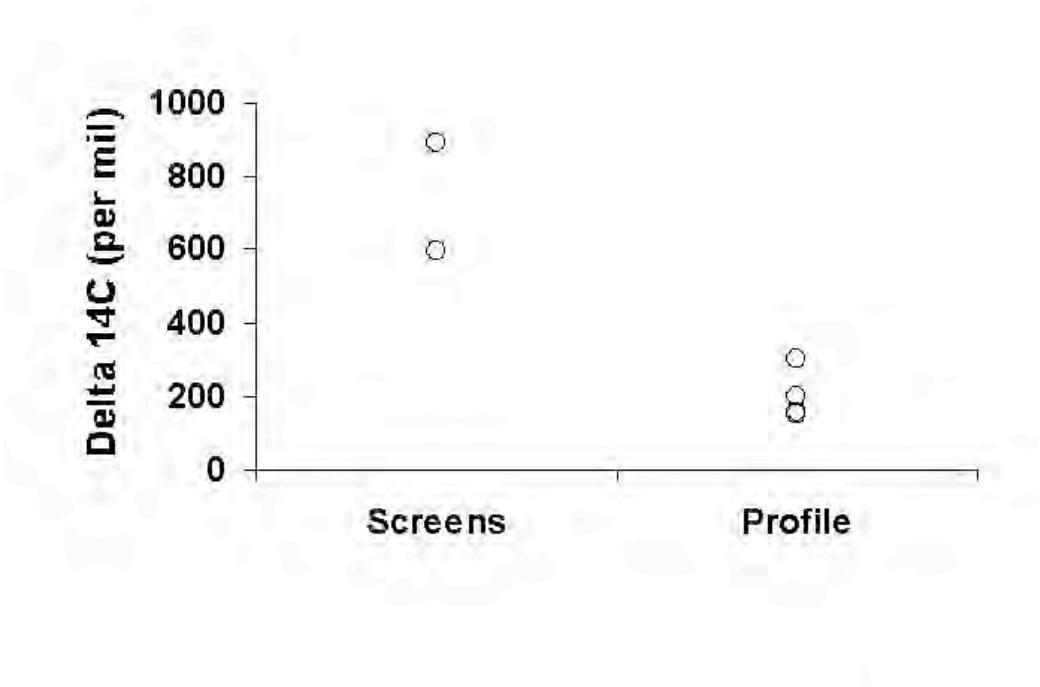

Figure 4. Radiocarbon values of fine roots of the Walker Branch Watershed sampled in 1999. Profile roots were grab samples from pits dug on July 23, 1999. "Screens" represent roots < 1 mm in diameter that grew through screens placed at the base of the O horizon at the beginning of the 1999 growing season and were subsequently harvested on Aiugust 20, 1999. "Profile" roots are < 1 mm in diameter and are grab samples from pits dug on July 23, 1999. Atmospheric $^{14}CO_2$ measured with two samples on May 7, and two on June 11, 1999 was 85 ‰ with a standard deviation of 10 ‰. The atmosphere on July 22, 1999 was 435 ‰.

At Walker Branch, using fine roots from composited soil cores on the treatment plots we will evaluate ^{14}C content based on four size classes, three depth intervals and branching order. With this approach and knowledge of the ^{14}C inputs for 1999 and 2000 we will be able to determine the lifetime and dynamics for the whole population of fine roots at Walker Branch. Additionally, the isotopic signature of root input (ΔR) to the total ^{14}C of soil respiration (ΔT) is very important in the overall mass balance equations discussed in section 3.2A. Thus, by knowing which size classes and branching orders are turning over the fastest at Walker Branch, we can apply this knowledge to the other three sites, decrease the size classes measured and still get a value for ΔR that is representative of what is actually contributing to the respiration flux instead of the more residual standing stock.

H1: *Because small diameter roots are commonly those most recently formed, diameter class can be correlated with $\Delta^{14}C$ values, and the finest diameter class (< 0.5 mm) should reflect the strongest signal from the 1999 ^{14}C release.*

H2: *Branching order (determined by proximity to root tips) will also be highly correlated with $\Delta^{14}C$ values. In fact, branching order may be a better predictor of $\Delta^{14}C$ values—and hence of root age—than diameter class.*

Methods for Task 2b

Each soil sample (obtained from the in-situ cores after compositing) will be sorted first into live versus dead roots and then by size class for three depth intervals (O horizon, A horizon, and 60-90 cm). At Walker Branch we will do the most detailed analysis of fine root lifetimes by sorting roots into four size classes (<.5 mm, .5-1 mm, 1-2 mm and > 2mm) for each depth interval. In addition, analysis of the relationship between branching order and fine root lifetime will be conducted on selected intact cohorts of extensively branched white oak roots sampled outside of the plots on roots at Walker Branch. Roots will be subdivided into branch order on intact representative root cohorts obtained by careful excavation in shallow pits.. These analyses will be performed during the first year only. Subsequently, Walker Branch will be treated like the other sites. The other sites (and Walker Branch post 2000) will have each sample sorted into live and dead root categories and two size classes (to be determined based on the detailed Walker Branch 2000 results).

In-growth root cores or root screens will be installed at the beginning of each growing season as described in section 3.1D in order to get the ^{14}C input signature for newly grown roots. Each site will have 8 ingrowth cores and/or root screens installed (for a total of 32). These cores will be installed outside the manipulated plots for the factorial study, as the root inputs should not be affected by the litter treatments. These cores will be collected at the end of the growing season, composited, sorted into two size classes and run for ^{14}C. Based on two previous samples from two root screens collected on the Walker Branch (600 and 900‰) we do expect a small degree of variation in isotopic signature even within roots grown in one growing season.

Prior to combustion and graphetization, all root samples will undergo a Soxhlet extraction and acid-base-acid treatment to obtain pure alpha-cellulose (after Roden and Ehleringer 1999).

3.3 Rate of subsurface tranport

3.3A Vertical transport within the surface soil horizons **(Task 3a - Jardine)**

Widespread, highly developed mature soils such as Ultisols and Oxisols have deep soil profiles that have a tremendous capacity to sequester organic C. The physical and chemical properties of the lower horizons (B-horizons) within these soils are ideal for maximizing organic C sorption to the solid phase (Sibanda and Young, 1986; Jardine et al., 1989a,b, 1990a; McCarthy et al., 1993; Benke et al., 1999). For example, these subsoil B-horizons have (1) the appropriate mineralogical components that strongly immobilize organic carbon; (2) ideal acidic pH and geochemical features for maximizing C sorption; (3) media that is highly structured with abundant microporosity that serves to enhance solute attenuation; (4) deep profiles which enhance C residence time prior to groundwater interception. The B-horizon carbon pool (passive C pool) is significantly less dynamic than the C in upper soil horizons since it is strongly stabilized on mineral surfaces with estimated turnover times of millennia and longer (Trumbore, 1997). Therefore, management methods for enrichment of subsoil organic carbon can be a favorable technique to sequester appreciable quantities of C. Although the mechanisms of C adsorption in some soils have been fairly well characterized in the laboratory (Tipping, 1981 a,b; Davis and Glour, 1981; Jardine et al., 1989b; Dunnivant et al., 1992; Baham and Sposito, 1994; Gu et al., 1995, 1996a,b; Wan and El-Swaify, 1998), only a handful of studies have demonstrated similar processes in the field (Jardine et al., 1989a, 1990b; McCarthy et al., 1993; Currie et al., 1996).

Key scientific questions remain regarding the magnitude of enhanced organic C sequestration that can be expected in subsoils. Namely:

1. What is the chemical nature and form of the dissolved organic material that is solubilized from surface organic matter?

2. How mobile is the dissolved organic material and how far can it migrate through the soil profile before it is immobilized by the solid phase?

3. What is the impact of preferential vertical and lateral flow on the transport of organic carbon in structured soils?

4. What role does the large surface area associated with soil microporosity play in the sequestration of subsurface C?

The answer to these important questions must be derived from field relevant, basic research strategies that emphasize the influence of coupled hydrological and geochemical processes on below ground C sequestration. The purpose of the research proposed here is to provide an improved understanding and predictive capability of the mechanisms that control the transport and immobilization of organic C through the soil profile. The study is motivated by the likelihood that deep clay and Fe-oxide rich subsoils of humid and tropical climates have a tremendous capacity to stabilize and accumulate organic C thus decreasing carbon turnover rates by orders of magnitude relative to surface soils. Application of the ^{14}C tagged litter allows us to address these issues since it serves as a well defined source. Coupling solid and solution phase

^{14}C analyses with conventional total organic C analyses, provides a powerful technique for assessing the sequestration potential of organic C in subsurface environments.

The task objectives are to (1) quantify the magnitude of enhanced carbon accumulation in the subsoil of a clay and Fe-oxide rich Ultisol and Inceptisol, (2) quantify the impact of coupled hydrological and geochemical processes on the fate and transport of solubilized organic C through the soil profile, (3) quantify the chemical nature of the sequestered C and the mechanisms responsible for immobilization by the solid phase. The proposed research is driven by the following hypotheses:

H1: *Subsoils rich in clay and Fe-oxides will sequester the majority of organic C that is solubilized and vertically transported from the upper O_e and A-horizons.*

H2: *Larger, more hydrophobic organic compounds will be preferentially sorbed by the solid phase during vertical transport through the soil profile.*

H3: *Hydraulic and concentration gradients will drive organic matter preferentially into micropores where it will be physically protected from microbes that cannot access this pore regime.*

H4: *Preferential flow during large storm events will diminish the potential for C sequestration in the subsoil due to significant bypass of the soil matrix and decreased C resident times in the soil profile.*

Methods for Task 3a

Each of the thirty-two (32) plots will contain four tension solution samplers and four free drainage (no tension) solution samplers. Two of each type of solution sampler will be instrumented within the A-horizon of the soils with the remaining samplers instrumented within the B-horizon of the soils. Tension free solution samplers will be designed to collect free flowing macro- and mesopore water, and tension solution samplers will be held at 250 cm suction for collecting pore water from the soil matrix (micropores). Tension free solution samplers will consist of coarse fritted glass plate lysimeters (Jardine et al., 1989b, 1990a,b). Installation will involve the removal of an undisturbed pillar of rotary cored soil, followed by lysimeter placement and packing, and repositioning of the original soil core minus a portion of its lower end. The annulus between the soil core and the surrounding soil media will be filled with soil slurry to eliminate potential infiltration between the soil masses. Tension solution samplers will consist of candle stick ceramic porous cups designed for vertical installation within predrilled holes. The annulus between solution sampler and the surrounding media is again filled with a soil slurry. Ceramic porous cup samplers have never been shown to adversely alter the composition of DOC in soil pore water and are suitable for sampling DOC within the matrix porosity (Litaor, 1988; McGuire et al., 1992). To quantify our input DOC source derived from the upper litter layer, two additional types of solution samplers will be installed directly beneath the O_e horizon and within the A-horizon. At select locations, leachate passing through the O_e horizon will be routed through small columns packed with XAD-8 and XAD-4 resin that are buried in the underlying A-horizon. The XAD-8 resin will concentrate hydrophobic DOC and the XAD-4 resin will concentrate hydrophilic DOC. The resin columns will be periodically changed out in the field (frequency to be determined in the laboratory) and the carbon mass will

be stripped from the resin and quantified. Miniature flow meters will be attached to the lower portion of the columns in the field and continuously monitored with a Campbell datalogging system so that the total organic C flux exiting the O_e horizon can be computed. Spatial and temporal monitoring of leachate moving through the O_e and A horizons will provide a known source term thus enhancing our ability to quantify DOC movement into the B-horizons.

Two nonreactive tracers (Br^- and Cl^-) will added to the soil surface using techniques described by Jardine et al. (1990b), in addition to the ^{14}C tagged litter and control litter. The Br^- tracer will be added to the ^{14}C tagged plots and Cl^- will be added to the control plots, with Cl^- being a suitable tracer since background concentrations are only 1-2 ppm. The tracers will serve two important roles; (1) they will provide a quantitative measure of solute transport rates, including preferential flow and matrix diffusion, and (2) they will provide a quantitative measure of any soil water mixing between treated and control plots. The various solution samplers will be continuously monitored before, during, and after at least 5 storm events and the cascading transfer of solute mass between small and large pores will be quantified with time as DOC moves from the A-horizon into the B-horizon. All samples will be analyzed for solution phase total organic carbon (TOC) using conventional combustion analysis. Due to cost considerations, 25-50 % of these samples will be analyzed for ^{14}C. It is thought that a correlation will emerge between the two analyses allowing for the distinction between the ^{14}C tracer C and indigenous organic C in solutions where ^{14}C is not actually measured. This approach will allow for a more thorough spatial and temporal analysis of C fate and transport mechanisms through the entire coarse of the study. All samples will be kept refrigerated and samples needed for isotopic analysis will be split and shipped to LLNL in a 50ml plastic centrifuge tube. The split will be freeze-dried in a vacuum freeze-drier, combusted, and cryogenically purified. One aliquot of CO_2 will be graphitized according to the methods described in Section 3.5. The other will be analyzed by a isotope ratio mass spectrometer for ^{13}C content.

The preferential mobility of specific DOC moieties through the soil profile and into the soil matrix will be assessed using fractionation techniques that separate the DOC components based on polarity and charge density differences (Leenheer, 1981; Aiken et. al., 1992; Jardine et al., 1989b; 1990a). Select samples will also be fractionated on the basis of molecular mass (Buffle, 1998; McCarthy et. al., 1993,1996). These techniques will quantify changes in hydrophobicity and molecular size as DOC components are selectively released or adsorbed during transport through the columns (Cronan and Aiken, 1985; Jardine et. al., 1989b, 1990b). Soil solution will also be characterized by spectral analysis where changes in absorptivity will indicate changing organic carbon functional groups and hydrophobicity. \

The tracers Br^- and Cl^- , as well as indigenous NO_3^- and SO_4^{2-} will be quantified on all solutions using ion chromatography. The anion SO_4^{2-} is of particular interest since it can compete effectively with DOC for solid phase sorption sites thus influencing organic C dynamics in soil (Jardine et al., 1990b; Kooner et al., 1995). Solution pH will also be measured on all samples using conventional methods.

As the investigation proceeds, sacrificial sampling of the solid phase (1" dia core) will occur with depth and analyzed for ^{14}C. Limited solid phase monitoring at this scale is essential to maintaining the integrity of the plot flow field for long-term monitoring of the organic C sequestration process. . Solid phase C sequestration tendencies will be supplemented with batch organic C sorption isotherms using techniques described in Jardine et al. (1989b). These experiments will provide a quantitative measure of solid phase organic C as a function of various organic C equilibrium solutions. Organic C sequestration tendencies should be very different for

Ultisols versus Inceptisols that are being investigated in this research. Solid phase sequestration potential can be estimated by comparing C breakthrough with nonreactive tracer breakthrough.

Changes in C concentration data determined from soil solution sampling at various times will be combined continuous water content monitoring to estimate C fluxes in large pore and small pore regimes. Since we have a good measure of our source term, combined with our use of nonreactive tracers, solid phase C analysis, and sorption isotherms, we can quantify the magnitude of C sequestration with depth. Also, data on ^{14}C adsorbed onto the soil phase will also provide insights into C sequestration in the subsoil. Aqueous fractionation and size separation techniques will further serve to identify the chemical form of organic C involved in the sequestration process (Jardine et al., 1989b, 1990b). The feasibility of calculating the amount of subsoil C sequestration due to either leaf or root decomposition will be determined. This may be accomplished by simulating changes in C concentration at various times by combining C concentration with current and historical water budget data (Luxmoore 1983) to estimate C flux in large and small pore regimes. Solid phase sequestration can be estimated by comparing C breakthrough with non-reactive tracer breakthrough.

3.3B Macrobiotic facilitated vertical transport **(Task 3b - Callaham)**

Soil invertebrates can be important regulators of several key processes in litter/soil ecosystems (Coleman and Hendrix 2000; Coleman and Crossley 1996; Swift et al. 1979). The degree to which these organisms influence such processes is largely dependent upon the taxa present and their densities. In forested systems, several taxonomic groups of soil and litter dwelling invertebrates are known to influence the rate of decomposition of leaf litter (Swift et al. 1979). Among these, earthworms are often the most abundant (at least in terms of biomass)(Coleman and Crossley 1996), but direct assessments of earthworm influences on litter and C dynamics in North American deciduous forest systems are scarce (Parkinson and McLean 1998) However, significant data for earthworms in agricultural ecosystems in North America are available (see Hendrix 1995). Earthworm activity in agroecosystems has been demonstrated to influence litter dynamics through burial and fragmentation of leaf litter by endogeic and epigeic species, or direct transport of litter into burrows by anecic species (terminology *sensu* Bouché 1977). Regardless of the mechanism, or ecosystem, the net result of earthworm activity is incorporation of leaf litter into mineral soil layers, and increased rates of decomposition. Following earthworm mediated incorporation of leaf litter material, microbial processing results in formation of SOM.

Most information regarding the influences of soil/litter invertebrates on litter decomposition comes from litter bag studies wherein the size of openings on litter bags is varied (in order to exclude invertebrate taxa of different sizes to determine the influence of those taxa on litter breakdown, as in references in Coleman and Crossley 1996). However, this method has certain limitations, and Coleman and Crossley (1996) specifically call for studies utilizing isotopic tracers to eliminate some of these limitations. Another method used to detemine influence of soil organisms on litter breakdown has been that of biocidal manipulation of the soil invertebrate community. In agroecosystems, earthworm-specific biocides, such as Carbofuran, have been used to experimentally decrease earthworm populations, but non-target effects of such chemicals are sometimes undesirable and may confound experimental results (Parmelee et al. 1990, Parkinson and McLean 1998). Alternatively, electroshocking of earthworms has been shown (again, in agroecosystems) to be an effective means of reducing earthworm populations with minimal non-target effects (Blair et al. 1995, Bohlen et al. 1995). Given the unique

opportunity afforded by the ecosystem-level ^{14}C labeling in this proposed study, we will combine some of the above-mentioned methods with ^{14}C tracer techniques to examine (in a directly quantitative manner) earthworm influences on litter breakdown/decomposition as well as their influence on movement of root derived C in soils. Ultimately, the ^{14}C tracer will allow direct measurement of earthworm mediated flows of C from litter and roots, through microbial pools, to SOM. Specific hypotheses to be addressed in the above outlined task are:

H1: *Earthworm abundance is positively correlated with the rate/magnitude of C flow from leaf litter to SOM. Reductions in earthworm populations will reduce the rate at which ^{14}C from labeled leaf litter will appear in mineral soil.*

H2: *Similarly, earthworm abundance is positively correlated with the rate/magnitude of C flow from root litter to SOM. Reductions in earthworm populations will reduce the rate at which ^{14}C from labeled roots appears in mineral soil.*

H3: *Earthworm species with different ecological strategies (i.e. epigeic, endogeic, anecic), will be differentially labeled, depending on litter treatment. Thus, ^{14}C signatures of epigeic and anecic earthworm tissues will parallel ^{14}C signatures of the applied litter, and signatures of endogeic earthworm tissues will resemble that of SOM ^{14}C signature.*

Methods for Task 3b

In valley bottom locations on Walker Branch (and/or Haw Ridge valleys) where significant earthworm populations were found (Callaham observations September 2000), we propose to establish a sequence of small 2X2 m plots for a parallel litter manipulation experiment designed to evaluate macrobiotic vertical transport. In plots that consist of either ^{14}C enriched litter on near background soils (east ORR) or near background litter on enriched soil (west ORR), we will manipulate earthworm populations using an electroshock method as described by Bohlen et al (1995). Reduction plots will be shocked 5-6 times annually, and control plots (ambient earthworm populations) will not be manipulated other than litter treatments. There will be 3 replicate control and earthworm reduction plots at each site. In these plots we will evaluate the accumulation of ^{14}C with depth by repeated sampling of small soil cores to a depth of 25 cm over 2 yr.

By sampling ^{14}C content of the bulk SOM pool at the time of litter application, and three times a year thereafter (November, February, and June), it will be possible to directly assess the influence of earthworms on the movement of litter derived C or root derived C into SOM. Specific pools to be sampled will include bulk SOM-^{14}C in the A and E horizons at each sampling date. Additionally, harvesting and analysis of ^{14}C signatures of different earthworm species will provide further insight into the relative contributions of specific taxa to this process.

3.3C Watershed scale transport of organic carbon **(Task 3c - Mulholland)**

In forested catchments, streamwater dissolved organic carbon (DOC) is primarily the result of leaching of soil organic matter and transport to the stream via surface and subsurface runoff (Hope et al. 1997, Hinton et al. 1998). However, there is considerable uncertainty as to whether the source of stream DOC is recently fixed carbon from the previous year's litterfall or older organic carbon from leached from the mineral soil. In a recent study using ^{14}C analysis, Schiff et al. (1997) reported that stream DOC varied from relatively old carbon at baseflow under

dry soil moisture conditions to relatively recent carbon during high flow or wetter soil conditions. The question of stream DOC source is important because its long-term fate and role as a sink in the global carbon cycle are related to its source. Although a portion of the recently-fixed stream DOC may be relatively labile, contributing to instream bacterial production and respiration, a significant portion may be preserved and transported downstream, eventually to the oceans where it contributes to an oceanic sink for atmospheric CO_2. In contrast, older DOC leached from the mineral soil is likely to be more refractory and a larger fraction transported to the oceans, but it does not represent a sink for CO_2 recently released to the atmosphere. Globally, the transport of organic carbon in streams and rivers to the ocean accounts for approximately 0.5×10^{15} grams of carbon per year (Meybeck 1993), and if primarily from recently-fixed sources, represents a modest additional sink for atmospheric CO_2.

We will address the question of stream DOC source using the differential ^{14}C labeling of soil organic carbon pools in catchments near the ^{14}C release. Because we have a much more temporally-distinct ^{14}C labeling of the soil litter pool, we will be able to distinguish old from recently-fixed organic carbon at a much finer temporal resolution than previous studies that relied on the early 1960's "bomb ^{14}C signal" (e.g., Schiff et al. 1997). If stream DOC is primarily from recently fixed soil organic carbon, we should observe significantly higher $DO^{14}C$ levels in catchments near the ^{14}C release compared with catchments at some distance from the ^{14}C release. Alternatively, if stream DOC is primarily from older, mineral soil DOC sources, then we should observe little difference in $DO^{14}C$ among all catchments. Stream DOC concentrations increase sharply during high flow events (Mulholland et al. 1990, Hornberger et al. 1994, Hinton et al. 1998) and we will determine whether the source of DOC changes between baseflow and stormflow conditions.

We will also evaluate whether recently-fixed terrestrial carbon is an important source of particulate organic carbon (POC) transported in streams, both under baseflow conditions seasonally and during storms when POC concentrations rise sharply. There are three possible sources of POC transported in streams: recently-fixed plant debris from terrestrial sources, older organic materials eroded from soils, and organic carbon derived from instream primary production. We will determine the relative importance of the first of these streamwater POC sources (carbon recently fixed in terrestrial environments) by measuring the ^{14}C content of streamwater POC in the same streams used for the DOC analysis.

In this task we will address the following hypotheses concerning the relative importance of recently fixed carbon to stream DOC and POC:

H1: *Stream DOC and POC are mixtures of recently-fixed carbon and older soil carbon and the relative fractions of the two sources vary with season, with recently-fixed carbon making up a higher fraction during the late autumn and winter after leaf-fall than during the spring and summer.*

H2: *The relative importance of recently-fixed carbon to stream DOC and POC increases and becomes the dominant source during storm events when the concentrations of stream DOC and POC increase sharply.*

Methods for Task 3c

Stream water samples will be collected quarterly at baseflow and during two stormflow periods (winter, spring) from 4 streams draining catchments at sites where extensive soil ^{14}C

measurements will be made. Two of the streams (unnamed) drain catchments at the West Pine Ridge ^{14}C-enriched site and the other two streams (East Fork of Walker Branch and an unnamed stream draining the East Haw Ridge site) drain catchments with little ^{14}C enrichment (Figure 3). Samples consisting of approximately 2L of stream water will be returned to the lab, filtered and subsampled for total DOC (measured by high temperature combustion, Shimadzu model 5000 TOC analyzer). The filtered sample will then be acidified to pH 3 and concentrated by rotary evaporation to 1 mL. The concentrated samples will be transferred to combustion tubes and processed for ^{14}C as described for the soil ^{14}C samples.
We will use an end-member mixing analysis to evaluate the contribution of recently-fixed organic carbon in stream DOC for each stream studied. Soil ^{14}C measurements (litter and A horizon soil) made in the same catchments drained by each stream will be used as ^{14}C source end members in the analysis. Stream DOC source will be determined by solving the following equations:

$$DO^{14}C_{stream} = (DO^{14}C_{litter} * F_{litter}) + (DO^{14}C_{soil} * F_{soil})$$
$$F_{litter} + F_{soil} = 1$$

where $DO^{14}C$stream is the measured ^{14}C value for stream DOC, $DO^{14}C_{litter}$ is the measured ^{14}C in litter, $DO^{14}C_{soil}$ is the measured ^{14}C in the A horizon soils, and F_{litter} and F_{soil} are the fractional contributions of litter and soil organic carbon to stream DOC. In the first year of the study, we expect that the ^{14}C enrichment will be limited to the litter layer and Flitter determined from the end-member mixing analysis will represent the fractional contribution of recently-fixed carbon to stream DOC for each stream. If the contribution of recently-fixed carbon to stream DOC is substantial, we should observe $DO^{14}C$ values that are elevated in the two ^{14}C-enriched catchment streams (West Pine Ridge streams) relative to $DO^{14}C$ values in the streams draining the catchments with minimal enrichment of ^{14}C (Walker Branch, East Haw Ridge).

We will collect stream POC by filtering water samples through pre-combusted glass fiber filters, acidify the filters to remove any carbonate particles, and analyze the residual material on the filters for ^{14}C using methods described for soil samples. The litter ^{14}C content in the catchments drained by each stream will be used as the end-member for recently-fixed terrestrial carbon and the other sources (older, soil-derived materials and instream primary production) will be treated as one pool (unenriched with ^{14}C) with the ^{14}C content of subsoil organic matter used as its end-member.

The work described for this task is focused on the first year of the study because that is when the ^{14}C signal will be strongest and confined primarily to the soil litter layer. Sampling of stream DOC and POC in future years of the study will be contingent on whether we are able to detect a strong ^{14}C signal in the stream water pools.

3.4 Soil C dynamics in unprotected and protected pools

The movement of C into and through measurable pools believed to represent unprotected and protected forms within the soil will be evaluated using two distinct approaches to fractionating the soil.. The first, termed the "Process/mechanistic-based approach," will distinguish C pools based on current conceptual models of the decomposition process and the mechanisms responsible for stabilizing soil C (Task 4a). In contrast, the "Density-based approach" will distinguish carbon pools based on the hypothesized role of soil clay minerals in stabilizing organic matter (Task 4b). We also propose to analyze microbial biomass pools of

carbon in conjunction with various soil fractions to evaluate the role microbial carbon plays in the pathway to the formation of protected C (Task 4c). Investment in all three of these approaches provides the opportunity to evaluate sources and relative turnover rates of protected and unprotected forms across a range of complexity for integration into different types of models of soil carbon cycling that accept different inputs (see also Section 3.6).

3.4A Process/mechanistic-based fractionation approach for soil C forms **(Task 4a – Jastrow)**

Leaf litter- and root-derived inputs are incorporated into mineral soil organic matter (SOM) by different pathways. Although some relatively undecomposed litter can be transported into the mineral soil via faunal activities (preliminary surveys by Callaham indicate earthworms are not abundant on the ridges where the primary study plots will be located) or mass flow during precipitation events, most litter decomposes unprotected on the surface. By the time a litter cohort moves through the Oi and Oe+Oa horizons to the surface of the A horizon, labile constituents are gone and any remaining materials are largely recalcitrant. In contrast, root-derived inputs decompose *in situ* where intimate contact with soil mineral surfaces affords opportunities for physical or chemical protection that can slow the decomposition process.

Despite these major differences in decomposition pathways, little information exists on the relative contributions of leaf litter- and root-derived inputs to forest SOM. Moreover, we know little about how C from these two sources cycles through the mineral soil horizons and the specific mechanisms protecting inputs from each source. Yet, basic information of this type is needed to determine if management strategies can be developed to move more inputs into protected pools in an effort to increase C sequestration in forest soils. Because tracers are required to identify and follow the fates of different organic matter sources, the *in situ* labeling of roots combined with the reciprocal transplant of litter in the proposed experimental design will enable us to address the following overarching question: **Do leaf litter- and root-derived inputs differ in how they cycle through unprotected and protected soil C pools?**

The mechanisms of soil organic C (SOC) stabilization may be categorized as (1) biochemical recalcitrance, (2) chemical protection, and (3) physical protection (Christensen, 1996). Biochemical recalcitrance may be due to the chemical characteristics of the substrate itself — e.g., lignin derivatives or fungal melanins (Stott et al., 1983; Martin and Haider, 1986) — or may result from transformations during decomposition, including incorporation into the excrement of soil meso- and microfauna (Kooistra and van Noordwijk, 1996). Chemical protection occurs because of chemical or physicochemical associations between decomposable compounds and soil mineral components (e.g., organics sorbed to clay surfaces by polyvalent cation bridges or intercalated between expanding layers of clays). In addition, the drying of relatively labile organics may cause them to be denatured or polymerized, thereby protecting them chemically from decomposition (Dormaar and Foster, 1991). Soil structure, however, plays a dominant role in the physical protection of SOM by controlling microbial access to substrates, microbial turnover processes, and food web interactions (Elliott and Coleman, 1988; van Veen and Kuikman, 1990). Relatively labile material may become physically protected from decomposition by incorporation into soil aggregates (Oades, 1984; Gregorich et al., 1989; Golchin et al., 1994) or by deposition in micropores inaccessible even to bacteria (Foster, 1985).

Significant interactions exist between SOC dynamics and the cycling of soil aggregates. Plant growth and the decomposition of organic inputs lead to the development of a hierarchical aggregate structure composed of macro- and microaggregates (Tisdall and Oades, 1982; Oades and Waters, 1991). Recent studies suggest that macro- and microaggregates are formed

sequentially as particulate matter is decomposed (Gale et al., 2000b; Six et al., in press). In addition, microaggregate-associated organic matter generally has a longer residence time and is more protected from decomposition than the organic matter in macroaggregates (Buyanovsky et al., 1994; Jastrow et al., 1996; Angers et al., 1997; Monreal et al., 1997).

Mineral-associated organic matter typically contains a mixture of very old and relatively young organic matter (Paul et al., 1997; Trumbore, 2000). The oldest pools may be biochemically recalcitrant organomineral complexes of plant or fungal origin, whereas more recent and labile organics (e.g., microbial byproducts) can be protected by their strong chemical associations with soil minerals. In temperate soils, silt-sized aggregates once formed appear to be very stable, exhibiting turnover times that are longer than those of other size fractions, including clays, probably because of the combination of biochemical stability and physical protection afforded by organomineral complexes at this scale (Christensen, 1996; Tisdall, 1996). However, the chemical forces holding organic films to silt-sized primary particles are probably weaker than those binding organic molecules to clay colloids and, hence, may have a shorter residence time (Buyanovsky et al., 1994).

Clay mineralogy also plays an important role in the physicochemical protection of SOM (Dalal and Bridge, 1996; Six et al., 2000). In soils dominated by 2:1 clay minerals, such as montmorillonite and illite, organic matter complexes with polyvalent cations form bridges between negatively charged clay platelets. In contrast, for soils dominated by 1:1 clays (kaolinite) and Fe- and Al-oxides, the electrostatic attractions between simultaneous positive and negative charges leave relatively fewer sites available for complexation with organics. In addition, pore size distributions of clay tactoids differ with clay mineralogy. For example, pore size in clay tactoids of Ca-illite is ~10 nm compared to ~100 nm in kaolinite. Because pore diameter controls microbial and extracellular enzymatic access, SOM should be more protected from decomposition in aggregates formed from Ca-illite than in similar aggregates of kaolinite.

We propose to evaluate the following hypotheses regarding the differential fate of root- and leaf litter-derived C in deciduous forest soils developed from two differing parent materials:

H1: *Because of the intimate contact of roots with soil minerals during decomposition, relatively greater proportions of labeled root inputs will be physically protected by incorporation into microaggregates. Furthermore, slowed decomposition of residues protected in microaggregates will enable relatively more root residues (or the byproducts of microbial activity on these residues) to be physically and/or chemically protected in the silt- or clay-sized fraction. Hence, a relatively small proportion of root-derived C will be transported as soluble C to deeper horizons. (See Section 3.6 Figure 7 for a related model simulation of the first portion of this hypothesis).*

H2: *A relatively small amount of particulate organic matter derived from leaf litter will be incorporated into stable microaggregates because the recalcitrance of most surface litter inputs to the mineral soil will not support sufficient microbial activity to bind soil minerals to residue surfaces. Precipitation events will leach soluble, labile organics and transport humified, colloidal material from the O horizon into the mineral soil where some of this surface litter-derived C will become associated with the silt- and clay-sized fraction. However, because the movement of much of this material will be relatively rapid, proportionally more litter-derived C will be transported to deeper soil horizons.*

H3: *Residue retention in microaggregates and the silt- and clay-sized fractions will be greater for the shale-derived Inceptisol than for the dolomite-derived Ultisol because of the dominance of 2:1 clay minerals in the Inceptisol and 1:1 clays in the Ultisol.*

These hypotheses are derived from current knowledge of protection mechanisms in soils and the pathways by which root and leaf litter residues enter the soil. However, several recent laboratory and field tracer studies lend further support. Work on the soil C dynamics of surface residue- and root-derived inputs under no-till agriculture found a clear partitioning of root- and surface residue-derived inputs of new C to soil and atmospheric sinks (Gale and Cambardella, 2000; Gale et al., 2000a,b). In this simulated no-till study, the fate of surface residue and *in situ* roots labeled with ^{14}C was followed for one year in laboratory microcosms. At the end of the experiment, the percentage of root-derived label in the soil was over 2.5 times that of the surface residue-derived label. Furthermore, the authors concluded that only root-derived inputs contributed significantly to aggregate stability. As a laboratory simulation, however, the effects of faunal mediated transport of surface residues belowground, alternate wetting/drying conditions, and leaching of soluble or particulate C through the soil profile on the fate of these two C sources were not examined. In another study, Magid et al. (1996) incubated soil with ^{14}C-labeled ryegrass and followed the migration of the label through multiple size and density fractions for 200 days. Although they used shoot material, it was thoroughly mixed with the soil, and the mixture was compressed to a bulk density of 1.3 g cm^{-3}. The label moved quickly from large, unprotected particulate fractions and soluble forms to heavy fractions that were finer than 100 μm. In a similar study, where $^{13}C^{15}N$-labeled wheat straw was uniformly mixed into surface soil and incubated under field conditions, almost 50% of the residual ^{13}C was in microaggregates after 574 days, with most of the rest of the label in the silt/clay fraction (Angers et al., 1997).

Methods for Task 4a

Soil from the A horizon of the composited soil cores taken in each plot will be physically and chemically fractionated to yield unprotected and protected particulate organic matter (POM) and four mineral fractions (Figure 5). Air-dried soil will be passed through a 2-mm sieve and dried at 55 °C. Rocks, coarse sand and large organic debris removed by sieving will be quantified to enable bulk density conversions of elemental concentrations to a volumetric basis.

Soil will be gently dispersed by shaking with glass beads over a 250-μm sieve in a microaggregate isolator developed by Six et al. (in press). A constant flow of water through the device will carry all soil fractions <250 μm through the sieve before further disruption occurs. Water and soil leaving the device will be passed through a 53-μm sieve and collected in a pan. Material retained on the 53-μm sieve will be manually wet-sieved in the pan to ensure that only water-stable microaggregates, unprotected fine POM, and sand remain. Soil passing the 53-μm sieve will be separated into silt- and clay- sized particles by sequential centrifugation. Clay-sized particles will be flocculated with $CaCl_2$ so that any soluble organics can be separated from the clay fraction. Coarse (>250 μm) and fine (53-250 μm) unprotected POM will be separated from mineral fractions (sand and microaggregates) by flotation in sodium polytungstate (density = 2.0 g cm^{-3}) and combined as one fraction for ^{14}C analysis. Microaggregates will be dispersed by shaking in water on a wrist-action shaker. Microaggregate-protected POM will be collected by sieving the dispersed aggregates through a 53-μm sieve and correcting to a sand-free basis by flotation. Silt- and clay- sized particles dispersed from the microaggregates will be separated as described above and combined with the free silt- and clay-sized fractions for ^{14}C determination.

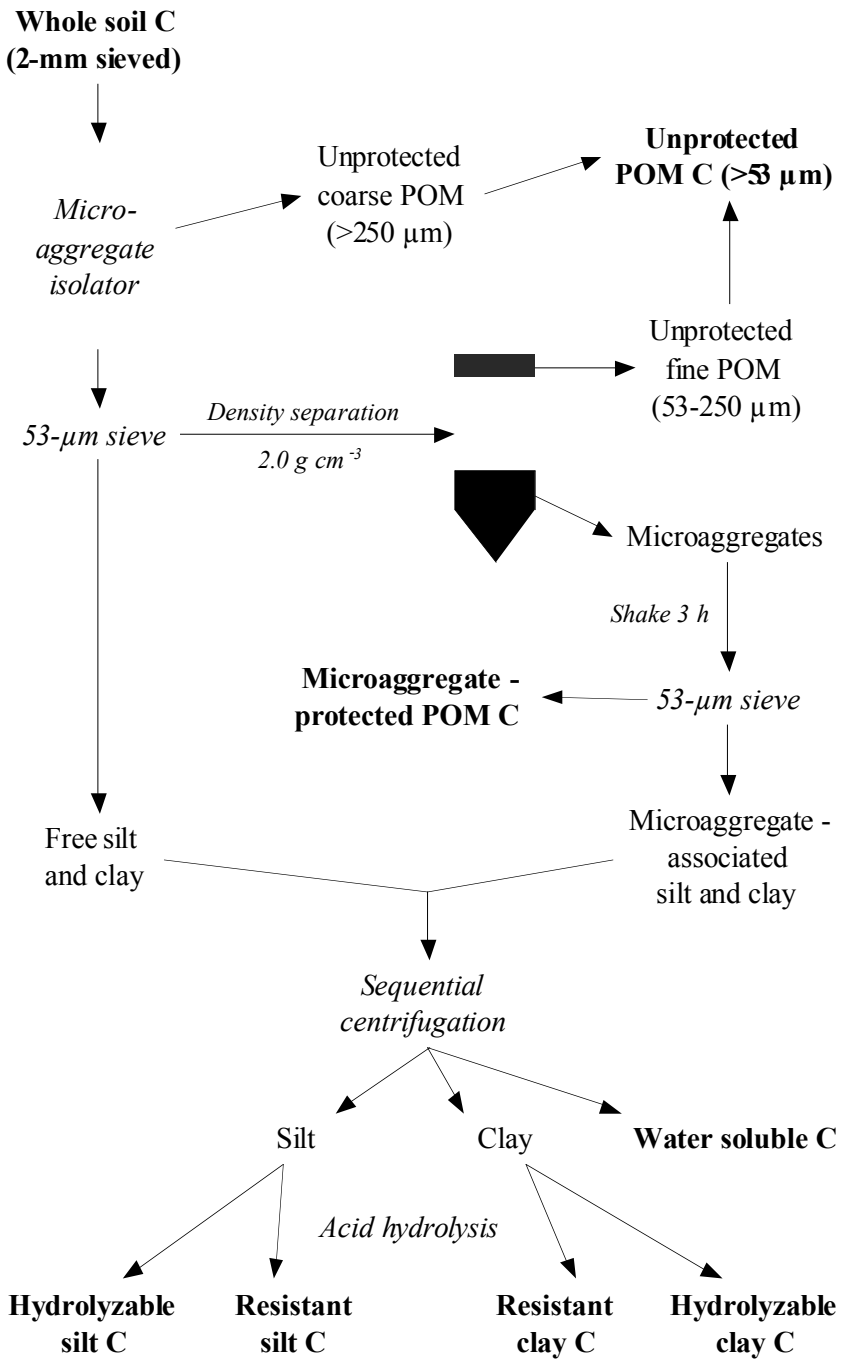

Figure 5. Process/mechanistic-based soil fractionation scheme for determination of protected and unprotected soil C. A horizon fractions for which ^{14}C will be determined are shown in bold. Water soluble ^{14}C will be determined by difference from whole soil ^{14}C (determined as part of Section 3.1D). For B horizon samples, all POM fractions will be combined and analyzed as one fraction.

Silt- and clay-sized fractions will be extracted by acid hydrolysis (Paul et al., 1997) to isolate old chemically resistant (non-hydrolyzable) organic C (aged ~1000 y or more) from more labile mineral-associated C, providing a more sensitive quantification of ^{14}C label movement into the mineral fractions. All fractions will be dried at 55°C, ground, analyzed for C (and N) by dry combustion with an elemental analyzer, and sent to CAMS for ^{14}C determinations.

In the first year, ^{14}C in hydrolyzable silt/clay will be determined by difference from measurements of ^{14}C in the total silt/clay and resistant (non-hydrolyzable) silt/clay fractions. After the first year, it will only be necessary to measure ^{14}C in total silt/clay fractions because once the size and ^{14}C signature of the resistant fractions are determined, the same values can be used in succeeding years. (The pool is so inert, it is not expected to change during the course of this study. However, a few measurements will be made each year to ensure that this assumption is valid.) Water soluble ^{14}C will be determined by difference from whole soil ^{14}C (determined as part of Section 3.1D). Hence, ^{14}C will be determined on six fractions the first year and four fractions thereafter.

Samples from the shallow B horizon of both soils and the deep B horizon of the dolomite-derived Ultisol will be similarly fractionated. However, because root growth is less extensive in these horizons, all POM fractions will be combined and analyzed as one fraction. Thus, for B horizon samples, ^{14}C will be measured on five fractions the first year and three fractions in later years.

We will track the movement of each labeled source into and out of the soluble pool, unprotected and microaggregate protected POM (total POM in the B horizon), hydrolysable clay, and hydrolysable silt and determine the relative partitioning of labeled C among these fractions for three of the treatment plots (enriched litter on low belowground label, unlabeled litter on high belowground label, and unlabeled litter on low belowground label). Samples from the treatment with both enriched leaf litter and a high belowground label will be archived for the purpose of selective comparative checks against the other plots. Simple estimates of turnover can be made by following the rate of label loss from each fraction after peak accumulation in that fraction (*sensu* Buyanovsky et al., 1994). More sophisticated analyses of C cycling, partitioning among fractions, and comparisons of the fate of above- and belowground C inputs will be accomplished in conjunction with the modeling efforts described in Section 3.6.

3.4B Density-based fractionation approach for soil C forms **(Task 4b - Torn)**

We propose to employ a second fractionation scheme to study soil organic matter dynamics, in addition to the multi-stage, process/mechanistic method described above. We will separate bulk soil into light and dense fractions, to separate the relatively labile (light) OM from the mineral-protected (dense) material (Trumbore and Zheng 1996). Unlike the multi-stage fractionation process described above, the simple density fractionation does not capture the mechanistic dynamics of soil organic aggregates and carbon protection. Nevertheless it has been shown to consistently separate SOM in different ecosystems into labile and recalcitrant pools for both experimental and modeling purposes (Trumbore and Zheng 1996). This is a commonly used, empirical approach to soil carbon cycle studies, including two studies of Oak Ridge forests, and well-suited to broad questions such as comparing many sites or many soil depths (e.g., Garten et al. 1999; Trumbore et al. 1996; Gaudinski et al. 2000). Our goal is to determine how effective this commonly- used technique is in describing carbon dynamics by comparing results of this technique with those generated in the approach based on processes and mechanisms in section 3.4A. In addition, density fractionations can help to elucidate the

influence on carbon storage of different soil minerals, for example within a single texture class (e.g., clay) identified in 3.4A.

Models of soil carbon cycling that are constrained by density fractionation data (e.g., Trumbore et al. 1996; Gaudinski et al. 2000) do a reasonably good job of characterizing soil carbon storage and the mass-weighted turnover times. These models are weaker, however, at predicting soil heterotrophic respiration. This is because the light fraction is a mixture of two types of organic matter. A small proportion is organic matter that cycles very rapidly and produces most respiration. The large proportion is slow–cycling and dominates the isotopic signature of the fraction, which is used to estimate the turnover time of the entire light fraction (Torn et al. 2000). In this project we will evaluate different modeling techniques, using data on soil respiration, multiple soil fractions, two-pool density fractions, microbial biomass and vertical transport of carbon.

Soil minerals are known to stabilize soil organic carbon, but how spatial and temporal variation in soil mineralogy controls the quantity and turnover of long residence-time organic carbon is not well known (Oades 1988). Recent research highlights the importance of non-crystalline and amorphous minerals in stabilization of plant inputs (e.g., Boudot et al. 1988; Torn et al. 1997; Saggar et al. 1994). It is likely that there are also differences in stabilization efficiency among different types of crystalline clays. The differences in parent material between the two ridges chosen for the EBIS study, provide sites with contrasting mineralogy while maintaining relatively constant climate and vegetation. As described in Section 3.1, the critical difference in mineralogy is the relative abundance of clays with 2:1 or 1:1 lattice structures. These clays differ in many attributes that are hypothesized to affect stabilization efficiency, including specific charge, surface area, and geometry (Martin and Haider 1986).

Objectives of the two-pool density fractionation:

(1) To generate data that is comparable (i.e., by same methods) to samples analyzed before the radiocarbon pulse. These data will thus continue a long-term data set and provide a pre-release control (i.e., an estimate of the "natural" background 14C content of SOM without the pulse), for data interpretation and modeling of soil organic matter turnover in the different treatments
(2) Compare the two-pool fraction results with the more complete multi-fraction results, to better understand the effectiveness and limitations of the simpler method. In particular, to understand how and when it is appropriate to use the two-pool data in modeling SOM cycling, and what kinds of SOM dynamics are best captured by each approach.
(3) Investigate the turnover time and root, leaf, and DOC sources of mineral-stabilized carbon, as they vary with soil depth.

Methods for task 4b:

The change in radiocarbon content of the organic matter will be used to constrain a model of turnover time. For the dense fraction, the results will be used to test the hypothesis that the amount and turnover time of organic C differs between shale and dolomite parent materials due to difference in stabilization efficiencies of the dominant soil minerals. Because the mineral-stabilized C pool is large and the annual inputs from roots and leaves may be small, our initial efforts will focus on the sites receiving the maximum ^{14}C enrichment (enriched litter and roots) to maximize the potential signal. When a signal is detected in the enriched roots + leaves plots, we will begin analysis on sites that have only one enriched input stream (i.e., leaf or root).

The proposed EBIS experiment provides a unique opportunity to evaluate the importance of leaf-derived DOC and root inputs to mineral-stabilized organic matter in soil. Carbon from litter arrives in the deeper, mineral-rich horizons through DOC transport. In the treatment with enriched litter and less enriched roots, nearly all enriched C the mineral horizons will come from DOC. By combining DO^{14}C measurements with density separations inputs in, we will be able to monitor the interaction of the hot DO^{14}C with different mineral fractions. We anticipate that we will see hot (enriched in ^{14}C) carbon appearing in the mineral-stabilized OM. If we do, we will be able to determine if this carbon is irreversibly bound over short time scales to the mineral fraction, or if it can slowly bleed on and off, exchanging with non-mineral bound C. If we do NOT see hot carbon appearing in the mineral-stabilized fraction, we will be able to address a key (and unspoken) assumption of current carbon sequestration programs: that new carbon added to a soil system can move quickly into the mineral-stabilized soil OM fraction. Either result will add substantially to our understanding of the interactions of minerals and soil C, and strategies for sequestration.

The DOC fraction will be collected from the lysimeters described in Section 3.3A. The soil water will be frozen, shipped to LLNL where it will be concentrated in a rotary evaporated and combusted to CO2 for target preparation as described in Section 5. Soils from the A, B1 and B2 horizons will be analyzed in the Dolomite-derived soils, and the A and B1 horizons in the shale-derived soils. Free particulate organic matter will be separated from mineral-stabilized organic matter by density separations. Soil is shaken into a sodium polytungstate solution and centrifuged to separate material that is more dense than the solution from the material that floats on the solution. The density of the solution depends on the mineral composition of the soil. Typical densities are 1.8-2.0 g/mL for crystalline minerals and 1.7 for amorphous minerals. For the dolomite-derived soils at Oak Ridge, 1.4 g/mL has been used to separate protected and labile carbon (Garten et al. 1999). For the two parent materials, we will evaluate which density of solution to use by inspecting fractionated material under a microscope for presence of minerals and by comparing the carbon and isotopic content. In future work on these soils, we propose to further isolate organic matter on different mineral forms, by using the graduated set of densities from 1.4 to 2.1 g/mL.

3.4C Soil microbial biomass in unprotected and protected pools **(Task 4c - Masiello)**

Soil microbes are the gateway for humification and heterotrophic respiration. In addition, bacterial and fungal biomass is an important source of recalcitrant or aggregate-forming organic matter. Because biomass should be one of the first components of the soil in which the elevated ^{14}C signal is detected, we expect to get useful results from the microbial biomass study after the first year of the experiment, whereas results based on more stable soil organic matter may take several years. In this project, measurements of the incorporation of ^{14}C into microbial biomass (total, fungal material, and selected bacterial compounds) will provide useful input data and constraints for our models of humification and heterotrophic respiration. Task 4a hypothesis 1 postulates that a substantial fraction of root material may become physically protected before microbial processing. In the Rothamsted model of soil C dynamics this can be simulated (Section 3.6) by moving a considerable fraction of root material directly to the HUM (humic) pool without going through the microbial pool. As a result, the amount of soil respiration predicted for root decomposition is lower than that predicted for comparable inputs of equally-labile leaf material, since leaf decay involves greater microbial processing and than root material

in this simulation (Figure 7). By comparing the isotopic enrichment of microbial biomass in the "enriched roots" and near background treatment plots, we will test this model structure. The ^{14}C enrichment combined with the leaf litter manipulations, allow us to directly examine the functioning of microbial biomass in soil C dynamics.

Analysis of microbial carbon will also be used to track sources of soil respiration and stable SOM. For example, turnover rates of the biomass or residual compounds of mycorrhizal fungi should be examined because mycorrhizal fungi are thought to receive about 10-20% of C fixed by plants (Allen 1991), and a significant portion of that C may be incorporated into fungal materials. If either live structures or residual compounds (e.g., chitin or glomalin) are relatively long-lived in the soil, their residence times may influence amounts and residence times of soil C. Research on microbial biomass will be focused on the following hypothesis:

H1: *A significant fraction of the root material that initially becomes stabilized in SOC does not first pass through the microbial biomass. In contrast, a significant fraction of leaf material that becomes stabilized is processed by microbes.*

H2: *The carbon exchanged between tree roots and mycorrhizal fungi (a) comes from recent photosynthate and looks isotopically like the current atmosphere, rather than being derived from older carbon stores from previous seasons and (b) the turnover time for live fungal biomass associated with roots is approximately annual.*

Methods for Task 4c:

Measurements will be made of three microbial carbon pools: total biomass, ectomycorrhizal fungi on root tips, bacterial cell walls and related specific compounds (e.g. glomalin). Total microbial biomass will be measured by chloroform fumigation (Jenkinson and Powlson 1976). In the incubation method, the evolved CO_2 is trapped cryogenically. In this study, we will also try the extraction method of recovering solubilized microbial C. Ectomycorrhizal material will be manually picked from roots (from frozen soil cores). Bacterial biomarkers and specific microbial compounds will be isolated through selective chemical analysis, purified, and made into radiocarbon targets (e.g, Rillig et al. in review).

Microbial biomass will be analyzed for the A horizon only, and for the dolomite parent material only, on the assumption that the role of microbes in these forests will be generalizable across parent materials. Soil for these measurements will be split from the cores taken for other soil organic matter analyses. In addition, in the first year, shallow (A horizon) soil cores will be collected 3 additional times (total of once each seasons) coincident with soil respiration measurements.

3.5 Radiocarbon measurements **(Task 5 - Southon)**

A major interest of the geosciences radiocarbon group at DOE's CAMS facility at LLNL is the use of ^{14}C as a tracer for the carbon cycle, and its application to research questions relevant to carbon sequestration and the interaction between the carbon cycle and climate change. CAMS scientists Southon and Masiello are collaborators in several projects with Trumbore's UC Irvine group and with Torn at LBNL, and the EBIS project represents a natural meeting point for these UC/LLNL/LBNL research interests with those of Hanson and colleagues at the Environmental Sciences division at ORNL and Jastrow at Argonne. CAMS will be an active participant in the EBIS research, not just a service center. Southon and Masiello will serve as radiocarbon liaison and quality control, assisting all laboratories on the project as required to ensure that EBIS ^{14}C sample preparation methods and measurement techniques are optimal. Masiello will assist in the field work, primarily in the collection and analysis of organic matter samples with an emphasis on DOC.

The EBIS project analytical costs for ^{14}C determinations can be compared in scope and cost with a FACE enhanced CO_2 study. The ca. 1500 annual ^{14}C measurements required represent a major logistical undertaking. At present, CAMS/LLNL is probably the only US AMS laboratory capable of carrying out such a program in a timely fashion. The lab currently provides about 10,000 high-precision AMS geosciences ^{14}C analyses per year (roughly half of the US total), using 2-3 days of beam time per week, and is unique among US AMS labs in having essentially zero measurement backlog. Approximately 5000 research unknowns (plus associated blanks and standards) are prepared in-house, with the remainder received as ready-to-measure prepared samples from seven other preparation laboratories. Although pressure for time on the CAMS spectrometer is increasing, commissioning of a dedicated AMS spectrometer for biomedical ^{14}C applications is presently underway. This migration of a major measurement program to a new instrument will ensure that the increases in the geosciences radiocarbon measurement program required by the EBIS experiment can be readily met.

Methods for Task 5

Sample pretreatment -- This study will require AMS ^{14}C measurements on at least six different types of samples: (1) CO_2 extracted from soil gas and atmospheric air, (2) leaf litter, (3) roots and tree cores, (4) mineral-associated soil carbon (5) dissolved organic carbon (DOC), and (6) particulate organic carbon (POC). CO_2 will be extracted from air and soil gas samples at UC Irvine using 13X molecular seive (Bauer et al., 1992; see also Section 3.2. Most other samples will require chemical and/or physical pretreatment prior to combustion to CO_2. Litter samples will be homogenized by grinding and given a brief acid treatment with warm 1N HCl to remove any carbonate dust. Roots will be treated with acid-base-acid (1N HCl, 1N NaOH) to isolate a fraction rich in structural carbon. Any samples needed for detailed chronologies (where it may be critical to remove all traces of translocated lignins, etc) will receive additional bleaching/oxidation steps using a 50:50 mixture of 3% sodium hypochlorite and 1N HCl to remove lignins, leaving a holocellulose fraction. Mineral-associated soil carbon samples will in most cases be density separated before samples arrive at LLNL and will require only acid treatment to remove any geological carbonate. Following the final acid treatment, all samples will be washed at least twice in deionized water and air dried. DOC samples in the form of 5-10ml extracts pre-concentrated by evaporation will be further concentrated to ca 1ml by vacuum centrifuging and then pipetted into combustion tubes for final drying *in vacuo*. POC samples on

glass or quartz fiber filters will require no pretreatment before combustion. Pretreatment is usually the rate-limiting step for the entire sample preparation process and throughput varies according to sample type, but typically, two people can pretreat 100 samples per week and prepare them for combustion.

Combustion/graphitization -- Samples will be combusted *in vacuo* in batches in sealed quartz tubes at 900°C with CuO oxidizer plus Ag powder to scavenge impurities such as sulphur and chlorine. CO_2 samples will be purified cryogenically and then converted to graphite using a hydrogen reduction catalyzed with Fe powder (Vogel et al, 1984) for samples graphitized at LLNL, or an equivalent zinc-based reduction for samples prepared at UI or LBL (We routinely measure samples prepared suing both techniques). The equipment currently in place at CAMS/LLNL can be used by two researchers or technicians to graphitize up to 48 samples per (long) working day. The batch processing line in place at UCI and the similar equipment to be installed at LBL together will handle another ca. 50 samples per day.

AMS measurements -- Approximately 48 samples, plus 12-15 standards, secondary standards and process blanks which must be prepared on the same equipment, make up one 64-sample ion source wheel. Samples will be measured by AMS on the CAMS/LLNL spectrometer using standard techniques (Southon et al, 1990), with researchers or students present during the measurements to monitor spectrometer performance and assure measurement quality. For samples such as these, which are close to Modern or contain elevated levels of ^{14}C, at least 2 wheels (100 research unknowns) can be measured to 5 per mil or better per 24-hour day of accelerator time. Results are reported as $\Delta^{14}C$, the per mil deviation from a standard normalized for ^{13}C content (Stuiver and Polach 1977). The stable isotope ratio of gas samples will be determined with either (1) a MicroMass Isoprime isotope ratio mass spectrometer (IRMS) configured with a Trace Gas cryogenic focusing unit or (2) a MicroMass dual inlet Prizm IRMS in LBNL's Center for Isotope Geochemistry or (3) an Isoprime at the Center for AMS, LLNL.

3.6 Integrating soil C cycling processes **(Task 6 - Post)**

The entire ecosystem pulse labeling of carbon inputs with ^{14}C combined with manipulations of aboveground inputs provides an opportunity for an integrated analysis of carbon fluxes through the soil of a temperate deciduous forest. Figure 6 indicates the time course of radiocarbon through various soil organic matter pools as modeled by the Rothamsted turnover model (Jenkinson 1990). This simulation indicates that the proposed manipulation experiment has the potential to utilize the ^{14}C tracer addition to resolve critical components of carbon stabilization. Using models as hypotheses of carbon dynamics in soil we will be able to synthesize measurements of radiocarbon in many forest ecosystem carbon pools through time and derive strong inferences concerning pools and fluxes of carbon between pools that are normally unmeasureable. Separate models have been developed and used for different aspect of soil carbon fluxes and resultant carbon pool dynamics. We will employ these models for data analysis and identify critical measurements of pools and fluxes that represent important linkages between models capturing processes at different time scales to improve our understanding of soil carbon cycle processes. Models to be applied include: multi-year models of soil accumulation based on empirically derived turnover times for operationally defined carbon pools; semi-empirical models operating at hourly to daily time steps driven largely by temperature, moisture, and root activity; and a third class of fast response models that combine biological respiration

data with physical diffusion estimates for the soil profile for CO_2 and hydrologic transport with biogeochemical kinetic and equilibrium reactions in soil for dissolved organic carbon.

Residence and turnover time of unprotected and protected SOM pools using multi-year models of soil accumulation --

The experimental manipulation of above ground inputs will allow us to estimate some model parameters and introduce new model components based on an improved understanding of partitioning, specific activity, turnover times, and detailed processes that following tracers through soil organic matter components allows. Figure 6 shows Rothamsted turnover model simulations that indicate the potential to differentiate between the relative contribution of leaf litter and root litter inputs into different soil organic matter pools. Other models based on soil carbon stocks having known turnover times include CENTURY (Parton et al. 1988), TerraFlux (Asner et al., submitted) and a 2 compartment model recently applied to a multi-site gradient of natural and plantation forests (Garten et al. 1999; Garten and Wullschleger in press). Such models contain assumptions about the relative partitioning of recent organic matter inputs into various soil organic matter pools. While operationally defined, these pools have been shown to be comparable, in agricultural and grassland soils, to the physically defined protected and unprotected fractions to be measured in Task 4 (Buyanovsky et al. 1994, Cambardella and Elliott 1994; Trumbore and Zheng 1996). This will be a unique opportunity to evaluate and improve these models for use in forest ecosystems and use them to expand our understanding of long-term soil carbon dynamics.

First, we will develop a simple model of forest litter and soil carbon pools to utilize the time-resolved quantity and radiocarbon content collected in Task 4 to estimate the partitioning and magnitude of leaf and root (and rhyzodeposits) plant inputs into stable structures in the soil and, at least for the relatively fast turnover pools, estimate the mean residence time of these pools. Humification of surface litter layers prior to incorporation into soil mineral layers will be modeled using a cohort based model of litter decomposition (LINKAGES, Pastor and Post 1985). Soil organic matter is stabilized through association with soil minerals, incorporation into aggregates, and microbial processing into small humic compounds. We will use the directly measured carbon pools separated by the fractionation scheme of Task 4 as the compartments of our new simple model. These include unprotected POM C, microaggregate protected POM C, silt-associated C, and clay-associated C. We will use soluble C from DOC measurements made in Task 3a. This model will be implemented for 3 depth increments for which these measurements are collected. We will explore generalizing the results from this model by comparing results with the CENTURY and Rothamsted models which have been validated for many sites under widely varying environmental, edaphic, and biological conditions. Relationships between the empirically defined pools of these models and the directly measured pools of our simple model will be analyzed.

Second, the paired sites with different parent materials will allow us to determine the effectiveness of carbonaceous (1:1 clay minerals) vs. shale-derived (2:1 clay minerals) soils in stabilizing recent plant inputs. Both the Rothamsted and the CENTURY models use clay content as a correlate of this clay-organic matter interaction. We will evaluate the theory behind these formulations and usefulness for explaining differences in carbon stabilization between our sites.

Vertical transport of organic C to mineral horizons --

We will quantify the time required for plant inputs (roots, leaf litter, DOC) to migrate to mineral horizons. Relative importance of DOC transport by leaching (Task 3a), direct root input to mineral horizons (Task 4), and bioturbation (Task 3a and 3b) resulting in downward diffusion of leaf material will be evaluated by developing alternative hypotheses as model constructs and simulating the resultant distribution of radiocarbon from these sources. Data collected in tasks 2, 3, and 4 will allow fluxes between various carbon pools in different soil layers to be hypothesized and estimated. Analysis of various model linkages between layers will allow us to differentiate among these hypotheses. The TerraFlux model already contains many processes known to be important in vertical transport of DOC, including aqueous advection and diffusion, adsorption and desorption, and microbial consumption. This model can therefore provide a starting point to constrain the possible pathways and magnitudes of fluxes between layers.

Identification of the components of soil respiration --

Models including biological consumption, release, aqueous chemistry, and diffusion of CO_2 and O_2 will be valuable in quantifying the influence of short term environmental conditions on decomposer and root respiration. Ouyang and Boersma (1992), Simunek and Suarez (1993), and Fang and Moncrieff (1999) have all recently reported on such models. The model of Fang and Moncrieff (1999) may be particularly suited to this project. These models can be constrained as a result of our experimental manipulations by amount and ^{14}C content of soil respiration. We will use measurements from Task 2a to examine the following influences on CO_2 and organic carbon fluxes: (a) Litter wetting and drying dynamics (Section 3.2A), (b) root metabolism (growth and maintenance, Section 3.2A), (c) macroinvertebrate metabolism (Section 3.2A), (d) macroinvertebrate facilitated vertical transport of organic matter and humus to soil depths (Section 3.3B), and (e) formation and transport of DOM through soil (Section 3.3A).

The response of the model to reasonable estimates of the litter and root enrichment that we expect to see in this study (Figures 6 and 7) clearly show the strength of this combined experimental and modeling approach for detailed studies of soil carbon cycling processes. Figure 7 shows model simulations for integrating measurements in Task 4 and testing hypotheses. Other models and model experiments suited for each task will be used in similar fashion for integration of measurements and conceptual formulations. The proposed research tasks described above will provide direct observations of the isotopic signatures of ecosystem carbon pools and facilitate the calculation of their rates of change and transport over time. Hypotheses proposed in individual tasks can be formulated as alternative model assumptions and accepted or rejected based on radiocarbon measurements resulting from the ^{14}C pulse and various manipulations.

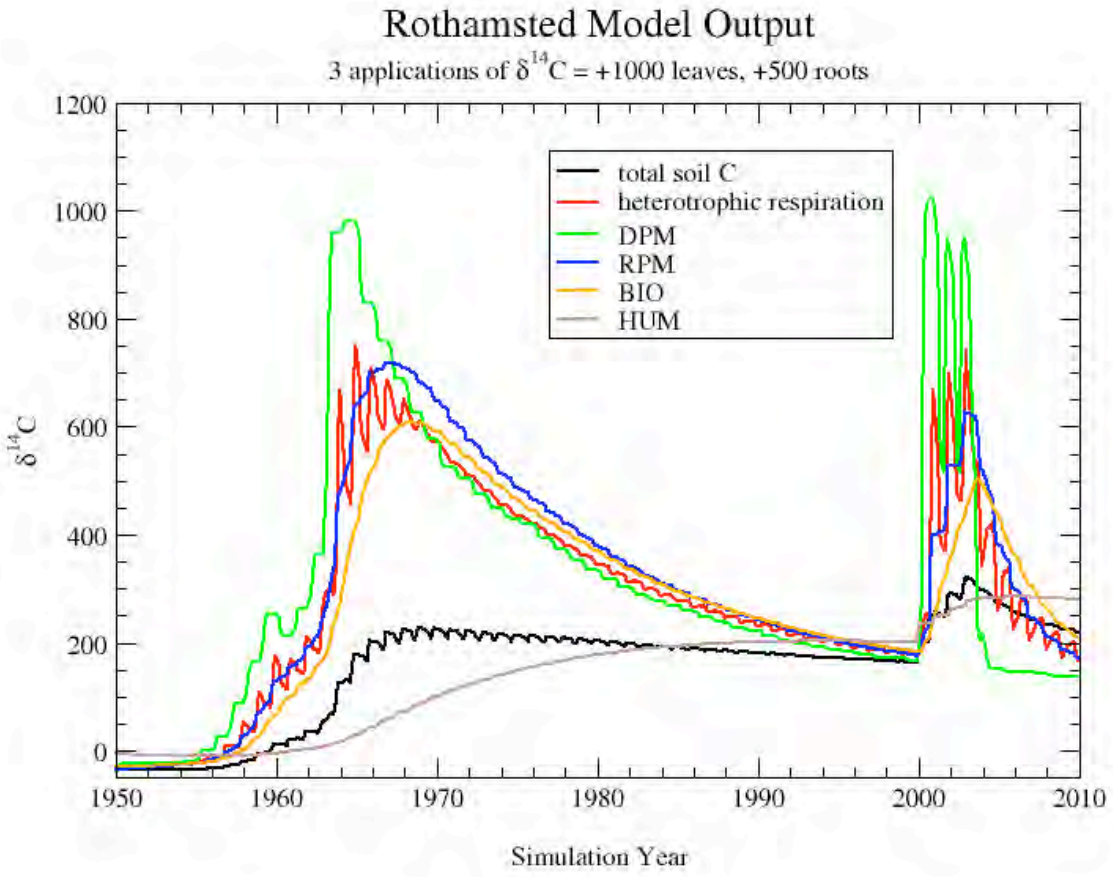

Figure 6. Simulation of the isotopic signature of soil carbon pools and fluxes from 1950 (pre-atmospheric bomb testing) through the hypothetical initiation and duration of the experiment described in this proposal. This demonstration run assumes both enriched leaf and root litter inputs. The enriched leaf litter inputs represent the 2000 cohort with a ^{14}C signature of 1000 per mill added in the fall of 2000, 2001, and 2002. Belowground enriched root inputs are assumed to be ~500 per mill in each year with ~1000 per mil inputs in late 1999. Components include the isotopic signatures of total soil C, heterotrophic respiration, decomposable plant material (DPM), resistant plant material (RPM), microbial biomass (BIO), and humified organic matter (HUM). The inert organic matter pool (IOM) is not shown since it has a turnover time of 1,500 years and remains constant during the simulation.

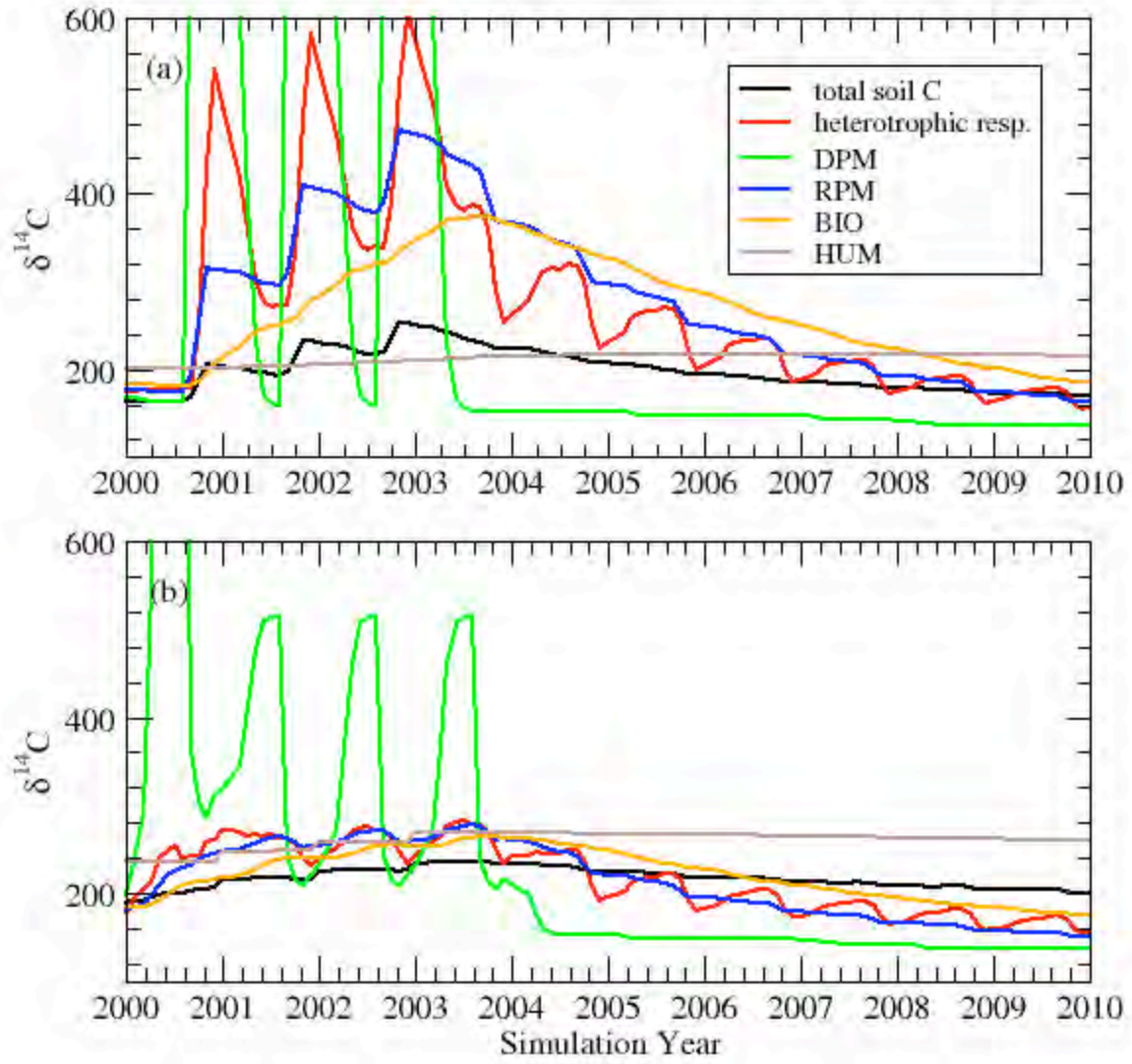

Figure 7. Individual simulations for the period following litter additions in the fall of 2000. The top figure represents a treatment where ^{14}C enriched litter is added to a site with near background root ^{14}C levels. The bottom figure would be an example for a site with near background litter, but having ^{14}C enriched roots. The y-axes are scaled the same to show the magnitude and thus resolution of the litter and root ^{14}C signature additions. Isotopic signatures of components are as described in the previous figure. While the magnitude of the leaf and root inputs are the same in these simulations, it is assumed in this simulation that half of the root inputs go directly to the HUM pool (microaggregate protection hypothesis - see Task 4a, Hypothesis 1) while the remainder enters the RPM and DPM pools in the same fashion as leaf inputs. As a result, the RPM pool (possibly equivalent to the POM C pool measured in Task 4) becomes more radiocarbon enriched in the only leaf labeled plots, and the HUM (possibly equivalent to the silt and clay associated C measured in Task 4) becomes more enriched in the only root labeled plots. The amount and 14C content of the microbial biomass pool and the respiration is dramatically different in the simulations of the 2 treatment plots and indicates the potential for evaluating hypotheses concerning microbial dynamics (Task 4c).

3.7 Research schedule and deliverables

Using the extremely unique opportunity of a whole-ecosystem label provided by the enriched isotopic release to the Oak Ridge atmosphere we will make significant inroads into understanding key issues in the terrestrial C cycle. Synthesis of the proposed research will lead to several peer-reviewed publications focusing on the following:

- The effects of seasonal and inter-annual climatic variation on the magnitude of total soil respiration and its partitioning between 1) autotrophic and heterotrophic respiration and 2) sources of heterotrophic respiration (above-ground litter decomposition versus below-ground root detritus decomposition)
- The pathways leading from leaf and root detritus to longer-term stabilization of soil organic matter, including the role of soil fauna
- The structure of fine root populations in terms of lifespan and turnover time
- The sources of dissolved organic carbon in streams and the role of DOC transport in distributing carbon within the soil profile
- Modeling net ecosystem productivity and carbon sequestration potential. This will include improvements made to existing models by facilitating better estimations of model parameters and introduction of new model components based on a more complete understanding of respiration partitioning, specific activity, turnover times, and detailed process representations.

The research tasks outlined in the project narrative (Sections 1 through Section 5) will be completed according to the schedule outlined below (Table 1). Topical reports will be provided as requested by DOE. A final project report is anticipated 6 months after the completion of all field data collection activities, but additional publications are likely to follow that report.

Table 1. Timetable of the proposed tasks for fiscal years 2000 (complete), 2001, 2002, and 2003 by month of the year. The 'X' indicates the approximate month of an activity.

Task	2000 F	2001 WSSF	2002 WSSF	2003 WSSF
Site setup	Complete			
1 Site characterization	X	X		
1 Environmental data		----------Automated----------		
1 Sampling C components	X	X	X	X
2a Components of soil respiration		XXXX	XXXX	XXXX
2b Fine root lifetimes		XXXX	XXXX	XXXX
3a Vertical transport		XXXX	XXXX	XXXX
3b Macrobiotic transport		XXXX	XXXX	
3c Watershed transport		XXXX	XXXX	
4 Forms of carbon		XXXX	XXXX	XXXX
5 ^{14}C analyses		XXXX	XXXX	XXXX
6 Inegration/modeling		XXXX	XXXX	XXXX
Deliverables				
Cooperators meeting		X	X	X
Presentations and publications		----------As prepared----------		
Final report			(6/1/2004)	

4.0 Research collaborations and consortium arrangements

The research proposed in this document builds on fundamental research being conducted under DOE's Program for Ecosystem Research (Throughfall Displacement Experiment; ERKP299), DOE's Carbon Cycle Program (Walker Branch Ameriflux sites) and fundamental results from this study will not only provide the information required to make informed terrestrial carbon management decisions, but provide information on the sensitivity of soil carbon sequestration processes to environmental changes. These data will be valuable for assessing the impact of changing future climates on long-term soil carbon stocks and nutrient cycling processes. Several projects funded under the Center for Research on Enhancing Carbon Sequestration in Terrestrial Ecosystems (CSiTE) will also benefit from the proposed work. The measurements and analysis proposed will provide complementary data for comparison with CSiTE's Task 1.1 Ecosystem and Landscape Scale Studies (Post and Izaurralde), and Task 1.2 Ecophysiological Scale Carbon Sequestration (Amthor et al.).

Consortium arrangements -- The field work proposed here will be undertaken within the boundaries of the National Environmental Research park on the Oak Ridge Reservation and will be dependent on the resources and facilities of the Department of Energy's Oak Ridge National Laboratory. Support is requested for Paul Hanson and ORNL technical staff to establish and maintain the experiment and provide support to all experimental groups through Task 1. Funding for the University of California - Irvine will support Susan Trumbore and a 3-year postdoc (splitting time between UCI and ORNL) to perform the components of soil respiration portion of this study (Task 2a). Support for Julia Gaudinski and Dev Joslin to define root lifetimes as a part of Task 2b will be provided through LLNL. ORNL will provide the lead role on the vertical transport C (Task 3a, Phil Jardine and a masters student), macrobiotic transport (Tasks 3b; Mac Callaham a postdoc), and watershed scale issues (Task 3c, Pat Mulholland). The lead role for studies of ^{14}C dynamics of unprotected and protected soil carbon pools (Task 4) will be provided by Julie Jastrow at ANL and by Margaret Torn of LBNL. The Center for Acceleratory Mass Spectrometry at Lawrence Livermore National Laboratory will provide all radiocarbon analyses for the EBIS efforts from samples prepared by the individual task investigators (Task 5). Integration and modeling of research results across the various activities (Task 6) will be lead by Mac Post (ORNL) with primary contributions from Margaret Torn (LBNL) and Susan Trumbore (UCI) and input from interactions with all other investigators.

LITERATURE CITED

Aiken,G.R., D.M. McKnight, K.A. Thorn and E.M. Thurman. 1992. Isolation of hydrophilic organic acids from water using nonionic macroporous resins. Org. Geochem. 18:567-573.

Angers, D.A., S. Recous, and C. Aita. 1997. Fate of carbon and nitrogen in water-stable aggregates during decomposition of $^{13}C^{15}N$-labeled wheat straw in situ. Eur. J. Soil Sci. 48:295-300.

Asner, G.P., A.R. Townsend, W.J. Riley, J.C. Neff, P.A. Matson, and C.C. Cleveland. (submitted). Physical and Biogeochemical Controls of Terrestrial Ecosystem Responses to Nitrogen Deposition. Biogeochemical Cycles. (submitted)

Baham, J. and G. Sposito. 1994. Adsorption of dissolved organic carbon extracted from sewage sludge on montmorillonite and kaolinite in the presence of metal ions. J. Environ. Qual. 23:147-153.

Bauer, J., P.M.Williams and E.R.M.Druffel. 1992. Recovery of sub-milligram quantities of carbon dioxide from gas streams by molecular sieve for subsequent determination of isotopic natural abundance; Anal. Chem. 64: 824-827.

Benke, M.B., A.R. Mermut and H. Shariatmadari. 1999. Retention of dissolved organic Carbon from vinasse by a tropical soil, kaolinite, and Fe-oxides. Geoderma 91:47-63.

Blair, J.M., P.J. Bohlen, C.A. Edwards, B.R. Stinner, D.A. McCartney and M.F. Allen. 1995. Manipulation of earthworm populations in field experiments in agroecosystems. Acta Zoologica Fennica 196:48-51.

Bohlen, P. J., R. W. Parmelee, J. M. Blair, C. A. Edwards and B. R. Stinner. 1995. Efficacy of methods for manipulating earthworm populations in large-scale field experiments in agroecosystems. Soil Biology and Biochemistry 27:993-999.

Boudot, J.P., Bel Hadi Brahim, A., and Chone, T. 1998. Dependence of carbon and nitrogen mineralisation upon amorphous metallic constituents and allophanes in highland soils. Geoderma, 42:245-260, 1988.

Bouché, M.B. Stratégies lombriciennes. Ecological Bulletin 25:122-132.

Buffle, J. 1988. Complexation reactions in aquatic systems: An analytical approach. Ellis Horwood Limited, Chichester, England.

Buyanovsky, G.A., M. Aslam, and G.H. Wagner 1994. Carbon turnover in soil physical fractions. Soil Sci. Soc. Am. J. 58:1167-1173.

Cambardella, C. A., and E. T. Elliott. 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Sci. Soc. Am. J. 56:777-783.

Christensen, B.T. 1996. Carbon in primary and secondary organomineral complexes. p. 97-165. In: M.R. Carter and B.A. Stewart (eds.) Structure and Organic Matter Storage in Agricultural Soils. CRC Press, Inc., Boca Raton, FL.

Coleman, D.C. and D.A. Crossley, Jr. 1996. Fundamentals of Soil Ecology. Academic Press, San Diego.

Coleman, D.C. and P.F. Hendrix (editors). 2000. Invertebrates as webmasters in ecosystems. CABI Publishing, New York.

Cronan, C.S. and C.R. Aiken. 1985. Chemistry and transport of soluble humic substances in forested watersheds of the Adirondack Park, New York. Geochim. Cosmochim. Acta. 49:1697-1705.

Currie, W.S., J.D. Aber, W.H. McDowell, R.D. Boone, and A.H. Magill. 1996. Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. Biogeochemistry. 35:471-505.

Dalal, R.C. and B.J. Bridge. 1996. Aggregation and organic matter storage in sub-humid and semi-arid soils. p. 263-307. In: M.R. Carter and B.A. Stewart (eds.) Structure and Organic Matter Storage in Agricultural Soils. CRC Press, Inc., Boca Raton, FL.

Davidson, E. A. and S. E. Trumbore. 1995. Gas diffusivity and production of CO_2 in deep soils of the eastern Amazon, Tellus. 47B:550-565.

Davidson, E.A., E. Belk, and R.D. Boone. 1998. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Global Change Biology 4:217-227.
Davis , J.A. and R. Glour. 1981. Adsorption of dissolved organics in lake water by aluminum oxide. Effect of molecular weight. Environ. Sci. Technol. 15:1223-129.
Dormaar, J.F. and R.C. Foster. 1991. Nascent aggregates in the rhizosphere of perennial ryegrass (Lolium perenne L.). Can. J. Soil Sci. 71:465-474.
Dunnivant, F.M., P.M. Jardine, D.L. Taylor, and J.F. McCarthy. 1992. Transport of naturally occurring dissolved organic carbon in laboratory columns containing aquifer material. Soil Sci. Soc. Am. J. 56:437-444.
Edwards NT 1975. Effects of temperature and moisture on carbon dioxide evolution in a mixed deciduous forest floor. Soil Sci. Soc. Amer. J. 39:361-365
Edwards NT and P. Sollins. 1973. Continuous measurement of carbon dioxide evolution from partitioned forest floor components. Ecology 54:406-412
Edwards NT, W.F. Harris and H.H. Shugart. 1977. Carbon cycling in deciduous forest. In The Belowground Ecosystem: A Synthesis of Plant-Associated Processes, J.K. Marshall ed., Range Science Department Science Series No. 26, Colorado State University, Fort Collins, Colorado. pp. 153-157
Elliott, E.T. and D.C. Coleman. 1988. Let the soil work for us. Ecol. Bullet. 39:23-32.
Foster, R.C. 1985. In situ localization of organic matter in soils. Quaest. Ent. 21:609-633.
Fang, C. and J.B. Moncrieff. 1998. An open-top chamber for measuring soil respiration and the influence of presure difference on CO2 efflux measurement. Functional Ecol. 12:319-325.
Gale, W.J. and C.A. Cambardella. 2000. Carbon dynamics of surface residue- and root-derived organic matter under simulated no-till. Soil Sci. Soc. Am. J. 64:190-195.
Gale, W.J, C.A. Cambardella, and T.B. Bailey. 2000a. Surface residue- and root-derived carbon in stable and unstable aggregates. Soil Sci. Soc. Am. J. 64:196-201.
Gale, W.J, C.A. Cambardella, and T.B. Bailey. 2000b. Root-derived carbon and the formation and stabilization of aggregates. Soil Sci. Soc. Am. J. 64:201-207.
Garten, C. T., Jr., and S. D. Wullschleger. (in press). Soil carbon dynamics beneath switchgrass as indicated by stable isotope analysis. J. Environ. Qual. 29:(in press).
Garten, C.T. Jr., W.M. Post III, P.J. Hanson, and L.W. Cooper. 1999. Forest soil carbon inventories and dynamics along an elevation gradient in the southern Appalachian Mountains. Biogeochemistry 45:115-145.
Garten, C.T., L.W. Cooper, W.M. Post III, and P.J. Hanson. 2000. Climate controls on forest soil C isotopes ratios in the southern Appalachian Mountains. Ecology 8(14): 1108-1119.
Gaudinski, JB, SE. Trumbore, PJ Hanson, and J Southon. 2000a. A whole ecosystem radiocarbon label in a southeastern hardwood forest near Oak Ridge, TN, USA. Abstract Amer. Geophys. Union, 2000 Fall Meeting.
Gaudinski, JB, S.E. Trumbore, E.A. Davidson and S. Zheng. 2000b. Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes. Biogeochemistry 51:33-69
Gaudinski JB, S.E. Trumbore, E.A. Davidson A. Cook, D. Markewitz, and D. Richter (in review). Heterogeneity of fine root dynamics measured by radiocarbon. Oecologia. (in review).
Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clark. 1994. Soil structure and carbon cycling. Aust. J. Soil Res. 32:1043-1068.
Gregorich, E.G., R.G. Kachanoski, and R.P. Voroney. 1989. Carbon mineralization in soil size fractions after various amounts of aggregate disruption. J. Soil Sci. 40:649-659.
Grigal, D.F. and R.A. Goldstein. 1971. An integrated ordination-classification analysis of an intensively sampled oak-hickory forest. J. Ecol. 59: 481-492.
Gu, B., J. Schmitt, Z. Chen, L. Liang, and J.F. McCarthy. 1995. Adsorption and desorption of different organic matter fractions on iron oxide. Geochim. Cosmochim. Acta. 59:219-229.

Gu, B., T.L. Mehlhorn, L. Liang, and J.F. McCarthy. 1996a. Competitive adsorption, displacement, and transport of organic matter on iron oxide: I. Competitive adsorption. Geochim. Cosmochim. Acta. 60:1943-1950.

Gu, B., T.L. Mehlhorn, L. Liang, and J.F. McCarthy. 1996b. Competitive adsorption, displacement, and transport of organic matter on iron oxide: II. Displacement and transport. Geochim. Cosmochim. Acta. 60:2977-2992.

Hanson, P.J., M.L. Chambers, J. S. Riggs, T.L. Misek and J.B. Stringfellow. 1996. Litter CO_2 Efflux: Daily Dynamics from Field Measurements and Model Extrapolations. Agronomy Abstracts, Agronomy Society of America, p. 300.

Hanson, P. J., S. D. Wullschleger, S. A. Bohlman, and D. E. Todd. 1993. Seasonal and topographic patterns of forest floor CO_2 efflux from an upland oak forest. Tree Physiology 13:1-15.

Hanson, P.J., N.T. Edwards1, C.T. Garten Jr. and J.A. Andrews. 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry 48:115-146.

Hanson, P.J., D.E. Todd, N.T. Edwards, and M.A. Huston. 1995. Field performance of the Walker Branch Throughfall Displacement Experiment, Pages 307-313, in A. Jenkins, R.C. Ferrier, and C. Kirby, editors, Ecosystem Manipulation Experiments: scientific approaches, experimental design and relevant results, Ecosystem Research Report #20, Commission of the European Communities.

Hanson, P.J., D.E. Todd, M.A. Huston, J.D. Joslin, Jennifer Croker, and R. M. Augé. 1998. Description and field performance of the Walker Branch Throughfall Displacement Experiment: 1993-1996, ORNL/TM-13586, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Harris, W. F., R. A. Goldstein, and G. S. Henderson. 1973. Analysis of forest biomass pools, annual primary production and turnover of biomass for a mixed deciduous forest watershed, pp. 41-64. IN (H. E. Young, ed.) Proceedings IUFRO Symposium Working Party on Forest Biomass. University of Maine Press, Orono, Maine.

Harris, W. F., R. S. Kinerson, Jr., and N. T. Edwards. 1977. Comparison of belowground biomass of natural deciduous forest and loblolly pine plantations. Pedobiologia 17:369-381.

Hendrix, P.F. (editor). 1995. Earthworm ecology and biogeography in North America. Lewis Publishers, Boca Raton.

Hinton, M. J., S. L. Schiff, and M. C. English. 1998. Sources and flowpaths of dissolved organic carbon during storms in two forested watersheds of the Precambrian Shield. Biogeochemistry 41:175-197.

Hope, D., M F. Billett, R. Milne, and T. A. W. Brown. 1997. Exports of organic carbon in British rivers. Hydrological Processes 11:325-344.

Hornberger, G. M., K. E. Bencala, and D. M. McKnight. 1994. Hydrological controls on dissolved organic carbon during snowmelt in the Snake River near Montezuma, Colorado. Biogeochemistry 25:147-165.

IGBP, International Geosphere-Biosphere Programme (IGBP) Terrestrial Carbon Working Group. 1998. The terrestrial carbon cycle: implications for the Kyoto protocol. Science 280: 1393-1394.

IPCC, Intergovernmental Panel on Climate Change. 1996. Climate change 1995: impacts, adaptations, and mitigation of climate change: scientific-technical analyses. R.T. Watson, M.C. Zinyowera, and R.H. Moss, eds., Cambridge University Press, Cambridge, pp. 745-798.

Jacobs, G.K. 1999. Center for Research on Enhancing Carbon Sequestration in Terrestrial Ecosystems. U.S. Dept. of Energy, Climate Change Technology Initiative.

Jardine, P.M., G.V. Wilson, and R.J. Luxmoore. 1988. Modeling the transport of inorganic ions through undisturbed soil columns from two contrasting watersheds. Soil Sci. Soc. Am. J. 52:1252-1259.

Jardine, P.M., G.V. Wilson, R.J. Luxmoore, and J.F. McCarthy. 1989a. Transport of inorganic and natural organic tracers through an isolated pedon in the field. Soil Sci. Soc. Am. J. 53:317-323.

Jardine, P.M. N.L. Weber, J.F. McCarthy. 1989b. Mechanisms of dissolved organic carbon adsorption by soil. Soil Sci. Soc. Am. J. 53:1378-1385.

Jardine, P.M., G.V. Wilson, and R.J. Luxmoore. 1990a. Unsaturated solute transport through a forest soil during rain events. Geoderma. 46:103-118.
Jardine, P.M., G.V. Wilson, J.F. McCarthy, R.J. Luxmoore, and D.L. Taylor. 1990b. Hydrogeochemical processes controlling the transport of dissolved organic carbon through a forested hillslope. J. Contaminant Hydrology. 6:3-19.
Jardine, P.M., G.K. Jacobs, and G.V. Wilson. 1993a. Unsaturated transport processes in undisturbed heterogeneous porous media. I. Inorganic Contaminants. Soil Sci. Soc. Am. J. 57:945-953.
Jardine, P.M., G.K. Jacobs, and J.D. O'Dell. 1993b. Unsaturated transport processes in undisturbed heterogeneous porous media II. Co-Contaminants. Soil Sci. Soc. Am. J. 57:954-962.
Jardine, P.M., R. O'Brien, Wilson, G.V., and J.P. Gwo. 1998. Experimental techniques for confirming and quantifying physical nonequilibrium processes in soils. p. 243-271. (In) H.M. Selim and L. Ma. Physical Nonequilibrium in Soils: Modeling and Application. Ann Arbor Press, Inc. Chelsea, Michigan.
Jastrow, J.D., T.W. Boutton, and R.M. Miller. 1996. Carbon dynamics of aggregate-associated organic matter estimated by carbon-13 natural abundance. Soil Sci. Soc. Am. J. 60:801-807.
Jenkinson, D.S. 1990. The turnover of organic carbon and nitrogen in soil. Phil. Trans. Royal Soc. London Series B - Biological Sciences 329:361-368.
Jenkinson, D. S. and D. S. Powlson 1976. The effects of biocidal treatments on metabolism in soil--V. A method for measuring soil biomass. Soil Biology and Biochemistry 8:209-213.
Johnson, D.W. and D.E. Todd Jr. 1998. Harvesting effects on long-term changes in nutrient pools of mixed oak forest. Soil Sci. Soc. Amer. J. 62:1725-1735.
Johnson, D. W., and R. I. Van Hook (eds.). 1989. Analysis of Biogeochemical Cycling Processes in Walker Branch Watershed. Springer-Verlag, New York. 401 p.
Johnson, D.W., and P.S. Curtis. (in press) The effects of forest management on soil carbon storage: Meta analysis. For. Ecol. Manag. (in press).
Johnson, M.G. 1995. The role of soil management in sequestering soil carbon. pp. 351-363. In: R. Lal, J. Kimble, E. Levine, and B.A. Stewart, eds. Soil management and greenhouse effect. Lewis Publishers, Boca Raton.
Joslin, J.D. and G.S. Henderson. 1982. A test of the budget method: A revised approach to the measurement of fine root turnover, In R. N. Muller (ed.), Central Hardwood Forest Conference, Proceedings of the Fourth Meeting, 1982, Univ. Kentucky, Lexington. p. 220-228.
Joslin, J. D. and M. H. Wolfe. 1998. Impacts of long-term water input manipulations on fine root production and mortality in mature hardwood forests. Plant and Soil 204:165-174.
Joslin, J.D., M.H. Wolfe, and P.J. Hanson. (in press) Factors controlling the timing of root elongation intensity in a mature upland oak stand. Plant and Soil (in press).
Joslin, J.D., M.H. Wolfe, and P.J. Hanson. 2000. Effects of shifting water regimes on forest root systems. New Phytologist 147:117-129.
Keeland, B.D. and R.R. Sharitz. 1993. Accuracy of tree growth measurements using dendrometer bands. Canadian Journal of Forest Research 23:2454-2457.
Kooner, Z.S., P.M. Jardine, and S. Feldman. 1995. Competitive surface complexation reactions of SO_4^{2-} and natural organic carbon on soil. J. Environ. Qual. 24:656-662.
Kooistra, M.J. and M. van Noordwijk. 1996. Soil architecture and distribution of organic matter. p. 15-56. In: M.R. Carter and B.A. Stewart (eds.) Structure and Organic Matter Storage in Agricultural Soils. CRC Press, Inc., Boca Raton, FL.
Leenheer, J.A. 1981. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. Environ. Sci. Technol. 15:578-587.
Litaor, M.I. 1988. Review of soil solution samplers. Water Resour. Res. 24:727-733.
Luxmoore, R.J. 1983. Water budget of an eastern deciduous forest stand. Soil Sci. Soc. Am. J. 47:785-791

Magid, J., A. Gorissen, and K.E. Giller. 1996. In search of the elusive "active" fraction of soil organic matter: Three size-density fractionation methods for tracing the fate of homogeneously ^{14}C-labelled plant materials. Soil Biol. Biochem. 28:89-99.

Martin, J.P. and K. Haider. 1986. Influence of mineral colloids on turnover rates of soil organic carbon. p. 283-304. In P.M. Huang and M. Schnitzer (eds.) Interactions of Soil Minerals with Natural Organics and Microbes. Spec. Publ. 17, Soil Sci. Soc. Am., Madison, WI.

Masiello, C.A., M.T. Torn, O.A. Chadwick and J.S. Southon. (in preparation) Amorphous mineral control on soil carbon storage across a northern California soil chronosequence.

McCarthy, J.F., T.M. Williams, L. Liang, P.M. Jardine, A.V. Palumbo, L.W. Cooper, L.W. Jolley, and D.L. Taylor. 1993. Mobility of natural organic matter in a sandy aquifer. Environ. Sci. Technol. 27:667-676.

McCarthy, J.F., B. Gu, L. Liang, J. Mas-Pla, T.M. Williams, and T.C.J. Yeh. 1996. Field tracer tests on the mobility of natural organic matter in a sandy aquifer. Water Resourc. Res. 32:1223-1228.

McGuire, P.E., B. Lowery, and P.A. Helmke. 1992. Potential sampling error: Trace metal adsorption on vacuum porous cup samplers. Soil Sci. Soc. Am. J. 56:74-82.

McLaughlin, S.B. and D.J. Downing. 1996. Interactive effects of ambient ozone and climate measured on growth of mature loblolly pine trees. Canadian Journal of Forest Research 26: 670-681.

McFee, W.W. and J. M. Kelly (eds.) 1995. Carbon Forms and Functions in Forest Soils, Soil Science Society of America, Inc., Madison, WI.

Meybeck, M. 1993. Riverine transport of atmospheric carbon: sources, global typology and budget. Water, Air and Soil Pollution 70:443-464.

Monreal, C.M., H.-R. Schulten, and H. Kodama. 1997. Age, turnover and molecular diversity of soil organic matter in aggregates of a Gleysol. Can. J. Soil Sci. 77:379-388.

Mulholland, P. J., G. V. Wilson, and P. M. Jardine. 1990. Hydrogeochemical response of a forested watershed to storms: effects of preferential flow along shallow and deep pathways. Water Resources Research 26:3021-3036.

Oades, J.M. 1984. Soil organic matter and structural stability, mechanisms and implications for management. Plant Soil 76:319-337.

Oades, J.M. 1988. The retention of organic matter in soils. Biogeochemistry, 5:35-70.

Oades, J.M. and A.G. Waters. 1991. Aggregate hierarchy in soils. Aust. J. Soil Res. 29:815-828..

Ouyang, Y. and L. Boersma. 1992. Dynamic oxygen and carbon dioxide exchange between soil and atmosphere: I. Model development. Soil Sci. Soc. Amer. J. 56:1695-1702.

Parkinson, D. and M.A. McLean. 1998. Impacts of earthworms on the community structure of other biota in forest soils. Pages 213-226 in C.A. Edwards (editor), Earthworm Ecology, St. Lucie Press, Boca Raton.

Parmelee, R.W., M.H. Beare, W. Cheng, P.F. Hendrix, S.J. Rider, D.A. Crossley, Jr., and D.C. Coleman. 1990. Earthworms and enchytraeids in conventional and no-tillage agroecosystems: a biocide approach to assess their role in organic matter breakdown. Biology and Fertility of Soils 10:1-10.

Parton, W.J., J.W.B. Stewart, and C.V. Cole. 1988. Dynamics of C, N, P and S in grassland soils: a model. Biogeochemistry 5:109-131.

Pastor, J. and W.M. Post. 1985. Development of a linked forest productivity-soil process model. ORNL/TM9519, Oak Ridge, TN.

Paul, E.A., R.F. Follett, S.W. Leavitt, A. Halvorson, G.A. Peterson, and D.J. Lyon. 1997. Radiocarbon dating for determination of soil organic matter pool sizes and dynamics. Soil Sci. Soc. Am. J. 61:1058-1067.

Paul, E.A., D. Harris, H.P. Collins, U. Schulthess, and G.P. Robertson. 1999. Evolution of CO_2 and soil carbon dynamics in biologically managed row-crop agroecosystems. Applied Soil Ecology 11:53-65.

Post, WM and KC Kwon 2000. Soil Carbon Sequestration and Land-Use Change: Processes and Potential. Global Change Biology 6:317-328.

Post, W. M., King, A. W., and S. D. Wullschleger 1996. Soil organic matter models and global estimates of soil organic carbon. pp. 201-222. IN (P. Smith, J. Smith and D. Powlson, eds.) {\sl Evaluation of Soil Organic Matter Models Using Existing Long-Term Datasets}. Springer-Verlag, Berlin.

Post, W.M., T-H Peng, W.R. Emanuel, A.W. King, V.H. Dale, and D.L. Deangelis. 1990. The Global Carbon Cycle. Amer. Sci. 78:310-326.

Reedy, O.C., P.M. Jardine, G.V. Wilson, and H.M. Selim. 1996. Quantifying diffusive mass transfer of non-reactive solutes in columns of fractured saprolite using flow interruption. Soil Sci. Soc. Am. J. 60:1376-1384.

Roden, JS; Ehleringer, JR. 1999. Hydrogen and oxygen isotope ratios of tree-ring cellulose for riparian trees grown long-term under hydroponically controlled environments. Oecologia, V121 N4:467-477.

Saggar, S., K. R. Tate, C. W. Feltham, C. W. Childs and A. Parshotam. 1994. Carbon turnover in a range of allophanic soils amended with 14C-labelled glucose. Soil Biology and Biochemistry 26(9): 1263-1271.

Schiff, S. L., R. Aravena, S. E. Trumbore, M. J. Hinton, R. Elgood, and P. J. Dillon. 1997. Export of DOC from forested catchments on the Precambrian Shield of central Ontario: clues from 13C and ^{14}C. Biogeochemistry 36:43-65.

Schimel, D.S. 1995. Terrestrial ecosystems and the carbon cycle. Global Change Biology 1:77-91.

Schimel, D. S., B. H. Brasswell, E. Holland, R. McKeown, D. S. Ojima, T. H. Painter, W. J. Parton and A. R. Townsend. 1994. Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils. Global Biogeochemical Cycles 8(3): 279-294.

Sibanda, H.M. and S.D. Young. 1986. Competitive adsorption of humus acids and phosphate on goethite, gibbsite and two tropical soils. Journal of Soil Science 37:197-204.

Simunek, J. and D.L. Suarez. 1993. Modeling of carbon dioxide transport and production in soil I. Model Development. Water Resources Res. 29:487-497.

Six, J., E.T. Elliott, and K. Paustian. Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. (in press).

Six, J., K. Paustian, E.T. Elliott, and C. Combrink. 2000. Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Sci. Soc. Am. J. 64:681-688.

Southon, J.R. M.W. Caffee, J.C. Davis, T.L. Moore, I.D. Proctor, B.Schumacher and J.S.Vogel, 1990. The new LLNL AMS spectrometer, Nucl. Instr. and Meth. B52:301-305.

Stott, E., G. Kassin, W.M. Jarrell, J.P. Martin, and K. Haider. 1983. Stabilization and incorporation into biomass of specific plant carbons during biodegradation in soil. Plant Soil 70:15-26.

Stuiver, M. and H. Polach. 1977. Reporting of ^{14}C data. Radiocarbon 19:355-363.

Swift, M.J., O.W. Heal, and J.M. Anderson. 1979. Decomposition in terrestrial ecosystems. University of California Press, Berkeley.

Tipping, E. 1981a. Adsorption by goethite (-FeOOH) of humic substances from three different lakes. Chem. Geol. 33:81-89.

Tipping, E. 1981b. The adsorption of aquatic humic substances by iron oxides. Geochim. Acta 45:191-199.

Tisdall, J.M. 1996. Formation of soil aggregates and accumulation of soil organic matter. p. 57-96. In: M.R. Carter and B.A. Stewart (eds.) Structure and Organic Matter Storage in Agricultural Soils. CRC Press, Inc., Boca Raton, FL.

Tisdall, J.M. and J.M. Oades. 1982. Organic matter and water stable aggregates in soils. J. Soil Sci. 33:141-163.

Todd, D.E. and P.J. Hanson. 1999. Sustained Precipitation Manipulations Alter Forest Litter Mass in an Oak Forest. Abstracts from the Annual Meeting of the Soil Science Society of America, October 31-November 14, 1999, Salt Lake City, Utah, p. 295.

Torn, M. S., S.E. Trumbore, O.A. Chadwick, P.M. Vitousek, and D.M. Hendricks. 1997. Mineral Control of Soil Organic Carbon Cycling. Nature, 389:170-173.

Torn, M.S. , E. Zavaleta, and C. Field. 2000. Quantifying carbon cycling dynamics using both 13C and 14C label from elevated CO2 experiments. Presented at the 17th International Radiocarbon Conference, Judean Hills, Israel.

Trettin, C.C., D.W. Johnson, and D.E. Todd Jr. 1999. Forest nutrient cycling and carbon pools at Walker Branch Watershed: Changes during a 21-year period. Soil Sci Soc. Amer. J. 63:1436-1448.

Trumbore, S.E. 1997. Potential responses of soil organic carbon to global environmental change. Proceedings of the National Academy of Sciences of the United States of America 94: 8284-8291.

Trumbore, S. 2000. Age of soil organic matter and soil respiration: Radiocarbon constraints on belowground C dynamics. Ecology Applications:10:399-411.

Trumbore, S.E., and S. Zheng. 1996. Comparison of fractionation methods for soil organic matter ^{14}C analysis. Radiocarbon, 38:219-229.

van Veen, J.A. and P.J. Kuikman. 1990. Soil structural aspects of decomposition of organic matter by micro-organisms. Biogeochem. 11:213-233.

Vogel, J. S. 1992. A rapid method for preparation of biomedical targets for AMS. Radiocarbon 34:344-350.

Vogel, J.S., J.R. Southon, D.E. Nelson, and T.A. Brown, 1984. Performance of catalytically condensed carbon for use in accelerator mess spectrometry, Nucl. Instr. and Meth. B5:289-293.

Wan, Y. and S.A. El-Swaify. 1998. Sediment enrichment mechanisms of organic carbon and phosphorous in a well-aggregated Oxisol. Journal of Environmental Quality 27:132-138.

Wilson, G.V. and R.J. Luxmoore. 1988. Infiltration, macroporosity, and mesoporosity distributions on two forested watersheds. Soil Sci. Soc. Am. J. 52:329-335.

Wilson, G.V., J.M. Alfonsi, and P.M. Jardine. 1989. Spatial variability of subsoil hydraulic properties of two forested watersheds. Soil Sci. Soc. Am. J. 53:679-685.

Wilson, G.V., P.M. Jardine, R.J. Luxmoore, and J.R Jones. 1990. Hydrology of a forested watershed during storm events. Geoderma. 46:119-138.

Wilson, G.V., P.M. Jardine, R.J. Luxmoore, L.W. Zelazny, D.A. Lietzke, and D.E. Todd. 1991a. Hydrogeochemical processes controlling subsurface transport from an upper subcatchment of Walker Branch watershed during storm events: 1. Hydrologic transport processes. J. Hydrology. 123:297-316.

Wilson, G.V., P.M. Jardine, R.J. Luxmoore, L.W. Zelazny, D.E. Todd, and D.A. Lietzke. 1991b. Hydrogeochemical processes controlling subsurface transport from an upper subcatchment of Walker Branch watershed during storm events: 2. Solute transport processes. J. Hydrology. 123:317-336.

Wilson, G.V., P.M. Jardine, J.D. O'Dell, and M. Collineau. 1993. Field-scale transport from a buried line source in unsaturated soil. J. Hydrology 145:83-109.

Wilson, G.V., J.P. Gwo, P.M. Jardine, and R.J. Luxmoore. 1998. Hydraulic and physical nonequilibrium effects on multi-region flow and transport. p. 37-61 (In) H.M. Selim and L. Ma. Physical Nonequilibrium in Soils: Modeling and Application. Ann Arbor Press, Inc. Chelsea, Michigan.

Wilson, K.B., P.J. Hanson, and D.D. Baldocchi. 2000. Factors controlling evaporation and energy partitioning beneath a deciduous forest over an annual cycle. Agric. For. Meteor. 102:83-103.

Zak, D.R., K.S. Pregitzer, J.S. King, and W.E. Holmes. 2000. Elevated CO2, fine roots and the response of soil microorganisms: a review and hypothesis. New Phytol. 147:201-222.

EFFORT BY TASK (Proposed 3-year totals)

The following table shows individual investigator responsibilities by task and the number of person months allocated.

Table 2. Proposed science effort (person months = PM) over the 3-year project broken down by investigator and task as defined above. Individual investigators are listed by last names and institutional affiliation. ORNL is Oak Ridge National Laboratory, UCI is University of California, Irvine, LLNL is Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, Livermore, LBNL is Lawrence Berkely National Laboratory, ANL is Argonne National Laboratory, and BFR is Belowground Forestry Research.

Investigator (Affiliation)	Person Months By Primary Tasks Over 3 years											3-year PM by Investigator
	1	2a	2b	3a	3b	3c	4a	4b	4c	5	6	
Scientific Staff												
Garten (ORNL)	--	--	--	--	--	--	1	--	--	--	0.5	1.5
Hanson (ORNL)	3	2	--	--	--	--	--	--	--	--	--	5.0
Jardine (ORNL)	--	--	--	1.5	--	--	--	--	--	--	--	1.5
Jastrow (ANL)	--	--	--	--	--	--	3	--	--	--	--	3.0
Joslin (LBNL-sub)	3	--	6	--	--	--	--	--	--	--	--	9.0
Mulholland (ORNL)	--	--	--	--	--	1.5	--	--	--	--	--	1.5
Post (ORNL)	--	--	--	--	--	--	--	--	--	--	4	4.0
Southon (LLNL)	--	--	--	--	--	--	--	--	--	9	--	9.0
Torn (LBNL)	--	--	--	--	--	--	--	2	1	--	1.5	4.5
Trumbore (UCI)	--	1.5	--	--	--	--	--	--	--	--	1.5	3.0
Postdoctoral Res. Assoc.												
Callaham (ORNL)	--	--	--	--	6	--	--	--	--	--	--	6.0
Gaudinski (LBNL)	--	0.5	4	--	--	--	--	--	--	--	--	4.5
Masiello (LLNL/LBNL)	--	--	--	--	--	--	--	--	16.5	9	--	25.5
Riley (LBNL)	--	--	--	--	--	--	--	--	--	--	6	6.0
Unnamed (UCI)	--	24	--	--	--	--	--	--	--	--	12	36.0
Unnamed (ANL)	--	--	--	--	--	--	18	--	--	--	--	18.0
Postmaster Res. Assoc.												
Unnamed (ORNL)	--	--	--	36	--	--	--	--	--	--	--	36.0
Technical staff												
Todd (ORNL)	3	--	--	2	--	--	--	--	--	--	--	5.0
Zheng (UCI)	--	6	--	--	--	--	--	--	--	--	--	6.0
Unnamed (ORNL)	6	--	--	4	--	2	--	--	--	--	--	12.0
Frantz/Zermeno (LLNL)	--	--	--	--	--	--	--	5	--	6	--	11.0
Unnamed (LBNL)	--	--	9	--	--	--	--	3	4	--	--	16.0
Unnamed (ANL)	--	--	--	--	--	--	12	--	--	--	--	12.0
3-year PM by Task	15	34	19	43.5	6	3.5	34	10	21.5	24	25.5	236