AN ABSTRACT OF THE DISSERTATION OF

Susan E. Crow for the degree of Doctor of Philosophy in Botany and Plant Pathology presented on March 16, 2006.

Title: Characteristics of Soil Organic Matter in Two Forest Soils

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Abstract approved _____

Kate Lajtha

Soil organic matter (SOM) is the terrestrial biosphere's largest pool of organic carbon (C) and is an integral part of C cycling globally. Soil organic matter composition typically can be traced directly back to the type of detrital inputs; however, the stabilization of SOM results as a combination of chemical recalcitrance, protection from microbial decomposition within soil structure, and organo-mineral interactions. A long-term manipulative field experiment, the Detrital Input and Removal Treatment (DIRT) Project, was established to examine effects of altering detrital inputs (above- vs. below-ground source, C and nitrogen (N) quantity, and chemical quality) on the stabilization and retention of SOM. Surface mineral soil was collected from two DIRT sites, Bousson (a deciduous site in western Pennsylvania) and H. J. Andrews (a coniferous site in the Oregon Cascade Mountains), to examine the influence of altering detrital inputs on decomposability and mean residence time of soil organic matter and different organic matter fractions.

Soil organic matter was physically separated into light fraction (LF) and heavy fraction (HF) organic matter, by density fractionation in 1.6 g mL^{-1} sodium polytungstate (SPT). Density fractionation in SPT resulted in the mobilization and loss of ~ 25 % of total soil organic C and N during the physical separation and rinsing of fractions during recovery, which was also the most easily decomposed organic matter present in the bulk soil. At H. J. Andrews, this mobilized organic matter had a short mean residence time (MRT),

indicating that it originated from fresh detrital inputs. In contrast, at Bousson, the organic matter mobilized had a long MRT, indicating that it originated from organic matter that had already been stabilized in the soil. Mean residence times of LF from Bousson varied widely, ~ 3 y from doubled litter and control plots and 78-185 y for litter removal plots, while MRT of HF was ~ 250 y and has not yet been affected by litter manipulations. Results from long term incubation of LF and HF material supported these estimates; respiration was greatest from LF of doubled litter and control plots and least from HF of litter removal plots. In contrast, MRT estimated for LF and HF organic matter from H. J. Andrews were similar to each other (~ 100 y) and were not affected by litter manipulation. These estimates were also supported by the incubation results; there was not a difference in cumulative respiration between detrital treatments or density fractions. The results from the coniferous site may be due to a legacy of historically large inputs of coarse woody debris on the LF and it may be decades before the signal of detrital manipulations can be measured. Alternatively, these highly andic soils may be accumulating C rapidly, yielding young HF ages and C that does not differ substantially in lability from coniferous litter-derived LF. The DIRT Project was intended to follow changes in soil organic matter over decades to centuries. As expected, manipulation of detrital inputs has influenced the lability and mean residence time of the light fraction before the heavy fraction organic matter; however, it will be on much more lengthy time scales that clear differences in organic matter stabilization in response to the alteration of detrital inputs will emerge.

Soil CO_2 efflux is a compilation of CO_2 from many sources, including root respiration and the decomposition of different organic matter fractions, roots, and exudates. If the sources of CO_2 have different isotopic signatures, the isotope analysis of CO_2 efflux may reveal the dominant sources within the soil profile. In a short incubation experiment of density fractions from both sites, respired CO_2 reflected the isotopic signature of the organic matter fraction after 30 days, but was more enriched in ^{13}C. Initially CO_2 was isotopically depleted in ^{13}C relative to the organic matter fraction and the period of depletion related to the amount of easily degraded organic matter present at H. J. Andrews only.

Characteristics of Soil Organic Matter in Two Forest Soils

by

Susan E. Crow

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented March 16, 2006

Commencement June 2006

Doctor of Philosophy dissertation of Susan E. Crow presented on March 16, 2006.

APPROVED:

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Susan E. Crow, Author

ACKNOWLEDGEMENTS

I thank my advisor, Kate Lajtha for her support and for giving me the independence to let this dissertation take its own path. Along with Kate, Bruce Caldwell and Phil Sollins have nurtured the DIRT project at H.J. Andrews and let me join the group, thanks for all the science, interactions, and fun. Likewise, Rich Bowden has nurtured the DIRT site at Bousson and was giving of his soil and time. I also thank my committee members Elizabeth Sulzman, Chris Swanston, Mark Johnson, Pat Muir, and David Hann for all their discussions, insights, and perspectives that made this a well-rounded, successful dissertation.

I've had the advantage of much help in the lab. Heather Carpenter, Dave Dinette, Katie Delaney, Dan Irvine, Annie Lawrence helped with lab work and data collection as undergraduates and Nella Parks helped design and execute an important incubation experiment. Cameron Bergen faithfully and lovingly maintained the HJA DIRT plots and Kim Townsend, Sarah Beldin, Heath Keirtstead, and Justin Brandt helped with various aspects of sample prep and lab work over the years. Julie Spears, Scott Holub, and Yuriko Yano passed the torch to me early in my time as a graduate student at OSU and helped me on my way. Tim Filley (Purdue University), Reneé Brooks (EPA, Corvallis), and Al Soeldner (SEM facility, OSU) provided me with access to unique facilities that added new dimensions to my work. Speaking of unique, the chance to work at the Lawrence Livermore National Laboratory Center for Accelerator Mass Spectrometry was incredible, I am very thankful for the opportunity made available through collaboration with Chris Swanston. The H.J. Andrews LTER group gave me support and access to facilities. Funding for the DIRT Project was provided by USDA and NSF grants. A National Academy of Science grant provided funding for travel to the Hungary DIRT sites. János Tóth (the Happy Hunter) and Mária Papp were gracious hosts in Hungary. Thanks to Zsolt and Zsolt for their hospitality and humor (take care of that Big Problem).

It would have been impossible to complete graduate school without my friends. John Bienapfl, Angel Saavedra, and Nate Miller: thanks for working late and living to tell about it (and to El Sol for providing the refreshments). Jenn Kucera and Sarah Beldin kept me sane. Bruce Caldwell, Kim Townsend, Willem Van Verseveld, Marloes Bakker, thanks for being cool and helping me see this through. Graduate school would have been even more impossible without my family. To my Grandparents and Uncle Clint; thanks for the respite and for dealing with the shameless consumption of food, drink, and laundry detergent. My extended family in Virginia kindly dubbed me Dr. Dirt (which suits me just fine) and has helped me keep things in perspective. Thanks to my sisters who, even though I am the oldest, have shown me how it's done a time or two. My parents gave me the genetic predisposition for science and the confidence to go all the way. Nature and nurture, how could I have failed? Dave Beilman always seemed to know from the tone of my hello when to put aside his own problems and listen to mine, we only made it because we did it together.

Egészségedre!

CONTRIBUTION OF AUTHORS

Dr. Rich Bowden established and maintains the Bousson DIRT site in Pennsylvania. He provided soil samples, elemental analysis and editorial help for Chapters 2 and 3. Dr. William Rugh provided stable isotope analysis, analytic assistance, and was involved in designing the study for Chapter 4. Dr. Renée Brooks provided stable isotope analysis and mathematical assistance necessary for the interpretation of data for Chapter 2. Heath Keirstead assisted in data collection and provided interpretation for Chapter 2.

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CHAPTER 1

INTRODUCTION

SOIL ORGANIC MATTER (SOM)

Non-living organic matter in soil is the terrestrial biosphere's largest pool of organic carbon (C) and is an integral part of the global C cycle (Schimel 1995). The amount of organic matter that enters the soil, as well as the chemical quality of those inputs, varies in response to environmental factors such as changes in climate and land-use (Jobbágy and Jackson 2003). Soil organic matter is important for enhancing soil fertility and functions by controlling soil structure, buffering capacity, metal binding capacity and water holding capacity (Wershaw 2004) and has been studied extensively. However, the relative importance and linkages of various biological, chemical, and physical factors regulating the balance of organic matter in soil are not yet understood despite its pivotal role in the global C cycle (Neff et al. 2002).

Stabilization of organic C in soil is dependant on a variety of factors including litter quality (Melillo et al. 1982), clay mineralogy (Balesdent et al. 1988), soil pH and redox conditions (Bunnel et al. 1977) as well as the formation and disruption of soil aggregate structure (Cambardella and Elliot 1994) and climate. Soil organic matter dynamics are governed by losses through microbial activity, leaching, and erosion and formation from fresh detrital inputs (both above- and below-ground) and microbial products (Sollins et al. 1996). Mechanisms controlling stabilization and destabilization of organic matter in soil can be divided into three categories along an increasing spatial scale: the chemical

recalcitrance of the present organic molecules, the **interaction** of these molecules and the mineral soil particles, and the **accessibility** of these molecules to microbial and chemical decomposition within the soil matrix (Sollins et al. 1996). During decomposition, the extent to which organic matter inputs are stabilized against degradation through these mechanisms regulates the rate of C loss from the soil and therefore the balance of the terrestrial C pool.

Carbon flux between the soil and the atmosphere is large and changes in the rate of SOM formation or decomposition, whether due to climate change, land management, fire, or other disturbance factors, may lead to either greater C sequestration in soil or greater C efflux to the atmosphere. A better understanding of the controls on stabilization and retention of soil C and nitrogen (N) is needed to improve predictions for changes in forest soil processes due to alternate management regimes, in global C budgets due to shifts in climate conditions, and in forest responses to atmospheric N inputs. In particular, a shift in detrital inputs may alter the net accumulation or loss of C in the soil organic matter pool (Boone et al. 1998) as well as the abundance and lability of specific organic constituents (Kögel-Knabner 2002). Many studies have considered aspects of litter decay, biota, organic composition, and turnover rate in soils however, few have conclusively determined links among detrital input quantity and quality and soil organic matter formation and stability.

APPROACH

Linking detrital inputs with organic matter stabilization in soil

Inspired by an ongoing experiment started in 1957 in forest and grassland ecosystems at the University of Wisconsin Arboretum (Neilson and Hole 1963), a collection of long-term manipulative field experiments were established to examine effects of differences in plant litter (above- vs. below-ground source, C and N quantity, and chemical quality) on the stabilization and retention of soil organic C and N. The central goal of this ongoing

experiment, called the Detrital Input and Removal Treatment (DIRT) Project, is to assess how rates and sources of plant inputs control the accumulation and dynamics of SOM and nutrients in forest soils over decadal timescales (Nadelhoffer et al. 2004). DIRT plots were established in an oak (*Quercus rubra*) forest at the Harvard Forest, MA, USA in 1990; a black cherry (*Prunus serotina)* and sugar maple (*Acer saccharum*) dominated forest at the Bousson Experimental Forest, PA, USA in 1991; a Douglas-fir/western hemlock (*Pseudotsuga menziesii/ Tsuga heterophylla)* forest at the H. J. Andrews Experimental Forest, OR, USA in 1997; a sessile/turkey oak (*Q. petraea and Q. cerris*) forest at the Síkfőkút Experimental Forest, Hungary in 2000; and bigtooth aspen and paper birch dominated (*Populus grandidentata and Betula papyrifera*) mixed deciduous forest at the University of Michigan Biological Station, MI, USA in 2005. All the sites differ in climate (temperature, precipitation), vegetation (type, succession stage), pedogenic state, and N deposition. Plots are replicated three times at each site and consist of treatments that either add or remove above-and below-ground litter inputs to the soil (Table 1-1).

Table 1-1. Treatments and methods of the Detritus Input and Removal Treatment (DIRT) experimental plots, each are replicated 3 times.

Treatment		Method
Control	(CTL)	Normal litter inputs are allowed.
No Litter	(NL)	Aboveground inputs are excluded from plots.
Double Litter	(DL)	Aboveground leaf/needle inputs are doubled by adding litter removed from No Litter plots.
Double Wood	(DW)	Aboveground wood inputs are doubled by adding large shredded wood pieces based on measured input rates of woody debris fall (H. J. Andrews only).
No Roots	(NR)	Roots are excluded with impermeable barriers extending from the soil surface to the top of the C horizon.
No Inputs	(NI)	Aboveground inputs are prevented as in No Litter plots, belowground inputs are prevented as in No Roots plots.

Early DIRT Project results

Soil CO_2 efflux from the DIRT plots at several sites changed rapidly in response to the detrital manipulation treatments. In the fifth year of detrital input manipulation at the DIRT site installed at Harvard Forest, respiration from the soil surface differed significantly between treatments especially in the warm summer months. Respiration rates were greatest in the plots with litter amendments and least in the plots with roots removed (Boone et al. 1998). At the H. J. Andrews site, similar patterns in soil respiration emerged and Sulzman et al. (2005) calculated that 22% of total efflux originated from root and rhizosphere respiration, 19 % from aboveground litter decomposition, and 58 % from belowground litter decomposition. Laboratory incubation of mineral soil from the site at Harvard Forest revealed that, after only two years of experimental treatment, detrital manipulations had already influenced the mineral soil degradability (Bowden et al. 1993).

The DIRT treatments also influenced soil solution chemistry and the form of dissolved organic carbon (DOC) in organic horizon leachates at H. J. Andrews within several years of initiating manipulations. Soil solution chemistry since 1999 from H. J. Andrews has shown a substantial increase in DOC concentration of the soil solution at 30 cm in the doubled wood plots compared to the controls (Lajtha et al. 2005). Also, all of the litter removal plots had lower DOC concentrations in soil solution at 30 cm than the control plots (Lajtha et al. 2005). At 1 m below the surface however, DOC concentration was low for all treatments, most likely due to retention of hydrophobic organic compounds via sorption to soil minerals, particularly for the doubled wood treatment (Lajtha et al. 2005). Hydrophobic organic acids were the dominant form of DOC at all soil depths at H. J. Andrews (Yano et al. 2004).

SOM conceptual model

Research at the DIRT sites is guided by a conceptual model developed to describe the potential stabilization and accumulation of C derived from detrital inputs in the soil profile over time (Figure 1-1). As plant litter enters the soil matrix, within a short time period (minutes to days) decomposition results in an efflux of CO_2. Simultaneously, DOC is produced and enters the soil solution and can either be lost from the soil pool via leaching to stream/ground water and further decomposition or retained in the soil matrix by interactions with both organic matter and mineral surfaces (adsorption). The soil C pool is conceptually divided into two distinct fractions with different recalcitrance and turnover times: the 'light fraction' which is composed of partially decomposed detrital material undergoing rapid decomposition and the 'heavy fraction' which is dominated by soil minerals in close association with a small amount of organic compounds considered to have a slow turnover rate. Organic matter that is not lost from the system via leaching or respiration remains in the soil matrix and may be stabilized against further degradation.

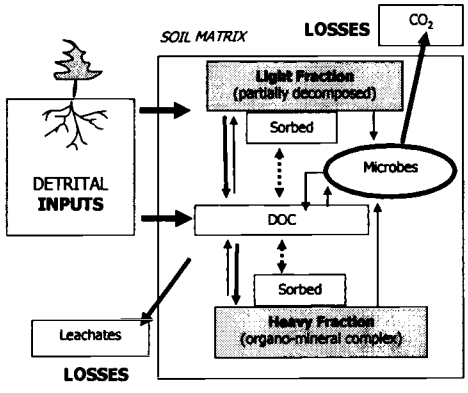

Figure 1-1. DIRT conceptual model for soil organic matter.

Dissolved organic carbon originating from recent inputs, decomposition of humic substances (already unrecognizable organic matter in soil), and rhizosphere deposition (root products, exudates, mycorrhizal metabolic products) can sorb to organic matter in the light fraction and to mineral surfaces in the heavy fraction. Decomposition of biomass (both plant and animal) begins with the most easily degradable compounds, resulting in an increasingly **recalcitrant** organic residue. Organo-mineral **interactions**, the association of organic compounds with clay mineral surfaces (through H-bonding, cation bridging, Van der Waal's forces, ligand exchange, and other mechanisms)

(Stevenson 1996), protect organic matter from further decomposition and leaching. Soil structure, promoted by the presence of clays and organic matter in undisturbed soil, forms very small pore spaces that can limit microbial **access** to organic material held within the aggregates. All three processes, as described, contribute to the accumulation of organic matter in the soil profile.

Ultimately, over longer periods of time (years to decades) changes in detrital inputs to the forest floor should be apparent in the soil composition itself. Since the light fraction is partially decomposed organic material, this change should be present in this organic matter pool first (Cromack et al. 1999). As decomposition of detrital inputs and DOC transport down the soil profile continues, the chemical properties of the inputs are transferred to the soil organic matter pools.

SITE DESCRIPTIONS

Bousson Experimental Forest, PA, USA

This DIRT experimental site is located in northwestern Pennsylvania, USA within the Bousson Environmental Research Reserve owned by Allegheny College (site characteristics are summarized in Table 1-2). The climate is humid and continental, winters are snowy and cold, spring and fall have wide fluctuations in temperature and moisture, summers are moderate and rainfall events are associated with afternoon thunderstorms (USDA-SCS 1979). The plots are at an elevation of 390 m on a gentle slope (5 %). The soils are fine loamy, mixed mesic Alfisols of the Cambridge B series and are moderately well drained with a bulk density of 0.52 g cm^{-3}. In the upper 15 cm of mineral soil, the texture is silty loam (20-50% sand, 50-90% silt, and 0-20% clay), pH is 4.0, and CEC is 3.73 cmolc kg^{-1} (Bowden et al. in press). The soil developed in material weathered from glacial till, which has origins of grey acid sandstone, siltstone and shale and is a compact mixture of material ranging from boulders and stones to fragments and particles. This series is characterized by deep deposits of moderately well

drained soils (USDA-SCS 1979). The depth to which roots can permeate Cambridge soil is often limited by a brittle fragipan layer at around 61 cm or shallow bedrock. The site has a history of agriculture, however evidence in the soil profile indicates plowing did not occur, and the site may have been used for grazing or wood production

Table 1-2. Comparison of H. J. Andrews Experimental Forest, OR (HJA) and Bousson Experimental Forest, PA (Bousson) DIRT sites. Data in table are compiled from many sources: USDA-SCS 1979; Bowden et al. 1993, Homman and Grigal 1996; Bowden et al. 2000; Vanderbilt et al. 2003; Lajtha et al. 2005; Sulzman et al. 2005; Crow et al. 2006; Sollins et al. 2006.

		HJA	Bousson
Mean annual T (°C)		8.8	8.3
Annual rainfall (cm y^{-1})		220	105
N input (g N m^{-2} yr^{-1})		0.2	10-12
Approximate stand age (yr)		500	80
Soil order		Andisol*	Alfisol
Litterfall (kg ha^{-1} y^{-1})			
C	Leaf and needle	4330	2110
	Wood	2600	
N	Leaf and needle	18	31
	Wood	3	
C:N	Leaf and needle	241	68
	Wood	897	
Forest floor (kg ha^{-1})			
C	Leaf and needle	25600	51275
	Wood	92500	
N	Leaf and needle	256	3539
	Wood	190	
C:N	Leaf and needle	100	14
	Wood	500	
Mineral soil			
C:N (0-5 cm)		35	13
Mineralogy		illite and vermiculite	plagioclase feldspar, quartz, smectite, ferrihydrite

* See text for discussion of classification

instead of crop production (Bowden et al. 2000). A typical profile has a 5 cm dark brown silt loam A horizon underlying 8 cm of leaves and organic debris.

Soil at Bousson has mixed clay mineralogy in an intermediate to advanced stage of weathering. Clays at the surface mineral horizons are approximately 30 % illite and 45 % vermiculite. The remaining clays are kaolinite, vermiculite, and illite interstratified with small amounts of chlorite and montmorillinite. The amount of montmorillinite is related to the chemical environment associated with soil formation in a humid environment and high moisture due to low permeability because of the fragipan. Montmorillinite and vermiculite have high CEC and exchange sites; the shrink-swell characteristic of montmorillinite is not seen in these soils because of the overall low clay content (USDA-SCS 1979).

All of the major clay mineral groups are present in Bousson soil:

1. Illite, 2:1, 1.4-2.0 layer charge
2. Vermiculite, 2:1, 1.2-1.8 layer charge
3. Kaolinite, 1:1, <0.01 layer charge
4. Chlorite, 2:1 with hydroxyl interlayer, variable charge
5. Smectite (montmorillinite) 2:1, 0.5-1.2 surface charge

(from Sposito 1989)

At Bousson, high aboveground biomass, litterfall, total soil N, and rates of N mineralization all indicate that this site is highly productive. The experimental plots are located in a nutrient rich mixed deciduous forest which was established approximately 80 years ago. The site is dominated by black cherry and sugar maple in the overstory and by small maple saplings (<5 cm diameter at 1.35 m height) in the understory. Total aboveground biomass is 434 Mg ha^{-1} of which 60 % is black cherry and 28 % is sugar maple. Groundcover is extensive and includes maple seedlings, mayapple (*Podophyllum spp.*) and troutlily (*Erythronium spp.*) (Bowden et al. 2000).

H. J. Andrews Experimental Forest, OR, USA

This DIRT experimental site is located on the western side of the Cascade Range in Oregon, USA (44°15'N, 122°10'W, 726 m elevation) (site characteristics are summarized in Table 1-2). The climate is Mediterranean with warm, dry summers and wet, mild winters which are typical of the maritime climate of the Pacific Northwest. Seventy percent of annual precipitation occurs during the wet season, between October and March, mostly as rain (Sollins et al. 1980). The plots are at an elevation of 726 m on a weathered terrace that is relatively flat and stone-free compared to the surrounding mountainous watersheds. The soil is a coarse loamy mixed mesic Andic Dystrudepts which are well drained with a bulk density of 0.83 g cm^{-3} (Dixon 2003). Soil pH is 5.2 in the 0-10 cm of mineral soil (Yano et al. 2004). The soil at this site is derived from volcanic parent material and is composed of mudflow, ash flow, and stream deposits, underlain by Oligocene and lower Miocene volcanic rocks. A stand replacing fire ca. 1500 was followed by the establishment of Douglas-fir trees which still are present in the overstory. Another large fire occurred ~250 y ago and large trees were selectively removed in the mid 1900's, but the stand remained largely intact and wildfire has been the primary disturbance throughout the natural history of the region.

The presence of non-crystalline clays gives Andisols many of their unique properties, including high organic matter content water holding capacity, porosity, and low bulk density (Tan 1984). Non-crystalline clays have a high surface area (exceeding montmorillinite) and variable charge but lack permanent charge. X-ray diffraction analysis (XRD) of soil mineralogy revealed that the bulk soil was predominantly plagioclase feldspar, quartz and smectite. While there was no indication of the presence of amorphous glass or substantial amounts of allophane as would be expected in an Andisol (Sollins et al. 2006), the soil has a high pH in 1 M NaF (10.7) (Yano et al. 2004), indicating the present of ferrihydrite, another type of non-crystalline clay that shares many properties with allophane (Sollins et al. 2006).

The experimental plots are located in a mature Doug-fir, western hemlock stand growing on relatively flat and stone-free soil compared to the surrounding hill slopes of the watersheds of the H. J. Andrews. Soils have a thin O-horizon (0-2 cm) which is interlaced with mosses and a 10-20 cm thick A horizon containing a large amount of fine root biomass and mycorrhizal mats. Coarse woody debris and moss layers cover extensive areas of the forest floor, as is typical of old-growth stands. Also, epiphytic lichens (primarily *Lobaria oregana*) fall from the forest canopy and litter the forest floor. The canopy is dominated by mixed old-growth Douglas-fir, western hemlock (*Tsuga heterophylla*) and western red cedar (*Thuja plicata*) and is relatively sparse (25 dominants ha^{-1}) (Sollins et al. 2006). Understory species include western hemlock, Pacific yew (*Taxus brevifolia*), vine maple (*Acer circinatum*), Pacific dogwood (*Cornus nuttallii*), huckleberry (*Vaccinium* spp.), and sword fern (*Polystichum munitum)* (Sollins et al. 2005).

FROM PLANT LITTER TO SOM

Litter quality and decomposition

The chemical composition of plant litter influences decomposition rates (Currie and Aber 1997, Almendros et al 2000). For example, decomposition generally correlates positively with initial N and P content (Berg 2000) and negatively with polyphenols (including tannins and lignin) (Palm and Sanchez 1991, Loranger et al. 2002) and lignin:N ratio (Mellilo et al. 1982). However, identifying litter quality characteristics that consistently relate to decomposability across litter types has been difficult (Lorenz et al. 2004). In a survey of litter types across Canada (CIDET, Canadian Intersite Decomposition Experiment) Preston et al. (2000) found that a combination of NMR, proximate analysis, and molecular level analysis is needed to further improve our understanding of the link between litter quality and decomposition rates across a wide range of vegetation. Overall, C:N ratio may simply be the best predictor of litter decomposition (Edmonds 1980, Enriquez et al. 1993) across a wide range of plant types.

Patterns in the relationship between litter quality and decomposition have not emerged across wide ranges of vegetation (Preston et al. 2000); however, on more localized scales, litter quality influences the chemical characteristics of soil organic matter and thus contributes to the stabilization or destabilization of C. For example, leaf litter from sugar maple has high concentrations of soluble carbohydrates and cellulose, which contribute to fast decay rates and rapid mineralization and immobilization of nutrients during decomposition. Litter from this species, which is abundant at the Bousson DIRT site, has high nutrient content that also contributes to fast decomposition rates, rapid release of nutrients into the soil, and high nitrification rates (Nadelhoffer et al. 2004). Changes in the amount and type of litter inputs to the forest floor are likely to impact nutrient cycling and organic matter formation.

Soil organic matter formation from litter

Cellulose is the predominant organic compound in soil (Wershaw 2004); however, residual polysaccharides and lignin from cell walls, long chain aliphatic molecules from cutin and suberin, and polyphenols from tannins also can be identified. Proteins are the most abundant polymer in living cells and amino acids, and proteins and amino sugars are important sources of organic N in soils (Wershaw 2004). Spectroscopic techniques, particularly ^{13}C NMR, have been used extensively to determine the organic composition of soil organic matter (Preston et al. 1994, Baldock et al. 1997). NMR techniques classify soil organic matter into functional groups that include: o-alkyls (polysaccharides, carbohydrates), alkyls (also referred to as aliphatics, cutin and suberin, lipids, waxes, and fatty acids), and aromatics (polyphenols such as hydrolysable and condensed tannins and lignin-degradation products).

Organic matter decomposition generally results in the reduction of carbohydrates (Golchin et al., 1997; Preston et al., 1998; Nierop et al. 2001; Qualls et al. 2003) and the accumulation of compounds with alkyl and aromatic structural units (Baldock et al. 1992;

Kögel-Knabner et al. 1992; Baldock and Preston 1995; Huang et al. 1999; Kramer et al. 2003), characteristics associated with recalcitrance (Marschner and Kalbitz 2003). In recognition of these concurrent processes, Baldock et al. (1997) defined aliphaticity as the ratio of alkyl:carbohydrates to be used as an indicator for the degree of decomposition. Kramer et al. (2004) found increased aliphaticity, decreased C:N, and isotopic enrichment along a sequence of increasing degree of decomposition with depth in the soil profile of several watersheds.

Correlations between classifications of organic compounds (i.e. amino acids, carbohydrates, and aliphatics) and recalcitrance should often be made with caution. A continuum of recalcitrance may exist as a result of decomposition; however, at any stage, interaction with soil minerals may interrupt the continuum by stabilization within the soil matrix (Baldock and Skjemstad 2000). In particular, labile organic matter components may be bound to more stable organic compounds (Guggenberger et al. 1994; Volk et al. 1997). Aliphatic molecules may appear more recalcitrant when extensively polymerized, oxidized, or bound to aromatic molecules such as lignocellulose (Guggenberger et al. 1994) or black carbon (Wershaw 2004). Also, plant polyphenols (including hydrolysable and condensed tannins) can form non-covalent bonds with proteins and polysaccharides thus resulting in precipitation of these compounds out of solution (Wershaw 2004). Over time, alterations in the quantity and quality of detrital inputs (both aboveground and belowground) to the soil should be reflected in the SOM organic composition. Although these changes in SOM may be complex in nature, ultimately, the net result may be apparent in the degree of organic matter stabilization against losses from the soil profile.

Organo-mineral interaction

Soil texture exerts substantial influence on organic C and N dynamics and storage (Kögel-Knabner 1997) and the physical interaction between OM and clays is crucial to SOM stabilization. Sand sized particles are associated with OM that closely resembles plant litter while the fine fractions of some soils have been associated with aliphatic

compounds (Baldock et al. 1992). Some young C is associated with clay-size particles due to sorption of fresh DOM, but refractory OM is associated primarily with fine silt and clays (Balesdent and Mariotti 1996). Overall, recalcitrant pools dominate C and N storage in soil (Kögel-Knabner 1997). The surface functional groups on organic compounds in soil may be associated with minerals either indirectly through binding to another layer of organic compounds or directly to the inorganic surface (Sposito 1989).

There is an active debate over the nature of the interaction between soil minerals and organic matter. Recent progress has been made by Sollins et al. (2006) in the development of a multi-layered "onion" model to describe organo-mineral interactions. Extensive evidence in the literature of an increase in C:N with increasing separation density pointed to the preferential protection of organic N within high density fractions of soil. The multi-layered model of organo-mineral interactions introduced by Sollins et al. (2006) and elaborated by Kleber et al. (in preparation for Biogeochemistry) proposes that fresh mineral surfaces become stably bound to peptidic organic matter forming an inner region characterized by a hydrophobic outer surface. To this new surface, other hydrophobic molecules may bond strongly forming an intermediate region characterized by reactive surfaces which can easy exchange with other ions in the soil solution. Sorption and desorption to this outer layer occurs readily and organic matter in soil solution will be in equilibrium with organic matter bound on the outer layer of the mineral surface. Over time, organic coatings accumulate on mineral surfaces and both mineralogy and plant input quality characteristics influence the degree to which a soil can retain organic matter.

DENSITY FRACTIONATION

Soil organic matter is often separated by physical means to simplify a complex substrate into fractions with different biological significance. A frequent approach is to isolate two or more fractions based on differing particle density by separation in a densimetric fluid. Soil fractions separated this way are often interpreted as organic matter pools with

different carbon turnover times, ranging from years to decades or centuries, and with different functional roles for nutrient dynamics *in situ*. To date, several fractionation schemes have been developed and propagated throughout the literature that differ in the theoretical framework underlying the density separation, including degree of organo-mineral interaction, extent of protection within aggregates, and association of SOM with different soil minerals. One method, initially used by Greenland and Ford (1964) and further developed by Spycher and Young (1977), Young and Spycher (1979), and Spycher et al. (1983) yields two fractions separated between 1.60-1.65 g cm^{-3}, which divide SOM according to the degree of organo-mineral association. The light fraction consists of partially decomposed detrital debris and the heavy fraction consists of organo-mineral and mineral particles. Strickland and Sollins (1987) refined the method by describing the aspiration of light fraction material as a way to increase recovery efficacy. The conceptual model used to develop DIRT hypotheses includes two conceptual SOM fractions which can be physically represented by dividing soil into light and heavy fractions by density.

RESEARCH QUESTIONS

By changing the quantity and quality of detrital inputs to the forest floor at several field sites, we hope to understand the mechanisms through which plant inputs become soil organic matter and are retained in or lost from the terrestrial carbon pool at each DIRT site. The suite of mechanisms controlling organic matter stabilization (recalcitrance, organo-mineral interactions, and microbial accessibility) is common across sites; however, the relative importance of each is different and will depend in part on the quality of detrital inputs, the transfer of those inputs to the mineral soil (by leaching and physical mixing), and the propensity for a soil minerals to interact with organic matter. **My goal was to characterize the soil organic matter at Bousson and H. J. Andrews and to determine whether detrital input manipulations had yet transferred to differences in organic matter quality (decomposability) in the 0-5 cm mineral soil at 2 of the DIRT sites: Bousson and H. J. Andrews.**

In the following three chapters, I address several research questions:

1. How does density fractionation impact the biological interpretation of SOM fractions?
2. Has altering detrital inputs influenced the decomposability of SOM fractions?
3. What influence has altering detrital inputs had on C and N dynamics of SOM fractions?
4. Can the isotopic signature of respired CO_2 be used to determine the SOM substrate utilized by microbial communities during decomposition?

Chapter 2 addresses question 1 in a short review of density fractionation as a method for physically separating SOM into fractions with biological significance in the function of a soil. In addition to the review, we report results from a series of experiments and analyses designed to determine why soil density fractions cannot be summed back to equal the bulk soil. This addresses a methodological problem that has affected many studies, yet has not yet been directly addresses in the literature. In addition, we report the first replicated radiocarbon-based mean residence times for the SOM fractions at both Bousson and H. J. Andrews, which helped lead to the discovery that microbes are utilizing an unexpected OM source at Bousson. Results from this experiment question the conceptual idea that the light fraction is "active" and readily decomposed and the heavy fraction is "passive" and not easily decomposed.

Chapter 3 covers the questions 2 and 3. The two study sites have fundamental differences in soil mineralogy, N deposition, precipitation regime, and vegetation type (deciduous versus coniferous), which all influence SOM stabilization. Radiocarbon based mean residence times of the SOM fractions reflect some of these differences, as do C, N and lignin concentrations from both sites. Other research at both sites has shown that the detrital inputs have influenced aspects of soil respiration and dissolved organic matter dynamics. However, results from a long term incubation of bulk soil and density

fractions show that bulk soil at neither site has (yet) shown any effect of altered detrital inputs on C or N dynamics. Light fraction material at Bousson is showing evidence of the influence of altered inputs, in particular, the mean residence time has changed drastically as a result of manipulating the quality and quantity of detrital inputs.

Chapter 4 addresses the final question and discusses results from a short incubation experiment of density fractions from the Double Litter, Control, and No Input DIRT treatments from H. J. Andrews and Bousson. The isotopic signature of the CO_2 respired during the incubation experiment led to an interesting discussion of the establishment of microbial communities following drying and rewetting of soil. The shift in community structure impacted the respired isotopic signature, which has implications for interpreting isotopic signatures of ecosystem fluxes.

CHAPTER 2

INTERPRETING DENSITY FRACTIONS: SEARCHING FOR MEANINGFUL SOIL ORGANIC MATTER POOLS

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Biogeochemistry

In Review

ABSTRACT

Soil organic matter (SOM) is often separated by physical means to simplify a complex substrate into fractions with different biological significance. A frequent approach is to isolate 2 or more fractions based on differing particle density by separation in a densimetric fluid. Soil fractions separated this way are often interpreted as organic matter pools with different carbon turnover times, ranging from years to decades or centuries, and with different functional roles for nutrient dynamics *in situ*. Here we discuss the development over the last half century of methods for dividing soil into distinct organic matter pools with methods that include density fractionation, including multiple density fractions and combinations of density fractionation and size separation. We focus on the use of sodium polytungstate (SPT) for density separation and address the potential effects of subjecting soil to a high density salt solution on data interpretation.

We separated soils collected from forested sites at H.J. Andrews Experimental Forest, Oregon and Bousson Experimental Forest, Pennsylvania into 2 density fractions based on floatation in a 1.6 g cm^{-3} solution of SPT. Soils from both sites exhibited an approximate 40 % reduction in cumulative respiration over 1 year from mathematically recombined density fractions compared to bulk soil that was not fractionated. The reduced respiration could be explained in part by several artifacts of the fractionation, including dissolved C and N loss, and tungsten contamination and toxicity. Mass balance calculations revealed that between 21 and 28 % of the original bulk soil C and N content was mobilized and subsequently lost during density fractionation for both soils. Based on estimates of the isotopic signature ($\delta^{13}C$ and $\delta^{15}N$) of the recombined density fractions, in some cases the light isotope is preferentially mobilized during density fractionation. Residual amounts of tungsten (W) present even in well-rinsed density fractions were enough to reduce microbial respiration by 27 % compared to the control in a 100 day incubation of O-horizon material. However, residual W was nearly eliminated by repeated leaching over the year-long incubation, and is not likely the primary cause of the decrease in respiration from the recombined fractions compared to bulk soil. Light fraction at Bousson, a

deciduous site developed on Alfisols, had a radiocarbon-based mean residence time (MRT) of 3 y while heavy fraction was 317 y. In contrast, both density fractions from H. J. Andrews, a coniferous site developed on andic soils, had approximately the same MRT (111 y and 93 y for light and heavy fractions). At H. J. Andrews, however, the organic matter lost during density separation had a short MRT (19 y), which could account for missing respired CO_2 from the recombined density fractions compared to the bulk soil. Careful consideration and characterization of the effects of the density separation procedure on the actual fractions separated can not only deepen our understanding of the role of the fractions *in situ*, but also help avoid misinterpretation of these fractions.

INTRODUCTION

Density fractionation has been used for nearly 45 years to physically separate soil organic matter (SOM) into discrete fractions thought to have differing stability. The method has gone through several metamorphoses during that time, yet remains true to its original intent. As with all methods that attempt to operationally define organic matter pools, there have been shortcomings and concerns, particularly with respect to the relationship between the conceptual organic matter pools and the actual characteristics of the resulting soil fractions. Christensen (1992) provided a detailed review of soil physical fractionation techniques, which included both size separation and density fractionation. However, in the years since, there has been a substantial increase in the use of density fractionation methods across a variety of ecological research fields and applications. Here we summarize trends in the density fractionation literature with a short review, emphasizing the expansion of work since the introduction of sodium polytungstate (SPT) as a densimetric liquid. Next, we consider the link between conceptualized organic matter pools and the physical, chemical, and biological properties of fractions isolated through density fractionation. Finally, we use examples from our own work to consider potential pitfalls in the methodology and suggest ways to avoid misinterpreting results.

Methods development

The use of densimetric techniques on soils, which divides material based floatation or sedimentation in a solution according to particle density, emerged initially as a means to separate both primary (Pearson and Truog 1937) and clay (Halma 1969; Francis et al. 1972) minerals. During the separation of soil minerals, it was necessary to disrupt soil aggregates and remove organic material. In the following decades, density fractionation techniques were developed with the specific goal to divide SOM into two discrete fractions (Monnier et al. 1962; Greenland and Ford 1964) that represented different stages of degradation, i.e. recent, partially decomposed organic matter versus thoroughly degraded organic matter already associated with minerals through organo-mineral complexes (Ford et al. 1968, Richter et al. 1975). Early developments in the technique were intended to improve separation of the fractions specifically by improving the disruption of soil structure by ultrasonic dispersion (Ford et al. 1968), surfactants (Ford et al. 1969), and boiling and grinding (Oades and Ladd 1977). A number of early studies were conducted with soil separated at ~2.0 g cm^{-3}, since most primary and secondary soil minerals are more dense than 2.0 g cm^{-3} (e.g., Laird and Dowdy, 1994; Arnarson and Keil 2001). The use of a separation density of 2.0 g cm^{-3} continued; however, a lower density 1.6 – 1.8 g cm^{-3} also became accepted as a way to exclude the most mineral and organo-mineral material from the light fraction while maximizing recovery of plant-like particulate organic matter (Ladd et al. 1977; Scheffer 1977; Young and Spycher 1979). The development of density fractionation as a means for physically separating soil had implications for identifying mechanisms of organo-mineral interactions and quantifying pools of organic matter with different ecological roles that are still relevant to soil organic matter research today.

To date, several additional fractionation schemes have been developed and propagated throughout the literature that differ in the theoretical framework underlying the density separation, including degree of organo-mineral interaction, extent of protection within aggregates, and association of SOM with different soil minerals. Golchin et al. (1994a,

b) adapted the 2-fraction method to divide soil into several fractions based on the degree of physical protection, or occlusion, within aggregates. The 3-fraction method divides SOM into a non-protected inter-aggregate ("free") light fraction separated from whole soil by floatation without sonication, a protected intra-aggregate ("occluded") light fraction separated from remaining sediment by sonication and floatation, and a residual organo-mineral fraction (Golchin et al.1994b; see also Swanston et al. 2005). A more complex method follows the steps of the first, but subsequently separates the residual organo-mineral fraction into multiple fractions by increasing density in increments (Golchin et al. 1994a). This sequential density fractionation, absent the separation of the occluded light fraction from the free light fraction, had been used for years with the intent to separate fractions primarily on the basis of mineralogy (Turchenek and Oades 1979; Skjemstad and Dalal 1987; Golchin et al. 1994b; Glaser et al. 2000). Arnarson and Keil (2001) and Sollins et al. (2006) specifically considered how organic coatings on different minerals could alter the particle density of the organo-mineral complexes (i.e., sequential density fractions), and how this might relate to C stabilization. Some methods combine size and density fractionation in an attempt to target multiple, spatially explicit SOM pools that relate to stable aggregates (Meijboom et al. 1995, Six et al. 2000). Based in part on earlier work by Cambardella and Elliot (1994) and Golchin et al. (1994a), Six et al. (2000) developed a complex method using a combination of size and density fractions to isolate fractions located within specific areas of the soil matrix that can have distinct functional roles in nutrient cycling or C dynamics within an ecosystem. These fractions include the 'enriched light fraction' (protected within macro-aggregates) and the micro-aggregates within macro-aggregates. None of these methods is monolithic: each should be applied with deliberation and carefully adapted to the given soil and research goals. In this spirit, the characterization of the simplest, 2-pool fraction is the primary focus of this paper.

Conceptualized organic matter pools

Density fractionation emerged as a useful methodological step in research seeking to quantitatively isolate SOM fractions with relevance to soil nutrient cycling and organic matter stabilization. During the development of methods over the last several decades, light fraction material has been described in a variety of ways including as partially degraded material not associated with minerals, as an "active" organic matter pool, and as a "labile" pool. Conceptually, since light fraction material is not protected by minerals, it was typically considered labile, with a short mean residence time (Young and Spycher 1979). Experimentally, Greenland and Ford (1964) supported this concept with evidence from a short incubation study. Soil with light fraction added consumed more O_2 than did soil with humic acids added but less than did soil with fresh plant material added, which was an indication that the light fraction material was readily decomposed (Greenland and Ford 1964). In forest and cultivated soils, Trumbore et al. (1995) defined the light fraction (< 2.0 g cm^{-3}) as "active C" having a turnover time of years (not decades); other soil organic matter pools were defined as "slow C" (decades) and "passive C" (centuries). The term labile was commonly used to describe the light fraction material in the agricultural literature (Ford and Greenland 1964; Janzen et al 1992; Dalal and Mayer 1986) where light fraction content had been found to be a reliable estimate for organic matter content and to be sensitive to cultivation practices and land use (Golchin et al. 1997; Alvarez et al. 1998). Boone (1994) extended the use of labile as a descriptor of the light fraction material to forest soils; but, ultimately concluded that density fractionation does not always provide a clear separation of forest soil OM into active and passive pools.

Exceptions to the conceptual division between biologically "active" light and "passive" heavy fraction organic matter were apparent in the early stages of density fractionation methods development. For example, it was recognized that low density, amorphous minerals float at densities <2.0 g cm^{-3}, resulting in the presence of mineral material with high adsorptive surface area in the light fraction, particularly in allophanic soils such as

Andisols (Spycher and Young 1979). Also, charcoal, which is recalcitrant, floats at low densities and is included within the light fraction material that was considered otherwise readily degradable by soil biota (Greenland and Ford 1964, Golchin et al. 1997). Strickland et al. (1992) incubated whole soil with $^{15}NH_4Cl$ for 60 days and expected to find little incorporation of the ^{15}N into heavy fraction material; however, the label was recovered within both physically protected and unprotected organic matter pools associated with the heavy fraction. The presence of the ^{15}N label indicated the potential for heavy fraction N to be chemically active, but physically protected (Strickland et al. 1992).

Other research also found that density fractions do not have the biologically "active" or "passive" characteristics conceptually attributed to them (Swanston et al. 2002; Swanston et al. 2004; Crow et al. 2006) and that the light and heavy fractions together do not cycle C and N as the bulk soil would (Sollins et al. 1984; Boone 1994; Bhupinderpal-Singh 2005). Sometimes only a small difference in substrate recalcitrance, as indicated by microbial respiration (g CO_2 g^{-1} $C_{initial}$), was observed between the light and heavy fractions during incubation studies (Swanston et al. 2002; Swanston et al. 2004). Rovira and Vallejo (2003) determined that the least chemically recalcitrant OM in Mediterranean calcareous forest soils was protected within aggregates in the "occluded" light fraction and the dense fraction (using the Golchin et al. 1994a, 1994b method). The free light fraction material (separated at 1.6 g cm^{-3}), although composed of recognizable plant material, was of an intermediate chemical recalcitrance and not always the most fresh and non-decomposed material (Rovira and Vallejo 2003). Swanston et al. (2005) found the heavy fraction to be initially more responsive to recent atmospheric enriched-radiocarbon inputs (EBIS project, Oakridge, TN) than the "occluded" light fraction. The heavy fraction may also be less recalcitrant than the light fraction (Crow et al. 2006). The free light fraction consistently responds most strongly to changes in environment and C inputs, but characterization of the "occluded" light fraction and the single-density heavy fraction are much less consistent. These fractions seem to vary most strongly with differences in soil structure and mineralogy, respectively.

Expansion of technique

Many heavy liquid solutions have been used for soil fractionation, i.e. bromoform ethanol (Monnier et al. 1962), bromoform-petroleum spirit mixture (Greenland and Ford 1964), and NaI (Sollins et al. 1983, Boone 1994); see Christensen (1992) for a review of methods. In the early 1980's, sodium polytungstate, $H_2Na_6O_{40}W_{12}$, (SPT, SOMETU, Berlin) was introduced as a safe, inorganic alternative to the toxic mixtures commonly used (Plewinksy and Kamp 1983). Previously, heavy liquid solutions were mostly halogenated-hydrocarbons that were highly toxic to humans (Torresan 1987). High viscosity reduces the utility of SPT at densities >2.7 g cm^{-3}; however, at densities typically used with soils, SPT is a non-corrosive, non-flammable, safe alternative to earlier methods (Skipp and Brownfield 1993). Another alternative heavy liquid was developed; Ludox™, an aqueous colloidal dispersion of silica particles in which the soil is suspended (Hassink 1995a; Hassink 1995b; Meijboom et al. 1995; van den Pol-van Dasselaar 1999; Accoe et al. 2004). The viscosity of Ludox is high, which prevents porous particles from becoming saturated and results in floatation. The maximum density of Ludox is 1.4 g cm^{-3} (lower even than most organic debris) and, since saturation of the pore space does not occur, the separation density is more representative of the soil bulk density than of particle density. Following the widespread acceptance of SPT as a high density liquid for the separation of soil fractions, research utilizing density fractionation began to increase substantially.

In recent years, density fractionation has increasingly been applied to a wide array of research questions ranging in context from the retention of organic pollutants within the soil matrix (Krauss and Wilcke 2005) to the incorporation of isotopically labeled material into various soil fractions (Bhudinderpal-Singh et al. 2005) and the influence changes in land use have on soil C and N dynamics (c.f. Homann et al. 2001; Lui et al. 2003; Wang et al. 2004; Ashagrie et al. 2005; Li et al. 2005; John et al. 2005). The focus of much of this research has been on understanding mechanisms of organic matter stabilization in

soils, i.e. the role of aggregate structure versus biochemical protection on soil organic matter stabilization (Accoe et al. 2004; Henry et al. 2005; Rovira and Vallejo 2003; Swanston et al. 2005). Evidence of material transfers between organic matter pools can be found using density fractionation (Swanston et al. 2005), which may contribute to the development of new theoretical ways to model SOM dynamics (Bruun et al. 2004).

Several widely used models over the past several decades, including BIOME-BGC and CENTURY, represent the heterogeneity of soil organic matter as discrete pools of various sizes and turnover times (Jenkinson and Raynor 1977, Running and Coughlin 1988; Parton et al. 1994). Attempts at correlating physically defined fractions with these conceptual pools have had little success (c.f. Six et al. 2002) and the models remain with immeasurable components. Six et al. (2002) put forth a model of SOM dynamics with measurable pools, facilitated in part by density fractionation, that are distinguished based on the degree to which organic matter is protected from degradation within macro- and micro-aggregates or in association with silt and clay particles. Although progress has been made, the ability to quantitatively separate and determine turnover rates of various soil organic matter pools remains elusive.

Interpreting density fractions within the method constraints

Breaking up soil structure has been central to the efficiency of separating organic matter pools by densimetric techniques since the earliest methods development. Protection within aggregate structure is one of the fundamental mechanisms for stabilizing organic matter in soils (Oades 1988, Sollins et al. 1996), thus it follows that density fractionation might alter the apparent properties of the fractions. Within this context, results from experiments with density fractions of a soil could be used to distinguish the role of protection within aggregates from organo-mineral interactions and chemical recalcitrance in stabilization of OM. Swanston et al. (2002) compared respiration from bulk soil and density fractions during 300-day incubation. Light and heavy fraction did not appear to differ in recalcitrance. Also, although density separation exposed previously protected

OM to microbial degradation and was expected to result in greater respiration, the mathematically summed fractions respired at the same rate as the whole soil. The authors proposed, as Magid et al. (1996) had previously, that the loss of DOC during density separation or the toxicity of residual Na or tungsten (W) had resulted in an inhibition of microbial communities resulting in less respiration from the density fractions than they had hypothesized.

We designed an incubation experiment similar to Swanston et al. (2002) to compare bulk soil to density fractions incubated in chambers crafted to allow respiration and leachate chemistry measurements repeatedly without destructive sampling (Nadelhoffer 1990). We sampled both an Andisol from H. J. Andrews Experimental Forest in Oregon and an Alfisol from Bousson Experimental Forest in western Pennsylvania, which are part of the ongoing Detritus and Input Removal Treatment (DIRT) Project. Experiments were designed to explore differences in the recalcitrance of density fractions compared to the bulk soil, as influenced by detrital treatment. In the present work we limit our discussion to the following hypotheses: 1) that the loss of soil structure during density fractionation led to changes in the accessibility of organic matter in the density fractions and contributed to the apparent biological functionality of the density fractions, 2) an inhibition of microbial respiration of light fraction material may have been induced as a result of the density separation procedure (i.e. either residual W or Na from fractionation; or nutrient limitation of light fraction, which has a high C:N), and 3) that density fractionation resulted in the loss of a labile organic matter pool that otherwise would have been used as substrate.

The mechanisms responsible for the differences between the conceptualized SOM pools and the physical, chemical, and biological characteristics of the density fractions need to be fully explored to successfully interpret results from any experiment using density fractionation. We discuss the potential for density fractionation of soil with SPT to physically alter the biological function of the resulting density fractions using results of

our experiments with soil from 2 forested sites. We further discuss how to appropriately interpret the isolated pools.

METHODS

Site description

Soil was collected from a coniferous site in the Cascade Range of western Oregon and from a deciduous site in western Pennsylvania. These sites are part of a larger on-going study to evaluate the long-term effects of changing detrital litter inputs on the accumulation and stabilization of carbon in soil (the Detrital Input and Removal Treatments or DIRT project, Nadelhoffer et al. 2004). The coniferous site is located in H. J. Andrews Experimental Forest (HJA) within an old-growth Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*) stand established approximately 500 years ago following a stand-replacing fire. The soils are andic, and coarse woody debris and a moss layer cover extensive areas of the forest floor. X-ray diffraction analysis (XRD) of soil mineralogy revealed that the bulk soil was predominantly plagioclase feldspar, quartz and smectite. While there was no indication of the presence of amorphous glass or substantial amounts of allophane as would be expected in an Andisol (Sollins et al. 2006), the soil has a high pH in 1 M NaF (10.7) (Yano et al. 2004), indicating the present of ferrohydrite, another type of non-crystalline clay that shares many properties with allophone (Sollins et al. 2006). The climate is Mediterranean with dry summers and a wet season from October to May in which 70 % of precipitation occurs (Sollins et al. 1980). Mean annual temperature is 8.8 °C, annual rainfall is 220 cm (Sulzman et al. 2005), and N deposition is 0.2 g N m^{-2} yr^{-1} (Vanderbilt et al. 2003).

The deciduous site is located in Bousson Experimental Forest (Bousson) within a nutrient-rich, mixed stand which is dominated by black cherry (*Prunus serotia*) and sugar maple (*Acer saccharum*) in the canopy and by small maple saplings in the understory and

is approximately 80 y old. An extensive ground cover of maple seedlings, mayapple (*Podophyllum sp.*) and troutlily (*Erythronium sp.*) is present. The soils are fine loamy, mixed mesic Alfisols of the Cambridge B series that are moderately well drained with a bulk density of 0.52 g cm^{-3}. Soil at Bousson has mixed clay mineralogy in an intermediate to advanced stage of weathering. Clays at the surface mineral horizons are approximately 30 % illite and 45 % vermiculite. The remaining is kaolinite, vermiculite, and illite interstratified with small amounts of chlorite and montmorillinite (USDA-SCS 1979). The climate is temperate with a 4 month growing season and 4 months of snow cover. Mean annual temperature is 8.3 °C, annual rainfall is 105 cm, and N deposition is 10-12 g N m^{-2} yr^{-1}. A more detailed description of both sites and soils can be found in Crow et al. (2006).

Soil collection

At HJA (the coniferous site), soil was collected in June 2002 from 3 different depths of the mineral soil: 0-5, 5-10, and 10-20 cm using a bucket auger. For each depth, 6 sub-samples were taken within each of the DIRT experimental plots and were composited in the field and mixed (one composite sample per plot) for a total sample size between 500-1000 g. Each composited sample of 0-5 cm soil was sieved moist to remove material >2 mm and stored at 4 °C in tightly sealed bags for several weeks before and during the density fractionation procedure. Due to high clay content and stickiness, soil samples from deeper depths could not be sieved moist and were dried overnight at 80 °C and then sieved to remove roots and material >2 mm. At Bousson (the deciduous site), soil was collected in June 2003 only from the 0-5 cm of the A horizon. As at HJA, 6 sub-samples were taken from each DIRT experimental plot and composited and mixed resulting in a total of ~ 1000 g of soil per plot. Soil was shipped overnight in a cooler, on ice, to Oregon State University where it was sieved moist to remove material >2 mm and stored at 4 °C in tightly sealed bags for several weeks during the density fractionation procedure. At both sites, 5-10 kg of 0-5 cm A-horizon soil was collected and composited from areas adjacent to the DIRT plots to use for methods validation.

Density fractionation method

Soil collected adjacent to the DIRT plots was used to determine the most appropriate density of sodium polytungstate (SPT, Sometu, Sherman Oaks, CA) at which to fractionate soil from each site. Fractionation of bulk soil was conducted at a range of densities (1.2, 1.4, 1.6, and 1.8 g cm^{-3}) to determine the point at which the light fraction contained the greatest amount organic matter with the least amount of mineral content. Following fractionation at each density (below), the fractions were oven-dried overnight at 105 °C to determine dry weight and were then combusted in a muffle furnace at 550°C for 4 hours to determine proportion lost on ignition (LOI). LOI (proportion organic matter) was used as a proxy for organic matter content and one minus LOI (proportion ash) for mineral content. For both sites, a density of 1.6 g cm^{-3} was determined to be the most appropriate, i.e. organic content of the heavy fraction stopped decreasing and the mineral content in the light fraction started increasing.

Moist soil from the DIRT plots was added to 1.6 g cm^{-3} density solution of SPT (1:3 soil to SPT ratio, such to ensure enough separation distance between fractions) and then separated into 2 fractions by aspirating the floating light fraction into a separate container, leaving the heavy fraction to be collected as sediment (Strickland and Sollins 1987). Moisture contents (after 18 h at 105 °C) were determined for each sample immediately prior to fractionation and extra SPT was added to each soil-SPT mixture to compensate for soil moisture so that the final density was 1.6 g cm^{-3}. Bottles containing soil and SPT were shaken while lying sideways on a bench top shaker for 1 hour. Following shaking, soil and debris stuck on the cap and sides were washed into the solution with 1.6 g cm^{-3} SPT and allowed to separate for 24-48 hr, depending on the clarity of the solution between floating light and fraction and the sediment. After the light fraction was aspirated from the surface of the SPT, the sediment was subjected to the shaking, separation, and aspiration steps twice more, except that the sediment was only shaken for 1 min.

The light fraction collected during the three separation cycles was combined, and the remaining sediment was heavy fraction. Light fraction was rinsed thoroughly on pre-combusted (550 °C) Whatman GF/F filters (0.7 μm pore size) by submerging the material at least 5 times with deionized water and removing the leachate with a vacuum filtration system. SPT was rinsed from the heavy fraction material by adding deionized water, shaking, and centrifuging at approximately 180 g for 15 min. Following centrifugation the supernatant was decanted, more deionized water was added to the bottle, and the heavy fraction was re-suspended before another round of centrifugation. Each bottle was centrifuged and decanted at least 3 times. If there was additional material liberated during centrifugation in the first rinse, when the density was still 1.6 g cm^{-3}, the supernatant was poured through a pre-combusted Whatman GF/F filter under vacuum pressure, rinsed thoroughly with deionized water, and collected as light fraction. Following fractionation, the light and heavy fractions were air-dried and stored at room temperature until the incubation experiment began.

Long incubation of mineral soils and density fractions

Bulk soil, light, and heavy fractions from each field site were incubated for 1 yr. Bench top filtration units (Falcon Filter, Becton Dickinson Labware) were modified according to Nadelhoffer (1990) to make microlysimeter chambers. Approximately 20 g of bulk soil, 6 g of light fraction, or 30 g of heavy fraction material was added to a chamber and acid-washed sand was mixed in equal amount to minimize anaerobic conditions during incubation (Swanston et al. 2002). At the start of the incubation, the sand and soil mixture was re-wetted by adding 10 mL of inoculum solution prepared from fresh soil of the respective site shaken in distilled water for 1 hr (1:10 soil:water). Moisture content was kept constant by adding distilled, deionized water to each chamber weekly to maintain a known weight over the incubation period.

CO_2 efflux from the substrates was measured for each chamber on days 3, 5, 8, 12, 17, 26, 53, 151, 267, and 361 for HJA and days 2, 5, 13, 20, 28, 64, 114, 208, 290, and 367 for BOU. Headspace was purged with CO_2-free air and sealed for approximately 240 minutes while respired CO_2 accumulated. A 500 µL-calibrated syringe was used to mix the headspace gas several times before extracting a sample, which was immediately injected into a 5700A Hewlett Packard gas chromatograph fitted with a Poropak R 80/100 column and thermal conductivity detector. Cumulative respiration was calculated for each substrate in SAS (SAS Institute, v. 9.1, Cary, NC) using PROC EXPAND to calculate and approximate area under the curve using the trapezoidal method. Respiration rate and cumulative respiration for the 'summed fraction' were calculated by mass weight using CO_2 efflux measured from the light and heavy fractions for a given soil sample.

Dissolved organic carbon (DOC) was measured in soil solution leached from each chamber on days 10, 35, 101, 151, 267, and 361 of the incubation for HJA, and 30, 118, 200, 301, and 371 for Bousson. One hundred mL of deionized water was added to the upper chamber of the microlysimeter and allowed to equilibrate with the soil for one hour. At the end of an hour, the solution was drawn through the soil and filtered through a pre-combusted Whatman GF/F filter (0.7 µm pore size). Soil solution samples were kept at 4 °C until analysis if within 48 h, or were otherwise were frozen at –20 °C. Dissolved organic carbon analysis was by Pt-catalyzed high-temperature combustion (Shimadzu TOC-V CSH analyzer). Cumulative DOC release for the 'summed fraction' were calculated by mass weight using DOC production measured from the light and heavy fractions for a given soil sample.

Extractable tungsten (W)

The amount of W residue in the density fractions immediately following density separation and after one year of incubation was determined by extraction of 0.5 g air dried substrate with 1 M HNO_3 (1:6 soil to acid ratio). Soil and acid were shaken on a

bench top shaker for 1 hr, allowed to settle for 30 min, and poured through a Whatman GF/F filter (0.7 μm pore size). Filtrate was collected and W concentration in solution was analyzed by ICP (Perkin Elmer Optima 3000DV with a diode array detector) at the Central Analytical Lab in the Crop and Soil Science department at Oregon State University. Results were used to design the short incubation experiment described below: Prior to incubation, extractable W was 40.8 $\pm$ 11.5 and 28.8 $\pm$ 10.0 mg W g^{-1} heavy fraction for Bousson and HJA, respectively. The amount of residual W remaining in the light fraction prior to incubation was similar, 20.4 $\pm$ 2.7 and 41.46 $\pm$ 5.71 mg W g^{-1} light fraction for Bousson and HJA, respectively.

Short incubation of Oa horizon material

Oa horizon material was collected near the DIRT field site at H. J. Andrews in Oregon from 3 points within an area of about 1 ha. Oi and Oe horizon material was carefully removed and the thin Oa horizon directly above the mineral A-horizon was collected in a large plastic bag and thoroughly mixed for homogeneity. The Oa samples were sieved moist to remove material >2 mm.

Two separate experiments were designed to determine whether microbial respiration was inhibited by nutrient limitation or residual amounts of Na and W present following density fractionation. In the first experiment, Oa horizon material was shaken in SPT, Na_2SO_4, or water and rinsed as described for the density fractionation procedure (5 replicate samples). As was done for the long incubation experiment, filtrate from the control samples was collected to use as inoculum for the experiment and the recovered Oa material was air dried at room temperature. Inoculum was added to the dry substrates in order to reach 40% water content, approximately the same as field conditions.

In the second incubation experiment, additions of various solutions were made to air dried Oa material in the second short incubation experiment to determine whether low nutrient availability or residual salts were inhibiting microbial respiration. Six different

treatments were used: one control, two nutrient treatments, two SPT treatments and a Na treatment. In one nutrient treatment, half-strength Hoagland's solution (Sigma Aldrich) was used as a general macro and micronutrient solution. In the second nutrient treatment, a 0.4151 g L^{-1} ammonium sulfate solution, $(NH_4)_2SO_4$ was used to add N to the substrate in the same concentration as the N in Hoagland's solution. In two SPT treatments, SPT solution was added such that the concentration of W corresponded to the highest and lowest residual concentration of extractable W present in our density fractions for the long-term incubation experiment (58.71 and 19.00 mg W g $soil^{-1}$). For the Na treatment, sodium sulfate (Na_2SO_4) was added so that the final concentration of Na was the same as in the high W treatment. Treatments were randomly assigned to each microlysimeter chamber so that there were 4 replicate samples for each and solutions were added to the dry substrates so that 40 % moisture content was attained.

Both short incubation experiments were conducted using the same microlysimeter chambers, gas sampling protocol, and calculations as previously described. Headspace gas sampling began the day following the wetting of soil with solution (day 1) and continued on days 2, 3, 5, 7, 11, 15, 21, 28, 48, and 90.

Elemental analysis and stable isotopes

Carbon concentrations of soil and density fractions were determined by dry micro-Dumas combustion (NA1500 C/H/N Analyzer, Carlo Erba Strumentazione, Milan) at the Stable Isotope/Soil Biology Laboratory of the University of Georgia, Institute of Ecology. Stable carbon and nitrogen isotope values ($\delta^{13}C$, $\delta^{15}N$) were determined on separate sub-samples for each light fraction, heavy fraction, and whole soil sample to ensure adequate N or C was present in the sample for accurate analysis. $\delta^{13}C$ and $\delta^{15}N$ were determined at the EPA Western Ecology Division's Integrated Stable Isotope Research Facility using an elemental analyzer (ECS 4010, Costech, Valencia, CA) coupled to a Isotope Ratio Mass Spectrometer (IRMS, Finnigan MAT Delta Plus XL, ThermoQuest Finnigan,

Bremen, Germany). Some $\delta^{13}C$ samples were analyzed at the Stable Isotope Lab at the College of Oceanic and Atmospheric Science at Oregon State University using a Carlo Erba continuous flow inlet with a Finnigan MAT Delta Plus XL.

A mass balance approach was used to determine if the density fractionation process caused any systematic shift in %C, %N, $\delta^{13}C$ or $\delta^{15}N$. The %C or %N was calculated for the light and heavy fraction by multiplying the concentration data obtained from the elemental analyzer for a particular fraction by mg fraction per gram of whole soil. Since $\delta^{13}C$ and $\delta^{15}N$ are ratios and are concentration dependent for scaling, the following mass balance equation was used for their comparison:

$$A_{blk} \approx \frac{(A_{lf} \cdot [E]_{lf}) + (A_{hf} \cdot [E]_{hf})}{[E]_{lf} + [E]_{hf}},$$

where A is the isotopic value in Atom% which is the ratio of heavy to total atoms (heavy + light) multiplied by 100. The conversion between δ and A was made according to the following equation:

$$\delta_{sample} = \left(\frac{A_{sample}}{R_{standard}(100 - A_{sample})} - 1 \right) 1000,$$

where $R_{standard}$ is 0.0112372 for PDB, and 0.0036765 for atmospheric N_2.

Black carbon quantification (BC)

Light fraction material (~ 1.5 g) from each treatment plot was digested in 175 mL of distilled, de-ionized water, 5 g $NaOCl_2$, and 5 mL acetic acid on a rotary shaker (240 rotations per minute) for 2 hr at room temperature for 3 digestion cycles (adapted from Simpson and Hatcher 2004). Following the final cycle, the residue was poured onto a pre-combusted Whatman GF/F (0.7 μm pore size) and rinsed thoroughly with distilled water (total rinse volume of 400 mL). The residue was then passed through a 500 μm sieve to separate fine particulate material and mineral soil from the larger

organic matter fragments. This step allowed the larger fragments to remain mostly free of mineral coatings that could make identifying BC, which is present at our sites in the form of charcoal with visible plant morphology, more difficult in subsequent steps. Radiocarbon analysis (below) determined that the < 500 μm material was modern (containing bomb-carbon in amounts similar to recent litter); therefore, we assumed that little BC material was lost during the size fractionation. Charcoal pieces were manually separated from 2 subsets of the >500 μm residue under a dissecting microscope with a pair of fine-pointed forceps. The subsets were averaged to calculate a total recovery of BC for each sample. Since the presence of charcoal in the soil pre-dated the establishment of our experiment, all recovered BC material from each site was combined together to have enough material for accurate radiocarbon analysis.

Mean residence time and radiocarbon dating

Radiocarbon concentration was measured on the Van de Graaff FN accelerator mass spectrometer (AMS) at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory, CA. Samples were prepared for analysis by combustion of organic C to CO_2 with CuO and powdered Ag in sealed evacuated tubes and subsequent reduction of the CO_2 onto iron powder in the presence of H_2 (Vogel et al. 1984). Radiocarbon data are expressed according to Stuiver and Polach (1977) as $\Delta^{14}C$, the deviation in parts per thousand from the absolute international standard activity (^{14}C:^{12}C ratio of oxalic acid corrected for decay since 1950). Independently measured $\delta^{13}C$ values for each fraction were used to adjust the $\Delta^{14}C$ values for mass-dependent fractionation. Mean residence times of density fractions were calculated with a time-dependent steady-state model (Trumbore et al. 1995, Gaudinski et al. 2000) (see Appendix). Three assumptions of the model are: 1) bulk C inputs equal loss in each pool through time, though $\Delta^{14}C$ varies; 2) the $\Delta^{14}C$ of inputs to the light and heavy fraction pools are equal to that of the atmosphere in the previous year (thus, the model does not account for multi-year lags before input or

transfer between pools and resulting MRTs should be considered maximum values); and 3) the $\Delta^{14}C$ of inputs to the pool attributed to OM lost during fractionation (SPT-loss) was mass-weighted between the light and heavy fractions. Yearly atmospheric values ($\Delta^{14}C$) used in the model were based on three chronologies, beginning in calendar year 1511 (Stuiver et al. 1998; Hua and Barbetti 2004; Levin and Kromer 2004). Since the radiocarbon in the bulk soil and light fraction were influenced by the inclusion of BC in the soil, BC was quantified for each sample, $\Delta^{14}C$ was determined, and bulk soil and light fraction were mathematically adjusted to exclude the influence of the BC on the C pool size and $\Delta^{14}C$.

RESULTS

Decomposition of bulk soil and density fractions

Cumulative respiration from bulk soil was substantially greater than from mathematically summed light and heavy fraction (summed fractions) from both H.J. Andrews and Bousson (Figure 2-1). Initially, respiration rate from the bulk soil was an order of magnitude greater than from the summed fractions at Bousson (Figure 2-1). By day 5 of the incubation period the rate of respiration from the bulk soil had dropped to nearly the same rate as the summed fractions and remained as such for the remainder of the incubation. On the final day of measurement the summed fractions respired at a greater rate than the bulk soil. At HJA, there was also an initial flush of respiration although not as great as had occurred with Bousson soil (Figure 2-1). Respiration rate of the bulk soil dropped after 26 days and leveled off after 150, yet remained elevated compared to the summed fractions throughout the incubation period. We found that cumulative respiration normalized by initial substrate mass (mg CO_2 g^{-1} substrate) was greatest from the light fraction, followed by the bulk soil and heavy fraction for both soils (Figure 2-2). However, when cumulative respiration was normalized by the initial amount of C present in the substrate (mg CO_2 g^{-1} $C_{initial}$), bulk soil respiration from H. J. Andrews (HJA) soils was much higher than either light or heavy fraction respiration (Figure 2-2).

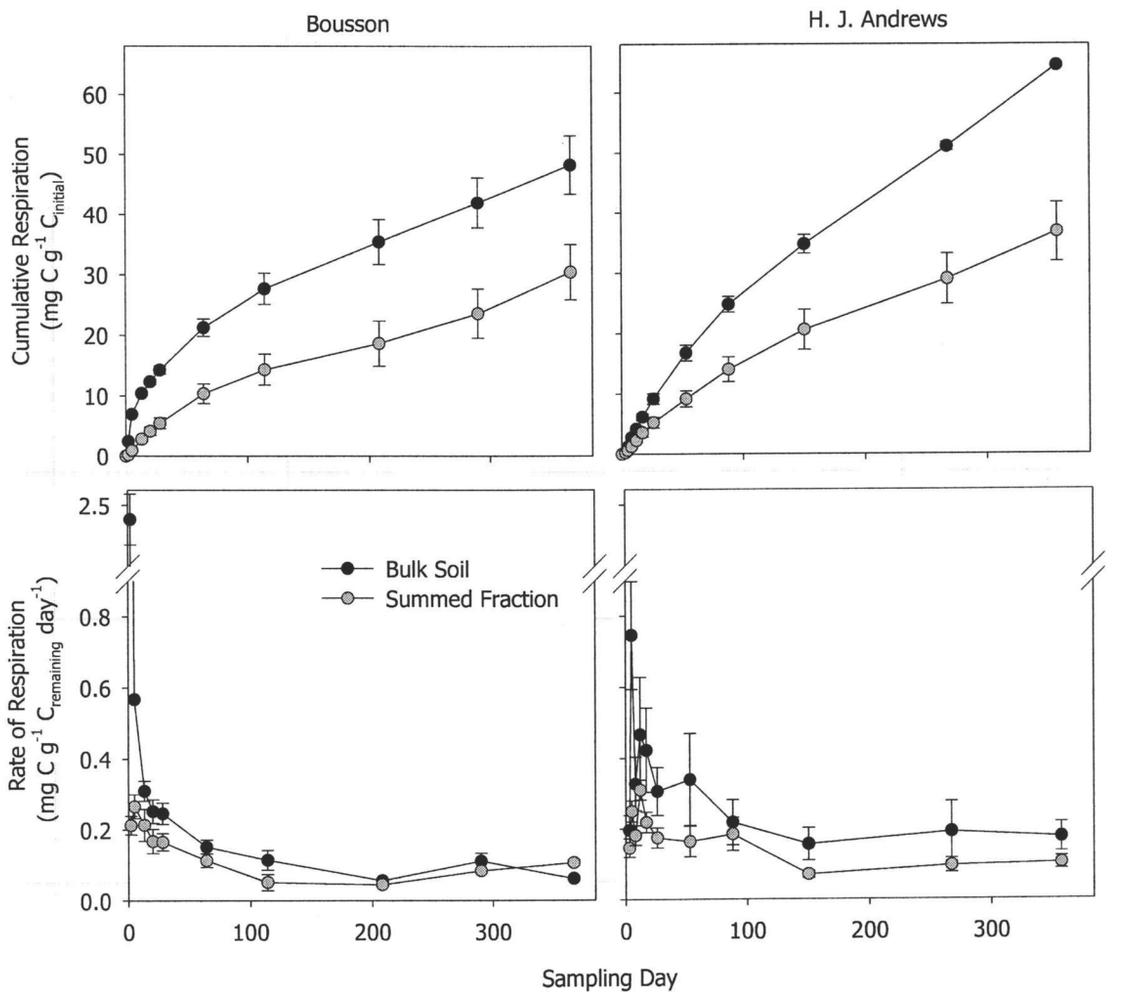

Figure 2-1. Cumulative respiration (upper panels) and rate of respiration (lower panels) during incubation from bulk soil and mathematically summed light and heavy fractions (summed fractions), values are means $\pm$ one standard error.

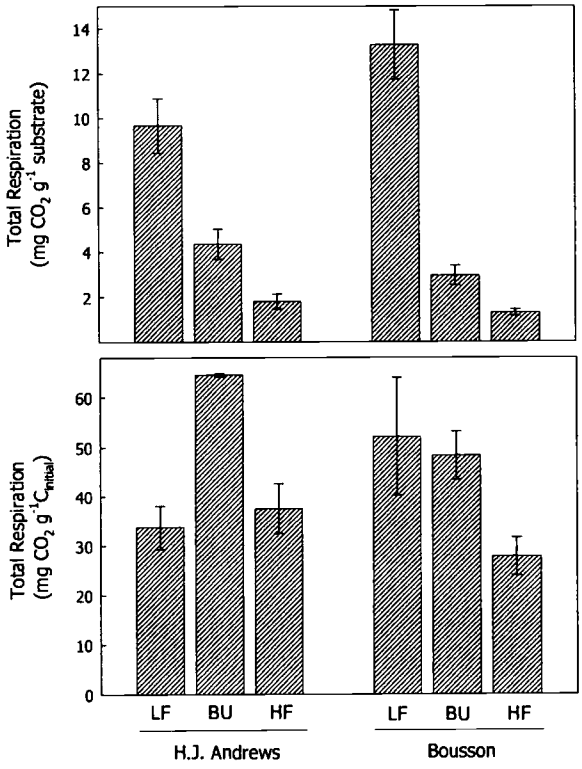

Figure 2-2. Comparing the apparent lability of density fractions and bulk soil (LF = light fraction, BU = bulk soil, HF = heavy fraction) when considered in units of respiration per gram of substrate (upper panel) or per gram of C initially present in the substrate (lower panel), values are means $\pm$ one standard error.

Disruption of soil structure

Scanning electron microscopy (SEM) images indicate the degree to which soil structure (Figure 2-3A) was disrupted during density fractionation and that the soil appears to be separated into different forms of organic matter at both sites (Figure 3B, C, D). Light fraction material, particularly at the old growth coniferous site, is composed primarily of woody debris including bark and roots, fungal fruiting bodies, and charcoal (Figure 3B). Light fraction material at the deciduous site appeared to originate mostly from deciduous

leaves and fine roots (Figure 2-3C). Heavy fraction material from both sites consists of mineral clays and, particularly at Bousson, sandy material which is present with organic debris coating portions of the surface (Figure 2-3D). Black carbon (BC) was abundant in the light fraction material at HJA and to a lesser extent at Bousson. Light fraction contained up to 0.100 g BC g^{-1} soil, which equated to 0.196 g BC g^{-1} total C at HJA while the greatest amount of charcoal at Bousson equated to 0.071 g BC g^{-1} total C. Both woody and amorphous forms that result from the combustion of plant material (Goldberg 1985) were present at both sites (Figure 2-4).

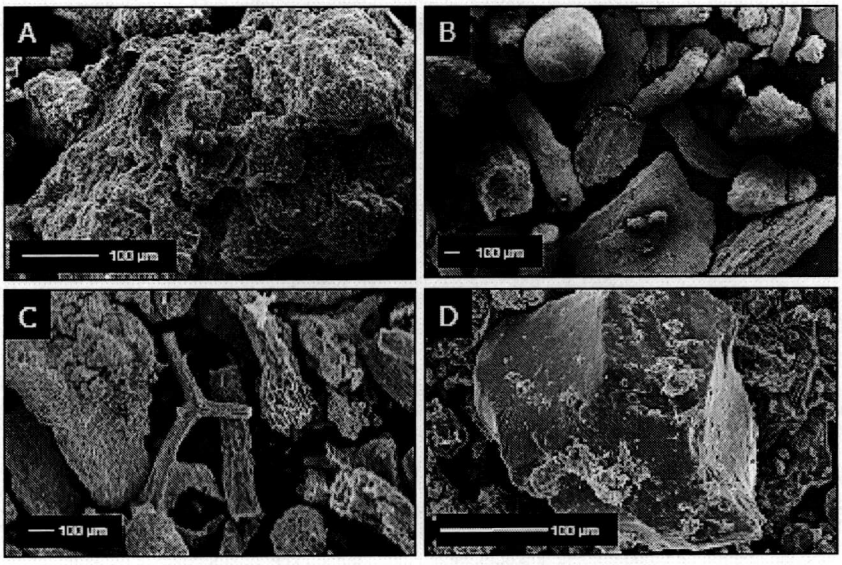

Figure 2-3. SEM images of a whole soil aggregate from Bousson (A), light fraction material from HJA (B), light fraction from Bousson (C), and heavy fraction from Bousson (D).

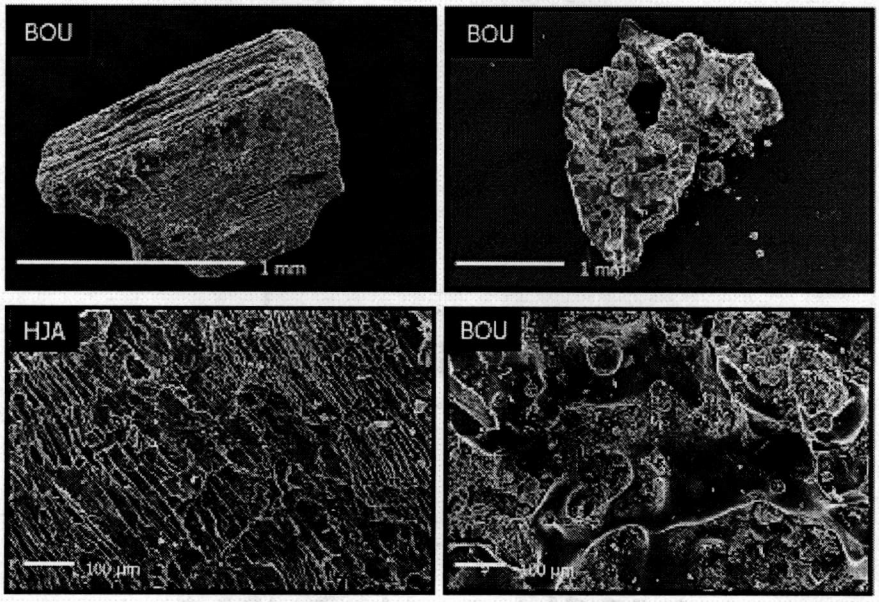

Figure 2-4. SEM images of different structures of charcoal made from plant biomass isolated from H.J. Andrews and Bousson soils; woody (right) and amorphous (left).

During the year incubation, losses of C as DOC in leachates were significantly greater for the summed fractions than the bulk soil at both sites during incubation (Figure 2-5). Total losses of C (DOC and respiration combined) were greater for the summed fractions than the bulk soil: summed fraction C losses were 70% of bulk soil at Bousson and 79% of bulk soil at HJA.

Inhibition of microbial respiration

In the first short incubation experiment (shaken in SPT, Na_2SO_4, or water and rinsed as for the density fractionation procedure) both the Na_2SO_4 and 1.6 g cm^{-3} sodium polytungstate (SPT) solutions resulted in significantly reduced soil respiration compared to the distilled water control ($p<0.001$, $F=31.80$) (Figure 2-6A). The greatest reduction in respiration occurred following shaking and rinsing with SPT (65 % of the control

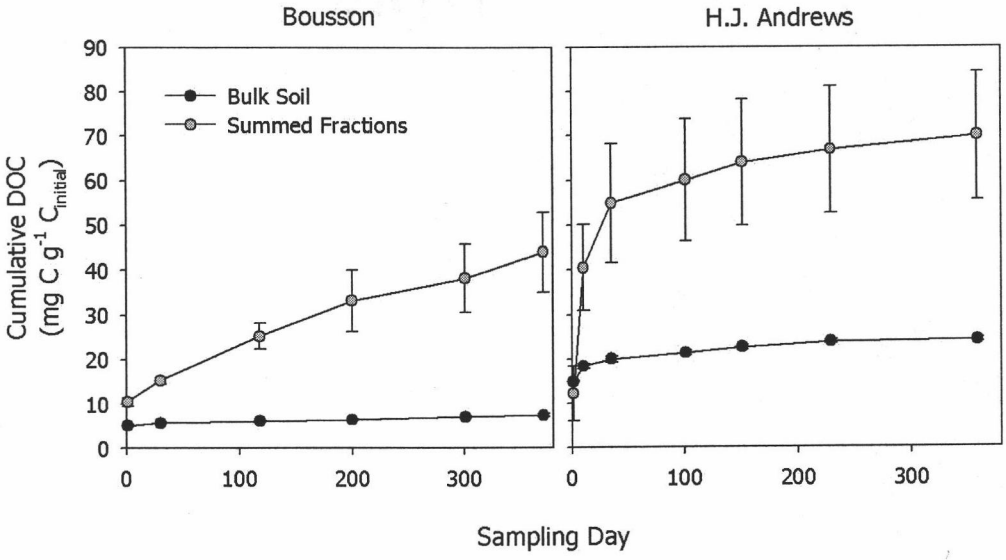

Figure 2-5. Cumulative DOC release from bulk soil and mathematically recombined density fractions during one year incubation, values are means ± one standard error.

value). Similar results were obtained from the second short incubation experiment when W was added to O_a horizon material. Both levels of W addition, which were in concentrations that spanned the amount of extractable W measured, resulted in reduced cumulative respiration compared to all other treatments ($p<0.001$, $F=249.04$) (Figure 2-6B); reduced to 35 % and 31 % of the control values low and high concentration of W respectively. In both experiments, the addition of a Na solution also significantly reduced respiration compared to the control (to 56 % of the control for the addition experiment and 85 % of the control for the shaken experiment).

The mean C:N of light fraction was 61.0 ± 6.1 at HJA, however, the addition of nutrient solutions to HJA Oa material did not result in increased respiration during the short incubation experiments. In fact, both the Hoagland's solution and the N solution significantly reduced respiration compared to the control ($p<0.001$, $F=249.04$) (Figure 6B).

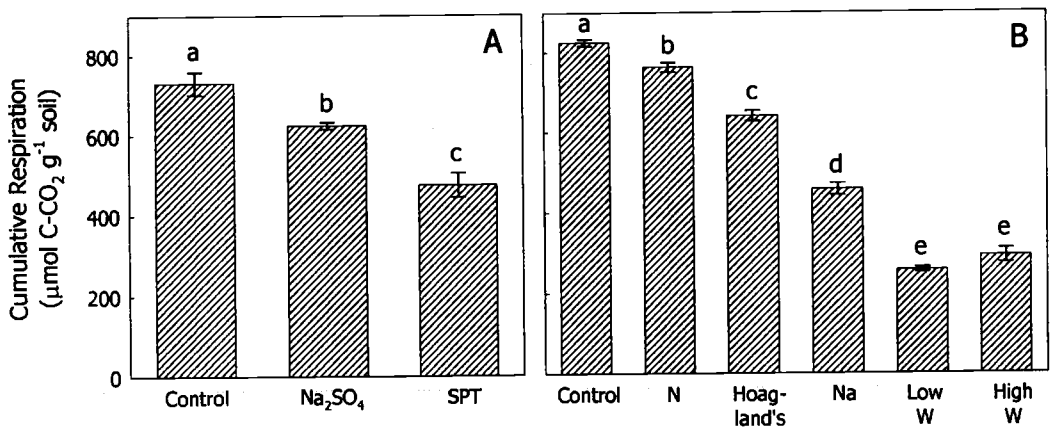

Figure 2-6. Cumulative respiration from O_a material from the H. J. Andrews field site during a 90- day incubation period following shaking and rinsing in solution (A) or adding nutrients or tungsten (B), values are means $\pm$ one standard error. Bars with different letters indicate significant differences between means; statistics were done separately for each experiment.

Recovery and mobilization in SPT solution

Soil from both sites had less than 4 % of the recovered mass in the light fraction (Table 2-1). After completing a mass balance accounting of the C and N, we found that at HJA 15 % of the C pool and 9 % of the N pool was recovered as LF (Figure 2-7). At Bousson, less of the C and N pools were present in LF than at HJA (9 and 5 % C and N respectively). A substantial pool of both C and N however, could not be accounted for in the recovered light or heavy fraction for either soil. We calculated that between 21 and 28 % of total soil C and N was mobilized in the SPT solution and subsequently lost during the density fractionation process in soils from both field sites (Figure 2-7).

Table 2-1. Percent light fraction (LF) and heavy fraction (HF) and C and N contents of the bulk soil (BU) and density fractions at both sites (n = 3, mean ± 1 standard error are reported).

	Bousson			H. J. Andrews		
% LF	2.2 ± 0.3			3.6 ± 0.6		
% HF	97.8 ± 0.3			96.4 ± 0.6		
	C	N	C:N	C	N	C:N
BU (%)	6.1 ± 0.3	0.5 ± 0.0	12.5 ± 0.6	6.8 ± 1.1	0.2 ± 0.0	34.3 ± 3.6
LF (%)	27.4 ± 4.3	1.1 ± 0.0	25.4 ± 5.4	28.7 ± 0.3	0.5 ± 0.0	60.7 ± 6.1
HF (%)	4.7 ± 0.3	0.4 ± 0.1	13.3 ± 1.8	4.9 ± 0.8	0.2 ± 0.0	29.9 ± 1.5

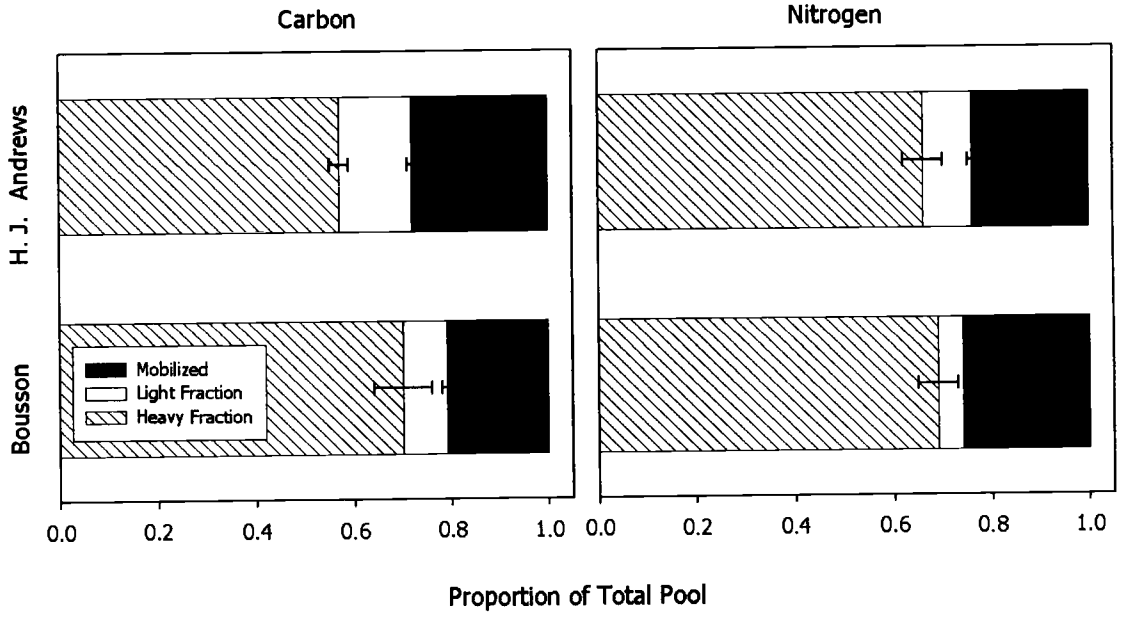

Figure 2-7. Mass balance of the total recovered and mobilized C and N pools, values are means ± one standard error.

Stable isotope fractionation

Stable isotopic analysis of the bulk soil and density separates indicate that, depending on the soil and the isotope, some degree of fractionation can occur during the density separation process. There was no difference in the C isotopic composition of the bulk soil and mathematically recombined density fractions from three different soil depths at HJA (Figure 2-8). Nor was there isotopic fractionation of either C or N during density fractionation of 0-5 cm mineral soil at Bousson. At HJA however, we found a consistent preferential loss of the light isotope (^{14}N) resulting in density fractions that were isotopically heavier than the original bulk soil from all depths, with an average shift of 1.3 ‰ (Figure 2-8). The change in the isotopic signal was related to the nitrogen concentration of the bulk soil ($p < 0.001$); fractionation was greater in samples with low nitrogen concentration (Figure 2-9).

Mean residence time of density fractions and mobilized organic matter

The mean turnover time of light fraction material, without BC included, at Bousson was 2.9 ± 0.7 y while heavy fraction was 317 ± 18 y ($n = 3$ for all means). At HJA, mean turnover time of light fraction was 111 ± 23 y and heavy fraction was 93 ± 12 y. The organic matter mobilized during density fractionation had a calculated turnover time much greater at Bousson (146 ± 52 y) than at HJA (19 ± 11 y).

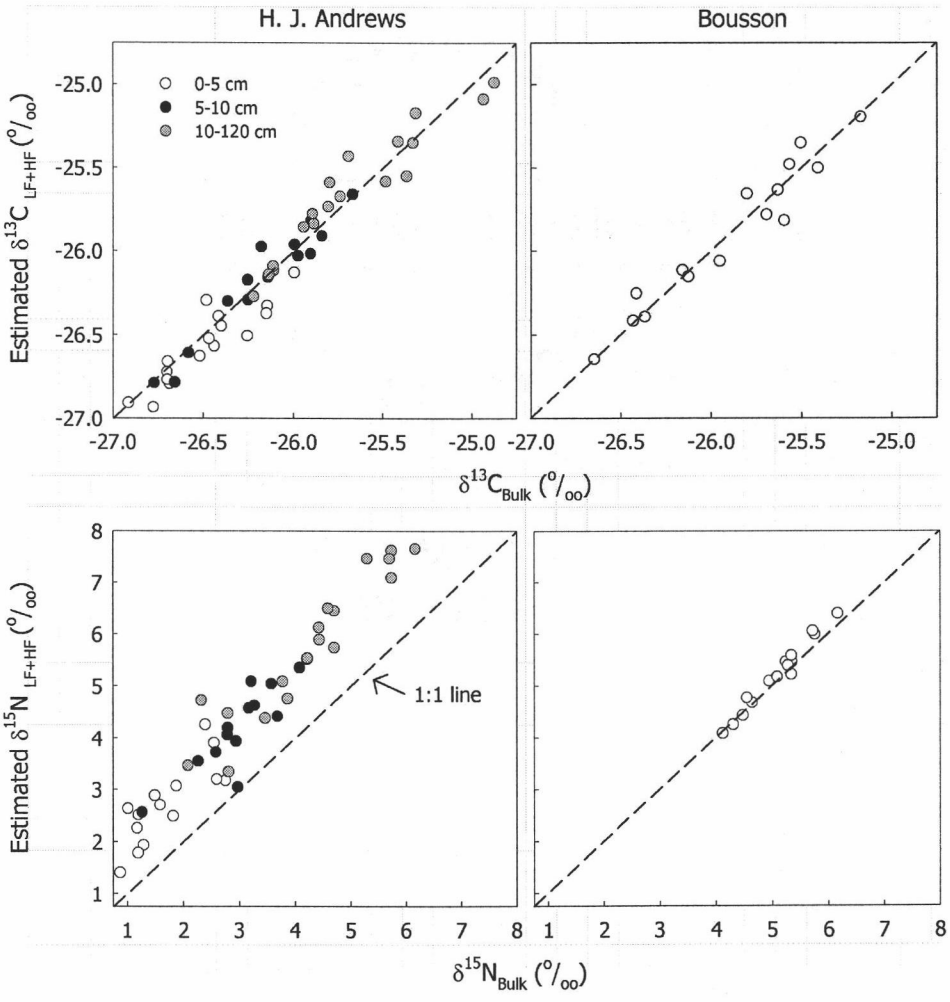

Figure 2-8. Isotopic composition of the bulk soil compared to the mathematically recombined density fractions for ^{13}C (upper panels) and ^{15}N (lower panels). Each point represents one sample.

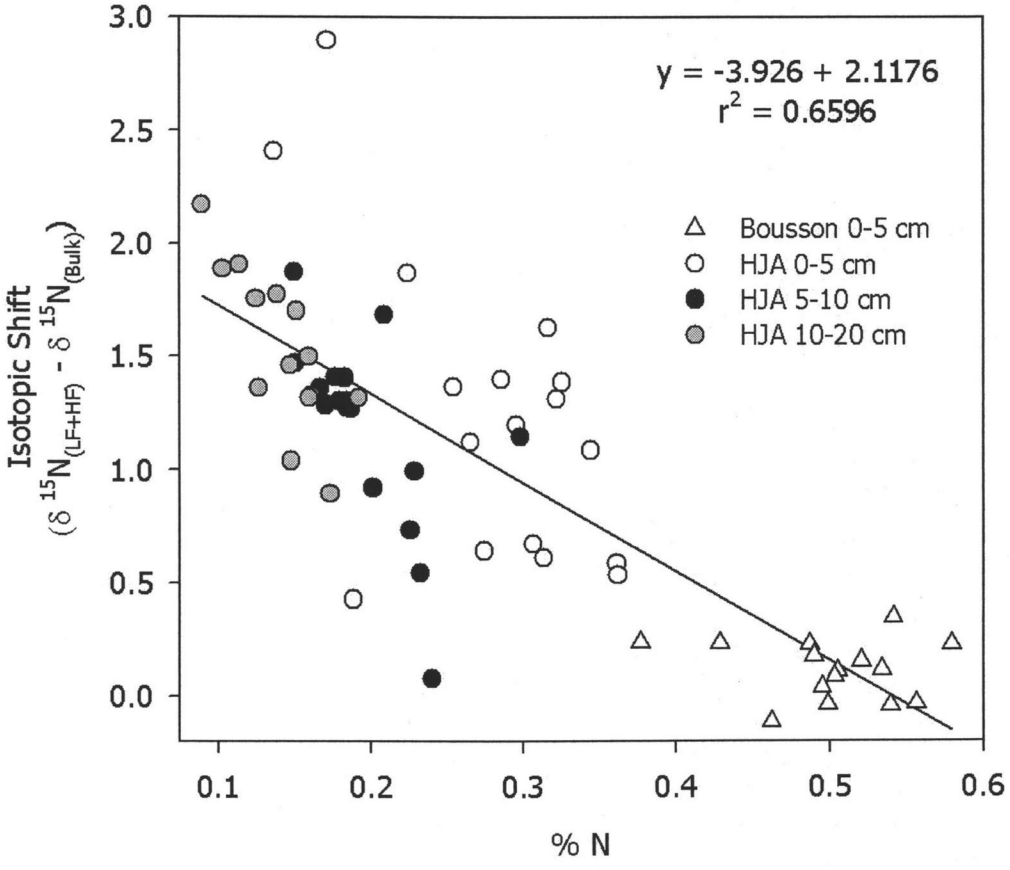

Figure 2-9. Relationship between bulk soil N content and isotopic fractionation during density separation.

DISCUSSION

Link to conceptual organic matter pools

During analysis of results from decomposition studies similar to ours, it is necessary to consider respiration results both in terms of the amount of a substrate incubated and in terms of the amount of organic carbon incubated. We found that cumulative respiration normalized by initial substrate mass (mg CO_2 g^{-1} substrate) was greatest from the light fraction, followed by the bulk soil and heavy fraction for both soils. This pattern is consistent with the conceptual definition of the light fraction as the most biologically active, and the heavy fraction as passive organic matter. However, when cumulative respiration was normalized by the initial amount of C present in the substrate (mg CO_2 g^{-1} $C_{initial}$), which is more indicative of the actual degradability of the C in the soil or fraction, bulk soil respiration from H. J. Andrews (HJA) soils was much higher than either light or heavy fraction respiration. Further, there was no longer any statistical difference between the respiration from light and heavy fractions. These trends are similar to those reported by Swanston et al. (2002), who incubated soils and density fractions from several Pacific Northwest forest soils for 300 d.

Patterns of respiration from the Bousson soils and fractions were similar to HJA when represented on the basis of initial substrate. Unlike HJA, however, Bousson light fraction respiration was clearly higher than the heavy fraction when normalized by initial substrate C. These results are more in keeping with the classic conceptual notion of how the fractions should differ (Greenland and Ford 1964). It is notable that the C-normalized respiration values from the fractions are more representative of the ^{14}C-based MRTs than the mass-normalized respirations: little difference between fractions at HJA, but faster turnover in the LF at BOU. We discuss below how the nature of the C inputs to the soil, and the nature of the soil itself, can influence the incubation and *in situ* turnover times.

Disruption of soil structure

At HJA, the present-day forest developed following a stand replacing fire approximately 550 y ago. Accordingly, soil contained up to 20% of total soil C in black carbon (BC), in the form of charcoal, which had a ^{14}C age of 770 y BP (± 45 y, analytical error). The fire history is not known at Bousson, but charcoal had a ^{14}C age of 630 y BP (± 40 y analytical error). Black carbon degrades at rates much slower than other light fraction material (Goldberg 1985), yet is recovered primarily in the light fraction during density separation. The presence of BC alters the apparent rate of respiration during incubation, particularly when using unit of CO_2 g^{-1} total C, since BC has a high carbon concentration. The appropriate method for the quantification of BC in forest soils depends on the form present, which can exist along a combustion continuum ranging from slightly burned biomass, to charcoal, to highly condensed soot (Hedges et al. 2000; and reviewed by Masiello 2004). Schmidt et al. (2001) found a 500-fold difference in black carbon content for the same sample quantified by several methods, and the issue of methodology is still being debated in the literature (Schmidt et al. 2001; Skjemstad et al. 2002; Masiello 2004; Simpson and Hatcher 2004).

We found that the light fraction at HJA was composed of woody debris (bark and roots), fungal fruiting bodies, and charcoal, which are typically considered recalcitrant materials. In 1964, Greenland and Ford described light fraction material as irregularly shaped plant fragments, small amounts of charcoal, and partially degraded plant material. Nonetheless, they measured the ash content of the light fraction at ~50 %, comprised of plant phytoliths, amorphous clay minerals, and quartz that had also floated during fractionation (Greenland and Ford 1964). In addition to mineral particles, light fraction material may also contain charcoal (Sollins et al. 1983; Golchin et al. 1994a; Baisden et al. 2002), which is typically chemically recalcitrant and has a high C concentration relative to the rest of the light fraction. In spite of the potential for charcoal to influence the actual and interpreted mean residence time of C in the light fraction, 'charcoal contamination' of light fraction has received little direct attention even in the current

literature. Ash content of the light fraction at Bousson was 42.5 % and at HJA was 31.0 %, indicating considerable mineral content in the light fraction at both sites. Organic coatings on the mineral particles in the light fraction may have had different chemistry and MRTs than the most of the light fraction (Sollins et al. 2006). Our modeled mean residence time (MRT) of 111 years for this fraction likely reflected the refractory nature of charcoal and some mineral-stabilized C; although woody inputs that are up to 500-y old (and could average 150-250 y) at this site also would increase the apparent MRT. In the Bousson light fraction, a MRT of 3 years confirmed the dominance of nutrient-rich leaf litter and fine root material inputs, which are more easily degradable than light fraction material at HJA. Black carbon may have also influenced the apparent recalcitrance of the light fraction at Bousson; however, the light fraction there totaled only ~15 % of total bulk soil C.

The role of soil structure in protecting C from loss as DOC was clear when incubating fractions and bulk soils. Dissolved organic carbon released during one year incubation of the summed density fractions was substantially greater than from bulk soil. Although DOC concentration was high in leachates from the density fractions, presumably the quality was low (since DOC leached from the fractions was not used by the microbial community as substrate) (Park et al. 2002). Dissolved organic carbon losses were greater from HJA (Andisols) than from Bousson (Alfisols) indicating a potentially larger pool of C which could be lost from soil following the disruption of soil structure or the displacement of organic matter from exchange sites by disruption of H-bonds or other electrostatic interactions between organic matter and minerals (Kleber et al. this issue).

Inhibition of microbial respiration

We hypothesized that some form of microbial inhibition contributed to the decrease in respiration from the summed density fractions relative to the bulk soil. Our results indicate that, although the C:N ratio of the light fraction is large (61.0 $\pm$ 6.1 at HJA), nutrient limitation was not likely to be a factor controlling the reduction of respiration

from the light fraction. In fact, during incubation of Oa material, we found a reduction of respiration in both nutrient addition treatments. H.J. Andrew's soil is dominated by a legacy of coarse woody debris inputs, sometimes creating organic horizons more than 50 cm deep. It is possible that the reduction in respiration when nutrients were added is due to deactivation the phenol-oxidase enzyme used by microbes for lignin degradation as a result of excess soluble N (Sinsabaugh et al. 2002; Waldrop et al. 2004) or a shift in allocation of metabolites from microbial respiration to microbial biomass or storage in the presence of available nutrients.

Results from the short incubation experiments suggest that residual Na and W reduce respiration in the initial stages of incubation, indicating that both Na and W present in the SPT solution may be influencing the ability of the microbial community to utilize the substrate, as previously suggested by Magid et al. (1996), Compton and Boone (2002), and Swanston et al. (2002). Extractable W from both soils was greatly reduced by the end of the incubation period, during which the substrates had been leached repeatedly. The greatest amount of extractable W left in any fraction was 1.2 ± 0.1 mg W g^{-1} soil, representing a 94.3 – 98.3 % decrease for all substrates. Thus, at some point during the incubation, the inhibition of respiration by residual W was most likely reduced or eliminated. This transition may have occurred around day 200 in the Bousson soils as indicated by a slight inflection upward of the cumulative C loss curve accompanied by an increase in the absolute rate of respiration. This shift is not obvious in the HJA soil, however, and there is no direct way to estimate the degree to which residual SPT reduced respiration over the course of the long-term incubation. Leachate chemistry showed measurable concentrations of NH_4^+ in the soil solution during the year long incubation period for all bulk soil and density fractions (data not shown). Therefore, any inhibition due to Na saturation of exchange sites was likely released over the course of the incubation period as Na was exchanged into solution with other cations release during decomposition.

During a similar long-term incubation of whole soil and density fractions, Swanston et al. (2002) found initially that microbial biomass was greater in the bulk soil than in the density fractions even though all substrates had been inoculated. By day 10 however, the light and heavy fraction had over 5 times the active microbial biomass of the bulk soil, although the rate of respiration (mg CO_2 g^{-1} $C_{remaining}$ d^{-1}) from the density fractions remained approximately half of the whole soil (Swanston et al. 2002). The disparity in respiration rate between the bulk soil and the fractions had disappeared by 120 d. It seems that although W is initially inhibitory, it may not have a lasting effect on the rate of respiration per unit C, though not necessarily per unit biomass. Nonetheless, the inhibition of respiration related to Na and W toxicity in our soils was probably not strong enough to account for the substantial reduction of cumulative respiration from the summed density fractions.

Organic matter mobilization

A significant amount of C and N was mobilized and subsequently discarded during the density separation process. Soil from HJA displayed a strong isotopic fractionation of N, with a preferential mobilization of the light isotope especially in soil with low N concentration, associated with the pool of OM lost during fractionation. We did not find a similar preferential mobilization of the light isotope of C at either site nor of the light N isotope at Bousson. It is not clear why the isotopic fractionation of N occurred in the HJA soil alone and not Bousson. Given that the same separation procedures were used on both soils, we speculate that the isotopic fractionation was soil dependent rather than methodologically dependent. Ignoring this potential for isotopic fractionation may lead to misinterpretation of N fluxes, and C and N interactions. Conversely, focus on this fractionation may reveal patterns that are otherwise obscured.

Overall, both of our soils demonstrated a reduction in cumulative respiration (mg C-CO_2 g^{-1} $C_{initial}$) between the bulk soil and recombined density fractions (summed fraction) following the one-year incubation (32.2-43.0 % lower than bulk soil at Bousson and 44.9

– 67.0 % lower at HJA). At Bousson, a large initial flush of respiration from bulk soil established the difference in the cumulative C loss curve, which then persisted for the duration of the incubation period. Without the initial flush of respiration from the bulk soil, most likely cumulative respiration would have been similar between bulk soil and summed fractions. Conversely, HJA bulk soil and summed fractions had different rates of respiration continuously during the one-year incubation and, as a result, cumulative C loss continued to diverge during the incubation.

We hypothesized that density fractionation caused the mobilization of a labile C fraction that originated from recent detrital inputs and would have otherwise easily decomposed during the incubation period. At Bousson, the mobilization of a labile C pool associated with the large initial flush of respiration from bulk soil could account for the decrease in respired C from the summed fraction. However, we found that the MRT of the mobilized organic matter pool was much longer at Bousson than at HJA (146 y compared to 19 y). Such a long MRT of this pool at Bousson indicates that the mobilized pool was not C primarily from fresh detrital inputs, as we had hypothesized. However, for the soil from HJA, the MRT of the mobilized pool was substantially shorter than either density fraction. At HJA, the mobilization and loss of this fast cycling pool may have contributed substantially to the difference in respiration from the summed fractions compared to the bulk soil over the course of the incubation. Conceptually, if 25 % of organic C had not been lost during fractionation and ~ 5 % (1/MRT) of this pool had been respired during the year incubation period (equation below), then an additional 29.3 mg $C\text{-}CO_2$ g^{-1} $C_{initial}$ would have been added to the cumulative C lost through respiration. This represents an amount approximately equal to the gap between respiration from bulk soil and summed fractions.

$$\text{Estimated total } CO_2 = \frac{CO_2 \text{ respired}_{SF} + \left[\left(\frac{1}{MRT}\right) * (\text{C lost})\right]}{[C]_{BU}},$$

where estimated total CO_2 is the total respiration expected if the organic matter mobilized in SPT during density fractionation had not been lost, and CO_2 respired $_{SF}$ is the cumulative mg C respired g^{-1} summed LF + HF soil, C lost is the amount of C (mg g^{-1} soil) lost during density fractionation, and $[C]_{BU}$ is mg C g^{-1} bulk soil.

Based on this estimate, summed fraction respiration at HJA would increase from being on average 63 % of bulk soil respiration to 102 % of bulk soil. By the same calculation, using MRT of 146 y, summed fraction respiration at Bousson would only increase from 57 % of bulk soil to 58 % of bulk soil. At HJA, the organic matter mobilized and lost during density fractionation was a labile organic matter pool originating from fresh inputs, which, in the bulk soil, was available for microbial decomposition throughout the incubation period. The loss of this organic matter pool is likely to be an important mechanism accounting for the difference in respiration between the bulk and summed density fractions at HJA. At Bousson, the organic matter mobilized during density fractionation may have been readily degraded, but not originating from fresh inputs. This scenario may indicate microbial usage of older organic matter for substrate (Trumbore 2006), particularly following soil drying and rewetting.

Andisols are characterized by the presence of alumino-silicate clays with high surface area, reactivity, and capacity to sorb organic anions (Tan 1984). Although the presence of allophane was not detected by Sollins et al. (2006), the soils at the HJA DIRT site were moderately reactive in NaF. Also, Lajtha et al. (2005) described a high capacity for the same soil to sorb and stabilize recent inputs of hydrophobic dissolved organic matter within the upper mineral horizons. Desorption of fresh, labile organic matter during density separation could account for the relatively short MRT of the OM pool lost from the Andisol compared to the Alfisol. The similarity in MRT of the light and heavy fractions at HJA is reflective of both the refractory material in the light fraction and the high capacity of the andic soil for sorption of fresh DOC inputs. This also implies a direct link between detrital inputs and minerals, perhaps at an intermediate region of organo-mineral contact (where hydrophobic interactions influence the structure of

organic matter binding to minerals) as described by Kleber et al (this issue). These hydrophobic bonds are strong and survived density separation intact, whereas all the easily exchangeable OM in the outer region (where electrostatic bonds between organic compounds allow for easy exchange with organic matter in solution) was lost during fractionation. At HJA, these compounds were from fresh inputs (MRT 19 y), though at Bousson, these bound outer region molecules were not.

Further considerations

Although the simple division into 2 density fractions appears to have resulted in quantifiable pools with different mean residence times for the Bousson soil, this method is clearly not appropriate for separating active and passive C fractions from the HJA soil. At our nutrient-rich, deciduous site, mean residence time of the light fraction reflected the traditionally defined "active" fraction while the heavy fraction reflected the "passive" heavy fraction. At HJA, both the light and heavy fractions are more like an intermediate cycling pool with a turnover time of around a century. Our results indicate that any method using a step of density fractionation will have the potential for large organic matter losses and for isotopic fractionation. Although we did not find isotopic fractionation of C isotopes at either site, the relationship between fractionation and low N concentration implies the possibility for C isotope fraction in soils with low C concentration. This possibility will complicate isotopic studies in soils using light and heavy fractions to interpret isotopic shifts with time.

Density fractionation is a powerful step to include in methods for quantitatively isolating SOM into fractions with relevance to the function of a soil and should not be disregarded. Particularly since the use of density fractionation has expanded since the introduction of SPT, the implications of dispersing soil in SPT should be addressed directly and used to our advantage in the interpretation of results. For example, even if direct measurements of the mobilized OM are not possible, properties should be estimated or calculated by difference and interpreted as an additional organic matter fraction. Organic matter lost

during dispersion in SPT will be a function of many aspects of a soil including the chemical nature of detrital inputs, soil structure, mineralogy, and land use history; interpretation of density fractions placed back into a biologically active environment should be done within the context of an individual soil.

Acknowledgments

We thank Sarah Beldin, Dave Dinette, Nella Parks for laboratory assistance. Al Soeldner provided access to SEM at the Oregon State University facility. Rich Bowden is the curator of the Bousson DIRT site and provided soil for this study. Elizabeth Sulzman provided mentorship to Heath Keirstead during her thesis work, some of which was presented within this manuscript. Bruce Caldwell, Phil Sollins, and Mark Johnson provided helpful comments on the manuscript. This project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2002-35107-12249 and by National Science Foundation (NSF), Division of Environmental Biology (DEB) grant number 0087081. Support to H.J. Andrews Experimental Forest and to this project was provided by H. J. Andrews Long Term Ecological Research program, funded by NSF-DEB. This work was performed in part under the auspices of the U.S. Department of Energy by University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48. This manuscript has been subjected to the Environmental Protection Agency's peer and administrative review, and it has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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CHAPTER 3

SOIL ORGANIC MATTER LABILITY AT TWO FORESTED SITES FOLLOWING MANIPULATION OF DETRITAL INPUTS

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~ Soil Biol & Biochem 2006 (38) 3279-3291

ABSTRACT

Changes in land use or environmental factors, such as atmospheric CO_2 concentration and temperature, will alter the quantity and quality of detrital inputs to the forest floor, which may translate into changes in soil organic matter (SOM) accumulation and stabilization over time. We determined the effect of manipulating the amount and type of detrital inputs on SOM lability and mean residence time at two forested sites that are part of the Detrital Input Removal Treatment (DIRT) Project. Surface mineral soil was collected from a deciduous site (Bousson) and a coniferous site (H.J. Andrews) and separated into organic matter fractions using density fractionation (in 1.6 g mL^{-1} sodium polytungstate solution). Bulk soil and density fractions were incubated for one year to determine organic matter lability. Radiocarbon-based estimates of mean residence time (MRT) of the organic matter fractions were made. Root exclusion treatments have increased the proportion of C and N within the light fraction material at Bousson but decreased the proportion of N within the light fraction at H.J. Andrews. The MRT of light fraction material at Bousson was short (~ 3 years) for the control and doubled aboveground litter treatments compared to the detrital removal treatments (78-185 years). Heavy fraction at Bousson had a MRT of 255 y. At Bousson, the greatest amount of cumulative CO_2 loss during incubation occurred from the light fraction of soil from the doubled litter treatments, while cumulative respiration was not different between the light and heavy fraction material of the litter removal treatments; these patterns are in accordance with the MRT estimates. In contrast, neither MRT nor cumulative respiration from density fractions at H.J. Andrews has changed in response to the manipulation of detrital inputs. In addition, cumulative respiration was not different between light and heavy fraction material from H.J. Andrews; patterns which also are in accordance with the MRT estimates for the fractions. At H.J. Andrews, light fraction organic matter from soil with both roots and aboveground litter excluded had the least amount of DOC release and CO_2 respired during incubation. As we expected, manipulations of detrital inputs has influenced the light fraction organic matter first, particularly at Bousson. The results from the coniferous site may be due to a legacy of historically large inputs of coarse

woody debris on the LF and it may be decades before the signal of detrital manipulations can be measured. Alternatively, these highly andic soils may be accumulating C rapidly, yielding young HF ages and C that does not differ substantially in lability from coniferous litter-derived LF.

INTRODUCTION

Non-living organic matter in soil is the terrestrial biosphere's largest pool of organic carbon (C) and is an integral part of C cycling globally (Schimel 1995). The amount of organic matter that enters the soil, as well as the chemical quality of those detrital inputs, varies in response to environmental factors such as changes in climate and land-use (Jobbágy and Jackson 2003). Changes in the rate of SOM formation or decomposition, whether due to climate change, land management, fire, or other disturbance factors, may lead to either greater C sequestration in soil or greater C efflux to the atmosphere. A better understanding of the controls on stabilization and retention of soil C and nitrogen (N) is needed to improve model predictions for changes in forest soil processes due to alternate management regimes, in global C budgets due to shifts in climate conditions, and in forest responses to atmospheric N inputs. In particular, a shift in detrital inputs may alter the net accumulation or loss of C in the soil organic matter pool (Boone et al. 1997) as well as the abundance and lability of specific organic constituents (Kögel-Knabner 2003). Many studies have considered aspects of litter decay, biota, organic composition, and turnover rate in soils; however, few have conclusively determined links among detrital input quantity and quality and soil organic matter formation and stability.

Soil organic matter composition can usually be traced directly back to the type of plant detrital inputs to the soil surface (Kögel-Knabner 2002). Generally, plant litter decomposition rate is determined by the chemical composition (Currie and Aber 1997, Almendros et al. 2000), for example, decomposition correlates positively with initial N and P content (Berg 2000) and negatively with polyphenols (including tannins and lignin) (Palm and Sanchez 1991, Loranger et al. 2002) and lignin/N ratio (Mellilo et al. 1982).

Overall, C/N ratio may simply be the best predictor of litter decomposition (Edmonds 1980, Enriquez et al. 1993). However, across wide ranges of vegetation types, patterns in the link between litter quality and decomposition have not emerged (Preston et al. 2000). On a localized scale, for example within a forest vegetation type, litter quality and decomposition influence the chemical characteristics of soil organic matter and contribute to the stabilization or destabilization of soil organic matter in the terrestrial pool. Changes in the amount and type of litter inputs to the forest floor will impact nutrient cycling and organic matter formation.

Inspired by an ongoing experiment started in 1957 in forest and grassland ecosystems at the University of Wisconsin Arboretum (Neilson and Hole 1963), a collection of long-term manipulative field experiments were established to examine effects of altering the quantity and quality of plant litter (above- vs. below-ground source, C and N quantity, and chemical quality) on the stabilization and retention of soil organic C and N. The central goal of this ongoing experiment, called the Detrital Input and Removal Treatment (DIRT) Project, is to assess how rates and sources of plant inputs control the accumulation and dynamics of SOM and nutrients in forest soils over decadal timescales (Nadelhoffer et al. 2004). Ultimately, as decomposition of detrital inputs and DOC transport down the soil profile continues, the chemical properties of the inputs are transferred to soil organic matter pools. By changing the quantity and quality of detrital inputs to the forest floor at several field sites, we hope to understand the mechanisms through which plant inputs become soil organic matter and are retained in or lost from the terrestrial carbon pool at each DIRT site. The suite of mechanisms controlling organic matter stabilization (recalcitrance, organo-mineral interactions, and microbial accessibility) is common across sites; however, the relative importance of each may be different and will depend in part on the quality of detrital inputs, the transfer of those inputs to the mineral soil (by leaching and physical mixing), and the propensity for a soil minerals to interact with organic matter.

We determined whether altering detrital inputs at two DIRT sites had yet resulted in changes in the amounts and decomposability of organic C and N within different organic matter pools. Density fractionation was used to separate soil into two organic matter fractions. A year-long incubation experiment was conducted to determine the decomposability of the bulk soil and density fractions and radiocarbon-based mean residence times were also calculated. We had several hypotheses: 1) The addition of litter inputs would result in greater amounts of C in the light fraction material and in greater lability of the light fraction material during decomposition. 2) Since, at H. J. Andrews, doubling wood inputs has resulted in an increase in soil solution DOC near the surface that is stabilized in the soil via abiotic sorption (Lajtha et al. 2005), we expected that soil with double wood inputs would be more resistant to decomposition, however, that the disruption of soil structure during density fractionation would result in a greater loss of DOC. 3) N leaching during incubation would be greatest from root removal treatments, which have resulted in elevated dissolved inorganic nitrogen in soil solution (Lajtha et al. 2005), but that SOM from root removal treatments would be the least labile and most resistant to decomposition. 4) Altering detrital inputs would result in differences in organic matter decomposability and mean residence time of the light fraction before the heavy fraction.

METHODS

DIRT Experimental Treatments

DIRT plots were established in a black cherry (*Prunus serotina*) and sugar maple (*Acer saccharum*) dominated mixed-deciduous forest in Bousson Experimental Forest, PA, USA in 1991and a coniferous Douglas-fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), and western red cedar (*Thuja plicata*) dominated forest in H.J. Andrews (HJA) Experimental Forest, OR, USA in 1997. The sites have different environmental (temperature, precipitation), biotic (vegetation type, succession stage), pedogenic, and N deposition characteristics. The treatment plots are replicated three

times at each site and consist of treatments that either add or remove litter inputs to the soil by screening and sweeping for removing aboveground inputs and trenching for removing root inputs (Table 3-1).

Table 3-1. Treatments and methods of the Detritus Input and Removal Treatment (DIRT) experimental plots, each are replicated 3 times.

Treatment		Method
Control	(CTL)	Normal litter inputs are allowed.
No Litter	(NL)	Aboveground inputs are excluded from plots.
Double Litter	(DL)	Aboveground leaf/needle inputs are doubled by adding litter removed from No Litter plots.
Double Wood	(DW)	Aboveground wood inputs are doubled by adding large shredded wood pieces based on measured input rates of woody debris fall (H.J. Andrews only).
No Roots	(NR)	Roots are excluded with impermeable barriers extending from the soil surface to the top of the C horizon.
No Inputs	(NI)	Aboveground inputs are prevented as in No Litter plots, belowground inputs are prevented as in No Roots plots.

Site Descriptions

The Bousson DIRT experimental plots are located in a nutrient-rich mixed deciduous forest located in northwestern Pennsylvania, USA within the Bousson Environmental Research Reserve owned by Allegheny College. The site is dominated by black cherry and sugar maple in the overstory and by small maple saplings (<5 cm diameter at 1.35 m height) in the understory (~ 80 y old stand). Total aboveground biomass is 434 Mg ha^{-1} of which 60 % is black cherry and 28 % is sugar maple. Groundcover is extensive and includes maple seedlings, mayapple (*Podophyllum*) and troutlily (*Erythronium*) (Bowden et al. 2000). Litterfall is 2.1 Mg C ha^{-1} y^{-1} (Bowden et al. 1993) and during the late summer, before annual leaf fall, the litter layer is 0-2 cm thick on the forest floor. Annual atmospheric N deposition for the site is 10-12 g N m^{-2} and total soil N is high, 10,000 kg ha^{-1} (Bowden et al. 1996). The experimental plots are located on a slight slope (~5 %) at

an elevation of 390 m. The climate is temperate with a 4 month growing season. Snow cover is also about 4 months annually and precipitation (105 cm yr^{-1}) is spread evenly throughout the year. Soils are derived from glacial till overlying shale and sandstone and are classified as Alfisols in the Cambridge series (USDA-SCS 1979). The soils have high sand and low clay content and the texture is silty loam (75% sand, 23% silt, 2% clay). A fragipan is present at 60 cm and the bulk density of the 0-10 cm depth increment is 0.5 g cm^{-3}. Soil pH in the top 6 cm of the A horizon is 4.0 and CEC of the upper 15 cm of mineral soil is 3.73 $cmol_c$ kg^{-1} (Bowden et al. 2000).

The HJA DIRT site is located in a temperate rainforest within the H.J. Andrews Experimental Forest in the Cascade Mountains of west-central Oregon, USA (44°15'N, 122°10'W). The canopy is dominated by mixed old-growth Douglas-fir, western hemlock and western red cedar (*Thuja plicata*). Understory species include western hemlock, Pacific yew (*Taxus brevifolia*), vine maple (*Acer circinatum*), Pacific dogwood (*Cornus nuttallii*), huckleberry (*Vaccinium* spp.), and sword fern (*Polystichum munitum)* (Sollins et al. 2006). A major stand-replacing fire ca. 1500 was followed by the establishment of Douglas-fir trees, many of which are still present in the overstory. Coarse woody debris and moss lcover extensive areas of the forest floor, as is typical of old-growth stands. Also, epiphytic lichens (primarily *Lobaria oregana*) fall from the forest canopy and litter the forest floor. Atmospheric deposition in this region is low, ~0.2 kg ha^{-1} y^{-1} (Vanderbilt et al. 2003). The experimental plots are located in a stand growing on relatively flat and stone-free soil compared to the surrounding hill slopes of the watersheds of the H.J. Andrews (726 m elevation). Temperatures averaged 8.8°C and annual precipitation was 2200 mm during 1974-2003 (Sulzman et al. 2005). The climate is Mediterranean, with dry summers and a cool wet season between October and March. Seventy percent of annual precipitation occurs during the wet season, mostly as rain (Sollins et al. 1980). The soils are derived from volcanic parent material, are andesitic/balsatic in chemistry and were recently described as coarse loamy mixed mesic Andic Dystrudepts (Sollins et al. 2006). Soil pH is 5.2 at 0-10 cm and soils from this site

have high amorphous Al hydroxide and aluminosilicate content and a high pH in 1 M NaF (10.7) at 40-50 cm (Yano et al. 2004).

Soil collection and density fractionation

Mineral soil (0-5 cm) was collected in June 2002 from HJA and in June 2003 from Bousson. Briefly, sub-samples were collected from six 5 cm^{-3} areas per each of the DIRT experimental plots and were composited for a total sample size between 500-1000 g from each plot (see Crow et al. 2006 for a detailed description). Each composited sample was sieved moist to remove material >2 mm and stored at 4 °C in tightly sealed bags for several weeks before and during the density fractionation procedure.

Soil was divided into two organic matter fractions based on floatation in a 1.6 g cm^{-3} solution of sodium polytungstate (SPT, SOMETU, Van Nuys, CA). Soil was added to the SPT solution in a ~ 1:3 soil:SPT ratio, shaken for one hour, and allowed to settle gravimetrically for 48 hr. Light fraction was aspirated (Strickland and Sollins 1987) and rinsed with distilled, deionized water on a pre-combusted Whatman GF/F (0.7 micron pore size) filter. The shaking, settling, and aspiration cycle was repeated until no light fraction remained floating, typically after three cycles. Heavy fraction was rinsed by repeated centrifugation and re-suspension of material in distilled-deionized water; typically for four cycles (see Crow et al. in review for a complete description of methods).

Incubation

Bulk soil, light, and heavy fractions from each plot (therefore n = 3 for each detrital treatment) at each site were incubated for 1 y in bench top filtration units (Falcon Filter, Becton Dickinson Labware) modified according to Nadelhoffer (1990). Approximately 20 g of bulk soil, 6 g of light fraction, or 30 g of heavy fraction material was mixed with acid-washed sand (Swanston et al. 2002) and incubated. At the start of the incubation,

the sand and soil mixture was re-wetted by adding 10 mL of inoculum solution prepared from fresh soil of the respective site shaken in distilled water for 1 hr (1:10 soil:water). Moisture content was kept constant by adding distilled, deionized water to each chamber weekly to maintain a known weight over the incubation period.

CO_2 efflux from the incubating soil and organic matter fractions was measured for each chamber on days 3, 5, 8, 12, 17, 26, 53, 151, 267, and 361 for HJA and days 2, 5, 13, 20, 28, 64, 114, 208, 290, and 367 for Bousson. Headspace was purged with CO_2-free air and sealed for approximately 240 minutes while respired CO_2 accumulated. A 500 µL-calibrated syringe was used to mix the headspace gas several times before extracting a sample, which was immediately injected into a 5700A Hewlett Packard gas chromatograph fitted with a Poropak R 80/100 column and thermal conductivity detector. Cumulative respiration was calculated for each substrate in SAS (SAS Institute, v. 9.1, Cary, NC) using PROC EXPAND to calculate and approximate the area under the curve using the trapezoidal method.

Water-soluble leachates (total dissolved nitrogen (TDN), dissolved organic carbon (DOC), nitrate (NO_3^-), and ammonium (NH_4^+)) were measured in soil solution leached under vacuum pressure from each chamber on days 10, 35, 101, 151, 267, and 361 of the incubation for HJA, and 30, 118, 200, 301, and 371 for Bousson. One hundred mL of deionized water was added to the upper chamber of the microlysimeter and allowed to equilibrate with the soil for one hour. At the end of an hour, the solution was drawn through the soil and filtered through a pre-combusted Whatman GF/F filter (0.7 µm pore size). Soil solution samples were kept at 4 °C until analysis if within 48 h, otherwise were frozen at –20 °C. Dissolved organic carbon analysis was by Pt-catalyzed high-temperature combustion (Shimadzu TOC-V CSH analyzer). The hydrazine sulfate reduction method was used for NO_3^- determination and the Berthelot reaction method with an Orion Scientific AC 100 continuous flow auto-analyzer (Westco Scientific Instruments, Inc., Danbury, CT) was used for NH_4^+. Total dissolved nitrogen was measured by high-temperature combustion (Shimadzu TOC-V CSH analyzer with TN

unit). Dissolved organic nitrogen (DON) was calculated as the difference between TDN and $NO_3^- + NH_4^+$).

Elemental analysis

Carbon concentrations of soil and density fractions were determined by dry micro-Dumas combustion (NA1500 C/H/N Analyzer, Carlo Erba Strumentazione, Milan) at the Stable Isotope/Soil Biology Laboratory of the University of Georgia, Institute of Ecology.

Black carbon quantification

Light fraction material (~ 1.5 g) from each treatment plot was digested in 175 mL of distilled, de-ionized water, 5 g $NaOCl_2$, and 5 mL acetic acid on a rotary shaker (240 rotations per minute) for 2 hr at room temperature for 3 digestion cycles (adapted from Simpson and Hatcher 2004). Following the final cycle, the residue was poured onto a pre-combusted Whatman GF/F (0.7 μm pore size) and rinsed thoroughly with distilled water (total rinse volume of 400 mL). The residue was then passed through a 500 μm sieve to separate fine particulate material and mineral soil from the larger organic matter fragments. This step allowed the larger fragments to remain mostly free of mineral coatings that could make identifying BC, which is present at our sites in the form of charcoal with visible plant morphology, more difficult in subsequent steps. Radiocarbon analysis (below) determined that the < 500 μm material was modern (containing bomb-carbon in amounts similar to recent litter); therefore, we assumed that little BC material was lost during the size fractionation. Charcoal pieces were manually separated from 2 subsets of the >500 μm residue under a dissecting microscope with a pair of fine-pointed forceps. The subsets were averaged to calculate a total recovery of BC for each sample. Since the presence of charcoal in the soil pre-dated the establishment of our experiment, all recovered BC material from each site was combined together to have enough material for accurate radiocarbon analysis.

TMAH

Tetramethylammonium hydroxide (TMAH) thermochemolysis gas chromatography / mass spectroscopy (GC/MS) was used to determine the chemical composition of light fraction material and charcoal. TMAH is a pyrolysis method that depolymerizes and methylates organic molecules present in organic material through hydrolyzation and methylation of esters and ether linkages. Methylated polar molecules can then be volatilized and quantified by GC-MS (Filley 2000). TMAH is highly specific for certain polar groups including esters, phenols and acids and results in information about the molecular composition of litter debris or soil organic matter (Chefetz et al. 2000).

TMAH thermochemolysis yields several classes of molecules: 1) Methylated *p*-hydroxyphenyl, guaiacyl, and syringyl compounds (from lignin biomacromolecule structures), 2) Non-lignin derived aromatics 3) Heterocyclic N (e.g., pyrroles, pyridines, and pyrazoles) compounds that appear to be either refractory or biologically unavailable in the soil and are protein derived and 4) FAMEs, fatty acid methyl esters, present as saturated, unsaturated or branched C chains of variable length (C_7-C_{27}) originating from aboveground litter inputs (cutin), belowground root inputs (suberin), or microbial byproducts. Aliphatics are often non-hydrolyzable and can be preserved in soils and sediments with minor structural alterations (Chefetz et al. 2000). Eleven lignin-derived TMAH GC-MS products (Table 3-2) and total FAMEs were identified and quantified.

Mean residence time and radiocarbon dating

Radiocarbon content was determined at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory, CA with a Van de Graaff FN accelerator mass spectrometer (AMS). Samples were prepared for analysis by combustion of organic C to CO_2 with CuO and powdered Ag in sealed evacuated tubes and subsequent reduction of the CO_2 onto iron powder in the presence of H_2 (Vogel et al.

Table 3-2. TMAH GC-MS products identified.

	Guaiacyl-derived compounds
G4	3,4-dimethoxybenzaldehyde
G5	3,4-dimethoxyacetophenone
G6	3,4-dimethoxybenzoic acid, methyl ester
G7	*cis*-1-(3,4-dimethoxyphenyl)-2-methoxyethylene
G8	*trans*-1-(3,4-dimethoxyphenyl)-2-methoxyethylene
G14	*threo/erythro* 1-(3,4-dimethoxyphenyl) 1,2,3-trimethoxypropane
G15	*threo/erythro* 1-(3,4-dimethoxyphenyl) 1,2,3-trimethoxypropane
	Syringyl-derived compounds
S4	3,4,5-trimethoxybenzaldehyde
S6	3,4,5-trimethoxybenzoic acid, methyl ester
S7	*cis*-1-(3,4,5-trimethoxyphenyl)-2-methoxyethylene
S8	*trans*-2-(3,4,5-trimethoxyphenyl)-1-methoxyethylene

1984). Radiocarbon data are expressed according to Stuiver and Polach (1977) as $\Delta^{14}C$, the deviation in parts per thousand from the absolute international standard activity (^{14}C:^{12}C ratio of oxalic acid corrected for decay since 1950). $\delta^{13}C$ values for each fraction was independently measured and used to adjust the $\Delta^{14}C$ values for mass-dependent fractionation. Mean residence times (MRT) of density fractions were calculated with a time-dependent steady-state model (Trumbore et al. 1995, Gaudinski et al. 2000). Three assumptions of the model are: 1) bulk C inputs equal loss in each pool at each time step; 2) the $\Delta^{14}C$ of inputs to the light and heavy fraction pools are equal to that of the atmosphere in the previous year; and 3) the $\Delta^{14}C$ of inputs to the pool attributed to OM mobilized during fractionation (SPT-loss) was mass-weighted between the light and heavy fractions. Yearly atmospheric $\Delta^{14}C$ values used in the model were based on three chronologies, beginning in calendar year 1511 (Stuiver et al. 1998; Hua and Barbetti 2004; Levin and Kromer 2004).

Since the radiocarbon content in the bulk soil and light fraction were influenced by the inclusion of BC in the soil, BC was quantified for each sample, BC $\Delta^{14}C$ was determined, and bulk soil and light fraction were mathematically adjusted to exclude the influence of the BC on the C pool size and $\Delta^{14}C$.

Statistical Analysis

Mean values of cumulative respiration and DOC, DON, NO_3^-, and NH_4^+ release in the laboratory incubation experiments for the treatments were compared using a completely randomized design ANOVA. For comparisons made between density fractions, a repeated measures ANOVA model was used. Before accepting the results of the statistical model, the model residuals were examined for constant variance. The Tukey-Kramer HSD method was used for comparison of means if a significant p-value was found. Significance for the contrasts was set at p=0.05; however, *P*-values up to 0.10 were also discussed. If the data to be analyzed was in the form of a proportion, an arcsine square-root transformation was used. Most calculations and all statistical analysis were completed using SAS v. 9.1 (SAS institute, Inc.).

RESULTS AND DISCUSSION

Effects of changing detrital inputs on %C and %N of SOM and OM fractions

Soil C/N ratio is different between sites and is driven by N content, which is greater at Bousson than at HJA. Both sites are C-rich compared to other forest soils and % C is approximately the same (Table 3-3). Even in the uppermost part of the A horizon, the majority of soil C and N is present within the heavy fraction at both sites. Comparing the Control plot soils of each site, the light fraction at HJA contains nearly twice as much of the total soil C as at Bousson. HJA light fraction contains more of the total soil N than at Bousson as well (Table 3-3). % C and % N of light fraction was greater than heavy

fraction and C/N ratio of light fraction was more narrow than heavy fraction (Table 3-4). Our results are in the middle of the range reported by Sollins et al. (2006) for % C of light fraction material separated at 1.6 g mL^{-1}-in a wide variety of soil types from around the world (16 – 40 % C). C/N ratio of Bousson light fraction is also in the middle of the range reported (10-40) but C/N ratio of the HJA light fraction is greater than other soils reported; however, is similar to the value Sollins et al. (2006) found for one soil sample from the same DIRT site.

Table 3-3. Percent C and N of 0-5 cm A horizon bulk soil and the % of total soil C and N present in the light fraction. Values are means ± one standard error in parentheses. Means with different letters in superscript are significantly different ($p < 0.05$, unless otherwise indicated) and means with no letters were not different. Statistical comparisons were done for the means of each variable measured at each site separately (n = 3). For overall means, n = 15 for Bousson and n = 18 for H.J. Andrews.

Description	% C		% N		C/N ratio		% of bulk soil C in LF		% of bulk soil N in LF	
Bousson										
Control	6.09	(0.31)	0.49	$(0.03)^{b}$	12.54	(0.64)	$^{*}11.44$	$(0.25)^{b}$	7.10	$(1.08)^{b}$
Double Litter	6.73	(1.02)	0.47	$(0.05)^{ab}$	14.12	(0.75)	16.31	$(3.46)^{ab}$	12.85	$(2.90)^{ab}$
No Inputs	7.78	(0.85)	0.52	$(0.03)^{a}$	15.00	(0.70)	18.06	$(2.61)^{ab}$	11.33	$(1.24)^{ab}$
No Litter	6.55	(0.44)	0.51	$(0.01)^{ab}$	12.81	(1.15)	12.26	$(0.78)^{ab}$	9.69	$(0.30)^{ab}$
No Roots	7.42	(0.40)	0.52	$(0.02)^{a}$	14.31	(0.50)	19.20	$(1.51)^{a}$	15.53	$(1.17)^{a}$
Mean	*6.91*	*(0.30)*	*0.50*	*(0.01)*	*13.75*	*(0.39)*	*15.45*	*(1.14)*	*11.30*	*(0.97)*
H.J. Andrews										
Control	6.79	(1.07)	0.20	(0.01)	34.31	(3.63)	$^{**}20.86$	$(0.44)^{ab}$	11.57	$(0.89)^{ab}$
Double Litter	9.59	(1.95)	0.25	(0.05)	38.43	(1.98)	32.01	$(4.93)^{a}$	21.85	$(2.84)^{a}$
Double Wood	11.77	(5.33)	0.33	(0.12)	33.45	(5.33)	25.72	$(3.56)^{ab}$	10.93	$(0.81)^{ab}$
No Inputs	11.19	(1.59)	0.26	(0.03)	43.18	(3.61)	14.19	$(2.34)^{b}$	6.97	$(0.41)^{b}$
No Litter	5.54	(0.99)	0.18	(0.02)	30.09	(2.08)	18.87	$(6.16)^{ab}$	11.28	$(5.22)^{ab}$
No Roots	8.83	(1.36)	0.29	(0.01)	31.14	(5.22)	19.83	$(1.11)^{ab}$	10.21	$(0.99)^{b}$
Mean	*7.88*	*(1.05)*	*0.23*	*(0.02)*	*30.84*	*(2.02)*	*19.29*	*(2.09)*	*10.37*	*(1.61)*

$^{*}p = 0.087$
$^{**}p = 0.071$

Altering detrital inputs has not yet affected the C concentration (% C) of the bulk 0-5 cm A horizon soil at either site (Table 3-3). Likewise, N concentration (% N) of the bulk soil has not been altered by either exclusion or addition of detrital inputs at HJA. However, both root exclusion treatments (No Roots and No Inputs) at Bousson have resulted in an increase in the % N of the bulk soil ($p = 0.065$), potentially as a result of the lack of

uptake of N by roots. Approximately 3 % and 5 % of total soil mass was recovered as light fraction material at Bousson and HJA respectively. Altering detrital inputs has not influenced the amount (percentage) of light fraction at HJA; however, at Bousson the No Roots treatment has resulted in significantly greater percentage of light fraction than the Control plots (Figure 3-1).

Table 3-4. Percent C and N and C:N of density fractions, values are means ± 1 standard error in parentheses.

	Light Fraction			Heavy Fraction		
Treatment	% C	% N	C:N	% C	% N	C:N
Bousson						
Control	27.4 (4.3)	1.10 (0.06)	25.4 (5.4)	4.56 (0.12)	0.35 (0.01)	13.3 (0.8)
Double Litter	25.4 (3.8)	1.28 (0.21)	19.9 (0.3)	4.51 (0.49)	0.33 (0.02)	13.8 (0.6)
No Inputs	31.0 (3.1)	1.21 (0.03)	25.5 (2.0)	5.08 (0.51)	0.37 (0.04)	13.7 (0.4)
No Litter	21.0 (0.8)	1.14 (0.06)	18.6 (0.8)	4.87 (0.08)	0.36 (0.01)	13.4 (0.1)
No Roots	23.0 (1.0)	1.18 (0.05)	19.4 (0.2)	4.90 (0.02)	0.35 (0.00)	14.0 (0.2)
H.J. Andrews						
Control	28.7 (0.3)	0.48 (0.04)	61.0 (6.1)	4.92 (0.83)	0.16 (0.02)	29.9 (1.5)
Double Litter	29.8 (1.4)	0.62 (0.03)	48.2 (2.7)	5.78 (1.05)	0.20 (0.04)	28.6 (0.9)
Double Wood	28.9 (1.1)	0.50 (0.06)	58.8 (6.9)	8.49 (3.17)	0.28 (0.05)	28.2 (5.7)
No Inputs	30.2 (1.9)	0.47 (0.05)	65.4 (4.4)	9.50 (1.59)	0.31 (0.02)	32.0 (7.4)
No Litter	30.9 (0.9)	0.66 (0.10)	49.4 (9.0)	4.61 (0.81)	0.20 (0.04	23.9 (2.5)
No Roots	32.0 (0.6)	0.53 (0.09)	64.7 (13.8)	6.03 (0.49)	0.21 (0.01)	28.8 (3.8)

Although total soil % C and % N have not yet changed in response to detrital treatments, there is evidence that changing detrital inputs influenced the distribution of C and N between the organic matter fractions. At Bousson, the exclusion of roots (No Roots) has resulted in a greater amount of total soil C and N within the light fraction ($p = 0.087$ and $p = 0.036$ for C and N) compared to Control plots. Under normal conditions, with intact roots and an active rhizosphere community, root inputs rapidly decompose. However, in

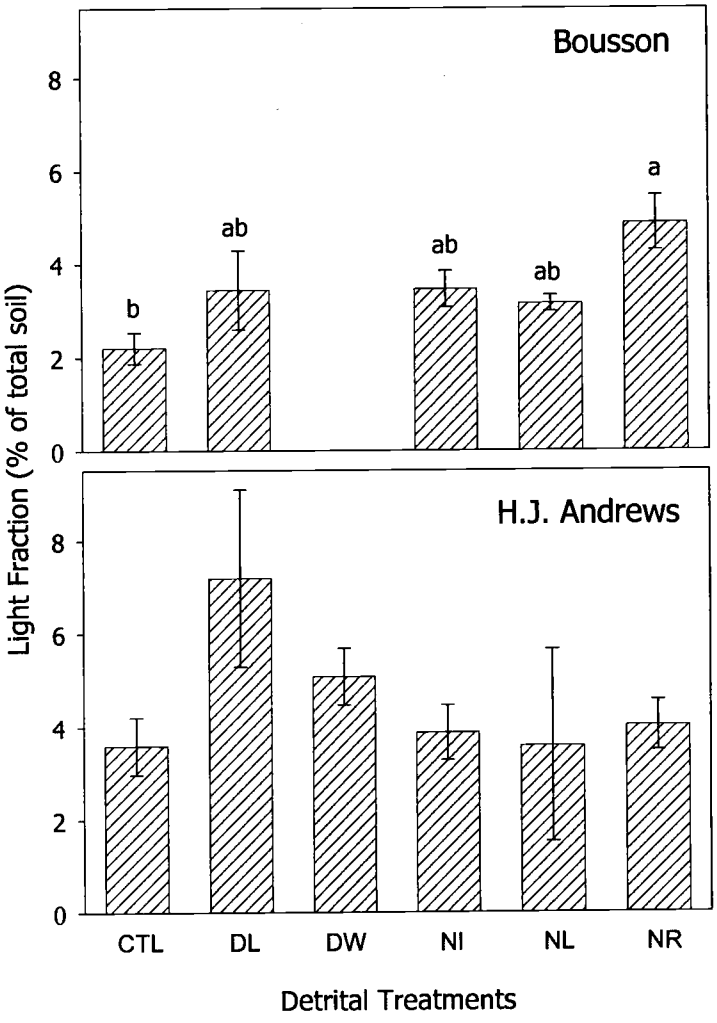

Figure 3-1. Proportion of bulk soil dry mass that was recovered as light fraction material.

the trenched treatments at Bousson, the rhizosphere community is absent and slowly decomposing root inputs were recovered as light fraction material. In contrast, doubled litter treatments have resulted in greater C within the light fraction organic matter at HJA (Double Litter) ($p = 0.071$). However, both root exclusion treatments (No Roots and No Inputs) have resulted in less N within the light fraction at HJA ($p = 0.035$). Although doubled litter has influenced nutrient pools in the light fraction, doubling wood inputs at HJA has not yet, potentially indicating a difference in the mixing and incorporation of different litter types into the A horizon by invertebrates. The dominant mechanism for movement of C from woody inputs into the mineral soil is by leaching of DOC and sorption to mineral surfaces (Lajtha et al. 2005).

Black carbon in the light fraction

Light fraction material contained up to 0.100 g black carbon g^{-1} soil, which equated to 0.196 g black carbon g^{-1} total C, or nearly 20 % of total C in the light fraction at HJA (Figure 3-2, upper). The amount of black carbon, which we refer to as "charcoal" since burned woody debris was the dominant form recovered, at Bousson was less than at HJA; however, still equated to, at most, 0.071 g black carbon g^{-1} total C, or ~ 7 % of total C in the light fraction (Figure 3-2, lower). Both woody and amorphous forms of charcoal that result from the combustion of plant material (Goldberg 1985) were present at both sites (Figure 3-3, upper). At HJA, the present-day forest developed following a stand replacing fire approximately 550 y ago and the recovered charcoal had a ^{14}C age of 770 y BP ($\pm$ 45 y, analytical error). The fire history is not known at Bousson, but charcoal had a ^{14}C age of 630 y BP ($\pm$ 40 y analytical error).

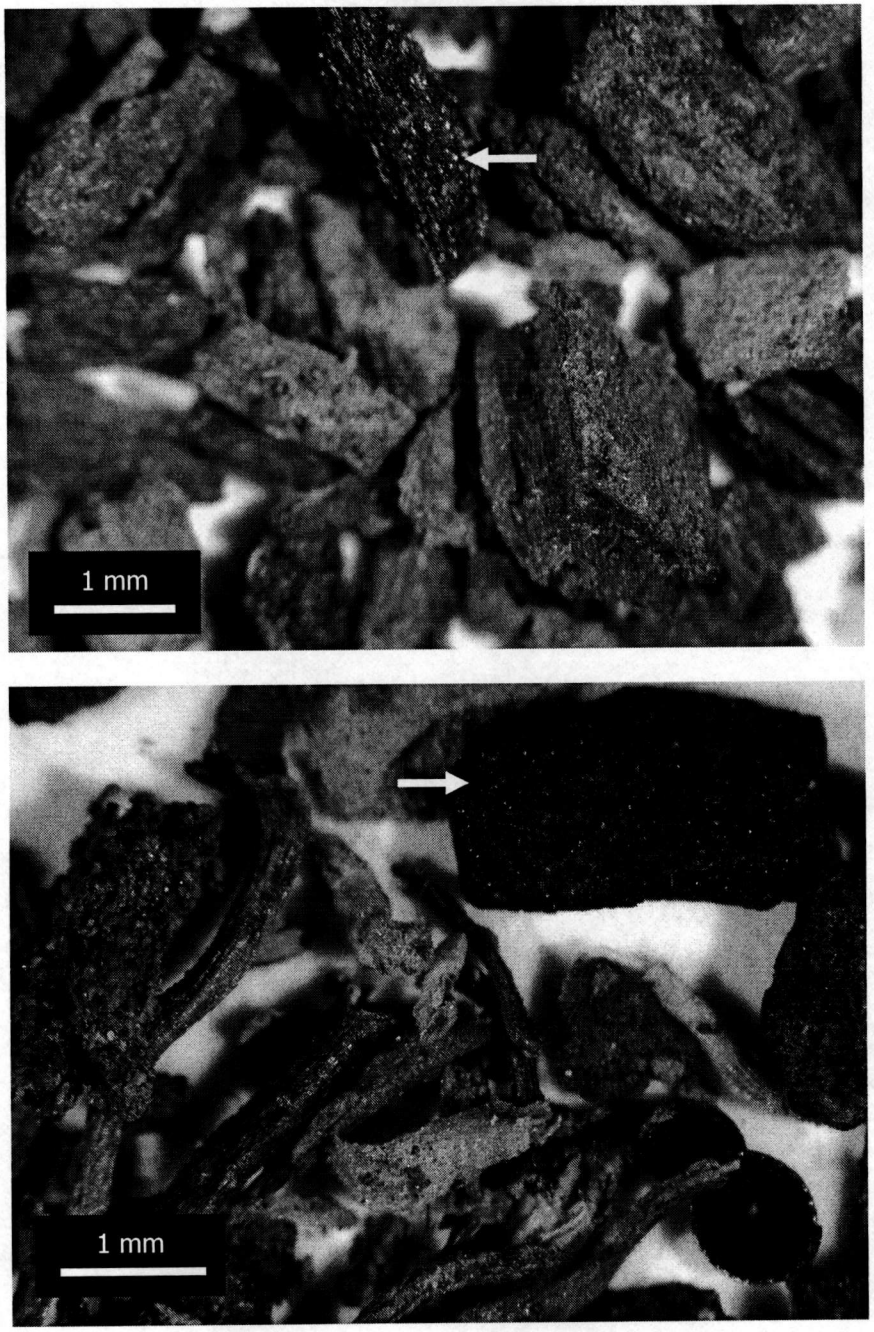

Figure 3-2. Light microscope images of light fraction material from both sites containing charcoal as indicated by arrows; H.J. Andrews (upper) and Bousson (lower).

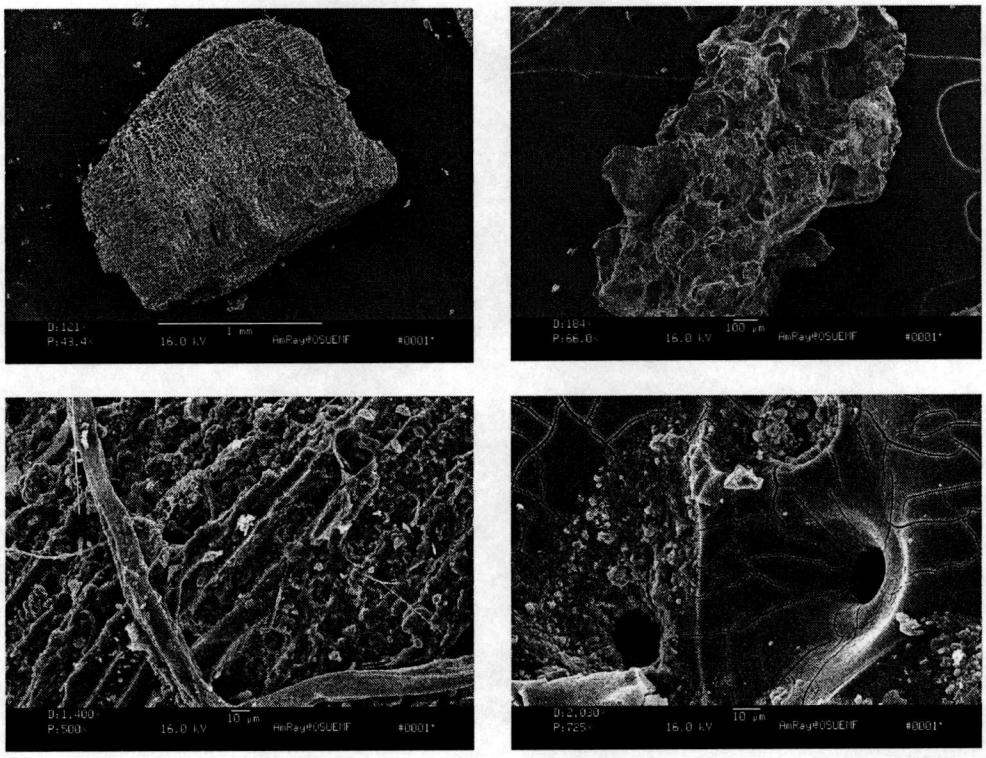

Figure 3-3. SEM images of different structures of charcoal from partially combusted plant biomass isolated from H.J. Andrews; woody (left) and amorphous (right).

TMAH spectra indicate the presence of all 11 lignin degradation products and aliphatic material in all our samples (Figure 3-4). As expected, the recovered charcoal had less guaicyl and syringyl lignin monomers than the light fraction material (Table 3-5). The Ac/Ad ratio was lower for the charcoal than the light fraction, which is indicative of a greater oxidation state. The S/G ratio of light fraction and charcoal is greater at Bousson than HJA, which is a reflection of the difference in the relative dominance of categories of lignin monomers between deciduous and coniferous vegetation. The relative amount of aliphatic compounds decreased between light fraction material and charcoal at Bousson; however, at HJA there was a substantial increase in the proportion of aliphatic compounds in the charcoal. Charcoal surfaces have ridges and holes where organic matter accretions may accumulate (Figure 3-3, lower). Aliphatic compounds may originate from the wood itself (and remained due to incomplete combustion), from fungal mycelia that have infiltrated the charcoal, or from hydrophobic DOM that had sorbed to the surface and remained intact during the digestion.

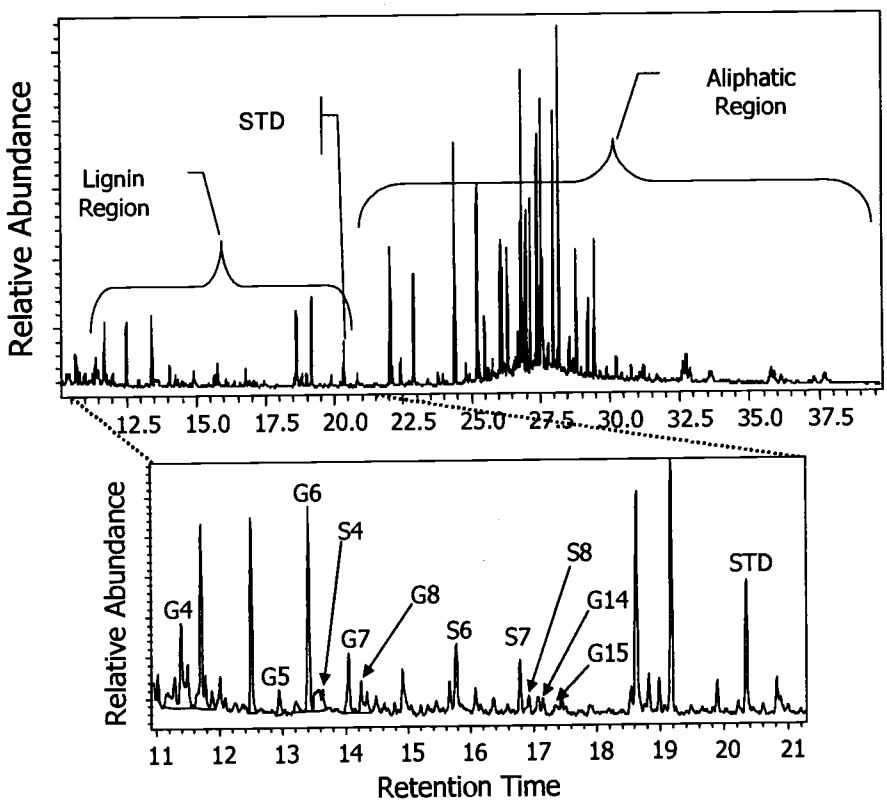

Figure 3-4. TMAH GC-MS spectra for light fraction material from HJA.

Table 3-5. Summary of TMAH oxidation products from the light fraction (LF) material and the recovered charcoal, yields are in mg 100 mg^{-1} total C.

Sample	Total G Yield	Total S Yield	Total G + S Yield	Total Aliphatic	S/G	Ac/Ad
Bousson LF	1.03	0.48	1.51	24.28	0.47	0.45
Bousson charcoal	0.10	0.02	0.12	0.68	0.21	0.16
HJA LF	0.99	0.03	1.03	1.44	0.03	0.57
HJA charcoal	0.41	0.00	0.41	10.89	0.00	0.14

G = Guaiacyl-derived compounds
S = Syringyl-derived compounds
Ac/Ad = Acid/aldehyde ratio

Mean residence time (MRT) of organic matter fractions

Charcoal, present in large amounts in some light fraction samples, has a different, older, radiocarbon signature than the rest of the light fraction material, which should reflect the incorporation of a considerable amount of C fixed as biomass following the spike in atmospheric radiocarbon concentration in the later 1960's. For this reason, the radiocarbon signature of the charcoal was mathematically removed from the radiocarbon signature of the whole light fraction. Even following this adjustment, the MRT of the light and heavy fractions at HJA are similar to each other, while at Bousson the MRT of light fraction is short and heavy fraction is long (Figure 3-5). The short MRT of light fraction at Bousson is reflected in its modeled radiocarbon content, which follows the pattern of the atmospheric curve closely. The curve of the heavy fraction radiocarbon content reflected little incorporation of bomb-origin ^{14}C, as expected in an organic matter fraction with a MRT of several centuries. The organic matter mobilized during density fractionation has a MRT intermediate to the light and heavy fractions. In contrast, at HJA, MRT of both organic matter fractions was approximately a century and the organic matter mobilized during density fractionation had the shortest MRT.

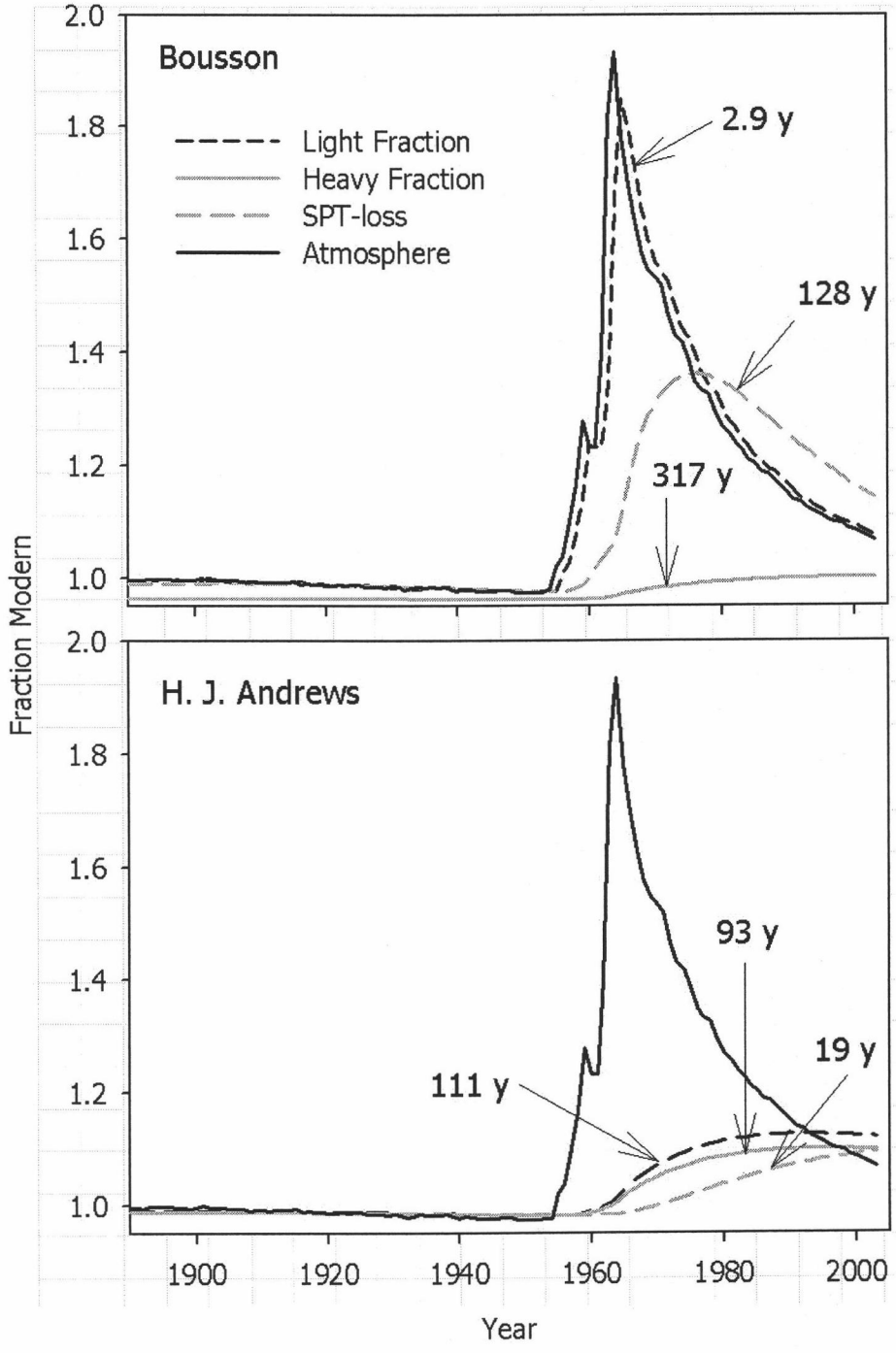

Figure 3-5. Modeled radiocarbon content for control soil organic matter pools and the atmosphere at Bousson and H.J. Andrews.

Twelve years of detrital input manipulations have had a significant influence on the mean residence time of the light fraction organic matter at Bousson, the deciduous site ($p = 0.001$, $F = 69.59$) (Table 3-6). Mean residence time of the light fraction from Double Litter and Control plots was short, 2.9 and 3.8 y respectively, compared to the MRT of the litter removal plots (78 – 185 y). Light fraction from the No Inputs plot had the longest MRT, followed by No Litter and No Roots. No significant differences in MRT of the heavy fraction were present among the detrital input treatments at Bousson, the mean MRT was 255 ± 15 y.

Table 3-6. Mean residence times of organic matter fractions following manipulation of detrital inputs. Values are means ± one standard error. Means with different letters in superscript are significantly different ($p < 0.05$, unless otherwise indicated) and means with no letters were not different. Statistical comparisons were done for the MRT means of each fraction measured at each site separately ($n = 3$)

Treatment	Light Fraction	Heavy Fraction	SPT-Mobilized
Bousson			
Control	2.9 ± 0.7^{c}	316.7 ± 18.0	127.7 ± 69.2
Double Litter	3.8 ± 0.9^{c}	232.7 ± 31.5	6.6 ± 5.0
No Inputs	184.8 ± 14.3^{a}	279.6 ± 16.1	145.5 ± 46.2
No Litter	108.8 ± 13.9^{b}	235.5 ± 39.8	7.6 ± 7.1
No Roots	78.4 ± 4.8^{b}	208.2 ± 33.3	1.1 ± 0.6
H.J. Andrews			
Control	111.2 ± 23.1	92.7 ± 12.1	19.1 ± 10.5
Double Litter	131.8 ± 34.7	106.9 ± 16.4	12.0 ± 7.9
Double Wood	108.1 ± 13.1	154.7 ± 76.6	0.5 ± 0.2
No Inputs	217.4 ± 69.5	134.1 ± 11.2	9.7 ± 9.2
No Litter	98.9 ± 15.5	132.5 ± 34.5	23.8 ± 7.3
No Roots	115.4 ± 29.3	211.8 ± 56.7	12.4 ± 3.0

At Bousson, high aboveground biomass, litterfall, total soil N, and rates of N mineralization all indicate that this site is highly productive. The average aboveground biomass for temperate deciduous forests (135 Mg ha^{-1}) is three times less than at the site in Bousson (Schlesinger 1991). Litterfall is in the upper range (1.1-2.8 Mg C ha^{-1}) of rates documented for similar aged stands (50-80 yr) of broad-leaved temperate forests (Raich and Nadelhoffer 1989). N mineralization rate is 121.0 kg ha^{-1} yr^{-1}, also in the upper portion of rates reported for temperate forests (20-200 kg ha^{-1} yr^{-1}) (Mcguire et al. 1992). Soil N is very high compared to other similar deciduous forests in the northeastern US with amounts nearly three times those reported at both Harvard Forest, MA (Magill et al. 1997) and Hubbard Brook, NH (Bormann et al. 1977). Foliage from sugar maple in particular has high concentrations of soluble carbohydrates and cellulose which contribute to fast decay rates and rapid immobilization and mineralization of nutrients during decomposition. Litter from the species present at this site has high nutrient content also contributing to fast decomposition rates and rapid release of nutrients into the soil (Nadelhoffer et al. 2004). Removal of litter inputs has drastically altered the MRT of light fraction material already, after only 13 y of detrital manipulations.

In contrast, six years of detrital input manipulation has not had an effect on MRT of either light or heavy fraction at HJA (no interaction effect of treatment and fraction or main effect of treatment) (Table 3-6). The average MRT of the light fraction from all treatments is 130.5 $\pm$ 15.8 y and the mean MRT of the heavy fraction is 138.8 $\pm$ 17.3 y. Mean residence times for the light and heavy fraction are not significantly different from each other; however, both are greater than the mean MRT of the organic matter mobilized and discarded during density fractionation (25.4 $\pm$ 12.8 y) ($p < 0.001$, $F = 27.33$). The lack of influence of altered detrital inputs is an indication that more time is necessary for alterations in litter to overcome the legacy of coarse woody debris that dominates the organic matter at HJA. Light fraction material is primarily woody debris and DOC originating from woody inputs is transported into the mineral soil and is

stabilized by sorption to mineral surfaces; indicating the influence the imprint of woody inputs on both organic matter fractions.

The MRT of the organic matter mobilized in SPT varied widely for soil from Bousson. Organic matter mobilized during the density fractionation from the Control and No Input plots had a longer MRT than the other treatments; however, because of high variability no significant difference was present and the mean MRT was 82.9 ± 27.8 y. We tested whether changing the source of the $\Delta^{14}C$ of the inputs to the SPT-loss pool within the model would alter the estimated turnover time (Table 3-7) and provide clues about the origin of the organic matter lost during density fractionation. When the model is changed so that the source of the SPT-loss pool is the light fraction, MRT increases by about 20 years for Bousson and decreases by a very small amount for HJA. When the model is changed so that the source of SPT-loss pool is the heavy fraction, the model fails to find a solution for Bousson indicating that, within the constraints of the model, it is not likely that the heavy fraction is the whole source of the SPT-loss pool. Changing the source of SPT-loss to the atmosphere, i.e. implying that the organic matter lost during fractionation is only from the most recent inputs to the soil, results in substantial increases in MRT at both sites but variability is high.

Table 3-7. Changes in the MRT of the SPT-mobilzed pool resulting from different $\Delta^{14}C$ input sources within the model. Values are means ± one standard error.

$\Delta^{14}C$ input source	Bousson	H. J. Andrews
Mass weighted	146 ± 52	19 ± 11
Light fraction	169 ± 58	18 ± 9
Heavy fraction	n/a*	20 ± 16
Atmosphere	171 ± 59	83 ± 81

* There was no feasible solution to the model for any of the plots when the entire source of SPT-loss was attributed to the heavy fraction.

Respiration during incubation

Respiration rates during incubation of bulk soil were high at the beginning of the incubation; however, by day 30 had decreased to lower rates, which were then maintained for the remainder of the incubation at both sites (Figure 3-6). Soil from Bousson had greater initial rates of respiration than HJA and had significantly greater cumulative C respired by day 30 than HJA (13.3 ± 0.7 mg $C\text{-}CO_2$ g^{-1} $C_{initial}$ for Bousson and 8.9 ± 0.6 7 mg $C\text{-}CO_2$ g^{-1} $C_{initial}$ for HJA, $p < 0.001$, $F = 21.17$). There were no significant differences in cumulative C respired by day 30 or during the year incubation period between detrital treatments at either site. After 1 y incubation, mean cumulative respiration during decomposition of bulk soil (averaged for all plots at each site) was not different between Bousson and HJA (47.9 ± 5.0 mg $C\text{-}CO_2$ g^{-1} $C_{initial}$ for Bousson and 58.2 ± 4.6 mg $C\text{-}CO_2$ g^{-1} $C_{initial}$ for HJA).

No significant differences in mean cumulative respiration were present between light and heavy fractions or between any of the detrital treatments at HJA (Table 3-8). At Bousson, cumulative respiration was greatest from the light fraction of the Double Litter plots ($p = 0.062$, $F = 3.20$, for the interaction effect of treatment and fraction)). Respiration from Double Litter light fraction was significantly greater than from heavy fraction of all treatments except No Litter. Light fraction from Control and Double Litter treatments had greater respiration than the heavy fraction within those treatments. Cumulative respiration from the light fraction of the No Litter treatments was significantly greater than from the heavy fraction of the Control and No Input treatments. Even at Bousson, where MRT of the light fraction varied widely between detrital treatments, there were no significant difference in the lability of light fraction between the treatments during incubation. However, generally, the incubation results agree with the MRT patterns at both sites.

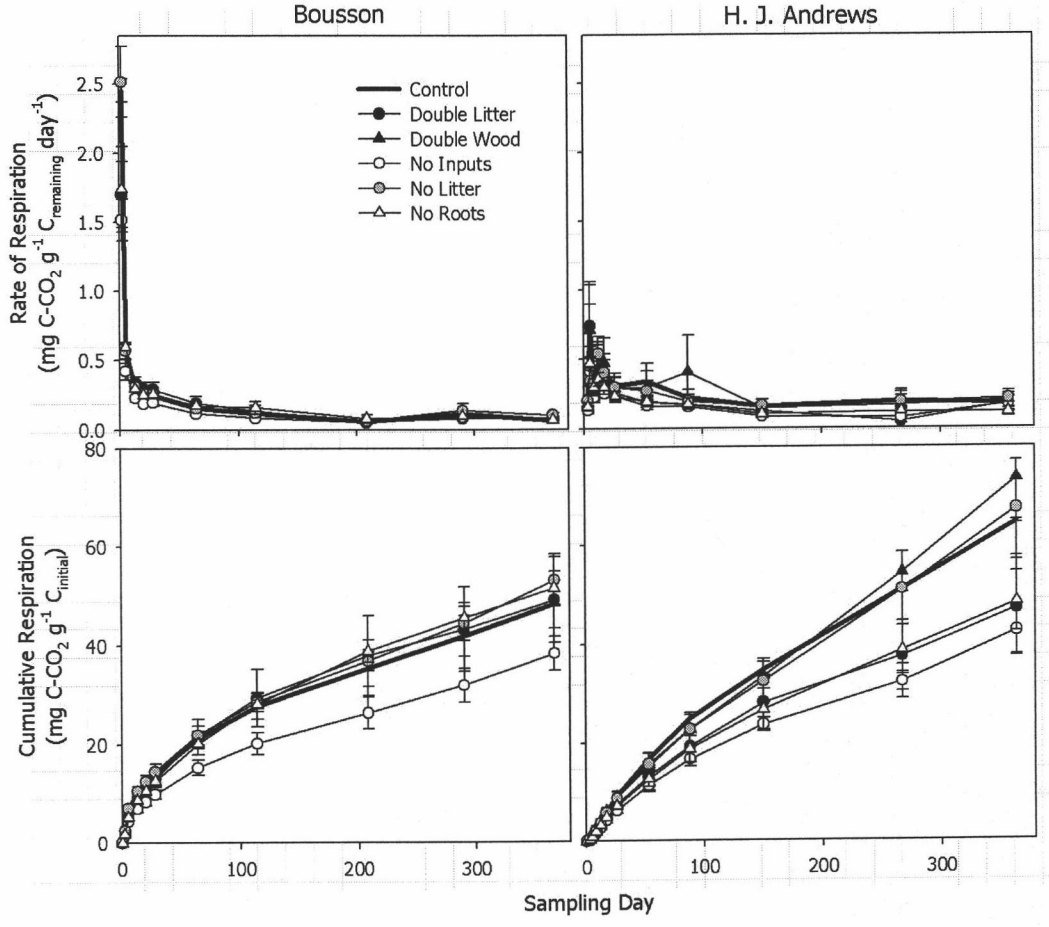

Figure 3-6. Respiration rates (upper) and cumulative respiration (lower) during one-year incubation of bulk soils from Bousson and H.J. Andrews. Points are means (n = 3) $\pm$ one standard error.

Table 3-8. Cumulative respiration (mg CO_2-C g^{-1} $C_{initial}$) during one-year incubation, values are means $\pm$ 1 standard error in parentheses, n = 3. Mean cumulative respiration values from the light and heavy fractions are compared statistically to each other; however, are not compared to the bulk soil values at each site.

Treatment	Bulk Soil		Light Fraction†		Heavy Fraction	
Bousson						
Control	*48.12	(4.84)	54.8	$(9.8)^{abc}$	27.62	$(3.85)^{d}$
Double Litter	49.02	(8.64)	57.1	$(10.0)^{a}$	34.66	$(3.71)^{bcd}$
No Inputs	38.16	(3.44)	34.2	$(4.9)^{abcd}$	28.23	$(6.13)^{cd}$
No Litter	52.93	(5.21)	54.8	$(1.5)^{ab}$	32.25	$(2.49)^{abcd}$
No Roots	51.39	(3.31)	51.2	$(5.5)^{abcd}$	26.43	$(1.41)^{bd}$
H.J. Andrews						
Control	*65.56	(0.25)	**35.8	(3.8)	38.17	(5.13)
Double Litter	47.82	(9.87)	35.2	(1.7)	32.40	(6.54)
Double Wood	74.86	(32.12)	35.5	(6.0)	37.45	(5.75)
No Inputs	43.37	(4.99)	32.6	(1.8)	26.59	(3.04)
No Litter	68.53	(9.90)	42.7	(6.4)	42.23	(4.01)
No Roots	49.18	(6.16)	31.6	(3.4)	39.15	(2.28)

† Light fraction cumulative respiration reported with black carbon content removed (see methods)
* No significant effect of detrital treatment
** No significant interaction effect of treatment x fraction or main effects

Production of DOC, DON, NO_3^-, and NH_4^+ during incubation

In addition to having the greatest amount of C and N present in the light fraction, during a year-incubation, light fraction material from the No Root treatment at Bousson released the greatest amount of DOC (cumulative DOC-C g^{-1} $C_{initial}$) and DON (cumulative DON-N g^{-1} $N_{initial}$) (Table 3-9) ($p = 0.066$ for DOC and $p = 0.065$ for DON). Dead roots remaining *in situ* at Bousson following trenching are still present in the light fraction organic matter and are providing substrate actively utilized during incubation. Aboveground litter inputs however are already decomposed by the time they enter the mineral soil and therefore do not show greater nutrient mineralization during incubation.

Table 3-9. Cumulative C and N losses during the year-long incubation of light fraction material. Values are means (n=3) with one standard error in parentheses except for overall means where n = 15 for Bousson and n = 18 for H.J. Andrews. Different letters indicate means that are significantly different ($p < 0.05$), statistical comparisons were not made between sites.

Treatment	DOC (mg C g^{-1} C)		DON (mg N g^{-1} N)		NO_3-N (mg N g^{-1}N)		NH_4-N (mg N g^{-1}N)	
Bousson								
Control	31.8	$(7.3)^{ab}$	40.8	$(5.6)^{ab}$	66.3	(5.0)*	4.75	(1.17)*
Double Litter	30.4	$(6.0)^{ab}$	36.0	$(3.3)^{b}$	45.0	(19.5)	2.18	(1.25)
No Inputs	19.3	$(2.8)^{b}$	36.8	$(2.5)^{b}$	41.0	(8.7)	1.54	(0.56)
No Litter	25.6	$(1.5)^{ab}$	39.5	$(4.7)^{ab}$	79.8	(6.1)	1.86	(1.30)
No Roots	41.5	$(1.4)^{a}$	53.9	$(2.9)^{a}$	50.5	(14.2)	2.81	(1.39)
Mean	*29.7*	*(2.6)*	*41.4*	*(2.3)*	*56.5*	*(6.0)*	*2.63*	*(0.54)*
H.J. Andrews								
Control	40.6	$(1.9)^{a}$	67.2	(8.6)*	20.1	$(3.0)^{a}$	3.45	(0.68)*
Double Litter	32.8	$(3.8)^{ab}$	42.7	(9.2)	8.27	$(2.21)^{ab}$	1.89	(0.76)
Double Wood	33.2	$(4.4)^{ab}$	39.6	(2.1)	16.5	$(1.3)^{ab}$	3.40	(0.65)
No Inputs	25.7	$(5.3)^{b}$	50.4	(13.7)	8.65	$(2.30)^{ab}$	3.78	(1.03)
No Litter	27.7	$(2.0)^{ab}$	38.3	(3.4)	5.85	$(3.55)^{b}$	1.71	(0.62)
No Roots	27.1	$(0.8)^{ab}$	47.5	(8.4)	9.37	$(4.41)^{ab}$	1.98	(0.97)
Mean	*31.2*	*(1.7)*	*47.7*	*(3.7)*	*11.5*	*(1.6)*	*2.70*	*(0.34)*

*No significant differences were present between means of detrital treatments.

At Bousson, aboveground litter and root inputs may decompose at different timescales, resulting in the apparent influence of root inputs 13 years after the initial disturbance from trenching. In contrast, at HJA, doubling litter resulted in greater C and N storage in the light fraction but DOC release was not greater for the Double Litter treatment than the others. Light fraction from the No Inputs treatment however had substantially less DOC release than control ($p = 0.077$) and the least cumulative respiration followed by the other roots removal treatment (No Roots), although not statistically different from the control (Table 3-9). Cumulative respiration and DOC, DON, NO_3^-, and NH_4^+ release from heavy fraction material during the year incubation period was not different between the detrital treatments at either site (Table 3-10).

Table 3-10. Cumulative C and N losses during the year-long incubation of heavy fraction material. Values are means (n=3) with one standard error in parentheses except for overall means where n = 15 for Bousson and n = 18 for H.J. Andrews. Different letters indicate means that are significantly different ($p < 0.05$), statistical comparisons were not made between sites.

Treatment	DOC		DON		NO_3-N		NH_4-N	
	(mg C g^{-1} C)		(mg N g^{-1} N)		(mg N g^{-1}N)		(mg N g^{-1}N)	
Bousson								
Control	33.8	(10.1)*	31.3	(7.2)*	43.3	(9.1)*	2.78	(0.85)*
Double Litter	46.5	(8.3)	52.9	(10.6)	45.1	(10.1)	3.80	(2.74)
No Inputs	35.1	(1.6)	41.8	(6.6)	40.5	(10.4)	2.35	(0.73)
No Litter	22.9	(1.6)	32.1	(7.7)	70.1	(7.3)	3.23	(2.19)
No Roots	39.2	(7.3)	42.8	(4.3)	64.0	(4.0)	5.84	(0.83)
Mean	*35.5*	*(3.3)*	*40.2*	*(3.6)*	*52.6*	*(4.5)*	*3.60*	*(0.72)*
H.J. Andrews								
Control	62.1	(20.8)*	46.8	(14.6)*	7.06	(2.01)*	3.94	(0.58)*
Double Litter	67.9	(22.4)	55.8	(17.4)	9.08	(3.78)	3.58	(0.89)
Double Wood	58.7	(17.5)	45.3	(12.4)	6.95	(3.24)	2.66	(0.58)
No Inputs	32.7	(2.2)	35.1	(5.0)	2.27	(0.32)	1.11	(0.43)
No Litter	64.7	(25.0)	40.0	(10.3)	5.37	(2.14)	2.92	(1.47)
No Roots	45.2	(6.0)	46.0	(17.5)	7.07	(0.42)	2.76	(0.35)
Mean	*55.21*	*(6.81)*	*44.8*	*(4.9)*	*6.30*	*(0.96)*	*2.83*	*(0.35)*

*No significant differences were present between means of detrital treatments.

The dominant form of cumulative N release during incubation of bulk soils was NO_3^- at Bousson and DON at HJA (Figure 3-7). Mean DON production (mg DON-N g^{-1} $N_{initial}$) was 3 times greater at HJA than at Bousson while NO_3^- (mg NO_3^--N g^{-1} $N_{initial}$) was nearly 20 times greater at Bousson (Table 3-11). The proportion of total N released as NH_4^+ was approximately the same for both sites (Figure 3-8) but the absolute amount was on average 3 times greater at Bousson than at HJA (Table 3-11). NH_4^+ release was the greatest at Bousson for the plots with no aboveground litter and least for the trenched plots ($p = 0.012$). Detrital treatment did not influence the amount of NH_4^+ released at HJA.

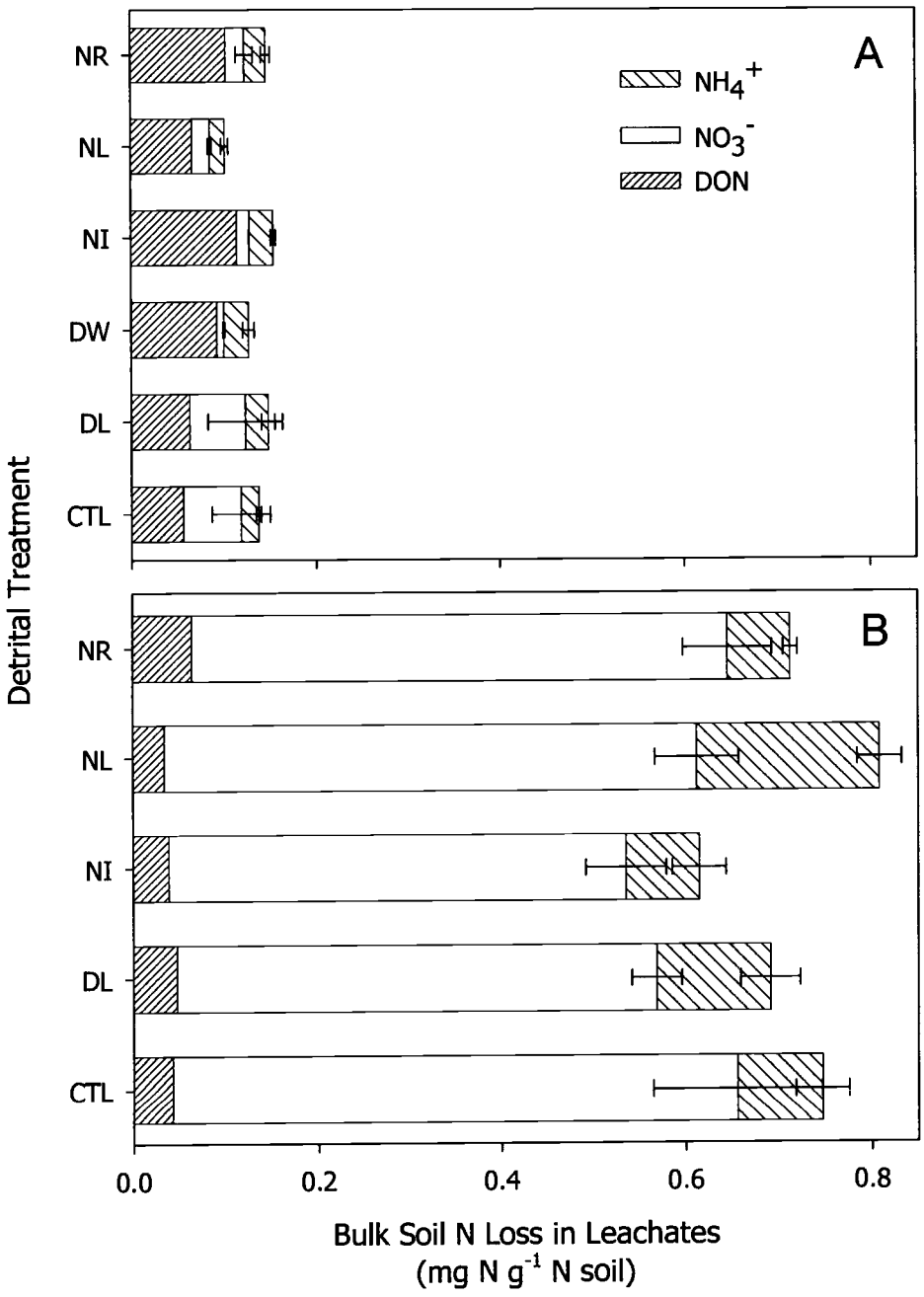

Figure 3-7. Forms of N release in leachates from bulk soils from H.J. Andrews (A) and Bousson (B). Bars are means $\pm$ one standard error.

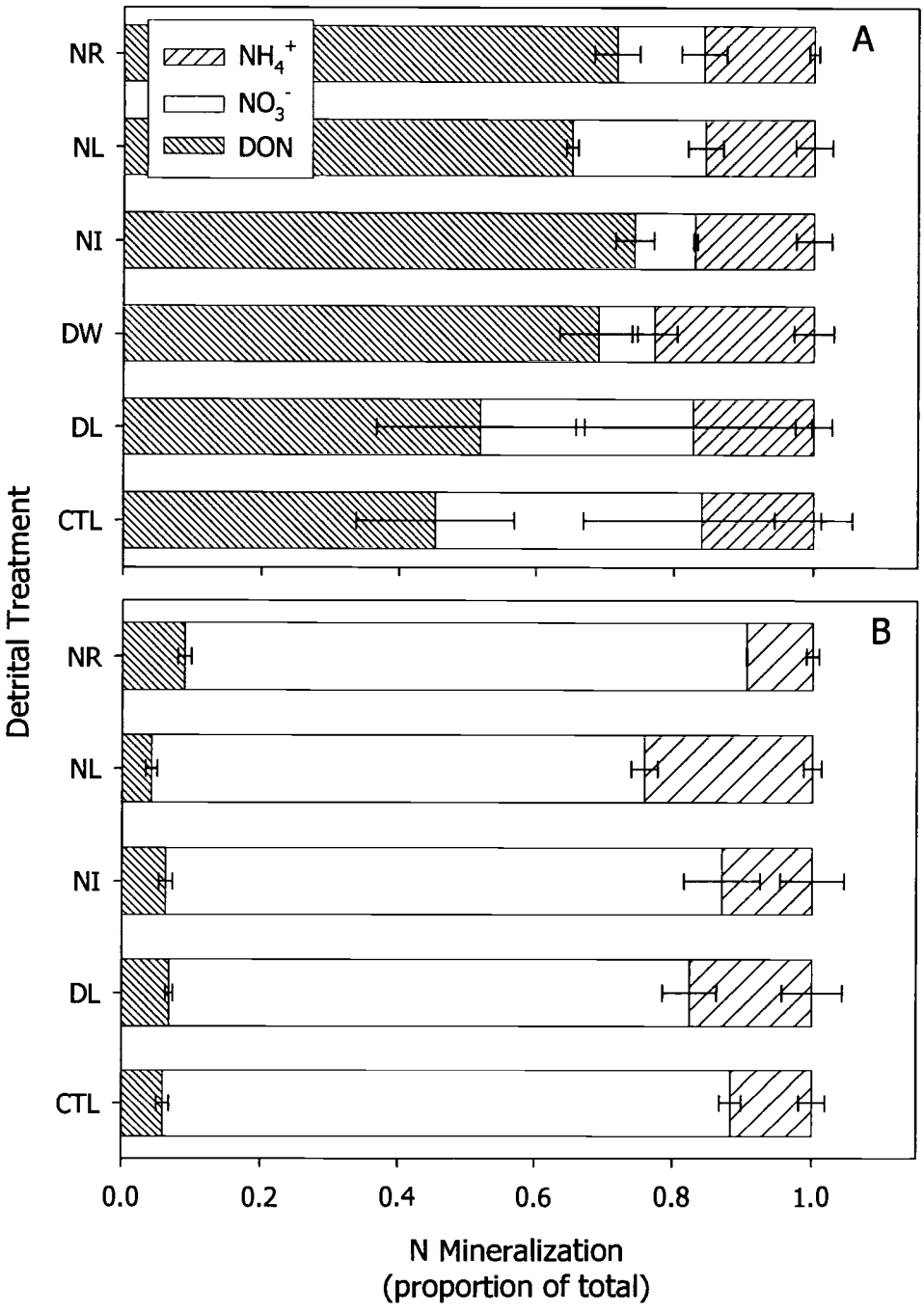

Figure 3-8. Relative proportion of N forms released during incubation of bulk soils from H.J. Andrews (A) and Bousson (B). Bars are means (n = 3) $\pm$ one standard error.

Table 3-11. Cumulative C and N release in leachates during the year-long incubation of bulk soils. Values are means (n=3) with one standard error in parentheses except for overall means where n = 15 for Bousson and n = 18 for H.J. Andrews. Different letters indicate means that are significantly different ($p < 0.05$), statistical comparisons were not made between sites.

Treatment	DOC (mg C g^{-1} C)		DON (mg N g^{-1} N)		NO_3-N (mg N g^{-1}N)		NH_4-N (mg N g^{-1}N)	
Bousson								
Control	2.34	$(0.24)^{b}$	2.97	(0.51)*	109	(8)*	18.0	$(4.7)^{ab}$
Double Litter	4.11	$(0.56)^{a}$	4.83	(0.19)	99.2	(9.7)	24.9	$(7.4)^{ab}$
No Inputs	2.35	$(0.09)^{b}$	3.79	(0.39)	82.7	(12.6)	15.0	$(5.7)^{b}$
No Litter	2.84	$(0.17)^{ab}$	3.76	(0.22)	96.9	(5.2)	36.7	$(4.3)^{a}$
No Roots	3.34	$(0.57)^{ab}$	4.96	(1.17)	92.9	(10.9)	12.9	$(1.8)^{b}$
Mean	*3.00*	*(0.23)*	*4.06*	*(0.31)*	*96.2*	*(4.31)*	*21.5*	*(3.0)*
H.J. Andrews								
Control	9.23	$(0.39)^{ab}$	13.6	(1.6)*	2.07	(0.12)*	8.48	(0.65)*
Double Litter	7.02	$(0.64)^{b}$	11.5	(3.0)	7.09	(4.54)	8.02	(1.58)
Double Wood	10.7	$(1.2)^{a}$	11.2	(3.0)	2.54	(0.90)	7.73	(1.23)
No Inputs	6.84	$(0.87)^{ab}$	9.69	(1.11)	2.12	(0.13)	8.52	(1.85)
No Litter	8.76	$(0.20)^{ab}$	14.2	(4.0)	9.96	(1.82)	7.65	(1.42)
No Roots	6.92	$(0.61)^{ab}$	12.3	(3.2)	5.67	(3.12)	6.99	(1.77)
Mean	*8.24*	*(0.43)*	*12.1*	*(1.2)*	*5.14*	*(1.11)*	*7.90*	*(0.52)*

*No significant differences were present between means of detrital treatments.

Soils at HJA have a thin O-horizon (0-2 cm) which is interlaced with mosses and a 10-20 cm thick A horizon which contains a large amount of fine root biomass. Different from other forested sites, net production of dissolved organic C and N is greater from the A horizon than from the O-horizon (Yano et al. 2004). Production of soluble organic matter may be from root exudation, fine root turnover, and rhizosphere microbial activity in the shallow mineral soil. Yano et al. (2004) found a large loss of free amino N to deep soil water and Sollins et al. (1980) demonstrated that organic nitrogen was the primary form of N loss from soil to stream flow in a watershed located near our site in the H. J. Andrews Experimental Forest.

The andic properties of the soil at HJA are conducive to sorption of organic matter to mineral surfaces, particularly for hydrophobic organic compounds. Doubling wood inputs resulted in a substantial increase in DOC concentration in soil solution in the upper 30 cm compared to Control soils (Lajtha et al. 2005). Yet, incubation of bulk soil from the Double Wood plots did not result in elevated DOC release or cumulative respiration indicating that the C originating from the added wood may be stabilized against microbial degradation. Nor has the addition of wood inputs resulted in an increase in C and N storage within the light fraction as doubling litter inputs has, further supporting the idea that DOM transport and sorption as the primary pathway for the incorporation of woody debris into soil organic matter. Generally, water soluble extracts of different litter types had different chemical characteristics; however, O-horizon leachates from the Double Litter, Double Wood and Control plots were not different after 4 years of treatments and all resembled extracts from decomposed litter (Yano et al. 2004).

Park et al. (2002) found that an increase in the available C led to a reduction in DON and DIN in leachates in an N-enriched forest floor and that DOC release was related to the amount of C sources (glucose, cellulose, leaf, wood additions to soils with no O_i or O_e layer). Fresh leaf litter was a more important source of DOC than more labile substrates (glucose and cellulose) and more stable substrates (forest floor materials and wood). (~130 y stand sessile oak and European beech). Amendment with NH_4NO_3 reduced cumulative DOC release but enhanced DON and DIN. We did not find these patterns at the Bousson, which indicates that litter manipulations are not yet translating into detectable alterations in the nutrient dynamics of the mineral soil and organic matter fractions.

The DOC/DON ratio of leachates was consistent over the course of the incubation and was not different between detrital treatments at both sites. At Bousson, DOC/DON ratio was 11.87 ± 0.69, 18.01 ± 1.77, and 26.43 ± 2.18 for bulk soil, heavy fraction, and light

fraction respectively (n = 15). At HJA, DOC/DON ratio was 35.87 $\pm$ 2.14, 33.37 $\pm$ 1.94, and 44.28 $\pm$ 2.59 in the same order (n = 18).

Conclusions

We expected that the litter addition treatments would result in greater amounts of C in the light fraction material and in greater lability of the light fraction material during decomposition. At Bousson, light fraction organic matter from the Double Litter treatments did have the greatest respiration and shortest mean residence time; however, at both sites, the root exclusion treatments had a greater influence overall on the lability of organic matter than the litter additions. Although doubled wood inputs at H. J. Andrews have resulted in an increase in DOC in soil solution near the surface, which is then retained in the soil via abiotic sorption, we did not find that this process had influenced the decomposability of soil organic matter. We expected the disruption of soil structure during density fractionation would result in a loss of DOC; however there was not significantly greater DOC loss from the Double Wood treatment resulting from the loss of organic matter that had been stabilized by sorption to mineral surfaces as we had hypothesized. The greatest differences in organic matter stability and mean residence time occurred between the two sites, which vary in climate, N-deposition, vegetation structure, and edaphic factors. It is not clear yet whether one or a combination of these factors is responsible for the observed patterns.

The DIRT Project was intended to follow changes in soil organic matter over decades to centuries. As expected, manipulation of detrital inputs has influenced the degradability and mean residence time of the light fraction before the heavy fraction organic matter, especially at Bousson; however, it will be on much more lengthy time scales that clear differences in organic matter stabilization will emerge in response to the alteration of detrital inputs.

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CHAPTER 4

ISOTOPIC ANALYSIS OF RESPIRED CO_2 DURING DECOMPOSITION OF SEPARATED SOIL ORGANIC MATTER POOLS

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Soil Biology and Biochemistry

In Press

ABSTRACT

A detailed understanding of the processes that contribute to the integrated $\delta^{13}C$ value of respired CO_2 is necessary to make links between the isotopic signature of CO_2 efflux from the soil surface and various sources within the soil profile. We used density fractionation to divide soils from two forested sites that are a part of an ongoing detrital manipulation experiment (the Detrital Input and Removal Treatments, or DIRT project) into two soil organic matter pools, each of which contributes differently to total soil CO_2 efflux. In both sites, distinct biological pools resulted from density fractionation; however, our results do not always support the concept that the light fraction is readily decomposable whereas the heavy fraction is recalcitrant. In a laboratory incubation following density fractionation we found that cumulative respiration over the course of the incubation period was greater from the light fraction than from the heavy fraction for the deciduous site, while the opposite was true for the coniferous site.

Use of stable isotopes yielded insight as to the nature of the density fractions, with the heavy fraction solids from both forests isotopically enriched relative to those of the light fraction. The isotopic signature of respired CO_2, however, was more complicated. During incubation of the fractions there was an initial isotopic depletion of the respired CO_2 compared to the substrate for both soil fractions from both forests. Over time for both fractions of both soils the respired $\delta^{13}C$ reflected more closely the initial substrate value; however, the transition from depleted to enriched respiration relative to substrate occurs at a different stage of decomposition depending on site and substrate recalcitrance. We found a relationship between cumulative respiration during the incubation period and the duration of the transition from isotopically depleted to enriched respiration in the coniferous site but not the deciduous site. Our results suggest that a shift in microbial community or to dead microbial biomass as a substrate could be responsible for the transition in the isotopic signature of respired CO_2 during decomposition. It is likely that a combination of organic matter quality and isotopic discrimination by microbes, in addition to differences in microbial community composition, contribute to the isotopic

signature of different organic matter fractions. It is apparent that respired $\delta^{13}CO_2$ can not be assumed to be a direct representation of the substrate $\delta^{13}C$. Detailed knowledge of the soil characteristics at a particular site is necessary to interpret relationships between the isotopic values of a substrate and respired CO_2.

INTRODUCTION

Soil organic matter (SOM) is a complex mixture of material from various sources that exists along a continuum of decomposition and stabilization in the soil profile. For simplicity and for modeling purposes, soil organic matter often is divided into several pools with different turnover times and recalcitrance. Each pool contributes to total efflux in different proportions depending on availability as a substrate for microbial decomposition (Parton et al. 1987; Coleman and Jenkinson 1996; Trumbore 1997). Many approaches have been taken to physically or chemically separate these pools in the laboratory (e.g., Strickland and Sollins 1987; Six et al. 2000; Six et al. 2001; Swanston et al. 2004). Analyses of the carbon isotopic composition of soil organic matter pools have yielded insight into turnover rates and microbial processing (e.g., Balesdent et al. 1987; Buchmann et al. 1998; Six et al. 2001) and some progress has been made to use $\delta^{13}C$ values of respired CO_2 to identify source pools for CO_2 efflux from the soil surface (Ehleringer et al. 2000). Often, these studies are in ecosystems where a shift between C_3 and C_4 vegetation has significantly altered the isotopic signatures of the C inputs to SOM pools. Consequent shifts of C isotopic inputs and accompanying changes in the isotopic signatures of SOM pools in these systems can help identify the sources of respiratory CO_2 (e.g. Rochette and Flanagan 1997). There is greater difficulty determining the source of respired CO_2, however, when only small variations in isotopic signatures of inputs exist, or when there is little difference in the isotopic composition between inputs and SOM pools. For these systems in particular, we need a more precise understanding of processes that control the C isotopic signature during respiration to make links between the isotopic composition of respired CO_2 and its source in the soil.

The differences between the $\delta^{13}C$ value of vegetation biomass, soil organic matter, and respired CO_2 have already been used to gain insight into biological processes that mediate C transfers among ecosystem pools (e.g. Nadelhoffer and Fry 1988; Šantrůčková et al. 2000b; Niklaus et al. 2001). Plant litter generally has lower $\delta^{13}C$ values than bulk soil and serves as continuous inputs into SOM in the form of both above and below ground sources (Accoe et al. 2003; Bird et al. 2003). Individual molecular components of these inputs have highly variable isotopic signatures; for example, lignin is depleted in ^{13}C content by 2-6 ‰ compared to the bulk plant material and by 4-7 ‰ relative to cellulose (Benner et al. 1987), and wood cellulose is 2 ‰ enriched compared to leaf cellulose (Gleixner et al. 1993). Invertebrates have been shown to excrete frass with lower $\delta^{13}C$ values compared to food (Šantrůčková et al. 2000a). Recent studies suggest that soil microorganisms may alter the isotopic composition of SOM during decomposition through mechanisms such as metabolic discrimination (Schmidt and Gleixner 1998, Šantrůčková et al. 2000b), selective consumption of substrates (Macko and Estep 1984), or preferential use of intramolecular position within substrates (Schweizer et al. 1999; Hobbie and Werner 2004). In general, processes that control the isotopic signature of CO_2 during decomposition and efflux from the soil back to the atmosphere are not well understood; indeed, whether isotopic fractionation during decomposition even occurs is currently under debate (Lin and Ehleringer 1997; Henn and Chapela 2000; Šantrůčková et al. 2000b; Fernandez et al. 2003; Klumpp et al. 2005).

As decomposition of fresh plant litter progresses and the decomposition products become incorporated into the soil profile, $\delta^{13}C$ content has been observed to increase (Buchmann et al. 1998). Multiple theories have been proposed to explain the observed trend of $\delta^{13}C$ with depth (Ehleringer et al. 2000), including changes in the atmospheric $\delta^{13}CO_2$ value since the Industrial Revolution, preferential feeding by microbes on isotopically light material, and metabolic fractionation during decomposition, among others. Boutton (1991) suggested that deeper SOM is older, and thus presumably more resistant to further decomposition than is surficial SOM. However, it is not clear that the observed pattern of increasing $\delta^{13}C$ value with depth necessarily means that more labile SOM is less ^{13}C

enriched. In addition to incomplete understanding of processes that contribute to isotopic fractionation, we also know little about differences in the degree of fractionation during decomposition from SOM pools of different ecosystems (Ehleringer et al. 2000).

Numerous authors have used density fractions of soil organic matter to represent different pools of soil organic matter that might turnover at different rates (c.f., Strickland and Sollins 1987; Trumbore 1997; Six et al. 2001; Baisden et al. 2002; Swanston 2002). Light fraction material (LF, < 1.6 g cm^{-3}) is composed of partially decomposed litter debris, charcoal, and humus. Heavy fraction material (HF, > 1.6 g cm^{-3}) consists of mineral clays and organic material in close chemical association with mineral surfaces. Heavy fraction material typically has a lower C:N than light fraction material and is thought to contain soil organic C that is more processed and stabilized (i.e., resistant to further decay). Heavy fraction SOM is generally found to be ^{13}C-enriched compared to the light fraction (Ehleringer et al. 2000; Six et al 2001; Fernandez et al. 2003). The purpose of this study was two-fold: (1) to follow the dynamics of respired $\delta^{13}CO_2$ during soil organic matter decomposition and (2) to determine whether, in a soil incubation system where root contributions to soil respiration are removed from heterotrophic decomposition of soil organic matter pools, respired $\delta^{13}CO_2$ is a reflection of substrate $\delta^{13}C$ in 2 very different forests. We expected greater cumulative respiration to occur during incubation of light fraction material than from heavy fraction material and that the isotopic signature of CO_2 respired during decomposition would be distinct between substrates of different recalcitrance for both forest soils.

MATERIALS AND METHODS

Soil from the 0-5 cm layer mineral A horizon was collected from two long-term experimental field sites at the H. J. Andrews Experimental Forest in the Cascade range of western Oregon in June 2002 (coniferous site) and at the Bousson Experimental Forest in western Pennsylvania in June 2003 (deciduous site). These sites have many contrasting characteristics (e.g., vegetation type, soil mineralogy, mineral soil C:N) (Table 4-1) and

are part of a larger on-going study to evaluate the long-term effects of changing detrital litter inputs on the accumulation and stabilization of carbon in soil (the Detrital Input and Removal Treatments or DIRT project; see Nadelhoffer et al. 2004 for a full description of the experimental design. The coniferous site is located in an old-growth Douglas-fir and western hemlock stand established approximately 500 years ago following a stand-replacing fire. The soils are andic; and coarse woody debris and a moss layer cover extensive areas of the forest floor. The climate is Mediterranean with dry summers and a wet season from October to May in which 70 % of precipitation occurs (Sollins et al. 1980). The deciduous site is located in a nutrient-rich, mixed stand dominated by black cherry and sugar maple in the canopy and by small maple saplings in the understory. Extensive ground cover of maple seedlings, mayapple (*Podophyllum*) and troutlily (*Erythronium*) is present. The mixed deciduous stand is approximately 80 years old and grows on Alfisols with a fragipan present at 60 cm. The climate is temperate with a 4 month growing season and 4 months of snow cover. In this study we focused on soils from three DIRT treatments: Double Litter, in which needle or leaf input rates are doubled annually; No Inputs, which excludes both aboveground litter (via screening and sweeping) and belowground root litter (via trenching); and Control. The plots have been maintained at the coniferous site since 1997 and at the deciduous site since 1991; each detrital treatment has been applied to three replicate plots at each site which are 10 x 15 m and 3 x 3 m, respectively.

Mineral soil was collected from 6 sub-samples within each plot, then composited and mixed (one composite sample per plot). Samples were collected in June 2002 at the coniferous site and June 2003 at the deciduous site. Following collection, soils were stored at field moisture content at 4 ^{o}C for up to 2 months prior to the start of the experiment while the density fractionation procedure was performed. Soil was separated into two pools by dispersion in a high density sodium polytungstate (SPT) solution (1.6 g mL^{-1}), in which the light fraction is collected after floating and the heavy fraction is collected as sediment (Strickland and Sollins 1987). Following fractionation, the light

and heavy fractions were air-dried and stored for an additional 1-2 months at room temperature until the incubation experiment began.

Table 4-1. Site descriptions.

	Bousson, PA Deciduous site	H. J. Andrews, OR Coniferous site
Dominant tree spp.	Black cherry, sugar maple[1] *Prunus serotina, Acer saccharum*	Doug fir, western hemlock[2] *Pseudotsuga menziesii, Tsuga heterophylla*
Approximate stand age (yr)	80	500
Soil order	Alfisol	Andisol
Mean Annual Temperature (°C)	8.3	8.8[3]
Mean Annual Rainfall (cm)	105[1]	220[3]
Litterfall C (kg ha^{-1} yr^{-1})	2110[4]	4330[5]
Litterfall N (kg ha^{-1} yr^{-1})	31.1[4]	17.5[5]
Mineral soil C:N (0-5 cm)	13[5]	35[6]
N Deposition (g N m^{-2} yr^{-1})	10-12	0.2[7]
Mineralogy	illite and vermiculite[8]	plagioclase feldspar, quartz, smectite[2]

[1]Bowden et al. 2000.
[2]Sollins et al., this issue.
[3]Sulzman et al. 2005
[4] Bowden et al. 1993.
[5]Unpublished data
[6] Lajtha et al 2005.
[7]Vanderbilt et al. 2003.
[8]USDA-SCS 1979.

Approximately 0.5 g of light fraction material and 2 g of heavy fraction material were weighed and mixed with 3 g of acid-washed quartz sand to increase aeration during the incubation. The soil and sand mixture was transferred into 12-mL exetainer vials (Labco Ltd, UK), which were prepared with 1 cm^3 of glass wool in the bottom to keep the soil from becoming anaerobic; the sample occupied roughly half the volume of each vial. A microbial inoculum was prepared with fresh soil from each field site by shaking 10 g of soil with 100 mL distilled water for 1 hr and filtering with Whatman GF/F filter paper. The filtrate was used to re-wet the soil to 25% water content on a mass basis. To ensure thorough wetting, the volume of inoculum was measured using a micro-syringe with a long needle that was inserted down into the soil in the exetainer. Starting from the bottom of the soil, the syringe plunger was slowly depressed and the needle raised so that the moisture would be mixed evenly with the soil. Care was taken so that no soil was removed with the needle or otherwise. Once the soils were moist, lids were loosely placed over the top to allow gas exchange while minimizing evaporative water loss. Distilled water was added approximately every 3 days to maintain near-constant moisture content.

On days 1, 3, 5, 8, 10, 12, 17, 30, and 65 since inoculation, lids (with septa) were sealed tightly and loaded onto a Combi-PAL, auto-sampler attached to a Finnigan Gas Bench II coupled to a Delta Plus XL Continuous Flow Mass Spectrometer. The gas bench used a continuous flow of helium to flush headspace gas from each vial through a 100 µl injection loop. The injection loop was alternately loaded and cycled 8 times through a Valco 8 port valve onto a 30 m long GC column, where CO_2 separated from the other soil gasses. The CO_2 then flowed into the mass spectrometer, where masses 44, 45 and 46 were collected simultaneously, and the ratios for $^{13/12}C$ in parts per thousand (‰) deviations from the defined international V-PDB standard were measured. External precision of the system for $\delta^{13}C$ was $\pm$ 0.06 ‰ at 300.6 ppm and $\pm$ 0.02 ‰ at 9,990 ppm.

The area under the mass 44 peak was used to calculate the concentration of CO_2. A tank of compressed air was measured at 300.6 ppm CO_2 with a LICOR Infra-Red Gas

Analyzer compared to a NOAA calibrated CO_2 standard. This tank was used to calibrate the low end of the concentration calibration and a 0.99 % (9,990 ppm) tank of CO_2 in He was used to calibrate the high end.

In addition to the soil headspace samples, a set of exetainers with working standards (9,990 ppm CO_2 in helium, and 300.6 ppm CO_2 in compressed air) was analyzed with each run for quality control. Vials were purged with CO_2-free air and left sealed for 4 hours while CO_2 accumulated in the headspace. Laboratory tests revealed that the mass spectrometer output was linear in $\delta^{13}C$ to $\pm$ 0.06 ‰ in the range 0.6 - 6.5 volts (in which the vast majority of our samples were analyzed) and to $\pm$ 0.3 ‰ in the range 0.3-6.5 volts (300-9,990 ppm) (Figure 4-1). Headspace samples that contained CO_2 concentrations outside this range were removed from the data set.

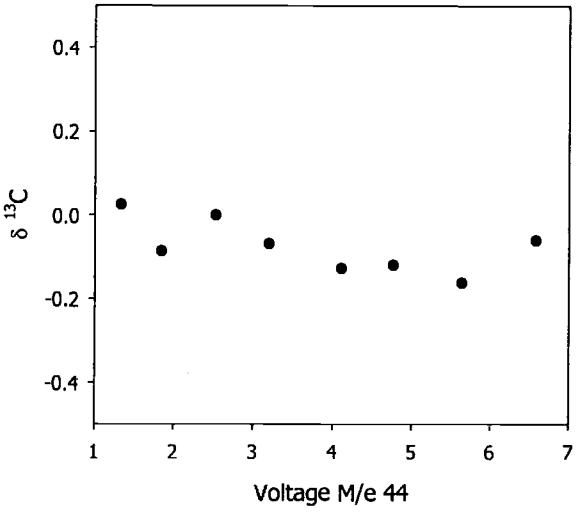

Figure 4-1. Relationship of voltage to isotope ratio across the range of voltages attained during the study. $\Delta^{13}C$ of the standard was evaluated relative to the 2.53 voltage level which was used for the reference gas calibration to V-PDB.

Carbon contents of litter material, soil, and density fractions were determined by dry micro-Dumas combustion (NA1500 C/H/N Analyzer, Carlo Erba Strumentazione, Milan) at the Stable Isotope/Soil Biology Laboratory of the University of Georgia Institute of Ecology. The $\delta^{13}C$ of these solid samples was measured on a Finnigan MAT Delta Plus XL (Breman, Germany) at the EPA Western Ecology Division's Integrated Stable Isotope Research Facility ($\delta^{13}C$ precision = $\pm$0.04‰). Some samples were analyzed at the Stable Isotope Lab at the College of Oceanic and Atmospheric Science at Oregon State University using a Carlo Erba continuous flow inlet with a Finnigan MAT Delta Plus XL. Calibration at

this facility was to NIST-8542 (Sucrose), and NIST 8541 (Graphite); external precision = ± 0.06 ‰.

Carbon isotopic discrimination between respired CO_2 and the initial substrate ($\Delta^{13}C_{(s/r)}$) was calculated using the following equation:

(1) $$\Delta^{13}C_{(s/r)} = (\alpha_{s/r} - 1) = (\delta_s - \delta_r)/(1 + \delta_r)$$

where $\alpha_{s/r}$ is R_s/R_r, and R_s is the $^{13}C/^{12}C$ molar ratio of the substrate (s) and R_r is that of the respiration. All δ values are expressed as absolute values; the total Δ is multiplied by 1000 for expression in units per mil (‰). If the fractionation factor, Δ, is positive, then the respired C is more depleted in ^{13}C than the substrate.

Comparisons between means of the detrital treatments and density fractions were made by repeated measures 2-way ANOVA in SAS v 9.2 (SAS Inc, Cary, NC) using PROC MIXED. Density fractions from each plot originated from the same bulk soil samples. Detrital treatments were classed as main plots, with density fractions treated as repeated measures (subplots). Since the deciduous and coniferous forest sites have very different characteristics, including having been experimentally manipulated for different amounts of time, no direct comparisons between the sites were made. Tukey-Kramer *a priori* adjustments were made for pair-wise comparisons so that experiment wise $\alpha = 0.05$. A comparison of pre-incubation and post-incubation soil fractions from the two sites was made with a paired Student's t-test. Linear regression was used to determine the nature of the relationship between cumulative respiration and isotopic zero point (SigmaPlot v 8.0).

RESULTS

Respiration rates peaked for all soil fractions on either day 5 or 8 of the incubation and decreased in subsequent weeks. Cumulative C loss via respiration continued to increase

during the 65-day incubation indicating a remaining supply of degradable organic substrate (Figure 4-2A). Cumulative respiration from the density fractions showed the light fraction exhibited greater respiration (mg C-CO_2 g C initial^{-1}) when compared to its heavy fraction counterpart at the deciduous site ($p < 0.001$, $F = 51.94$). The opposite was true for soils of the coniferous site; respiration from heavy fraction samples was higher than all light fraction samples of the same detrital treatment ($p < 0.001$, $F = 96.90$). Cumulative respiration from the detrital treatments showed the greatest cumulative respiration occurred in the Control plots at the coniferous site ($p = 0.042$, $F = 5.66$). There was not a significant effect of detrital treatment on respiration at the deciduous site ($p = 0.191$, $F = 2.21$); however, for both forest types the No Inputs treatment substantially reduced 65-d cumulative respiration from both density fractions, although the reduction was greater in soils from the coniferous site. The patterns observed for light and heavy fraction respiration, as well as the detrital treatments, are consistent with those from a related 1-year incubation experiment of the same soils and density fractions where greater amounts of substrate (~6 g light fraction and ~25 g heavy fraction) were incubated in chambers of greater headspace volume (~450 mL) than during this experiment (Crow, unpublished data). On day 64 of the one-year incubation of the density fractions from the deciduous soil, cumulative respiration (mg C-CO_2 g C initial^{-1}) from the light fraction was 3.32 ± 0.32, 3.91 ± 0.35, and 2.84 ± 0.16 from the Control, Double Litter, and No Inputs treatments respectively; heavy fraction cumulative respiration was 0.47 ± 0.06, 0.47 ± 0.02, and 0.38 ± 0.03 for the same treatments. On day 70 of the one-year incubation of the density fractions from the coniferous soil, cumulative respiration (mg C-CO_2 g C initial^{-1}) from the light fraction was 9.98 ± 0.67, 10.60 ± 0.63, and 8.44 ± 0.23 from the Control, Double Litter, and No Inputs treatments respectively; heavy fraction cumulative respiration was 12.02 ± 1.97, 11.84 ± 2.94, and 9.48 ± 1.09 for the same treatments. At the end of the one year period, the relationship between the treatments and fractions remained generally consistent with that at days 64 and 70 for the 2 soils.

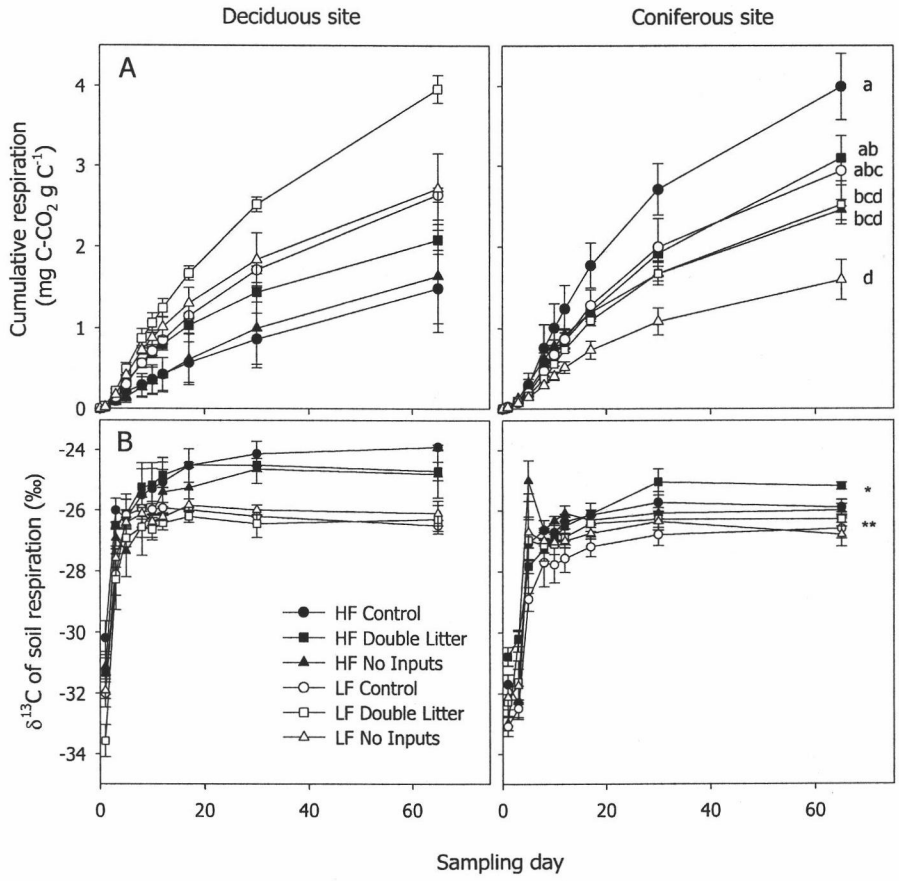

Figure 4-2. A. Cumulative CO_2 efflux from incubated soil density fractions from the deciduous and coniferous DIRT sites. B. $\delta^{13}C$ value of respiration from incubated soil density fractions from the deciduous and coniferous sites. All values are mean $\pm$ 1 standard error, n = 3, means for values at day 65 of the incubation with different letters are significantly different. While there was no significant effect of detrital treatment on $\delta^{13}C$ CO_2 values at either site, stars indicate significant differences between the density fractions (n = 9).

Isotopic composition of litter inputs varied over a wide range of $\delta^{13}C$ values, particularly at the coniferous site; however, the detrital treatments have not yet altered the isotopic signatures of either density fraction. Two common moss species and the most common epiphytic lichen species (*Lobaria oregano*) at the coniferous site were substantially depleted in $\delta^{13}C$ compared to the other litter inputs (Table 4-2), while a second common lichen species at this site, *Platismatia glauca*, was ^{13}C-enriched. Other above and below ground detrital inputs at both sites were consistent with $\delta^{13}C$ values typical for fresh C_3

plant material. Senescent leaves of black cherry and sugar maple, dominant species at the deciduous site, were isotopically depleted relative to branches and roots (Table 4-2). Five years of detrital manipulation at the coniferous site and 13 years at the deciduous site have not significantly affected $\delta^{13}C$ values of the density fractions. Unexpectedly, although there have been no fresh, isotopically depleted inputs for 13 years to the No Inputs plots at the deciduous site, light fraction from these plots had the most depleted $\delta^{13}C$ (Table 4-3). At both sites, all heavy fraction samples were more enriched than the light fraction samples. This enrichment was present to a greater degree at the deciduous site than at the coniferous site.

Table 4-2. $\delta^{13}C$ values (‰) for primary inputs to the forest floor and mineral soil at each study site. Each value is the mean of analytical duplicates.

H. J. Andrews, Coniferous site	
Lichen sp. (*Lobaria oregano*)	-33.1
Lichen sp. (*Platismatia glauca*)	-22.9
Moss sp. (*Euryhynchium oreganum*)	-30.8
Moss sp. (*Isothecium myosuroides*)	-31.4
Needles (mixed western hemlock/Doug-fir)	-26.6
Branches (mixed western hemlock/Doug-fir)	-26.4
Fine roots (< 2mm)	-27.4
Bousson, Deciduous site	
Senescent foliage (mixed black cherry/sugar maple)	-29.6
Branches (deciduous)	-27.2
Fine roots (< 2mm)	-26.9

Table 4-3. $\delta^{13}C$ values of the initial substrate, mean $\pm$ one standard error. Means were compared within each site for differences due to detrital treatment and density fraction; means that are significantly different are indicated with different letters.

		Double litter	Control	No inputs
Deciduous site	Light fraction	-27.2 ± 0.1^{cd}	-26.8 ± 0.3^{bcd}	-27.5 ± 0.5^{d}
	Heavy fraction	-25.6 ± 0.2^{ab}	-25.3 ± 0.2^{a}	-25.8 ± 0.2^{abc}
Coniferous site	Light fraction*	-26.7 ± 0.2	-26.9 ± 0.3	-27.1 ± 0.1
	Heavy fraction*	-26.3 ± 0.1	-26.6 ± 0.2	-26.6 ± 0.1

* No significant differences between detrital treatments were present at the coniferous site; stars indicate a significant difference between the overall mean $\delta^{13}C$ values for light and heavy fraction.

A comparison of $\delta^{13}C$ of the substrate from the two sites before and after the incubation revealed that the heavy fraction samples from the deciduous site became ^{13}C depleted ($p = 0.014$) and the light fraction samples from the coniferous site became ^{13}C enriched ($p = 0.022$) compared to their starting values over the course of the incubation (Table 4-4). A comparison of the isotopic signature of the substrate and respiration ($\Delta\ ^{13}C_{(s/r)}$, equation 1) at 2 points in time revealed that isotopic discrimination was greater for light fraction samples than for heavy fraction samples on both day 1 and 65 of the incubation. This disparity between discrimination of the density fractions was greatest at the coniferous site, where initially there was hardly any difference between heavy and light substrate $\delta^{13}C$ values. Discrimination at day 1 was much greater than at day 65. At both sites, respiration from the heavy fraction was enriched relative to the substrate while the respiration from the light fraction was isotopically indistinguishable from the substrate or still depleted on day 65.

Table 4-4. $\delta^{13}C$ values of soil fractions on days 1 and 65 of the incubation for the two forested sites. $\Delta^{13}C_{(s/r)}$ values represent discrimination against the heavy isotope in the conversion of substrate to product (see text). Data are for Control plots only, n=3; values are means ± one standard error. Substrate $\delta^{13}C$ mean values on day 1 and 65 were compared with paired t-tests for each site and density cut, significant differences are indicated with *.

		Substrate $\delta^{13}C$ (‰)		$\Delta^{13}C_{(s/r)}$ (‰)	
Site	Density	Day 1	Day 65	Day 1	Day 65
Deciduous	Light fraction	-26.8 ± 0.3	-26.2 ± 0.3	5.4 ± 0.3	0.0 ± 0.3
	Heavy fraction	-25.3* ± 0.2	-26.9* ± 0.3	5.0 ± 0.6	-3.0 ± 0.2
Coniferous	Light fraction	-26.9* ± 0.3	-25.3* ± 0.1	6.4 ± 0.2	1.1 ± 0.2
	Heavy fraction	-26.6 ± 0.2	-27.0 ± 0.3	5.2 ± 0.5	-1.5 ± 0.1

By day 65, there was a significant enrichment of $\delta^{13}CO_2$ in the heavy versus light fraction samples at both sites ($p < 0.001$ at the deciduous site, $p = 0.014$ at the coniferous site) (Figure 4-2B). Detrital treatments have not yet affected the isotopic signature of CO_2 respired from the density cuts at either site (deciduous: $p = 0.685$, $F = 0.40$; coniferous: $p = 0.3514$, $F = 0.40$). Regardless of site, density fraction, or initial substrate $\delta^{13}C$, the first CO_2 respired was more isotopically depleted than the starting substrate (Figure 4-3). However, this trend was reversed in all samples within the first 25 days, after which the respired CO_2 was more ^{13}C-enriched than the initial substrate. The isotopic signatures of respired CO_2 from all samples stabilized in the second half of the incubation period.

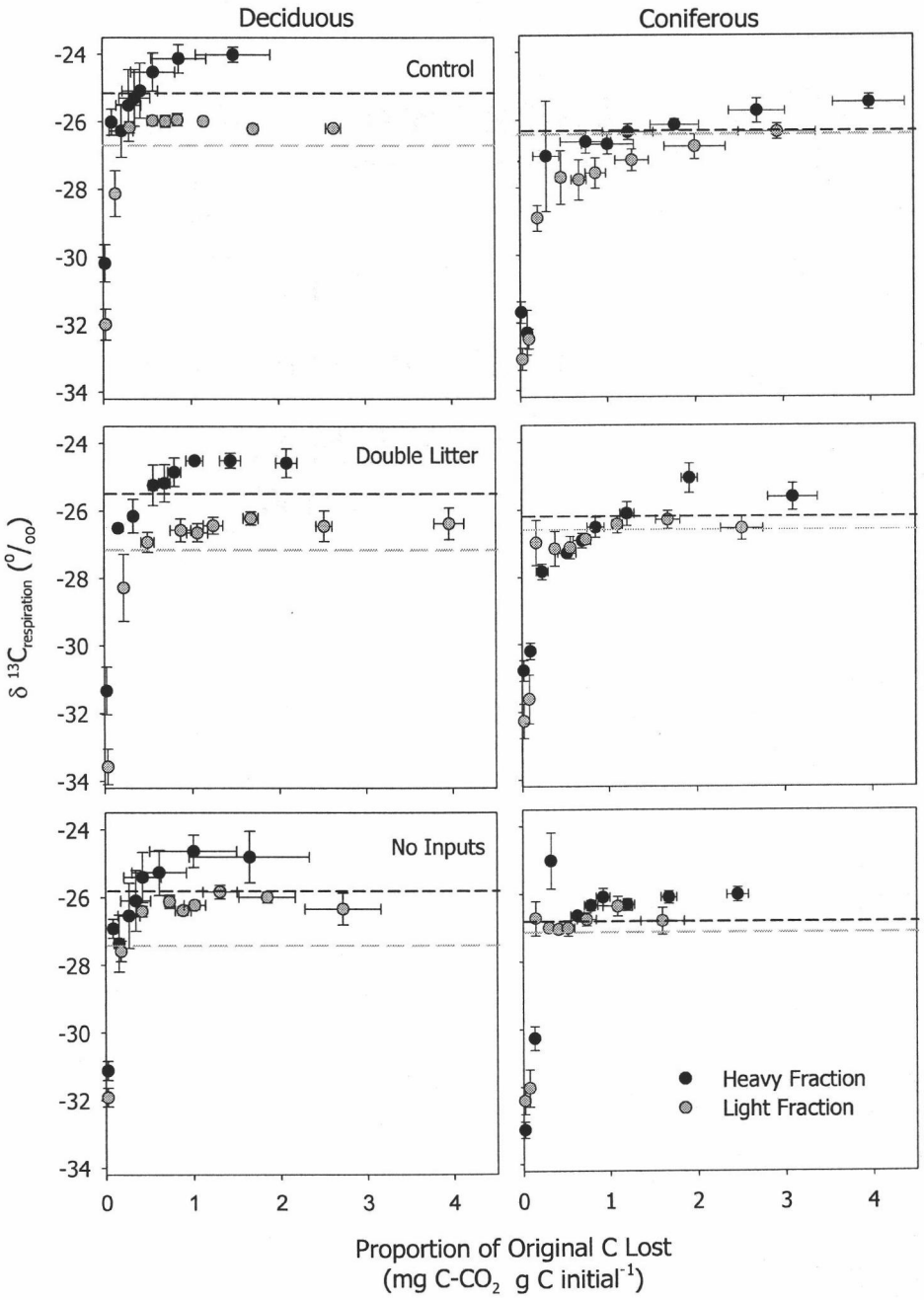

Figure 4-3. $\delta^{13}C$ CO_2 signature during the incubation period as a function of the amount of original C loss (mg C-CO_2 g C initial^{-1}). Initial isotopic signature of the substrate is referenced for both light fraction (gray dotted line) and heavy fraction (black dotted line). Values are means $\pm$ 1 standard error along both axes.

DISCUSSION

Due to more fresh plant material and low mineral interactions of the organic matter in the light fraction, we expected these substrates to exhibit greater cumulative respiration than the heavy fraction. Similarly, we expected soils from the input-addition treatment (Double Litter) to have the greatest cumulative respiration, as they have received additional inputs of the most labile organic matter (fresh litter) compared to soils from control or input removal plots. The soils from the deciduous site did in fact show increased respiration in response to the Double Litter treatment while those from the coniferous site did not (Figure 4-2A). The deciduous site is characterized by a lower soil C:N than the coniferous site (Table 4-1), which has a large amount of background C from the legacy of coarse woody debris inputs to the forest floor, and productivity and microbial activity is not generally limited by nitrogen (Bowden et al. 2000). Doubling leaf litter at the deciduous site adds fresh, labile carbon that may contribute to an increase in the rate of decomposition from the light fraction organic matter pool. We found no increase in respiration in response to the Double Litter treatment in the heavy fraction, even though the influence was strong in the light fraction at the deciduous site. A lack of similar influence of doubling litter inputs on the heavy fraction may be due to low interaction between fresh organic inputs and mineral surfaces, rapid decomposition of litter and little or no movement into deeper soil horizons, or to a very strong chemical recalcitrance of the C entering the mineral fraction possibly as a result of chronic high N deposition in the region (Fog 1988, Bowden et al. 2000, Swantson et al. 2004). Possibly, over a longer period of time, DOC transported to deeper soil will accumulate enough to be detectable against the background soil C. Studies have suggested that decomposed litter already present as O-horizon material, as opposed to fresh litter, is the primary source of DOC leached into the A-horizon (Frögberg et al. 2003, Yano et al. 2005). In this case, there will be a lag time between litter manipulation and resulting changes in organic matter pool size and dynamics (Yano et al. 2005) and the length of the lag time will be dependant on site characteristics which influence decomposition rates as well as the sorption capacity of a particular soil.

Density fractions from the coniferous site yielded different patterns of respiration during incubation than those observed at the deciduous site (Figure 4-2A, B). Incubation of the light fraction at the coniferous site resulted in lower rates of respiration than expected. Soil at this site is characterized by a legacy of woody debris, charcoal inputs from periodic fires, and low atmospheric N deposition, conditions that have contributed to high soil C:N and potential N-limitation of microbial activity (Myrold et al. 1989). The low degradability of these components contributes to the chemical recalcitrance of this fraction, which likely resulted in low cumulative respiration. Conversely, incubation of the heavy fraction at the coniferous site produced more respired CO_2 than expected, possibly due to a rapid-turnover pool within the heavy fraction that may have been made freshly-available for microbial degradation during the density fractionation procedure. Swanston et al. (2002) incubated light and heavy fractions of several soils from western Oregon and Washington, and also found no difference in the cumulative respiration at of density fractions at 60 d. Yano et al. (2005) found root litter to be the dominant producer of dissolved organic matter at this site. The removal of root inputs, both as fine root litter and root exudation, caused a substantial decrease in respiration in the No Inputs treatment for both light and heavy fractions even after only 5 years of manipulation

Soils at the coniferous site derive from volcanic parent material and have strong andic properties, such as high amorphous Al hydroxide and aluminosilicate content (oxalate-extractable Al = 1.1%) and pH in 1M NaF (10.7) (Yano et al. 2004). Andic soil characteristics likely contribute to high sorption of fresh inputs to the mineral fraction at the coniferous site and help account for the apparent differences in the recalcitrant nature of the heavy fraction C between sites. In addition, clay content of the mineral soil is lower at the deciduous (2%) than the coniferous site (13%) (Bowden et al. 2000 and Dixon 2003, respectively) giving less surface area for sorption to occur. Lajtha et al. (2005) demonstrated the potential for sorption of fresh DOC inputs to mineral surfaces at the coniferous site as a mechanism for long-term stabilization of soil organic C. Our data supported those findings: the coniferous heavy fraction contained significant amounts of

labile C that became available for microbial degradation once soil structure was disrupted during density fractionation. However, in andic soils C stabilized by sorption to mineral surfaces may not ultimately increase SOM storage if the system is disturbed and soil aggregate structure is lost.

Many authors have observed a pattern of $\delta^{13}C$ enrichment of SOM with depth in the soil profile (e.g., Nadelhoffer and Fry 1988, Boutton 1991, Trumbore et al. 1995, Boutton et al. 1998, Bowling et al. 2002), consistent with the observation that as decomposition of fresh plant litter progresses and the decomposition products become incorporated into the soil profile, ^{13}C content increases (Buchmann et al. 1997; Buchmann et al. 1998). Thus, we expected heavy fraction and samples from No Inputs plots to be isotopically enriched relative to light fraction and to samples from the Double Litter treatment. Indeed, heavy fraction samples were isotopically enriched relative to light fraction samples at both sites; however, the $\delta^{13}C$ values of the density fractions are more similar to each other at the coniferous site than at the deciduous site (Table 4-2 and Figure 4-3, dashed lines). The similarity of isotopic values for the density fractions at the coniferous site could be further evidence of the close association between inputs and mineral soil through sorption of dissolved organic matter. No change occurred yet in the isotopic value from the input removal treatments compared to control at either site despite the fact that the treatments have been in place for 5 and 13 years for the coniferous and deciduous sites respectively (Table 4-3). A large background C content, particularly at the coniferous site, may mask any subtle changes in isotopic signature resulting from our experimental treatments.

All of our substrates lost between 0.14-0.40 % of the initial C content through respiration as a result of a combination of mineralogy, nutrient status, and SOM quality. Regardless of these characteristics or the initial $\delta^{13}C$ of the substrate, the $\delta^{13}C$ CO_2 of every sample shifted from depleted $^{13}C_{(s/r)}$ to enriched within the first 30 days of incubation (Figures 4-2B and 4-3). Many similar studies with incubations of chemically separated SOM fractions and plant material have also documented the same shift in respiratory $^{13}CO_2$ from depleted to enriched relative to the initial substrate (e.g., Mary et al. 1992;

Schweizer et al.; 1999; Plante and McGill 2002; Fernandez et al. 2003; Fernandez and Cadisch 2003). Fernandez et al. (2003) observed that the transition between depleted and enriched $\delta^{13}CO_2$ respired from various plant materials did not occur either simultaneously or at the same degree of decomposition of the original material. To equalize the transition time to account for differences between the substrates in respiration rates and in amount of total C initially present, a quantification of the transition period was calculated by Fernandez et al (2003) as a measure of the degree of decomposition that had occurred when $\Delta^{13}C_{(s/r)} = 0$ (Equation 1). Likewise, we calculated this transition period as the proportion of initial C loss from the density fraction via respiration at the point when $\Delta^{13}C_{(s/r)} = 0$ (i.e., where there is no difference between the isotopic composition of the substrate and respired CO_2). This estimate was made for each sample using a non-linear regression (three parameter, exponential decay). Similar to the findings of Fernandez et al. (2003), the proportion of decomposition that had occurred when $\Delta^{13}C_{(s/r)} = 0$ for the density fractions from the coniferous site were variable (Figure 4-4A). However at the deciduous site the transition period was not different between the light and heavy fraction substrates (Figure 4-4B).

Fernandez et al. (2003) found a positive correlation between the percentage of decomposition at the transition from depleted to enriched respired CO_2 and several measures of C quality of the plant litter, including a modeled estimate of labile C and an acid-detergent extractable fraction (sugars, starch, hemicellulose, lipids, proteins and nucleic acids). We also found a relationship between the cumulative loss of C over the course of the entire incubation period (an indication of the overall decomposability of the organic matter present in the substrate) and the proportion of decomposition that had occurred when $\Delta^{13}C_{(s/r)} = 0$ for the substrates at the coniferous site. Although the total cumulative C loss during the incubation period varied within the same range for the substrates from both sites, there was no relationship between cumulative C loss and the transition period from depleted to enriched respired CO_2 in the substrates from the deciduous site (Figure 4-5).

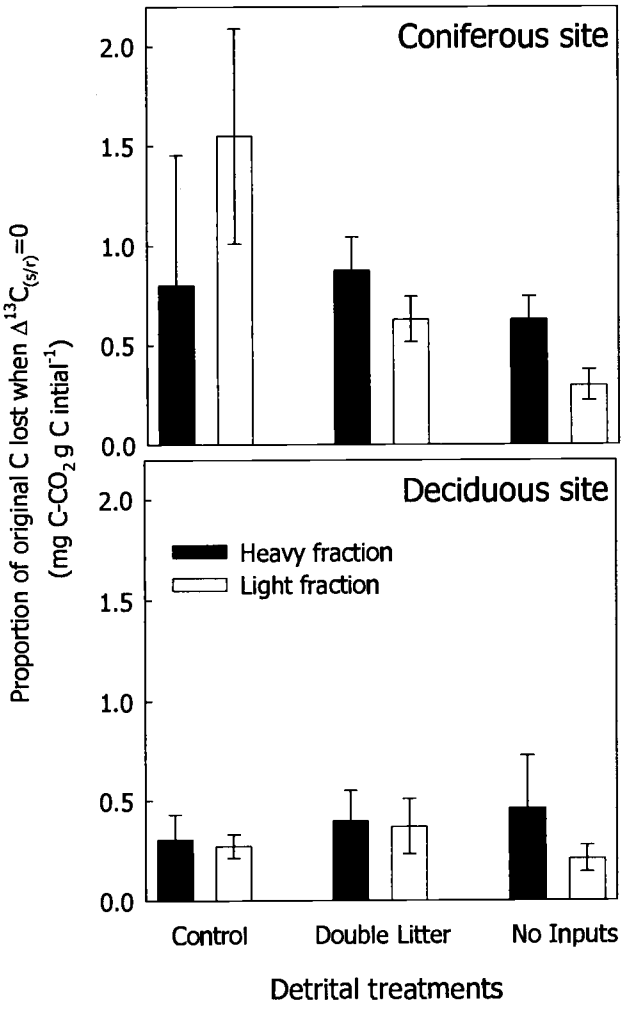

Figure 4-4. The transition period between isotopically depleted and enriched respiration, represented as amount of original C loss from the density fractions $\Delta\ ^{13}C_{(s\,/\,r)} = 0$ for each detrital treatment at both sites (see text for explanation of calculation). Bars are means (n=3) $\pm$ 1 standard error.

A possible explanation for the presence of a relationship between measures of lability and the transition from depleted to enriched respired CO_2 is that more labile C pools are isotopically lighter than less labile pools and are utilized first by the microbial community. However, Fernandez et al. (2003) did not find a depleted δ^{13} C in the acid-detergent extractable C pool compared to the whole plant material. This indicates that the initial depletion of respired CO_2 compared to the substrate was not a direct result of isotopic discrimination of chemically-defined labile material. Plant storage compounds, including starch and sugars, are typically enriched compared to mean plant biomass (Gleixner et al. 1993), while more recalcitrant structural molecules such as lignin are depleted (Benner et al. 1987). Schweizer et al. (1999) also found no relationship between the isotopically depleted CO_2 phase and the $\delta^{13}C$ of several labile C pools (including non-acid detergent fiber and cellulose), further supporting the idea that labile C pools are not necessarily isotopically depleted and that substrate pool switching from depleted to enriched is not solely driving respired CO_2 isotopic signature during decomposition.

While chemically-defined labile C pools may not be isotopically depleted, it is still possible that certain detrital inputs with fast turnover times are isotopically depleted and decomposition of these inputs contribute to the early phase of depleted respiration. An extensive ground cover of bryophytes is present at the coniferous site and epiphytic lichens are ubiquitous in the forest canopy. Thus, inputs of lichens and mosses contribute substantially to aboveground litter inputs at this site. A common lichen species and both common moss species analyzed have isotopic values between -33.1 and -31.4 ‰ (Table 4-2) which is substantially more isotopically depleted than either soil density fraction. The epiphytic lichen species *Lobaria* is quickly shredded by herbivores and detritivores once on the forest floor and then decomposed further by small arthropods and microbes. Mean turnover time for *Lobaria* is 7 months (McCune and Daly 1994). At the deciduous site, senescent leaves also have a low isotopic signature (-29.6 ‰) and have high

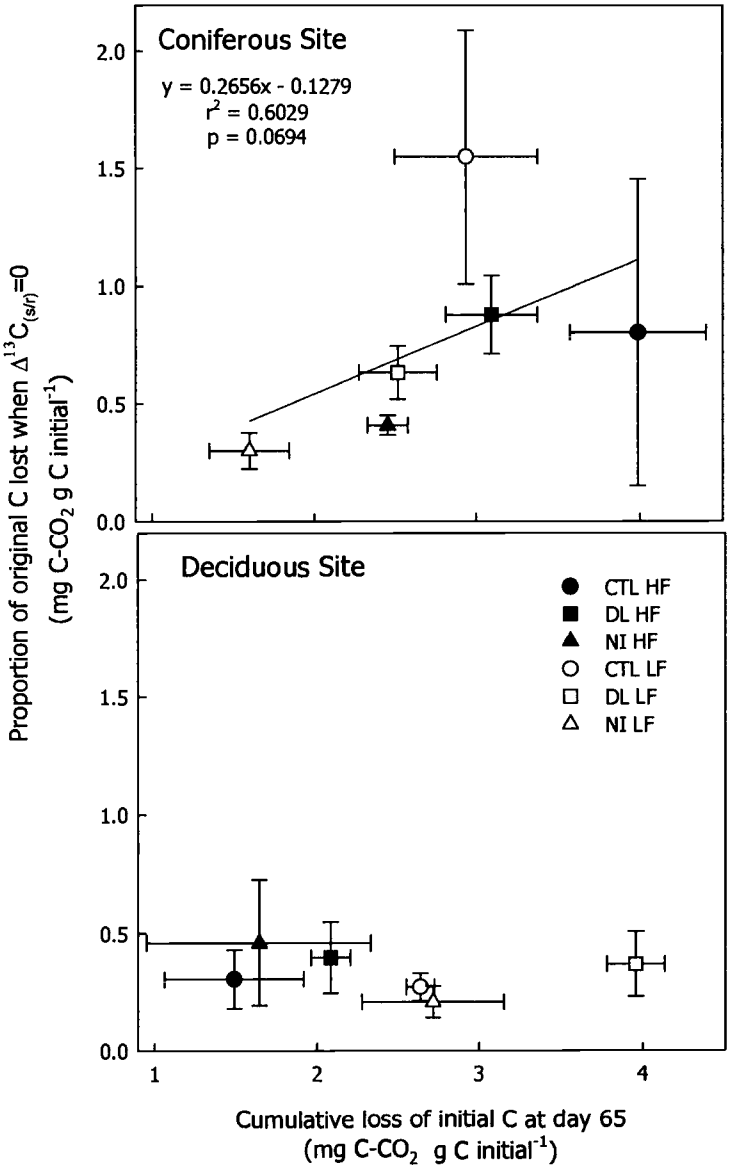

Figure 4-5. The relationship between the amount of original C loss when $\Delta^{13}C_{(s/r)} = 0$ and the overall lability (measured as cumulative C loss during 65 days of incubation) of the soil fractions at both study sites.

concentrations of soluble sugars and cellulose (Nadelhoffer et al. 2004), which contribute to a fast turnover rate (50-60% mass loss over 1 year for mixed black cherry and sugar maple foliage) (Bowden, unpublished data). Particularly at the coniferous site, where high annual rainfall constantly carries soluble material from the forest floor to the mineral horizons and properties of the mineral soil promote sorption of dissolved organic matter (as discussed above), decomposition of detrital inputs with fast turnover times and depleted isotopic signature early in the incubation may contribute the early depleted stage of the respiration. However, at both sites, there is a disparity of the isotopic signature between the lichens, mosses, and deciduous leaves and the respective light fraction material. This isotopic evidence further supports Yano et al. (2005) conclusion that most decomposition of these materials occurs before entering the mineral horizon and may not exert a great influence on A-horizon soil. Therefore, decomposition of these isotopically light inputs is not likely to be wholly responsible for the depleted phase of respiration.

Microbial discrimination against isotopically heavy C molecules in the most labile C pool is yet another possible explanation for the transition from isotopically depleted to enriched respired CO_2. With more complex substrates made of material with different isotopic signatures all contributing differently to CO_2 efflux, this process is difficult to quantify (Schweizer et al. 1999). However, in simple one-substrate and one-species aerobic incubations, there is evidence for the selective use of isotopically light C molecules from a uniformly labeled glucose substrate (Blair et al. 1985). Species-specific discrimination patterns, while still under debate, may also make the interpretation of these data complicated. Fernandez and Cadisch (2003) found that two species of white rot fungi discriminated against ^{13}C to different degrees, and that the degree of discrimination changed over the course of a 76-d incubation. In addition, Henn and Chapela (2000) found species-specific isotopic fractionation during sugar uptake that was mediated by the intra-molecular distribution of C atoms and by micro-environmental conditions in three species of basidiomycetes.

As decomposition continues and pools of readily degradable organic matter are depleted, alterations in microbial community can occur. Plante and McGill (2002) found that the labile portion of a particulate organic matter amendment to agricultural soils was decomposed and respired after 2 weeks of incubation. At approximately the same time, $^{13}CO_2$ also made the transition from depleted to enriched. Our results from the coniferous site, like those of Fernandez et al. (2003), showed a relationship between substrate C quality and the duration of the transition from depleted to enriched respiration. These patterns suggest that a shift in the microbial community following consumption of easily decomposed C pools occurs, and may contribute to the change from depleted to enriched respiration. As the readily available pool of substrate is depleted, the colonizing microbial community may become the food for subsequent populations; however, our data did not allow us to directly test this hypothesis.

Recent studies have documented consistent enrichment of $\delta^{13}C$ values between food sources and fungal biomass (Gleixner et al; 1993, Hobbie et al. 1999; Högberg et al. 1999; Kohzu et al. 1999). Early research using stable isotopes to study trophic levels demonstrated that heterotrophs are on average 1‰ enriched in ^{13}C relative to their diet (DeNiro and Epstein 1978). Enrichment occurs during transport from foliage to roots and during fungal chitin bio-synthesis (Hobbie et al. 1999). Gleixner et al. (1993) proposed that fractionation during glycolysis causes an intramolecular enrichment of the glucose molecules at the C3 and C4 position, contributing to this biomass enrichment. Saprotrophs are more enriched than mycorrhyzae (Hobbie et al. 1999; Högberg et al. 1999) and further enrichment may be due to selective use of isotopically enriched C molecules such as starches, as is common for mycorrhyzal species. Kohzu et al. (1999) found enrichments factors of 1.4 for ectomycorrhizal fungi / wood and 3.5 for wood decaying fungal species / wood for 115 species across diverse tropical ecosystems in Japan and Malaysia (Kohzu et al. 1999). In cases where the decomposer population is dominated by fungi, as it is at our coniferous site (Brandt et al., in review Oecologia) alteration in the species composition or increased reliance on dead biomass for food would eventually lead to an overall enrichment of respired CO_2 during decomposition.

On average, the transition from isotopically depleted to enriched respiration at the deciduous site (0.33 ± 0.06 mg C-CO_2 g^{-1} C initial, n=18) was shorter than that at the coniferous site (0.76 ± 0.16 mg C-CO_2 g^{-1} C initial, n=18) ($p = 0.0150$), indicating that different mechanisms may have controlled the shift from isotopically depleted to enriched respiration. It is likely that a combination of selective use of isotopically light, readily available organic inputs and a shift in microbial community, including the fast development of a fungal-dominated microbial community, contributed to the initial depletion of respired CO_2 compared to the substrate $\delta^{13}C$. This concept is supported by our results from the density fractions from the coniferous forest showing a relationship between substrate quality and duration of the depleted phase. The variable transition times from depleted to enriched respiration were directly related to the lability of the organic matter present, regardless of the detrital treatment or density fraction, suggesting that shifts in the microbial community occurred as the easily degraded organic matter pools were used. In contrast, the transition between isotopically depleted and enriched respiration came uniformly quickly for all substrates at the deciduous site. The reasons for the difference in pattern between the sites are not easily discernable; however, microbial community composition may have driven differences in respiratory isotopic pattern between the substrates at the coniferous and deciduous sites. Using phospholipid fatty acid analysis to identify relative abundances of functional groups within the microbial communities at the two study sites, Brant et al. (2006) found distinct communities, with fungi dominant at the coniferous site and gram negative bacteria dominant at the deciduous forest site. Because the incubated soils were inoculated with soil slurry from each respective site, it also is possible that the mechanisms causing isotopic shifts during decomposition are related to the differences in dominant members and shifts in community composition between the sites.

CONCLUSION

Large scale C-balance models recognize that above- and belowground ecosystem components contribute to total CO_2 efflux and that the relative contributions of these components depend on pool sizes and retention time of C within those pools. The variations in isotope discrimination between substrate and respiration in our sites suggests that detailed knowledge of factors influencing the differences in ^{13}C signatures of SOM and respired CO_2 are needed to properly constrain large scale C balances that link terrestrial $\delta^{13}C$ values and $\delta^{13}C$ CO_2 efflux (Ehleringer et al. 2000). While respired $\delta^{13}C$ reflected more closely the initial substrate value by the end of our incubation, it is apparent that respired $\delta^{13}CO_2$ should not be assumed to be a direct reflection of the pool $\delta^{13}C$. The relationship between respired $\delta^{13}CO_2$ and the $\delta^{13}C$ of the substrate is complex, and further knowledge of stable isotope composition of specific compounds or classes of compounds and microbial communities during decomposition is needed to understand processes which together determine the isotopic composition of soil organic matter pools and respired CO_2 from forest soils.

Acknowledgements. We thank Sarah Beldin, Katie Delaney, Heath Keirstead, Dave Dinette, and Tim Filley for help in the sample preparation and isotopic analysis and Nick Baldauf for collection of Bousson soils. Chris Swanston and Phil Sollins gave helpful comments on early drafts of the manuscript, for this we are very grateful. We also thank Paul Rygiewicz for first suggesting the microbial connection following an early presentation of the data. Funding was provided by NSF DEB-0087081 and USDA NRICGP 97-35101-4256 awarded to Kate Lajtha and Bruce Caldwell, and by Oregon State University Research funds through the Crop and Soil Science Department.

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CHAPTER 5

CONCLUSIONS

The SOM conceptual model used to guide research at the DIRT sites describes the potential stabilization and accumulation of C derived from detrital inputs into two organic matter pools over time. These are two distinct fractions with different recalcitrance and turnover times: the 'light fraction', composed of partially decomposed detrital material undergoing rapid decomposition and the 'heavy fraction', dominated by soil minerals in close association with a small amount of organic compounds considered to have a slow turnover rate. This conceptual division of organic matter pools holds true for soil from the Bousson DIRT site; however, is not reflective of the nature of soil from H.J. Andrews. At HJA, adsorption of DOM is a direct pathway for fresh detrital inputs to interact with the heavy fraction, which is reflected in the short mean residence time compared to Bousson heavy fraction. In addition, the legacy of coarse woody debris dominates C in the soil and contributes to the apparent old age of the light fraction material. The end result is light and heavy fraction at HJA that do not differ in degradability or mean residence time. Yano et al. (2005) calculated that only ~ 1/70 of total A horizon organic C content is produced as Oa horizon leachates annually. Particularly at a site where abiotic adsorption is a dominant pathway for OM stabilization, many more decades beyond the current six years of manipulation will be needed to for clear differences in organic matter stabilization in response to the alteration of detrital inputs to emerge

At Bousson, the leaf litter from the two dominant deciduous tree species is characterized by high nutrient content, which contributes to fast decomposition rates, rapid release of nutrients into the soil, and high nitrification rates. For example, leaf litter from sugar maple has high concentrations of soluble carbohydrates and cellulose, which contribute to fast decay rates and rapid immobilization and mineralization of nutrients during decomposition. Eleven years of litter manipulation, both above- and below-ground, has

had profound effect on the mean residence time of the light fraction material. No effect is apparent in the heavy fraction yet, indicating a degree of separation between light and heavy fraction organic matter in terms of the time scale for C cycling. The longer MRT of heavy fraction at Bousson than at HJA may also be indicative of the longer geologic history at that site.

Ultimately, over longer periods of time (years to decades) a change in detrital inputs to the forest floor should be apparent in the soil composition itself. The suite of mechanisms controlling organic matter stabilization (recalcitrance, organo-mineral interactions, and microbial accessibility) is common across sites; however, the relative importance of each is different and will depend in part on the quality of detrital inputs, the transfer of those inputs to the mineral soil (by leaching and physical mixing), and the propensity for a soil minerals to interact with organic matter. All the DIRT sites have been ongoing for different amounts of time and vary along climate, pedogenic, and N-deposition gradients, making direct comparisons between sites difficult. For all sites, baseline information is needed in order to fully understand the complexity of SOM stabilization.

Several widely used models over the past several decades, including BIOME-BCG and CENTURY, represent the heterogeneity of soil organic matter as discrete pools of various sizes and turnover times. Attempts at correlating physically defined fractions with these conceptual pools have had little success and the models remain with immeasurable components. Although progress has been made, the ability to quantitatively separate and determine turnover rates of various soil organic matter pools remains elusive. Future work with archived DIRT samples (ex. 0, 5, 10 y collections from all sites) and common measurements can begin to develop links between various biological, chemical, and physical factors regulating the balance of organic matter which hold true across sites and potentially make progress towards the validation of these already well developed, process-based models.

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APPENDIX

Radiocarbon-based mean residence time modeling:

Steady state assumption: $I = \frac{C}{\tau}$

I = Inputs
C = Carbon pool
τ = Mean residence time (MRT)

$\tau = \frac{1}{\kappa}$, κ = loss via decomposition

A 3-pool model: 3 pools were measured and a fourth was estimated, the SPT-mobilized organic matter (OM).

$$C_{SPT}F_{SPT} = C_{BU}F_{BU} - C_{LF}F_{LF} - C_{HF}F_{HF}$$

$$F_{SPT} = \left(\frac{1}{C_{SPT}}\right)\left(C_{BU}F_{BU} - C_{LF}F_{LF} - C_{HF}F_{HF}\right)$$

C = the proportion of total carbon within each OM fraction
F = the measured or estimated radiocarbon signature (fraction modern)

Radiocarbon signature of the charcoal was removed from LF and BU F.

Example for LF: $C_{LF-char}F_{LF-char} = C_{LF}F_{LF} - C_{char}F_{char}$

Modeling F for each OM pool pre-1890 (pre-Seuss effect and bomb carbon):

$F = \left(\frac{\kappa}{\kappa + \lambda}\right)$, $\lambda = 0.0001245$ decays per year

Modeling F for each OM pool post-1890:

$$C_t F_t = IF_I + C_{t-1}F_{t-1} - \kappa C_{t-1}F_{t-1} - \lambda C_{t-1}F_{t-1}$$

Where:
C_tF_t = measured C fraction at some time point with a measured F value
IF_I = inputs with a fraction modern value (assumed to equal to the atmosphere)
$C_{t-1}F_{t-1}$ = plus the OM that was already there
$\kappa C_{t-1}F_{t-1}$ = minus what was lost through decomposition during that time-step
$\lambda C_{t-1}F_{t-1}$ = minus loss through radioactive decay

$$C_t F_t = IF_I + C_{t-1}F_{t-1} - \kappa C_{t-1}F_{t-1} - \lambda C_{t-1}F_{t-1}$$

$$F_t = \left(\frac{1}{C_t}\right)\left(IF_I + C_{t-1}F_{t-1} - \kappa C_{t-1}F_{t-1} - \lambda C_{t-1}F_{t-1}\right)$$

$$F_t = \left(\frac{1}{C_t}\right)\left[IF_I + C_{t-1}F_{t-1}(1 - \kappa - \lambda)\right]$$

For each OM pool at each time-step in the model, F_t is calculated
F_I is the atmospheric F value at each time-step.

SOIL: HJA 4 NR

Model calculates cells colored this color

Total __ C inventory:	100			
Pool:	1: Fresh LF	2: HF	3: SPT sol	
Fraction of no-char C in pool:	0.1097	0.7123	0.1780	1.0000
Fraction of total C in pool:	0.1092	0.7092	0.1772	0.9955
Turnover time	173.62	306.57	237.19	change these so calc FM = meas FM
				alt answer on bomb curve

Remove char from LF		
<1.65 FM	1.0392	
Prop. total soil C in LF	0.1137	
tot C =	100	
	LF w/o char	char
frac of tot	0.960655	0.0393
FM	1.0448	0.9024
	enter this value	
	calculated value	

Remove char from bulk soil		
bulk FM	1.0002	
percent of soil C:		100
tot C =	100	
	w/o char	char
frac of tot	0.9955	0.0045
FM	1.0006	0.9024

2002

	Calculated FM	Calculated input	Measured FM
Bulk	0.0000	0.371	1.0006
Pool 1:	0.0000	0.063	1.0448
Pool 2:	0.0000	0.232	1.0039
Pool 3:	0.0000	0.075	0.9602

SOLVER 1.00118

Prop. LF	Prop. HF
0.1381	0.8619

*assuming loss came equally from LF & HF

Steady-state: Inputs = outputs

Year	Delta 14C	R-ATM	Pool 1 size	Pool 2 size	Pool 3 size	Total inven	Total loss	FM Pool 1	FM Pool 2	wtd avg	FM Pool 3	Total FM
1511	6.9	1.0069	10.9675	71.23458	17.79795	100		0.978842	0.963235	0.976686	0.971317	0.966385
1512	10	1.01	10.967476	71.23458	17.79795	100	0.370567	0.978882	0.963258	0.976723	0.971219	0.966388
1513	10.5	1.0105	10.967476	71.23458	17.79795	100	0.370567	0.978939	0.96329	0.976777	0.971121	0.9664
1514	10	1.01	10.967476	71.23458	17.79795	100	0.370567	0.978999	0.963324	0.976834	0.971024	0.966414
1515	8.5	1.0085	10.967476	71.23458	17.79795	100	0.370567	0.979056	0.963357	0.976887	0.970927	0.966426
1516	9.2	1.0092	10.967476	71.23458	17.79795	100	0.370567	0.979103	0.963384	0.976932	0.970832	0.966434
1517	11.6	1.0116	10.967476	71.23458	17.79795	100	0.370567	0.979155	0.963413	0.97698	0.970737	0.966443
1519	6.4	1.0064	10.967476	71.23458	17.79795	100	0.370567	0.97922	0.963451	0.977042	0.970642	0.96646

etc...

Pool HF	FM HF	% LF	% HF	FM LF	% C in LF	% char in LF	FM BU
0.7092	1.0038	0.1381	0.8619	1.0392	0.1137	0.0393	1.0002