UNIVERSITY OF CALIFORNIA,
IRVINE

Using a ^{14}C Release to Partition Soil Respiration Sources in a Southeastern Hardwood Forest

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Earth System Science

by

Luz Maria Cisneros Dozal

Dissertation Committee:
Professor Susan E. Trumbore, Chair
Professor William S. Reeburgh
Professor Michael L. Goulden

2005

The dissertation of Luz Maria Cisneros Dozal
is approved and is acceptable in quality
and form for publication on microfilm:

_____

_____

_____

Committee Chair

University of California, Irvine
2005

DEDICATION

To

My Family
for their support and
encouragement

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the excellent guidance and support from my advisor Dr. Susan Trumbore. Her enthusiasm for science and dedication greatly motivated me to go forward every time I left her office. I thank Sue for always finding the time to advice me and for providing the help needed in the field and laboratory, as well as for her constant solidarity. Her kindness and disposition to recognize the efforts of the people in her team work are a great example to follow.

I thank Dr. William S. Reeburgh and Dr. Michael Goulden for their valuable advice and insightful comments on the first draft of this dissertation, and for their time and good disposition. I am very grateful to many other people who were involved in this work. My deepest appreciation to Shuhui Zheng for providing me with valuable training, who showed me the discipline and insight needed to be a scientist and who helped me to solve problems in countless times during my research work. I thank Xiaomei Xu for training in mass spectrometry and for her support and willingness to help at any time. I thank Nicole Nowinski and Kelsey McDuffee for help in the field and in the laboratory.

I thank Dr. Paul Hanson at Oak Ridge National Laboratory for his support since the very first minute I joined the EBIS project, for his help and supervision in the field, for always finding the time to assist me, for the revision of my first two manuscripts and for providing insightful ideas. I thank Dr. Donald Todd for his valuable assistance in the field and for those chats that made the preparation for field work more pleasant. Many thanks to Dr. Christopher Swanston, Dr. Thomas Guilderson and Dr. Michaele

Kashgarian for their work at Lawrence Livermore National Laboratory on measuring my sample targets and providing me with data in a timely manner. Thanks to Dr. John Southon and Dr. Guaciara Dos Santos for training in mass spectrometry and for being always available to help at the Keck Carbon Cycle Mass Spectrometer facility in UCI. I thank Gregory Winston for help with instrumentation in the field and for revision of my second manuscript. I would also like to thank the administrative personnel for their valuable assistance, Department Manager Elizabeth Ford, Payroll Coordinator Jeffrey Beckwith and Student Affairs Coordinator Cynthia Dennis.

I thank my family for their love and for being closer than ever, in spite of the distance. Their endless support in the bad and in the good times and those conversations over the phone imparted me with the strength necessary to reach the goal. I thank my dear friend Tibisay for her advice and support throughout graduate school and since we met in 1998, and for her friendship. Thanks to Christa and Margaret for their friendship and for those fun times on Friday nights. Thanks to Steve, Chia-Chi (my only other classmate) and Adrian for nice conversations, and to students in earlier years whom I did not get the chance to know better but certainly enjoyed chatting with, Kai, Gopi, Aparna, Fengpeng and Leah.

The text of Chapter 2 of this dissertation is a reprint of the material as it appears in *Global Change Biology* (doi:10.1111/j.1365-2486.2005.01061.x, 2005). I thank Blackwell Publishing Ltd for granting me permission to include this text. The co-author Susan E. Trumbore listed in this publication directed and supervised research which forms the basis for this dissertation. The co-author Paul J. Hanson also listed in this publication contributed significantly to this paper.

The EBIS project is sponsored by the U.S. Department of Energy, Office of Science, Biological and Environmental Research Program as a part of the program on Terrestrial Carbon Processes (TCP). Work conducted by Oak Ridge National Laboratory personnel was conducted under contract No. DE-ACO5 00OR22725 with UT-Battelle, LLC. Financial support for my graduate studies was provided by the Department of Earth System Science in the University of California, Irvine, by a NASA Earth System Science Graduate Student Fellowship (2003-2006) and by NSF EAR-0223514 for carbon cycle research.

CURRICULUM VITAE

Luz Maria Cisneros Dozal

EDUCATION

Ph.D. in Earth System Science, University of California, Irvine, California, USA (2005)

M.S. in Earth System Science, University of California, Irvine, California, USA (2002)

B.S. in Chemical Engineering, Instituto Tecnologico de Sonora, Cd. Obregon, Sonora, Mexico (1992)

RESEARCH EXPERIENCE

Research Assistant, Department of Earth System Science, University of California, Irvine, California, USA (1999-2005)

Research Assistant, International Maize and Wheat Improvement Center (CIMMYT), Cd. Obregon, Sonora, Mexico (1994-1999)

TEACHING EXPERIENCE

Teaching Assistant, Department of Earth System Science, University of California, Irvine, California, USA (2001-2002)

FELLOWSHIP
NASA Earth System Science (ESS) Fellowship (2003-2006)

FIELD OF STUDY

Radiocarbon measurements for the study of soil respiration sources

PEER REVIEWED PUBLICATIONS

Cisneros-Dozal LM, Trumbore S and Hanson PJ (2005) Partitioning Sources of Soil-Respired CO_2 and Their Seasonal Variation Using a Unique Radiocarbon Tracer. *Global Change Biology* 11, 1-11, doi: 10.1111/j.1365-2486.2005.01061.x

Cisneros-Dozal LM, S. Trumbore, P. J. Hanson and G. Winston (in revision). The Effect of Moisture on Leaf Litter Decomposition and its Contribution to Soil Respiration in a Temperate Forest. For submission to Journal of Geophysical Research-Biogeoscience

CURRICULUM VITAE

Luz Maria Cisneros Dozal

PUBLISHED ABSTRACTS AND PRESENTATIONS

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

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

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

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

ABSTRACT OF THE DISSERTATION

Using a ^{14}C Release to Partition Soil Respiration Sources in a Southeastern Hardwood Forest

By

Luz Maria Cisneros Dozal

Doctor of Philosophy in Earth System Science

University of California, Irvine, 2005

Professor Susan E. Trumbore, Chair

Soil-respired CO_2 represents a large flux of carbon from land ecosystems to the atmosphere. Relative contributions from autotrophic and heterotrophic sources and factors causing their temporal variation are not well understood, which limits the ability to predict soil respiration fluxes and their response to future climate change. This thesis documents a new method to estimate soil respiration sources and elucidate seasonal patterns using radiocarbon. The work was conducted in a temperate forest near Oak Ridge, Tennessee, USA, that was subjected to a whole-ecosystem ^{14}C label in 1999. Near background (~230‰) and enriched (~1000‰) ^{14}C levels in roots and leaf litter were combined as part of the Enriched Background Isotope Study (EBIS).

Leaf litter decomposition contributed ~ 1 ±3 to 42 ±16% (6 ±3 to 96 ±38 mg C $m^{-2}h^{-1}$ respectively) to soil respiration, and near-zero when leaf litter was extremely dry. The contribution from leaf litter decomposition increased on average from 8 ±8% during drought to 32 ±12% following wetting (11 ±11 and 53 ±25 mg C $m^{-2}h^{-1}$

respectively), representing a main source of temporal variation in soil respiration fluxes and consequently in net ecosystem production in this forest. Decomposition of leaf litter ≤3 years old accounted for at least 50% of overall leaf litter decomposition (>15-30% of soil respiration) with little contribution (6 ±4%) to overall mineral soil decomposition.

Autotrophic respiration contributed 16 ±10% to 64 ±22% of soil respiration during the growing season (34 ±14 to 40 ±16 mg C $m^{-2}h^{-1}$ respectively), with one exception in September 2003 (88 ±35 mg C $m^{-2}h^{-1}$). The substrate for autotrophic respiration was comprised of a slow and a fast cycling pool corresponding to stored (> 1 year) nonstructural starch and recently fixed (days to weeks earlier) carbon respectively. Stored pools contributed ~70% early in the growing season (March) and <20% in the second half (August to October).

The radiocarbon methods developed here can be applied to ecosystems lacking an isotopic label provided significant isotopic differences between sources. The work presented demonstrates both the utility and some of the pitfalls of using isotopic mass balances to quantify relative contributions of different respiration sources to soil respiration.

CHAPTER 1

INTRODUCTION

The increase in atmospheric CO_2 concentration observed in the past few decades (Figure 1.1.) is predicted to continue throughout the 21st century with implications for global climate change [Prentice et al., 2001]. Although there is no doubt that anthropogenic emissions of CO_2 will be the main driver for higher atmospheric concentrations in the future, considerable uncertainty exists regarding the actual concentrations that will prevail in the second half of this century, mainly due to gaps in the knowledge of the terrestrial and oceanic carbon cycles. In the global carbon budget, the amount of terrestrial uptake is inferred from the difference between the sources (fossil fuel burning and land use change) and the sinks (atmosphere accumulation and ocean uptake) and for the 1990's, this residual has been estimated to be an uptake of 2.9 ± 1.1 Pg C yr^{-1} (Table 1.1.) [Houghton, 2003; Plattner et al., 2002; Prentice et al., 2001]. The uncertainty on this estimate clearly includes uncertainties on the estimates of ocean uptake and carbon release by land use change. Although significant efforts have been made to explain the estimated terrestrial uptake, the mechanisms involved are still under debate [Cao et al., 2005; Houghton, 2002; Valentini et al., 2000]. Predicting the future role of terrestrial biomes in counteracting the continued increase in atmospheric CO_2 is crucial but it requires a better understanding of the terrestrial carbon cycle.

Carbon in terrestrial ecosystems is assimilated by photosynthesis and lost by respiratory processes. Ecosystem assimilation is usually termed gross primary production (GPP). Ecosystem respiration includes aboveground autotrophic respiration

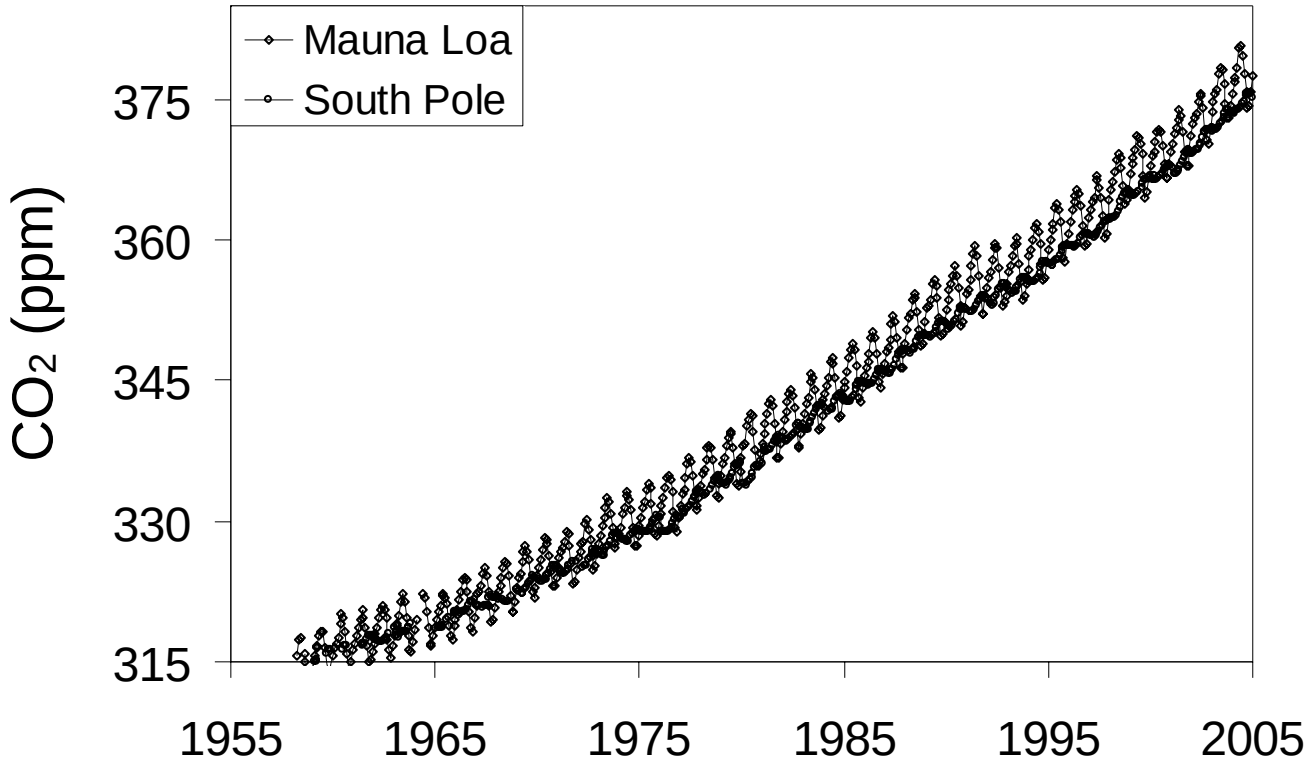


Figure 1.1. Atmospheric CO_2 concentrations measured in the Northern (Mauna Loa) and Southern (South Pole) hemispheres.
Source: Keeling, C.D. and T.P. Whorf. 2005. Atmospheric CO2 records from sites in the SIO air sampling network. In Trends: A Compendium of Data on Global Change. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tenn., U.S.A. (http://cdiac.esd.ornl.gov/trends/co2/sio-keel.htm).

Table 1.1. The estimated global carbon budget for the periods of 1980-1989 and 1990-1999 based on atmospheric measurements of CO_2, O_2, O_2/N_2 and ocean heat uptake. Positive values indicate emissions to the atmosphere; negative values indicate uptake from the atmosphere.

Global Carbon Balance (Pg C yr^{-1})		
	1980's	1990's
Atmospheric increase[1]	3.3 ± 0.1	3.2 ± 0.2
Emissions (fossil fuel, cement)[1]	5.4 ± 0.3	6.3 ± 0.4
Ocean-atmosphere flux[2]	-1.7 ± 0.6	-2.4 ± 0.7
Land-atmosphere flux[2]	-0.4 ± 0.7	-0.7 ± 0.8
Land-use change[3]	2.0 ± 0.8	2.2 ± 0.8
Residual terrestrial sink	-2.4 ± 1.1	-2.9 ± 1.1

[1] [Prentice et al., 2001]
[2] [Plattner et al., 2002]
[3] [Houghton, 2003]

and belowground autotrophic and heterotrophic respiration; the last two are usually combined and referred to as soil respiration. The net ecosystem carbon balance is the difference between GPP and ecosystem respiration and on an annual basis, this balance, usually referred to as net ecosystem production (NEP), represents the net gain or loss of carbon, i.e. whether an ecosystem represents a source or a sink for atmospheric carbon. Micrometeorological techniques have been used to estimate NEP directly, although some limitations associated with the methodology require that it be combined with other approaches to estimate stand-level C balance [Miller et al., 2004; Wilson and Baldocchi, 2001]. Estimates of NEP can also be derived from the difference between net primary production (NPP) which represents the net carbon gain by autotrophic organisms (including litterfall and losses to herbivores), and heterotrophic respiration which refers to carbon loss during decomposition of soil organic matter (e.g. [Chambers et al., 2004]). In theory, NPP is the difference between GPP and the autotrophic portion

of ecosystem respiration but approximate field estimates can be derived from biometric measurements [Curtis et al., 2002]. Heterotrophic respiration however, has to be derived from soil respiration measurements in the field, which is not a straightforward determination. Hence the adequate measurement or alternatively, the adequate prediction of soil respiration fluxes in terrestrial ecosystems is indispensable in estimating the net carbon gain or loss from ecosystems.

Soil respiration is the largest terrestrial source of carbon to the atmosphere, accounting for ~80 Pg C (10^{15}g C) annually [Raich et al., 2002]. Additionally, in forests, soil respiration constitutes ~70% of ecosystem respiration [Granier et al., 2000; Janssens et al., 2001]. Field measurements have shown that soil respiration is highly correlated with temperature on an annual basis, although its variability can also be driven by changes in precipitation [Raich et al., 2002; Yuste et al., 2003; Davidson et al., 2005]. Thus, the prediction of soil respiration and its interannual variability has proven challenging. Complications further arise given that soil respiration is not a single process but, as mentioned above, the CO_2 efflux arising from the combined activity of two different processes, microorganisms decomposing organic matter (the heterotrophic component) and live roots metabolizing photosynthetic products (the autotrophic component). These processes consume different carbon pools and consequently return carbon to the atmosphere on different timescales. Additionally, each process may be controlled by different factors and thus there is as yet limited understanding for prediction of fluxes derived from heterotrophic and autotrophic sources. These predictions become even more challenging in the context of how ecosystem carbon balance will respond to future climate change.

Some insight into the different responses of autotrophic and heterotrophic respiration to climate change has already emerged although in some cases with contradictory results, emphasizing the need for a process-based understanding. Increased surface temperatures associated with global warming are expected to enhance microbial activity resulting in greater soil CO_2 fluxes [Davidson et al., 2000; Kirschbaum, 1995] although the contrary has also been proposed [Giardina and Ryan, 2000]. With regard to root respiration, the same discussion prevails as to whether root respiration will increase with temperature [Pregitzer et al., 2000; Zogg et al., 1996] or roots will acclimate to warmer temperatures [Atkin et al., 2000]. The effects of increased CO_2 and nutrient availability have also been investigated and some studies have found positive feedbacks between CO_2 fertilization and soil respiration sources [Heath et al., 2005; King et al., 2004], which may be related to autotrophic activity [Soe et al., 2004]. On the other hand, the combined effect of CO_2 fertilization and increased nutrient availability seems more complex [Heath et al., 2005]. Monitoring stored nonstructural carbohydrates in roots may be the key on assessing the role of roots in the response of plants to atmospheric CO_2 increase [Norby, 1994] and to temperature increase [Pregitzer et al., 2000].

Hence, it is clear that the study of soil respiration must start by differentiating heterotrophic and autotrophic sources. This allows investigation and quantification of the effect of various perturbations or environmental factors on these two respiration sources. This is considered a key aspect to improve current models of terrestrial carbon cycle and even partitioning the heterotrophic component into a leaf litter and mineral soil compartment has been recommended [Pendall et al., 2004]. Partitioning of

autotrophic versus heterotrophic respiration sources has the potential to improve the ability to predict future changes in carbon storage in forests by determining how they affect the overall residence time of C in the ecosystem. Similarly, the incorporation of carbon allocation patterns to roots into models are crucial for improving predictions but these patterns are currently poorly understood [Ryan and Law, 2005; Trumbore, 2005].

The differentiation between heterotrophic and autotrophic respiration in the field, however, is not straightforward, and measuring the relative contributions to total soil respiration requires a specific technique and/or manipulated experiments. Several approaches have been applied including: physical separation of the components (e.g. leaf litter, soil, live roots), elimination of the autotrophic component by either trenching or girdling, and the labeling of plants with stable (^{13}C) or radioactive (^{14}C) isotopes. For radiocarbon, 'labeling' can mean either ^{14}C that was created in the 1950s- early 1960s by atmospheric weapons testing (or 'bomb' ^{14}C) or subsequent addition of radiocarbon (either purposeful or serendipitous). For stable isotopes, 'labeling' is accomplished either by a change in land cover from vegetation with predominantly C3 photosynthetic pathway to one with predominantly C4 or vice versa**.** Purposeful labeling of ^{13}C and ^{14}C usually accompanies Free Air Carbon-dioxide Enrichment (FACE). Isotopic methods have the advantage that they disturb the soil environment the least, although measurements of isotopes are relatively expensive. If the approaches to separate autotrophic from heterotrophic respiration are combined with manipulation of environmental variables such as temperature and moisture, then the individual response of each source of soil respiration to these variables can be captured. Additionally, the isotopic techniques offer the potential to distinguish recent (since the time of the

labeling) from old (pre-treatment) carbon sources thus providing information on carbon allocation patterns as well as on the impact of each source of soil respiration on the residence time of carbon pools of different ages. This knowledge can advance the current understanding of carbon sequestration potential in forests and perhaps provide direction towards beneficial land management.

This dissertation concerns the study of soil respiration sources in a temperate forest in eastern Tennessee, USA. The investigation of the response of heterotrophic and autotrophic respiration sources to temperature and moisture changes as well as carbon allocation patterns was performed using ^{14}C measurements. The application of the isotopic technique was made possible as a result of a labeling event at the whole-ecosystem scale that was discovered at this temperate forest in 1999 [Trumbore et al., 2002]. The unexpected event presented the unique opportunity to study carbon cycling by using levels of radiocarbon far greater than background. The research comprising this dissertation formed part of the Enriched Background Isotope Study (EBIS; http://ebis.ornl.gov/), a multi-institutional effort established in 2000 to study several crucial aspects of terrestrial carbon processes. The EBIS project involved the manipulation of ^{14}C inputs through leaf litter and roots at four sites in the temperate forest which is located near the Oak Ridge National Laboratory, Oak Ridge, Tennessee. The resulting experimental design combined enriched and near background ^{14}C levels in roots with enriched and near background ^{14}C levels in leaf litter.

The specific questions addressed in this dissertation are:

(1) What are the relative contributions of heterotrophic (leaf litter, mineral soil) and autotrophic sources to soil respiration over the course of the growing season?

(2) How are these relative contributions affected by phenology and changes in soil moisture and soil temperature?

(3) What is the relative importance of recently fixed leaf litter (1-3 years old) versus older carbon substrates as a substrate for heterotrophic respiration?

(4) What are the responses of heterotrophic (specifically leaf litter decomposition) and autotrophic respiration to increased levels of moisture?

(5) What are the effects of moisture and temperature changes on the type of substrate (recent versus old carbon) used in heterotrophic respiration?

(6) What is the role of stored nonstructural carbohydrates (>1 year) versus recently assimilated carbon (few days old) as substrate for autotrophic respiration during the growing season?

The first three questions are addressed in the second chapter. The initial goal with applying litter labeled with different levels of ^{14}C at two sites that had experienced different initial levels of ^{14}C exposure in 1999, was to discern autotrophic from heterotrophic respiration sources in the field by comparing the ^{14}C of CO_2 respired in high versus low ^{14}C label litter addition plots, and high versus low ^{14}C root exposure

plots. Specifically, we partitioned soil respiration into (1) recently fixed carbon which dominates autotrophic sources, (2) leaf litter decomposition and (3) decomposition of carbon substrates older than 3 years. The approach involved the measurement of the ^{14}C signature in the CO_2 derived from (1) total soil respiration, (2) live roots and microorganisms acting on the same carbon substrate in the rhizosphere (all comprising autotrophic respiration), (3) the decomposition of leaf litter and (4) the decomposition of organic matter in the mineral soil. In order to estimate the relative contributions of these sources to soil respiration, a mass balance for ^{14}C was applied to compare the ^{14}C of different sources with the ^{14}C of respired CO_2.

Chapter 2 demonstrates how the ^{14}C measurements are used to partition total soil respiration into component sources. Leaf litter decomposition was found to be a major contributor to soil respiration fluxes (up to 42 ±16%) but its contribution was highly variable in time, with variation linked to changes in moisture availability. In contrast, autotrophic respiration was not as variable. However, we noticed a seasonal variation in the radiocarbon signature of root respired CO_2 that revealed a few clues as to the use of stored versus recent photosynthate as substrate. Finally, carbon fixed 3-5 years earlier dominated heterotrophic fluxes from shallow mineral soil.

Questions 4-6 above were formulated based on these initial results. The third chapter addresses questions 4 and 5. In order to investigate the role of moisture as a control of leaf litter decomposition and as a source of temporal variability in soil respiration,, we observed the response to a manipulation of litter moisture content. As part of this study, we compared two chamber designs to measure soil respiration: manual and automated chambers. The experimental design of EBIS was used to

estimate fluxes derived from leaf litter decomposition only, based on the difference in ^{14}C content of soil respiration between plots containing different leaf litter (enriched and near background). Applying the ^{14}C-based mass balance methods developed in Chapter 1, we estimated that leaf litter decomposition accounts for the all of the increase in soil respiration normally observed following an increase in moisture (e.g. precipitation). Pre-existing moisture conditions were important in determining the magnitude of the response. We further investigate the importance of moisture and temperature variations on heterotrophic CO_2 fluxes by manipulating incubations. The type of substrate used for decomposition (i.e. recent versus old carbon) was unaffected by changes in moisture and temperature confirming that the cause of increased soil respiration fluxes was rather the enhanced activity of decomposers.

Question 6 is addressed in the fourth chapter and was based on the observations of the ^{14}C content of root respiration that we report in Chapter 2. Before attempting to answer these questions, analytical techniques for the isolation of nonstructural and structural carbohydrates for the purpose of measuring radiocarbon were developed and tested. Plant material with natural levels of radiocarbon (leaves and stems collected from a tree located on the UCI campus), were selected to test the reproducibility of the methods. The precision varied from 2‰ to 7‰, only slightly greater than the accuracy of the mass spectrometry technique of 2-5‰. Radiocarbon signatures of carbohydrate fractions (cellulose, bulk water soluble compounds and starch) extracted from roots that were collected at different times during the growing season were compared to the radiocarbon signatures of autotrophic respiration to elucidate patterns of carbon allocation. Two pools of carbon were identified as potential substrates for autotrophic

respiration through the growing season: a slow and a fast cycling pool, and preliminary estimates were made regarding the relative contributions of each of them. The slow pool, made up of stored (longer-lived) nonstructural carbohydrates, dominated root respiration sources in the early part of the growing season while the fast pool, derived from recent photosynthetic products, dominated later in the growing season.

In the final section of this dissertation, concluding remarks and some recommendations for improvement as well as suggestions for future work regarding the research topics investigated are summarized.

Reprinted with permission from *Global Change Biology*, **11**, 1-11,
doi:10.1111/j.1365-2486.2005.01061.x, 2005.

CHAPTER 2

PARTITIONING SOURCES OF SOIL-RESPIRED CO_2 AND THEIR SEASONAL VARIATION USING A UNIQUE RADIOCARBON TRACER

Abstract

Soil respiration is derived from heterotrophic (decomposition of soil organic matter) and autotrophic (root/rhizosphere respiration) sources, but there is considerable uncertainty about what factors control variations in their relative contributions in space and time. We took advantage of a unique whole-ecosystem radiocarbon label in a temperate forest to partition soil respiration into three sources: (1) recently photosynthesized carbon (C), which dominates root and rhizosphere respiration; (2) leaf litter decomposition, and (3) decomposition of root litter and soil organic matter >1-2 years old.

Heterotrophic sources and specifically leaf litter decomposition were large contributors to total soil respiration during the growing season. Relative contributions from leaf litter decomposition ranged from a low of ~1 ±3% of total soil respiration (6 ±3 mg C m^{-2} hr^{-1}) when leaf litter was extremely dry, to a high of 42 ±16% (96 ±38 mg C m^{-2} hr^{-1}). Total soil respiration fluxes varied with the strength of the leaf litter decomposition source, indicating that moisture-dependent changes in litter

decomposition drive variability in total soil respiration fluxes. In the surface mineral soil layer, decomposition of C fixed in the original labeling event (3-5 years earlier) dominated the isotopic signature of heterotrophic respiration.

Root/rhizosphere respiration accounted for 16 ±10% to 64 ±22% of total soil respiration, with highest relative contributions coinciding with low overall soil respiration fluxes. In contrast to leaf litter decomposition, root respiration fluxes did not exhibit marked temporal variation ranging from 34 ±14 to 40 ±16 mg C m^{-2} hr^{-1} at different times in the growing season with a single exception (88 ±35 mg C m^{-2} hr^{-1}). Radiocarbon signatures of root respired CO_2 changed markedly between early and late spring (March vs. May), suggesting a switch from stored nonstructural carbohydrate sources to more recent photosynthetic products.

1. Introduction

A key area of uncertainty in the terrestrial C cycle concerns the processes, referred to collectively as respiration, that control the vast majority of C lost annually. Carbon is added to ecosystems through photosynthesis and lost either rapidly, by fueling plant metabolism and growth, or more slowly, through the decomposition of dead plant organic matter. The portion of total ecosystem respiration that takes place below-ground, soil respiration, has been estimated in forests to be as much as 69% of the total ecosystem respiration (plant and soil) and 55% of the carbon assimilated through photosynthesis annually [Janssens *et al.*, 2001]. Besides representing a large loss of carbon, soil respiration is also a major contributor to inter-annual variability in the net ecosystem balance [Goulden *et al.*, 1996; Valentini *et al.*, 2000].

Soil respiration is derived from autotrophic and heterotrophic sources. Autotrophic respiration includes CO_2 derived from root metabolism and the activity of microorganisms in the rhizosphere. Heterotrophic respiration includes CO_2 released during microbial decomposition of soil organic matter. While autotrophic respiration is linked to the supply of photosynthetic products from plants, heterotrophic respiration is derived from several different kinds of dead plant material (leaf litter, root detritus, soil organic matter) available to decomposers. Knowledge of the relative contributions of the two sources is relevant to studies of C cycling as each source returns C to the atmosphere on different time scales (years or less through root/rhizosphere respiration vs. decades to centuries through soil organic matter decomposition). Furthermore, we need to better understand all potential factors (environmental and/or phenological) controlling their seasonal variation to make accurate predictions of future C sequestration in forests. Important questions include: (1) how much of total soil respiration comes from autotrophic versus heterotrophic sources? (2) what portion of heterotrophic respiration comes from decomposition of different substrate types? and (3) how do changes in phenology, soil moisture and temperature, affect each of the sources of soil respiration?.

Different approaches have been used to find the relative contributions to soil respiration. Three widely applied methods include component separation, root removal and the use of isotopes, described in detail by Hanson *et al.* [2000]. Component separation extrapolates measurements of CO_2 flux for components (e.g. incubations, excised roots) to the soil volume, but can be subject to artifacts associated with respiration rates measured under non-field conditions and ultimately limited by

knowledge of factors like the quantity of roots in the soil. Root removal is commonly accomplished by trenching or girdling trees, and is limited in the short term by the time it takes for roots to die and in the long term by enhanced decomposition of severed or dead roots. Isotope studies offer the advantage that measurements are made in intact systems, but they tend to be relatively expensive and typically require lumping respiration sources that have similar isotopic signatures.

Estimates of the relative contribution of root respiration to soil respiration vary considerably. Kelting *et al.* [1998] estimated root respiration to account for 32% of soil respiration by trenching in a red oak stand (*Quercus rubra* L.). Also using trenching methods, Epron *et al.* [1999] estimated root respiration to contribute 60% of the annual total CO_2 from soils in a beech stand (*Fagus sylvatica* L.). A large scale girdling experiment in a boreal forest (*Pinus sylvestris* L.) was used to estimate that root respiration accounted for up to 56% of total soil respiration in the middle of the summer, but recognized that the contribution could be even higher given the longevity of starch reserves in recently-severed roots [Högberg *et al.*, 2001]. Another trenching experiment at a smaller scale in a Mediterranean forest (*Quercus* spp.), indicated an annual contribution of 23%, but other factors such as enhanced decomposition in trenched plots and recent logging of the site could have affected the results [Rey *et al.*, 2002]. The reporting of root respiration as a percentage of total respiration rather than an absolute quantity may be masking differences in root inventory, allocation, and total soil respiration among sites.

In this paper, we report on the application of a unique ^{14}C tracer to the characterization of soil respiration components. Large enrichments of the ^{14}C

signatures of plants, litter and soil resulted from a release of ^{14}C in 1999 from one or more hazardous waste incinerators near our study site on the Oak Ridge Reservation, Oak Ridge, Tennessee. This isotope label was incorporated into living plants to varying degrees according to distance from the source [Trumbore *et al.*, 2002]. We used this unique ^{14}C-tracer to describe how respiration sources vary with season and plant activity.

2. Methods

2.1. Site description

The study site is located in the Oak Ridge Reservation (ORR) of the Oak Ridge National Laboratory in East Tennessee, USA (35°58 N and 84° 17 W). Mean annual precipitation is 1358 mm and mean annual temperature is 14.1°C. Vegetation is dominated by *Quercus* spp and *Acer* spp [Johnson and Van Hook, 1989].

While the large ^{14}C release occurred in July/August 1999, leaves did not show a strong increase in radiocarbon content until the following spring. We observed a large gradient in radiocarbon content of this new leaf growth, with highest amounts of incorporated label in the western portion of the reserve [Trumbore *et al.*, 2002]. In the fall of 2000, leaf litter from two areas in the western and eastern portions of the ORR was collected, mixed (to homogenize), dried and stored. Repeated measurements of aliquots of the stored leaf litter showed that leaves collected from the western portion of the reserve had ^{14}C values ranging from +952 ‰ to +1055 ‰, while the ones collected from the eastern reserve had values from +215 ‰ to +230 ‰. Hereafter, these will be referred to as "HL" (high labeled) and "LL" (low labeled) litter, respectively.

The Enriched Background Isotope Study (EBIS) established a total of four sites, two on Inceptisols and two on Ultisols, along the gradient of ^{14}C label from the western to the eastern side of the reserve. The sites provided a gradient of differential labeling of plants, including roots, with high levels of label on the western side and low levels on the eastern side, hereafter referred to as "HR" (high labeled root) and "LR" (low labeled root) sites, respectively. After preliminary measurements showed no large differences in the isotopic signature of soil respiration with soil type, we limited our measurements to two sites: Tennessee Valley Authority, hereafter referred to as "TVA" (HR site) and Walker Branch (LR site) both on the Ultisol soil type.

At each site, a total of eight 7 m x 7 m plots were established to manipulate the ^{14}C content of leaf litter. Natural leaf litter was excluded by covering plots with landscape cloth from October to November, and ambient litter was replaced with HL or LL litter over three consecutive years. Litter exclusions occurred in the fall of 2000, 2001, and 2002, and leaf litter additions took place in May 2001, February 2002 and February 2003, with 4 plots at each site receiving HL vs. LL litter. We use this factorial design, with combinations of HR-HL, HR-LL, LR-HL, LR-LL, together with the large ^{14}C differences in sources among plots and sites, to partition soil respiration into root/rhizosphere respiration and decomposition sources.

2.2. Moisture and temperature measurements

Litter-layer water content and soil water status were measured at long-term reference plots at each site. Continuous direct measurements of forest litter water content were based on the electrical resistance characteristics of wet vs. dry litter

[Hanson *et al.*, 2003b]. Buried frequency domain reflectometer waveguides (CS615; Campbell Scientific, Inc., Logan, Utah) were installed in the A and B horizons (~30 cm). All data were logged as hourly means and stored on a data logger (CR10; Campbell Scientific, Inc., Logan, Utah). Additional soil temperature (between 0 and 5 cm depth into the mineral soil) and air temperature data were collected during chamber flux measurements.

2.3. Determination of ^{14}C signatures

2.3.1. Total soil respiration

Soil respiration was measured concurrently with sampling for isotopes in the months of May, July and September of 2002, March, May, September and October of 2003 and March of 2004 at the two sites. The isotopic signatures measured in March 2004 at the TVA site were not used as they were confounded by a unexpected new and localized release of ^{14}C on the date of our measurements. This new release event did not affect the isotopic signatures measured at the Walker Branch site. Measurements were carried out in 6 plots per site including 3 plots with HL and 3 with LL litter treatment.

Closed dynamic chambers were used to measure total soil respiration and to collect CO_2 for radiocarbon analysis as described by Gaudinski *et al.* [2000]. Briefly, an infrared gas analyzer (LI-800, LiCor, Inc. Lincoln, Nebraska) was attached to a pumping system that circulated air to and from the chamber, and the CO_2 concentration increase was monitored for approximately 5 to 10 min. During this time, CO_2 concentrations rose from 380-400 ppm to 500-900 ppm, depending on the respiration rates. The rate of increase was usually linear with a few exceptions due to either pump

failure or to the effect of placing the chamber lid on the collar which sometimes caused an initial perturbation of the CO_2 flux that lasted for 40 seconds to 1 minute. Non-linear rates of CO_2 increase were not considered in the estimation of fluxes. Once the flux was measured, the air in the headspace of the chamber was scrubbed of CO_2 initially present (which has a different radiocarbon signature from that of soil respiration) by passing the air coming from the chamber through soda lime. The scrubbing was complete when 3 times the volume of the chamber passed through soda lime which took ~40 minutes at flow rates of ~0.8 L min^{-1}. Low flow rates (<1 L min^{-1}) were desirable for preventing disturbance of the CO_2 concentrations in the soil profile beneath the chamber. The concentration of CO_2 in the chamber reached a steady state value at the end of the scrubbing period that depended on the soil respiration and air circulation rates, but usually remained higher than ambient CO_2 concentrations (between 500 and 600 ppm). When scrubbing was complete, the flow was redirected to a U-shaped tube filled with molecular sieve (mesh size 13X; Advanced by UOP, Specialty Gas Equipment) for trapping of the CO_2 emitted inside the chamber. A container filled with drierite (calcium sulfate) was placed before the trap to remove water that would decrease the efficiency of the molecular sieve [Gaudinski *et al.*, 2000]. Activated molecular sieve traps CO_2 at ambient temperatures and releases it when heated at 475^{o}C [Bauer *et al.*, 1992]. The trapping time depended on the respiration rates, with the goal of collecting sufficient carbon for ^{14}C and ^{13}C analysis (about 2-3 mg C total). CO_2 collection from all 6 plots at each site took place between 10 am and 5 pm.

Molecular sieve traps were taken to the University of California, Irvine (UCI) where CO_2 was released and the traps reactivated by baking at 610 ºC. The released

CO_2 was purified cryogenically and converted to graphite using the Zn reduction method [Vogel, 1992]. An aliquot of each ^{14}C sample was analyzed for ^{13}C using continuous flow isotope ratio mass spectrometry at UCI. Approximately 0.1 micro liters of purified CO_2 was removed from the vacuum line with a syringe and injected into a He-flushed septum-capped vial. The isotopic signature of the CO_2 was measured using a Gas bench II inlet to a Delta-plus stable isotope mass spectrometer.

Graphite targets were sent to Lawrence Livermore National Laboratory (LLNL) for ^{14}C analysis by accelerator mass spectrometry. The results are expressed in $\Delta^{14}C$ the deviation from a standard (oxalic acid) in per mil (‰):

$$\Delta^{14}C = \left[\frac{\left[\frac{^{14}C}{^{12}C} \right]_{sample}}{\left[\frac{^{14}C}{^{12}C} \right]_{standard}} - 1 \right] 1000$$

where the $^{14}C/^{12}C$ ratio of the sample is corrected for mass-dependent isotope fractionation to a common value of -25 ‰ in $\delta^{13}C$ [Stuiver and Polach, 1977]. Overall accuracy of our Zn-reduced targets measured at LLNL, based on repeated measures of secondary standards (oxalic acid 2, ANU, Tiriwood), is ± 5 per mil for modern samples.

At each site, we obtained the ^{14}C signature of total soil respiration (hereafter referred to as $\Delta^{14}C_{total\ respiration}$) from each of the 6 plots. The values per treatment reported here and used in the calculations are the means (± standard deviation) of three measurements. We tested for significance of differences of these means between treatments using one-way ANOVA.

2.3.2. Heterotrophic respiration

We collected leaf litter (Oi plus Oe/Oa horizons) and soil cores representing the top 5 cm of mineral soil from each of the six plots (3 plots with HL and 3 with LL litter treatment) at each site. We did not sample deeper soil layers because heterotrophic respiration sources decrease sharply with depth and only ~25% of total fluxes originated in the mineral soil comes from layers between 8 to 70 cm depth (E and B horizons) at this site [Gaudinski and Trumbore, 2003]. Soil and leaf litter samples were incubated separately as to obtain independent radiocarbon signatures for each horizon. To calculate the isotopic signature of heterotrophic respiration ($\Delta^{14}C_{heterotrophic}$), we weighted the isotopic signature of litter and soil layers according to their contribution to the total decomposition flux (see below).

Leaf litter was sampled by collecting all material above the mineral soil in a 15 x 15 cm area. The sample was placed in an airtight plastic bag and refrigerated during transport and storage. Soil samples were collected using a core (4.7cm diameter by 5 cm long) inserted vertically into the soil. The core was carefully removed and the soil extruded with a minimum of disturbance into a flask, which was capped and refrigerated for transport and storage. Samples of soil and leaf litter were collected on all sampling dates except in July 2002. The isotopic signature of heterotrophic respiration used for July was calculated as the mean of the signatures obtained in May and September at each site. No data are available for October 2003 at both sites.

The leaf litter and soil samples were refrigerated for at least 1 week (up to a month) prior to further analysis. While this amount of time is dictated by the time of transport from the field, it also allows time for fine roots that can be present in the

samples to die so that their autotrophic respiration no longer contributes significantly to the CO_2 evolved in the incubation. One liter jars (Mason) were used to separately incubate soil cores and leaf litter samples. Humidity inside the jar was maintained by pouring approximately 100 ml of water over glass beads placed in the bottom of the jar. The flasks containing the soil cores were uncapped, weighed (for gravimetric determination of moisture content after the incubation was finished) and placed inside the 1 L jars over the glass beads. Leaf litter samples were weighed and a sub-sample was placed in perforated aluminum foil packets before sealing in the Mason jar.

For approximately 1 week after sealing the jars, we measured the rate of accumulation of CO_2 in the jar headspace using an infrared gas analyzer (LI-6252, Inc. Lincoln, Nebraska). After CO_2 fluxes were determined, air inside the jars was scrubbed of CO_2, and CO_2 was allowed to re-accumulate until there was sufficient concentration (1-2% CO_2) for ^{13}C and ^{14}C analysis. CO_2 samples for isotope analyses were collected by connecting the jars to evacuated stainless steel canisters (of 0.5 liters in volume). The ^{14}C and ^{13}C signatures were determined as described above. At each site, we report soil and leaf litter decomposition signatures ($\Delta^{14}C_{\text{soil decomposition}}$ and $\Delta^{14}C_{\text{leaf litter decomposition}}$ respectively) as the mean (± standard deviation) of three plots with identical litter addition treatments.

2.3.3. Autotrophic respiration

To determine the radiocarbon signature of autotrophic respiration ($\Delta^{14}C_{\text{autotrophic}}$), we incubated live roots in the field. Since this ^{14}C signature is dependent on photosynthetic carbon supplies, and is independent of leaf litter carbon inputs, roots for

analysis were collected outside the experimental plots to avoid within-plot disturbance. At two or more randomly chosen locations, we excavated roots mostly in the upper 5 cm of mineral soil, though some may be from slightly deeper as root networks were pulled out without distinction of depth, size (most were <5mm diameter), or species.

After sufficient roots for three replicates were collected, they were shaken and washed free of soil, divided into 3 sub-samples and placed immediately in airtight containers. Roots were normally collected at 5-6 pm local time. Isotopes were sampled using the same methodology described for total soil respiration, though we did not measure CO_2 flux rates as we assumed manipulation of the roots may have altered the fluxes [Rakonczay *et al.*, 1997]. Air inside the containers was scrubbed of CO_2, CO_2 was allowed to re-accumulate, and was collected by trapping on molecular sieve. The accumulation of CO_2 for trapping started within an hour after roots had been removed from the soil and lasted for an amount of time from 10 min to an hour depending on the respiration rate of the sub-sample. Molecular sieve traps were transported to UCI and the ^{14}C and ^{13}C signatures of the CO_2 collected determined as described above. At each site we report the mean (± standard deviation) of three replicates.

2.4. Calculation of respiration components

2.4.1 Fraction of total soil respiration coming from leaf litter decomposition (FLD)

Contrasting the levels of ^{14}C in CO_2 respired from HL and LL treatments at a given site allowed us to uniquely determine the contribution of leaf litter decomposition to total soil respiration using isotope mass balance:

$$\Delta^{14}C_{\text{total respiration}} = \Delta^{14}C_{\text{autotrophic}} \cdot FRR + \Delta^{14}C_{\text{leaf litter decomposition}} \cdot FLD + \Delta^{14}C_{\text{soil decomposition}} \cdot FSD \quad \text{(Eq. 2.1.)}$$

where FRR, FLD and FSD are the fractional contributions of autotrophic respiration, leaf litter decomposition and soil decomposition, respectively, to total soil respiration. The $\Delta^{14}C_{\text{autotrophic}}$, $\Delta^{14}C_{\text{leaf litter decomposition}}$ and $\Delta^{14}C_{\text{soil decomposition}}$ values were derived from our field and laboratory incubations, as described above. Since we incubated not only the labeled leaf litter added to plots, but the entire litter layer (Oi + Oe/Oa), FLD as calculated above reflects the contribution from the decomposition of the entire O horizon. At a given site, $\Delta^{14}C_{\text{autotrophic}}$ and FRR should be the same for both treatments. The difference between HL and LL treatments in $\Delta^{14}C_{\text{soil decomposition}}$ was generally very small compared to the difference in $\Delta^{14}C_{\text{leaf litter decomposition}}$ (see the results section). Therefore, we could assume that the differences in ^{14}C between HL and LL treatments were overwhelmingly caused by leaf litter decomposition. Applying Eq. (2.1.) to each treatment and subtracting LL from HL we solved for FLD as follows:

$$FLD = (\Delta^{14}C_{\text{total})\mathbf{HL}} - \Delta^{14}C_{\text{total})\mathbf{LL}})/(\Delta^{14}C_{\text{ leaf litter decomposition})\mathbf{HL}} - \Delta^{14}C_{\text{ leaf litter decomposition})\mathbf{LL}}) \quad \text{(Eq. 2.2.)}$$

We report one single value of FLD for each site (HR and LR) and sampling date. The associated uncertainty was estimated by error propagation.

2.4.2. Fraction of total soil respiration coming from autotrophic respiration (FRR)

To estimate the fraction of total respiration derived from autotrophic respiration using ^{14}C, we applied the mass balance approach described in Trumbore *et al*., [2002]:

$$\Delta^{14}C_{\text{total respiration}} = \Delta^{14}C_{\text{autotrophic}} \cdot FRR + \Delta^{14}C_{\text{heterotrophic}} \cdot (1\text{-}FRR) \qquad \text{(Eq. 2.3.)}$$

Here, FRR includes metabolic respiration from live roots as well as anything with a similar ^{14}C signature, such as symbiotic mycorrhizal fungi and the activity of microorganisms decomposing recent photosynthetic products. While we will hereafter refer to FRR as the fraction coming from root respiration, it should be recognized that it refers to the sum of all of these processes.

The radiocarbon signature of heterotrophic respiration ($\Delta^{14}C_{\text{heterotrophic}}$) was determined as the weighted contribution of CO_2 derived from leaf litter and soil organic matter decomposition:

$$\Delta^{14}C_{\text{heterotrophic}} = \Delta^{14}C_{\text{leaf litter decomposition}} \cdot L + \Delta^{14}C_{\text{soil decomposition}} \cdot (1\text{-}L) \qquad \text{(Eq. 2.4.)}$$

where L is the fractional contribution of leaf litter decomposition to total heterotrophic respiration. One way to determine L is to compare the relative rates of CO_2 evolution from our incubations of leaf litter and soil. However, the temperature and moisture conditions of our incubations differed from field conditions, and if we attempt to scale the rates of heterotrophic respiration derived from laboratory incubations to conditions

in the field, we overestimate observed soil respiration by at least a factor of two. Hence, we could not use our laboratory incubations to derive weighting factors with any confidence.

A second method of determining L is obtained by assuming that FRR and L are the same for both HL and LL treatments for a given site. Since L and FLD represent the contribution of decomposing litter to heterotrophic and total respiration, respectively, they are related by:

$$FLD = L \cdot (1\text{-}FRR) \qquad \text{(Eq. 2.5.)}$$

Substituting Eqs. (2.4.) and (2.5.) into Eq. (2.3.) results in an equation with two unknowns, FRR and FLD. Since we have calculated FLD using Eq. (2.2.) above, we can solve this equation for FRR, with the requirement that FRR and FLD be identical for both HL and LL treatments within a given site. We only report values of FRR that satisfied this assumption within the error we calculated for FLD using Eq. (2.2.). We report the uncertainty in FRR by treatment calculated using the standard deviations of total soil respiration, root and heterotrophic respiration according to Phillips and Gregg, [2001].

3. Results

3.1. Total soil respiration fluxes

Although there was high spatial variability in soil respiration fluxes, we saw no systematic differences in CO_2 fluxes between the two sites (Figure 2.1.; data for March 2004 at the TVA site were not reported). The lower fluxes measured at TVA in July

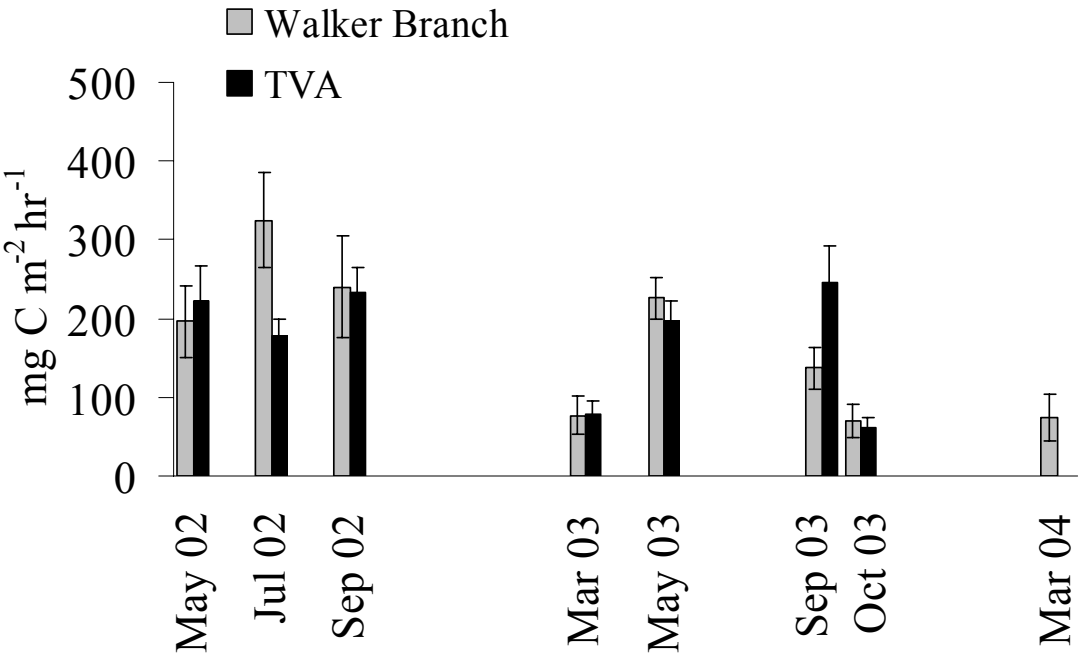


Figure 2.1. Total soil respiration fluxes at the Walker Branch and TVA sites on each sampling date. At each site we report the mean (±standard deviation) of 6 chamber measurements including 3 per litter treatment. No data are available for March 2004 at the TVA site

2002 and Walker Branch in September 2003 were most likely due to moisture differences between the sites. Since the two sites were sampled on different days and rainfall from isolated summer precipitation events is very unevenly distributed across the landscape, the two sites do not always replicate each other. Soil moisture varied from 20 to 35% volumetric water content (at approximately 10 cm depth) across sites during the three sampling periods in 2002, except in July, where moisture at TVA site fell to 11%. In September 2003, we have no data for soil moisture content, but leaf litter at Walker Branch was extremely dry and crunchy, while soil respiration measurements at TVA were carried out the day after a heavy rain event.

3.2. ^{14}C signatures of total soil respiration, root respiration and heterotrophic sources

We observed a marked difference in the radiocarbon signatures of leaf litter decomposition between HL and LL treatments at both TVA (HR) and Walker Branch (LR) sites (Figure 2.2.). The ^{14}C-labeled litter addition imparted a higher radiocarbon signature to total soil respiration on all dates except those where litter was extremely dry (July 2002 and September 2003). The isotopic signature of decomposing soil organic matter increased slightly with time, though differences between HL and LL treatments remained small compared to those associated with leaf litter decomposition. Across sites and litter treatments, the radiocarbon signatures were overall higher at TVA (HR) than at Walker Branch (LR), reflecting the continued influence of ^{14}C-enriched carbon acquired by plants during the 1999 labeling event.

The ^{14}C signature of root respiration varied with time (Figure 2.2.). Temporal variations between sites followed very similar patterns, although values in the HR site remained ~100‰ higher than the LR site throughout 2002-2003. The very depleted values that we observed at the Walker Branch site in July 2002 (-0.28 ‰) and May 2003 (2 ‰) were attributable to a local release of fossil fuel derived CO_2, which has been verified by measurements of reduced radiocarbon content in ambient air sampled at this site since 2000.

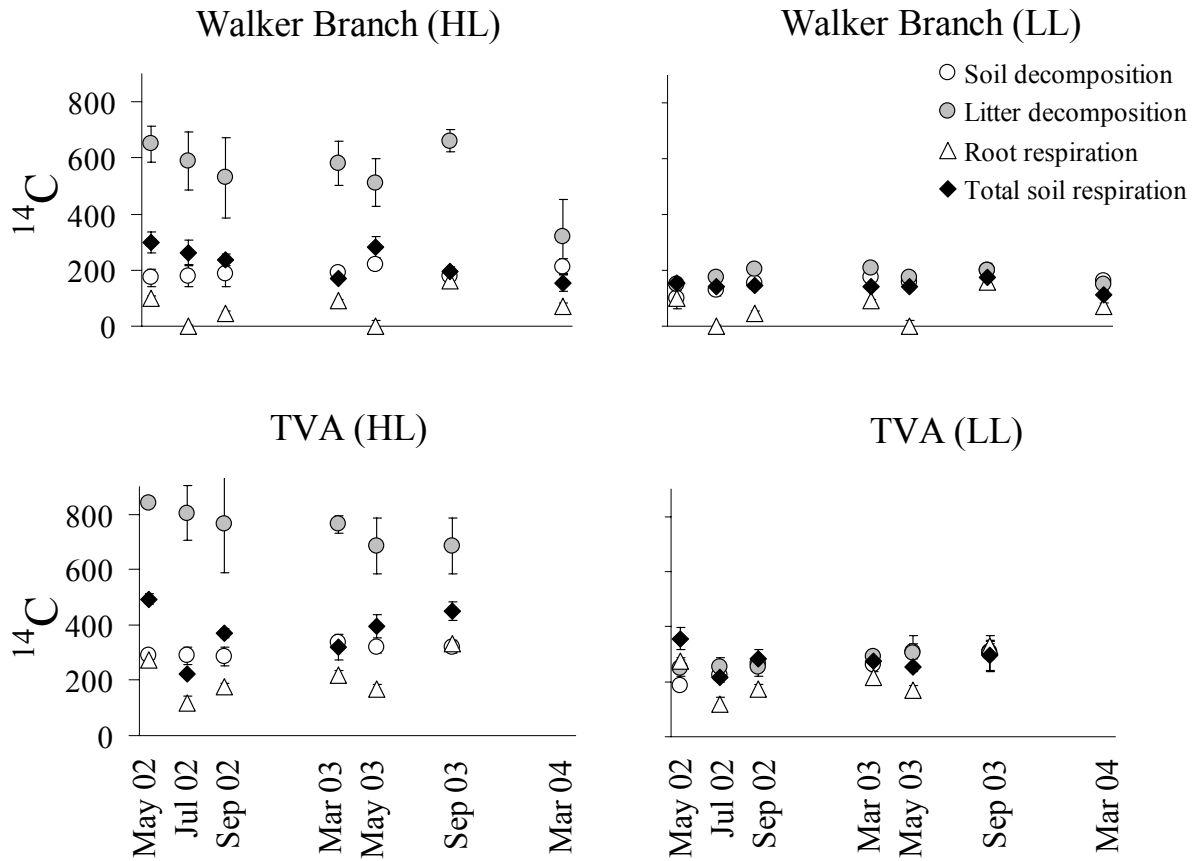


Figure 2.2. ^{14}C signatures (in per mil, ‰) of total soil respiration, root respiration, soil decomposition and leaf litter decomposition by litter treatment, HL and LL (high and low ^{14}C labeled leaf litter respectively) for the Walker Branch and TVA sites. Each value represents the mean of 3 replicates (±standard deviation). The isotopic signatures of soil decomposition and leaf litter decomposition that we report for July 2002 at both sites are the means of the signatures measured in May and September 2002

3.3. Fraction of total soil respiration coming from leaf litter decomposition (FLD)

According to Eq. (2.2.), the fraction of total soil respiration derived from leaf litter decomposition (FLD) was proportional to the difference in the ^{14}C signature of total soil respiration between HL and LL treatments. FLD ranged from near zero in September 2003 to 0.42 ±0.16 in May 2003 (Figure 2.3.). Lowest FLD values were found on July 2002 (TVA; HR) and September 2003 (WB; LR).

3.4. Fraction of total soil respiration coming from root respiration (FRR)

Our approach of solving for FRR by requiring FLD and FRR to be identical in HL and LL plots at a given site produced consistent results for the Walker Branch site on most dates (Table 2.1.). We had greater problems with the TVA site and our constraints were met only on May 2003. When the isotopic differences among root respiration, heterotrophic respiration and total respiration were small compared to their respective standard deviations, HL and LL treatments did not yield consistent results (blank entries in Table 2.1.). On several occasions the $\Delta^{14}C$ of total soil respiration fell outside the range of the $\Delta^{14}C$ of its heterotrophic and autotrophic sources and thus the mass balance approach could not be applied (dates marked "na" in Table 2.1.). Increased uncertainties in FRR calculated from the HL treatment at the Walker Branch site on September 2002 and 2003 resulted from small isotopic difference between autotrophic respiration and the estimated signature of heterotrophic respiration. For subsequent calculations, we have used the Walker Branch results, assuming the largest error among the two treatments, except for September 2002 and 2003, when we used the LL treatment values only.

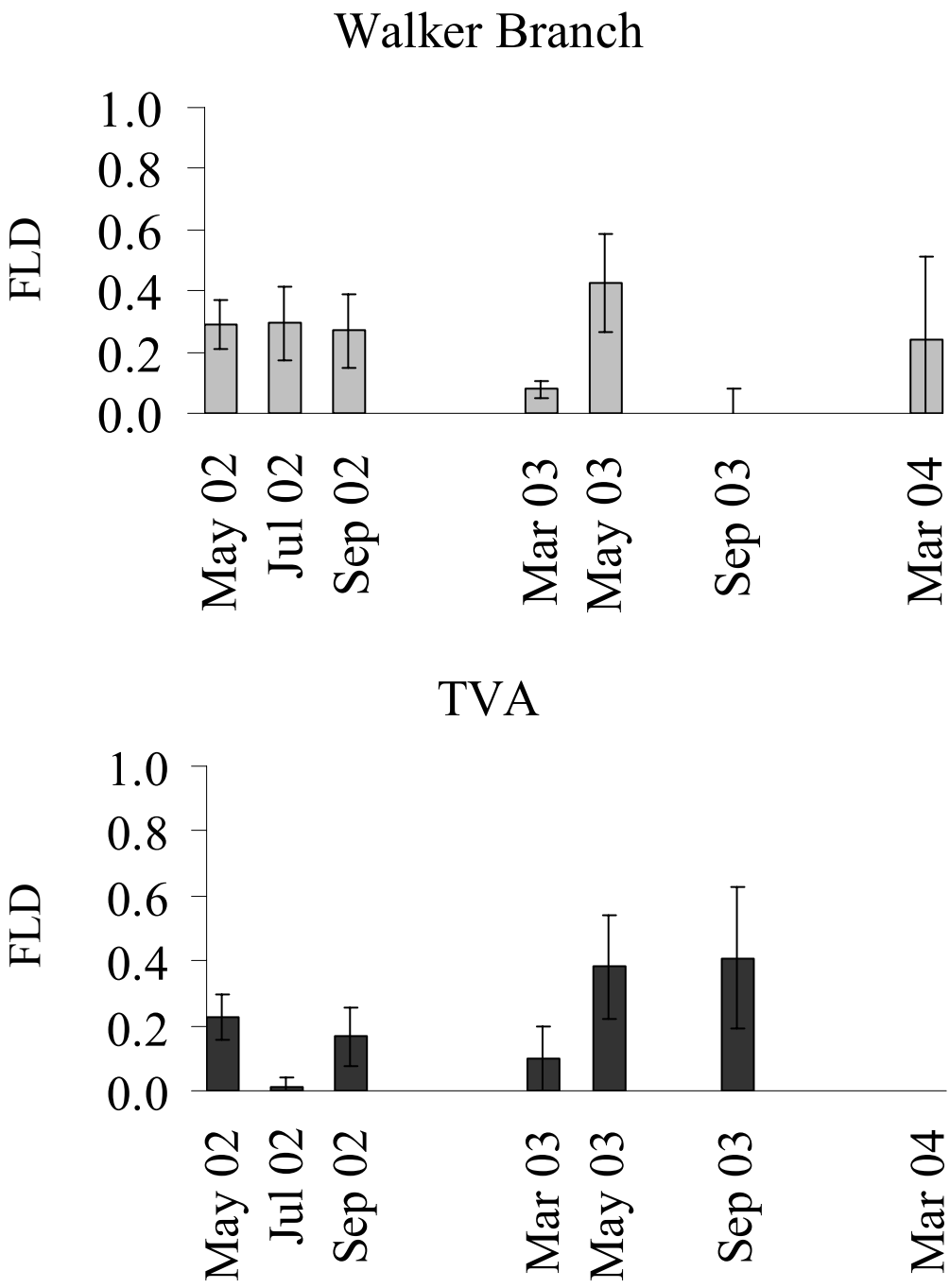


Figure 2.3. Fraction of total soil respiration coming from leaf litter decomposition (FLD) at the Walker Branch and TVA sites by sampling date. The fractions were calculated using Eq. (2.2.). No data are available for March 2004 at the TVA site.

Table 2.1. Fraction of total soil respiration coming from root respiration (FRR) for HL and LL (high and low ^{14}C labeled leaf litter) treatments at the Walker Branch (WB) and Tennessee Valley Authority (TVA) sites. Dates when the isotopic signatures did not satisfy the mass balance are indicated as "na"; blank entries indicate times when HL and LL treatments did not satisfy assumptions on which the calculations were based.

		2002			2003			2004
Site	Treatment	May	Jul	Sep	Mar	May	Sep	Mar
WB	LL	na		0.17	0.45	0.16	0.64	0.53
				(0.05)	(0.11)	(0.08)	(0.22)	(0.12)
	HL			0.16	0.45	0.16	0.63	0.53
				(0.21)	(0.10)	(0.10)	(0.61)	(0.19)
TVA	LL	na		na		0.40	na	na
						(0.18)		
	HL					0.40		na
						(0.10)		

Numbers in brackets represent the standard error according to Phillips and Gregg, (2001)

3.5. Partitioning of total soil respiration

A summary comparing the absolute contributions (in mg C m^{-2} hr^{-1}) of root respiration and leaf litter decomposition for the Walker Branch site is shown in Figure 2.4. Fluxes labeled as "other" were calculated from mass balance (Total respiration *minus* root respiration *minus* leaf litter respiration), and have isotopic signatures close to those evolved in soil incubations (FSD; Eq. 2.1.).

Heterotrophic respiration, especially leaf litter decomposition, constituted a large portion of the total CO_2 respired in the growing season (May and September), though it was also the most variable component in space and time, ranging from nearly

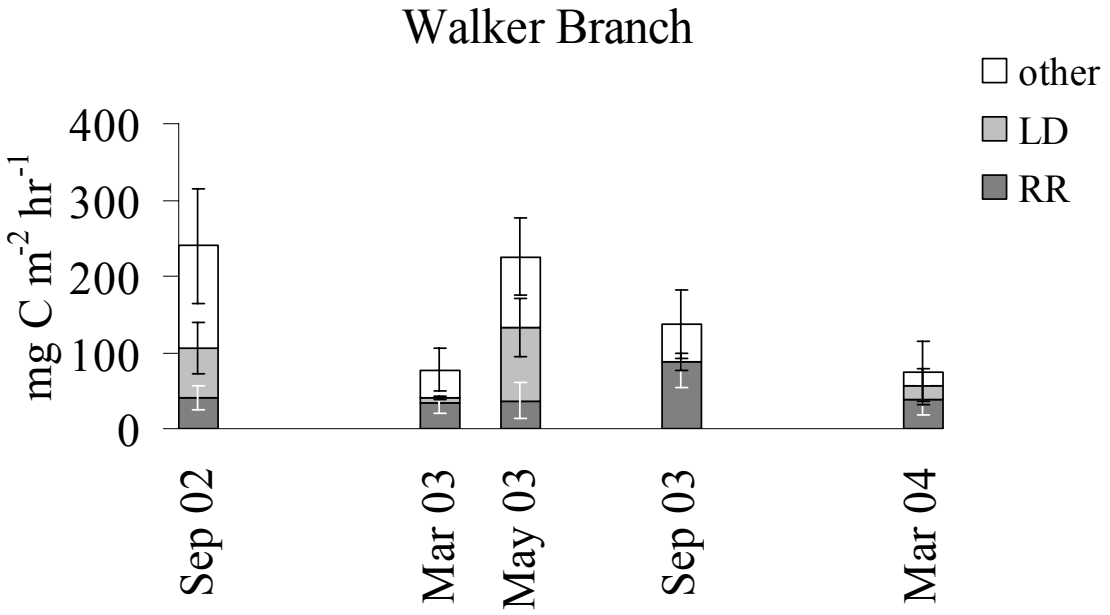


Figure 2.4. Absolute contributions from leaf litter decomposition (LD), root/rhizosphere respiration (RR) and other heterotrophic sources (other) to total soil respiration for the Walker Branch site. Fluxes shown under "other" were calculated as the remaining difference and are assumed to have the isotopic signature of the incubated soil organic matter.

zero in September 2003 and ~6 ±3 mg C m^{-2} hr^{-1} in March 2003, to 96 ±38 mg C m^{-2} hr^{-1} in May 2003. Root respiration fluxes remained fairly constant ranging from 34 ±14 to 40 ±16 mg C m^{-2} hr^{-1} on the different sampling dates with the exception of September 2003 (88 ±35 mg C m^{-2} hr^{-1}). Heterotrophic respiration was larger than root respiration on the days we sampled during the peak of the growing season, but in early spring (March 2003 and 2004), root respiration and heterotrophic sources contributed equally to total soil respiration.

4. Discussion

Large differences in the radiocarbon signatures of leaf litter between treatments allowed us to successfully apply a ^{14}C mass balance approach to separate sources of soil respiration into components derived from root/rhizosphere respiration, leaf litter decomposition, and other heterotrophic sources (Figure 2.4.). We were able to partition total soil respiration at different times in the growing season and identify the effect of moisture on heterotrophic sources. We achieved consistent results across sites in many cases, indicating that our methods of determining isotopic signatures for respiration components do not have significant systematic errors (though our methods for determining fluxes, especially in incubations, do). The partitioning approach gave consistent results even when the isotopic signature of root respiration varied by over 100‰ from one sampling date to the next. Our method does have the problem that if the separation between isotopic signatures of respiration components is not larger than their respective standard deviations (Figure 2.2.), we cannot solve for FRR in any meaningful way.

4.1. Leaf litter decomposition

Estimates of the relative contribution of decomposing leaf litter to total soil respiration (FLD) showed good agreement across sites when moisture was not a limiting factor (Figure 2.3.). The greater standard deviations of $\Delta^{14}C_{\text{leaf litter decomposition}}$ characteristic of HL treatments (Figure 2.2.) translated into large uncertainties in the estimates of FLD on some dates. In March 2004, greater uncertainty for FLD was expected as isotopic differences in $\Delta^{14}C_{\text{leaf litter decomposition}}$ between treatments were

reduced by dilution of the ^{14}C label in leaf litter in the fall of 2003 (the first fall season that native litter fall was allowed into the plots after 3 years of ^{14}C labeled litter additions).

The strong dependence of FLD on moisture conditions was evidenced by the marked difference between sites in July 2002 and September 2003. The link between leaf litter decomposition and moisture has been documented by Lee *et al.* [2004] who found a linear decrease in the relative contribution of the litter layer with lower litter water contents in a manipulated study in a mixed forest. In a tropical forest, an indication of the dependence of FLD on litter moisture content was observed by Goulden *et al.* [2004] when patterns of soil respiration rates varied in concert with the moisture content of the surface leaf litter.

In our study, times of lower total soil respiration fluxes (July 2002 at TVA and September 2003 at Walker Branch; Figure 2.1.) coincided with times of lower relative contributions from leaf litter decomposition (Figure 2.3.). As a result of the dependence on moisture conditions, and in contrast with root respiration, fluxes from leaf litter decomposition were highly variable in time. This suggests that litter decomposition fluxes can dictate the temporal variation in total soil respiration in response to changing moisture conditions, in agreement with findings from other studies [Hanson *et al.*, 2003b; Borken *et al.*, 2005]. Hanson *et al*. [2003b] developed a model to predict total soil respiration validated against repeated measurements of litter fluxes and moisture. Model predictions were found to improve when the response of litter fluxes to variations in litter moisture contents were taken into account. In addition of being a

considerable fraction of soil respiration (up to 42%), the response to moisture conditions emphasizes the relevance of this component of total soil respiration.

4.2. Other heterotrophic sources

Across sites and same litter treatments, the radiocarbon signature of soil decomposition was always higher at TVA (HR) than at Walker Branch (LR) (on average by ~120 ‰, Figure 2.2.). In contrast, the difference in the radiocarbon signature of soil decomposition between HL and LL treatments within a given site remained small (~40 ‰). This indicates that a major source of decomposing material in the 0-5 cm soil layer is derived from carbon fixed in the original 1999 ^{14}C release, rather than the labeled litter treatment applied subsequently. Dead roots are the likely source since differences in soil organic matter in this layer remained small in 2002-2003 (J. Jastrow, 2004, personal communication). In theory, we should have been able to perform a similar analysis to that of FLD to calculate the fraction of heterotrophic respiration coming from labeled root decomposition from the difference between HR and LR sites in $\Delta^{14}C_{\text{soil decomposition}}$. However, since root respiration radiocarbon values differed between sites, we could not independently solve for the root decomposition end member as we could for FLD.

4.3. Root respiration sources

The accurate estimation of $\Delta^{14}C_{\text{heterotrophic}}$ is obviously important in the calculation of FRR (Eq. 3) and this in turn depends strongly on the value of "L" (Eq. 4). Overestimation of "L", as would have occurred with the use of our incubation data as

weighting factors, would have led to an overestimation of $\Delta^{14}C_{heterotrophic,}$ especially in HL treatments (Figure 2.2.). This in turn would have translated into an overestimation of FRR. We are confident that the isotopic signatures measured in incubations ($\Delta^{14}C_{leaf\ litter\ decomposition}$ and $\Delta^{14}C_{soil\ decomposition}$) are unlikely to be a cause of error as radiocarbon values of CO_2 derived from incubated material have been shown to be independent of temperature or moisture variation even with very large changes in CO_2 evolution rate [Dioumaeva *et al*., 2003; Cisneros-Dozal unpublished data].

The estimates of the fractional contribution of root respiration to total respiration (FRR) reported here are not easily compared to the annual means commonly reported in literature. We have not attempted to estimate annual root respiration contributions because of the limited nature of our sampling. However, FRR ranged from a low of 16% to a high of 64% in our study, within the range of published estimates. The annual mean contribution of root respiration to soil respiration has been estimated at ~60% from trenching experiments [Epron *et al.*, 1999, Högberg *et al*., 2001]. A lower estimate by Kelting *et al.* [1998] of 32% in early June was recognized to be a minimum since the sampling did not take place during the period of maximum root growth. In addition, the use of excised roots to measure rates of root respiration remains questionable, because their function has been observed to decrease rapidly after excision [Rakonczay *et al*., 1997]. An earlier study carried out in the ORR estimated that respiration from live roots accounted for 35% of total soil respiration annually for *Liriodendron tulipifera* L. spp. [Edwards and Harris, 1977]. Rey *et al*. [2002] reported an annual contribution from root respiration of 23% although previous disturbance at the site were thought to be the cause of this low estimate. It is important to note that in our study, times of highest

relative contributions (FRR) were not necessarily times of high root respiration fluxes and thus both quantities should be reported. For instance, FRR values in March 2003 and 2004 were higher in comparison to other dates (with the exception of September 2003, Figure 2.3.), however the absolute fluxes were not different (Figure 2.4.).

In contrast to other studies [Epron *et al.*, 2001; Högberg *et al*., 2001; Rey *et al*.,2002], we did not observe marked temporal variation in root respiration fluxes with the exception of the increase in September 2003 (Figure 2.4.). Several studies indicate a link between soil respiration and plant activity through the phenological control on root respiration fluxes [Högberg *et al*., 2001; Janssens *et al.*, 2001; Reichstein *et al.*, 2003; Curiel *et al.*, 2004]. For this forest, a correlation between root growth and leaf area expansion was observed by Joslin *et al.* [2001], with the highest root elongation intensity taking place after the completion of leaf area expansion. In terms of root respiration fluxes however, we did not see an increase during the periods of maximum root growth observed in May, June and July for *Quercus* spp [Joslin and Wolfe, 2003]. We did observe an increase from May to September in 2003; however an earlier increase from March to May would have been expected in accordance with the timing of root growth. More frequent measurements (which may obviate some of the differences in soil moisture and temperature conditions between widely spaced sampling intervals) may be required to capture the relationship between root growth and root respiration patterns.

The observed large differences in the ^{14}C signature of root respiration between sampling dates (Figure 2.2.) was an unexpected result. These temporal changes were very similar at both sites, which would seem to rule out sampling or measurement errors

as the cause. Other causes could be related to changes in the internal sources of C being utilized, or to fluctuations in the radiocarbon content of atmospheric CO_2 (i.e., perhaps further local releases). Trumbore *et al*. [2002] showed that nonstructural carbohydrate pools were labeled in the 1999 event. Depending on the carbohydrate turnover rate, the ^{14}C content of this storage pool could be greater than that of more recent photosynthetic products. For example, leaf buds grown each spring have higher ^{14}C values than atmospheric CO_2, and parasitic plants growing in early spring also have ^{14}C signatures close to our root respiration ^{14}C measurements for the same time period [Trumbore, unpublished data]. The observed declined in the ^{14}C of CO_2 respired by roots at both HR and LR sites from May to July in 2002 and March to May in 2003 indicates the use of stored C as an energy source prior to leaf-out, with a change to current photosynthetic C after the forest canopy matures and the root growth peaks in July. A further indication that roots use more recently fixed carbon during the summer is the observation of very low ^{14}C values for root respiration observed at Walker Branch in July 2002 and May 2003, (i.e., a site under the influence of recent local fossil fuel burning). However, root respiration values remain higher at the HR site (TVA) than the LR site (Walker Branch) throughout the summer, indicating that either some portion of root respiration is derived from storage pools or that differences in atmospheric $^{14}CO_2$ persist between sites. We have taken bi-weekly time averaged samples of air at 1m height above-ground at both HR and LR sites to monitor for events like ^{14}C releases, but it is difficult to link these to values we observe in root respiration measured on one day. Further analysis of radiocarbon in carbohydrates of root tissues is needed in order to better elucidate the source of carbon respired throughout the growing season.

5. Conclusions

We have used ^{14}C labeled leaf litter and roots together with a mass balance approach to quantify heterotrophic and autotrophic (root and rhizosphere) respiration sources in a temperate forest. Heterotrophic sources, especially leaf litter decomposition, account for a large fraction of total soil respiration in this temperate deciduous forest. Heterotrophic respiration in the surface mineral soil layer (0-5 cm) was dominated by the decomposition of C fixed 3-5 years earlier (during the 1999 labeling event). Leaf litter decomposition is a major contributor to overall heterotrophic CO_2 fluxes, but it is also the most variable in space and time in response to changing moisture conditions. The instantaneous relative contributions from leaf litter decomposition ranged from ~1 ±3% to 42 ±16%, corresponding to absolute fluxes of 6 ±3 to 96 ±38 mg C m^{-2} hr^{-1}, and decreased to near zero when leaf litter was extremely dry. As a result of its dependence on moisture conditions, leaf litter decomposition is apparently the main source of temporal variation in total soil respiration in this forest.

In contrast, root/rhizosphere respiration fluxes did not exhibit marked temporal variation, ranging from 34 ±14 to 40 ±16 mg C m^{-2} hr^{-1} with a single exception of increased fluxes (88 ±35 mg C m^{-2} hr^{-1}) in September 2003. The relative contributions from root/rhizosphere respiration ranged from 16% ±10 to 64% ±22. Times of highest relative contributions did not necessarily coincide with times of highest absolute fluxes, underscoring the importance of reporting quantities. The radiocarbon signatures of root respiration provided preliminary information on the source of C respired by roots and indicated that sources of C respired may shift from stored C pools in early spring to recent photosynthetic products as the summer progresses.

The 1999 ^{14}C-pulse being studied in the EBIS project provided a unique opportunity for rapid evaluation of the components of soil respiration. The same isotope mass balance approaches can presumably be applied in other ecosystems where the sources of soil respiration differ substantially in age (such as in boreal forests). While similar studies can not be done for all established forest ecosystems, other situations where anthropogenic activities may have perturbed the background isotopic ^{14}C status may exist and should be investigated.

CHAPTER 3

THE EFFECT OF MOISTURE ON LEAF LITTER DECOMPOSITION AND ITS CONTRIBUTION TO SOIL RESPIRATION

Abstract

We used ^{14}C-enriched leaf litter and different temperature and moisture conditions (both natural and manipulated) together with ^{14}C measurements in CO_2 from soil, heterotrophic and autotrophic respiration, to show that increased soil respiration following precipitation is accounted for by increased decomposition of the leaf litter layer, predominantly from leaf litter that is ≤3 years old. Leaf litter decomposition fluxes increased on average from 11±11 mg C m^{-2} hr^{-1} during a transient drought to 53±25 mg C m^{-2} hr^{-1} immediately after water addition corresponding to an increase in the contribution to soil respiration from 8±8% to 32±12%. Pre-wetting conditions determined the degree of increase; litter CO_2 efflux shows the largest increase when very dry litter is wetted. At least 50% of the enhanced leaf litter decomposition flux was derived from ^{14}C-enriched material ≤3 years old which translates into a contribution from this recent C substrate of >15% to a maximum of 30% of soil respiration fluxes without moisture limitation. Heterotrophically respired CO_2 from the mineral soil horizons contained very little recently leached carbon (≤3 years old) from leaf litter (6 ±4%). Temperature (13ºC versus 25ºC) and moisture (dry versus field capacity) conditions dramatically influenced the decomposition rates for organic matter in

mineral and litter layers but did not change the ^{14}C signature of the evolved CO_2. This indicated that the relative contributions of different decomposition substrates, which have a range of isotopic compositions, remain constant and all sources were enhanced or declined to the same degree.

1. Introduction

Net ecosystem productivity (NEP) is the difference between ecosystem uptake of CO_2 by photosynthesis and CO_2 loss by respiration. Most of the uncertainty associated with NEP estimates is contributed by soil respiration. Soil respiration is a major component of ecosystem respiration [Janssens et al., 2001] and consists of CO_2 derived from both plant (autotrophic) and microbial (heterotrophic) sources. Hence uncertainties in estimates of NEP reflect the difficulty of separating heterotrophic from autotrophic respiration in the field [Hanson et al., 2000].

Soil respiration fluxes are highly variable in space and time, even within a single ecosystem. While temperature can apparently account for much of this variability, factors such as the covariance of soil temperature with moisture and with phenological patterns (i.e. root growth, seasonal availability of substrate) confound the temperature-CO_2 flux relationship [Davidson et al., 1998]. In addition, sudden and transient changes in moisture availability are a source of temporal variability. Hanson et al., [2003b] estimated that a failure to incorporate short-term enhancement in soil respiration following precipitation can lead to underestimation of the annual soil respiration flux in forested ecosystems.

A number of field studies have observed a rapid increase in soil respiration fluxes with increased water availability (i.e. precipitation, snow melt), particularly those that follow a period of drought [Borken et al., 1999; Borken et al., 2002; Savage and Davidson, 2001; Scott-Denton et al., in press]. These variations have been shown to be better correlated with leaf litter moisture rather than soil moisture [Borken et al., 2003; Hanson et al., 2003b], suggesting a heterotrophic response. However, addition of moisture to dry soils may stimulate both autotrophic [Borken et al., 2005; Burton et al., 1998] and heterotrophic components of soil respiration.

Identifying autotrophic and heterotrophic responses to changes in soil moisture is a difficult task to perform under field conditions. Three general methods have been used: (1) correlation of soil respiration fluxes with moisture and temperature conditions [Epron et al., 2004; Goulden and Crill, 1997; Yuste et al., 2003] (2) manipulations such as girdling [Bhupinderpal-Singh et al., 2003; Hogberg et al., 2001] or trenching to remove the heterotrophic component [Bond-Lamberty et al., 2004; Boone et al., 1998; Jiang et al., 2005; Lavigne et al., 2003; Lee et al., 2003] or removal of the leaf litter layer to determine leaf litter decomposition contribution [Lee et al., 2004; Rey et al., 2002] and (3) isotopic mass balance approaches that take advantage of differences in the C isotopic signature of autotrophic and heterotrophic (including litter versus mineral decomposition) sources [Borken et al., 2005; Cisneros-Dozal et al., 2005; Subke et al., 2004]. In addition, leaf litter decomposition has been studied on time scales of months to years using litter decomposition bags which are periodically monitored for changes in mass loss. The litterbag approach has been combined with rainfall exclusions to estimate the effect of moisture on decomposition but the detection of drought effects on

decomposition has been difficult by measuring mass loss alone contrasting with nitrogen indexes which do show a reduction with drought [Emmett et al., 2004; Hanson et al., 2003b].

When performed in combination with measurements of leaf litter and/or soil moisture changes (either natural or induced) these methods allow estimations of the effect of changes in moisture availability on soil respiration (or its components) and the spatial and temporal variability. For example, in a deciduous forest, induced drought affecting mainly the water content of the O horizon reduced total soil respiration rates, reflecting mostly a lower contribution from decomposition of leaf litter [Borken et al., 2005]. Similarly, Goulden et al., [2004] observed a clear strong influence of leaf litter moisture on total soil respiration in a tropical forest when soil respiration rates (measured with automated chambers) increased approximately 2-fold following rain events that increased leaf litter moisture from ~15% to 20 - 40% moisture by volume. Keith et al., [1997] also observed a correlation between soil respiration fluxes and the water content of leaf litter measured gravimetrically, although only above a soil temperature greater than 10ºC. Lee et al., [2004] combined soil CO_2 flux measurements with and without leaf litter removal with an irrigation treatment to determine the effect of moisture on the litter contribution to soil respiration. They found that leaf litter decomposition in a mixed forest varied almost linearly with leaf litter moisture (fractional contributions ranged from ~0.35 to 0.55 when leaf litter moisture varied from 5 to 15% by volume).

Combining soil respiration and isotopic measurements has the advantage of minimizing disturbance. This has been successfully applied with ^{13}C- [Subke et al.,

2004] and ^{14}C-labeled leaf litter [Cisneros-Dozal et al., 2005] to estimate the fraction of leaf litter decomposition to the soil respiration flux in boreal and temperate environments respectively. Cisneros-Dozal et al., [2005] showed that leaf litter decomposition contributed from ~1 to 42% of the total soil respiration flux in a temperate deciduous forest, with lowest contribution coinciding with extremely dry leaf litter conditions.

Under laboratory conditions, a number of studies have shown that the decomposition of leaf litter increases with increasing moisture. Borken et al., [2003] observed increased CO_2 production from incubated leaf litter samples (representing the entire O horizon) with higher leaf litter moisture content almost immediately after water addition. Using measurements of respiration from leaf litter samples and of water content in laboratory conditions, Hanson et al., [2003b] observed that changes in respiration rates from leaf litter were highly variable and followed the changes in leaf litter water content with minimum respiration rates at water contents < 0.5 g g^{-1} or approximated leaf water potential < -2.5 MPa. In another laboratory experiment, Van Cleve and Sprague, [1971] also observed reduced respiration rates (measured as O_2 uptake) from leaf litter samples of birch and aspen forests at levels of moisture content < 0.5 g g^{-1}, although data on leaf water potential was not provided.

In this study, we used ^{14}C labeled leaf litter and moisture manipulations to assess the role of moisture in controlling the amount of soil respiration derived from leaf litter decomposition. We combined measurements of ^{14}C in CO_2 evolved from litter and mineral soil under different temperature and moisture conditions with field measurements of changing soil respiration with a moisture manipulation and following

a natural rainfall event. We used both manual and automated chamber techniques for sampling CO_2 fluxes and radiocarbon signatures in the field. Our measurements use an isotope mass balance (^{14}C and ^{13}C) method to determine the role of heterotrophic versus autotrophic activity in causing the observed increase in soil respiration fluxes after rain events.

2. Methods

2.1. Site description and experimental design

This study was conducted as part of the Enriched Background Isotope Study (EBIS) experiment [Cisneros-Dozal et al., 2005; Hanson et al. 2005; Trumbore et al., 2002] which uses labeled leaf litter produced after a whole ecosystem ^{14}C label caused by releases from hazardous waste incinerators in 1999. The site is a temperate deciduous forest located in the U.S Department of Energy's Oak Ridge Reservation near Oak Ridge, Tennessee at 35°58 north and 84°17 west [Johnson and Van Hook, 1989]. Mean annual precipitation is 1352 mm and mean annual temperature is 14.2°C. The soils are Ultisols and the forest vegetation is dominated by Quercus spp and Acer spp [Hanson et al., 2003; Huston et al., 2003]. All measurements reported in this chapter were made at the Walker Branch site which was exposed to low levels of plant labeling in the 1999 ^{14}C release.

A detailed description of the EBIS project and experimental design can be found in Hanson et al. [2005] with additional details at http://ebis.ornl.gov/. Briefly, 8 plots of 7 by 7 meters received ^{14}C-labeled leaf litter over three consecutive years replacing local litter fall. 4 plots received near background or 'low' ^{14}C-labeled litter (LL) that

was ~215 per mil and the other 4 plots received enriched or 'high' ^{14}C-labeled leaf litter (HL) that was ~971 per mil. Starting in the fall of 2000, natural leaf litter fall was excluded from the plots. Labeled leaf litter additions took place in May 2001, February 2002 and February 2003.

2.2. Moisture and temperature measurements

Soil (0 to 15 cm) and leaf litter (Oi horizon) moisture and temperature were monitored with multiple sensors and a data logger (CR10X, Campbell Scientific, Inc.) coupled to a multiplexer (AM 16/32 Relay Analog Multiplexer, Campbell Scientific, Inc). Readings from all sensors were stored every 12 minutes. Litter water content of the Oi horizon was measured using the half bridge approach [Hanson et al., 2003b] as modified by Borken et al., [2003]. A total of 9 half bridge (HB) sensors were installed inside the plots in 3 different areas (3 sensors per m^2) and irrigated in the same manner as the collars used for soil respiration on day 250 (see below). As a control, 7 HB sensors were placed in an area with no irrigation. The sensors were placed in the Oi horizon only. The voltage output from the HB sensors was converted to litter water content (LWC) using the calibration curve for the Oi horizon from Borken et al., [2003]. While this calibration curve is site-specific, the LWC we calculated provided a way to monitor the changes in litter moisture conditions and relate them to measured radiocarbon signatures of soil respired CO_2. Long-term LWC data from the Oi layer is also available at this site on an hourly basis from a different set of HB sensors [Hanson et al., 2003b] for the control sites. Additionally, moisture changes in the Oi layer were monitored with a Fuel Moisture Sensor (CS505 Campbell Scientific, Inc.) placed

horizontally on the forest floor and manually irrigated on day 250 (see below). Litter temperature in the Oi was measured with a Humidity and Temperature Probe (Vaisala HMP44) in an area without manual irrigation.

Soil moisture changes in the upper ~15 cm of mineral soil were monitored using Water Content Reflectometers (CS616 Campbell Scientific, Inc.) installed at angle of ~20 degrees with the surface, using one sensor in dry or irrigated areas. Soil temperature was measured with four thermistors per either dry or irrigated area installed immediately below the O horizon.

2.3. Measurement of soil respiration and its ^{13}C and ^{14}C signature

Measurements of soil respiration (CO_2) fluxes and collection of CO_2 for analyses of ^{13}C and ^{14}C were carried out at three levels of leaf litter moisture, on days 243, 248 and 250 in 2003, using manual and automated chambers in parallel to evaluate agreement between methods. The initial and drier moisture contents measured on day 243 resulted from a transient late summer drought; surface leaf litter was very dry and brittle and rain had not reached the forest floor for 13 days prior to sampling. Field measurements were carried out just before a heavy rain event that occurred at the end of the afternoon (see below). Less dry conditions (our 'intermediate' moisture content) were attained on day 248 after a few days with precipitation had wetted the litter layer (32 mm total) and it was starting to dry out again. On day 250, we manually irrigated the area inside and around the collars (a total area of 1 m^2) used for soil respiration measurements, 6 collars for manual chambers and 4 collars for automated chambers. The irrigation took place after leaf litter moisture had decreased to levels close to those

on day 243 (see below). We irrigated all collars with an amount of 7.5 litters of water per m^2 (7.5 mm) to simulate a heavy rain. The water used was commercially available purified water and was sprayed uniformly over and around the collar. Measurements took place a few minutes after the addition of water to allow for draining.

Collars for the manual chamber measurements (3 per litter treatment) were inserted ~2-5 cm into the mineral soil at least 24 hours before measurements began. We used closed dynamic chambers attached to an infrared gas analyzer (LI-800, LiCor, Inc. Lincoln, Nebraska) [Davidson et al., 2002]. After placing the chamber lid on the collar, we monitored and recorded (LI-1400, data logger, LiCor, Inc. Lincoln, Nebraska) the increase in the CO_2 concentration in the headspace of the chamber for about 10 min. Initial unstable portions of the plot of CO_2 flux over time (usually the first 40-60 seconds after placing the lid) were not included in regressions used for flux estimation. Following soil respiration measurements, we collected headspace CO_2 for ^{14}C and ^{13}C analysis using molecular sieve traps (mesh size 13X; Advanced by UOP, Specialty Gas Equipment). The procedure for CO_2 collection using manual chambers and the ^{14}C and ^{13}C analyses, are described in Cisneros-Dozal et al., [2005] and Gaudinski et al., [2000]. All measurements with manual chambers took place between 11:00 and 16:00 hours.

A total of 8 automated chambers were installed in two treatment plots in August 2003 (HL and LL, 4 chambers per plot). The automated closed chamber system is described in detail elsewhere [Winston, in preparation]. Soil respiration fluxes were measured continuously from day 242 through 252 using an infrared gas analyzer (LI-820, LiCor, Inc. Lincoln, Nebraska) and recorded with a data logger (CR10X, Campbell Scientific, Inc.). The automated chamber system was programmed to sequentially close

the eight chambers after flushing tubing lines, while monitoring the CO_2 concentration continuously, along with chamber air temperature and atmospheric pressure. The system includes a sampling manifold downstream of the IRGA on the chamber return line, for the purpose of selectively trapping CO_2 for isotopic analysis. Nine molecular sieve 13X traps for CO_2 collection (8 for the automated chambers and one for ambient air) were connected to the solenoid valves which were programmed to sequentially open the air flow coming from the headspace of the chamber into the molecular sieve trap. The trapping program for each chamber comprised a total of 12 minutes: 2 min of monitoring ambient air CO_2 concentration before the chamber closed, 7 min of measuring the rate of CO_2 emission after closing the chamber followed by 2 minute of CO_2 collection (circulating air from the chamber into the molecular sieve trap) (Appendix 1). A complete cycle to sample the 8 chambers lasted 96 minutes with some time allotted for trapping of CO_2 from ambient air and for flushing the system (IRGA, tubing, etc.). CO_2 was collected over 3 to 4 complete cycles in order to get enough carbon for ^{14}C and ^{13}C analysis. The ^{13}C of CO_2 from chambers and the ^{14}C and ^{13}C of CO_2 from ambient air were used to correct the ^{14}C signature of CO_2 collected from the chambers, which included both, the signature soil respiration and the signature of the ambient air initially present in the chamber at the time of closing [Gaudinski et al., 2000].

2.4. ^{14}C and ^{13}C signatures of heterotrophic decomposition sources

To estimate the isotopic signatures of leaf litter and soil organic matter decomposition (0-5 cm) and their dependence on moisture and temperature, we carried out incubations of leaf litter samples and soil cores combining two levels of temperature and moisture. Six samples (3 per HL and LL treatment) of the entire O horizon (including the added ^{14}C-enriched litter plus more decomposed parts of the O horizon that predate the litter addition experiments) in an area of 0.021 m^2 were collected on day 243 when leaf litter moisture was the driest (on the first day of CO_2 collection for radiocarbon analysis). Soil samples representing the upper 5 cm of mineral soil were collected from the same area using a core (4.7 cm diameter by 5 cm long). The soil cores were placed in glass jars of similar dimensions trying to preserve the cores intact. The soil and leaf litter samples were refrigerated for transport to the laboratory and until the time of incubation. At the time of the incubation, 4 sub-samples of each of the six leaf litter samples were placed in aluminum foil (perforated) and inside sealed jars avoiding unnecessary disturbance. The soil cores were divided in 4 sub-samples and each was placed in a glass jar. The open-mouth glass jars with soil sub-samples were sealed inside 1 L Mason jars outfitted with inlet and outlet valves on the lids. The 4 sets of leaf litter and the mineral soil sub-samples were incubated at 25ºC for 6 days in order to estimate the rates of CO_2 production which were used for normalization of the rates under manipulated temperature and moisture conditions [Dioumaeva et al., 2002]. After this initial incubation, two sets of leaf litter sub-samples received water to field capacity allowing for draining before placing them back inside the jars. Similarly, two sets of soil samples received water to reach a moisture content of 20% by volume (chosen

arbitrarily). One set of moistened leaf litter and soil sub-samples was then placed at 25ºC and the other at 13ºC; we also incubated control samples that remained at original field moisture conditions at the same two temperatures to provide the full factorial of temperature and moisture conditions. For the 13ºC temperature we used small commercial refrigerators equipped with temperature controllers as described in Dioumaeva et al., [2002]. For the incubations at 25ºC, the samples were placed at room temperature inside a closed cabinet to prevent sudden temperature fluctuations associated with building air handing changes. Temperatures were monitored with Onset (R) temperature loggers.

Analysis of CO_2 for ^{14}C and ^{13}C content was carried out as described by Cisneros-Dozal et al., [2005]. The ^{14}C signatures of leaf litter and soil organic matter decomposition (hereafter referred to as $^{14}C_{\text{leaf litter decomposition}}$ and $^{14}C_{\text{soil decomposition}}$ respectively) were estimated by litter treatment as the mean ± standard deviation of 3 separate incubations at each temperature and moisture condition. ^{13}C signatures were estimated as the mean ± standard of 6 separate incubations (there were no ^{13}C leaf litter treatments) at each temperature and moisture condition. ^{14}C signatures were expressed in $\Delta^{14}C$, the deviation of the $^{14}C/^{12}C$ ratio of the sample from that of the standard (oxalic acid) in per mil (‰), normalized to a common value of -25 ‰ in $\delta^{13}C$.

2.5. Contribution of leaf litter decomposition to total soil respiration

The experimental design combined with radiocarbon measurements in CO_2 from soil respiration allowed us to estimate the fraction of total soil respiration coming from leaf litter decomposition (hereafter referred to as FLD) since the additions began

[Cisneros-Dozal et al., 2005]. In order to calculate this fraction, we assigned the difference in the radiocarbon signatures of total soil respiration ($^{14}C_{total}$) between HL and LL plots to be derived from the difference in the radiocarbon signature of CO_2 evolved in incubation of the leaf litter ($^{14}C_{\text{leaf litter decomposition}}$) from HL and LL plots:

$$FLD = (\Delta^{14}C_{total)\mathbf{HL}} - \Delta^{14}C_{total)\mathbf{LL}})/(\Delta^{14}C_{\text{leaf litter decomposition})\mathbf{HL}} - \Delta^{14}C_{\text{leaf litter decomposition})\mathbf{LL}}) \quad \text{(Eq. 3.1.)}$$

This method assumes that radiocarbon signatures of other potential soil respiration sources, including autotrophic respiration and the CO_2 evolved from mineral soil organic matter decomposition, do not differ between treatment plots. Our measurements show this to be the case [Cisneros-Dozal et al., 2005]. Hence if there is no isotopic difference in the CO_2 respired from HL and LL plots, there is no contribution from leaf litter decomposition; while a large difference would indicate that leaf litter as an important source of respired CO_2.

2.6. Contribution of the labeled leaf litter to heterotrophic respiration sources

Given the distinct ^{14}C signatures of the low- and high-labeled leaf litter added, we estimated the relative contribution of the labeled leaf litter to heterotrophic decomposition in the O horizon and the 0-5 cm of mineral soil (hereafter referred to as FLL_O and FLL_M respectively). These fractions would represent the decomposition of leaf litter that is ≤3 years old. The mass balance equations for the O horizon and mineral soil respectively are:

$$FLL_O = (\Delta^{14}C_{\text{leaf litter decomposition})\mathbf{HL}} - \Delta^{14}C_{\text{leaf litter decomposition})\mathbf{LL}})$$
$$*(1/(\Delta^{14}C_{\text{labeled litter})\mathbf{HL}} - \Delta^{14}C_{\text{labeled litter})\mathbf{LL}})) \quad \text{(Eq. 3.2.)}$$

$$FLL_M = (\Delta^{14}C_{\text{soil decomposition})\mathbf{HL}} - \Delta^{14}C_{\text{soil decomposition})\mathbf{LL}})$$
$$*(1/(\Delta^{14}C_{\text{labeled litter})\mathbf{HL}} - \Delta^{14}C_{\text{labeled litter})\mathbf{LL}})) \quad \text{(Eq. 3.3.)}$$

In this mass balance we assume that the ^{14}C signatures of CO_2 evolved in leaf litter and soil incubations ($^{14}C_{\text{leaf litter decomposition}}$ and $^{14}C_{\text{soil decomposition}}$ respectively) reflect the ^{14}C signature of the combined heterotrophic sources in the sample (labeled and unlabeled); any mass dependent fractionation effects will be accounted for in the ^{13}C correction in the calculation of ^{14}C. We used the amount of ^{14}C label added each year as the actual ^{14}C content in the incubated leaf litter ($^{14}C_{\text{labeled litter}}$) but recognize that these values may differ from each other due to decomposition and possibly leaching, as has been suggested by Hanson et al., [2005]. Leaching of more ^{14}C-enriched material (i.e. leaving a ^{14}C-depleted residue; [Hanson et al., 2005] would imply that our calculation underestimates the labeled litter contribution to total soil respiration; hence, our estimates of FLL_M should be considered the minimum fraction of soil respiration derived from decomposition of the labeled litter.

2.7. Contribution of autotrophic respiration to soil respiration

The ^{14}C signature of autotrophic respiration ($\Delta^{14}C_{\text{autotrophic}}$), defined here as the CO_2 evolved from live roots and rhizosphere respiration as well as the respiration of any

other recent C substrate of similar radiocarbon content, was measured from excised live roots in the field as described by Cisneros-Dozal et al., [2005]. The ^{13}C signature of autotrophic respiration was also measured in the CO_2 collected for radiocarbon analysis. CO_2 from autotrophic respiration was only collected on day 243.

The mass balance approach used to calculate its relative contribution to soil respiration (hereafter referred to as FRR) is as follows and was also applied to ^{13}C signatures:

$$\Delta^{14}C_{\text{total respiration}} = \Delta^{14}C_{\text{autotrophic}} \cdot FRR + \Delta^{14}C_{\text{heterotrophic}} \cdot (1\text{-}FRR) \qquad \text{(Eq. 3.4.)}$$

In the case of ^{14}C signatures, problems with the flux-weighting of mineral and litter incubation fluxes to derive $\Delta^{14}C_{\text{heterotrophic}}$, led us to calculate FRR and $\Delta^{14}C_{\text{heterotrophic}}$ simultaneously using HL and LL plots measured on the same day and assuming the breakdown of soil respiration into FRR, FLD, and 'other' sources was the same for both treatment plots on the same day [Cisneros-Dozal et al., 2005]. We used a similar constraint here when using ^{13}C, with the exception that FRR was only constrained by FLD estimates as there are no ^{13}C leaf litter treatments.

3. Results

3.1. Moisture and temperature

Leaf litter temperature exhibited a distinct diel pattern (attenuated only by rainfall) with highest temperatures in the afternoon (~3 pm) and lowest in the early morning (5 to 8 am). Soil temperature followed a similar pattern with smaller oscillations (Figure 3.1a). Leaf litter moisture was influenced by precipitation and the manual irrigation on day 250 (Figure 3.1b). Data from our newly installed half bridge (HB) litter moisture sensors were not reliable before precipitation on day 243 due to

non-equilibrium moisture conditions. The pattern of response to wetting, starting with the rain event at the end of day 243, however, agreed well with that of the long-term HB sensors installed at the site (data not shown). For the very dry conditions on the first day of radiocarbon sampling (day 243, before the rain event) data from the long-term sensors indicated constant and low levels of litter water content around 0.34 g g^{-1} during the time of sampling.

Litter water content as measured with the HB sensors on the second sampling day (day 248) over the time of soil respiration measurements (over approximately 3 hours) declined continuously throughout the day from 0.9 to 0.1 g g^{-1} and thus it was difficult to assign one single value of litter water content for this period. The same pattern of decreasing leaf litter moisture was observed on this day with the long-term HB sensors with hourly values ranging from 1.2 to 0.6 g g^{-1}over the same period. Readings from the Fuel Moisture Sensor placed on the forest floor also showed a decrease in its moisture content over the same period of time on day 248 (Figure 3.1c). Among the 16 HB sensors, some showed a continuous decline, while others remained fairly constant. Large variation among sensors was also observed with values ranging from 2.7 to 0.0 g g^{-1}. Differences between the HB outputs reflect real heterogeneity in the field.

The manual irrigation on day 250 increased moisture levels of leaf litter (Figure 3.2a) and the moisture content of the subtending mineral soil (Figure 3.2b). Litter water content increased from 0.2 to 1.7 g g^{-1}. Light precipitation at the end of the day after the field measurements were finished and low vapor pressure deficits, maintained

Figure 3.1. a) Temperature of the Oi horizon (solid line) measured with a Temperature Probe (Vaisala HMP44) and of the upper 5 cm of mineral soil (dotted line) measured with thermistors, b) Moisture content of the Oi horizon measured with 9 half bridge sensors (solid line) and precipitation data (diamonds), days of CO_2 collection for radiocarbon analysis are indicated with arrows, c) Moisture changes in the Oi horizon (solid line) measured with a ponderosa pine dowel (Fuel Moisture Sensor, CS505 Campbell Scientific, Inc.) and in the upper 15 cm of mineral soil (dotted line) measured with a Water Content Reflectometers (CS616 Campbell Scientific, Inc.),

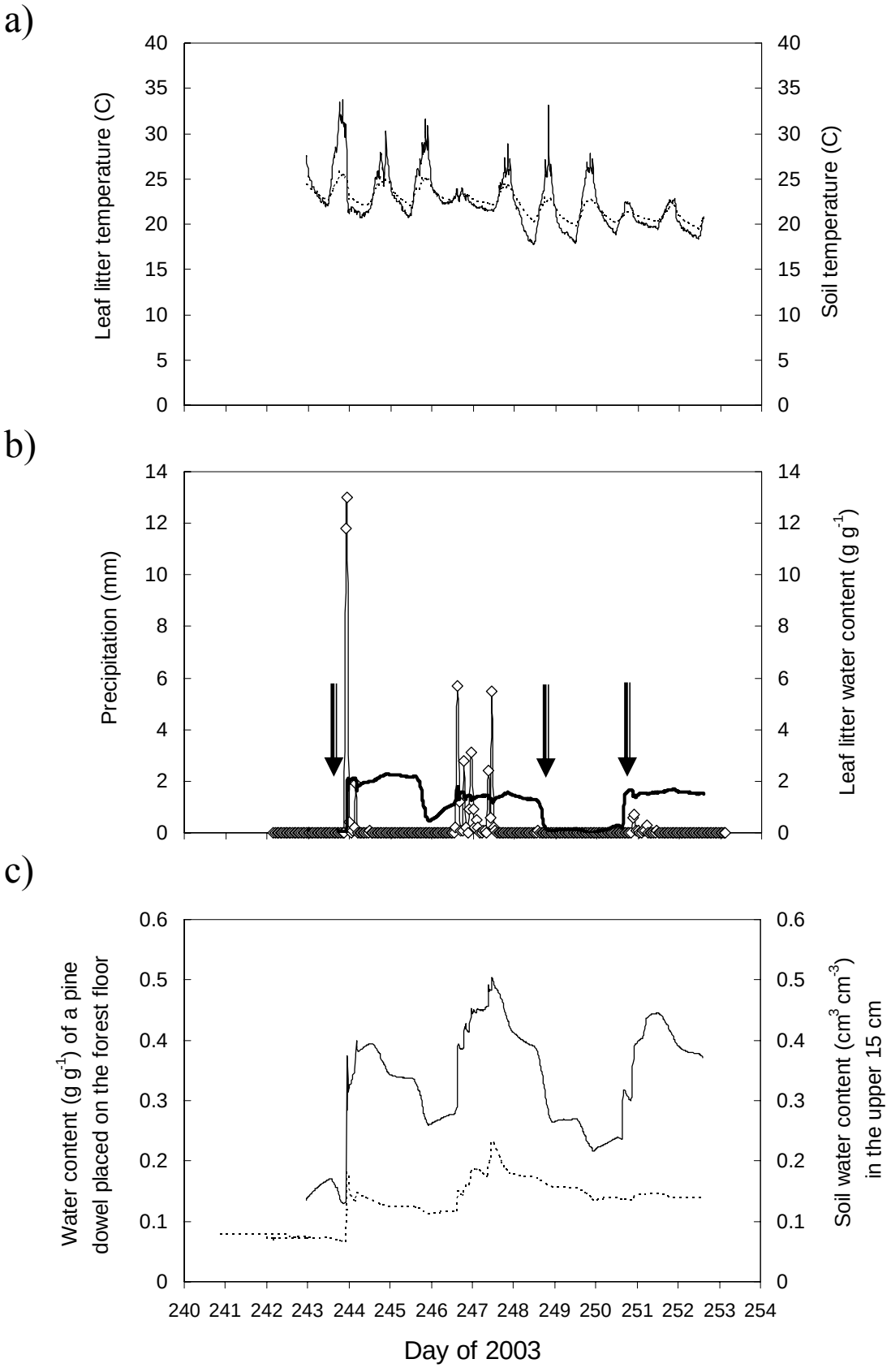
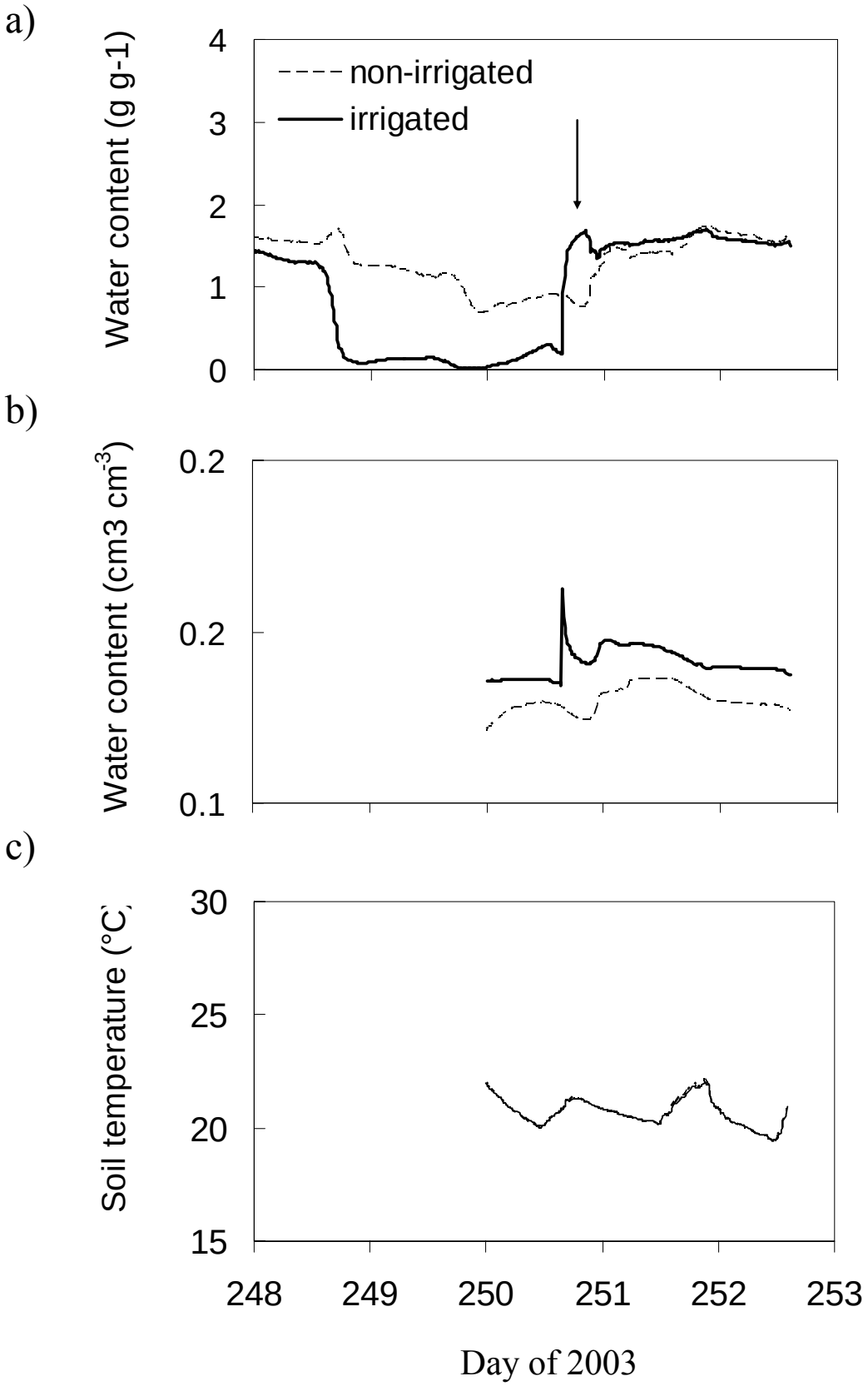

a)
Leaf litter temperature (C)
Soil temperature (C)
b)
Precipitation (mm)
Leaf litter water content (g g-1)
c)
Water content (g g-1) of a pine dowel placed on the forest floor
Soil water content (cm3 cm-3) in the upper 15 cm
Day of 2003

Figure 3.2. Effect of the manual irrigation on: a) Moisture content in the Oi horizon (arrow indicates approximate sampling time for radiocarbon), b) Moisture content in the mineral soil (0-15 cm depth) and c) Soil temperature in surface mineral soil.


a)
Water content (g g-1)
non-irrigated
irrigated
b)
Water content (cm3 cm-3)
c)
Soil temperature (°C)
Day of 2003

the litter water content at around 1.5 g g^{-1}. The mean value of leaf litter moisture in non-irrigated areas (measured with 7 HB sensors) is provided as well for comparison (Figure 3.2a); litter moisture in these areas continued to decrease until the light precipitation event when it reached a similar value to that of the irrigated areas. Soil moisture of the surface 15 cm increased only slightly after manual irrigation (from 0.13 to 0.16 cm/cm^3; Figure 3.2b) while temperature in the surface mineral soil remained unaffected (Figure 3.2c).

3.2. Soil respiration fluxes and isotopic signatures

CO_2 fluxes measured with the automated chambers ranged from ~120 to 300 mg C m^{-2} hr^{-1} (Figure 3.3.); due to leaks in two of the chambers, we report only six fluxes here. CO_2 fluxes increased immediately after rainfall on days 243, 246 and 250. The response after the first rainfall was most pronounced, likely due to the combined effect of a more intense rainfall and to preceding drier conditions (as mentioned above, surface leaf litter was very dry and brittle at the time of sampling for radiocarbon on day 243). During days 243, 248 and 250 when manual soil respiration measurements were obtained, CO_2 fluxes measured with the 6 automated chambers averaged from 142 ±16, 160 ±28 and 161 ±41 (average of 4 irrigated chambers) on each sampling day respectively. The means were not significantly different ($p > 0.05$) between sampling days. We observed high variability among chambers within a treatment plot (likely reflecting real heterogeneity in the field) that made it difficult to quantify differences between sampling days using automated chambers. CO_2 fluxes measured with the manual chambers averaged 137 ±27, 173 ±16 and 170 ±29 mg C m^{-2} hr^{-1} on days 243,

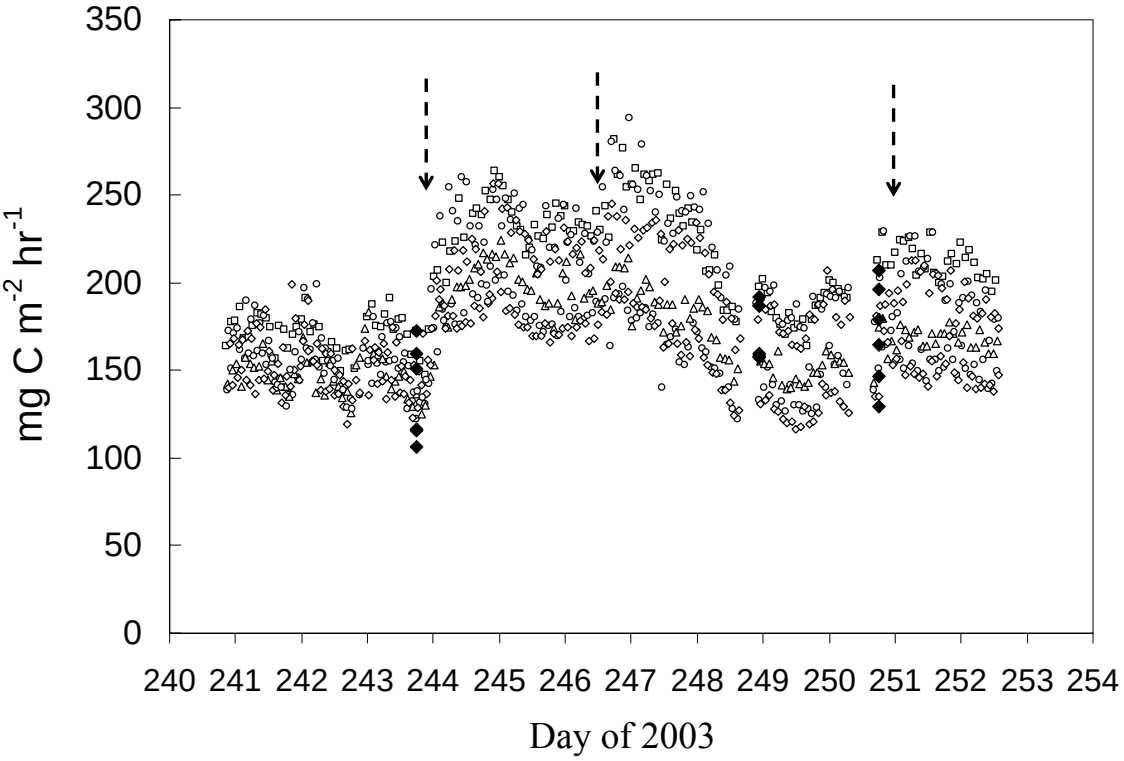


Figure 3.3. Soil CO_2 fluxes measured with 6 automated chambers (white symbols) and manual chambers (black diamonds); dotted arrows indicate precipitation.

248 and 250 respectively (Figure 3.3.). The means were significantly different ($p < 0.05$) between the first and second sampling day only. Again, natural variability in the field accounted for similarities in the mean fluxes between day 243 and 250 when leaf litter moisture differed the most. Fluxes measured with the manual chambers and automated chambers were not significantly different ($p > 0.05$) from each other in any sampling day.

Radiocarbon signatures collected using the automated and manual chamber techniques were similar, and exhibited the same temporal pattern of increasing difference between litter treatments with higher leaf litter moisture on days 243, 248 and 250 (Figure 3.4.). Due to the large standard deviation in the HL treatment as measured with the manual chambers on day 248 (Figure 3.4a), the difference between treatments was only statistically significant on day 250 ($p < 0.05$). Radiocarbon signatures measured with the automated chambers were significantly different between treatments on the three sampling days ($p < 0.05$).

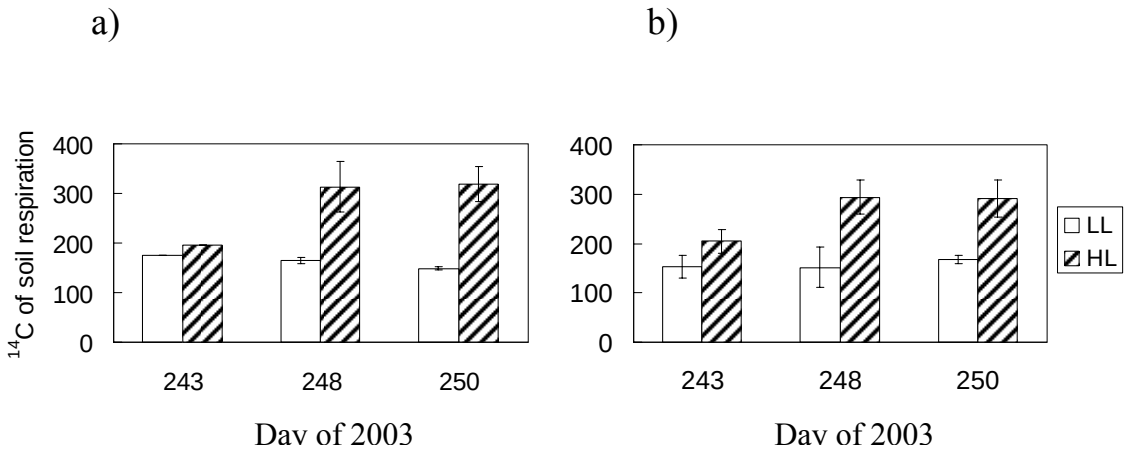


Figure 3.4. Radiocarbon signatures of soil respiration by leaf litter treatment, HL and LL (high and low ^{14}C-labeled leaf litter respectively) measured using (a) manual and (b) automated chambers. Each value from manual chambers is the mean of 3 measurements ± standard deviation (except for LL treatment on day 243 with only one value). Values from automated chambers are the mean of 3 to 4 measurements on days 243 and 248, and of 2 measurements on day 250, ± standard deviation.

The ^{13}C signature of soil respiration measured with manual chambers was -24.4 ±0.2, -25.9 ±0.3 and -26.2 ±0.3‰ for low, intermediate and high moisture conditions respectively; ^{13}C signatures measured with automated chambers reflect the mixture of ambient air and soil-respired CO_2 and were used to determine the fraction of air in the sample for correction of radiocarbon signatures as mentioned above.

3.3. Dependence of the ^{14}C and ^{13}C signatures of heterotrophic decomposition sources on temperature and moisture

The radiocarbon signatures of leaf litter and mineral soil decomposition derived from incubations were unaffected by temperature and moisture (Figure 3.5.). The mean ^{14}C signature at each of the manipulated incubation conditions (at 13ºC, at higher moisture content and at the two conditions combined), was not significantly different ($p>0.05$) from the mean signature of the CO_2 evolved at 25ºC. In contrast, the rates of CO_2 evolution inside the jars did show the effect of change in temperature and moisture, with fluxes increasing by 260% at highest moisture levels (Figure 3.6a), and decreasing by 27% of base values at lower temperature (Figure 3.6c) in the case of leaf litter. The combined effect of lower temperature and increased moisture had a mixed effect with some rates remaining unchanged (Figure 3.1e). Similar effects were observed in the incubated mineral soil (Figure 3.1b, d, f) although the increase in the rates of CO_2 production after water addition was of lesser magnitude.

The mean ^{13}C signature of the CO_2 evolved in the manipulated leaf litter incubations increased ~1‰ with a change in the incubation temperature from 25ºC to 13ºC at both, field moisture and field capacity conditions (Table 3.1.). In mineral soil incubations, the mean ^{13}C signatures of respired CO_2 were only significantly different at the combined 13ºC and increased moisture condition with respect to the incubation at 25ºC and field moisture.

Figure 3.5. Radiocarbon signature of heterotrophic decomposition measured as the ^{14}C signature of the CO_2 evolved during incubations of leaf litter and soil cores, at field moisture (open symbols) and at field capacity (closed symbols). a) leaf litter samples incubated at 25°C, b) leaf litter samples incubated at 13°C, c) soil cores incubated at 25°C and d) soil cores incubated at 13°C. A total of 6 samples, 3 per litter treatment, HL and LL (high or low ^{14}C-labeled leaf litter respectively) were incubated in each condition. The ^{14}C signatures of the O horizon and upper 15 cm of mineral soil (measured in February 2003 before the third litter addition) are indicated with lines as follows: in a) and b), coarse and fine solid lines correspond to the Oi horizon in HL and LL treatments respectively and coarse and fine dotted lines correspond to the Oe/Oa horizon in HL and LL treatments respectively; in c) and d), solid and dotted lines correspond to the mineral soil in HL and LL treatments respectively. Stars denote the amount of ^{14}C label added to the HL and LL treatments, ~1000‰ and ~230‰ respectively.

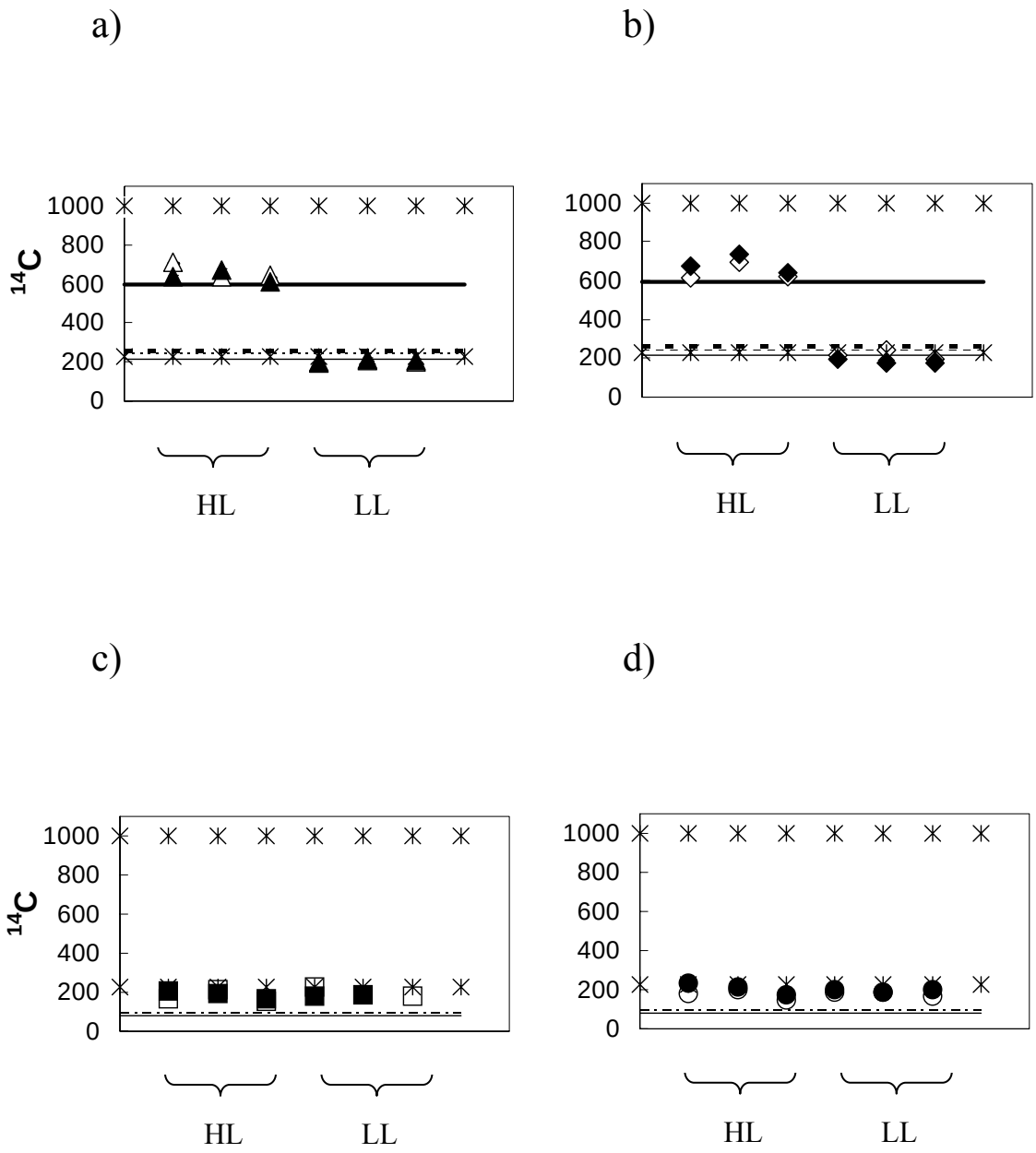

a)
b)
c)
d)
1000
800
600
400
200
0
^{14}C
HL
LL
Treatment plots

Figure 3.6. Daily increase in CO_2 concentration (expressed in $\%CO_2$) in the headspace of sealed jars containing leaf litter and mineral soil (0-5 cm depth) samples (n=6 for each). The rates from incubations of leaf litter are shown on the left hand side with the arrows indicating: a) water addition to field capacity, c) decrease in the incubation temperature from 25ºC to 13ºC and e) both a) and c). The rates from incubations of soil samples are shown on the right hand side with the arrows indicating: b) water addition to 20% by volume, d) decrease in the incubation temperature from 25ºC to 13ºC and f) both b) and d).

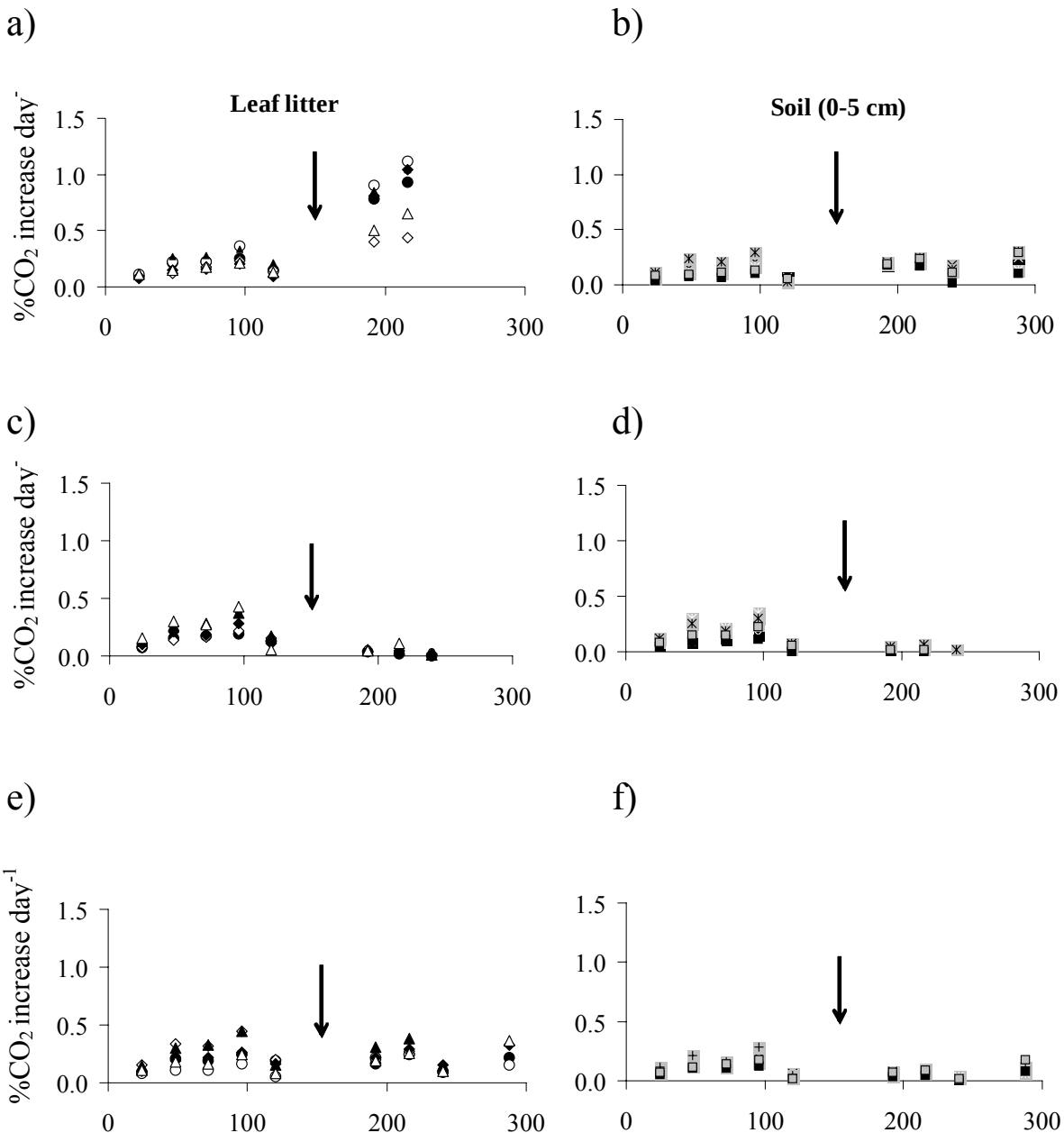

a)
b)
Leaf litter
Soil (0-5 cm)
%CO2 increase day-
c)
d)
%CO2 increase day-
e)
f)
%CO2 increase day-1
1.5
1.0
0.5
0.0
0
100
200
300
Incubation time (hours)

Table 3.1. $\delta^{13}C$ signatures (‰) of the CO_2 evolved in manipulated incubations of leaf litter and soil samples (0-5 cm). Data are the mean ± standard deviation (n=6 separate incubations except when indicated otherwise). Numbers in bold indicate values that are significantly different ($p<0.05$) from those measured at field moisture and 25ºC.

Leaf litter

	25ºC	13ºC
Field moisture (dry)	-28.0 ±0.4	**-27.3** ±0.6
Field capacity	-27.7 ±0.8	**-27.1** ±0.8

Soil

	25ºC	13ºC
Field moisture (dry)	-27.0 ±0.5	-26.7 ±0.4
Field capacity	-26.6 ±0.4 (n=3)	**-26.3** ±0.5

3.4 Relative contribution from leaf litter decomposition to soil respiration (FLD)

As mentioned earlier, the difference in radiocarbon content of soil respiration between HL and LL treatment plots is proportional to the fractional contribution from leaf litter decomposition (Eq. 3.1.). Using Eq. 3.1. and radiocarbon signatures measured with each chamber technique, we estimated leaf litter decomposition under dry conditions on day 243 to account for 11 ±7% for the automated chamber sampling and 5±2% using manual chambers, where no significant difference in the radiocarbon signature of CO_2 respired was observed using between HL and LL treatments. The contribution of leaf litter decomposition increased to 27 ±9% and 37 ±8% for automated and manual chambers respectively with higher levels of leaf litter moisture (up to 1.7 g g^{-1}). The corresponding absolute contributions ranged from 16 ±11 to 43 ±18 mg C m^{-2} hr^{-1} using fluxes measured with automated chambers and from 6±3 to 63 ±18 mg C m^{-2} hr^{-1} using soil respiration fluxes measured with manual chambers (Figure 3.7).

3.5. Relative contribution from the labeled leaf litter (FLL)

We estimated that recent leaf litter (≤3 years old) contributed at least 50% to the total decomposition in the O horizon with little or no contribution to CO_2 efflux from downward transport of dissolved organic compounds in the mineral soil (0-5 cm). When we applied equations (2) and (3) to data collected over the last two years of litter additions (2002 and 2003, no data were available for 2001) we found that the contribution from the labeled leaf litter did not vary much throughout these years, with a mean value of 54 ±12% in the O horizon and 6 ±4% in the 0-5 cm of mineral soil. Given that the mean contribution from leaf litter decomposition to soil respiration

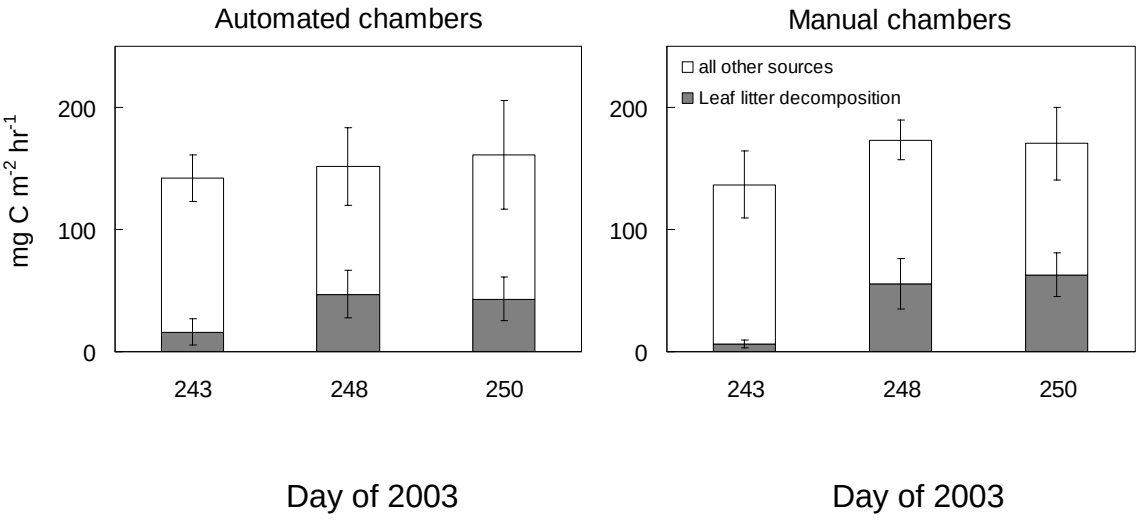


Figure 3.7. Absolute contributions of leaf litter decomposition to total soil respiration as estimated using measurements from each chamber technique. Fluxes were calculated as the product of soil respiration fluxes times the relative contribution from leaf litter decomposition (FLD). FLD was estimated using equation 1 and radiocarbon signatures of soil respiration and leaf litter decomposition.

without moisture limitation was 32 ±12% (mean value of manual and automated chamber techniques, see above), C≤3 years old contributes at least 15% to a maximum of 30% to total soil respiration fluxes. It should be pointed out that these averages are for sampling dates during the growing season, and are not annual averages.

3.6. Relative contribution from autotrophic respiration to soil respiration (FRR)

The relative contribution of autotrophic respiration to soil respiration was estimated for each sampling day using Equation (4) and both, ^{14}C and ^{13}C signatures. The isotopic signatures of heterotrophic sources and autotrophic respiration were assumed to be constant through the sampling period as documented in Figure 3.5. and Table 1. The validity of assuming constant isotopic signature for autotrophic respiration is discussed below. The isotopic signatures used in Eq. (3.4.) are as follows: ^{14}C and ^{13}C signatures of heterotrophic respiration were estimated using the signatures of CO_2 evolved in incubations of leaf litter and soil samples at 25ºC and field moisture, as shown in Figure 3.5a and c for ^{14}C and Table 1 for ^{13}C; ^{14}C and ^{13}C signatures of total respiration were as measured with manual chambers on each sampling day and the ^{14}C and ^{13}C signatures of autotrophic respiration were as measured on day 243.

Radiocarbon signatures yielded a FRR of 0.64 on day 243 [Cisneros-Dozal et al., 2005] but discrepancies between HL and LL treatments on days 248 and 250 did not permit calculation of FRR. However, ^{13}C signatures allowed us to estimate FRR for these two days yielding fractions of 0.60 and 0.50, though the signatures did not satisfy the mass balance on day 243.

4. Discussion

The increase in soil respiration after precipitation was evident from the automated measurements and has been observed in several other studies [Borken et al., 1999; Borken et al., 2002; Goulden et al., 2004; Savage and Davidson, 2001; Scott-Denton et al., in press]. However, spatial variability in the fluxes among chambers was considerable (Figure 3.3); although this likely reflects the spatial heterogeneity in the field, it accounted for the lack of significant differences between the mean fluxes at very different litter moisture conditions (i.e. days 243 vs. 250). CO_2 fluxes measured on day 243, the day with driest leaf litter, were less variable than those measured on days 248 and 250. Hence, much of the spatial variability may be due to heterogeneity in the wetting and drying of the litter layers. Another potential reason may be a predominance of mineral soil flux, given that the leaf litter layer flux only impacts ~5 to 30% of total soil respiration, the correlation of total flux to rainfall should not be expected strong.

Despite the spatial and temporal variability, fluxes measured with the manual chambers agreed well with those measured over the same period of time with the automated chambers as have been observed in other studies [Edwards and Riggs, 2003; Savage and Davidson, 2003]. The agreement in the radiocarbon signatures of total soil respiration measured with both chamber techniques was also good. Sampling for radiocarbon using the automated system provided an independent way of testing the protocol followed with the manual chambers. The agreement between techniques ruled out major artifacts in the CO_2 collection using manual chambers such as the effect of scrubbing the headspace of the chamber.

The increase in leaf litter water content caused an increase in the radiocarbon signature of soil respiration, mainly noticeable in HL plots (Figure 3.4.). The resulting greater isotopic difference between litter treatments, HL and LL, indicated greater contribution from leaf litter decomposition according to Eq. 3.1.. Furthermore, results from the manipulated incubations indicated no change in the ^{14}C signature of leaf litter decomposition alone (Figure 3.5a and b) upon addition of water, meaning no shift in C substrate within the litter layer could be responsible for the observed isotopic shift in total soil respiration. The ^{14}C-signatures obtained from the manipulated incubations did not change even with the extreme change in moisture content that resulted from adding water in excess (to field capacity) to the very dry leaf litter samples that were collected on day 243. In contrast, CO_2 fluxes did show a pronounced increase after water addition as well as a decrease with temperature (Figure 3.6 a, b, c, d) in agreement with observations from other incubation studies [Borken et al., 2003; Dioumaeva et al., 2002; Fierer and Schimel, 2003; Kelliher et al., 2004; Lomander et al., 1998; Orchard and Cook, 1983; Waldrop and Firestone, 2004] and indicated greater decomposition activity with greater water content. We conclude that the isotopic shift in soil respiration associated with increased soil moisture is due to an increase in the CO_2 derived from heterotrophically respired CO_2 from the litter layer.

Dioumaeva et al., [2002] observed a similar pattern: large changes in respiration rates with no change in the radiocarbon signature of the evolved CO_2 for boreal forest peat mosses incubated over a range of temperature conditions. Other studies however, have observed changes in the isotopic signature of the CO_2 evolved during incubations with changes in moisture or temperature, which the authors have associated with a shift

of C substrate for decomposition. Using soil samples from a pineapple plantation and ^{13}C measurements, Waldrop and Firestone, [2004] inferred the utilization of older C (>14 years) at 20ºC versus 5ºC although no change was observed with addition of water. Their incubations lasted for 103 days but an isotopic difference in the CO_2 produced between the two temperature conditions was seen throughout the experiment. Samples in their experiment were homogenized and stored for 9 months before the incubations started. By contrast we incubated leaf litter as well as mineral soil samples, handled the samples with as little disturbance as possible (cores, including roots, were left intact), and the time elapsed between collection and the start of incubations was only ~3 months. Our results did not indicate a difference in the C substrate mineralized at 25ºC versus 13ºC in leaf litter or soil samples based on radiocarbon (Figure 3.6). Andrews et al., [2000] found an isotopic enrichment (2.2-3.5‰) in the ^{13}C of the CO_2 evolved during incubations at 4ºC versus 22ºC and attributed it to the different type of microbial species present at each temperature. However in their experiment, there was a disturbance of the soil samples by removing roots and homogenizing which again differed from our incubation protocol.

In a different incubation study that involved ^{14}C measurements and samples from a deciduous forest, Borken et al., [2005] inferred the decomposition of older C in leaf litter after addition of water. Their protocol for radiocarbon sampling did not differ from that used in our experiment (except for the removal of roots from one set of their samples) but the addition of water was done for a period of time of 1 to 2 days. Water addition over a longer period of time could have accounted for the observed change in the ^{14}C signature of the CO_2 evolved during their incubations but this needs to be

further investigated. Similarly the effect of incubating homogenous versus intact samples and/or doing short- versus long-term incubations on C isotopes of the CO_2 evolved from decomposition deserves further research.

In our study, a shift to older pools of C for decomposition upon water addition and/or lower temperature would have been indicated by a significant decrease (or increase) in the ^{14}C signature of the CO_2 evolved in the incubated high-labeled leaf litter (or low-labeled leaf litter) samples. That is, we would have seen the decomposition of pre-treatment (> 3 years) leaf litter with a ^{14}C signature of ~200‰ in the HL plots or alternatively, the decomposition of leaf litter with a ^{14}C signature higher than 215‰ in the LL plots. In fact, the decomposition of incubated mineral soil (0-5 cm) did not reflect changes in C substrate either, at any of the manipulated moisture and temperature conditions from any of the litter treatments (Figure 3.6c and d). The instantaneous increase in CO_2 fluxes observed in the incubations accompanied by no change in their radiocarbon signature indicated an increase in the rate of organic matter decomposition upon water addition, but not a shift in C substrates. This could be due to one of two causes: (1) the substrates for decomposition all have uniform isotopic signature or (2) the decomposition rates of all substrates increase in proportion with temperature and moisture changes (i.e. the relative contribution of different substrates being decomposed is not altered). Option (1) may be discarded as the incubated leaf litter included both, the recent leaf litter or Oi and the more decomposed leaf litter or Oe/Oa and ^{14}C bulk signatures indicate a marked difference between these two pools in the HL treatments (Figure 3.5a and b). Even though we see that, at 25ºC and field moisture, the major contributor to decomposition in the O horizon is the Oi layer

(Figure 3.5a), some flux must be derived from the Oe/Oa layer as the CO_2 evolved is lower in radiocarbon than the original amount added. Unless this difference (~400‰) can be accounted for by decomposition and leaching, the contribution from the Oe/Oa layer although minimal has to be present. Option (2) seems likely with the overwhelming contribution to decomposition from the Oi horizon regardless of moisture content in the sample.

The increase in radiocarbon content of total soil respiration observed in the field after increases in leaf litter moisture reflected the increase in decomposition of leaf litter and not the decomposition of a different substrate that was unavailable to decomposers under dry conditions. Furthermore, the increase in total soil respiration fluxes after water addition was proportional to the increased contribution from leaf litter decomposition (Figure 3.5b), which seems to rule out a strong response of autotrophic origin at this site. It is possible that more severe and prolonged droughts are needed for autotrophic (root/rhizosphere) respiration to be affected as deep tree roots retrieve water from deeper than the O horizon. In addition autotrophic respiration may be more strongly controlled by aboveground factors such as site productivity [Janssens et al., 2001; Reichstein et al., 2003], photosynthetic activity [Craine et al., 1998; Hogberg et al., 2001] and/or environmental conditions [Ekblad et al., 2005; Irvine and Law, 2002]. Interestingly and in agreement with other studies [Borken et al., 2003; Goulden et al., 2004], the pattern of soil respiration fluxes (Figure 3.2d) followed those of the ponderosa pine wood sensor (Figure 3.2b) that represented the dynamic of moisture changes in the Oi horizon. Our results confirmed that, in this forest, the cause for the

usually observed increase in soil respiration fluxes after rain events following transient drought is of heterotrophic origin and is derived from enhanced decomposition of the O horizon.

Lee et al., [2004] estimated a linear relationship between the relative contribution from leaf litter decomposition to total soil respiration and leaf litter water content in a temperate forest. The contribution comprised approximately an additional 20% of total soil respiration when moisture levels increased from 6 to 14% by volume. Their measurements of water content however were recognized to be only an approximation of that of the leaf litter layer as they spanned a depth comprising the entire O horizon plus ~3 cm centimeters of mineral soil. While it is difficult to assign a value of increase in leaf litter decomposition to a specific increase in litter water content (due to real heterogeneity in the field litter moisture and different water holding capacities between sites), our data suggests an increase of approximately 25% more relative contribution with ~1.4 g g^{-1} increase in surface litter water content during the peak of the growing season. The increase could be lower during spring when cool temperatures limit decomposition as has been observed from field measurements [Cisneros-Dozal et al., 2005] and in model predictions [Hanson et al., 2003b]. Lastly, the radiocarbon signature of leaf litter decomposition in HL plots shows that the Oi layer (i.e. leaf litter ≤3 years old) contributes more to decomposition fluxes from the O horizon than the Oe/Oa layer (Figure 3.6a and b).

There is no consensus on the specific mechanisms linking increased moisture to enhanced decomposition, but several have been proposed including: high metabolic activity of young bacteria [Birch, 1958], the decomposition of microbial biomass killed

under dry conditions [Bottner, 1985] and/or C release by microbial biomass perhaps through catabolism of internal solutes, transport of solutes out of cells [Fierer and Schimel, 2003] or cell lysis [Kieft et al., 1987]. It is difficult to identify the mechanism responsible for the increased decomposition of the O horizon from our data, though we can definitely state that the increase is due to enhanced decomposition of the Oi layer in particular. Perhaps no single mechanism can account for the increased decomposition but a combination of all is required. Additional analyses of radiocarbon in microbial biomass and leaf litter will be needed to elucidate the underlying mechanisms. The fact that the combination of substrates does not change with added moisture may indicate a storage mechanism at work in microbes that buffers the change in substrate supply when moisture is reduced. Changes in ^{13}C of evolved CO_2 were observed only with temperature change in incubations indicating a change in the microbial fractionation factor rather than a change in substrate, since ^{14}C, which had much larger source variations, remained the same.

4.1. Relative contribution from the labeled leaf litter (FLL)

The contributions of the decomposition of the ^{14}C label in the O horizon and mineral soil should be viewed as lower limits as the amount of label present in the sample (denominator in Equations 2 and 3) could be lower than what was originally added, as mentioned above. In part this should be expected due to the decomposition that occurred between the time of litter addition (March 2003) and the time of sample collection (August 2003). Furthermore, there is an indication of lost of recent leaf litter derived-C perhaps through leaching as the bulk ^{14}C signatures of the Oi and Oe/Oa

horizons measured yearly did not show the expected increase in ^{14}C content after two years of litter addition (bulk 14C signatures in fact, decreased). A much greater contribution than 50% from the recent leaf litter to decomposition in the O horizon can be inferred from comparing the measured bulk ^{14}C signatures of the Oi and Oe/Oa horizons with that of the CO_2 evolved in incubations. This is particularly clear in HL treatments where these signatures clearly denote that the Oi layer (recognizable leaf litter) is the main substrate for decomposition in the O horizon with negligible contribution from the Oe/Oa layer (more decomposed and humidified leaf litter) (Figure 3.5a) as mentioned above.

Over the EBIS study period (2001-2003), the bulk ^{14}C signatures of the Oe/Oa layer and mineral soil (0-15 cm) have not changed markedly indicating little incorporation of the label into these horizons. This was not surprising given that the mean turnover time for leaf litter has been estimated between 5 and 13 years, with longer turnover times for the humified material [Gaudinski et al., 2000].

4.2. Autotrophic contribution to soil respiration (FRR)

Although it was not possible to estimate FRR using both isotopes on a given day due to discrepancies with either isotopic signatures, constraining the estimates with FLD values calculated independently provides confidence on the FRR estimates. One source of uncertainty may be the assumption that autotrophic respiration maintains a constant isotopic signature throughout the three sampling days. In the case of radiocarbon, this assumption may not be valid if changes in moisture conditions cause a change in the proportion of stored versus recent C as the source for respiration.

Although such changes seem to be feasible seasonally [Cisneros-Dozal et al., 2005; Hogberg et al., 2001], it is unknown if they could occur in a time frame of 7 or less days (day 243 to 250) as a result of transient drought and rain events. Transient drought may not strongly influence photosynthesis [Goulden et al., 1996], which is tightly coupled to root respiration, but in the event that stored C was being respired in greater proportion by roots during drought, there is the possibility that any change would not have been reflected in root respiration immediately after the heavy rain on day 243 as a time lag from 1 to 6 days has been found between photosynthesis and root respiration [Ekblad et al., 2005].

With regard to ^{13}C signatures, there is no direct evidence on isotopic shifts in root respiration associated with drought and although links between photosynthetic activity and ^{13}C signature of soil and ecosystem respiration in relation to air humidity or drought have been found [Ekblad et al., 2005; McDowell et al., 2004], ^{13}C autotrophic signatures are also subjected to the time lags between C assimilation and lost through root respiration.

Without direct measurements of the isotopic signatures of autotrophic respiration on our second and third sampling days, the assumption of constant isotopic signature at the pre-rain value remains a source of uncertainty in our estimates of FRR. However, the fact that we can explain the entire change in total CO_2 flux with the increase in decomposition of the O horizon suggests that changes in root respiration must have been small.

Borken et al., [2005] observed a decrease in both autotrophic and heterotrophic respiration with simulated drought in the Harvard Forest Massachusetts, whereas we

seem to see a change only in heterotrophically respired CO_2 at a deciduous forest in Tennessee. One explanation for this may lie in the vertical distribution of roots between the two sites: at the Harvard forest, the litter layer is thicker and contains ~ 50% of the fine root biomass whereas at Oak Ridge, the litter layer is thin and the majority of fine roots are found in the 0-30 cm layer of the soil [Joslin et al., 2003].

5. Conclusions

We combined ^{14}C labeled leaf litter with a moisture manipulation and radiocarbon measurements in CO_2 from soil, heterotrophic and autotrophic respiration to assess the role of leaf litter decomposition on the response of soil respiration to wetting. Leaf litter decomposition fluxes increased from 11±11 mg C m^{-2} hr^{-1} during a transient drought to 53±25 mg C m^{-2} hr^{-1} after wetting, corresponding to relative contributions to soil respiration of 8±8% and 32±12% respectively. Pre-wetting conditions determined the response of leaf litter decomposition to water addition. Wetting of litter that had been recently dry (i.e. following a transient drought) increased fluxes markedly in contrast to wetting of litter that had been recently wet.

C substrate ≤3 years contributed at least 50% to leaf litter decomposition corresponding to a contribution of >15-30% to soil respiration fluxes without moisture limitation. We observed little contribution of C leached from litter layers (6±4%) in heterotrophically respired CO_2 from mineral soil horizons. We did not observe a change of C substrate (i.e. older C) within the leaf litter layer with a change in moisture (dry versus field capacity) or temperature (13ºC versus 25ºC). These results disagreed with those of other incubation studies that involved measurements of either ^{13}C or ^{14}C

but differed from our incubation approach by using disturbed (homogenized, roots removed) leaf litter and soil samples, doing long-term incubations or adding water for a long period of time. Therefore the effect of these incubation conditions on the C isotopes of the CO_2 evolved need to be further investigated.

Manual and automated CO_2 collection techniques for radiocarbon measurements in soil respiration in the field gave similar results, further providing confidence on our routinely used manual measurements as well as showing the potential of the new automated technique.

The isotopic mass balance approach applied did not indicate a strong response of autotrophic origin to wetting although our assumption of no shift in isotopic signatures (^{14}C and ^{13}C) immediately after rain events following transient drought, is an uncertainty in our estimates of the relative contribution of autotrophic respiration. While we cannot absolutely rule out a contribution from root respiration to changes in soil respiration following wetting, a significant contribution would require a shift in the isotopic signature of root-respired CO_2.

Temporal variability in soil respiration fluxes was accounted for by increased decomposition of leaf litter following precipitation. Models of soil respiration should account for this heterotrophic source and its response to moisture availability.

CHAPTER 4

SEASONALLY CHANGING SOURCES OF CARBON FOR ROOT RESPIRATION

Abstract

Carbon allocation patterns belowground are not well understood representing a source of uncertainty in models of terrestrial carbon cycling [Ryan and Law, 2005; Trumbore, 2005]. Carbon allocation to roots can account for 35-50% [Davidson et al., 2002a; Giardina et al., 2003] of gross primary productivity and ~40% of this allocation is lost as respiration [Farrar and Jones, 2003]. We have attempted to identify the source of carbon fueling root/rhizosphere respiration by using ^{14}C labeling at the whole-ecosystem scale in a temperate forest in Oak Ridge, Tennessee. Previous measurements of the radiocarbon signature of root/rhizosphere respiration performed at this temperate forest revealed a potential shift in the substrate for respiration from stored carbon in the first half of the growing season to recent photosynthetic products in the latter part [Cisneros-Dozal et al., 2005]. In the present study, we aimed to estimate the seasonal contribution of recent versus stored carbon as substrate for root/rhizosphere respiration by using the ^{14}C signatures of ambient air, the starch pool and root/rhizosphere respiration. The radiocarbon signature of sub-canopy air was measured on a bi-weekly basis. Measurements of the radiocarbon signature of root/rhizosphere respiration were performed in the 2003 and 2004 growing seasons along with collection of root tissue for extraction of carbohydrates. Methods were developed and tested to isolate nonstructural (water soluble extracts and starch) and structural (cellulose) carbohydrates from plant

material. The precision achieved with regard to radiocarbon analyses of these carbohydrate fractions extracted from standard plant material (having background levels of radiocarbon) ranged from 3-6 ‰ for bulk water soluble compounds, 7 ‰ for starch and 2 ‰ for cellulose. These levels of precision were slightly larger than the reported accuracy for the ^{14}C measurement itself (2 to 4.5‰) with regard to nonstructural carbohydrates. The relative contribution from stored pools to root/rhizosphere respiration was calculated using ^{14}C signatures of starch and atmospheric CO_2 as end-members. We estimated starch pools to contribute ~70% in March, decreasing to <20 % later in the growing season as fresh photosynthetic products begin to dominate root respiration sources. We estimated that the source of carbon for root/rhizosphere respiration is comprised of two pools: a slow pool derived from stored nonstructural starch reserves and a fast cycling pool derived from carbon fixed in the past few days to weeks. Starch reserves represent a pool with relatively long (order of years) turnover time that is a significant (though seasonally variable) contributor to root respiration

1. Introduction

Carbon allocated to roots is used for structural growth, respiratory processes (both for growth and maintenance purposes), storage, exudation and symbiotic activity [Farrar and Jones, 2003]. Knowledge of the factors controlling seasonal and annual patterns of carbon allocation belowground is currently lacking [Ryan and Law, 2005]. The two main sinks for carbon in roots are structural growth and respiratory losses each accounting for up to 30-35% of the carbon fixed through photosynthesis [Lambers, 1987]. Of the pathways through which carbon can be lost from the root system,

respiration is the most important quantitatively, accounting for ~40% of inputs [Farrar and Jones, 2003]. Research on crop plants has shown similar quantitative losses of photosynthetically fixed carbon through exudation [Whipps and Lynch, 1986] although conservative estimates are in the range 0.5-5% of net fixed carbon [Farrar and Jones, 2003]. Root respiration is the source of energy for various metabolic processes upon decomposition of the carbohydrates acquired from aboveground plant parts and since it depends strongly on carbon assimilated through photosynthesis, its characterization is of relevance on assessing fast (within days) loss of carbon from ecosystems.

Sugars, in particular sucrose, are the main source of energy in root metabolism, used in the synthesis of new tissue, maintenance, symbiotic activity and transport of substances [Gašparíková, 1992]. As a continuous metabolic process, and when supplies are greater than demands [Chapin et al., 1990], carbon is stored in the form of carbohydrates, specifically starch [Gašparíková, 1992] although some species store carbon in the form of fructose-derived compounds [Meier and Reid, 1982]. In most tree species (including the dominant species in the present study, *Quercus* spp and *Acer* spp) the main form of carbon storage is as nonstructural carbohydrates namely starch and soluble sugars (e.g. glucose, sucrose and fructose) [Chapin et al., 1990; Hoch et al., 2003]. Several plant organs can serve as storage reservoirs for carbon and which plays a major role depends on species and environmental factors, for example frequent exposure to disturbance. Trees use aboveground woody tissues as well as roots as storage reservoirs [Dickson, 1991]. Nonstructural carbohydrates have been extensively measured in bole, stem and leaves of forests trees [Hoch et al., 2003; Körner, 2003; Tschaplinski and Hanson, 2003] as they are more easily sampled in the field and since it

is believed that concentrations in stem are similar to that of coarse roots [Tschaplinski and Hanson, 2003]. While nonstructural carbohydrates expressed as concentrations and size pools of aboveground parts are good indicators of tree health and carbon status [Hoch et al., 2003; Körner, 2003], relating these compounds to respiration fluxes provides information on their role in supporting carbon loss from ecosystems, seasonally and interannually.

Deciduous trees rely on stored carbon over the dormant season to support growth at the onset of the next growing season [Dickson, 1991]. Nonstructural carbohydrates as reserve carbon are also crucial in sustaining tree growth under adverse environmental conditions such as drought, increased temperature, pollution [Grulke et al., 2001], nutrient stress [Ericsson et al., 1996; Weinstein et al., 1991] and fire occurrence [Langley et al., 2002], all of which many forests are already experiencing and will continue to experience in the future. Hence, given the importance of stored nonstructural carbohydrates for the sustainable growth of trees, it is of interest to assess their role versus current photosynthates for sustaining root/rhizhosphere respiration.

Current knowledge about the timing and magnitude of the allocation of carbon reserves to root growth and respiration in terrestrial ecosystems is limited and in some cases site-specific. For instance, shrub oaks were observed to use stored carbon over recent photosynthate for root growth after fire disturbance [Langley et al., 2002] but the opposite has been observed in root growth of sugar maple (*Acer saccharum* Marsh.) under normal conditions [Wargo, 1979]. With regard to root/rhizosphere respiration, the general consensus until very recently has been that currently fixed carbon acts as the substrate for this process given its dependence on aboveground supplies. This concept

has been supported by recent studies [Bhupinderpal-Singh et al., 2005; Bowling et al., 2002; Ekblad et al., 2005; Hogberg et al., 2001] although insight into the seasonal use of stored nonstructural carbohydrates has also been gained [Hogberg et al., 2001].

Radiocarbon labeling is a helpful tool to track carbon uptake, incorporation and release from ecosystems [Kandiah, 1979; Dickson et al., 1990; Dickson et al., 2000; Nishizawa et al., 1998]. A unique opportunity to study carbon cycling using radiocarbon emerged following a ^{14}C release at the whole-ecosystem level that took place in a temperate forest of eastern Tennessee in the Oak Ridge National Laboratory (ORNL) reserve, during the growing season of 1999 [Trumbore et al., 2002]. The Enriched Background Isotope (EBIS) study was established in 2001 (see http://ebis.ornl.gov/ for further details) to follow carbon transformations in plant biomass, soil organic matter, soil solution and soil respiration. As part of EBIS, we began measuring soil respiration and its radiocarbon signature at different times in the growing season of 2002 [Cisneros-Dozal et al., 2005] at two of the sites selected for EBIS. These sites differed from each other in the amount of ^{14}C label incorporated into roots: Tennessee Valley Authority (TVA) with high amounts of ^{14}C label in roots (~1000‰) and Walker Branch (WB) with low amounts (~200‰); these will also be referred to as "HR" and "LR" site respectively. During our soil respiration measurements, we observed wide seasonal variations in the ^{14}C signature of the CO_2 evolved from root/rhizosphere respiration during 2002 that appeared to be of phenological origin (e.g. the same pattern was observed at the two sites). These variations suggested the use of mainly stored carbon in the first half of the growing

season and a greater proportion of current photosynthate in the latter part [Cisneros-Dozal et al., 2005].

In the present study, we investigated these allocation patterns further by looking at the ^{14}C signature of carbohydrate fractions in root tissue to try to elucidate the source of carbon for respiration. The specific goals of the present study are: (1) to isolate fractions of nonstructural carbohydrates from root tissue, specifically soluble sugars and starch, for measurement of ^{14}C signatures and (2) to estimate the contribution of recent versus stored photosynthate as source of carbon for root/rhizosphere respiration seasonally at this temperate forest by using the ^{14}C signatures of ambient air, the starch pool and root/rhizosphere respiration. We hypothesized that (1) the ^{14}C signature of root/rhizosphere respiration will follow the same seasonal patterns as that of the bulk soluble sugars as these constitute the main source of energy in root metabolism and (2) the source of carbon for root/rhizosphere respiration is derived from stored carbon in starch pools (which are expected to be enriched in ^{14}C with respect to current photosynthate as they were fixed prior to the current growing season) early in the growing season and from current photosynthate in the latter part (August).

We are unaware of other measurements of ambient levels of radiocarbon in carbohydrates extracted from root tissue, though measurements of different chemical fractions in highly labeled plant materials have been made [Dickson et al., 1990; Dickson et al., 2000]. We therefore undertook as part of our investigation a development of methods for isolation and measurement of the radiocarbon content of water soluble extracts and starch.

2. Methods

2.1. ^{14}C and ^{13}C signatures of ambient air

The isotopic signatures of sub-canopy air at the western and eastern sides of the Oak Ridge reserve (ORR) corresponding to local ambient air at TVA and Walker Branch sites respectively, have been measured during the months of the growing season, from April to October on a bi-weekly basis. Air was collected by slowly filling an evacuated 32-liter canister over a two-week period through a capillary restrictor. The air inlet was located at approximately 1.5 m above the soil surface. Air samples were sent to UCI for cryogenic purification of CO_2 and preparation of ^{14}C targets via the zinc reduction method [Vogel, 1992]. Radiocarbon measurement of the graphite targets was performed by accelerator mass spectrometry at Lawrence Livermore National Laboratory (LLNL). An aliquot of each purified CO_2 sample was collected and analyzed for ^{13}C using continuous flow isotope ratio mass spectrometry at UCI as described by Cisneros-Dozal et al., [2005].

2.2. ^{14}C signatures of root/rhizosphere respiration

Collection of CO_2 from root/rhizosphere respiration for measurement of ^{14}C signatures was performed at different times during the growing seasons of 2002 (May, June and September), 2003 (March, May, August and October) and 2004 (March, June and August) at TVA and Walker Branch sites as described by Cisneros-Dozal et al., [2005]. Briefly, live roots (black colored and brittle roots were avoided) cut from intact root networks were excavated at two or three random locations mostly from the upper 5 cm of mineral soil, excised without distinction of species or size (most were <5 mm in

diameter) and washed or shaken (if soil was very dry) free of soil. Three sub samples were placed separate in air tight containers and the CO_2 evolved was collected for isotopic measurements. Samples were processed for ^{14}C analyses as described above. Field samplings were performed at the HR and LR sites on consecutive days or within two to four days between sampling of CO_2 fluxes at each site.

2.3. Extraction of carbohydrate fractions

2.3.1. Analytical precision

To test the reproducibility of our methods, we collected a large amount of leaf and stem material from a tree located on the UCI campus. This material was vacuum dried, ground and homogenized to make a standard material for multiple analyses of water soluble extracts and starch extracts and thus determine the precision of our methods.

2.3.1. Collection and preparation of root tissue

During 2003 and 2004 and prior to the collection of CO_2 for radiocarbon measurement, one subsample of live roots was compiled from all random excavations and placed in a cooler for transport to the ORNL facilities. On a few dates, samples from each excavation (each from a random location) were kept separated to assess heterogeneity. Root samples at the ORNL facilities were maintained frozen until the time of transport to the laboratory at UCI for the extraction of carbohydrates.

Upon arrival at UCI, root samples were dried under vacuum until complete removal of H_2O (which usually took 3-4 hours). Fine (<2 mm diameter) were separated

from coarse (>2 mm diameter) roots and ground to 40 mesh. Isolated fractions of carbohydrates for radiocarbon measurement included bulk soluble compounds (comprised mostly of soluble sugars), starch and cellulose. Additionally, we measured radiocarbon in bulk root tissue.

2.3.2. Extraction of soluble sugars and starch

Bulk soluble sugars were obtained from the supernatant following boiling of the root material in H_2O and centrifugation. The supernatant was dried in the vacuum concentrator and analyzed for radiocarbon. Starch was extracted from the residue following a method adapted from Brugnoli et al., [1988] for isotopic analysis. Refer to Appendices 2B and 2D for a detailed description of the methods used.

2.3.3. Extraction of cellulose

The procedure followed was adapted from Green, [1963] and Leavitt and Danzer, [1993] for the extraction of holocellulose which is commonly referred to as the "Jayme-Wise" technique [Gaudinski et al., in press]. Hereafter, this method will be referred to as the conventional method. On some samples we performed an alternative procedure to obtain holocellulose in sequence after starch extraction. Preliminary tests indicated good agreement in the radiocarbon signatures between the two methods on most of the dates on fine roots (see results section), and thus we continued to use the alternative method on the rest of the samples (which were mostly coarse root samples) since it minimized the time involved in laboratory extractions. Comparison of the two methods on coarse roots was not performed directly however. The alternative procedure

is described in Appendix 2E and consisted of the bleaching treatment performed in the "Jayme-Wise" technique and described in Appendix 2 (reaction in a solution of sodium chlorite and glacial acetic acid at 70ºC) of the root material residue following extraction of starch.

2.4. ^{14}C signatures of carbohydrate fractions

Depending on the carbon content on each of the isolated carbohydrate fractions (varying from 35-40% carbon by weight) and on the bulk root material (~40% carbon by weight), an amount equivalent to ~1mg of carbon was combusted with copper oxide and the resulting CO_2 was cryogenically purified and converted to graphite via the zinc reduction method [Vogel, 1992]. Radiocarbon graphite targets were measured by accelerator mass spectrometry at the Keck Carbon Cycle Accelerator for Mass Spectrometry Laboratory (KCCAMS) at UCI and at Lawrence Livermore National Laboratory (LLNL). On some samples, especially from starch, little carbon yield was obtained from extractions and thus ^{14}C targets were prepared from smaller amounts (usually $\leq$ 0.5 mg of carbon). Alternatively, when the amount of sample allowed, replicated analyses of the carbohydrate fractions were performed.

Radiocarbon signatures are expressed in delta notation (Δ), the deviation of the $^{14}C/^{12}C$ ratio in the sample with respect to that of the standard (oxalic acid) corrected for mass-dependent isotope fractionation to a common value of -25 ‰ in $\delta^{13}C$ and in parts per thousand (‰). ^{13}C signatures are expressed in delta notation (δ), the deviation of the $^{13}C/^{12}C$ ratio of the sample with respect to that of the standard (Pee Dee belemnite) (in parts per thousand (‰).

2.5. Fraction of root/rhizosphere respiration derived from stored carbon

To estimate the fraction derived from stored carbon versus current photosynthate in the respired CO_2 by live roots and microorganisms operating on the same substrate in the rhizosphere, a mass balance was applied to the ^{14}C signatures of root/rhizosphere respiration, ambient air and starch pool at each site:

$$^{14}C_{RR} = {}^{14}C_{STARCH} * F + {}^{14}C_{AIR} *(1-F) \qquad \text{(Eq. 4.1.)}$$

The ^{14}C signature of root/rhizosphere respiration ($^{14}C_{RR}$) and that of starch ($^{14}C_{STARCH}$) were point-in-time measurements as described above. The ^{14}C signature of local ambient air ($^{14}C_{AIR}$) used in Equation (1) was taken from the biweekly measurements. To assume a value for $^{14}C_{AIR}$ representative of the ^{14}C signature of current photosynthate, we chose the two-week period falling within the 10 days preceding the date of root/rhizosphere respiration measurements. This time window was chosen according to the lag times that have been observed between photosynthetic activity and root respiration and that span from hours to approximately 10 days [Bhupinderpal-Singh et al., 2005; Bowling et al., 2002; Ekblad and Hogberg, 2001; Ekblad et al., 2005].

3. Results

3.1. Radiocarbon signature of ambient air

Radiocarbon measurements of CO_2 in ambient air revealed several local releases of both ^{14}C–enriched and ^{14}C–depleted CO_2 in 2002-2005. These releases led to large

changes in the ^{14}C of CO_2 between subsequent two-week samples (Figure 4.1.). In 2002, the first two-week averages spanning April 5th to 18th were highly enriched at both eastern and western portions of the ORR. The rest of the growing season averaged $\Delta^{14}C$ values of 46‰ and 60‰ at eastern and western sides respectively; these values are below those for background air at similar latitudes ($\Delta^{14}C$ of ~78‰; [Levin and Kromer, 2004]). During August 1st through the 19th and October 1st to the 21st of 2003, elevated ^{14}C levels were measured at the western end only with a mean value the rest of the time of 65‰. The mean value at the eastern side for the entire 2003 growing season was only 2‰, representing a large decline from the previous year and indicating strong influence from local fossil fuel emissions. A fossil-fuel burning plant opened upwind of the Walker Branch area during this time interval, and could account for the change from 2002-2003 in air at this site. Fossil-fuel derived CO_2 was also evident at the eastern side during the growing season of 2004 although events of unprecedented magnitude of ^{14}C-depletion in CO_2 were measured over during six weeks from June to July. The rest of the growing season averaged 52‰, which is lower than background clean air ^{14}C values for the northern hemisphere (which average between 60 and 65‰; Xiaomei Xu, unpublished data). By contrast, local ^{14}C releases at the western end of the Oak Ridge reserve (located nearest to the hazardous waste incinerator that is the presumed source of the label) continued to take place in the growing season of 2004 from May 20th to June 18th. The mean value the rest of the 2004 growing season at the western site was 155‰.

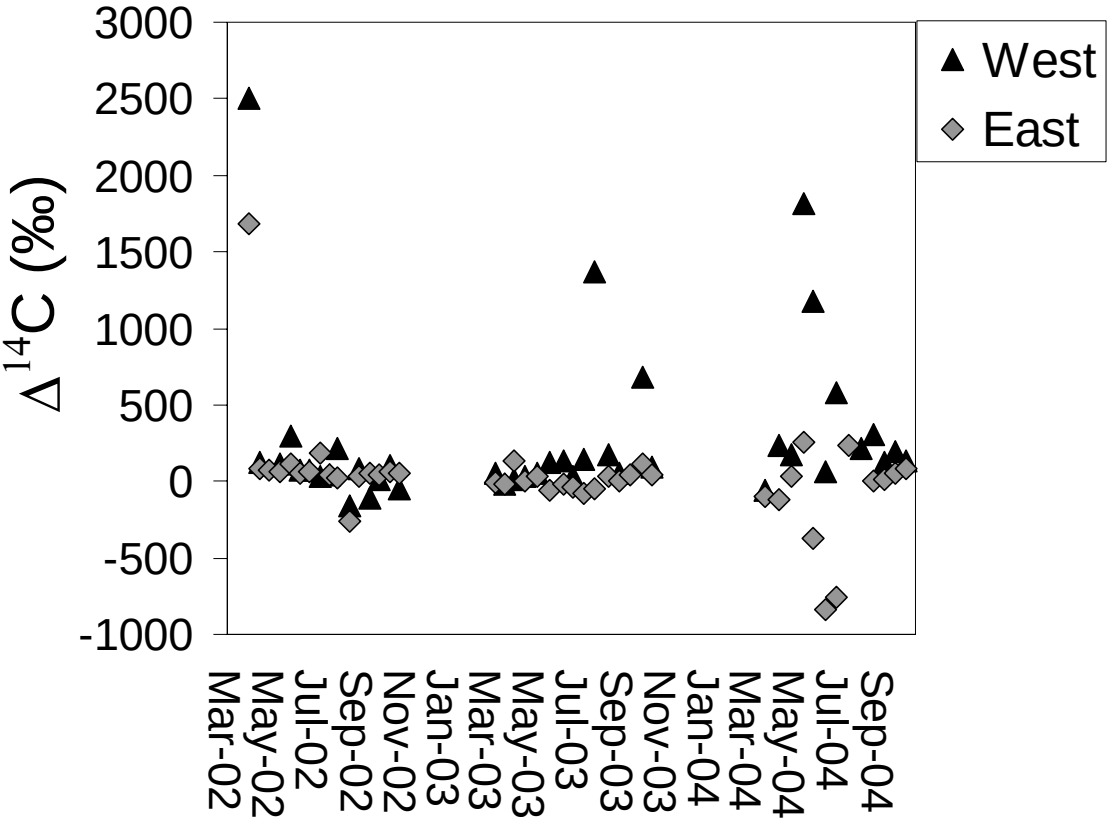


Figure 4.1. Mean radiocarbon signature of CO_2 in ambient air over two-week periods at the western and eastern sides of the Oak Ridge Reservation (corresponding to ambient air at TVA and Walker Branch sites respectively). Ambient air was withdrawn into an evacuated 32-liter canister 24 hours a day during two weeks. Air sampling each year took place during the growing season only (April to October).

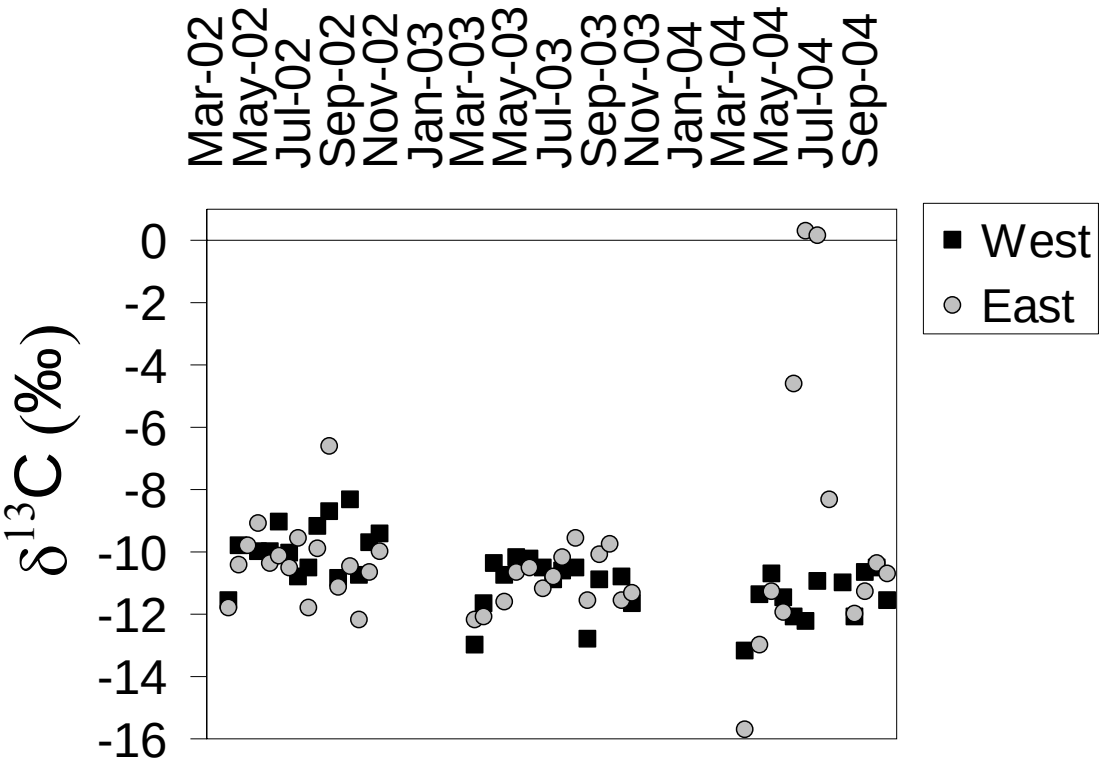


Figure 4.2. Mean ^{13}C signatures of CO_2 in ambient air over two-week periods at the western and eastern sides of the Oak Ridge Reservation (corresponding to ambient air at TVA and Walker Branch sites respectively). Ambient air was withdrawn into an evacuated 32-liter canister 24 hours a day during two weeks. Air sampling each year took place during the growing season only (April to October).

3.2. ^{13}C signature of ambient air

^{13}C levels in air ranged between 8 and 12‰ at both sites with a few exceptional events with ^{13}C-enriched CO_2 in 2004 on the eastern end (Figure 4.2.) The dates for which we measured very enriched ^{13}C levels were also those when air CO_2 was most ^{14}C-depleted at this site, for instance the ^{13}C signature in 2002 (close to -6‰) and in 2004 (close to -4‰) had ^{14}C values of -262‰ and -373‰ respectively (Figure 4.1.).

3.3. ^{14}C signature of root/rhizosphere respiration

The radiocarbon content of respired CO_2 by live roots and any microorganisms operating in the rhizosphere that were still attached to roots in our incubations exhibited large variations at both sites during the three growing seasons (Figure 4.3.). The trends for eastern and western site root respiration ^{14}C parallel each other, though the TVA ^{14}C values were always higher than those at WB.

The relationships between root respiration ^{14}C values and those of the air samples are complex. The most enriched ^{14}C values observed at both sites were likely caused by a localized release of ^{14}C prior to the sampling dates of June 16-18, 2004. Elevated levels of ^{14}C in air were measured on the west end of the ORR during the preceding month (see Figure 4.1.). We have omitted from Figure 3.3 data from the TVA (western) site for March 2004 because very high levels of ^{14}C (similar in magnitude to those of June 2004) were measured in the 3 replicates which suggested another local release. Air measurements did not show this release but it was likely missed since air sampling did not start until a couple of days before respiration measurements.

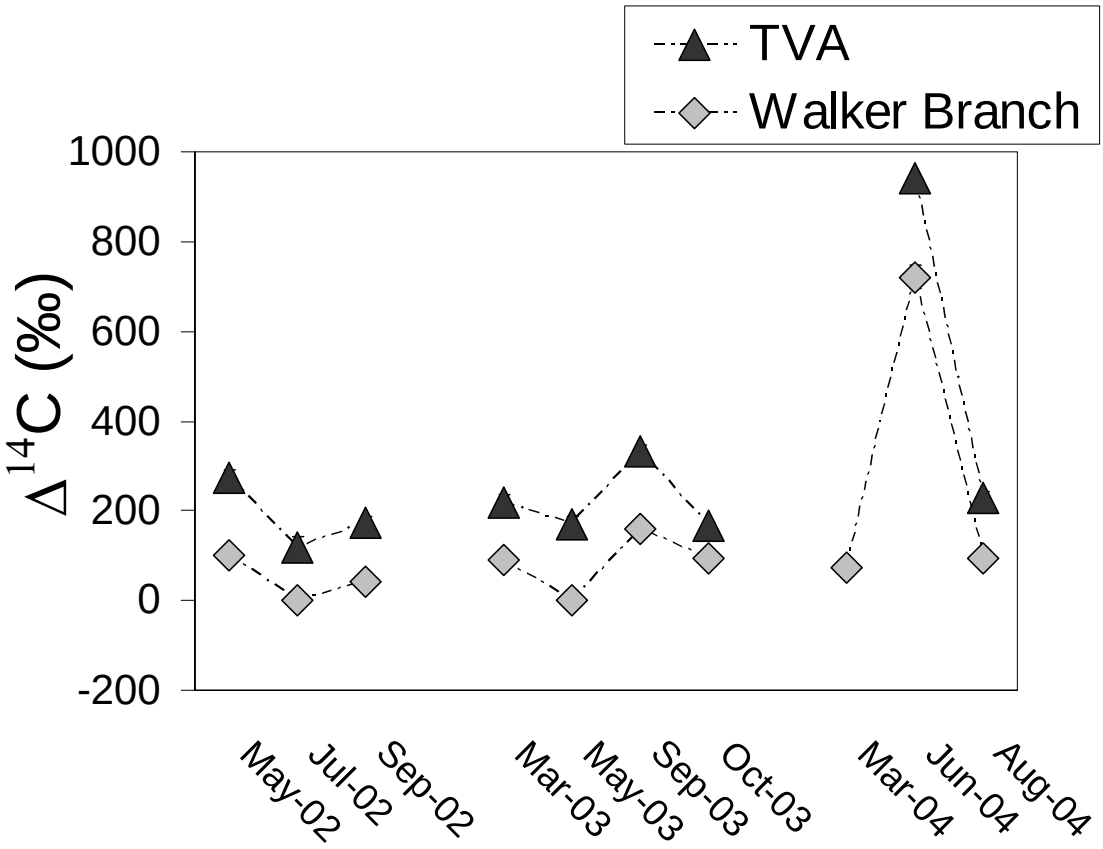


Figure 4.3. Radiocarbon signature of root/rhizosphere respiration measured in CO_2 evolved from incubated live roots collected at TVA and at Walker Branch sites. TVA on western side of the Oak Ridge Reservation (ORR) was exposed to high amounts of ^{14}C in 1999 (~ 1000‰) while Walker Branch on the eastern side was only exposed to near background levels (~230‰). The value for TVA on March 2004 is not available.

3.4. Results from the carbohydrate extractions

3.4.1. Analytical precision

Preliminary tests of our methods which consisted of replicated extractions on samples of leaves and stems showed a precision for radiocarbon analyses of 3-6 ‰ for bulk water soluble compounds, 7 ‰ for starch and 2 ‰ for cellulose (Table 4.1). The standard deviation for multiple analyses was slightly larger than the reported accuracy for the ^{14}C measurement itself (2 to 4.5‰) with regard to nonstructural carbohydrates, which indicates that our processing of the samples introduced some error.

A comparison between the conventional method to isolate cellulose (the "Jayme-Wise" technique) and the alternative method (bleaching of the samples after starch extraction) done on fine roots showed good agreement with regard to ^{14}C signatures for 3 out of 4 samples (Table 4.2). For the sample that did not produce similar results ("j-cell") between methods, the value obtained with the conventional method was used in subsequent calculations. No test was performed on coarse root samples.

To assess the heterogeneity in our root samples, which are usually a composite of three samples excavated at three random locations, we extracted carbohydrate fractions from the individually located samples on two sampling dates and measured radiocarbon in all of the carbohydrate fractions. Heterogeneity in fine root samples ranged from 9 to 24‰ for all the carbohydrate fractions and approximately from 6 to 50‰ for all the carbohydrate fractions in coarse root samples (Table 4.3.).

Table 4.1. Isotopic signatures ± standard deviation of bulk H_2O soluble compounds, starch and cellulose extracted from plant material collected from a tree located in the park of the University of California, Irvine.

Analysis Type	Sample Type	$\Delta^{14}C \pm 4.5$ ‰[1]	$\delta^{13}C \pm 0.2$ ‰
Bulk H_2O soluble compounds			
	Leaves	46 ± 6 (n=3)	-31.6 ± 0.1 (n=3)
	Stems	52 ± 3 (n=3)	-31.1 ± 0.1 (n=3)
Starch			
	Leaves	29 ± 7 (n=3)	-27.5 ± 0.8 (n=3)
	Stems	42 ± 7 (n=3)	-29.3 ± 0.1 (n=3)
Cellulose (alternative method[2])			
	Leaves	42 ± 2 (n=3)	-31.8 ± 0.3 (n=3)
	Stems	50 ± 2 (n=3)	-31.4 ± 0.3 (n=3)

[1]Accuracy varied as follows: ± 4.5 ‰ for bulk H_2O soluble compounds, ± 1.8 ‰ for starch and ± 1.9 ‰ for cellulose
[2] See Appendix 2 and Table 3.2.

Table 4.2. ^{14}C signatures of root cellulose (fine roots, <2 mm in diameter) isolated using two different procedures. Standard deviations of replicated analyses are shown when applicable (n=3).

		$\Delta^{14}C \pm 5$ ‰	
Sample ID	Sampling date / Site	Conventional method[1]	Alternative method[2]
m2-cell	03-31-04 / TVA	303	307 ± 3
i-cell	03-30-04 / WB	214 ± 17	188 ± 22
j-cell	08-14-04 / WB	102	172
l-cell	03-25-03 / TVA	231	228

[1]Adapted from the "Jayme-Wise" technique, refer to Appendix 2 for a detailed description of the method
[2] See Appendix 2 for a description of the method

Table 4.3. (page I of 2). ^{14}C signatures of carbohydrate fractions extracted from different root samples collected on the same day at random locations. Standard deviations of replicated analyses are shown when applicable (n=3).

Analysis Type	Sampling date / Site	Sample ID and number	Root type[1]	$\Delta^{14}C \pm 5$ ‰	Mean	Standard deviation
Cellulose[2]	03-31-04 / TVA	m-conv-1	Fine	279		
Cellulose[2]	03-31-04 / TVA	m-conv-2	Fine	303		
Cellulose[2]	03-31-04 / TVA	m-conv-3	Fine	317	300	19
Cellulose[3]	03-31-04 / TVA	m - 1	Fine	322		
Cellulose[3]	03-31-04 / TVA	m - 2	Fine	307 ± 3		
Cellulose[3]	03-31-04 / TVA	m - 3	Fine	306	312	9
Cellulose[2]	03-30-04 / WB	i - 1	Fine	233 ± 6		
Cellulose[2]	03-30-04 / WB	i - 2	Fine	207 ± 11		
Cellulose[2]	03-30-04 / WB	i - 3	Fine	203 ± 6	214	16
Cellulose[3]	03-30-04 / WB	i - 1	Fine	205 ± 4		
Cellulose[3]	03-30-04 / WB	i - 2	Fine	173 ± 21	189	23
Cellulose[2]	03-30-04 / WB	I-1	Coarse	249		
Cellulose[2]	03-30-04 / WB	I-2	Coarse	239		
Cellulose[2]	03-30-04 / WB	I-3	Coarse	250	246	6
Starch	03-30-04 / WB	i - 1	Fine	144 ± 8		
Starch	03-30-04 / WB	i - 2	Fine	173 ± 3	159	21
Starch	03-31-04 / TVA	m-2	Fine	331 ± 12		
Starch	03-31-04 / TVA	m-3	Fine	298	315	23
Starch	03-31-04 / TVA	M-2	Coarse	351		
Starch	03-31-04 / TVA	M-3	Coarse	272	312	56

[1] Fine roots (<2 mm in diameter) and coarse roots (>2 mm in diameter)
[2] Conventional method (see appendix 2)
[3] Alternative method (see appendix 2)

Table 4.3. (page 2 of 2). ^{14}C signatures of carbohydrate fractions extracted from different root samples collected on the same day at random locations. Standard deviations of replicated analyses are shown when applicable (n=3).

Analysis Type	Sampling date / Site	Sample ID and number	Root type[1]	$\Delta^{14}C \pm 5$ ‰	Mean	Standard deviation
Bulk H_2O soluble	03-30-04 / WB	i - 1	Fine	188 ± 3		
Bulk H_2O soluble	03-30-04 / WB	i - 2	Fine	188 ± 4	188	0
Bulk H_2O soluble	03-31-04 / TVA	m-1	Fine	330		
Bulk H_2O soluble	03-31-04 / TVA	m-2	Fine	335		
Bulk H_2O soluble	03-31-04 / TVA	m-3	Fine	291	319	24
Bulk H_2O soluble	03-31-04 / TVA	M-2	Coarse	300		
Bulk H_2O soluble	03-31-04 / TVA	M-3	Coarse	229	265	50

[1] Fine roots (<2 mm in diameter) and coarse roots (>2 mm in diameter)
[2] Conventional method (see appendix 2)
[3] Alternative method (see appendix 2)

3.4.2. Radiocarbon signatures of carbohydrate fractions

The ^{14}C signatures of starch and bulk water soluble compounds extracted from fine roots are compared with ambient air CO_2 and root respiration time series in Figures 4.4. and 4.5. for Walker branch and TVA sites, respectively. As noted previously, the relationship between the ^{14}C of root respiration and the ^{14}C of air continuously sampled over two week periods is complex. Overall, starch and water soluble extracts had ^{14}C values that were less variable in time and generally (with one exception) higher than those of atmospheric CO_2 or root respiration. Patterns of variation with time in the carbohydrate fractions were similar at the WB and TVA sites, though again, ^{14}C values in general were greater at TVA.

The ^{14}C signatures of root cellulose and bulk root tissue varied more with time than those of starch or water soluble pools (Figure 4.6.) Again, it is difficult to discern clear seasonal patterns with time, and correspondence is less good between the WB and TVA sites.

Figure 4.3 shows that changes in root respiration ^{14}C occur in concert at TVA and WB sties. In order to eliminate variability caused by the short-term changes in the ^{14}C signature of local ambient air at each of the sites, Figure 4.7 shows the difference in ^{14}C signatures between the TVA and WB for all the carbohydrate components in 2003. All components show a similar pattern, with the difference in ^{14}C between the two sites increasing from March to May and subsequently decreasing from May to October. The largest difference in ^{14}C between the two sites is in the starch (although some key data are missing from the figure); the smallest differences between the two sites are in the

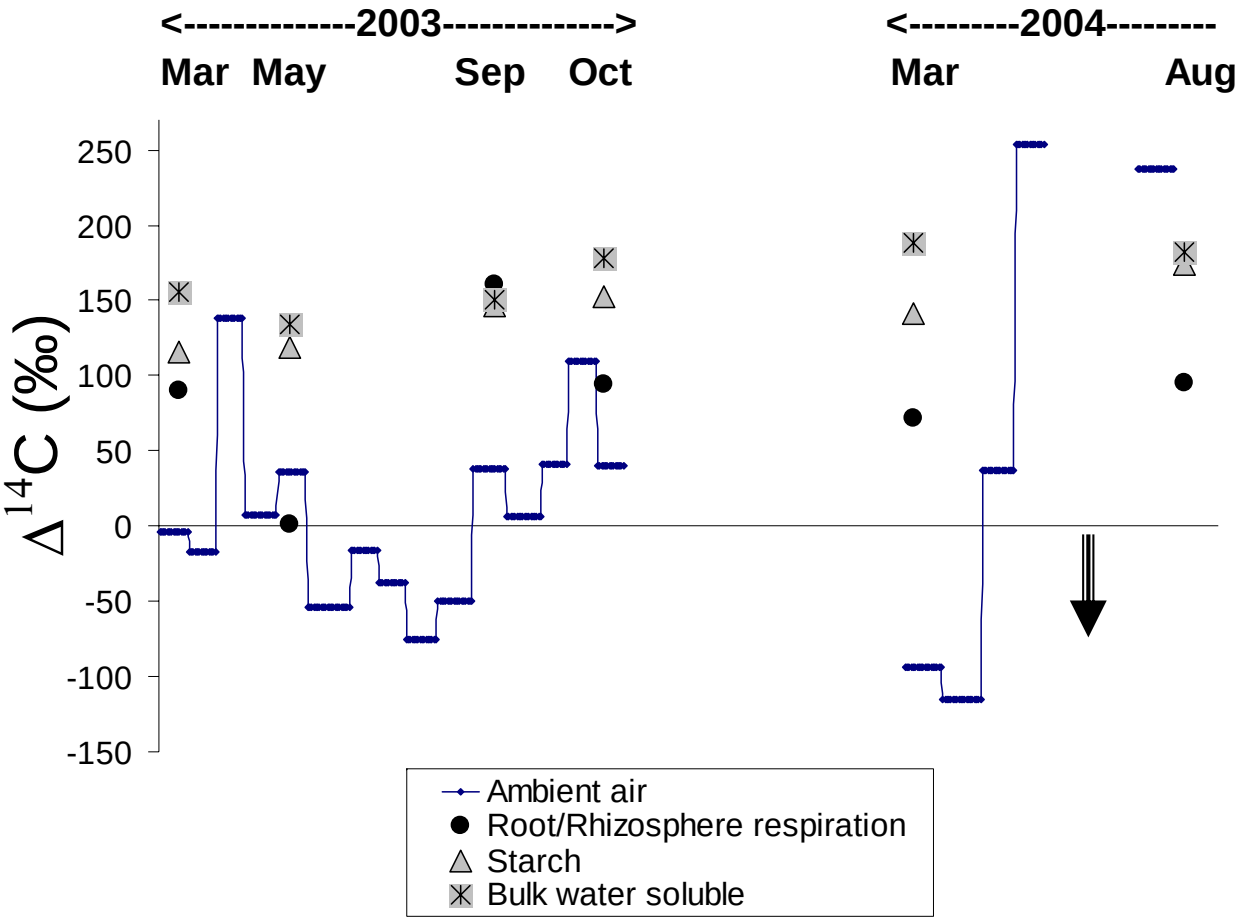


Figure 4.4. Radiocarbon signatures of ambient air, root/rhizosphere respiration, and starch and bulk water soluble compounds extracted from roots (<2 mm in diameter) collected at the Walker Branch site. Values of ambient air are averages over a period of two weeks; other values are point-in-time measurements. Starch and bulk water soluble compounds were extracted from roots collected on the same days of respiration measurements. Arrow indicates lower than -150 ‰ ^{14}C levels in ambient air (see Figure 3.1.).

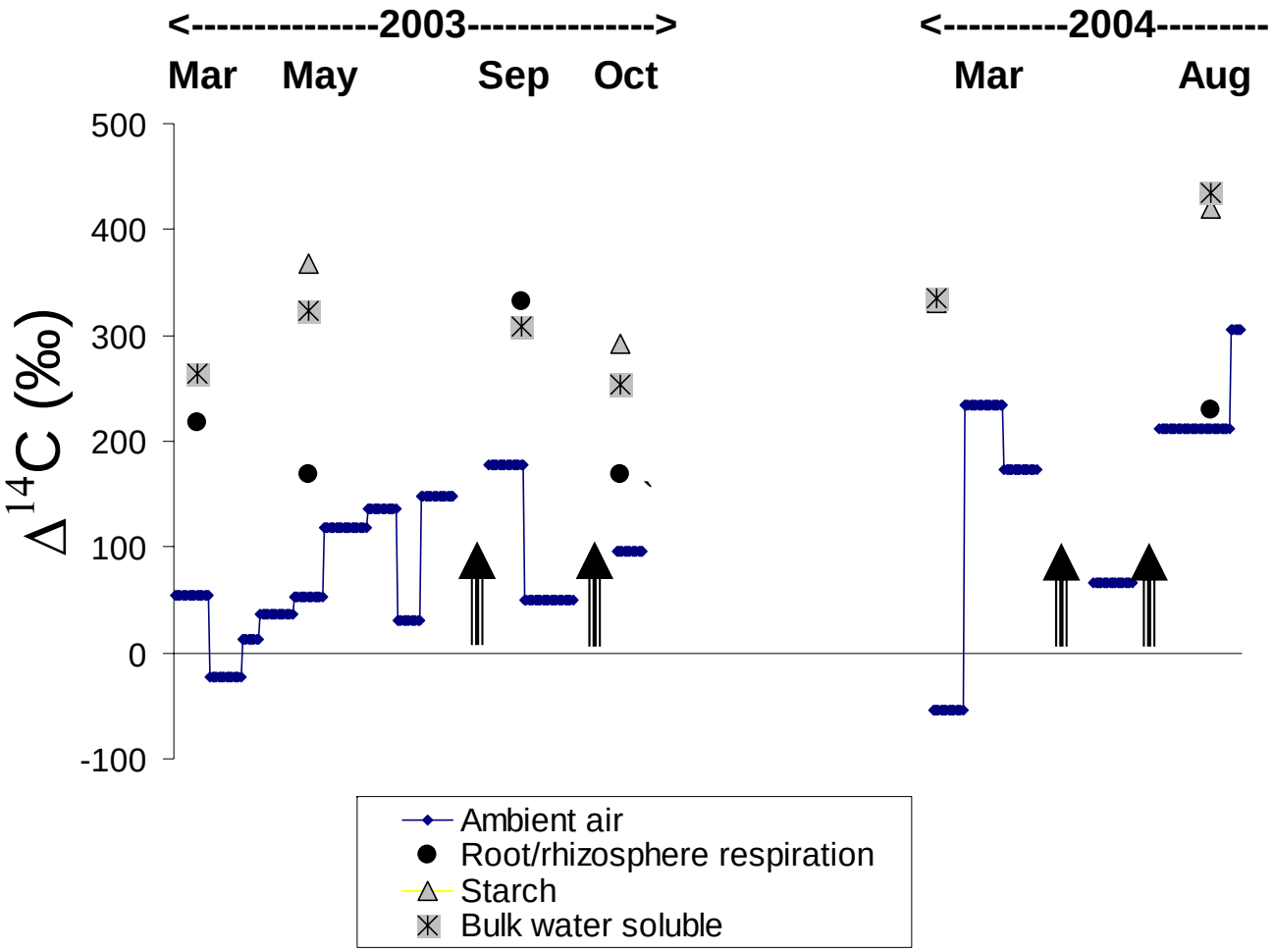


Figure 4.5. Radiocarbon signatures of ambient air, root/rhizosphere respiration, and starch and bulk water soluble compounds extracted from roots (<2 mm in diameter) collected at the TVA site. Values of ambient air are averages over a period of two weeks; other values are point-in-time measurements. Starch and bulk water soluble compounds were extracted from roots collected on the same days of respiration measurements. Arrows indicate greater than 500 ‰ ^{14}C levels in ambient air (see Figure 3.1.).

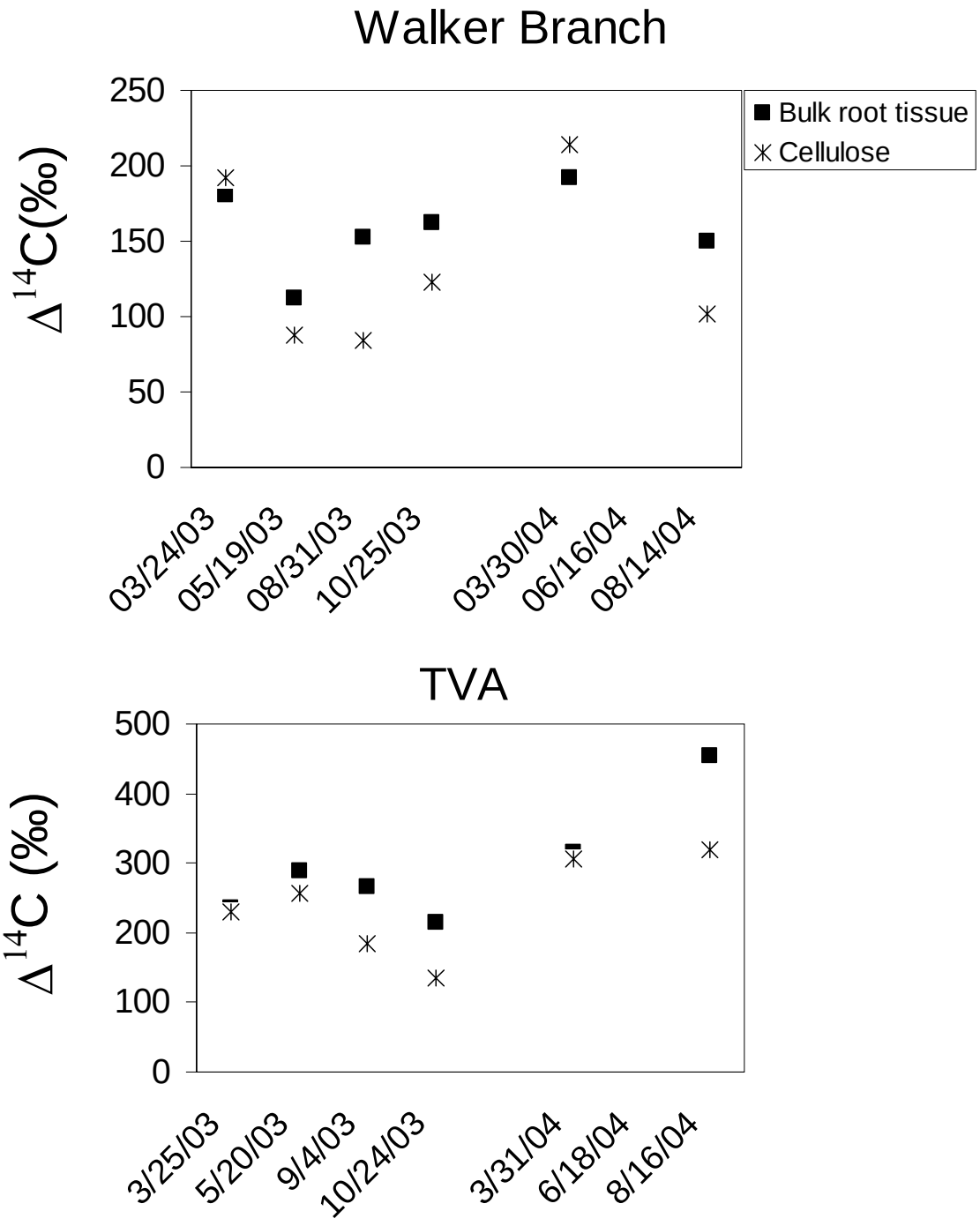


Figure 4.6. Radiocarbon signatures of bulk root tissue and root cellulose (roots <2 mm in diameter) at each site. Notice the different scale on the ordinates.

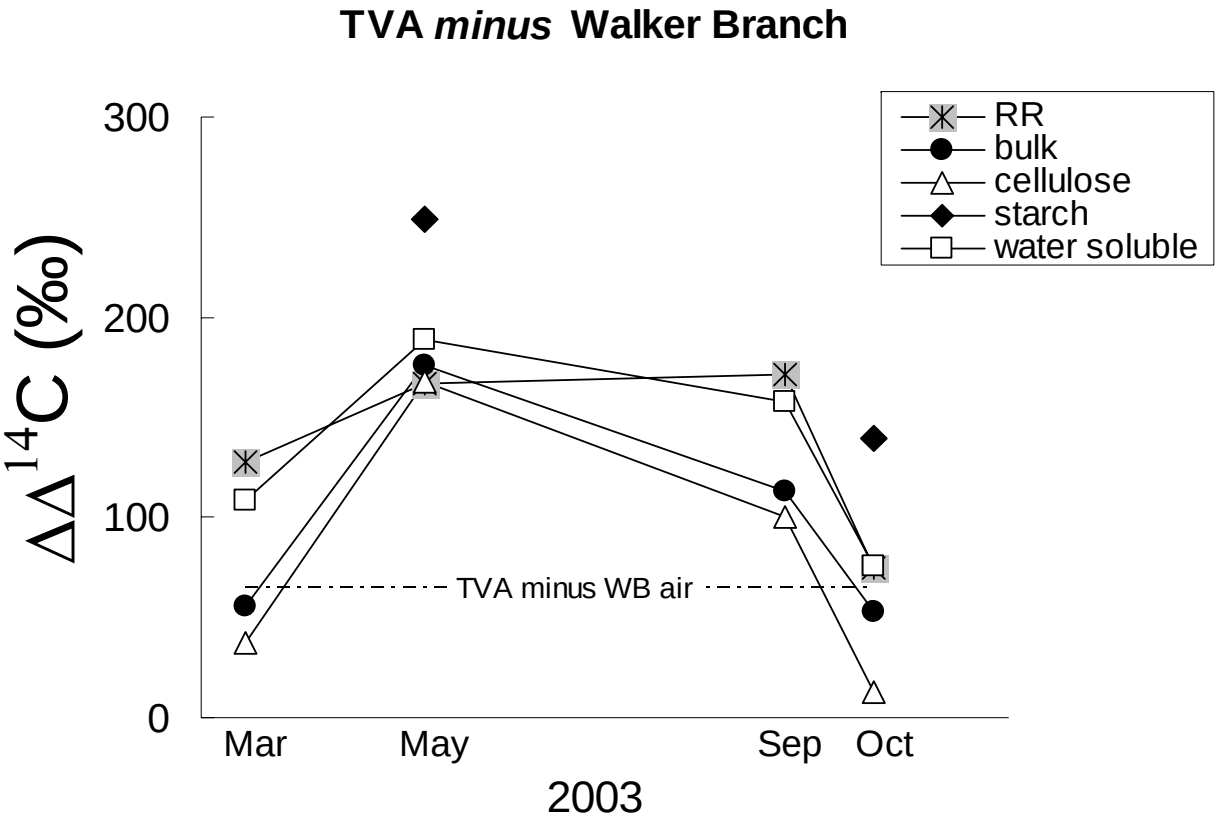


Figure 4.7. The difference in 2003 of ^{14}C signatures for (1) root/rhizosphere respiration (RR), (2) bulk tissue, (3) cellulose and (4) nonstructural carbohydrates (starch + water soluble compounds) between TVA (high initial ^{14}C label in 1999) and Walker Branch (low initial ^{14}C label in 1999). All data refers to fine roots (<2 mm in diameter) and mostly fine roots (≤90%) in the case of RR. Actual variations in the ^{14}C signature of ambient air at the sites are large, related to short term releases of both ^{14}C-enriched and ^{14}C–depleted CO_2; the 'normal' range of variation excludes these short term imprints (see Figure 3.1. for their magnitude and timing).

cellulose fraction. Differences in the ^{14}C signatures of root/rhizosphere respiration and bulk water soluble compounds lie in between cellulose and starch values.

Starch extracted from coarse roots was higher in ^{14}C signature than that extracted from fine roots collected on the same day at the WB site, particularly in the beginning of the growing season (Figure 4.8). However given the heterogeneity in our root samples (Table 4.3.), this difference should not be taken at face value. Additionally we should consider the small degree of error that is introduced with our procedures as indicate above. As for starch extracted from root samples collected at TVA and bulk water soluble compounds extracted from samples collected at both sites, no clear difference with root diameter was observed (Figure 4.8.). Since only the alternative method was used for the isolation of cellulose from coarse root samples (with the exception of the heterogeneity test, Table 4.3.), these results were not compared against those of fine roots.

3.5. Stored versus recent photosynthate as substrate for root/rhizosphere respiration

To assess the contribution of stored carbon versus current photosynthate as a source for root/rhizosphere respiration, we applied Eq. (4.1.) to signatures measured on any given day (Figures 3.4. and 3.5.). On some dates, this calculation was not possible due to either missing data or to the signatures of the source components being out of balance (e.g. May and September 2003, Walker Branch). Nevertheless, estimations could be made for key months in the growing season, i.e. March, August and October.

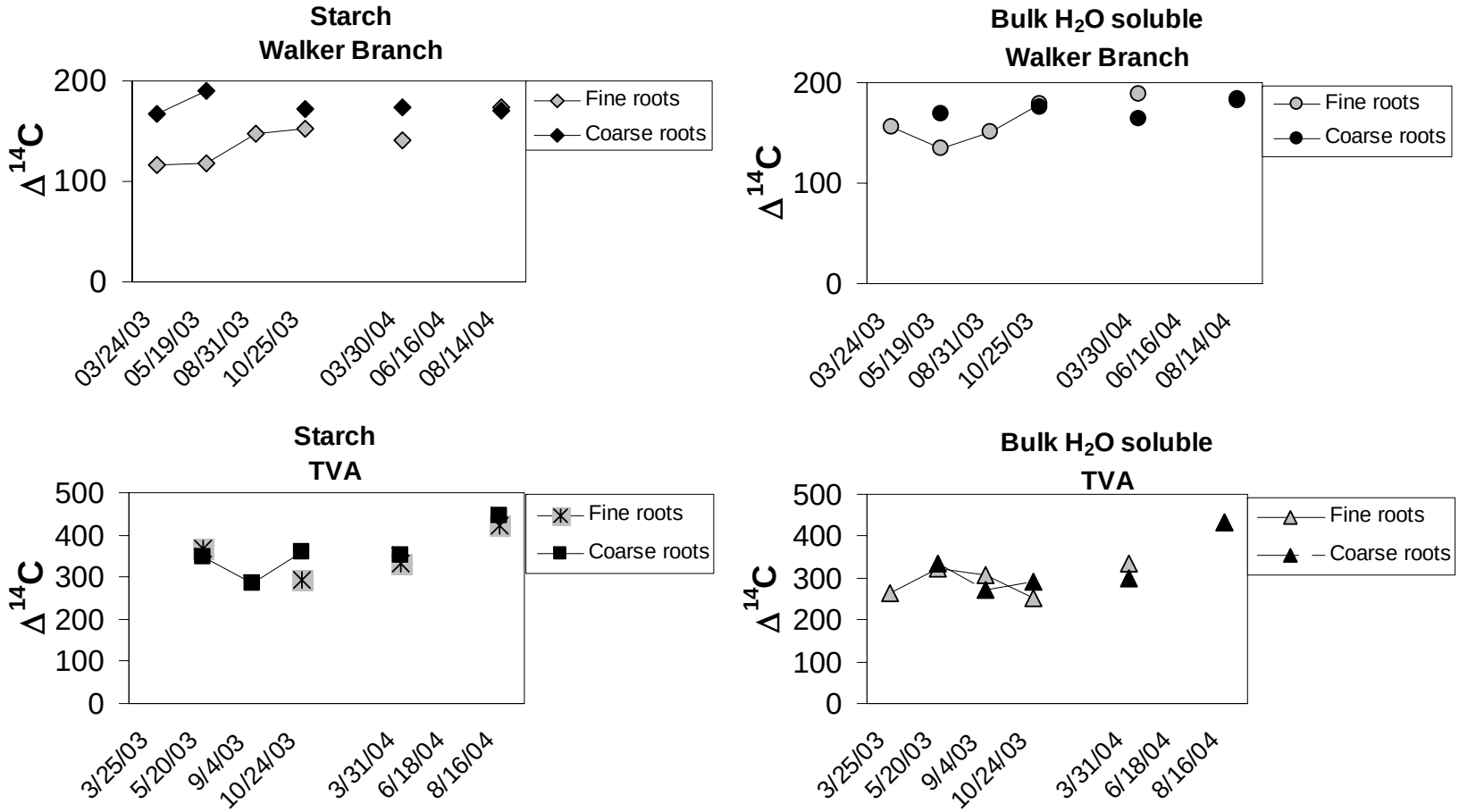


Figure 4.8. Radiocarbon signatures (‰) of starch and bulk water soluble compounds extracted from fine (< 2 mm in diameter) and coarse (> 2 mm in diameter) roots collected at Walker Branch (low initial ^{14}C label in 1999) and TVA (high initial ^{14}C label in 1999) sites (notice the different scale on the ordinates).

Stored carbon in the starch pool was estimated to account for approximately 70% of the carbon source for root/rhizosphere respiration in March and for lower amounts in August and October (from less than 10% to up to 30%; Table 4.4.).

Table 4.4. Fraction of root/rhizosphere respiration derived from stored carbon (starch pool) in fine roots (<2 mm in diameter) estimated using Eq. (4.1.) and ^{14}C signatures from Figures 3.4 and 3.5 for Walker Branch (WB) and TVA sites respectively. The cases when ^{14}C signatures of the components in Eq. (4.1.) did not satisfy the mass balance are indicated by "n/a". No data available is indicated by hyphens.

Site	Mar-03	May-03	Sep-03	Oct-03	Mar-04	Aug-04
WB	0.78	n/a	n/a	0.24	0.71	-
TVA	-	0.38	-	0.37[1]	-	0.08

[1] This value is uncertain as a local ^{14}C release preceding respiration measurements (see Figure 3.5.) may have enriched the ^{14}C of root/rhizosphere respiration thus yielding an erroneous higher contribution from starch which is normally enriched in ^{14}C with respect to ambient air.

4. Discussion

4.1 Precision of analytical methods

Repeated measures of homogenized standard materials (leaves and stems), indicated that we can measure the radiocarbon signature of starch and water soluble extracts with an overall precision of ±7 ‰. The reproducibility in root samples collected in the field was sometimes larger than ±7 ‰ (Table 4.3.). Nevertheless, in our study, deviations in the range of 10-20‰ in $\Delta^{14}C$ are not too problematic, given the very large range in ^{14}C values we observe at the EBIS sites.

The overall accuracy of our methods is somewhat more difficult to assess. We were able to show (data not included here) that putting a standard sucrose (ANU sucrose) through our extraction procedure for water soluble compounds did not change its radiocarbon content. Similarly, we attempted to use a purchased starch compound for our starch extraction, but it had different solubility characteristics than the starch we isolate with our procedure. Our starch and water soluble extract fractions are operationally defined fractions; as yet we do not know exactly what compounds are in them and how they might vary in composition from one plant to another. However, we are confident based on our ability to reproduce the values for samples from the same site on the same day that these operationally defined fractions can have some physiological meaning.

In addition to demonstrating that existing methods for extracting starch can produce meaningful radiocarbon data for samples at natural ^{14}C levels, we have shown a potential alternative method for isolating a "cellulose" component that can be

performed easily in sequence after starch extraction. Gaudinski et al., [in press] point out that "cellulose" as it is commonly extracted from woody materials, is an operationally defined, and not a pure, substance. Our method reproduces the ^{14}C signature obtained with the standard ("Jayme-Wise") technique for samples collected in March but not for those collected in August (Table 4.2.). One possible cause for the discrepancy between methods may be the presence of lipids and waxes not removed during the starch extraction procedure. Another source of error may be partial removal of lignin with our alternative method [Gaudinski et al., in press]. We are confident in our data on the $\Delta^{14}C$ signatures of cellulose in fine roots, as they were derived from the conventional method on the dates when the alternative method did not show good agreement.

4.2. Comparison of carbohydrate fractions with root respiration

The correspondence of variations in the ^{14}C content of root respiration at the TVA and WB sites (Figure 4.3.) indicates that there must be a rapidly-metabolized component that varies across both sites, most likely the ^{14}C content of CO_2 in air. Recently fixed carbon (fixed within days to a month earlier) clearly also provides an important source for root/rhizosphere respiration, as evident from our respiration measurements of elevated ^{14}C signatures in root respiration in June 2004, following the earlier release of ^{14}C observed in air sampled during the preceding two-week period (June 4th to June 18th). The fact that the signatures returned to the usual levels in August at both sites seems also to reflect the large changes in the signatures of ambient air usually observed at these sites. Our first hypothesis was that the bulk water soluble

compounds would be the closest analog to this rapidly-metabolized component. However, the ^{14}C content of bulk water soluble compounds did not consistently follow those of root/rhizosphere respiration at either site (Figure 3.4 and 3.5); and indeed was closer to the ^{14}C signature of the starch than root respiration. We found no extractable component that varied in ^{14}C in concert with root respiration; hence we must infer that either it was lost before our extractions (perhaps it was respired before the samples could be frozen, or was lost in freeze-drying), or that it makes up a minor component of one of our extracts. Because our water soluble extract is a mixture of compounds, containing pigments as well as sugars, further work will be required to isolate sugars from water extracts as described on Appendix 2B. In addition, isolation of specific compounds (i.e. sucrose, glucose) may be needed to further investigate the role of soluble sugars as source for respiration.

Nonstructural carbohydrates (water soluble extract and starch) were distinctly enriched in ^{14}C at both sites compared to root/rhizosphere respiration on all dates with the exception of September 2003 (Figure 4.4. and 3.5.). The September 2003 values likely reflect incorporation by the plants of an earlier ^{14}C release measured at the TVA site (Figure 3.1), although the time elapsed between the release and root respiration measurements was at least 16 days at TVA (12 days at Walker Branch). The temporal link between photosynthesis and root/rhizosphere respiration is a topic of current research and the information that is available suggest lag times from a few hours to up to 10 days although a fixed lag time should not be expected [Bhupinderpal-Singh et al., 2005; Bowling et al., 2002; Ekblad and Hogberg, 2001; Ekblad et al., 2005]. It is clear that our air sampling measurements, which integrate air over two weeks, for 24 hours a

day, can be biased by incorporation of respired CO_2 (and fossil fuel CO_2) that does not get mixed out of the nocturnal boundary layer. For instance, the incorporation of soil CO_2 flux can be inferred from the ^{13}C signatures of the air samples (Figure 4.2.) which are slightly depleted than background air levels (-8.5‰; NOAA ESRL, Global Monitoring Division) indicating the incorporation of depleted soil respired CO_2 (usually around -26‰ at both sites). To better understand how ^{14}C in atmospheric CO_2 affects the root respiration isotopic signatures requires air sampling at canopy height and during the daylight hours when plants are actively fixing carbon.

The ^{14}C signature of cellulose was highly variable at both sites but did not follow a similar pattern (Figure 3.6). The variation and magnitude of the ^{14}C signature of bulk tissue though was very similar to that of cellulose on all dates at both sites which may seem to rule out analytical errors given that cellulose constitutes ~40% of carbon in plant tissues. New root growth has been observed to be derived from current photosynthate in sugar maple (*Acer saccharum* Marsh.) and if this is a characteristic pattern of deciduous trees [Dickson, 1991], then lower ^{14}C signatures of cellulose (assuming background levels in ambient air) should be expected during periods of root growth. This seems to be the case at the TVA site where the ^{14}C content of cellulose was the lowest on September and October of 2003, (the former apparently unaffected by the earlier release in ambient air mentioned above), dates immediately following the time of maximum root growth at this temperate forest [Joslin et al., 2001]. In 2004, releases during this time may be responsible for the higher signature of cellulose measured on August. In the case of the Walker branch site, the same pattern of lower signatures was observed on August in both years. Variations in the temporal trends in

root ^{14}C can also reflect heterogeneity associated to our sampling procedures, since we did not attempt to separate roots by species or separate roots according to branching order.

In addition to the parallel variations in root respiration ^{14}C at Walker Branch and TVA sites that may reflect changes in the ^{14}C signature of local ambient air, there is a consistent tendency for the ^{14}C signature of root respiration at TVA to be higher than that at Walker Branch. Further, a visual comparison of the ^{14}C signature of root/rizhosphere respiration with that of the mean signature of air in days preceding respiration measurements indicates, on some dates, a source of carbon for root/rizhosphere respiration with a higher ^{14}C signature, i.e. stored carbon must be a source of root-respired CO_2 (Figures 3.4. and 3.5.). Expressing the seasonal variations in the ^{14}C signatures as the difference between sites (e.g. Figure 4.7.) eliminates some of the short-term variability in local air and emphasizes the changing importance of nonstructural carbohydrates as sources of carbon for root/rhizosphere respiration over the course of the growing season. During 2003, the decrease in the difference in ^{14}C signatures between TVA and Walker Branch sites later in the growing season (September-October) may indicate the dilution of these pools with recent photosynthate of ^{14}C content similar to that of the mean signature in ambient air (Figure 3.7). Although the difference between TVA and Walker Branch ^{14}C signatures also increased in the spring (from March to May), the site-to-site differences in March exceeded those in October by 25 ‰ with regard to cellulose and by 33 ‰ with regard to water soluble compounds. In addition, we lack data on the starch pool for March, which we hypothesize should demonstrate the largest difference between sites.

For a difference between the TVA and WB sites (HR and LR respectively) in the ^{14}C signature of starch pools to be maintained over two years of this study (2002-2003; Figures 3.4. and 3.5.) requires that the turnover time of this pool be relatively long. In particular, if the starch pool is not greatly affected by ^{14}C releases that occurred after the original 1999 event (the lack of response to releases in the growing season of 2003 indicates this may be the case), the difference must have been maintained for at least 4 years. Data on the residence time of starch reserves in roots to which we can compare our data against to is not available in literature. Such data does exist though with regard to structural carbon in fine roots (< 2 mm in diameter) which has been estimated to have a residence time of 4-6 year in forest tress [Matamala et al., 2003].

The relative contribution from stored pools to root/rhizosphere respiration calculated using ^{14}C signatures of starch and atmospheric CO_2 as end-members confirmed a decreasing importance in the starch pools through the growing season (Table 3.4). These estimates are in agreement with observations from a pine forest (*P. sylvestris* L.) that showed greater dependence of root respiration on current photosynthate late in the growing season (August) relative to earlier (June) [Hogberg et al., 2001]. Our estimates of a greater proportion of stored carbon as a source for root/rhizosphere respiration early in the growing season is in line with the growth pattern of deciduous trees, where carbon reserves are used for the initial part of the growing season and until new leaf growth is completed [Dickson, 1991]. Wargo, [1979] observed that starch reserves in roots of sugar maple (*Acer saccharum* Marsh.), white oak (*Quercus alba* L.), red oak (*Q. rubra* L.) and black oak (*Q. velutina* Lamarc) were almost depleted by mid-May following the onset of leaf expansion and started to be

replenished in June when current photosynthetic products began to be allocated to roots. Dickson, [1991] observed that export of new carbon from leaves of a northern red oak (*Quercus rubra* L.) started only after at least 70% of leaf expansion had been completed. Leaf expansion at the temperate forest in our study is near to completion (95%) on average on mid-May [Joslin and Wolfe, 2003] when we estimate ~30% use of stored carbon in contrast with 70% in March.

Our second hypothesis was that the source of carbon for root/rhizosphere respiration is derived from stored carbon in starch pools early in the growing season (March) and from current photosynthate in the latter part (August). This is partially true in the sense that the source for respiration early in the growing season is not exclusively from the starch pool we were able to isolate, though that pool can constitute the major (~70%) source of root respiration in spring, and contributes much less later in the growing season. A few aspects associated to our methods should be pointed out. The starch signatures used in Equation (1) correspond to those extracted from fine roots and our respiration measurements were performed on samples that contained both fine and (in lesser proportion) coarse roots. Although the difference measured in the ^{14}C signatures of starch between coarse and fine roots (Figure 4.8.) might just have been the result of the heterogeneity of our root samples (particularly of coarse root samples) as mentioned above, we cannot rule out that the ^{14}C signature of starch would be higher had we extracted it from the entire (coarse + fine root) sample, especially during the early part of the growing season when the differences with root diameter were the greatest at the Walker Branch site. In this case, we would be overestimating the contribution of starch pools to root/rhizosphere respiration. However the magnitude of

any correction would depend on whether coarse roots respire at similar or lower rates than fine roots. We did not test the respiration rates of roots with different diameters although coarse roots have been observed to respire at much lower rates (on a per unit mass basis) than fine roots in other forest stands [Desrochers et al., 2002; Pregitzer et al., 1998].

A second issue arises from questions about our method for measuring root respiration, which involves excision of roots and measurement of CO_2 respired over the next 1-2 hours. If roots start using starch reserves at a greater rate upon excision, we might also be overestimating the contribution of starch pools to root respiration. Although this should be tested at our EBIS sites, excision did not cause a change in the substrate used for respiration immediately after detachment for roots of Ponderosa pine even after 24 hours [Lipp and Andersen, 2003]. In addition, if excision indeed affected the type of substrate used for respiration towards starch reserves, then we would expect to see less seasonal variations in the ^{14}C signature of root/rhizosphere respiration and levels closer to those we measured in starch pools.

5. Conclusions

Two major lines of evidence suggest that the starch pool we have extracted represents a pool with relatively long (order of years) turnover time that is a significant (though seasonally variable) contributor to root respiration. First, the ^{14}C signature of the extracted starch varies much less over time than its potential source (atmospheric $^{14}CO_2$), indicating that it is replenished slowly compared to atmospheric variations. Second, the difference in ^{14}C in this pool between TVA and Walker Branch sites is

maintained over the period of several years after the major release that labeled both pools initially.

The source of carbon for root/rhizosphere respiration is comprised of two pools: a slow pool derived from stored nonstructural starch reserves and a fast cycling pool derived from carbon fixed in the past few days to weeks. Both pools are used as a source for respiration throughout the growing season but their relative contributions vary seasonally, with highest contribution of ~70% from the slow-cycling pool we have identified as starch in March, decreasing to <20 % later in the growing season as fresh photosynthetic products begin to dominate root respiration sources. It is important to note, however, that we estimate that the stored carbon pools are always a source of some of the root-respired carbon.

Isotope labeling of plants, either with ^{13}C or ^{14}C, is a powerful tool to track physiological processes such as patterns of carbon allocation. Therefore efforts should be made to apply these techniques, particularly in forested ecosystems in order to advance the understanding of terrestrial carbon cycling. While serendipitous discoveries like the one made at the temperate forest in Oak Ridge, Tennessee may not occur at other ecosystems, experiments like those comprising the Free Air Carbon-dioxide Enrichment (FACE) project demonstrate the feasibility of the approach for the study of carbon cycling in established forests and thus emphasizes the importance of the continued application of these techniques.

CHAPTER 5

CONCLUSION

The research performed as part of this dissertation was aimed to the study of soil respiration sources in a temperate forest located near Oak Ridge, Tennessee, USA. Radiocarbon labeling at the whole-ecosystem scale combined with a litter manipulation experiment (the Enriched Background Isotope Study, EBIS; http://ebis.ornl.gov/) provided the basis for the application of an isotopic approach. Two sites differing in the amount of ^{14}C label incorporated into roots were selected for soil respiration measurements: TVA and Walker Branch, corresponding to high (~1000‰) and low (~230‰) label in roots respectively. At each site, experimental plots were established as part of the EBIS project to manipulate ^{14}C inputs through leaf litter with either enriched or near background levels. The resulting experimental design combined high and low ^{14}C inputs through roots and leaf litter.

The specific topics addressed in this dissertation were: (1) the use of the ^{14}C label to partition soil respiration into heterotrophic (leaf litter and mineral soil decomposition) and autotrophic sources, (2) the investigation of the response of these sources to changes in temperature and moisture (3) the assessment of the relative importance of carbon pools of different ages as substrates for heterotrophic respiration and (4) the role of stored nonstructural carbohydrates as substrate for autotrophic respiration. The isotopic approach involved the measurement of the ^{14}C signature in the CO_2 derived from (1) total soil respiration, (2) live roots and microorganisms acting on

the same carbon substrate in the rhizosphere (all comprising autotrophic respiration), (3) the decomposition of leaf litter and (4) the decomposition of organic matter in the mineral soil.

The application of isotope mass balance approaches to partition soil respiration into its different source components was demonstrated. By comparing the different ^{14}C signatures of CO_2 evolved from high versus low ^{14}C label litter plots at each of the two sites studied, it was possible to estimate the proportion of soil respiration derived from the decomposition of leaf litter only. The estimates of the contribution from leaf litter decomposition were consistent across sites indicating the robustness of the approach. Leaf litter decomposition contributions to soil respiration ranged from ~ 1 ±3% to 42 ±16% corresponding to absolute fluxes of 6 ±3 to 96 ±38 mg C $m^{-2}h^{-1}$ and near zero when leaf litter was extremely dry. These results showed the quantitative importance of this source of soil respiration as well as its strong dependence on moisture conditions. We further investigated the dependence of litter decomposition on moisture availability, using natural and manipulated moisture. Leaf litter decomposition fluxes accounted for the entire increase in soil respiration fluxes following an increase in moisture (i.e. precipitation), increasing in contribution from 8 ±8% during drought to 32 ±12% after wetting, corresponding to 11 ±11 and 53 ±25 mg C $m^{-2}h^{-1}$ respectively. However, the type of carbon substrate used for decomposition (i.e. recent versus old carbon), was unaffected by changes in temperature and moisture. These results confirmed that litter water content effects on decomposition are a main source of temporal variation in

growing season soil respiration fluxes in this forest and underscored the need to incorporate this component of soil respiration into models.

By applying a mass balance to the ^{14}C signatures of total soil respiration, heterotrophic and autotrophic fluxes, the autotrophic portion of soil respiration was estimated at each site. Due to the problem we encountered with systematic overestimation of the litter contribution to heterotrophic respiration fluxes using our incubation methods, it was necessary to constrain our estimates of the autotrophic contribution with our estimates of leaf litter decomposition (which we know are robust) on each sampling date and for each litter treatment plot. The relative contribution of autotrophic respiration was estimated to vary from 16 ±10% to 64 ±22% during the growing season corresponding to absolute contributions of 34 ± 14 to 40 ± 16 mg C $m^{-2}h^{-1}$ with a single exception in September 2003 (88 ±35 mg C $m^{-2}h^{-1}$). There was no correspondence between times of highest relative and highest absolute contributions, emphasizing the need to report both quantities. By contrast with leaf liter decomposition fluxes, autotrophic respiration did not exhibit marked temporal variation although more frequent measurements may be required to capture seasonal variations in response to phenology and environmental factors.

By constrasting the effects of two levels of ^{14}C in leaf litter across treatment plots at each site, it was possible to estimate the contribution of the litter added (≤3 years old at the point when the calculation was made) to total heterotrophic fluxes. This recent leaf litter was estimated to contribute at least 50% to leaf litter decomposition fluxes, which translated into an overall contribution of >15-30% to total soil respiration fluxes. With regard to decomposition fluxes in the mineral soil, recent leaf litter was

estimated to contribute only 6 ±4%, which indicated little decomposition of carbon leached from leaf litter layers in shallow mineral soil (0-5 cm) during the growing season.

The different amounts of ^{14}C label in roots across sites were useful in elucidating the source of carbon for root/rhizosphere respiration. The ^{14}C signatures of root/rhizosphere respiration, nonstructural carbohydrates in root tissue and CO_2 in ambient air were combined to estimate the relative contribution of stored versus recently fixed carbon as substrate for root/rhizosphere respiration. Analytical techniques were developed and tested to isolate carbohydrate fractions from plant material for radiocarbon measurement. The precision achieved was higher than the accuracy of the radiocarbon measurement itself (7‰ versus 4.5‰) with regard to nonstructural carbohydrates (water soluble compounds and starch). The source of carbon for root/rhizosphere respiration was comprised of two carbon pools: a slow and a fast cycling pool. The slow pool, which we assign to stored nonstructural carbohydrates in starch pools, was estimated to have a relatively long (order of years) turnover time while the fast pool appeared to be derived from recently fixed carbon (days to weeks earlier). The relative contributions of each of these pools as substrates for respiration (as estimated by isotope mass balance) varied seasonally, with stored carbon constituting ~70% early in the growing season (March) and <20% in the latter part (August-October) as fresh photosynthetic products begin to dominate.

The utility of the isotope techniques for partitioning soil respiration sources in forests has been demonstrated. Isotopic techniques do have the limitation that if the signatures of the sources in a mixture are not significantly different from one another,

then an isotopic mass balance can not be applied with confidence. Aside from errors associated to measurement and analytical procedures, this issue may be found in ecosystems with background isotopic signatures. We often encountered this case in the 'near background' litter plots because the +250‰ of applied leaf litter caused the ^{14}C signature of leaf litter decomposition to be nearly equal to that of root respiration in the years we made measurements. To ensure good isotopic separation of sources, it is important to add relatively high levels of label, and in the case of using labeled plant material, to make sure it is uniformly labeled. Experiments like those comprising the Free Air Carbon-dioxide Enrichment (FACE) project or similar approaches in forested ecosystems, could be adapted for labeling of control plots (elevated CO_2 plots already have an isotope label). Field measurements that can differentiate autotrophic from heterotrophic respiration are needed to validate model simulations of the response of these soil respiration sources to various environmental factors such as increased atmospheric CO_2 concentrations, increased temperatures, nutrient availability, pollution and drought.

FUTURE WORK

Future work should involve the testing of model predictions of soil respiration fluxes as well as leaf litter decomposition fluxes. The data generated as part of chapter 2 and 3 (including environmental data) provide a basis to perform such testing and this is currently under progress. Long-term deployment of the automated soil respiration system to link variations in the radiocarbon signature of soil respiration with changes in soil respiration fluxes should be pursued in order to increase the frequency but also with the purpose of capturing extreme environmental conditions such as transient droughts and precipitation events. Future studies of autotrophic respiration should include more frequent measurements performed at key times during the growing season as well as during the dormant season. This offers the potential of capturing the relationship between root respiration and root growth patterns and the effect of transient environmental changes.

Some specific tests have high priority. One is the evaluation of root respiration rates with time following excision to asses the effect on the CO_2 evolved and its isotopic signatures, as compared to that of nonstructural carbohydrates. The goal is to identify the period of time that may be allowed to elapse before there is a statistically significant change in the isotopic signatures in these measurements rather than to asses the effect on the respiration rates because as pointed out in the second chapter, these may not necessarily reflect those of live roots *in situ*. With regard to the collection of root tissue for isotopic analyses, live roots may be preferably placed in liquid nitrogen immediately after excision to further assure that the measured isotopic signatures of the different

carbohydrates are truly representative of the time of interest. On the other hand, the direct evaluation of the effect of excision on the isotopic signature of autotrophic respiration (implying thus a change in substrate) should be evaluated as well by measuring both, attached and excised roots. With regard to the sampling protocol of live roots for respiration measurements, this may be performed by species and/or including roots of the dominant species which are expected to dominate autotrophic fluxes. All these tests should be performed at the specific site under investigation as the same pattern should not be expected across different ecosystems (i.e. boreal versus temperate versus grasslands).

With regard to incubations of plant material performed in the laboratory, these require measurements of fluxes over the exact same temperature and moisture conditions as those encountered in the field at the time of sample collection. Additionally, the effect of disturbing the samples (i.e. removing roots, homogenizing) on the carbon isotopes of the CO_2 evolved during the incubation must be assessed beforehand.

With regard to air sampling with the purpose of relating isotopic signatures of air to those of autotrophic respiration, air samples may be collected at the canopy level or, even better, at different heights through the canopy allowing enough distance from the ground as to avoid incorporating soil-derived fluxes. This sampling may be performed at high frequency (e.g. daily) or during key times in the growing season (e.g. weeks before and after the start of leaf development and root growth) if the seasonal dynamics are to be evaluated. Higher sampling frequency (e.g. hours) may be chosen to evaluate day versus night time dynamics.

One important result of this thesis is the determination that spatial and temporal variability in soil respiration can be traced to heterotrophic respiration sources, specifically to decomposition of leaf litter. The effect of moisture content on leaf litter decomposition needs to be incorporated into models that attempt to reconstruct short-term soil respiration.

Another relevant result is the finding that stored nonstructural carbohydrates play a substantial role supporting autotrophic respiration in the onset of the growing season. Given that stored nonstructural carbohydrates are essential for tree survival under stress conditions (i.e. drought, nutrient depletion), the patterns of carbon allocation to roots under these conditions may differ from those studied here and should be investigated.

REFERENCES

Andrews, J. A., R. Matamala, K. M. Westover and W. H. Schlesinger (2000) Temperature effects on the diversity of soil heterotrophs and the delta C-13 of soil-respired CO_2, *Soil Biology & Biochemistry, 32*(5), 699-706.

Atkin, O. K., E. J. Edwards, and B. R. Loveys (2000) Response of root respiration to changes in temperature and its relevance to global warming, *New Phytologist, 147*(1), 141-154.

Bauer J, P. M. Williams and E. R. Druffel (1992) Recovery of sub-milligram quantities of carbon dioxide from gas streams by molecular sieve for subsequent determination of isotopic natural abundance. *Analytical Chemistry,* 64, 824-827.

Bhupinderpal-Singh, A. Nordgren, M. O. Lofvenius, M. N. Hogberg, P. E. Mellander and P. Hogberg (2003) Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year, *Plant Cell and Environment, 26*(8), 1287-1296.

Bhupinderpal-Singh, M. J. Hedley, and S. Saggar (2005) In situ dynamics of recently allocated C-14 in pasture soil and soil solution collected with Rhizon Soil Moisture Samplers, *Australian Journal of Soil Research, 43*(5), 659-666.

Birch, H. F. (1958) The effect of soil drying on humus decomposition and nitrogen availability, *Plant and Soil, X*(1), 9-9-31.

Bond-Lamberty, B., C. K. Wang and S. T. Gower (2004) Contribution of root respiration to soil surface CO2 flux in a boreal black spruce chronosequence, *Tree Physiology, 24*(12), 1387-1395.

Boone, R. D., K. J. Nadelhoffer, J. D. Canary and J. P. Kaye (1998) Roots exert a strong influence on the temperature sensitivity of soil respiration, *Nature, 396*(6711), 570-572.

Borken, W., E. A. Davidson, K. Savage, J. Gaudinski and S. E. Trumbore (2003) Drying and wetting effects on carbon dioxide release from organic horizons, *Soil Science Society of America Journal, 67*(6), 1888-1896.

Borken, W., K. Savage, E. A. Davidson and S. E. Trumbore (2005) Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil, *Global Change Biology* (in press)..

Borken, W., Y. J. Xu, E. A. Davidson and A. Beese (2002) Site and temporal variation of soil respiration in European beech, Norway spruce, and Scots pine forests, *Global Change Biology., 8*(12), 1205-1216.

Borken, W., Y. J. Xu, R. Brumme and N. Lamersdorf (1999) A climate change scenario for carbon dioxide and dissolved organic carbon fluxes from a temperate forest soil: Drought and rewetting effects, *Soil Science Society of America Journal, 63*(6), 1848-1855.

Bottner, P. (1985) Response of Microbial Biomass to Alternate Moist and Dry Conditions in a Soil Incubated with C-14-Labeled and N-15-Labelled Plant-Material, *Soil Biology & Biochemistry, 17*(3), 329-337.

Bowling, D. R., N. G. McDowell, B. J. Bond, B. E. Law, and J. R. Ehleringer (2002) C-13 content of ecosystem respiration is linked to precipitation and vapor pressure deficit, *Oecologia, 131*(1), 113-124.

Brugnoli, E., K. T. Hubick, S. Von Caemmerer, S. C. Wong, and G. D. Farquhar (1988) Correlation between the Carbon Isotope Discrimination in Leaf Starch and Sugars of C-3 Plants and the Ratio of Intercellular and Atmospheric Partial Pressures of Carbon-Dioxide, *Plant physiology, 88*(4), 1418-1424.

Burton, A. J., K. S. Pregitzer, G. P. Zogg and D. R. Zak (1998) Drought reduces root respiration in sugar maple forests, *Ecological Applications, 8*(3), 771-778.

Cao, M. K., S. D. Prince, B. Tao, J. Small, and K. R. Li (2005) Regional pattern and interannual variations in global terrestrial carbon uptake in response to changes in climate and atmospheric CO2, *Tellus Series B-Chemical and Physical Meteorology, 57*(3), 210-217.

Chambers, J. Q., E. S. Tribuzy, L. C. Toledo, B. F. Crispim, N. Higuchi, J. dos Santos, A. C. Araujo, B. Kruijt, A. D. Nobre, and S. E. Trumbore (2004) Respiration from a tropical forest ecosystem: Partitioning of sources and low carbon use efficiency, *Ecological Applications, 14*(4), S72-S88.

Chapin, F. S., E. D. Schulze, and H. A. Mooney (1990) The Ecology and Economics of Storage in Plants, *Annual Review of Ecology and Systematics, 21*, 423-447.

Cisneros-Dozal, L. M., S. E. Trumbore and P. J. Hanson (2005) Partitioning sources of soil-respired CO_2 and their seasonal variation using a unique radiocarbon tracer, *Global Change Biology* (in press).

Craine, J. M., D. A. Wedin and F. S. Chapin (1998) Predominance of ecophysiological controls on soil CO2 flux in a Minnesota grassland, *Plant and Soil, 207*(1), 77-86.

Curiel Yuste J, Janssens IA, Carrara A and Ceulemans R (2004) Annual Q_{10} of soil respiration reflects plant phonological patterns as well as temperature sensitivity. *Global Change Biology*, 10, 161-169.

Curtis P. S., P. J. Hanson, P. Bolstad, C. Barford, J. C. Randolph, H. P. Schmid, K. B. Wilson (2002) Biometric and eddy-covariance estimates of annual carbon storage in five eastern North American deciduous forests. *Agricultural and Forest Meteorology 113*, 3-15.

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*(2), 217-227.

Davidson, E. A., K. Savage, P. Bolstad, D. A. Clark, P. S. Curtis, D. S. Ellsworth, P. J. Hanson, B. E. Law, Y. Luo, K. S. Pregitzer, J. C. Randolph, D. Zak (2002a) Belowground carbon allocation in forests estimated from litterfall and IRGA-based soil respiration measurements, *Agricultural and Forest Meteorology, 113*(1-4), 39-51.

Davidson, E. A., I. Janssens, and Y. Luo (2005) On the variability of respiration in terrestrial ecosystems: moving beyond Q_{10}, *Global Change Biology, (in press)*.

Davidson, E. A., K. Savage, L. V. Verchot and R. Navarro (2002) Minimizing artifacts and biases in chamber-based measurements of soil respiration, *Agricultural and Forest Meteorology, 113*(1-4), 21-37.

Davidson, E. A., S. E. Trumbore, and R. Amundson (2000) Biogeochemistry – Soil warming and organic carbon content, *Nature, 408*(6814), 789-790.

Desrochers, A., S. M. Landhausser, and V. J. Lieffers (2002) Coarse and fine root respiration in aspen (Populus tremuloides), *Tree physiology, 22*(10), 725-732.

Dickson, R. E. (1991) Assimilate distribution and storage, in *Physiology of trees* edited by A. S. Raghavendra, pp. 51-51-86, John Wiley & Sons, Inc., New York, .

Dickson, R. E., J. G. Isebrands, and P. T. Tomlinson (1990) Distribution and Metabolism of Current Photosynthate by Single-Flush Northern Red Oak Seedlings, *Tree physiology, 7*(1-4), 65-77.

Dickson, R. E., P. T. Tomlinson, and J. G. Isebrands (2000) Partitioning of current photosynthate to different chemical fractions in leaves, stems, and roots of northern red oak seedlings during episodic growth, *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 30*(8), 1308-1317.

Dioumaeva, I., S. Trumbore, E. A. G. Schuur, M. L. Goulden, M. Litvak and A. I. Hirsch (2003) Decomposition of peat from upland boreal forest: Temperature dependence and sources of respired carbon, *Journal of Geophysical Research-Atmospheres, 108*(D3), 8222.

Edwards NT and Harris WF (1977) Carbon cycling in a mixed deciduous forest floor. *Ecology*, 58, 431-437.

Edwards, N. T. and J. S. Riggs (2003) Automated monitoring of soil respiration: A moving chamber design, *Soil Science Society of America Journal, 67*(4), 1266-1271.

Ekblad, A., and P. Hogberg (2001) Natural abundance of C-13 in CO_2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration, *Oecologia, 127*(3), 305-308.

Ekblad, A., B. Bostrom, A. Holm and D. Comstedt (2005) Forest soil respiration rate and delta C-13 is regulated by recent above ground weather conditions, *Oecologia, 143*(1), 136-142.

Emmett, B. A., C. Beier, M. Estiarte, A. Tietema, H. L. Kristensen, D. Williams, J. Penuelas, I. Schmidt and A. Sowerby (2004) The response of soil processes to climate change: Results from manipulation studies of shrublands across an environmental gradient, *Ecosystems, 7*(6), 625-637.

Epron D, V. Le Dantec, E. Dufrêne and A. Granier (2001) Seasonal dynamics of soil carbon dioxide efflux and simulated rhizosphere respiration in a beech forest. *Tree Physiology,* 21, 145-152.

Epron D, L. Farque , E. Lucot and P. M. Badot (1999) Soil CO_2 efflux in a beech forest: the contribution of root respiration. *Annals of Forest Science,* 56, 289-295.

Epron, D., J. Ngao and A. Granier (2004) Interannual variation of soil respiration in a beech forest ecosystem over a six-year study, *Annals of Forest Science, 61*(6), 499-505.

Ericsson, T., L. Rytter, and E. Vapaavuori (1996) Physiology of carbon allocation in trees, *Biomass & Bioenergy, 11*(2-3), 115-127.

Farrar, J. F., and D. L. Jones (2003) The control of carbon acquisition by and growth of roots, in *Root Ecology* edited by H. de Kroon and E. J. W. Visser, pp. 91-91-124, Springer-Verlag, Berlin, .

Fierer, N. and J. P. Schimel (2003) A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil, *Soil Science Society of America Journal, 67*(3), 798-805.

Gašparíková, O. (1992) Root metabolism, in *Physiology of the plant root system* edited by J. Kolek and V. Kozinka, pp. 82-82-127, Kluwer Academic Publishers, Dordrecht, .

Gaudinski J.B. and S. E. Trumbore (2003) Soil carbon turnover. In *North American Temperate Deciduous Forest Responses to Changing Precipitation Regimes*, Vol. 166 (Eds, Hanson, P. J. and Wullschleger, S. D.) Springer, New York, pp. 190-209.

Gaudinski, J. B., S. E. Trumbore, E. A. Davidson and S. H. Zheng (2000) Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes, *Biogeochemistry, 51*(1), 33-69.

Gaudinski, J. B., T. E. Dawson, S. Quideau, E. A. Schuur, J. S. Roden, S. E. Trumbore, D. R. Sandquist, S. Oh, and R. E. Wasylishen (in press) A comparative analysis of cellulose preparation techniques for use with ^{13}C, ^{14}C and ^{18}O isotopic measurements.

Giardina, C. P., and M. G. Ryan (2000) Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature, *Nature, 404*(6780), 858-861.

Giardina, C. P., M. G. Ryan, D. Binkley, and J. H. Fownes (2003) Primary production and carbon allocation in relation to nutrient supply in a tropical experimental forest, *Global Change Biology, 9*(10), 1438-1450.

Goulden, M. L., S. D. Miller, H. R. da Rocha, M. C. Menton, H. C. de Freitas, A. M. E. S. Figueira and C. A. D. de Sousa (2004) Diel and seasonal patterns of tropical forest CO2 exchange, *Ecological Applications, 14*(4), S42-S54.

Goulden, M. L., J. W. Munger, S. M. Fan, B. C. Daube and S. C. Wofsy (1996) Exchange of carbon dioxide by a deciduous forest: Response to interannual climate variability, *Science, 271*(5255), 1576-1578.

Granier, A., E. Ceschia, C. Damesin, E. Dufrêne, D. Epron, P. Gross, S. Lebaube, V. Le Dantec, N. Le Goff, D. Lemoine, E. Lucot, J. M. Ottorini, J. Y. Pontailler and B. Saugier (2000) The carbon balance of a young Beech forest, *Functional Ecology, 14*(3), 312-325.

Green, J. W. (1963) *Methods in carbohydrate chemistry* edited by R. L. Whistler, pp. 9-9-21, Academic Press, New York.

Grulke, N. E., C. P. Andersen, and W. E. Hogsett (2001) Seasonal changes in above- and belowground carbohydrate concentrations of ponderosa pine along a pollution gradient, *Tree physiology, 21*(2-3), 173-181.

Hanson P. J., E. G. O'Neill, L. S. Chambers, J. S. Riggs, J. D. Joslin and M. H. Wolfe (2003b) Soil Respiration and Litter Decomposition, in *North American Temperate Deciduous Forest Responses to Changing Precipitation Regimes,* edited by P. J. Hanson and S. D. Wullschleger, pp. 163-189, Springer-Verlag, New York.

Hanson P. J., M. A. Huston and D. E. Todd (2003) Walker Branch throughfall displacement experiment, in *North American temperate deciduous forest responses to changing precipitation regimes,* edited by P. J. Hanson and S. D. Wullschleger, pp. 8-8-31, Springer-Verlag, New York.

Hanson P. J., N. T. Edwards, T. J. Tschaplinski, S. D. Wullschleger and J. D. Joslin (2003a) Estimating the Net Primary and Net Ecosystem Production of a Southeastern Upland *Quercus* Forest from an 8-Year Biometric Record, in *North American Temperate Deciduous Forest Responses to Changing Precipitation Regimes,* edited by P. J. Hanson and S. D. Wullschleger, pp. 378-378-395, Springer-Verlag, New York.

Hanson PJ, Edwards NT, Garten CT and Andrews JA (2000) Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry, 48*, 115-146.

Hanson, P. J., C. W. Swanston, C. T. Garten Jr, D. E. Todd and S. E. Trumbore (2005) Reconciling change in Oi-horizon ^{14}C with mass loss for an Oak forest, *Soil Science Society of America Journal* 69:1492-1502.

Heath, J., E. Ayres, M. Possell, R. D. Bardgett, H. I. J. Black, H. Grant, P. Ineson, and G. Kerstiens (2005) Rising atmospheric CO_2 reduces sequestration of root-derived soil carbon, *Science, 309*(5741), 1711-1713.

Hoch, G., A. Richter, and C. Körner (2003) Non-structural carbon compounds in temperate forest trees, *Plant Cell and Environment, 26*(7), 1067-1081.

Hogberg, P., A. Nordgren, N. Buchmann, A. F. S. Taylor, A. Ekblad, M. N. Hogberg, G. Nyberg, M. Ottosson-Lofvenius and D. J. Read (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration, *Nature, 411*(6839), 789-792.

Houghton, R. A. (2002) Magnitude, distribution and causes of terrestrial carbon sinks and some implications for policy, *Climate Policy, 2*(1), 71-88.

Houghton, R. A. (2003) Revised estimates of the annual net flux of carbon to the atmosphere from changes in land use and land management 1850-2000, *Tellus Series B-Chemical and Physical Meteorology, 55*(2), 378-390.

Huston M. A., D. E. Todd and D. G. Barlar (2003) Long-term forest dynamics and tree growth at the TDE site on Walker Branch Watershed, in *North American temperate deciduous forest responses to changing precipitation regimes,* edited by P. J. Hanson S. D. Wullschleger, pp. 417-417-432, Springer-Verlag, New York.

Irvine, J. and B. E. Law (2002) Contrasting soil respiration in young and old-growth ponderosa pine forests, *Global Change Biology, 8*(12), 1183-1194.

Janssens, I. A., H. Lankreijer and G. Matteucci (2001) Productivity overshadows temperature in determining soil and ecosystem respiration across European forests, *Global Change Biology* (7), 269-278.

Jiang, L. F., F. C. Shi, B. Li, Y. Q. Luo, J. Q. Chen and J. K. Chen (2005) Separating rhizosphere respiration from total soil respiration in two larch plantations in northeastern China, *Tree Physiology, 25*(9), 1187-1195.

Johnson D. W. and R. I. Van Hook (1989) Analysis of biogeochemical cycling processes in Walker Branch Watershed, edited by D. W. Johnson and R. I. Van Hook, pp. 14, Springer-Verlag, New York.

Joslin J. D. and M. H. Wolfe (2003) Fine root growth response. In North American Temperate Deciduous Forest Responses to Changing Precipitation Regimes edited by P. J. Hanson and S. D. Wullschleger, pp. 274-302, Springer-Verlag, New York,

Joslin, J. D., M. H. Wolfe, and P. J. Hanson (2001) Factors controlling the timing of root elongation intensity in a mature upland oak stand, *Plant and Soil, 228*(2), 201-212.

Kandiah, S. (1979) Turnover of Carbohydrates in Relation to Growth in Apple-Trees .2. Distribution of C-14 Assimilates Labeled in Autumn, Spring and Summer, *Annals of Botany, 44*(2), 185-&.

Keith, H., K. L. Jacobsen and R. J. Raison (1997) Effects of soil phosphorus availability, temperature and moisture on soil respiration in Eucalyptus pauciflora forest, *Plant and Soil, 190*(1), 127-141.

Kelliher, F. M., D. J. Ross, B. E. Law, D. Baldocchi and N. J. Rodda (2004) Limitations to carbon mineralization in litter and mineral soil of young and old ponderosa pine forests, *Forest Ecology and Management, 191*(1-3), 201-213.

Kelting D. L., J. A. Burger and G. S. Edwards (1998) Estimating root respiration, microbial respiration in the rhizosphere, and root-free soil respiration in forest soils. *Soil Biology and Biochemistry,* 30, 961-968.

Kieft, T. L., E. Soroker and M. K. Firestone (1987) Microbial Biomass Response to a Rapid Increase in Water Potential when Dry Soil is Wetted, *Soil Biology & Biochemistry, 19*(2), 119-126.

King, J. S., P. J. Hanson, E. Bernhardt, P. DeAngelis, R. J. Norby, and K. S. Pregitzer (2004) A multiyear synthesis of soil respiration responses to elevated atmospheric CO2 from four forest FACE experiments, *Global Change Biology, 10*(6), 1027-1042.

Kirschbaum, M. U. F. (1995) The Temperature-Dependence of Soil Organic-Matter Decomposition, and the Effect of Global Warming on Soil Organic-C Storage, *Soil Biology & Biochemistry, 27*(6), 753-760.

Körner, C. (2003) Carbon limitation in trees, *Journal of Ecology, 91*(1), 4-17.

Lambers, H. (1987) Growth, respiration, exudation and symbiotic associations: the fate of carbon translocated to the roots, in *Root development and function* edited by P. J. Gregory, J. V. Lake and D. A. Rose, pp. 125-125-145, Cambridge University Press, Cambridge.

Langley, J. A., B. G. Drake, and B. A. Hungate (2002) Extensive belowground carbon storage supports roots and mycorrhizae in regenerating scrub oaks, *Oecologia, 131*(4), 542-548.

Lavigne, M. B., R. Boutin, R. J. Foster, G. Goodine, P. Y. Bernier and G. Robitaille (2003) Soil respiration responses to temperature are controlled more by roots than by decomposition in balsam fir ecosystems, *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 33*(9), 1744-1753.

Leavitt, S. W., and S. R. Danzer (1993) Method for Batch Processing Small Wood Samples to Holocellulose for Stable-Carbon Isotope Analysis, *Analytical Chemistry, 65*(1), 87-89.

Lee, X., H. J. Wu, J. Sigler, C. Oishi and T. Siccama (2004) Rapid and transient response of soil respiration to rain, *Global Change Biology, 10*(6), 1017-1026.

Lee, M. S., K. Nakane, T. Nakatsubo and H. Koizumi (2003) Seasonal changes in the contribution of root respiration to total soil respiration in a cool-temperate deciduous forest, *Plant and Soil, 255*(1), 311-318.

Levin, I., and B. Kromer (2004) The tropospheric (CO_2)-C^{14} level in mid-latitudes of the Northern Hemisphere (1959-2003), *Radiocarbon, 46*(3), 1261-1272.

Lipp, C. C., and C. P. Andersen (2003) Role of carbohydrate supply in white and brown root respiration of ponderosa pine, *New Phytologist, 160*(3), 523-531.

Lomander, A., T. Katterer and O. Andren (1998) Carbon dioxide evolution from top- and subsoil as affected by moisture and constant and fluctuating temperature, *Soil Biology & Biochemistry, 30*(14), 2017-2022.

Matamala, R., M. A. Gonzalez-Meler, J. D. Jastrow, R. J. Norby, and W. H. Schlesinger (2003) Impacts of fine root turnover on forest NPP and soil C sequestration potential, *Science, 302*(5649), 1385-1387.

McDowell, N. G., D. R. Bowling, A. Schauer, J. Irvine, B. J. Bond, B. E. Law and J. R. Ehleringer (2004) Associations between carbon isotope ratios of ecosystem respiration, water availability and canopy conductance, *Global Change Biology, 10*(10), 1767-1784.

Meier, H., and J. S. G. Reid (1982) Reserve polysaccharides other than starch in higher plants, in *Encyclopedia of plant physiology, Plant carbohydrates I. Intracellular carbohydrates* edited by F. A. Loewus and W. Tanner, pp. 418-418-471, Springer-Verlag, Berlin.

Miller, S. D., M. L. Goulden, M. C. Menton, H. R. da Rocha, H. C. de Freitas, A. M. E. S. Figueira, and C. A. D. de Sousa (2004) Biometric and micrometeorological measurements of tropical forest carbon balance, *Ecological Applications, 14*(4), S114-S126.

Nishizawa, T., Y. Shishido, and H. Kumakura (1998) Mobilization of C-^{14}-carbohydrate reserves in relation to vegetative growth and inflorescence development in June bearing strawberry plants, *Journal of Horticultural Science & Biotechnology, 73*(4), 499-505.

Norby, R. J. (1994) Issues and Perspectives for Investigating Root Responses to Elevated Atmospheric Carbon-Dioxide, *Plant and Soil, 165*(1), 9-20.

Orchard, V. A. and F. J. Cook (1983) Relationship between Soil Respiration and Soil-Moisture, *Soil Biology & Biochemistry, 15*(4), 447-453.

Pendall, E., S. Bridgham, P. J. Hanson, B. Hungate, D. W. Kicklighter, D. W. Johnson, B. E. Law, Y. Q. Luo, J. P. Megonigal, M. Olsrud, M. G. Ryan, S.Q. Wan (2004) Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. *New Phytologist 162*(2), 311-322.

Phillips DL and Gregg JW (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia,* 127, 171-179.

Plattner, G. K., F. Joos, and T. F. Stocker (2002) Revision of the global carbon budget due to changing air-sea oxygen fluxes, *Global Biogeochemical Cycles, 16*(4), 1096.

Pregitzer, K. S., J. A. King, A. J. Burton, and S. E. Brown (2000) Responses of tree fine roots to temperature, *New Phytologist, 147*(1), 105-115.

Pregitzer, K. S., M. J. Laskowski, A. J. Burton, V. C. Lessard, and D. R. Zak (1998) Variation in sugar maple root respiration with root diameter and soil depth, *Tree physiology, 18*(10), 665-670.

Prentice, I. C., G. D. Farquhar, M. J. R. Fasham, M. L. Goulden, M. Heimann, V. J. Jaramillo, H. S. Kheshgi, C. Le Quéré, R. J. Scholes, and D. W. R. Wallace (2001) The Carbon Cycle and Atmospheric Carbon Dioxide, in *Climate Change 2001: The Scientific Basis,* edited by J. T. Houghton, L. Pitelka and A. Ramirez Rojas, pp. 185-225, Cambridge University Press, Cambridge, UK.

Raich, J. W., C. S. Potter, and D. Bhagawati (2002) Interannual variability in global soil respiration, 1980-94, *Global Change Biology, 8*(8), 800-812.

Rakonczay Z, John R S and Kelting DL (1997) Carbon efflux rates of fine roots of three tree species decline shortly after excision. *Environmental and Experimental Botany,* 38, 243-249.

Reichstein, M., A. Rey, A. Freibauer, J. Tenhunen, R. Valentini, J. Banza, P. Casals, Y. F. Cheng, J. M. Grunzweig, J. Irvine, R. Joffre, B. E. Law, D. Loustau, F. Miglietta, W. Oechel, J. M. Ourcival, J. S. Pereira, A. Peressotti, F. Ponti, Y. Qi, S. Rambal, M. Rayment, J. Romanya, F. Rossi, V. Tedeschi, G. Tirone, M. Xu and D. Yakir (2003) Modeling temporal and large-scale spatial variability of soil respiration from soil water availability, temperature and vegetation productivity indices, *Global Biogeochemical Cycles, 17*(4), 1104.

Rey, A., E. Pegoraro, V. Tedeschi, I. De Parri, P. G. Jarvis and R. Valentini (2002) Annual variation in soil respiration and its components in a coppice oak forest in Central Italy, *Global Change Biology, 8*(9), 851-866.

Ryan, M. G., and B. E. Law (2005) Interpreting, measuring, and modeling soil respiration, *Biogeochemistry, 73*(1), 3-27.

Savage, K. E. and E. A. Davidson (2001) Interannual variation of soil respiration in two New England forests, *Global Biogeochemical Cycles, 15*(2), 337-350.

Savage, K. E. and E. A. Davidson (2003) A comparison of manual and automated systems for soil CO2 flux measurements: trade-offs between spatial and temporal resolution, *Journal of Experimental Botany, 54*(384), 891-899.

Scott-Denton, L. E., T. N. Rosenstiel and R. K. Monson (in press) Differential controls by climate and substrate over the heterotrophic and rhizosphere components of soil respiration, *Global Change Biology*.

Soe, A. R. B., A. Giesemann, T. H. Anderson, H. J. Weigel, and N. Buchmann (2004) Soil respiration under elevated CO2 and its partitioning into recently assimilated and older carbon sources, *Plant and Soil, 262*(1-2), 85-94.

Stuiver M and Polach HA (1977) Reporting of 14C data. *Radiocarbon,* 19, 355-363.

Subke, J. A., V. Hahn, G. Battipaglia, S. Linder, N. Buchmann and M. F. Cotrufo (2004) Feedback interactions between needle litter decomposition and rhizosphere activity, *Oecologia, 139*(4), 551-559.

Trumbore, S. E., J. B. Gaudinski, P. J. Hanson and J. R. Southon (2002) Quantifying ecosystem-atmosphere carbon exchange with a ^{14}C label, *EOS, Transactions, American Geophysical Union, 83*(265), 267-267-268.

Trumbore, S. E. (2005) Carbon respired by terrestrial ecosystems - recent progress and challenges, *Global Change Biology,* (in press).

Tschaplinski, T. J., and P. J. Hanson (2003) Dormant-season nonstructural carbohydrate storage, in *North American temperate deciduous forest responses to changing precipitation regimes* edited by P. J. Hanson and S. D. Wullschleger, pp. 67-67-84, Springer-Verlag, New York, .

Valentini R, G. Matteucci, A. J. Dolman, E. D. Schulze, C. Rebmann, E. J. Moors, A. Granier, P. Gross, N. O. Jensen, K. Pilegaard, A. Lindroth, A. Grelle, C. Bernhofer, T. Grunwald, M. Aubinet, R. Ceulemans, A. S. Kowalski, T. Vesala, U. Rannik, P. Berbigier, D. Loustau, J. Guomundsson, H. Thorgeirsson, A. Ibrom, K. Morgenstern, R. Clement, J. Moncrieff, L. Montagnani, S. Minerbi, P. G. Jarvis (2000) Respiration as the main determinant of carbon balance in European forests. *Nature, 404*(6780), 861-865

Van Cleve, K. and D. Sprague (1971) Respiration rates in the forest floor of birch and aspen stands in interior Alaska, *Arctic and Alpine Research, 3*(1), 17-17-26.

Vogel, J. S. (1992) Rapid Production of Graphite without Contamination for Biomedical Ams, *Radiocarbon, 34*(3), 344-350.

Waldrop, M. P. and M. K. Firestone (2004) Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions, *Biogeochemistry, 67*(2), 235-248.

Wargo, P. M. (1979) Starch Storage and Radial Growth in Woody Roots of Sugar Maple, *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 9*(1), 49-56.

Weinstein, D. A., R. M. Beloin, and R. D. Yanai (1991) Modeling Changes in Red Spruce Carbon Balance and Allocation in Response to Interacting Ozone and Nutrient Stresses, *Tree physiology, 9*(1-2), 127-146.

Whipps, J. M., and J. M. Lynch (1986) The Influence of the Rhizosphere on Crop Productivity, *Advances in Microbial Ecology, 9*, 187-244.

Wilson, K. B., andD. D. Baldocchi (2001) Comparing independent estimates of carbon dioxide exchange over 5 years at a deciduous forest in the southeastern United States, *Journal of Geophysical Research-Atmospheres, 106*(D24), 34167-34178.

Winston G. (in preparation) Automated chamber estimates of forest floor CO_2 efflux from two stands within a Canadian boreal forest fire chronosequence.

Yuste, J. C., I. A. Janssens, A. Carrara, L. Meiresonne, and R. Ceulemans (2003) Interactive effects of temperature and precipitation on soil respiration in a temperate maritime pine forest, *Tree physiology, 23*(18), 1263-1270.

Zogg, G. P., D. R. Zak, A. J. Burton, and K. S. Pregitzer (1996) Fine root respiration in northern hardwood forests in relation to temperature and nitrogen availability, *Tree physiology, 16*(8), 719-725.

APPENDIX 1

TIMING IN THE PROGRAM OF THE AUTOMATED CHAMBER SYSTEM

Time into cycle (minutes)	Action
0	Open stream to chamber[1] Open trap manifold common[1]
1	Trap ambient sample
2	End ambient trap Sample ambient concentration
3	Close chamber
10	Trap chamber CO_2
12	End chamber CO_2 trap Raise chamber Open stream to next chamber[2]

[1] Flush of the plumbing system
[2] Start of a new cycle

APPENDIX 2

PROCEDURE FOR THE EXTRACTION OF SOLUBLE SUGARS, STARCH AND CELLULOSE FROM PLANT MATERIAL

A. Sample preparation

1. Dry and grind samples to at least 40 mesh size.
2. Weigh 300 to 1000 mg (depending on how much sample is available) in a 100ml beaker.

B. Extraction of soluble compounds

1. Add 35ml of deionized water, cover with a watch glass and bring to a boil for 30min.
2. After cooling, centrifuge for 10 min. The supernatant contains a mixture of soluble sugars and other compounds (see below). Save the sample residue for starch extraction.
3. If no isolation of soluble sugars is desired, dry the supernatant thoroughly in the vacuum concentrator at low temperature. Homogenize the dry sample and load a sub sample into a quartz tube for combustion and graphitization.
4. Follow instructions below for isolation of soluble sugars.

C. Isolation of soluble sugars

After boiling in water, a lot of materials other than soluble sugars are extracted too, such as pigments, alkaloids and phenols. Extra steps should be taken to isolate soluble sugars; the following procedure was adapted from Brugnoli et al., [1988] and involves the use of ion-exchange resins, cation (AG 50W-X8 – hydrogen form) and anion (AG 1-X8 –chloride form):

1. Pretreatment of cation and anion exchange resins: soak the resins in 6NHCl, wash (use the ion exchange column) several times until the supernatant is colorless. Wash with deionized water until PH~4. Keep the resins in water when not in use.
2. Put 10ml of wet **cation** exchange resin in the ion-exchange tube and pour the sugar solution in the tube, use the cap of the tube to control the rate of elution (~ 1 ml/min).
3. Put 10ml of wet **anion** exchange resin in another ion exchange tube. Pour the solution (after cation-exchange) into the tube. Collect the eluant (should be colorless).
4. If the eluant has color, it may be necessary to pass through C18 (Sep-Pak C18 Cartridges for solid phase extraction), a reagent used to get ride of small amounts of chlorophyll, most of the time, it is not necessary. Pour the eluant into the syringe tube, Connect the syringe tube to the C18 tube and collect the solution passing through C18.
5. Dry the solution in the vacuum concentrator at low temperature. Homogenize and load a sub sample into a quartz tube for combustion and graphitization.

D. Starch extraction

The following procedure was adapted from Brugnoli et al., [1988].

1. Add ~40ml of ethanol (HPLC grade) to the sample residue, cover with the watch glass and boil for 30 min. Sudden bursts (that can turn over the beaker spilling the sample) can normally be avoided at temperatures no higher than ~130 ºC.
2. After cooling discard the supernatant (centrifuge if necessary; use a pipette to avoid disturbance and/or lost of plant material).
3. Repeat steps 1 and 2 until the supernatant is colorless.
4. Add 10ml of 20% HCl to the residue and place the beaker on a warm plate (~50ºC) for 30 min. Let stand overnight.
5. Centrifuge the solution (~10 min) and transfer the supernatant to a 50 ml centrifuge tube (carefully to avoid bringing plant material along as it will precipitate along with the starch); save this solution. Add 10 ml of 20%HCl to the sample residue again, and place in the beaker on a warm plate for 30 min.
6. After cooling centrifuge and combine the supernatant with the previous one. Add 35 ml of ethanol (HPLC grade) to the combined supernatants and let this solution stand overnight. Save the sample residue for further extraction of cellulose if needed.
7. The loose white precipitation represents starch. Centrifuge for ~10 min and discard the supernatant with a pipette.
8. Wash the starch with a bit of ethanol (~1 ml) HPLC grade by gently pouring ethanol and taking it out with a pipette. Wash a couple of times to eliminate the

remaining HCL. Note: the extracted starch is water soluble, thus do not try to wash the precipitate with water.

9. Dry the starch thoroughly in the vacuum concentrator at low temperature; the drying does not take more than a few hours (check every 1 or 2 hours), starch becomes loose when completely dry. Note: a complete drying is very important as to prevent contamination with carbon from the ethanol.
10. Load a sub sample (approximate C yield from starch samples extracted from root, stems and leaves is 35%) into a quartz tube for combustion and graphitization.

E. Cellulose extraction

1. After starch extraction, let the sample air dry in the hood. Load enough sample to get 1-2 mg C (carbon content is normally 40%) into a polyester filter bag (Ankom Technology, Macedon, New York), seal the bag (use a specific pattern for identification).
2. Soak bags in deionized water a couple of times and proceed to bleach the sample. The following bleaching treatment was adapted from Green, [1963] and Leavitt and Danzer, [1993]
3. Prepare a solution in 1L beaker with 600 ml of deionized water, 4 g of sodium chlorite and 2 ml glacial acetic acid.
4. Place bags in the solution and place the beaker in a hot plate for 3-4 hrs, adjust the temperature of the solution to 70ºC.

5. Add 4 g of sodium chloride and 2 ml glacial acetic acid every 3-4 hrs until the samples turn white (3 times is normally enough).
6. Soak bags in deionized water 2-3 times
7. Let the bags air dry thoroughly.
8. Load a sub sample (carbon content in cellulose is ~40%) into a quartz tube and combust and graphitize.