AN ABSTRACT OF THE THESIS OF

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Abstract approved:

Elizabeth W. Sulzman

The mechanisms governing short- and long-term belowground carbon dynamics need to be understood. As part of a larger project developed to assess the effect of quantity and quality of litter inputs on the rate of soil organic matter (SOM) formation, I examined SOM in the H. J. Andrews Detritus Input and Removal Treatments (DIRT) plots.

This study was designed to: (1) determine how five years of treatment had changed the SOM in the reduced input plots and the added input plots relative to the control plots; (2) determine if the more labile (light) fraction of the soil had changed more from the manipulations than the more recalcitrant (heavy) fraction of the SOM; (3) document how the light and heavy fractions changed with depth in this coniferous forest relative to the published trends in other forest types; and, (4) determine if density fractionation conserved the C and N of the sample, or if the method resulted in any losses or transformations that might yield the method untrustworthy. To accomplish these objectives, I measured carbon and nitrogen concentrations and isotope values of SOM at different depths in the soil profile and by density fraction because previous work has shown that these parameters are good indicators of soil age/ recalcitrance.

I separated soil into labile (light) and more recalcitrant (heavy, mineral-bound) fractions from three depths in each of 18 treatment plots at the H. J. Andrews Forest, Cascades, OR using sodium polytungstate. Soil light fraction averaged 5.3% of whole

soil at 0-5 cm, 3.3% at 5-10 cm, and 1.3% at 10-20 cm. Light fraction from Control plots contained less carbon than light fraction from No Inputs and No Roots plots, and No Litter plots contained less carbon than No Roots plots. No other treatment differences were observed.

In a second set of analyses, the treatments were combined and treated as replicate samples to quantify depth and density trends. Light fraction C and N concentrations were greater than heavy fraction concentrations. For example, the light fraction contained 25.6% more C and 0.3% more nitrogen at 0-5 cm than the heavy fraction. The heavy fraction was more ^{13}C and ^{15}N enriched than the light fraction ($P<0.001$). Heavy fraction $\delta^{13}C$ values ranged from -26.5 to -25.3‰, whereas light fraction values ranged from -27.0 to -26.8‰ with increasing depth. Heavy fraction $\delta^{15}N$ values ranged from 3.1 to 6.3‰ with depth compared to a range of 0.1 to 0.7‰ in the light fraction. Bulk soil carbon and nitrogen concentrations generally decreased with depth while bulk C and N isotope values increased with depth. Lower concentrations of more isotopically enriched carbon and nitrogen indicate more decomposed soil organic matter. Because the density trends followed the bulk depth trends (and were even more pronounced), I conclude that heavy fraction SOM is more decomposed than light fraction material.

In summary, the findings were: (1) five years of treatment did not change the SOM in the reduced input plots and the added input plots relative to the control plots, with the one exception of light fraction carbon concentration differences between control and rootless plots, suggesting a root mediated priming effect; (2) the light fraction did not change more from the manipulations than the heavy fraction of the SOM (with the one exception mentioned above), indicating that the turnover times of both pools are greater than five years; (3) the trends with depth in this coniferous forest were similar to the published trends in other forest types despite the fact that this forest had greater carbon concentrations at all depths than SOM from other forests; and, (4) based on mass balance analysis, density fractionation conserved the C and N of the samples, so the method can be used to separate the labile and recalcitrant pools of SOM.

Quantifying C and N Contents and Isotope Signatures of SOM pools in the H. J. Andrews DIRT plots

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APPROVED:

Major Professor, representing Soil Science

Head of the Department of Crop and Soil Science

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Heath Keirstead, Author

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1.1 Introduction: Carbon and Nitrogen Cycles

Carbon dioxide (CO_2) and methane (CH_4) are greenhouse gases that have been linked to global warming. As a result of the Industrial Revolution, humans are releasing elevated levels of fossil fuel derived-CO_2 (Bird et al. 1996) significantly increasing the CO_2 concentration of the atmosphere. In order to assess the potential future greenhouse effect, sources and sinks for CO_2 need to be identified and quantified and the mechanisms driving the CO_2 fluxes into and out of these pools must be understood. Carbon moves between three major pools: the oceans, the atmosphere and the terrestrial biosphere. At least twice as much carbon is stored in soils as is stored in the atmosphere and three times as much as in terrestrial vegetation, but much less is known about carbon cycling in soil than about either of the other carbon pools (Townsend et al. 1995, Ehleringer et al. 2000). To slow climate change, one possible solution would be to sequester more carbon in the soil. To accomplish this goal, the mechanisms governing short- and long-term belowground carbon dynamics need to be understood.

The major processes regulating the flow of carbon between the soil and the atmosphere are photosynthesis and respiration. During photosynthesis plants fix carbon dioxide into sugars. Plant material enters the soil when plants or plant parts die and are shed, or through the process of exudation. Soil microorganisms use this detritus as a food source. They break down more complex hydrocarbons into simpler ones, and release carbon dioxide as they respire. Up to 80% of organic matter entering the soil is respired as CO_2, 3-8 % is incorporated into microbial biomass, and the remainder (13-38%) remains in the soil as humus (Brady and Weil 1999). About two-thirds of the carbon consumed by microbes is respired as CO_2. Roughly 30-50 % of soil respired carbon comes from roots (Schimel et al. 1994). The rest of soil respiration is a mixture of detrital turnover (25%), microbial turnover (15%), and turnover of soil organic carbon (SOC; approximately 10%). Some of the detritus turns

over rapidly, and some enters long-term storage pools. Separating these different carbon cycling processes and understanding the controls that regulate their rates of respiration is central to understanding soil carbon cycling.

The rate of soil carbon cycling is clearly interlinked with the nitrogen cycle because of plant and microbial stoichiometry. In many ecosystems, nitrogen is limiting to both photosynthesis and respiration. Generally, organic material added to the soil is much lower in nitrogen content than is needed by the respiring microbes. Plant leaves have a C:N of 30-80, a number that varies widely by species (Townsend et al. 1996). Tree bark and wood can have a C:N of ~200-500. Microbes (bacteria and fungi) have a C:N of 8-10 (Brady and Weil 1999). This means that for each gram of N in the detritus, the microbes can incorporate 8 g of C into their bodies. If the C:N of the detritus is higher than 25:1, N is immobilized into microbial biomass and is unavailable for plant uptake. If an area is N rich (due to fertilizer application or N deposition) more C will be respired, but it will also lead to increased humus formation (Brady and Weil 1999). Because nitrogen plays a role in the process of organic matter formation, I will highlight the more significant transformations it undergoes in the soil.

Nitrogen (N_2) is the most abundant gas in the atmosphere, but it is a limiting nutrient for vegetation. The dinitrogen bond is exceedingly strong, and can only be broken naturally by lightning and N-fixing bacteria (Sylvia et al. 1998). Nitrogen fixation is an anaerobic process that usually occurs in root nodules. When nitrogen fixing bacteria transform nitrogen gas into usable forms, they first incorporate it into their biomass. This organic nitrogen might then be released through the process of ammonification, or conversion to ammonium (NH_4^+). Any process that converts organic nitrogen to inorganic nitrogen is called mineralization. Conversely, immobilization is any process that converts inorganic nitrogen to organic nitrogen. The two major forms of inorganic nitrogen in the soil are NH_4^+ and nitrate (NO_3^-). Ammonium is the form plants can use immediately, whereas nitrate must be stored for later use or converted to NH_4^+ before it can be used. Sometimes NH_4^+ is converted to nitrite (NO_2^-) and subsequently to NO_3^- in the microbially-mediated process of

nitrification. Because of its negative charge, nitrate is more mobile in the soil and can be lost through the process of leaching. The amount of nitrogen present in the soil is dependent on transformative processes such as those described above.

Carbon and nitrogen concentrations, as well as C:N and lignin:N ratios, have been used as indicators of the extent of organic matter (OM) degradation. These measurements help scientists develop models and theories to describe the role of soil organic matter (SOM) in global nutrient cycles.

1.2 Soil Organic Matter Pool Theories

A large number of models have been developed to help explore and explain the transformations that take place as organic matter decomposes. The following models and theories deserve some attention because they are used to make predictions about global climate change and other large scale issues. To date, no general consensus exists as to the most realistic number of SOM pools, in part because of a lack of agreement as to the appropriate laboratory methods to separate and identify distinct SOM pools.

Current models contain anywhere from one to a continuum of SOM pools and have varying degrees of success in matching how physically separable SOM responds to environmental conditions. Giardina et al. (2000) chose a one-pool soil carbon model to examine the influence of temperature on forest soil decomposition predicted by models such as Century. They suggest that the use of only one pool did not influence their results, but Davidson et al. (2000) argue that the one-pool model is too simplistic because it underestimates the cycling rates of labile soil carbon pools. Torn et al. (1997) use two pools differentiated by vertical location within the soil. The surface pool represents fast cycling SOM, and the subsurface pool represents SOM with cycling rates varying from 10,000 to 20,000 years. They have suggested that as a soil ages, its mineral stability increases and its ability to stabilize organic matter decreases. Studies such as these are important for determining the appropriate number of soil organic matter pools to use in models.

Soil organic matter (SOM) is more commonly divided into three pools that vary in length of residence within the pedosphere. One of the earliest SOM models is the Rothamsted Model (RothC), which was based on research plots that have been maintained for over 100 years at Rothamsted, England (Coleman and Jenkinson 1996). In this model SOM is categorized into one inert organic matter pool and one of 4 active pools assumed to have differing turnover times: microbial biomass, decomposable plant material, resistant plant material, and humified organic matter. Century is another model, which has been widely used and adapted for studies of feedbacks between the carbon cycle and future climate (Parton 1996). The soil organic matter subcomponent of Century is comprised of three pools that differ in turnover time. The active pool, consisting of microbes and microbial products, has turnover times ranging from months to a few years. The turnover rate depends on soil texture, with higher rates for sandy soils and lower rates for clayey soils. The slow soil C pool has turnover times of 20 to 50 years. It receives inputs from the active pool and the surface microbial pool. The passive pool can have a turnover time ranging from 400 to 2000 years, depending on clay content of the soil. Depending on the source of information, labile carbon makes up approximately 5% to 10% of the total soil carbon pool, 40-85% is in the intermediate pool, and the remaining 10-50% is in the passive pool (Townsend et al. 1995, Trumbore 1997).

Baldock et al. (1992) proposed a conceptual model describing the process of decomposition based on the results of ^{13}C-NMR analysis. The Baldock model is a decay continuum beginning with fresh plant matter of large size, which loses O-alkyls as it begins to decompose. Organic matter then enters a partially degraded OM pool that consists of smaller size material (Baldock et al. 1992). During this intermediate phase lignin is degraded, and this process causes a loss of aromatic carbon. Finally, the most decayed material enters an organo-mineral pool of clay-associated particles, with an increased alkyl signature, possibly due to microbial synthesis.

Another conceptual model of decomposition is based on aggregate stability, or the tendency of particles to clump together. This theory predicts that well-aggregated soils will provide physical protection for otherwise labile OM, and so will store more

C for longer than similar but unaggregated soils (Golchin et al. 1994, Six et al. 1999, Puget and Drinkwater 2001).

Perhaps the primary reason why so many models have been developed is because a disjunct exists between the theoretical soil carbon pools used in models and the data available to calibrate the models which consists of methodologically derived pools. Several procedures are used to separate SOM pools in the laboratory, but lack of consensus remains as to the soil separation method that yields the most biologically meaningful results. Below I discuss some of the soil separation techniques and various analytical tools used to clarify belowground SOM processes.

1.3 Soil Separation Techniques

Any number of combinations of methodological and analytical techniques can be used to separate and describe soils (Evans et al. 2001). The justification for separating soil into component pools is to clarify the varying rates of decomposition of the pools, which is fundamental for understanding SOM dynamics (Sohi et al. 2001). Some of the methods are much better for studying short-term carbon dynamics, while others attempt to define longer-term carbon storage pools. The classical fractionation method entails extracting soil fractions based on their solubility in acid and base. It yields fulvic acids with turnover times of hundreds of years, as well as humic acids and humin, which probably have longer residence times ranging up to thousands of years (Evans et al. 2001). Physical separation of SOM by size and/or by density, followed by various analyses of the chemical constituents can help elucidate the number of pools necessary for a realistic assessment of SOM dynamics.

1.3.1 Size Fractionation

Some researchers separate soils by particle size (Bird et al. 2002, Bird et al. 2003). Based on chemical analyses that will be discussed later in this review, sand-associated carbon is more labile than silt and clay-associated carbon whereas the silt sized fraction has the slowest turnover time (Balesdent et al. 1987, Evans et al. 2001). Another technique commonly used is the separation of soil by aggregates (Sollins et al. 1999). Use of this method is necessary for studies using the aggregate stability

models detailed above. However, slight variations in aggregate separation methodology lead to dissimilar chemical and/or biological properties, indicating that aggregate separation is arguably not a good method for describing soil organic matter pools (Ashman et al. 2003).

1.3.2 Density Fractionation

Soil is a heterogeneous mixture that can be separated based on the density of its constituents. Density fractionation separates soil by immersion in a solution with a specific density. The theory behind density fractionation is that the light fraction (LF), which floats on the solution surface, is less cycled and more labile than the heavy fraction (HF), which sinks to the bottom of the solution. In other words, the turnover time of the LF should be less than the turnover time of the HF (Evans et al. 2001). The heavy fraction is denser because it is mineral-associated, whereas the light fraction is relatively mineral-free (Evans et al. 2001). Theoretically, the material that floats on water is the most recent, because it has not lost its water-repellency (Magid et al. 2002). As it decays and loses its water-repellent properties, its density changes (air leaves the interstitial spaces). The material >1.6g/cm^3 is generally termed the "organomineral fraction," meaning it is associated with mineral particles (Magid et al. 2002).

Swanston (2000) argues that the density fractionation method has major advantages over particle separation methods. First, the method is based on the premise that what floats in the dense solution is functionally different than what sinks. Second, the solution can be adjusted to any density necessary to allow maximum separation between the light and heavy pools. Low density organic matter has been shown to have shorter turnover times than high density SOM based on radiocarbon data (Trumbore 2000). The above traits of density fractionation allow a researcher to separate the light and heavy fractions of any soil based on the unique characteristics of that soil, whereas particle size separation is restricted by the arbitrarily assigned cut-off values for sand, silt, and clay and the rather limited availability of different mesh sizes.

Density fractionation protocols often entail the use of multiple density solutions, usually ranging from 1.6 to 2.2 g/cm^3. Sometimes the lowest density used is water (1.0 g/cm^3), based on the observation that fresh, intact plant debris is water-resistant (Magid et al. 2002). Aside from water, there are three commonly used density solutions (Evans et al. 2001): sodium polytungstate (Golchin et al. 1994, Magid et al. 2002, Rovira and Vallejo 2003) , sodium iodide (Sohi et al. 2001), and Ludox, a stable silica suspension (Accoe et al. 2002).

Sohi et al. (2001) used density fractionation followed by ^{13}C-NMR analysis to find the best methodology for separating soils into pools that match the pools in theoretical models. They found more decomposed organic matter in the intra-aggregate light fraction than in the free light fraction. They suggested that density separation to remove the free light fraction, followed by sonication to remove the intra-aggregate light fraction from the mineral-associated organic matter produces three chemically distinct SOM pools, ideal for use in models. Golchin et al. (1994) performed a similar separation and contend that the two light fraction pools are distinct enough that they should not be combined.

Puget and Drinkwater (2001) also separated organic matter based on its location relative to aggregates, yielding an occluded and a free particulate organic matter (POM) fraction. They suggested that occluded POM is protected from microbial processes and so it belongs to the intermediate carbon storage pool, whereas the free POM is more labile. They claimed that roots promote increased aggregation, and, based on ^{13}C evidence concluded that root-derived SOC has longer residence times than shoot-derived SOC.

Rovira and Vallejo (2003) were interested in comparing the recalcitrance of free light fraction to occluded light fractions. Using the ratio of cellulose to total carbohydrates as an indicator of recalcitrance, they discovered that the dense fraction was the least recalcitrant, the free light fraction had an intermediate recalcitrance, and the occluded light fractions were the most recalcitrant. The greatest amount of organic carbon was found in the coarse free light fraction, followed by the dense fraction.

1.3.3 Combining Size and Density Separation

It is not unusual for the size and density fractionation techniques to be used in combination. Combining these techniques has been used to identify the pool of SOC with the greatest capacity to stabilize C for the long term, to isolate the fraction with the highest N mineralization potential, to study the mechanisms of soil carbon sequestration, and to determine the particle size most important in promoting physical protection of SOM (Rodionov et al. 2000, Puget and Drinkwater 2001, Six et al. 2001, Sohi et al. 2001, Accoe et al. 2002, Magid et al. 2002, Six et al. 2002, Rovira and Vallejo 2003). Unfortunately, this can lead to an unwieldy number of fractions of questionable distinction that make statistical analyses unnecessarily complicated. For instance, Rodionov et al. (2000), developed a fractionation scheme resulting in 27 fractions. Six et al. (2001) size-density fractionated forest soils into 18 pools, which they later were able to reduce to five (Six et al. 2002). Magid et al. (2002) investigated a tropical soil that was converted from rainforest (C_3) to pasture (C_4) 16 years earlier. Their size-density fractionation scheme yielded a total of ten fractions.

The size-density fractionation procedure has other methodological problems as well. Magid et al. (2002) contend that the location of soil components in one of the SOC pools depends on the soil treatment prior to density separation. For instance, sonication may move some "light" particles into the organomineral fraction. Dispersal with sodium hexametaphosphate may not be enough treatment to get all of the light fraction into the light fraction. For instance, Magid et al. (2002) found that fecal pellets and cemented aggregates remained in the heavy fraction in their study, even though those materials are defined as light fraction. To avoid these issues, Magid et al. (2002) outline some key criteria for deriving multiple soil carbon pools. These are: (1) there should be a small number of fractions with consistent chemical composition; (2) they should be quantifiable; and (3) relate to SOM pools that differ in turnover time.

1.4 Techniques Used to Investigate the Separated Fractions

1.4.1 An Overview of Three Common Tools

To understand SOM dynamics, separation of pools needs to be coupled with at least one descriptive analytical technique. Nuclear magnetic resonance (NMR) differentiates between carbon compounds by detecting the resonance frequency of the carbon nuclei (Evans et al. 2001). Signal intensities relate to different carbon compounds, such as carbonyl, aromatic, O-alkyl and alkyl groups (Froberg et al. 2003). The chemical composition of SOM changes over the course of decomposition. Several authors have noted that as material decomposes it loses O-alkyls, and the proportion of alkyl groups increases (Baldock et al. 1992, Golchin et al. 1994, Zech et al. 1997).

The measurement of stable and radioactive isotopes can also be used to understand the processes involved in carbon storage. Radioactive ^{14}C has a half-life of 5,730 years and can be used to estimate the age of soil carbon (Trumbore 2000). Natural levels of ^{14}C can be measured and, if the annual variation in atmospheric ^{14}C is known, an estimated mean residence time of SOC pools can be calculated using a technique developed by Hsieh (Hsieh 1993, Evans et al. 2001). This technique can be used to calculate residence times between 1 and 80 years. In general, ^{14}C content decreases with depth in the profile (Trumbore 1997).

An additional benefit of radiocarbon studies stems from the bomb ^{14}C spike from the atmospheric nuclear weapons testing that took place in the late 1950's and early 1960's (Trumbore 1997). Bomb ^{14}C can be used to study the fast-cycling soil carbon pool by comparing current SOM ^{14}C measurements to pre-bomb data. This technique is only useful if archived soil samples are available for comparison. A number of other techniques have been developed to explore SOC using radiocarbon (Trumbore 1997). For example, the $\Delta^{14}C$ of soil-respired CO_2 can be used to split the fast-cycling OM into active and intermediate pools. Incubation experiments with ^{14}C-labeled substrate have been undertaken to study the movement of carbon from the coarse light fraction to the fine heavy organo-mineral fraction (Trumbore 1997).

The stable C isotopic signature seems to be linked to the process of decomposition, with more positive $\delta^{13}C$ values associated with more decomposed substrates (Bird et al. 2003). Studies using isotopic analysis can illuminate ecological processes, but isotope notation is complex and isotopic fractionation can be caused by a wide variety of mechanisms. Therefore, an overview of how they are reported and used follows.

1.4.2 Stable Isotope Basics

Stable isotopes of an element such as carbon have the same number of protons, but differ in number of neutrons. Different isotopes of an element have different atomic masses. The two stable isotopes of carbon are ^{12}C and ^{13}C. Carbon-12 is far more abundant than carbon-13, making up about 98.9% of all carbon in the biosphere. The relatively rare ^{13}C has a natural abundance of about 1.1% (Farquhar et al. 1989). Since the concentration of the rare isotope is so low relative to the more abundant isotope, the δ (delta) notation is used to report its abundance in a sample and is calculated using the following equation:

$$\delta X = [(R_{sample}/R_{standard})-1] \times 10^3$$

where X is the rare isotope (in this case ^{13}C), R is the ratio of the rare to the more abundant isotope ($^{13}C/^{12}C$), and for carbon the international standard is PeeDee belemnite (limestone). The δ value for the standard is, by definition, zero. A positive δ value for a sample means the sample is heavier than the standard, and a negative value means the sample is lighter than the standard (Peterson and Fry 1987). Because these values are so small, they are multiplied by a thousand and reported in units of permil (‰). The $\delta^{13}C$ value of a sample is sometimes referred to as its isotopic signature.

Although differences in isotopic signature may be small, they can be used to make comparisons between samples and inferences concerning the mechanisms of ecological processes. The mass balance approach, sometimes referred to as a mixing model, is a method that has been used in a variety of ecological studies. For example, it has been used to calculate how much carbon in soil samples is derived from C_4

versus C_3 sources (Boutton 1996), to determine the proportion of SOM contributed by stable and labile pools (Bernoux et al. 1998), and to calculate the C and N isotopic composition of litter extractions (Nadelhoffer and Fry 1988). The general form of the equation is

$$\delta_s[C_s] = \delta_a[C_a] + \delta_b[C_b]$$

where δ represents the isotopic signature of the material, [C] represents the concentration of an element in the material, and the subscripts s, a, and b represent the sample and its components respectively.

By calculating the $\delta^{13}C$ value of soil-respired CO_2, researchers can determine the degree to which each component is contributing to the process of respiration (Yakir and Sternberg 2000). The components, in the case of soil respiration, refer to roots, fungal hyphae, and microbes. Each of these constituents respires CO_2 with a distinct signature. When measurements of $\delta^{13}C$ and $\delta^{18}O$ are taken, they are plotted with the $\delta^{13}C$ of the organic form of the individual components. The contributing component with the highest $\delta^{13}C$ value and the one with the lowest $\delta^{13}C$ value become the end members. The respired signature should fall somewhere between the two end members, and the distance from one end member or the other indicates the relative contribution of that end member to the total system respiration. Isotopically distant or distinct sources give more reliable answers than sources that are isotopically similar. This approach works well for simple systems with two distinct end members, but gives less meaningful information in systems with multiple end members. However, the IsoSource program developed by Phillips and Gregg (2003) enables the user to determine a range of possible combinations of sources that could lead to the observed isotopic values of a sample. This model is especially useful in systems with more than two or three contributors where unique solutions are not possible. For example, IsoSource has been used to determine sources of air pollution and relative contributions of different prey species in the diets of predators and gives the bounds of potential contributions for each source (Phillips and Gregg 2003).

1.4.3 Stable Isotopes in Soil Organic Matter

Many studies have used the natural abundance of carbon and nitrogen isotopes to determine turnover rates of SOM in various ecosystems (Trumbore 1997, Accoe et al. 2002). Stable isotopes do not decay, so they can't be used to determine turnover times directly. However, their measurement can indicate the relative degree of SOM decomposition in any particular system, because the process of decomposition leads to increased (heavier) isotopic signatures (Nadelhoffer and Fry 1988). Their measurement can be especially useful in systems that have undergone a shift in vegetation from C_3 to C_4 plants.

1.4.3.1 Using Vegetation Shifts

A number of studies use the transition from C_3 to C_4 species to trace the flow of carbon through the soil (Balesdent et al. 1987, Accoe et al. 2002, Magid et al. 2002). All plants use the enzyme Rubisco to convert atmospheric carbon dioxide into a 3-carbon compound (Boutton 1996). However, C_4 plants have evolved an additional mechanism for concentrating the CO_2 before Rubisco converts CO_2 into a 3-carbon compound; this mechanism initially creates a 4-carbon compound, thus the name C_4. C_4 plants, which are mostly tropical and subtropical grasses, make up about 5% of plant species. They evolved during a time when atmospheric carbon dioxide was relatively scarce (Cerling et al. 1997). C_4 plants use the enzyme PEP-carboxylase to convert CO_2 into 4-carbon compounds, such as Malate, which are then transported into the bundle sheath cells where Rubisco completes the normal C_3 process. The C_4 plants are more efficient than C_3 plants and therefore less discriminatory against ^{13}C than C_3 plants. As a consequence, C_4 and C_3 plants have non-overlapping ranges of carbon isotopic signatures: C_3 plants have signatures ranging from -24‰ to -34‰, whereas C_4 plants have signatures ranging from -6‰ to -16‰ (Smith and Epstein 1971). C_3 vegetation has typical isotopic signatures ranging from whereas C_4 vegetation ranges from The fractionation that takes place during litter decomposition is relatively small compared to the fractionation of photosynthesis, so isotopic studies of SOM can be used to track past vegetation shifts.

C_3-C_4 vegetation shifts have been used in conjunction with size and density fractionation to estimate turnover times of the multiple SOM fractions (Balesdent et al. 1987). Accoe et al. (2002) applied a combination density-size fractionation scheme to a soil that had experienced a vegetation shift from C_3 to C_4 vegetation. The isotopic signature of the different fractions was used to study the path of decomposition and rate of incorporation of organic matter into SOC pools. Accoe et al. (2002) found lower turnover rates in the higher density fractions and in the smaller particle size fractions. They also found that organomineral complexes are more resistant to losses associated with soil disruption than organic carbon present in larger size fractions. To study the intermediate and short term dynamics of SOM pools, Magid et al. (2002) used ^{13}C analysis based on the vegetation shift and the addition of ^{14}C labeled plant matter, respectively. Their results support the hypothesis that over time C moves from large, light fractions into fine organomineral fractions.

1.4.3.2 $\delta^{13}C$ trends with depth

Even in soils that have not experienced a vegetation shift, isotopic trends in SOM shed light on the process of decomposition. Soil organic matter $\delta^{13}C$ values tend to increase (more positive values) with depth (Nadelhoffer and Fry 1988, Trumbore et al. 1995, Flanagan et al. 1996, Ehleringer et al. 2000, Bowling et al. 2002). Trumbore et al. (1995) sampled to a depth of 800 cm in an Amazonian forest and found a change from -27.3 ‰ at 0-10 cm to -23.6‰ at 500-800 cm. Soil profiles across Oregon also exhibit the pattern of ^{13}C enrichment with depth (Bowling et al. 2002). Sampling to a depth of 25 cm, Bowling et al. (2002) found the most negative $\delta^{13}C$ signatures in the fresh litter of the wettest site (-29.6‰) and the least negative $\delta^{13}C$ value in the 20-25 cm deep mineral soil of the driest site (-24.7‰). Nadelhoffer and Fry (1988) sampled SOM from a forested site in Wisconsin to a depth of 20 cm, and reported $\delta^{13}C$ values of -27.3‰ for leaf litter, -25.2‰ for 0-10 cm mineral soil, and -23.6‰ for 10-20 cm mineral soil.

A number of hypotheses have been generated to account for this depth-related variation, but as yet, the mechanisms for enrichment with depth have not been shown

conclusively. Ehleringer et al. (2000) summarized four hypotheses generated to account for the commonly reported ^{13}C enrichment with depth. The first one is called "influence of atmospheric change," and it concerns the decrease in atmospheric $\delta^{13}C$ values as a consequence of increased fossil fuel combustion since the dawn of the Industrial Revolution. Fossil fuels are depleted in ^{13}C, so the carbon incorporated into biomass now is isotopically lighter than pre-industrial biomass. This atmospheric depletion of $\delta^{13}C$ would only account for 1.3‰ difference between surface soil and that at depth, whereas the observed enrichment with depth may be greater than 3‰.

The second hypothesis concerns the phenomenon of microbial fractionation during decomposition of litter. Microbial fractionation would occur if microbes preferentially used lighter carbon for metabolic processes. A preference for lighter C would lead to a gradual shift in residual soil organic matter towards heavier $\delta^{13}C$ values. However, Lin and Ehleringer (1997) found no fractionation during autotrophic mitochondrial respiration, and claim the same would hold for heterotrophic respiration.

The third hypothesis entails the preferred digestion of lighter litter fractions. Some litter components, such as lignins, remain in the soil for longer than other components. However, lignins are lighter than their more digestible counterparts, which would lead to the opposite pattern. For example, *Acer rubrum* wood cellulose has a $\delta^{13}C$ of -25.9‰, whereas its lignin has a lighter value of -29.2‰ (Benner et al. 1987). Therefore, the isotopic difference between digestible and indigestible components does not generally support this hypothesis.

Soil carbon mixing is the fourth hypothesis. Studies have found a microbial ^{13}C enrichment relative to SOC by an average of 2‰ (Santrucková et al. 1998, Accoe et al. 2002). Microorganisms may become enriched relative to SOM if they incorporate soil CO_2, which is enriched relative to soil-respired CO_2, into their biomass during catabolism (Cerling et al. 1991). As microbes are recycled, they would then contribute a heavier isotopic signature to the SOM pools (Wedin et al. 1995). The crux of this hypothesis is that changes in $\delta^{13}C$ values with depth in a soil

profile result from an increase in the contribution of microbial biomass to the SOM pool with time.

In addition to the four hypotheses above, Nadelhoffer and Fry (1988) put forward a number of hypotheses to account for ^{13}C enrichment trends. According to these authors enrichment could be due to changes in litter inputs from sources enriched in ^{13}C content to those of low ^{13}C content. As discussed above, C_3 vegetation has typical isotopic signatures ranging from -24‰ to -34‰ whereas C_4 vegetation ranges from -6‰ to -16‰ (Smith and Epstein 1971). Nadelhoffer and Fry (1988) also mention the possibility of illuviation of dissolved organic matter enriched in ^{13}C into soil at depth, but one study reported that dissolved organic matter from spruce and beech forest soils became depleted in ^{13}C as a consequence of incubation (Kalbitz et al. 2003). The *in situ* measurement of DOM with depth in the profile would be interesting to pursue; as of yet it has not been studied.

A number of other reasons could explain why $\delta^{13}C$ becomes more positive with increasing soil depth. For example, if clays preferentially immobilize ^{13}C-enriched SOC, then translocation of clays down the soil profile could lead to ^{13}C enrichment with depth. Research designed to clarify the mechanisms of ^{13}C enrichment with depth is needed.

Low C and N concentration, low C:N ratio, and less negative isotopic ratios have been used as indicators of older SOM (Baisden et al. 2002b). The $\delta^{13}C$ evidence available suggests that deeper SOM is less negative and therefore older than SOM closer to the surface (Nadelhoffer and Fry 1988). Patterns of $\delta^{15}N$ with depth also support the argument that deeper SOM is generally heavier as do patterns of $\Delta^{14}C$ with depth (Boutton 1991).

1.4.3.3 $\delta^{15}N$ trends with depth

Nitrogen isotope natural abundance studies, which have the advantage of being practicable *in situ,* help elucidate the mechanisms of SOM decomposition. In most systems, soil $\delta^{15}N$ values increase with depth (Cheng et al. 1964, Karamanos et al. 1981, Tiessen et al. 1984, Garten and Miegroet 1994, Johannisson and Hogberg 1994,

Högberg et al. 1996, Kerley and Jarvis 1997, Högberg et al. 1999, Baisden et al. 2002a, Koba et al. 2003). For example, the $\delta^{15}N$ may increase 5-10‰ or more within the top 10 cm of forest mineral soil (Högberg 1997). An undisturbed grassland in southwest England experienced an increase in $\delta^{15}N$ from 1.9‰ at 1.5 cm depth to 8.5‰ at 20-30 cm depth (Kerley and Jarvis 1997). Soils of Saskatchewan also exhibit the trend of ^{15}N enrichment with depth in the top 50 cm of soil (Karamanos et al. 1981).

As with $\delta^{13}C$ profile trends, various theories have been put forth to explain the commonly observed pattern. Dijkstra et al. (2003) suggest that N assimilation enzymes discriminate against ^{15}N, causing roots to be heavy and vacuole-stored N to be light leading to the relative ^{15}N depletion of surface soils compared to those at depth. However, the most widely accepted theory suggests that mycorrhizae fractionate soil nitrogen, causing enrichment of ^{15}N within the mycorrhiza and movement of ^{15}N-depleted nitrogen to the plant (Högberg et al. 1996, Stewart 2001). The mycorrhizal theory would account for the relatively light $\delta^{15}N$ of plants and litter. As the litter is added and incorporated into soil surfaces, the top of the soil becomes isotopically lighter relative to the deeper soil. Since fungal material eventually enters the pool of recalcitrant organic matter, this theory helps explain the relatively ^{15}N-enriched values of that pool. The deeper soils become enriched due to mycorrhizal turnover, accentuating the difference in $\delta^{15}N$ values within the profile. This theory is supported by the NIFTE model developed by Hobbie et al. (1999b), which has the best fit to the observed trends when isotopic fractionation is set to occur as nitrogen moves from mycorrhizae to their plant partner. This pattern is more pronounced in forest soils, where plant litter is added to the soil surface, than in agricultural systems (especially tilled systems), where there are fewer mycorrhizae and the majority of plant litter is removed at the end of the growing season.

Ammonia volatilization, which occurs under conditions of high pH, is another explanation for the ^{15}N enrichment trends with depth (Högberg 1997). The loss of mostly $^{14}NH_3$ during litter decomposition would tend to leave behind a ^{15}N-enriched

pool of nitrogen. The process of nitrification, or microbially mediated conversion of NH_4^+ to NO_3^-, may have some associated isotopic fractionation. If the remaining ammonium is enriched in ^{15}N and the nitrate product is depleted, then leaching of depleted NO_3^- could cause an enrichment of the soil nitrogen (Högberg et al. 1996). Denitrification (conversion of nitrate to dinitrogen gas), which occurs under anaerobic soil conditions, could also cause enrichment of soil nitrogen pools, if the N_2 product is depleted in ^{15}N (Högberg et al. 1996).

Nitrogen is a limiting nutrient in most forest soils. Many tree species prefer to take up NH_4^+ over NO_3^-, and even if that NH_4^+ is not enriched by nitrification, a progressive shift toward lighter (depleted) surface soils could occur, since depleted litter is the source of plant uptake. Ammonium taken up by plant roots is derived from decomposed plant litter in surface soils. In a system with minimal new nitrogen inputs, (such as a forest with few N-fixers) the following scenario could account for relatively depleted surface soils compared to those at depth: if some of the soil nitrogen is shunted to a recalcitrant pool (perhaps through the mycorrhizal enrichment phenomenon), then a progressive depletion of plant litter and thus of surface soils could occur. The uptake of progressively lighter NH_4^+ could cause a shift in soil N from heavier to lighter isotopic values (Högberg 1997).

The natural processes of decomposition seem to be correlated with enrichment of SOM in the heavier isotopes ^{13}C and ^{15}N. The rate of SOM formation is heavily dependent on the biota, including plant and animal life, as they are the donors of litter, the precursor of SOM.

1.5 The Effect of Quality and Quantity of Inputs on SOM Storage

1.5.1 Input Effects on SOM Turnover and Storage

The quantity and quality of litter inputs greatly influence the rate of soil organic matter formation. A number of studies explore the effects of input quality and quantity on SOM. For instance, doubling litter inputs seems to cause increased decomposition of more recalcitrant organic matter (Nadelhoffer et al. 2004).

Parker (2002) suggests that litter quality is more important than litter quantity in determining SOM turnover. In a study of reforested agricultural fields in Massachusetts, Compton (1998) concluded that net nitrogen mineralization depended on tree species, which differed in litter quality. Sites dominated by conifers with low litter quality and high C:N ratios had the lowest rates of mineralization. The C:N ratio of softwood soils is wider than that of hardwood soils, which leads to slower rates of decomposition in the softwood soils. Several studies have found that low C:N ratio soils have less light fraction (labile SOM) than high C:N ratio soils (Boone 1994, Parker et al. 2002). Perhaps the microbes in the low C:N ratio soils are more active and thus respiring more of the light fraction C.

The Detritus Input and Removal Treatments (DIRT) plots are a long term study of the effects of quantity and quality of litter inputs on the rate of soil organic matter formation (Nadelhoffer et al. 2004). DIRT plots have been established in Harvard Forest, MA (1990), Bousson Experimental Forest, PA (1991), H. J. Andrews Experimental Forest (1997), and Síkfökút Forest, Hungary (2000). All the DIRT sites have the following treatments: no inputs (roots and aboveground litter excluded), no roots, no aboveground litter, control, and doubled aboveground litter. The developers of the DIRT experiment hypothesized that gross mineralization and nitrification rates are related to organic nitrogen inputs, with mineralization directly related to the quality of litter (Nadelhoffer et al. 2004). This hypothesis can be tested more extensively at the Andrews DIRT plots where a seventh treatment of doubled wood has been installed. Wood, with a C:N ratio of 897, is a lower quality input than litter, which has a C:N ratio of 119 (Sollins et al. 1980).

During the first decade of the DIRT experiment at the oak-maple-birch dominated Harvard Forest, root inputs appear to be approximately equal in magnitude to aboveground inputs, but effects of root inputs dominate during the first five years of treatment (Nadelhoffer et al. 2004). During these early years contributions of roots to the soil ecosystem appear to play a large role in mineralization. Belowground litter could be more important than aboveground litter simply due to the location of the roots within the mineral soil (Langley and Hungate 2003).

Roots are recalcitrant, probably due to the presence of ectomycorrhizae, which have a high percentage of chitin and may produce anti-microbial substances that inhibit decomposition (Simard et al. 1997, Langley and Hungate 2003). Root exudates, however, probably stimulate microbial activity and lead to increased rates of decomposition. In a trenched plot study, Simard et al. (1997) found mycorrhizal richness and diversity was only half as high in plots without roots as in rooted plots. Roots and rhizodeposition are central to overall community structure. Roots and root exudates thus have great potential to affect decomposition and nutrient cycling.

1.5.2 The Priming Effect

The priming effect is a change in soil organic matter mineralization caused by a manipulation of litter or other substrate (Kuzyakov et al. 2000). The change could be either an increase (positive priming) or decrease (negative priming) in the rate of SOM mineralization. The addition of mineral N fertilizers, easily decomposable organic substances, rhizodeposition, and the cycle of soil drying and rewetting could lead to an increased rate of mineralization (Kuzyakov et al. 2000). Microbial activity is integral to the phenomenon of priming because microbial populations respond to added substrates with increased activity.

Roots have been implicated as a major cause of the priming effect (Kuzyakov et al. 2000, Fontaine et al. 2003). Rhizodeposition stimulates microbial activity, which leads to increased nitrogen mineralization. This additional mineralized nitrogen can be taken up by the plants and thus lost from the soil stores. One study found that after three months with maize roots, the total soil organic carbon decreased 5-7%, assumedly because the presence of roots enhanced microbial activity and increased decomposition of SOM (Kuzyakov et al. 2000).

Because the DIRT plots manipulate quantity and quality of inputs to the soil, they may be subject to the priming effect. The complexity and diversity of chemical components may differ, in which case the priming effect could be greater in the treatment with more complex inputs (Fontaine et al. 2003). The rooted treatments might have less carbon than the No Inputs and No Roots treatments, which exclude

roots, if root exudates are stimulating microbial populations. The DIRT experiment is an ideal field setting for studying how the priming effect might change soil's role as a source or sink of atmospheric carbon dioxide.

1.5.3 Input Effects on Isotopic Signatures

nHow will inputs affect the isotopic signatures of soil organic matter? On a global scale, the C and N signatures of SOM should reflect the vegetative inputs of an ecosystem. C isotope signatures of a C_4 ecosystem should be heavier than C isotope signatures of a C_3 ecosystem. However, all inputs are not created equal. Ågren et al. (1996) predicted that high quality litter (low C:N) would lead to the formation of more ^{13}C-enriched SOM than low quality litter (high C:N), since decomposers can grow more rapidly on high quality litter.

The typical pattern of $\delta^{13}C$ for SOM is a rapid increase from the forest floor to the mineral horizon, which could be due to the presence of leaf litter (lighter) on the surface and the presence of roots (heavier) in the mineral soil (Bird et al. 2003). Bird et al. (2003) found that root $\delta^{13}C$ values are an average of 1.1 ±0.5‰ heavier than litterfall of the same species. Similarly, Schweizer et al. (1999) found the roots of a legume were 1.5‰ enriched in ^{13}C compared to its leaves. Data for *Pseudotsuga menziesii* in the Oregon Cascades suggest that needles, though quite variable, are lighter than fine roots (Bowling et al. 2002). It follows that the signature of the OM will depend on the relative contribution of different litter pools. Fine roots and root exudates are the only inputs directly entering the mineral soil. If belowground inputs have a greater influence on decomposition than aboveground inputs, then the signature of SOM should be more similar to that input (Dijkstra et al. 2003). This hypothesis has been tested in our study by altering the amount of different litter pools, and by varying the contribution of above vs. below ground inputs.

Nadelhoffer and Fry (1988) examined the isotopic signatures of inputs and SOM in plots with aboveground litter manipulations. Their two oak forest sites, established in 1956, included no litter, double litter, and normal litter treatments. The double litter treatment resulted in lighter $\delta^{13}C$ and $\delta^{15}N$ values than in the control

(normal litter treatment). This result was stronger for $\delta^{15}N$ than for $\delta^{13}C$ and more noticeable in the 0-10 cm depth than in the 10-20 cm depth. Fresh litter had the lightest signatures, whereas the heaviest isotopic values were in the no litter plots at 10-20 cm.

1.6 Objectives

Carbon storage in soils has been called the black box of the global carbon cycle. Shang and Tiessen (2000) emphasize the problematic nature of trying to separate real soil into carbon pools that match up with model-designated carbon pools. Developing universal methodologies for separating SOM into biologically meaningful pools is an important step in clarifying the role of soils in global carbon storage. The DIRT projects described above are advancing our understanding of the mechanisms driving belowground carbon dynamics. As part of the DIRT project, my research examines how SOM pools have changed after five years of manipulations to the organic matter inputs. My project was designed to examine the four following objectives:

- Determine how five years of treatment changed the SOM in the reduced input plots and the added input plots relative to the control plots.
- Determine if the more labile fraction of the soil changed more from the manipulations than the more recalcitrant fraction of the SOM.
- Document how the light and heavy fractions changed with depth in this coniferous forest relative to the published trends in other forest types.
- Determine if density fractionation conserved the C and N of the sample, or if the method resulted in any losses or transformations that might yield the method untrustworthy.

To accomplish these objectives, I examined the isotopic signature of the carbon and nitrogen in soil organic matter (SOM) as a function of depth in the soil and by treatment. The DIRT treatments of interest are: no inputs (roots and aboveground litter excluded), no roots, no aboveground litter, control, doubled aboveground litter,

and doubled woody debris. Because we are interested in the time course of organic matter formation and decomposition, I split each soil sample from each treatment into two "fractions," the labile, light fraction, and the recalcitrant, heavy fraction. I also compared these fractions to the bulk soil. I measured carbon and nitrogen concentrations and isotope values of SOM at different depths in the profile because changes in these parameters reflect the degree of SOM decomposition. Decomposition leads to decreasing carbon and nitrogen concentrations and increasing $\delta^{13}C$ and $\delta^{15}N$ values of soil organic matter.

This research project should contribute information to the extant body of research into soil organic matter decomposition. It should help answer the questions of how a change in inputs to the soil system will affect SOM dynamics, and how different pools of SOM will respond to such manipulations.

2 Changes in SOM after Five Years of Input Manipulations

2.1 Introduction

Carbon dioxide (CO_2) and methane (CH_4) are greenhouse gases that have been linked to global warming. As a result of the Industrial Revolution, humans are releasing elevated levels of fossil fuel derived-CO_2 (Bird et al. 1996), significantly increasing the CO_2 concentration of the atmosphere. In order to assess the potential future greenhouse effect, sources and sinks for CO_2 need to be identified and quantified and the mechanisms driving the CO_2 fluxes into and out of these pools must be understood. Carbon moves between three major pools: the oceans, the atmosphere and the terrestrial biosphere. At least twice as much carbon is stored in soils as is stored in the atmosphere and three times as much as in terrestrial vegetation, but much less is known about carbon cycling in soil than about the other carbon pools (Townsend et al. 1995, Ehleringer et al. 2000). To slow climate change, one possible solution would be to sequester more carbon in the soil. To accomplish this goal, the mechanisms governing short- and long-term belowground carbon dynamics need to be understood.

One approach to understanding these mechanisms is through experimental manipulations in the field to assess the effect of quantity and quality of litter inputs on the rate of soil organic matter formation. The DIRT plots are a series of long term Detritus Input and Removal Treatments that employ such field manipulations. DIRT plots have been established in Harvard Forest, MA (1990), Bousson Experimental Forest, PA (1991), H. J. Andrews Experimental Forest, OR (1997), and Síkfökút Forest, Hungary (2000). All the DIRT sites have the following treatments: exclusion of roots and aboveground litter (no inputs, NI), no roots (NR), no aboveground litter (NL), control (CTRL), and doubled aboveground litter (DL). The developers of the DIRT experiment hypothesized that gross mineralization and nitrification rates are related to organic nitrogen inputs, with mineralization directly related to the recalcitrance or quality of litter (Nadelhoffer et al. 2004)

The rate of soil carbon cycling is clearly interlinked with the nitrogen cycle because of plant and microbial stoichiometry. In many ecosystems, nitrogen is limiting to both photosynthesis and respiration. Generally, organic material added to the soil is much lower in nitrogen content than is needed by the respiring microbes. Plant leaves have a C:N of 30-80, a number that varies widely by species (Townsend et al. 1996). Tree bark and wood can have a C:N of ~200-500. Microbes (bacteria and fungi) have a C:N of 8-10 (Brady and Weil 1999). This means that for each gram of N in the detritus, the microbes can incorporate 8 g of C into their bodies. If the C:N of the detritus is higher than 25:1, N is immobilized into microbial biomass and is unavailable for plant uptake. If an area is N rich (due to fertilizer application or N deposition) more C will be respired, but it will also lead to increased humus formation (Brady and Weil 1999). The process of decomposition leads to decreasing carbon and nitrogen concentrations and increasing $\delta^{13}C$ and $\delta^{15}N$ values of SOM (Nadelhoffer and Fry 1988).

In this study, one research objective was to determine how five years of treatment had changed the SOM in the reduced input plots and the added input plots relative to the control plots. One might expect to see more carbon in the added input plots, and less in the reduced input plots. If the presence of roots stimulates the decomposition of SOM, then plots with roots might have less SOM than plots without roots. Over time, the plots should all begin to take on the C and N signatures of their dominant inputs as observed by Nadelhoffer and Fry (1988). If wood is the isotopically heaviest input, as data from Bowling et al. (2002) suggest, then Double Wood plots should have less negative $\delta^{13}C$ and $\delta^{15}N$ signatures than Double Litter or Control pots. Microbes in the No Input plots, faced with a lack of fresh litter, might start decomposing older SOM, and this process of continued humification could cause SOM signatures in No Input plots to become even heavier, as suggested by Bird et al. (2003). I hypothesized that treatments would cause isotopic shifts in near-surface soils, with No Inputs heaviest and Double Litter lightest. Because the treatments have only been going for 5 years, treatment effects might only be observed in the most active soil pools, so labile and recalcitrant pools were examined separately.

A second research objective was to determine if the labile soil pool had changed more from the manipulations than the recalcitrant SOM fraction. I split each soil sample into two "fractions", light (presumably fast turnover pool), and heavy (presumably recalcitrant, not likely to be influenced by treatments on decadal timescales). Theoretically, light fraction (LF) represents less decomposed SOM than heavy fraction (HF) material, so one would expect to see more carbon in the light fraction than in the heavy fraction. Another possibility might be a ^{13}C-enrichment in the heavy fraction, indicating that the heavy fraction is older or has been subjected to more decomposition than the light fraction, as suggested by Nadelhoffer and Fry (1988).

A third research objective was to document how the light and heavy fractions changed with depth in this coniferous forest relative to the published trends in other forest types. Based on a survey of the literature, I expected the soil organic matter to exhibit trends of decreasing C and N concentrations and increasing isotopic signatures with increasing depth in the profile. SOM should lose carbon and nitrogen with increasing depth and become more isotopically enriched, as seen in other ecosystems, because detritus collects on or near the soil surface, and decreasing C and N concentrations as well as increasing isotopic values indicate increasing stages of decomposition (Nadelhoffer and Fry 1988, Högberg et al. 1996, Kerley and Jarvis 1997, Garten and Ashwood 2002) The direction of trends with depth should be the same at the Andrews as in other forests, but the actual values could differ since detrital inputs in the Andrews Douglas fir forest have relatively large carbon concentrations (Sollins et al. 1980).

The fourth objective was to determine if density fractionation conserved the C and N of the sample, or if the method resulted in any losses or transformations that might yield the method untrustworthy. I used a mass balance approach to determine if the fractionation procedure resulted in a loss of C or N, or skewed the results of the isotopic analysis.

The Andrews DIRT plots were only five years old when this study was undertaken. In terms of soil processes these plots are very young and treatment effects

may not, as yet, be reflected in SOM. Therefore, a major goal of my study was to characterize the SOM profile at the DIRT plots and make this data available for future reference.

2.2 Materials and Methods

2.2.1 Research Site

The H.J. Andrews Experimental Forest (HJA) DIRT plots are located in the Lookout Creek Valley (N44º 13'51.71, W122º 13'16.21) at an elevation of 531 m in Central Cascade Range of Oregon. The plots are distributed across a relatively flat river terrace. The dominant overstory vegetation includes old growth Douglas fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla)*. Western red cedar (*Thuja plicata*) and vine maple (*Acer circinatum*) are also present. Mean annual temperature at the Andrews headquarters is 8.7 °C (1973-2002) and mean annual precipitation over the same period is 2370 mm yr^{-1}, mostly as rain. In general over 70 percent of the precipitation occurs during a "wet season" between November and March.

The soils are classified as coarse loamy mixed mesic Typic Hapludands. Small areas of Andic Dystrudepts and Vitrandic Dystrudepts also underlie the treatment plots (Dixon, 2004). The soils have strong andic properties: high amorphous Al hydroxide and aluminosilicate contents (oxalate-extractable Al= 1.1 %) and a pH near 11 in 1N NaF (Yano 2002). The mineral surface horizon of the DIRT site has an average pH of 5.4 and a bulk density of 0.82 Mg/ha (Dixon 2003). The average C:N ratio of the top 0-5 cm is 28.6. The texture is loam and contains 9-20% clay (Dixon, 2004). The mean soil temperature at 5 cm was 9.8ºC in 2002, and the mean soil moisture at 10 cm was 25.9% for the same year (Sulzman et al. 2004).

2.2.2 Experimental Manipulations

Detritus Input and Removal Treatments (DIRT) is a long term study designed to elucidate how the quantity and quality of soil organic matter inputs are related to the rate and variability of decomposition processes. The six treatments include double litter (DL), double wood (DW), no aboveground inputs (NL), no belowground inputs

(NR), no inputs (NI) and control (CTRL). In 1997 the litter input/exclusion treatments, replicated three times, were randomly assigned to the 10 x 15 m plots (Figure 1). The litter is chronically excluded from No Litter plots with 1 mm-mesh screens and transferred to Double Litter plots four to five times per year: at the end of the dry season, twice or more during the wet season (November–March), and at the beginning of the dry season (typically June). Large branches and stems or lichen/moss masses that fall on screens are removed. A mix of extremely decomposed woody debris and the chips of large pieces of intact Douglas-fir, with a ratio of decomposed to intact woody debris of 4:1, are added every other year to the forest floor of Double Wood plots. For the past six years, 1,794 kg litter C/ha/y have been added to the Double Litter plots in addition to the natural litter fall, and 5,760 kg wood debris C/ha/y have been added to the Double Wood plots. No Root and No Input plots were established by trenching the perimeter to 1 m, inserting a 10 mil (127 micron) thick polyethylene sheet along the bottom and sides of the trench, then back-filling. The same mesh screen as for the No Litter plots was also used for the No Input plots.

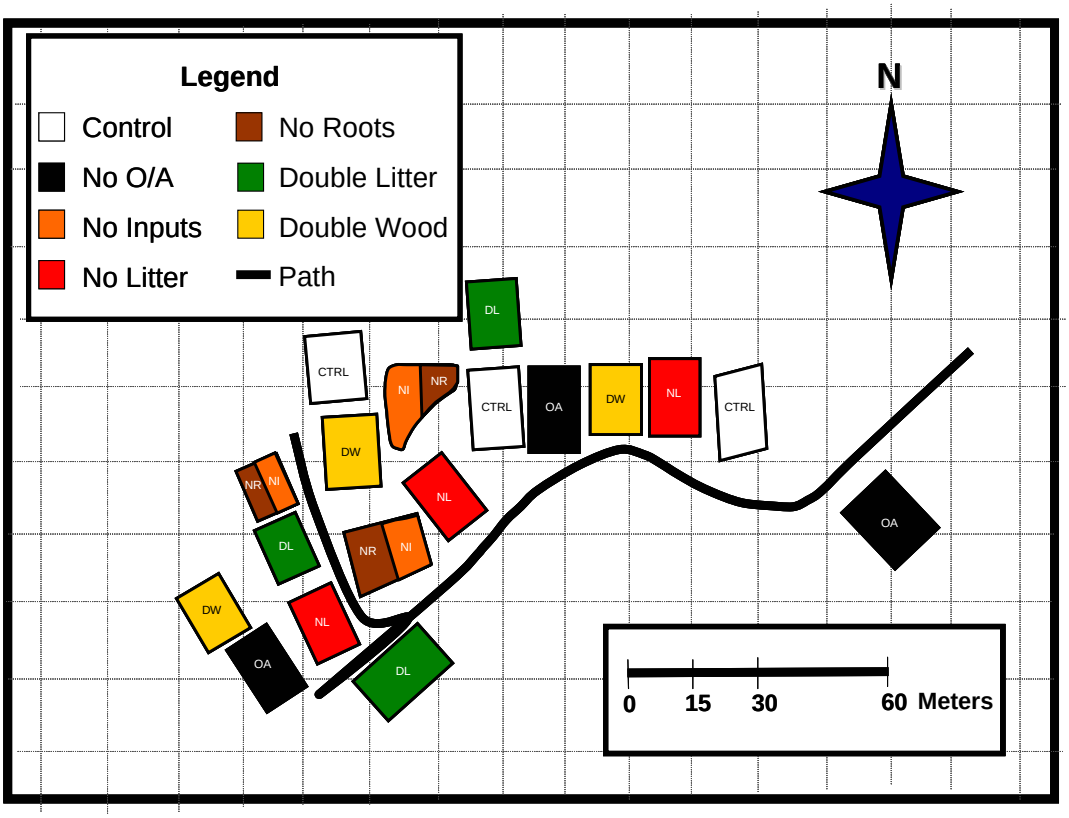

Figure 1. Map of H. J. Andrews DIRT plots showing location of plots and respective treatments.

2.2.3 Soil Collection

The 0-5, 5-10 and 10-20 cm samples were collected with a bucket auger. I composited soil from six locations within each plot for approximately 500 to 1000 g of bulk soil. The soils from 90-100 cm were collected using an Environmentalist's Sub-Soil Probe (JMC, Clements Associates Inc, Newton, IA). For the 90-100 cm samples, one core was taken per plot in an effort to minimize destructive sampling. The soils were placed in Ziploc bags, labeled and stored in a 5ºC refrigerator until they were analyzed. The field moist soils were sieved with a 2mm sieve and roots were removed.

2.2.4 Density Fractionation

Half of each soil sample was density separated into light and heavy soil fractions and the remainder of the sample was analyzed as bulk soil. The fractionation

procedure followed the method used by Swanston (1996) with some modifications. See Appendix A for step-wise directions. The first step in fractionation was to determine the correct density of sodium polytungstate (SPT) (Sometu, Sherman Oaks CA) solution to use for a particular soil. The typical density is between 1.2 and 2.2 g/cm^3. For the HJA soil, initial fractionation was conducted at densities of 1.2, 1.4, 1.6, and 1.8 g/cm^3 using an off-plot sample of control soil. The goal was to determine the density at which the light fraction contained the most organic matter and the least mineral content. The fractionated subsamples were heated in a combustion oven at 500ºC for 30 minutes to determine percent loss on ignition (% LOI). The proper density was the one at which the amount of organic matter (OM) left behind in the HF leveled off (density where % LOI stopped changing) and the amount of mineral soil started to increase in the LF (% ash in LF increased).

For the H. J. Andrews soil, a density of 1.6 g/cm^3 maximized differences between LF and HF. The 0-5 cm samples were processed field moist as required for a separate incubation experiment. Approximately 260 g moist soil were placed in 1L Nalgene bottles with SPT solution that was already at a density of 1.6. Soil volume did not always justify using the 1 L Nalgene bottles, in which case 500 ml bottles were substituted. Extra dry SPT was added to the bottle to cancel out the effect of the water in the soil. Water content for each sample was determined on subsamples of each 0-5 cm soil by weighing, drying at 105ºC overnight, and re-weighing. The amount of extra dry SPT to maintain a solution density of 1.6 g/cm^3 was calculated from the soil water content and the density chart provided with the SPT. The samples from all other depths were density fractionated using oven dried samples rather than field moist. Samples were dried overnight at 80ºC to avoid C volatilization. For the remainder of the 5-10 and 10-20 cm samples, 50 grams of dry soil and 100 ml of SPT solution were added to a 250 ml wide mouth Nalgene bottle.

Fifty to 100g of bulk sieved soil was set aside for future analysis. It was oven dried at 80ºC but it was not rinsed or subjected to any of the other processes involved in density fractionation.

Once all the samples were in bottles with the appropriate SPT solution, all bottles were shaken for one hour on a shaker table. The sides and caps of the bottles were then rinsed down with SPT, and allowed to settle for 24 hours. After settling, the light fraction was aspirated from the surface of the solution (Strickland and Sollins 1987). The remaining sample was then reshaken by hand, rinsed down and allowed to settle for another 24 hours. This process was followed by a second aspiration. The aspirated light fraction was filtered using ashed glass fiber filters, and rinsed multiple times with distilled deionized water to remove all SPT from the sample. Best recoveries were obtained when samples were scraped off filters while still wet. The heavy fraction was centrifuged for 25 minutes to separate soil from SPT. The supernatant was discarded and replaced with ultra pure water after each centrifugation. The bottle was then shaken to resuspend the soil, and the sides rinsed down. Each sample was centrifuged a total of three times, then the bottle of heavy fraction was uncapped and air dried for 24-48 hours before HF retrieval.

Soil from 90-100 cm was not fractionated because it was very low in light fraction material. Based on the light fraction obtained in the 10-20 cm soils (1.4%±0.06), and the trend of decreasing LF with increasing soil depth, I determined that I would need more than 100 g of bulk soil from 90-100 cm in order to retrieve 1 g of LF. Because I had a maximum of 20 g of 90-100 cm soil from each plot I did not density fractionate those samples.

2.2.5 Chemical Analysis

Oven-dried samples were ball milled to a fine powder and sent to the Stable Isotope/Soil Biology Laboratory of the University of Georgia Institute of Ecology for analysis of total C and N using the Micro-Dumas combustion technique (NA1500 C/H/N Analyzer, Carlo Erba Strumentazione, Milan).

Sample carbon and nitrogen contents were used to determine the amount of soil to weigh out for isotopic analysis so that samples contained the same amount of carbon or nitrogen to avoid potential linearity problems on the Isotope Ratio Mass Spectrometer (IRMS). Sample weights ranged from 1 to 75 μg. Light fraction, heavy

fraction, and bulk soils were run for $\delta^{13}C$ and $\delta^{15}N$ determination at the EPA Western Ecology Division's Integrated Stable Isotope Research Facility using an IRMS **(**Finnigan MAT Delta Plus XL, Breman, Germany); precision = ±0.04‰ $\delta^{13}C$; ± 0.09‰ $\delta^{15}N$; ±0.08% C; and ±0.04% N as determined by the standard deviation of repeated measurements of the standards (NIST Peach and Pine) and selected sample replicates.

2.2.6 Mass Balance Analysis

To compare the fractionated soil to the bulk soil, I used the following mass balance equation:

$$A_{blk}*[C]_{blk} = (A_{lf}*[C]_{lf}) + (A_{hf}*[C]_{hf}),$$

where A represents atom percent ^{13}C or ^{15}N, [C] stands for concentration of carbon or nitrogen in the fraction, specified by $_{blk}$ (bulk), $_{lf}$ (light fraction) or $_{hf}$ (heavy fraction). A simplified version of this equation: $[C]_{blk} = [C]_{lf} + [C]_{hf}$ was used to determine if the carbon or nitrogen concentrations were different between the bulk and fractionated soil.

The first mathematical step was to compute percent LF by dividing g LF by (g LF plus g HF) for each sample and multiplying by 100. I multiplied the percent light fraction by the elemental concentration in the light fraction. I then converted each sample's δ value to atom percent using the following equation:

$$A_{sample} = 100*(R_{standard}*(\delta_{sample}+1000))/(1000+ R_{standard} *(\delta_{sample} +1000)).$$

Next, I multiplied the elemental concentration from light fraction by its atom percent value. I used the same procedure to calculate what elemental concentration of the recombined soil originated in the HF. If the method was conservative, the two sides of the mass balance equation should be equal.

2.2.7 Statistical Analysis

Treatment analyses were conducted using PROC MIXED in SAS (version 8.0) because the measurements were repeated over space (at different depths) and assumed to be correlated. The use of a repeated measures statistical model accounted for the variation associated with the plots. I performed the mixed procedure for each

measurement (%C, %N, $\delta^{13}C$, $\delta^{15}N$, and C:N) for each density fraction (bulk soil, HF and LF) for a total of 15 iterations. I assumed the density parameter was less correlated than depth, so I could use the repeated measure structure. The repeated factor was depth in the soil profile, and the repeated subject was the plot. I acquired AICc values (Akaike's Information Criterion) for the eight following covariance structures: Compound Symmetry (CS), Unstructured with 0-3 correlated depths (UN 1, UN 2, UN 3), Banded Toeplitz with 0-3 correlated depths (TOEP 1, TOEP 2, TOEP 3), and Autoregressive Order 1 (AR 1). For each iteration, the covariance structure with the lowest AICc value was used. This analytical framework indicated the level of significance of depth, treatment, and the interaction of depth * treatment. I used the conservative Bonferroni method for pair-wise comparisons.

For the depth and density analyses, I used the same SAS mixed procedure with the repeated measure structure as described above. Because the treatments were not in place long enough to become significantly different, I collapsed them for the depth and density analyses. For each measured parameter (%C, %N, $\delta^{13}C$, $\delta^{15}N$, and C:N), the covariance structure with the lowest AICc value was used. This analytical framework indicated the level of significance of depth, density, and the interaction of depth * density. It also indicated which depths, densities or depth*density interactions were significantly different. Again, I used the conservative Bonferroni method for pair-wise comparisons. I sought additional statistical advice from Dr. Alix Gitelman, a professional statistician at Oregon State University.

No statistics were performed on the litter or O horizon because fresh litter was only collected from four of the treatments, and O horizon material from two. The 90-100 cm bulk soil samples were not analyzed statistically because these soils were not density fractionated, and insufficient nitrogen was present in the deep soils for isotopic analysis.

2.3 Results

2.3.1 Treatment Effects

2.3.1.1 Light Fraction Yields

Light fraction as a percent of bulk soil ranged from a maximum of 11.7% (Double Litter plot 2) in the 0-5 cm samples to a minimum of 0.4% (No Litter plot 7) in the 10-20 cm samples (Figure 2). Treatments did not significantly alter the amount of light fraction present (P=0.57; Table 3). Double wood plot 16 had more light fraction than any of the other plots (46.6%, 12.15%, and 4.5% in order of increasing depth), so the cross treatment mean percent light fraction was calculated without plot 16. The amount of light fraction significantly decreased with depth (Table 1). Although significantly more light fraction was present in the 0-5 and 5-10 cm samples than in the 10-20 cm samples, no interaction between treatment and depth existed (Figure 2).

Table 1. Cross-treatment mean proportion light fraction (% ±1 SE) at the H.J. Andrews DIRT plots, Oregon. Letters next to depths represent significant depth differences.

Depth (cm)	% Light Fraction
0-5 (a)	5.3 (0.16)
5-10 (a)	3.3 (0.15)
10-20 (b)	1.3 (0.07)

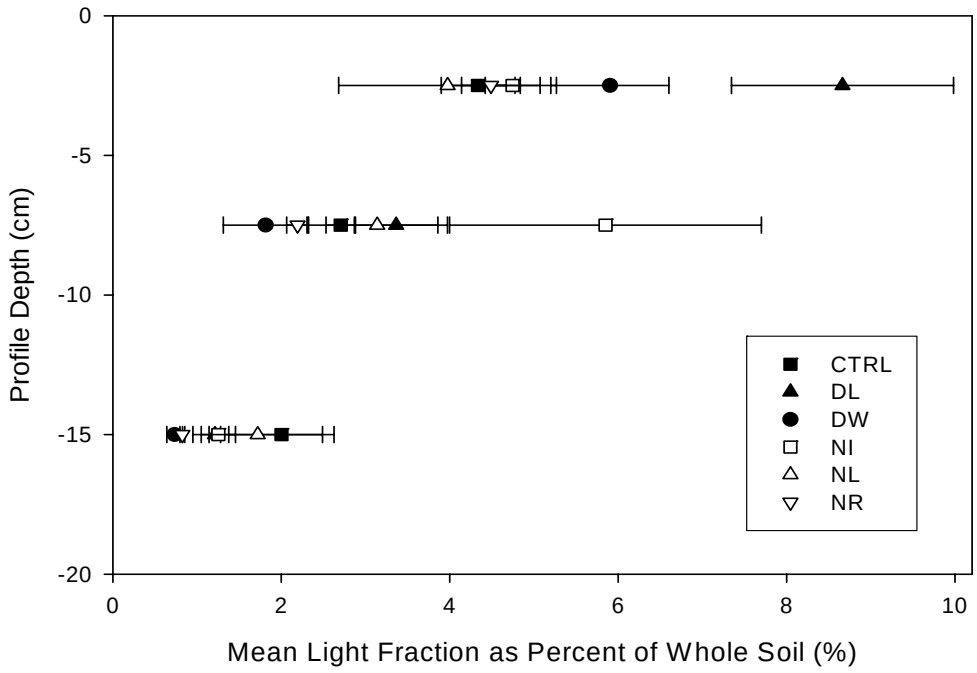

Figure 2. Mean light fraction as a percent (%) of whole soil for each treatment (± 1 standard error). Each point is placed at the midpoint of the mineral soil horizon depth interval it represents.

2.3.1.2 Treatment Comparisons

Mean carbon concentrations ranged between 29.1 and 40.0% in the light fraction, 1.5 and 10.8% in the heavy fraction, and between 1.9 and 13.1% in the bulk soil (Table 2). Carbon concentration in the light fraction was the only variable that showed significant differences across treatments (P= 0.002, Table 3). Light fraction from Control plots contained less carbon than LF from both No Input and No Root plots. Light fraction from No Litter plots also had less carbon than LF from No Root plots. Plots without roots generally contained more C than plots with roots at all depths.

Table 2. Mean carbon concentration (±1SE) for light fraction (<1.6 g/cm^3) of treatments. Letters next to treatments represent significant treatment differences, and letters next to depths represent significant depth differences.

Depth (cm)	Control (a)	Double litter (abc)	Double Wood (abc)	No Inputs (bc)	No Litter (ab)	No Roots (c)
0-5 (a)	29.7 (0.17)	31.0 (1.27)	30.9 (0.88)	34.3 (0.98)	33.8 (0.89)	35.0 (0.48)
5-10 (b)	31.9 (0.61)	38.9 (0.32)	34.7 (0.72)	38.7 (0.70)	34.0 (0.20)	38.8 (0.67)
10-20 (ab)	30.4 (0.80)	34.7 (0.19)	35.6 (0.26)	40.0 (1.13)	29.1 (3.09)	39.6 (1.49)

Table 3. P-values for treatment, depth, and the treatment *depth interaction, as well as the covariance structure used for each analysis. Significant p-values are listed in bold. Analyses were conducted for percent light fraction, carbon and nitrogen concentration C:N ratio, and carbon and nitrogen isotope values on heavy, light and bulk soil samples.

	Treatment	Depth	Treatment* Depth	Covariance Structure
%LF	0.57	**0.007**	0.41	UN3
$\%N_{LF}$	0.42	**0.005**	0.099	CS
$\%N_{BLK}$	0.75	**<0.001**	0.66	UN3
$\%N_{HF}$	0.68	**<0.001**	0.23	UN3
$\%C_{LF}$	**0.002**	**<0.001**	0.17	UN1
$\%C_{BLK}$	0.60	**<0.001**	0.56	UN3
$\%C_{HF}$	0.43	**<0.001**	**0.046**	UN3
$C:N_{LF}$	0.13	0.089	0.64	UN2
$C:N_{BLK}$	0.24	**<0.001**	0.96	CS
$C:N_{HF}$	0.55	**<0.001**	**0.002**	CS
$\delta^{15}N_{LF}$	0.54	**<0.001**	**<0.001**	AR
$\delta^{15}N_{BLK}$	0.19	**<0.001**	0.84	UN3
$\delta^{15}N_{HF}$	0.79	**<0.001**	0.28	CS
$\delta^{13}C_{LF}$	0.083	0.42	0.67	CS
$\delta^{13}C_{BLK}$	0.98	**<0.001**	0.41	CS
$\delta^{13}C_{HF}$	0.62	**<0.001**	0.63	Toep1

Interactions between depth and treatment for HF %C, HF C:N and LF $\delta^{15}N$ were found (Table 3). No other interaction effects were found. The DW and NI treatments had the most carbon in the surface of the heavy fraction. As depth increased, less carbon was present in all treatments (Figure 3a). However, the severity of the change with depth was different for different treatments. For example, the DW plots had the most carbon at the surface, but the least carbon in the following two depths. This could indicate that the surficial wood inputs were locking up all the new OM in the surface, causing a steep decrease in %C with a small change in depth. The decline in carbon concentration was most gradual in the CTRL, NI and NL plots. DL, DW and NR treatments reached the lowest carbon concentrations by 10-20 cm (Figure 3a).

The trends in HF C:N ratio were similar to the carbon concentration trends (Figure 3b). For example, the No Input plots had the highest C:N ratio at the surface but not at depth. This similarity was caused by the inextricable linkage of %C with the calculation of C:N. Since the nitrogen concentration was not causing a treatment* depth interaction (Table 3), it can be assumed that the carbon concentration interaction led to the interaction effect in the C:N ratio.

The interaction of treatment and depth for the light fraction nitrogen isotopic signature was significant. The lightest values occurred in the CTRL, DW, NL and NR treatments (Figure 3c). This could indicate a lesser degree of decomposition in these plots, or it could indicate that the inputs in these plots had a different signature than the inputs in the other plots. The NL treatment had the heaviest isotopic signature. A high level of variability at all depths within treatments could cause an interaction effect with no biological significance. If the differences were biologically meaningless they could have been due to the highly heterogeneous nature of soil.

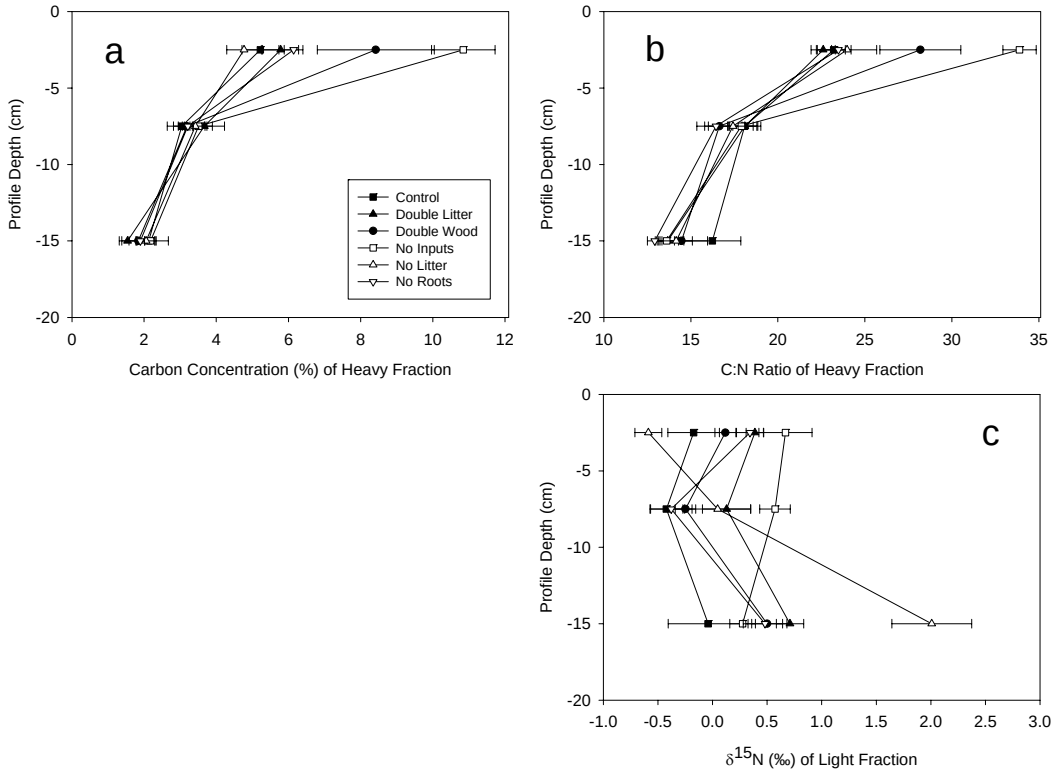

Figure 3a-c. Treatment graphs where a significant treatment * depth interaction was found: (a) Carbon concentration (%) of heavy fraction, (b) C:N ratio of heavy fraction, (c) $\delta^{15}N$ (‰) of light fraction. Points are plotted at midpoint of depth interval and represent treatment averages ± 1 SE.

The respiration and microbial PLFA data collected by other members of the DIRT team (Sulzman et al. 2004, Brant in prep) indicate that the most important inputs to the mineral soil are those mediated by roots. To determine the effect of roots, I specifically compared the Control plots to the No Root plots using the same SAS mixed effects procedure as for the treatment and depth interaction analysis (Table 4). Although Control and No Roots did not differ statistically in percent nitrogen, C:N ratio, $\delta^{13}C$, or $\delta^{15}N$, the light fraction of the No Roots plots had significantly more carbon than the Control plots ($p=0.003$). Changes were visible in the light fraction, a relatively small pool (~1-5% of bulk soil), but not in the other soil pools. Mean carbon concentration increased from 35.0% to 39.6% with increasing depth in the No Roots treatment, and from 29.7% to 30.4% in the Control treatment (Figure 4).

Although more carbon was in the light fraction of the No Root plots (e.g. NR 0-5 cm =35.0% C; CTRL 0-5 cm = 29.7% C), the relative amount of light fraction in the two groups was the same (e.g. NR 0-5 cm =4.5% LF; CTRL 0-5 cm = 4.3% LF). The No Roots treatment contained approximately 5-9 % more carbon than Control (P=0.003); however, depths did not differ significantly (P= 0.12) and the roots*depth interaction for LF %C was insignificant (P=0.40).

Table 4. P-values for roots, depth, and the roots *depth interaction, as well as the covariance structure used for each analysis when comparing the Control to the No Roots treatment. Significant p-values listed in bold. Analyses were conducted for carbon and nitrogen concentration, C:N ratio, and carbon and nitrogen isotope values on heavy, light and bulk soil samples.

	Roots	Depth	Roots* Depth	Covariance Structure
$\%N_{LF}$	0.57	0.36	0.83	CS
$\%N_{BLK}$	0.061	**<0.001**	0.89	UN2
$\%N_{HF}$	0.25	**<0.001**	0.79	CS
$\%C_{LF}$	**0.003**	0.12	0.40	Toep1
$\%C_{BLK}$	0.23	**<0.001**	0.83	UN1
$\%C_{HF}$	0.71	**<0.001**	0.39	CS
$C:N_{LF}$	0.21	0.56	0.73	UN1
$C:N_{BLK}$	0.26	**<0.001**	0.58	CS
$C:N_{HF}$	0.41	**<0.001**	0.37	AR1
$\delta^{15}N_{LF}$	0.52	**0.026**	0.39	CS
$\delta^{15}N_{BLK}$	0.14	**0.003**	0.83	Toep2
$\delta^{15}N_{HF}$	0.11	**0.009**	0.24	CS
$\delta^{13}C_{LF}$	0.37	0.44	0.32	Toep2
$\delta^{13}C_{BLK}$	0.87	**0.001**	0.61	CS
$\delta^{13}C_{HF}$	0.24	**<0.001**	0.53	AR1

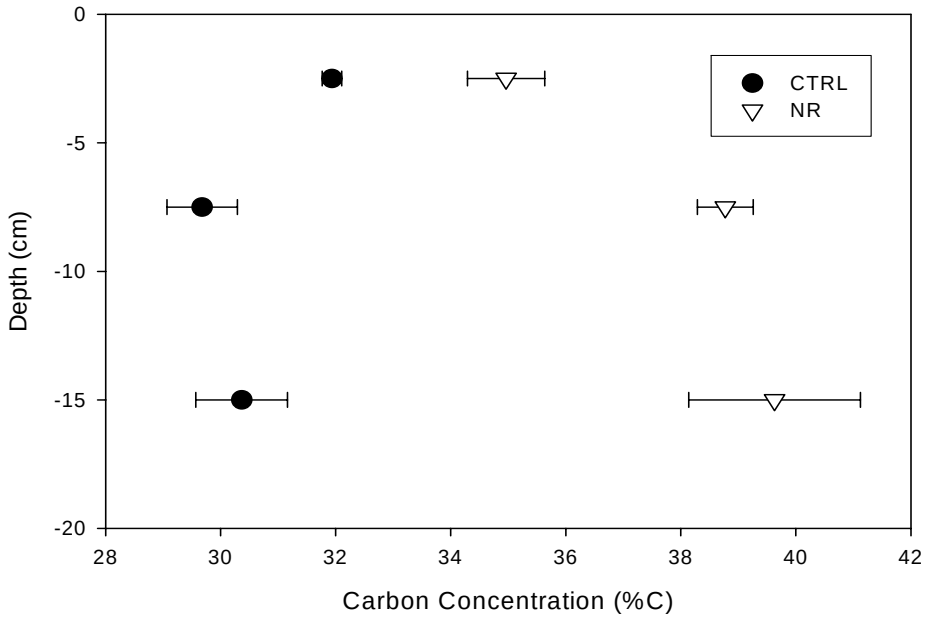

Figure 4. Mean carbon concentration (± 1 SE) in Light Fraction of Control and No Root Treatments. No Roots treatment contains more carbon than Control at all depths. Points are plotted at midpoint of depth interval (0-5 cm, 5-10 cm or 10-20 cm) and represent treatment averages ± 1 SE.

2.3.2 Depth and Density Trends

Since there were only limited trends with treatment, the processes that change the concentrations and isotopic signatures of OM must take longer to show up than 5 years. I therefore collapsed the treatments and continued the analyses based solely on depth and density criteria. The treatments were combined and treated as replicate samples for analyses of depth and density.

2.3.2.1 Elemental Concentration

The carbon concentration of the light fraction was significantly greater than that of the heavy fraction (Table 5, $P<0.001$). The light fraction contained between 32 and 36 %C while the heavy fraction ranged between 2 and 7 %C (Table 6). Fresh litter and O horizon had higher C concentrations than all mineral soil fractions with mean concentrations of 47.1 and 47.7%, respectively (Figure 5a). As depth increased, heavy fraction carbon concentration decreased, and although light fraction carbon concentration did not, density and depth interacted significantly ($P<0.001$; Table 5).

Nitrogen concentration was significantly higher in the light fraction when compared to the heavy fraction (e.g. mean 0-5 cm %N 0.6 vs. 0.3%, respectively; $P<0.001$; Table 5). The litter and O horizons contained more nitrogen than the mineral soil samples (Figure 5b) with means of 0.8 and 0.6%, respectively. Nitrogen concentration decreased significantly ($P<0.001$) in all depths but most markedly in the HF from 0.3% at 0-5 cm to 0.1% at 10-20 cm (Table 6). The LF 5-10 cm depth (0.6%N) was greater than LF 10-20 (0.5%N). The depth * density interaction was significant for nitrogen concentration ($P<0.001$, Table 5).

The litter and O horizon had the widest C:N ratios (59.5 and 152.7 mean C:N, respectively; Figure 5c). As with carbon and nitrogen concentration, the light fraction had a greater C:N ratio than the heavy fraction ($P<0.001$; Table 5). Depth did not have a statistically significant effect on C:N ratio ($P = 0.056$), although an interaction between density and depth occurred ($P<0.001$). As depth increased, light fraction ratios ranged from 60.7 to 68.2, whereas heavy fraction ratios ranged from 25.9 to 14.2 (Table 6).

2.3.2.2 Isotope Values

The O horizon and fresh litter were ^{13}C depleted relative to the mineral soil (Figure 5d), with mean signatures of -26.9 and -27.6‰, respectively. Mean carbon isotope values ranged from -27.0‰ at 0-5 cm and -26.8‰ at 10-20 cm in the light fraction (Table 6). Heavy fraction mean carbon isotope values fell over a slightly wider range from -26.5‰ at 0-5 cm to -25.3‰ at 10-20 cm. Both depth and density were significant factors, with the heavier density and deeper depths more ^{13}C enriched ($P<0.001$) than their lighter, shallower counterparts (Table 5). The interaction between depth and density was significant for $\delta^{13}C$ ($P <0.001$). The heavy fraction became more greatly enriched with depth than the light fraction (Figure 5d).

The fresh litter (-3.7‰) and O horizon (-3.0‰) material had less ^{15}N than mineral soil horizons (Figure 5e). The most ^{15}N-depleted density fraction was the light fraction (Table 5), and the heavy fraction was the most ^{15}N-enriched. The soil became progressively enriched in ^{15}N with increasing depth ($P<0.001$, Table 5). An

interaction between depth and density was present for $\delta^{15}N$ ($P<0.001$). All LF values were close to 0‰ (0.1 to 0.7‰ mean $\delta^{15}N$), whereas heavy fraction became progressively more enriched with depth (3.1 to 6.3‰ mean $\delta^{15}N$; Table 6).

Table 5. P-values for density, depth, and the density *depth interaction, as well as the covariance structure used for each analysis when comparing the light fraction to the heavy fraction. Significant p-values listed in bold. Analyses were conducted for carbon and nitrogen concentration, C:N ratio, and carbon and nitrogen isotope values.

	Density	Depth	Density* Depth	Covariance Structure
%N	**<0.001**	**<0.001**	**<0.001**	CS
%C	**<0.001**	<0.055	**<0.001**	UN3
C:N	**<0.001**	<0.056	**<0.001**	UN3
$\delta^{15}N$	**<0.001**	**<0.001**	**<0.001**	UN3
$\delta^{13}C$	**<0.001**	**<0.001**	**<0.001**	CS

Table 6. Mean cross-treatment carbon concentration, nitrogen concentration, C:N ratio, $\delta^{13}C$, and $\delta^{15}N$ (± 1 SE) for each depth (0-5 cm, 5-10 cm, and 10-20 cm) and density (light fraction, heavy fraction, and bulk soil samples). Sample size =18. Letters next to fractions correspond to significantly different densities; letters next to depths correspond to significantly different depths found using SAS mixed procedure. Bulk soil not included in statistical analysis.

Carbon Concentration (%)			
Depth (cm)	Light Fraction (a)	Heavy Fraction (b)	Bulk
0-5	32.4 (0.17)	6.9 (0.17)	9.3 (0.28)
5-10	36.2 (0.18)	3.3 (0.06)	4.7 (0.09)
10-20	34.9 (0.32)	1.9 (0.05)	2.5 (0.06)
Nitrogen Concentration (%)			
Depth (cm)	Light Fraction (a)	Heavy Fraction (b)	Bulk
0-5 (a)	0.6 (0.01)	0.3 (0.00)	0.3 (0.01)
5-10 (a)	0.6 (0.00)	0.2 (0.00)	0.2 (0.00)
10-20 (b)	0.5 (0.01)	0.1 (0.00)	0.1 (0.00)
C:N ratio			
Depth (cm)	Light Fraction (a)	Heavy Fraction (b)	Bulk
0-5	60.7 (0.80)	25.9 (0.30)	30.1 (0.32)
5-10	60.7 (0.57)	17.4 (0.14)	22.9 (0.21)
10-20	68.2 (1.13)	14.2 (0.16)	18.6 (0.23)
$\delta^{15}N$ (‰)			
Depth (cm)	Light Fraction (a)	Heavy Fraction (b)	Bulk
0-5 (a)	0.1 (0.03)	3.1 (0.06)	1.6 (0.04)
5-10 (b)	-0.1 (0.03)	4.6 (0.04)	3.0 (0.04)
10-20 (c)	0.7 (0.05)	6.3 (0.07)	4.5 (0.07)
$\delta^{13}C$ (‰)			
Depth (cm)	Light Fraction (a)	Heavy Fraction (b)	Bulk
0-5 (a)	-27.0 (0.02)	-26.5 (0.01)	-26.5 (0.02)
5-10 (b)	-26.9 (0.02)	-25.9 (0.02)	-26.2 (0.02)
10-20 (c)	-26.8 (0.02)	-25.3 (0.02)	-25.6 (0.02)

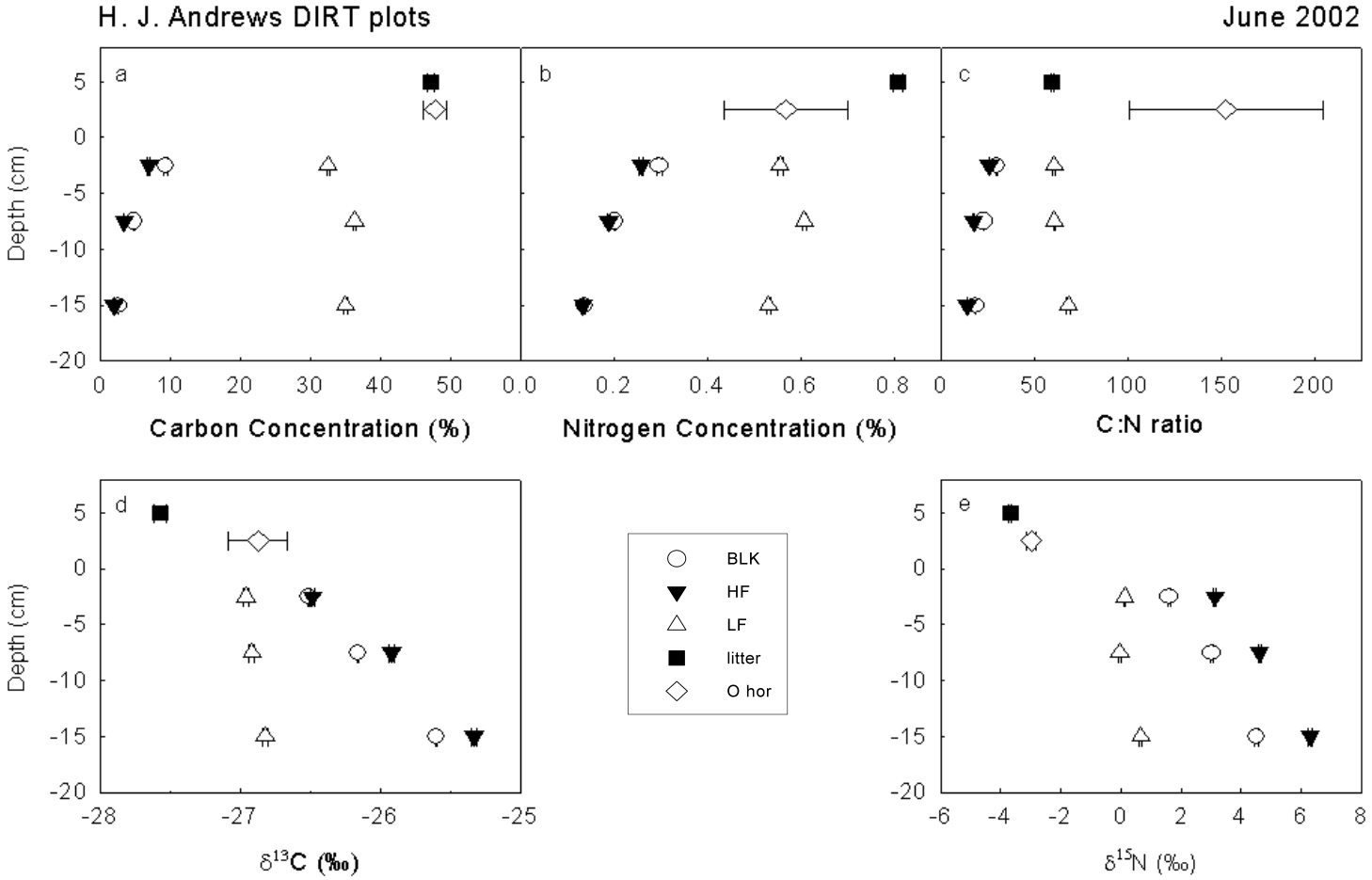

Figure 5. (a) Carbon concentration, (b) nitrogen concentration, (c) C:N ratio, (d) $\delta^{13}C$ isotope ratio, and (e) $\delta^{15}N$ isotope ratio for litter, O horizon, light fraction, heavy fraction and bulk soil. Each point is the mean ±1S.E. of 18 samples and is plotted as the midpoint for the following intervals: litter layer, O horizon, 0-5 cm, 5-10 cm, and 10-20 cm.

2.3.3 Mass Balance Comparisons

One of the objectives of this study was to determine if the density fractionation method, in combination with measurement of the natural abundance of carbon and nitrogen isotopes, is a useful method for isolating distinct pools with different biological availability. A crucial test for the method is determining if the density fractionation process results in any loss of the original material. If the density fractionation procedure was conservative, the bulk soil calculation would equal the recombined light + heavy fraction calculation. If the method is not conservative, I was further interested in determining whether the difference was due to a loss of mass or an isotope shift. If the fractionation method was conservative for carbon, then the data points would fall on a one to one line with $A^{13}C_{blk}*[C]_{blk}$ plotted on one axis and $(A^{13}C_{lf}*[C]_{lf}) + (A^{13}C_{hf}*[C]_{hf})$ plotted on the other. As Figure 6a illustrates, the bulk and recombined heavy and light fractions did not differ statistically in carbon isotopes (P= 0.66; Table 7). For analysis on carbon concentration alone, the bulk C concentration was not significantly different from the recombined HF + LF C concentration (P=0.65; Figure 6b; Table 7). For nitrogen, the mathematically recombined heavy and light fraction was statistically similar to the bulk soil material for all depths (P=0.81, Table 7). Furthermore, bulk N concentration was not significantly different from the recombined HF + LF N concentration (P=0.80; Table 7; Figure 6d).

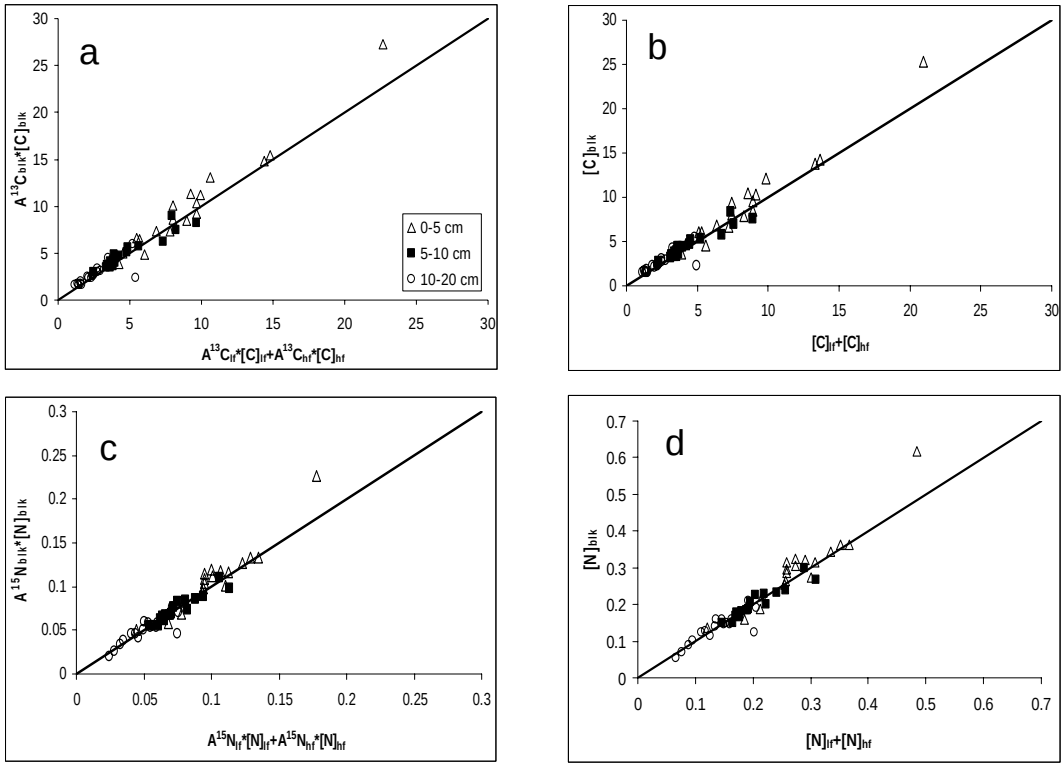

Figure 6 a-d. Mass balance comparisons of bulk soil to recombined heavy +light fraction with 1:1 line for (a) carbon atom percent, (b) carbon concentration, (c) nitrogen atom percent, and (d) nitrogen concentration. Points represent data for each plot at each depth.

Table 7. P-values for recombination, depth, and the recombination *depth interaction, as well as the covariance structure used for each analysis when comparing bulk soil to mathematically recombined heavy fraction + light fraction. "Recombination" represents the comparison of bulk, unfractionated soil to mathematically recombined HF+LF. Significant p-values listed in bold. Analyses were conducted for carbon and nitrogen concentration mass balance, and carbon and nitrogen isotopic atom percent mass balance.

	Recombination	Depth	Recombination* Depth	Covariance Structure
%N	0.80	**<0.001**	0.61	UN3
%C	0.65	**<0.001**	0.87	UN3
A%^{15}N	0.81	**<0.001**	0.61	UN3
A%^{13}C	0.65	**<0.001**	0.87	UN3

2.4 Discussion

2.4.1 C and N Concentration Trends

After 5 years, the only difference between treatments was in amount of C in the light fraction. All other measurements ($\delta^{13}C$, $\delta^{15}N$, %N) were similar. This indicates that the light and heavy fractions have average turnover times greater than five years. Light fraction carbon concentration did not decrease with depth whereas light fraction N concentration did, causing an increase in the C:N ratio with depth. Similarly, another study of density fractionated soils from the H. J. Andrews revealed no change in LF carbon concentration with depth (33.23-34.87%) but a decrease in LF nitrogen concentration (0.57-0.42%), leading to a greater C:N ratio at a depth of 5-15 cm than at 0-5 cm (Swanston 1996). The light fraction in this study contained between 25 and 33% more carbon and from 0.29 to 0.42% more nitrogen than the heavy fraction. The findings of other studies also support these results. Swanston (1996) found 29.5 and 32.3% higher concentrations of carbon and 0.41 and 0.30% more nitrogen in the light fraction than in the heavy fraction at 0-5 and 5-15 cm depth intervals, respectively.

In this study, bulk carbon concentrations ranged with increasing depth from 9.3% to 2.5%, bulk nitrogen concentrations ranged from 0.3% to 0.1%, and bulk C:N ratios varied from 30.1 to 18.6. Oak forests in Wisconsin have decreasing C and N concentrations with increasing soil depth, as well as decreasing C:N ratios (Nadelhoffer and Fry 1988). Nadelhoffer and Fry (1988) found carbon concentrations of 3.03 and 0.82%, nitrogen concentrations of 0.25 and 0.08%, and C:N ratios of 12 and 9 at 0-10 and 10-20 cm depth intervals, respectively. Although the nitrogen concentrations they found were similar to those of the Andrews, the carbon concentrations were much lower, which led in turn to lower C:N ratios. Bulk evergreen forest soils in Tennessee have decreasing C:N ratios from 17.0 at 0-20 cm to 12.3 at 20-40 cm (Garten and Ashwood 2002). However, these C:N ratios are both lower than the average C:N ratio of 23.8 from 0-20 cm for the Andrews DIRT plots. Despite the fact that oak forests and eastern evergreen forests have less carbon and

lower C:N ratios than the Andrews DIRT plots, the trend of decreasing values with increasing depth is apparent in all cases. Nadelhoffer and Fry (1988) suggest that C:N ratio and OM size decrease as a result of decomposition; therefore, the trend with depth can be used as a proxy for degree of degradation.

The first five years of DIRT at the Andrews resulted in less carbon in the light fraction of Control plots than in No Input and No Root plots (Table 2). No Litter plots also contained less carbon than No Root plots. Others have found that the presence of roots can influence SOM decomposition. As described by Kuzyakov et al. (2000), Helal and Sauerbeck (1986) found that after three months with maize roots, total soil organic carbon decreased 5-7%, presumably because the presence of roots enhanced microbial activity and increased decomposition of SOM. Slow root exudation and addition of complex mycorrhizal and root-derived litter might stimulate increased microbial activity, which in turn leads to increased nitrogen mineralization (Fontaine et al. 2003). When roots are absent or non-functioning, microbial populations might languish, resulting in a build-up of SOM. An important finding of the first 10 years of the Harvard Forest DIRT plots is that root inputs appeared to be approximately equal in magnitude to aboveground inputs, but effects of root inputs dominated during the first five years of treatment (Nadelhoffer et al. 2004). During these early years contributions of roots to the soil ecosystem played a large role in mineralization (Nadelhoffer et al. 2004).

Based on the results of a trenching experiment, Simard et al. (1997) noted that severed roots were slow to decompose. Data from Chen et al. (2002) indicate 30.3% of Douglas-fir and 27.8% of western hemlock roots still exist five years post-trenching. Langley et al. (2003) contribute the recalcitrant nature of roots to the presence of ectomycorrhizae, which have a high percentage of chitin, and may produce anti-microbial substances that inhibit decomposition. They propose that ectomycorrhizae are a precursor of recalcitrant soil N pools. The slow decay rate of roots, in addition to the lack of root exudates to prime the microbial populations in the No Root plots, could cause the difference in carbon between the Control and No Root treatments.

Alternatively, the additional carbon in the light fraction of the No Root plots could be indicative of the type of carbon compounds present. One trend several authors have noted is a loss of O-alkyls as material decomposes, and a concomitant increase in the proportion of alkyl groups (Baldock et al. 1992, Golchin et al. 1994, Zech et al. 1997). Since no new root exudates were entering the trenched plots, the remaining carbon compounds should have been predominantly alkyls. In contrast, microbes in the plots with roots had a continuous supply of new carbohydrates, and presumably would have had a greater concentration of O-alkyls than the No Root plots. Carbon is a smaller proportion of O-alkyls ($C_nH_{2n+1}O$) than of alkyls (C_nH_{2n+1}), so the loss of O-alkyls would mean a greater concentration of carbon in alkyl-dominated (trenched plot) SOM.

No concentration or isotopic differences between the doubled aboveground input plots and any of the other treatments were found. However, respiration data suggest increased decomposition (or priming) of older SOM in Double Litter plots compared to Control plots (Sulzman et al. 2004). Evidence for priming was observed after the first ten years of DIRT at Harvard Forest, where a doubling of aboveground litter caused increased decomposition of more recalcitrant organic matter (Nadelhoffer et al. 2004). The fact that SOM measurements show priming due to belowground inputs whereas respiration measurements show priming due to aboveground inputs may indicate each method (respiration vs. direct soil analysis) is useful for studying a different piece of the SOM cycling puzzle. Respiration measurements may be more useful for looking at organic horizon dynamics and SOM measurements may be better for studying mineral horizon dynamics.

2.4.2 Carbon and Nitrogen Isotope Trends

In the HJA DIRT plots $\delta^{15}N$ and $\delta^{13}C$ increase with depth in bulk soil as well as in the light and heavy fractions (Figure 5d&e). These results concur with results of other studies of soil organic matter (Sollins et al. 1980, Nadelhoffer and Fry 1988, Flanagan et al. 1996, Högberg et al. 1996, Neill et al. 1996, Hobbie et al. 1999a, Ehleringer et al. 2000, Pardo et al. 2001, Bowling et al. 2002, Garten and Ashwood

2002, Koba et al. 2003). The typical pattern of $\delta^{13}C$ and $\delta^{15}N$ for SOM is a rapid increase from the forest floor to the mineral soil (an increase of approximately 2.2‰ $\delta^{13}C$ in the Oregon Cascades: Bowling et al. 2002), which could be due to the presence of leaf litter on the surface and the presence of roots in the mineral soil (Bowling et al. 2002, Bird et al. 2003). Data for *Pseudotsuga menziesii* in the Oregon Cascades suggest that shade needles, though quite variable, are lighter than fine roots (Bowling et al. 2002).

A number of hypotheses have been generated to account for the depth-related variation in $\delta^{13}C$. Fractionation could occur during microbial respiration, or enrichment of SOM might be due to the formation of ^{13}C-enriched carbohydrates (Hobbie et al. 2004). Hobbie et al. (2004) suggested that aboveground inputs would have the greatest influence in the organic horizons, whereas inputs to the A horizon would be dominated by litter layer and root material, and inputs to deeper horizons would likewise come from roots and overlying horizons. Since roots and root exudates have a greater influence on mineral horizon SOM than aboveground inputs, their signatures should be more similar to that of SOM (Dijkstra et al. 2003). Leaf litter is generally lighter than roots, so A horizon SOM, as expected, was lighter than deeper soil. However, five years of altering root and litter inputs had no effect on the isotopic ratio of SOM even in the labile light fraction. This indicates that either these isotopic differences between pools are so small that it would take considerable time for the isotopic signal to be observed, or the turnover time of the labile fraction is sufficiently long that five years has led to a very small change in the labile pool, or both. Furthermore, many soils experience a translocation of clays down the soil profile. It has been suggested that clays help stabilize high ^{13}C SOC. The longer SOC resides in the soil, the greater the opportunity for re-metabolism of microbial products, which would lengthen the decomposition pathway and result in increasing $\delta^{13}C$ values (Bird et al. 2003).

Nitrogen follows a similar isotopic pattern of enrichment with soil depth (Figure 5e), although the mechanisms suggested to account for the trend are different. In forest soils, the $\delta^{15}N$ may increase 5-10‰ or more within the top 10 cm of the

mineral soil (Högberg 1997). Soils of Saskatchewan also exhibit the trend of ^{15}N enrichment with depth in the top 50 cm of soil (Karamanos et al. 1981). The most widely accepted N-fractionation theory suggests that mycorrhizae fractionate soil nitrogen, causing enrichment of ^{15}N within the mycorrhiza and movement of ^{15}N-depleted nitrogen to the plant (Högberg et al. 1996, Stewart 2001). The mycorrhizal theory accounts for the relatively light $\delta^{15}N$ of plant litter. As the litter is added and incorporated into soil surfaces, the top of the soil becomes isotopically lighter relative to the deeper soil. Since fungal material eventually enters the pool of recalcitrant organic matter, this theory helps explain the comparatively ^{15}N-enriched values of the refractory pool. The deeper soils become enriched due to mycorrhizal turnover, accentuating the difference in $\delta^{15}N$ values within the profile.

In this study, the isotopic trends with depth matched the predictions; however, although I expected lower $\delta^{15}N$ and $\delta^{13}C$ values in Double Litter plots and higher values in No Inputs plots, there were no significant treatment effects on isotopic composition of SOM. At the ecosystem level, Schweizer et al. (1999) predict that high quality litter (low C:N) will lead to the formation of more ^{13}C-enriched SOM than low quality litter (high C:N). In coniferous forests of the Pacific Northwest, fine roots (C:N=36) are a higher quality input than aboveground litter (C:N=119)(Sollins et al. 1980, Chen et al. 2002). One would expect to see changes in trenched plots before, or of a greater magnitude than, changes in any of the plots where aboveground litter has been manipulated. However, I found no statistical differences in SOM $\delta^{15}N$ or $\delta^{13}C$ across treatments. Although the results of this study do not match the expectations, the spatial heterogeneity and young age of the treatment plots (five years) might explain the similarity among treatments. Another consideration is the enormity of the background signature of the pre-treatment SOM.

Nadelhoffer and Fry (1988) reported isotopic differences between Double Litter and Control plots at 0-10 and 10-20 cm depths in an oak site measured approximately 30 years after establishment. In their study the double litter treatment resulted in approximately 1‰ lower $\delta^{13}C$ and 2‰ lower $\delta^{15}N$ values than in the

control treatment. The more pronounced change in $\delta^{15}N$ values compared to $\delta^{13}C$ values was attributed to the smaller pool size of N relative to C. Hobbie et al. (2004) calculated $\delta^{13}C$ enrichment factors for SOC from needle and root inputs of 4‰ and 2‰, respectively. In the future, we might expect to see the lightest $\delta^{15}N$ values in the NR treatment as pre-trenching rhizodeposits diminish and aboveground inputs become a more important source of energy for decomposers. We might also expect the DL plots to become lighter than Control plots as the additional litter is incorporated into the new SOM.

2.4.3 Comparing Whole Soil to Density Fractions

There were no concentration or isotopic differences between the bulk and fractionated soil, indicating that any loss during fractionation was minimal and did not affect the analytical results. Studies have shown that nitrogen can be lost from soil during the process of drying and rewetting (Kuzyakov et al. 2000). The 0-5 cm soils were processed field moist in order to avoid any loss due to the drying-rewetting phenomenon, whereas the 5-10 and 10-20 cm soils were dried first. However, the mass balance analyses revealed that the mathematically recombined heavy and light fraction was statistically similar to the bulk soil material for all depths (Table 7). This suggests that the density fractionation procedure is conservative for both carbon and nitrogen.

2.4.4 Comparing Trends with Depth to Trends with Density

The results of this study form a cohesive story about SOM trends consistent with the findings of Nadelhoffer and Fry (1988). In the H. J. Andrews DIRT plots, soil C and N concentration decrease as the relative abundance of ^{13}C and ^{15}N increase, and both of these conditions occur as soil depth increases (Figure 7a-f). Based on these same trends in their data, Nadelhoffer and Fry (1988) concluded that the process of decomposition led to increased (heavier) isotopic signatures. Low C and N concentration, low C:N ratio, and less negative isotopic ratios have all been used as indicators of older SOM (Baisden et al. 2002b).

The use of radiocarbon to determine the age of SOM supports the view that SOM ages with increasing depth in the soil profile (Boutton 1996). For instance, most of the carbon (>800g $C/m^2 \cdot cm^{-1}$ depth) in a temperate forest in Massachusetts is present above 10 cm, and has turnover times between 1 and 35 years (Trumbore 2000). Below a depth of 20 cm, <200g $C/m^2 \cdot cm^{-1}$ depth is present and has turnover times predominantly greater than 300 years (Trumbore 2000).

Although the direction of trends with depth is the same for both density fractions in the Andrews DIRT plots, the light fraction is significantly different from the heavy fraction and the bulk soil. Carbon and nitrogen are a greater proportion of the light fraction than of the heavy fraction. Likewise, the carbon and nitrogen in the heavy fraction are isotopically heavier than the C and N in the light fraction. Since more positive C and N isotope ratios and smaller C and N concentrations both indicate an increase in SOM age, I suggest that the heavy fraction is older than the light fraction. This concept is also supported by the work of Trumbore (2000), who measured ^{14}C of density fractionated temperate forest soil from Harvard Forest, MA. Trumbore (2000) calculated turnover times from 160 to 400 years for high density A horizon soil, whereas low density A horizon humics had turnover times from 50 to 160 years. Based on the evidence that surface soils are younger than deep soils, and that low density SOM is younger than high density SOM, the combination of density and depth measurements can be used to divide the soil into biologically meaningful SOM pools.

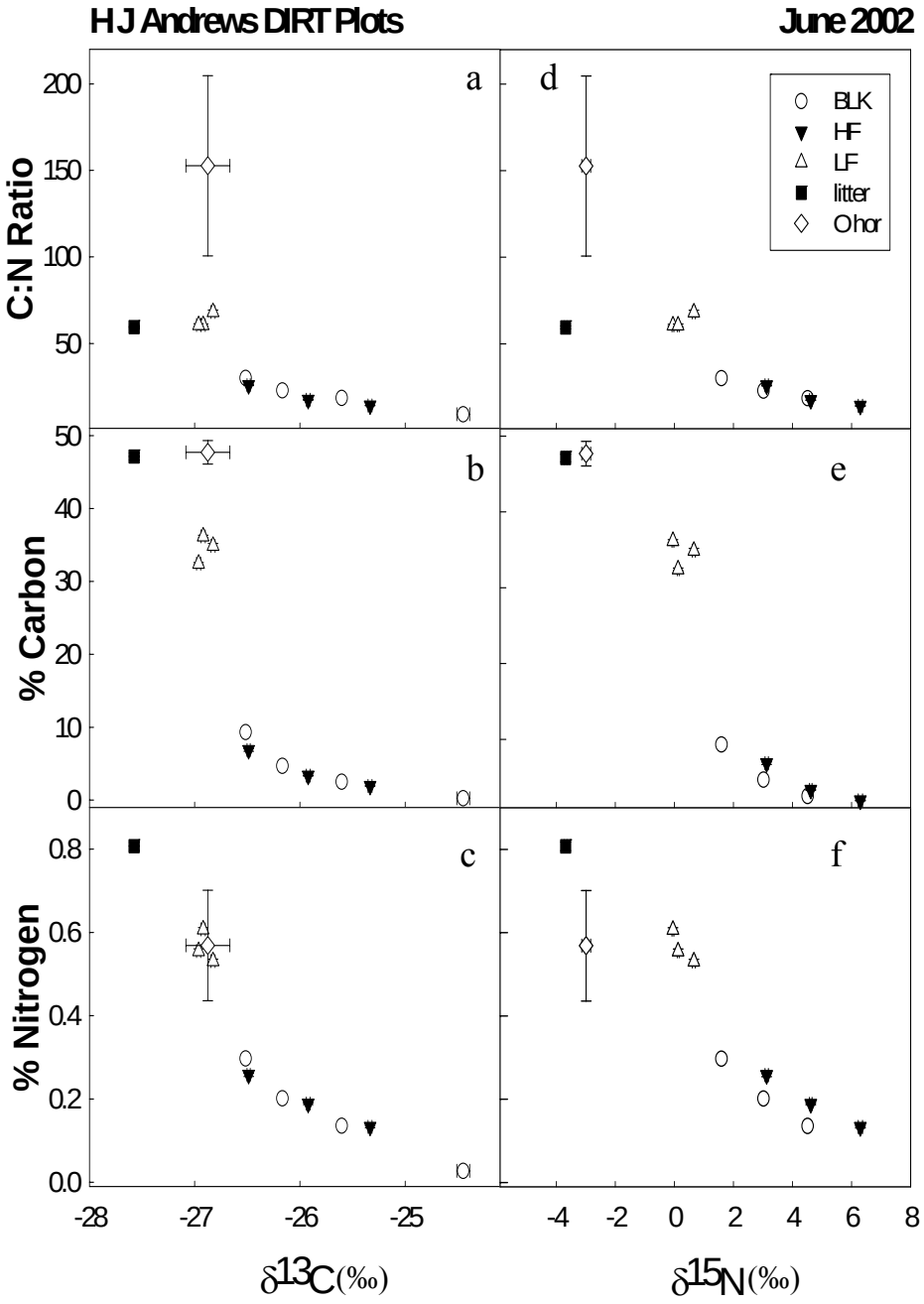

Figure 7a-f. Elemental concentration trends with respect to isotopic trends. Points represent mean cross-treatment values ±1 SE for fresh litter (n=3) and O horizon material (n=13) as well as bulk, light and heavy fraction samples at 0-5 cm, 5-10 cm, 10-20 cm depths (n=18). (a) $\delta^{13}C$ (‰) vs. C:N ratio, (b) $\delta^{13}C$ (‰) vs. carbon concentration (%), (c) $\delta^{13}C$ (‰) vs. nitrogen concentration (%), (d) $\delta^{15}N$ (‰) vs. C:N ratio, (e) $\delta^{15}N$ (‰) vs. carbon concentration (%), and (f) $\delta^{15}N$ (‰) vs. nitrogen concentration (%).

2.4.5 The Melding of Theory and Method

Magid et al. (2002) outlined three key criteria for deriving multiple soil carbon pools: (1) there should be a small number of fractions with distinct chemical composition, (2) the fractions should be quantifiable, and (3) they should relate to SOM that differs in turnover time. According to these criteria, the density fractionation method in combination with isotopic analysis is a useful technique for determining SOM pools. The density fractionation method is based on the premise that what floats in the dense solution is functionally different than what sinks. Swanston (2000) argues for density fractionation because the solution can be adjusted to any density necessary to allow maximum separation between the free and mineral-associated organic matter. Density fractionation allows a researcher to separate the labile and mineral-associated fractions of any soil based on the unique characteristics of that soil.

There may be other fractionation schemes that are better suited to the study of SOM dynamics, such as separating the soil at multiple densities. Multiple separations might reveal significant differences between all of the fractions. The drawback of further complicating the separation scheme is that it becomes more energy intensive to perform analyses, and it is possible that the fine gradations of SOM you would detect are not useful theoretically. For example, Six et al. (2002) size-density fractionated forest soils and measured carbon concentrations to study the mechanisms of soil C sequestration and to identify the pool with the greatest potential to stabilize C. They determined that microaggregate protected particulate organic matter (mpPOM) is the soil C pool most sensitive to ecosystem changes as well as the pool able to obtain long-term stability. However, their fractionation scheme is exceedingly complex and thus does not meet the criterion outlined above for deriving a small number of SOM pools.

The fractionation procedure of Baisden et al. (2002b) did not meet the criterion of relating to pools that differ in turnover time. Baisden et al. (2002b) separated grassland soils into five density fractions, and concluded that the four mineral associated fractions (primarily >1.6 g/cm^3) contained similar decadal and millennial

SOM pools. Therefore, fractionation at one density is likely enough to separate the dominant SOM pools, and is a procedure which meets the criteria outlined by Magid et al. (2002).

Based on the results of the fractionation scheme used in this study, I contend that two to three SOM pools are enough for soil carbon models. The short term pool can be represented by the light fraction, and would consist of 1-6% of the top 20 cm of soil. An intermediate pool could be represented by the heavy fraction in the top 0-5 or 0-10 cm. Depending on the ecosystem, the long term storage pool would correspond to the HF SOM below 5 or 10 cm. This type of SOM density fractionation/isotopic analysis could be performed on representative soil ecosystem types to determine where to draw the cut-offs for the different SOM pools. It would be highly clarifying to support such characterization with radiocarbon data to determine the turnover times assigned to each pool. While soil separation is useful in elucidating belowground SOM dynamics for the purpose of modeling, I suspect there is a continuum of SOM, and that any breaks drawn between "pools" of SOM oversimplify the complex and dynamic system.

2.5 Future Directions

The results of this study bring up a number of methodological and mechanistic questions for future studies. It could be useful to determine how long it will take to see changes in the carbon and nitrogen signatures of the SOM pools due to changes in inputs. Based on the available evidence, the inputs should have different isotopic signatures. Wood is probably isotopically heavier than roots and roots are heavier than litter. Mycorrhizae are heavier than roots, but possibly not heavier than wood. We need to better measure $\delta^{13}C$ and $\delta^{15}N$ of the inputs in order to calculate projected δ values for LF, HF and BLK at future time steps. I attempted this calculation with the available data and found several important pieces of information were missing. The belowground inputs are difficult to measure, especially since it is unknown if and/or which components of rhizodeposition are preferentially degraded. The variability of the inputs is possibly greater than signature differences between the inputs, in which

case the calculation would not be useful. If the SOM becomes progressively more similar to the signature of the inputs, then we should be able to calculate how much new input is needed to shift the signature of the SOM to the input signature. But we also need to account for the isotopic fractionation associated with decomposition. Research needs to be done to determine fractionation factors for different depths and the time scales on which these processes happen for the different density materials.

The first ten years of DIRT at Harvard Forest point to noticeable changes in decomposition rates between the treatments (Nadelhoffer et al. 2004). Based on C:N ratios, the litter quality in the Andrews Forest is lower than the litter quality at Harvard Forest, so it may take longer for changes to become apparent at the Andrews. It would be illuminating to perform this analysis again in 10 years. If this study were repeated, I recommend the following modifications: (1) at least 200 g of 90-100 cm soil should be extracted from each plot to yield a gram of light fraction for further analysis; (2) soil from intermediate depths (between 20 and 90 cm) should be collected to develop a more complete profile and more accurately model SOM pools; (3) the procurement of bulk density data for all depths would lead to more accurate calculation of C and N stocks.

Since the presence of roots seems to have the greatest impact on decomposition in the mineral horizons, we need to design studies to look specifically at the priming effect in these soils. Separating the effect of roots from the effect of mycorrhizae is difficult, but doing so may be enlightening. The exudate elution method outlined by Kuzyakov (2002) separates soil respiration into root respiration and microbial respiration, and might help in an examination of priming. Mycorrhizae are an important factor in the study of roots' role. Butler (2003) performed a ^{13}C pulse-chase labeling technique followed by phospholipid fatty acid analysis (PLFA) to follow the flow of root C into different microbial communities. A labeling study could also help clarify the role of mycorrhizae in the process of nitrogen isotope fractionation.

The difference in carbon concentration between the control plots and reduced input plots is intriguing. Because the amount of light fraction did not differ between

these treatments, the difference in %C may have been due to the types of compounds present. Performing ^{13}C-NMR on the different fractions and depths (especially the LF and HF of CTRL and NR plots) could be conducted to determine if the presence of different compounds was responsible for the discrepancy in % C. Unfortunately, the presence of paramagnetics in the Andrews soil may complicate the performance of the NMR procedure.

This study confirmed the pattern of heavy isotope enrichment with increasing depth and density, but it did not lend insights into the mechanisms controlling fractionation. To address this problem, more data on inputs is required. The isotopic signatures of fine and coarse roots, mycorrhizae, wood, litter and, if possible, of microorganisms for all plots could help tease apart their roles in the process of decomposition. To examine the hypothesis that enriched DOC is being translocated down the profile, samples of soil solution need to be analyzed isotopically. Finally, I encourage the undertaking of a labeling study to track the flow of new C into SOM pools.

The density fractionation method is gaining popularity among the scientific community. More research needs to be done to develop a universal fractionation scheme so comparisons can be made between studies. Comparing a number of different fractionation schemes using the same soil and comparing the results could help determine a procedure resulting in a small number of chemically distinct, biologically meaningful pools. I suggest comparing the fractionation method I used to techniques that entail particle size separation and sonication to separate free from occluded light fraction. I also strongly recommend combining the fractionation-isotope technique with radiocarbon analysis in order to determine not only the size of the pools but also their turnover times, and to examine whether deep SOM has longer turnover times than surface SOM.

2.6 Conclusion

In summary, I found that:

- Five years of treatment did not change the SOM in the reduced input plots and the added input plots relative to the control plots, indicating that the turnover times of the light and heavy pools is greater than five years. The one exception was that light fraction from No Roots and No Inputs plots had more C than light fraction from Control plots, possibly signifying a root-mediated priming effect.
- The labile fraction of the soil changed more from the manipulations than the recalcitrant fraction of the SOM, but only slightly. Light fraction carbon concentration was the only significantly different measurement between treatments.
- The trends with depth in this coniferous forest were similar to the published trends in other forest types; however, this forest had greater carbon concentrations at all depths than SOM from other forests.
- Density fractionation conserved the C and N of the samples, so the method can be used to separate the labile and recalcitrant pools of SOM.

Although I expected to see a build up of light fraction SOM in Double Litter plots compared to Double Wood plots due to litter quality, no difference was observed. This finding was probably due to the limitations of my methodology in detecting changes due to aboveground manipulations. The absence of roots seemed to reduce SOM decomposition, because No Roots plots had more C in the light fraction than Control plots, a finding suggestive of a root-mediated priming effect. Only three light fraction %C pairs were different. Since they all occurred in the same fraction for the same variable, these results are probably not just random error. However, from an ecological perspective, the heterogeneous nature of forest soil could cause the observed differences. The Andrews DIRT plots were established in 1997, only five years before the samples were taken. On the time scale of soil processes, the plots are

young and input differences may not, as yet, be reflected in soil organic matter. The lack of distinction among treatments in the light fraction indicates that the average turnover time of this labile pool is probably greater than five years.

We hypothesized that treatments would cause isotopic shifts in near surface soils, with No Inputs heaviest and Double Litter lightest. The plots should begin to take on the C and N signatures of their dominant inputs, as observed by Nadelhoffer and Fry (1988). If wood is the isotopically heaviest input as data from Bowling et al. (2002) suggest, then Double Wood plots should have more positive $\delta^{13}C$ and $\delta^{15}N$ signatures than Control and Double Litter plots. However, there were no isotopic differences among treatments. Although no differences in $\delta^{13}C$ and $\delta^{15}N$ signatures were observed after five years of treatment, this trend should become more apparent with time as post-treatment derived SOM becomes a greater proportion of total SOM.

The results of this study showed trends of isotopic enrichment and decreasing C and N concentrations with increasing depth and density in the H.J. Andrews DIRT plots. The deeper soil appeared to be older or more decomposed than the surface soil. Similarly, the heavy fraction appeared more decomposed than the light fraction.

The single density fractionation procedure used in this study successfully separated SOM into isotopically distinct pools in different stages of decomposition. Based on mass balance comparisons of recombined heavy and light fractions to bulk soil, the density fractionation procedure conserves SOM carbon and nitrogen. The C and N data accrued during this experiment can be considered accurate and will be available to future researchers participating in the DIRT long term experiment.

3 Summary

In order to assess the potential future greenhouse effect, sources and sinks for CO_2 need to be identified and quantified and the mechanisms driving the CO_2 fluxes into and out of these pools must be understood. To slow climate change, one possible solution would be to sequester more carbon in the soil. To accomplish this goal, the mechanisms governing short- and long-term belowground carbon dynamics need to be understood.

As part of a larger project developed to use experimental manipulations in the field to assess the effect of quantity and quality of litter inputs on the rate of soil organic matter formation, I examined soil organic matter in the H. J. Andrews Detritus Input and Removal Treatments (DIRT) plots. The DIRT treatments of interest are: no inputs (roots and aboveground litter excluded), no roots, no aboveground litter, control, doubled aboveground litter, and doubled woody debris.

This study was designed to address the following objectives:

- Determine how five years of treatment changed the SOM in the reduced input plots and the added input plots relative to the control plots.
- Determine if the more labile (light) fraction of the soil changed more from the manipulations than the more recalcitrant (heavy) fraction of the SOM.
- Document how the light and heavy fractions changed with depth in this coniferous forest relative to the published trends in other forest types.
- Determine if density fractionation conserved the C and N of the sample, or if the method resulted in any losses or transformations that might yield the method untrustworthy.

To accomplish these objectives, I measured carbon and nitrogen concentrations and isotope values of SOM at different depths in the profile because changes in these parameters reflect the degree of SOM decomposition. Decomposition leads to decreasing carbon and nitrogen concentrations and increasing $\delta^{13}C$ and $\delta^{15}N$ values of

soil organic matter (Nadelhoffer and Fry 1988). Because I was interested in the time course of organic matter formation and decomposition, I separated each soil sample into two "fractions," the labile, light fraction (LF), and the recalcitrant, heavy fraction (HF).

All treatments contained the same amount of light fraction with cross-treatment means of 5.3% at 0-5 cm, 3.3% at 5-10 cm, and 1.3% at 10-20 cm. Light fraction from Control plots contained less carbon than light fraction from No Inputs and No Roots plots ($P= 0.002$). For example, at 0-5 cm Control averaged 29.7 % carbon, No Inputs averaged 34.3%C, and No Roots averaged 35.0% carbon. Light fraction from No Litter plots had less carbon than light fraction from No Roots (29.1%C vs. 39.6%C at 10-20 cm, respectively; $P= 0.002$). No other treatment differences were observed. Because the data did not indicate any strong treatment effects, the treatments were combined and treated as replicate samples for the depth and density analyses.

Light fraction C concentrations were greater than heavy fraction concentrations by 25.6% at 0-5 cm, 32.8% at 5-10 cm, and 32.9% at 10-20 cm ($P<0.001$). At 0-5 cm, 0.3% more nitrogen was found in light fraction than heavy fraction, 0.4% more at 5-10 cm, and 0.4% more at 10-20 cm ($P<0.001$). Nitrogen concentrations decreased significantly with increasing depth ($P<0.001$) although C concentrations did not. The heavy fraction was statistically more ^{13}C and ^{15}N enriched than the light fraction ($P<0.001$). Heavy fraction $\delta^{13}C$ values ranged from -26.5 to -25.3‰, whereas light fraction values ranged from -27.0 to -26.8‰ with increasing depth. Heavy fraction $\delta^{15}N$ values ranged from 3.1 to 6.3‰ with depth compared to a range of 0.1 to 0.7‰ in the light fraction. Fractions became slightly heavier (more ^{13}C and ^{15}N) with depth ($P<0.001$), and this pattern was more pronounced in the heavy than in the light fraction.

Bulk soil carbon and nitrogen concentrations decreased with depth while bulk C and N isotope values increased with depth. These depth trends indicate the degree of SOM degradation, with lower concentrations of more isotopically enriched carbon and nitrogen indicating more decomposed soil organic matter. Similar depth trends

can be found in evergreen forest soils of the Eastern United States as well as in oak forests of Wisconsin (Nadelhoffer and Fry 1988, Garten and Ashwood 2002). However, the Andrews soils contained greater carbon concentrations than either of the other forest types. Because the density trends followed the bulk depth trends (and were even more pronounced), I conclude that heavy fraction SOM is more decomposed than light fraction material.

Using a mass balance equation, I compared the bulk soil to the mathematically recombined heavy + light fraction to determine if C and N were conserved during the density fractionation process. There was no concentration or isotopic difference between the bulk and fractionated soil, indicating that any loss during fractionation was minimal and did not affect the analytical results.

Thus, in summary, the findings were:

- Five years of treatment did not change the SOM in the reduced input plots and the added input plots relative to the control plots, with the one exception of light fraction carbon concentration differences between control and rootless plots. The additional carbon in the light fraction of the rootless plots suggests a root mediated priming effect.
- The light fraction did not change more from the manipulations than the heavy fraction of the SOM (with the one exception mentioned above), indicating that the turnover times of both pools are greater than five years.
- The trends with depth in this coniferous forest were similar to the published trends in other forest types; however, this forest had greater carbon concentrations at all depths than SOM from other forests.
- Based on mass balance analysis, density fractionation conserved the C and N of the samples, so the method can be used to separate the labile and recalcitrant pools of SOM.

The Andrews DIRT plots were established in 1997, only five years before the samples were taken. On the time scale of soil processes, the plots are young and input

differences were not reflected in soil organic matter. Aside from a possible priming effect due to roots and rhizodeposition, the data do not indicate any strong treatment effects. The processes that change SOM isotopic signatures must take longer to show up than five years.

I contend that two to three SOM pools are enough for soil carbon models. The short-term pool can be represented by the light fraction, and would consist of 1-6% of the top 20 cm of soil. An intermediate pool could be represented by the heavy fraction in the top 0-5 or 0-10 cm, and the long-term storage pool would correspond to the HF SOM below 5 or 10 cm.

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5 APPENDICES

5.1 Appendix A. Density Fractionation Protocol for Sieved Soil

1. Determine the correct density to use for your soil.
    a. Mix up SPT solution at multiple densities (i.e.-five or six densities between 1.2 and 2.2). Use the attached chart to determine ratio of distilled water to dry SPT to make solutions. ∗Always check density volumetrically before using. ∗
    b. Process subsamples of a soil using the different densities as described below.
    c. Weigh 1-3 g of dry subsamples and heat in combustion oven at 500ºC for 30 minutes, turn down to 100ºC for about 3 hours, then remove and weigh.
    d. Plot the density versus the % loss on ignition. Look for the density at which the amount of OM left behind in the HF levels off (density where % LOI stops changing) and the density at which the amount of mineral soil starts to increase in the LF (% ash in LF increases). They should be about the same, and that is the density to use in fractionating your soils.
2. IF you are processing the soil when it is field moist, determine water content of each sample you are fractionating. Skip this step if you are processing dry soil!
    a. Dry a small subsample overnight at 105ºC. Determine % moisture.
    b. Calculate how much moist soil you need to weigh out in order to have 50 g of dry soil in each 250 ml Nalgene bottle.(i.e.-If water content(WC) =30%, then 50g dry soil/(1-.3)= 71.4g wet soil)
    c. Use the g of water (in the above example=21.4 g) and the attached chart to calculate how much extra dry SPT to add to each bottle to maintain a solution density of 1.6 g/ml (or whatever density you are using).
        i. Example: The chart says to add 741 g SPT to 859 ml H_2O to arrive at a solution density of 1.6 g/ml. So, if you have 21.4 g H_2O in your sample (and using 741gSPT/859ml H_2O= 0.863 g SPT/ml H_2O): $\frac{21.4 ml H_2O}{} \times \frac{0.863 g SPT}{ml H_2O} = 18.47 g SPT$
        ii. You need to add an extra 18.47 g SPT to your 250 ml Nalgene of soil.
3. Density Fractionating
    a. Weigh 50 g dry soil into a 250 ml wide mouth Nalgene bottle.
    b. Add 100 ml of SPT solution to bottle.
    c. Repeat for all subsamples of a sample, or for multiple samples.
    d. Shake on shaker table for one (1) hour.

e. Rinse down cap and sides of Nalgenes with SPT solution.
f. Let settle for 24 hours.
g. Aspirate light fraction from surface of solution.
h. Re-shake and repeat steps **e** through **g** one or two more times, depending on how much light fraction seems to be left after second round of settling. (As a rule of thumb, if you have a bunch of stuff floating in solution when you centrifuge the heavy fraction, you may want to aspirate 3x instead of 2.)
i. Filter light fraction using ashed glass fiber filters. (If you use three large buchner filters for each sample, it should take about a half hour per sample.) Rinse the light fraction several times with DDW to remove all SPT.
j. Scrape light fraction off of filters while still wet.
k. Centrifuge heavy fraction (HF) for 25 minutes. (15 minutes to start up, 10 minutes to centrifuge, 15 minutes to slow down). All bottles being centrifuged at the same time should be within 1 g of each other.
l. Pour off supernatant, add DDW, resuspend HF, rinse cap and sides, and repeat centrifugation 2 more times. (This should remove all SPT from HF.)
m. Uncap HF bottles and let air dry for 24-48 hours.
n. Scoop out HF.

Suggested Density Fractionation References

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5.2 Appendix B. Master Data Table