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Kate J. Lajtha

Globally, the forestry sector is the second largest contributor of greenhouse gases, and sustainable forest management is a major target of international environmental policy. However, there is the assumption underlying many policy recommendations that an increase in above-ground carbon stocks correspond to long term increases in ecosystem carbon stocks, the majority of which is stored in soils. We analyzed soil carbon and nitrogen dynamics in forest soils that had undergone twenty years of organic inputs manipulations as part of the Detritus Input and Removal Treatment (DIRT) network. There was no statistically significant effect of the rate of litter or root inputs on the carbon or nitrogen in bulk soil, on respiration rates of soil in laboratory incubations, on the non-hydrolyzed fraction of soil organic matter, or on any organic matter associated with any density. However, there is evidence for positive priming due to increased litter inputs; doubling the rate of litter inputs decreased C and N contents of bulk soil and decreased respiration rates of soil. Furthermore, there is evidence that roots influence soil organic matter dynamics more
strongly than above-ground inputs. Both of these results trends match data from other DIRT sites, and are supported by the literature.
Effects of 20 Years of Litter and Root Manipulations on Soil Organic Matter Dynamics

by
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Jennifer D. Wig, Author
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CONTRIBUTION OF AUTHORS

Kate Lajtha was the primary adviser to the author and editor of the manuscript, and Alain Plante offered his time and equipment for many of the analyses.
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1 ORGANIC MATTER DYNAMICS IN FOREST SOILS

1.1 FORESTS AND GLOBAL BIOGEOCHEMISTRY

Globally, soils store more than three times the amount of carbon as the atmosphere, and four and a half times the amount of carbon as the world’s biota (Lal 2004). Soil degradation, land use change, particularly to agricultural systems, and unsustainable forest management have substantially decreased soil carbon stocks (Lal 2004, Vagen et al 2005). Managing forests to maximize the sequestration of atmospheric carbon into the terrestrial ecosystem is often suggested as a management technique to reduce atmospheric CO$_2$ concentrations. However, the mechanisms of soil carbon sequestration and the amounts of carbon potentially sequestered on short and long timescales, and therefore the long-term implications of these management techniques on diverse soils and ecosystems, are poorly understood (Baldock and Skjemstad 2000, Six et al 2002, von Luztow et al 2006).

1.1.1 Forests in environmental policy

Despite this gap in knowledge, using forests as a potential terrestrial carbon sink is a top priority for many international organizations. The Intergovernmental Panel on Climate Change (IPCC) is one such organization, with a mission to “provide the world with a comprehensive assessment of the current state of knowledge of climate change and its potential environmental and socioeconomic impacts.” According to the most recent report by the IPCC (2000), the forestry sector is responsible for 17% of greenhouse gas (GHG) emissions, making it the second largest source of greenhouse gas emissions due to activities such as deforestation, forest
degradation, and burning practices. Forests cover nearly a third of the earth’s land surface, but this area is decreasing, prompting the IPCC to spend a considerable amount of energy encouraging forest management for carbon sequestration. The IPCC explicitly promotes afforestation, reforestation, and reduction of deforestation to increase global forest area; reduction of forest degradation; and promotes silvicultural practices aimed to increase stand- and landscape-level C density. Specifically, site preparation, tree improvement, fertilization, uneven-aged management, longer forest rotations, management for fire and insects, and forest conservation are encouraged. The IPCC admits a lack of knowledge about the impacts of management on soil, and a lack of integration with climate impact studies, social issues, and sustainable development. To address these fundamental issues, we need to synthesize current knowledge on multiple scales and design interdisciplinary studies to elucidate the effects of silvicultural management on total ecosystem carbon storage, especially in the long term.

In response to findings of the United Nations Framework Convention on Climate Change, the Kyoto Protocol was created to set binding targets for industrialized countries to stabilize GHG emissions (UNFCCC 1998). Due to widespread participation in the Kyoto Protocol, its policy recommendations bear significant weight for governments and the managers they hire. One of the eight policy recommendations of the Kyoto Protocol is to promote afforestation, reforestation, and sustainable forest management (article 2). It allows carbon sequestered as a result of forest management to be used towards a country’s GHG reduction commitment. Like the IPCC, the Kyoto Protocol acknowledges technical
and methodological issues in applying specific silvicultural tools designed to maximize terrestrial carbon storage to diverse ecosystems over long timescales.

In theory, these policy recommendations increase carbon input into soils, where it can be stored. Afforestation, reforestation, and sustainable forest management increase C uptake in biomass, and therefore should lead to an increased organic inputs into the soil as litter, woody debris, and roots. In the policies described above, the increased C inputs to soil are assumed to increase the short-term and long-term soil carbon stocks. Soil carbon stocks will only increase if the increase in organic inputs is larger than soil carbon losses. In forest ecosystems, major soil carbon losses are due to microbial respiration, dissolution and leaching through the soil profile, and disturbance, such as erosion (Yanai et al 2003). Although site and soil properties that increase carbon uptake and promote storage in the biosphere are assumed by policy makers to result in an increase in soil C, a differentiation must be made between short-term accumulation and long-term stabilization of soil organic matter (SOM). There are different mechanisms controlling the short-term and long-term fates of SOM, and if not addressed, the distinction between the two may not be obvious.

1.1.2 Definitions

For the purposes of this review, I use “stabilization” to mean any process, thermodynamic or kinetic, that makes SOM stay in the soil longer, and “destabilization” to mean any process which decreases the amount of time SOM stays in soil. For example, long-term stabilization of additional organic inputs would lead to
long-term C increases. Alternatively, the input may be lost to decomposition by microorganisms and respired back into the atmosphere within hours or days to a few years (i.e. Johnson et al 2002, see later discussion) or removed from the soil matrix by other methods. This would obviously not increase the SOM over time, and may even decrease SOM, as can be seen in priming effects.

To discuss potential gains and losses of SOM in relation to forest management, an understanding of organic matter dynamics in the soil is important. While there is some debate of the importance of controls on SOM stabilization, without understanding how organic matter interacts with soil, it is impossible to accurately predict long-term consequences of any policy on carbon sequestration, so I will begin with a brief review of proposed models of SOM decomposition. After establishing mechanisms of SOM stabilization on the molecular level, I will scale up spatially to review literature on controls of SOM stabilization in forest ecosystems in relation to forest management. I will conclude by describing the network of Detritus Input and Removal Treatment (DIRT) studies, in which root, litter, and woody inputs into soils are experimentally manipulated in situ, providing a way to analyze different SOM pools in a natural environment.

1.2 SOIL ORGANIC MATTER

1.2.1 Development of a Model of Soil Organic Matter

The specific mechanisms of carbon stabilization in the soil are not completely understood. Two theories have dominated the literature: the humification model, in which a stable fraction of SOM is created by transformations of organic molecules by
microbial enzymes, and the molecular aggregate model, in which climate, the chemistry of the organic inputs, associations with aggregates, and complexation with soil minerals control the length of time SOM lasts in soils.

The humification model was developed in the late eighteenth and nineteenth centuries. The theory is that microbial enzymes break down organic substrates and repolymerize them to create very large molecules called humic substances. Humic substances were divided into three classes based on their solubility in acids and bases to determine pools of humin (insoluble in alkali, soluble in acid), humic acid (soluble in alkali, insoluble in acid), and fulvic acid (insoluble in both acid and alkali.)

In the 1970’s and 1980’s people started to question the existence of humic molecules, especially with the advent of new analytical techniques. In particular, NMR provided opportunities to examine molecular structure of SOM accurately (Bortiatynski 1996). Studies of this kind gave rise to several alternative mechanisms of soil organic matter stabilization, collectively known as the molecular aggregate model of SOM stabilization.

1.2.2 Supramolecular Aggregate Model

In 1986, Warshaw proposed the supramolecular aggregate model. In this model, humic substances had low molecular mass, were amphiphilic, formed micelle-like structures in aqueous solution, and were made of recognizable component biomolecules. They were formed during oxidative depolymerization of organic matter. The contrasted directly with the humic polymers that had been proposed were
large polyanionic polymers with many heterocyclic functional groups, formed by secondary synthesis.

Since this model was first proposed, many studies of SOM chemistry and its interaction with the soil matrix have expanded on definition to propose mechanistic controls on SOM. Four commonly accepted factors that contribute to SOM dynamics according to this model are climate, biochemical recalcitrance, accessibility, and interactions with secondary minerals and metal oxides (Sollins et al. 1996, Baldock and Skjemstad 2000, Eusterhues et al. 2003, von Lutzow et al. 2006, Jastrow et al. 2007, Crow et al. 2009, Eriksson 2009, Verchot et al. 2011).

**Climate.** Frozen soils and constantly waterlogged soils store large amounts of carbon. Soil organic matter pools with slow turnover times are associated with frozen saturated soil, promoting the accumulation of organic matter in boreal soils (Trumbore 2000).

**Biochemical protection (chemical recalcitrance of substrate).** The concept of biochemical protection of a molecule stems from basic enzyme kinetics, where the rate of a reaction depends on a constant, which depends on the potential change in energy, the environment, and the concentrations of enzyme and substrate. SOM is not a single, homogenous substrate, however, but different inputs have widely ranging chemistries (Kogel-Knabner 2002). The number of different bonds in a molecule, the activation energy associated with each of those bonds, and their orientation all affect how long it will take for enzymes to decompose that molecule (Sollins et al. 1996). A relationship between chemistry and initial decomposition rate of litter has been
observed but does not hold for later stages of decomposition (von Lutzow et al 2006, Schmidt et al 2011).

*Physical protection.* Physical protection of SOM results in inhibition of decomposition by physically separating the organic substrate from microbes and their enzymes due to aggregates (Tisdall and Oades 1982, Sollins et al 1996, Six et al 2004, Verchot et al 2011). This may be due to several processes. Aggregates, and especially microaggregates, already present in the soil may accumulate a large amount of stable SOM (Six et al 2000, Jastrow 1996) within themselves, and organic material can be surrounded by fine mineral particles, creating new aggregates (Tisdall and Oades 1982, Jastrow 1996, Six et al 1998).

*Association with secondary minerals and metal oxides.* Organic matter interacts with mineral surfaces via hydrophobic interactions with uncharged surfaces (ie 1:1 clays), cation bridging with permanently charged surfaces (2:1 clays with isomorphic substitution,) and through ligand exchange with hydroxylated surfaces (metal oxides) (Baldock and Skjemstad 2000, Kaiser and Guggenberger 2003, Kleber et al 2007, Kogel-Knabner 2008). The pool of SOM associated with mineral surfaces is often large (Kogel-Knabner 2008, von Lutzow et al 2008) and it has a slow turnover time compared to other SOM pools and can controls SOM on long time scales (Torn et al 1997, Mikutta and Kaiser 2011).

1.3 **SOIL ORGANIC MATTER DYNAMICS IN MANAGED FORESTS**

In forest ecosystems, major carbon inputs to the soil include woody debris, leaf litter and needle fall, and decomposing root biomass; major outputs include microbial
respiration, dissolution and leaching through the soil profile, and disturbance, such as erosion (Kogel-Knabner 2002, Yanai et al 2003, Lajtha et al 2005). As previously discussed, the quantification of these processes does not necessarily describe the resultant amount of C in the soil or its residence time. Organic matter turnover in forest soils also depends on chemical properties of the input, association with aggregates, and complexation with secondary minerals and metal oxides, as described in the molecular aggregate model (Baldock and Skjemstad 2000, Six et al 2002, Eusterhues et al 2003, von Lutzow et al 2006, Jastrow et al 2007, Crow et al 2009, Verchot et al 2011). Forest management, or lack thereof may theoretically affect the stabilization of SOM by influencing those controls. For example management can control the overall quantity of organic inputs and the ratio of above to below ground inputs, (Kogel-Knabner 2002, Crow et al 2009). The amount and size of soil aggregates is affected by soil texture, moisture, pH, and microbial dynamics (Six 2002, von Lutzow 2008, Verschot et al 2011), as well as the human use and interaction with soil (Six et al 2002). Input chemistry, the amount of Fe- and Al- oxides, and soil texture (clay) control the amount of organic matter stabilized by complexation (Baldock and Skjemstad 2000, Kalbitz et al 2005, von Lutzow 2008).

The most common management techniques include physical or chemical preparation of the site, varying harvesting intensity, varying rotation length, and managing slash residues. Each of these is discussed below.

*Site Preparation.* The objective of site preparation is to promote the rapid establishment and growth of the desired tree species (Jandl et al 2007). Methods of site preparation are manual, mechanical, or chemical, and aim to control competing
vegetation, minimize the potential for disease and pests, and improve soil properties by alleviating compaction and improving soil moisture and sub-soil drainage (Lal 2005, Tappeiner et al 2007).

The intended effect of site preparation is the rapid accumulation of aboveground biomass, specifically in trees of the desired species. This implies sequestration of carbon from the atmosphere for biomass production and as the forest becomes re-established inputs of roots and litter to the soil. However, many site preparation techniques disturb the soil significantly (Jandl et al 2007), which can have a negative effect on soil carbon accumulation.

The assumption is that with effective site preparation, new trees regenerate more quickly than without site preparation. Without rapid regeneration, there is a potential decrease in carbon stored in live biomass and woody detritus (Harmon and Marks 2002). Harmon and Marks (2002) showed a decrease in landscape level carbon storage as regeneration time increased. This would imply that effective site preparation would increase landscape level carbon relative to no site preparation, although the specific proportion of carbon stored in the soil was not investigated. While site preparation can stimulate aboveground carbon sequestration, the disturbance it can cause has a potential negative effect on soil carbon storage. Jandl et al (2007) reported a net loss of soil carbon following site preparation, the magnitude of which increased as the intensity of the soil disturbance increased. The most intense disturbances can result in nutrient losses in the soil and a subsequent decrease in long term productivity (Jandl 2007). However, the increase in above ground productivity
can offset these losses, and enhancing soil quality following site preparation is considered “crucial to increasing terrestrial C pool in forest plantations” (Lal 2005).

**Harvesting and Harvest Intensity.** Harvesting temporarily decreases aboveground biomass, and thus litter and root inputs, but allows new biomass to accumulate and sequester carbon. Furthermore, slash left behind post-harvest can be incorporated into the soil. Harvesting also disturbs the soil and changes the microclimate, both of which can influence microbial respiration rates. The classic example of soil carbon dynamics post-harvest is the “Covington Study” (Lal 2005, Yanai et al 2003). In this study Covington found a large decrease in forest floor organic matter in harvested stands, which reached a minimum after 18-22 years with a 50% decrease in carbon, some of which was gained back in the oldest stands. He attributed this to changes in wood and litter inputs, and a post-harvest acceleration of decomposition (Covington 1981, Yanai et al 2003). Resampling of Covington’s study sites has confirmed a loss of carbon after harvest, but did not conform to his model, and the authors have suggested other possible causes of this decrease, notably the mixing of organic material on the forest floor into the mineral soil and losses due to erosion and accelerated decomposition following disturbances caused by harvesting (Federer 1984, Yanai et al 2003, Lal 2005, Elliot 2003)

Some studies have shown that amount of carbon sequestered due to biosynthesis is smaller than that released to the atmosphere following soil disturbances caused by harvesting (Jandl et al 2007). Regenerating forests following clear-cuts with slash removal in Saskatchewan showed decreases of 11% to 36% in soil carbon stores to a depth of 45 cm (Pennock and van Kessel 1997) and Olsson et al (1996) saw
similar decreases in soil carbon in regenerating forests 15-16 years after clear-cutting in plantations in Sweden. However, following decreases in stands 20 years after harvest, Covington observed increases in soil carbon to pre-harvest levels after several decades. Modeling studies have addressed this: harvesting can be expected to increase carbon stores temporarily if slash is left on site, and after the slash decomposes there is a decrease in soil carbon to a temporary minimum after which it recovers partially. After two harvests the soil carbon stock decreased significantly, and continued to decrease with continued harvest for 2000 years (Liski et al 1998).

Other studies have shown the opposite. Harvesting had little lasting effects on soil carbon after 15 years in three forests in the southeastern United States (Johnson et al 2002). A meta analysis of 26 studies on the effects of harvest on soil carbon storage showed that stocks of soil carbon were either only slightly affected by harvesting or weren’t affected at all (Johnson and Curtis 2001). There may be a smaller, or non-existent, effect of harvesting on soil carbon if a decrease in microbial activity and soil moisture causes litter decomposition rates to decrease, if it is done in a way that minimizes disturbance, or if there are large amounts of residues left behind (Yanai et al 2003).

The literature has not shown any long-term effect of harvest intensity on soil carbon stores. Although Johnson and Curtis (2001) discovered a significant difference in the effects of sawlog harvesting (where the tree above ground was harvested), which increased soil carbon, and whole tree harvesting (where the entire tree, including slash and roots, was harvested), which decreased soil carbon (resulting in no net change to soil carbon following harvest in the meta analysis), this effect disappears
with time as the residues left from the sawlog treatment decomposed. Other studies failed to find effects of harvest intensity 15 years after clear-cutting (Olsson et al 1996, Johnson et al 2002).

**Rotation Length.** When managing a stand for wood harvest, rotation length is often determined to keep the stand producing biomass near its maximum annual increment (MAI). However, the Kyoto Protocol allows countries to increase rotation intervals as a means to reduce greenhouse gases (UNFCCC 1998). Increasing rotation length allows trees to grow larger, increasing carbon stored in above-ground biomass. The effects on soil carbon dynamics and ecosystem carbon balance are less understood (Liski et al 2001, Kaipainen et al 2004). Relatively short, MAI-targeting rotation intervals maximize above ground production, and thus carbon sequestration into biomass. However, shorter intervals do not necessarily maximize the amount of carbon stored in the soil, and they increase the frequency of disturbance. Conversely, increasing rotation lengths decreases soil disturbance frequency and stand productivity, resulting in carbon accumulation until a certain rotation length when the amount of carbon sequestered in biomass production is smaller than that lost to the atmosphere by respiration (Jandl et al 2007).

Models have suggested that the amount of detritus and harvest residues are controlling factors in carbon storage when rotation length is changed. In one study, the soil carbon stock decreased in two out of seven forest stands when rotation length was increased and decreased in the others. Ecosystem carbon stocks and ecosystem carbon stocks plus wood product carbon stocks increased with increasing rotation length (Kaipainen et al 2004). This was because soil carbon stocks were less sensitive
to changing rotation length than were trees, due to inputs of litter and harvest residues, which changed in opposing directions as rotation length changed. Harmon and Marks (2002) also found an increase in ecosystem carbon following an increase in rotation length, but it was much more substantial than the increase Kaipainen et al (2004) found. Rotation length was more important than the intensity of the harvest or the effect of burning slash, and it increased the carbon stored in detrital and live biomass pools. Although detritus can be quickly decomposed and oxidized to CO$_2$, the amount of carbon released by decomposition is less than that which would be released by other forest practices and processing of forest products.

*Slash Management.* Although there is doubt about Covington’s conclusion that the large decrease he saw in soil carbon was due litter inputs and inputs of live biomass, the litter effects were likely significant. Model simulations suggest that detritus can have a significant effect on the amount of carbon stored in forests, however, studies on the effects of slash or forest floor removal suggest that it may be less important for ecosystem carbon storage than the models suggest. Removal of slash and stumps decreased the amount of carbon stored in the soil but was less important to total ecosystem carbon storage than the amount of carbon emissions reduced by the use of removed slash and stumps for biofuels (Eriksson 2007). There is evidence that any effects of logging residues decrease over time, and that logging residues do not affect long term carbon storage (Johnson et al 2002). Effects of removing forest floor material have been inconsistent: it can cause decreases in soil respiration rates, probably due to decreases in moisture and organic matter content, but has also been shown to increase soil temperatures enough to increase soil respiration
rates (Mallik and Hu 1997, Fleming et al 2006) Removal of detritus significantly affects plant productivity and seedling survival, most likely due to changes in microclimate and nutrient availability (Fleming et al 2006), however, carbon stabilization in soil is not related to site productivity. These studies suggest that positive effects on live biomass production due to the removal of slash may be more important to ecosystem carbon sequestration than effects on soil carbon stabilization.

1.4 DIRT: Experimental Manipulation of Organic Inputs

None of the studies described above chronically manipulated the inputs of litter to the forest soil, although many took repeated measurements over time after a harvest. Furthermore, although they measured and discussed bulk soil carbon, none of them described the organic matter lost or gained, for example there were no measurements of labile or stable fractions of carbon before and after the event, except for in situ respiration measurements. All of the studies described above were either model-based or were observational; none designed an experiment to test a specific hypothesis about SOM dynamics related to harvesting.

1.4.1 The DIRT network and sites

The Detritus Input and Removal Treatment (DIRT) study was designed to assess how rates and sources of plant litter inputs control the accumulation and dynamics of organic matter and nutrients in forest soils over decadal time scales (Lajtha et al 2005). The current DIRT network is based on a study design by Francis Hole at the University of Wisconsin Arboretum in 1957. The original DIRT treatments consisted of chronically altering plant inputs to forest soils by regularly
removing surface litter from some permanent permanent plots and adding it to others. Currently there are also manipulations of root inputs by preventing root growth in some plots and of woody debris. Because these are chronic, experimental manipulations and not observations of existing sites, we can follow specific pools of SOM over. These manipulations are summarized in table 1.

<table>
<thead>
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<th>Treatment</th>
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<tr>
<td>Control</td>
<td>Normal litter inputs are allowed.</td>
</tr>
<tr>
<td>Double Litter</td>
<td>Aboveground leaf inputs are doubled by adding litter removed from No Litter plots.</td>
</tr>
<tr>
<td>No Litter</td>
<td>Aboveground inputs are excluded from plots. Roots are excluded with impenetrable barriers extending from the soil surface to the top of the C horizon.</td>
</tr>
<tr>
<td>No Roots</td>
<td>Aboveground inputs are excluded as in No Litter plots; Belowground inputs are prevented as in No Roots plots.</td>
</tr>
<tr>
<td>No Inputs</td>
<td>Top 30 cm of soil was replaced once with mineral soil.</td>
</tr>
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*Table 1: DIRT input manipulations*

DIRT sites have been established in eight locations in North America and Europe. In 1990, a DIRT site was created by Knute Nadelhoffer in the Harvard Forest, MA (figure 1). I have analyzed the soils from the sampling at twenty years and will present the results in the following chapter.
1.4.2 Current Study

The first objective of this study was to determine how SOM dynamics are affected by different detritus input sources and amounts in the bulk soil, and through
the more labile and more recalcitrant pools. We hypothesize that after twenty years, increases in high quality above-ground inputs (litter) result in a) small increases in accumulation of the most labile carbon, because as SOM quantity is increased, easily degradable microbial substrate is also increased and increased respiration would counteract the accumulation of new inputs; b) moderate increases in intermediate carbon pools, because additional inputs into this pool are less accessible to microbes and thus are not respired as quickly as additions to the labile pool; and c) no affect on the stable carbon pool, because the timescale of the study is significantly shorter than the residence time of the carbon in the stable pool. Furthermore we hypothesize that the elimination of above-ground inputs will mirror the above pattern, but will have a more significant negative effect on long-term carbon stabilization because continued microbial respiration without continued inputs necessarily degrades the intermediate and stable carbon pools after the labile pool becomes exhausted.

Alternatively, additions of high-quality above-ground inputs may result in priming, where the increased litter inputs cause an increase in microbial respiration and thus release both newly added carbon and stored SOC at increased rates. If the rate of microbial respiration exceeds the rate of organic inputs, there will be a decrease in the amount of carbon in the labile pool and no changes, or slight decreases to the intermediate and stable carbon pools. Also, even if elimination of above-ground inputs has a negative effect on all three soil pools, the effects take longer than 20 years to manifest significantly, so we may not observe them. Finally, the methods we have chosen may not accurately partition SOM into functional pools, and thus they may not reflect real changes to the functional SOM pools.
To test these hypotheses, we a) analyzed bulk soil for C and N contents, b) incubated the soil for one year to measure rate of respiration and cumulative amount of C respired, a measure of the most labile soil carbon pool, c) performed acid hydrolysis to obtain the fraction of carbon that was resistant to hydrolysis, which corresponds the most stable fraction of soil carbon, and d) performed sequential density fractionation, isolating fractions that differ in properties related to residence time and stability (Sollins et al 2009)

If our initial hypotheses are correct, we expect to see an increase in total carbon content of bulk soil as litter inputs increase from the no litter to the control to the double litter treatments. We also expect to see an increase in respiration rates and cumulative carbon respired with increasing litter inputs, because respiration rate is indicative of the proportion of total carbon that is the most easily accessible and easily decomposable by microbes. We also expect to see an increase in the amount of carbon associated with the lightest density fraction, (<1.85 g cm\(^{-3}\)) because this is another measure of non-decomposed organic matter that has not been stabilized by associations with aggregates or minerals. We expect the amount of carbon in heaviest fractions isolated from sequential density fractionation (>2.8 g cm\(^{-3}\)), corresponding to the oldest, most microbially processed soil fraction, not to differ between treatments. The percentage of non-hydrolyzable C (%NHC) and non-hydrolyzable N (%NHN) also represent the most stable C pool. Since I don’t expect the stable SOM pool to change, but I do expect the total C and N to increase with increased inputs, I expect the %NHC and %NHN to decrease with increased inputs.
The second objective of this study was to determine the relative effects of above-ground and below-ground inputs on SOM accumulation and stabilization. We hypothesize that due to their position in the soil profile, below-ground inputs are more quickly associated with aggregation processes and with mineral complexes and are therefore less easily respired. We expect the effects of below-ground inputs on both short-term SOM accumulation and long-term SOM stabilization will be more pronounced than the effects of above-ground inputs.

Alternatively, below-ground inputs of organic matter may be equally as or less important for the accumulation and stabilization of SOM than above-ground inputs due to decreased microbial processing at depth, since microbial processing has been seen to increase with residence time in soils. If we don’t observe differences in any of the carbon pools dependent on position of the plant input, the large number of interacting controls on SOM dynamics may mask the relatively small effect of the source of the OM, or the timescale of the study may not be large enough to observe the effects of manipulating input position in the soil profile.
2 Twenty Years of Litter and Root Manipulations: Insights into Multi-Decadal SOM Dynamics and Controls

2.1 INTRODUCTION

Globally, soils store more than three times the amount of carbon as the atmosphere, and four and a half times the amount of carbon as the world’s biota (Lal 2004). Soil degradation, land use change, and unsustainable forest management have substantially decreased soil carbon stocks (Lal 2004, Vagen et al 2005). While carbon sequestration in soil is often suggested as a management technique to reduce atmospheric CO$_2$ concentrations the mechanisms of soil carbon sequestration, the amount of carbon potentially sequestered, and the long-term implications for carbon sequestered in the soil are poorly understood (Baldock and Skjemstad 2000, Six et al 2002, VonLuztow et al 2006).

Many international organizations are promoting afforestation and sustainable forest management to create carbon sinks. According to the Intergovernmental Panel on Climate Change (IPCC), the forestry sector is the second largest source of greenhouse gas emissions due to activities such as deforestation, forest degradation, and burning practices. The IPCC specifically promotes afforestation, reforestation, and reduction of deforestation to increase global forest area; as well as the use of silvicultural tools to increase C density on the stand and landscape scale (IPCC 2000). In response to the United Nations Framework Convention on Climate Change, most nations have either signed or are considering participation in the Kyoto Protocol to counteract increasing atmospheric greenhouse gas (GHG) concentrations. The Kyoto protocol allows carbon sequestered as a result of forest management to be used
towards a country’s GHG reduction commitment, making forest management and afforestation important tools for many governments.

While increasing above-ground biomass necessarily sequesters C from the atmosphere, changes in C stored in biomass does not lead to immediate or long-term changes in soil C storage (Sulzman et al 2005, Crow et al 2009). This can be seen in studies of the effects of tree harvesting: even though tree harvesting clearly decreases the C content of the stand, there was no change in soil organic carbon (SOC) content for at least 15 years post-harvest (Olsson et al 1996, Johnson and Curtis 2001, Johnson et al 2002, Yanai et al 2003), even though harvesting clearly decreases the C content of the stand, at least in the short-term. Similarly, chronic manipulation of above and below ground organic inputs into two forest soils had no effect of SOM after four or eleven years (Holub et al 2005). In addition to land use change, factors such as fire repression, longer growing seasons, N deposition, and CO₂ fertilization are responsible for sequestering CO₂ from the atmosphere into terrestrial ecosystems and there is disagreement over the role of forest ecosystems (Townsend et al 1996, Korner et al 2000, Barford et al 2001, De Vries et al 2008, De Vries et al 2009).

In forests, the sources of plant inputs into soil can be altered by management or disturbance, and these can have significant effects on soil organic matter (SOM) dynamics. Studies have shown a greater impact of roots than above-ground inputs on SOM stability than above ground inputs (Rasse et al 2005, Mueller et al 2009, Six et al 2001). Altering above and below ground inputs can affect microbial respiration rates (Fontaine et al 2003, Sulzman et al 2005, Brant et al 2006, Crow et al 2009), and alter soil solution chemistry (Park and Matzner 2003, Latha et al 2005, Yano et al 2005).
Increases in plant inputs into soil can cause disproportionate increases in microbial respiration rates, known as positive priming, or they can have the opposite effect, a decrease in microbial respiration rate in response to organic matter additions, known as negative priming. Sulzman et al (2005) saw a positive priming effect of 187% in response to litter additions in an old growth forest after 13 years, which agrees with other studies in forest ecosystems. Carbon and nitrogen is lost from soils as dissolved organic matter (DOM), but the direct effects of management practices on DOM are not well understood. Roots have an important effect on dissolved organic nitrogen (DON) chemistry (Yano et al 2005), and DOM retention may increase in response to increases in litter inputs (Park and Matzner 2003), although other studies did not see this effect (Yano et al 2005, Lajtha et al 2005). There is a clear need for more long-term, comprehensive studies to elucidate controls on soil C and N cycling over short and long time scales.

Vocabulary is important and easily confused, especially in such diverse disciplines as biogeochemistry where scientists have many different backgrounds. Here, I define some vocabulary that is often confused or ambiguous in manuscripts. I use “stabilization” to mean any process, thermodynamic or kinetic, that makes SOM stay in the soil longer, and “destabilization” to mean any process which decreases the amount of time SOM stays in soil. For example, long-term stabilization of additional organic inputs would lead to long-term C increases. Alternatively, the input may be lost to decomposition by microorganisms and respired back into the atmosphere within hours or days to a few years (i.e. Johnson et al 2002) or removed from the soil matrix by other methods. This would obviously not increase the SOM over time, and may
even decrease SOM, as can be seen in priming effects. I refer to SOM that has gone through some stabilization process and “stable” and that which has not as “labile.” I am not referring to the chemistry of the organic molecule or a specific process; I’m describing the relative mean residence time of the SOC in the environment (Note that I am not associating specific MRT values to different SOM pools. I use “fraction” to refer to a part of the soil that corresponds to a direct measurement, and “pool” to describe a hypothetical, relative subset or SOM. For example, the labile fraction of SOM in this study refers to the C respired in incubations, and the light-fraction obtained from sequential density fractionation. The labile SOM pool, however, is the real proportion of SOM that has not gone through significant stabilization processes. Generally the fraction isolated from an analysis is believed to represent a real SOM pool.

Many recent studies trying to define the mechanisms of long-term dynamics of carbon in soil focuses on the molecular scale. For example, there has been significant focus on the roles of aggregates (i.e. Six 2002,) and organo-mineral complexes (i.e. Kleber et al 2007, Mikutta and Kaiser 2011) in SOM stabilization. Once thought to control SOM dynamics, the importance of chemical recalcitrance is now widely questioned (i.e Marschner et al 2008, von Lutzow et al 2008, Schmidt et al 2011). This has important consequences for how we conceptualize and model the influence of different organic inputs on SOM stocks and fluxes over different spatial and temporal scales. However, to elucidate the real effects of land use on total ecosystem carbon storage, we need to synthesize this mechanistic knowledge with stand- and ecosystem-scale studies.
The Detritus Input and Removal Treatment (DIRT) study was designed to assess how rates and sources of plant litter inputs control the accumulation and dynamics of organic matter and nutrients in forest soils over decadal time scales (Lajtha et al 2005). DIRT sites consist of plots with different amounts of roots, litter, and wood allowed to interact with the soil. Because the manipulation of these inputs is experimental and (relatively) consistent, we can examine pools of carbon through time more accurately than observational studies of tree harvests, but still observe stand level effects with large plots located within forest stands. The current network of DIRT sites is based on the original study design by Francis Hole at the University of Wisconsin Arboretum in 1957. The DIRT network consists of eight sites in North America and Europe, the oldest of which, besides the original WI site, was founded in 1990 in the Harvard Forest, a transitional hardwood forest in MA, USA. Data from the WI site suggests that after fifty years, chronic manipulations of inputs may have affected long-term SOM dynamics (unpublished data). Here we present data on C and N dynamics of SOM after twenty years of litter and root manipulation at the Harvard Forest.

The objectives of this study were to a) determine the influence of quantity of above- and below-ground plant inputs into soil on SOM dynamics, and b) to determine the relative effects of above-ground vs below-ground inputs on SOM dynamics on a decadal time scale in a transitional/mixed hardwood forest in Massachusetts.

We hypothesize that: 1) as high-quality above-ground inputs (litter) increase, we will see increases in C and N in the most labile organic fractions (though these effects may be muted by an increase in microbial respiration), 2) we will not see
corresponding increases in C and N content of stable C pools with increasing litter inputs because of the relatively short timeframe of the study, 3) the effects of removal of below-ground inputs on both short-term SOM accumulation and long-term SOM stabilization will be more pronounced than the effects of above-ground inputs because below ground inputs will be more easily protected by the soil matrix and will be less easily respired by microbes.

Alternatively, additions of high-quality above-ground inputs may result in priming, in which case there may be a decrease in the amount of carbon in the most labile organic fractions. Furthermore, the large number of interacting controls on SOM dynamics may mask the relatively small effect of the source of the OM, or the timescale of the study may not be large enough to observe the effects of manipulating input position in the soil profile.

2.2 MATERIALS AND METHODS

2.2.1 Site Description

Vegetation. Soils were collected from the DIRT site located in the Tom Swamp tract at the Harvard Forest in Massachusetts (42.49°N, 72.20°W, 320 mas; figure 1), which is a transition/mixed hardwood-white pine-hemlock forest. Dominant tree species at the DIRT site are northern red oak (*Quercus borealis*), red maple (*Acer rubrum*), and paper birch (*Betula papyrifera* Marsh.), which represent 43, 19 and 15 percent of the total basal area of the stand, respectively. From 1733 to 1850 the site was permanent pasture. In 1908 it was classified as old-field white pine, and then as a white pine transition-hardwood in 1923 (Bowden et al 1993).
Soil and climate characteristics. The soil is a moderately well-drained sandy-loam inceptisol, from the Charlton series. Forest floor depth is 3-8 cm. Average soil depth is 3m, with a thin Oa horizon (1-3 cm). Roots have not been observed below 70 cm. Bedrock is primarily granite, gneiss, and schist. Mean temperature ranges from -7°C in January to 20°C in July, and mean annual precipitation is 110cm (Nadelhoffer et al 1999).
Figure 1: The Tom Swamp Tract in Harvard Forest, showing the location of the DIRT experiment
2.2.2 Study design

Litter and root manipulations began in September 1990, and include five input/exclusion treatments and a control (C), each replicated three times. Plots are 3mx3m, and none include trees or saplings. Treatments are double litter (DL), no litter (NL), no roots (NR), no input (NI), and Oa-less (OA), as summarized in table 1. To exclude litter from NL and NI plots, they are screened with 2mm mesh, and the litterfall is swept off plot, collected and quantified. Litter removed from NL plots is then transferred to DL plots by area. While a certain amount of DOM will pass through the screens between screen cleaning events, the vast majority of litter C inputs to the plots will be excluded through this screening technique. Trenches one meter deep were dug around the NR and the NI plots to prevent roots from surrounding trees to penetrate the soil beneath the plots. In the OA plots, the O and A horizons of soil were removed and replaced with mineral soil from the B-horizon when they were first established in 1990, and since then have been allowed normal inputs. We are not analyzing the OA plots and looking only at plots with manipulated inputs and the control.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal litter inputs are allowed.</td>
</tr>
<tr>
<td>Double Litter</td>
<td>Aboveground leaf inputs are doubled by adding litter removed from No Litter plots.</td>
</tr>
<tr>
<td>No Litter</td>
<td>Aboveground inputs are excluded from plots.</td>
</tr>
<tr>
<td>No Roots</td>
<td>Roots are excluded with impenetrable barriers extending from the soil surface to the top of the C horizon.</td>
</tr>
<tr>
<td>No Inputs</td>
<td>Aboveground inputs are excluded as in No Litter plots; Belowground inputs are prevented as in No Roots plots.</td>
</tr>
<tr>
<td>OA-less</td>
<td>Top 30 cm of soil was replaced once with mineral soil.</td>
</tr>
</tbody>
</table>

*Table 1: Descriptions of treatments at the Harvard Forest DIRT site*
2.2.3 Soil collection and treatment

Soils were collected in October 2010. O-horizons were collected as 20cm x 20cm brownies and mineral soil was collected with a diamond bit corer. Two cores were removed from each plot to account for spatial variation, one to 30cm below the soil surface and the other as deep as possible. Cores were then separated by depth into 0-10 cm, 10-20 cm, 20-30 cm, and >30 cm samples.

Samples were kept in airtight baggies at 4°C for transport to Oregon State University (OSU). Subsamples used for the year-long incubation remained field moist, at 4°C until measurements began. The remaining soil was air dried and stored in airtight plastic bags until analysis.

2.2.4 Chemical and physical analyses

Carbon and Nitrogen. Organic carbon and total nitrogen concentrations were determined by dry micro-Dumas combustion with a Costech Instruments Elemental Combustion System (NA1500 C/H/N Analyzer, Carlo Erba Strumentazione, Milan) in the Department Earth and Environmental Sciences, University of Pennsylvania.

Long-term incubation. We measured CO$_2$ respiration rates in a laboratory incubation of the soils from the O-horizon, 0-10 cm, and 10-20 cm depths. 70g dry-weight equivalent of moist soil from each soil core of the mineral horizons and 30g dry-weight equivalent from the o-horizon was placed in microcosms based on Nadelhoffer (1990) Soils were saturated with a leaching solution and drained to field capacity. This moisture level was maintained throughout the experiment. We measured respiration on days 1, 3, 7, 15, 25, 33, 55, 63, 96, 250, and 370 with a LiCor
LI-6400 Portable using the soil respiration attachment that was customized to be fit our microcosms. Target CO\textsubscript{2} concentrations were set to the ambient level and were reset periodically throughout sampling, delta values were between 2ppm and 15ppm, the minimum sampling time was 30 seconds, and each sample was measured three times. Between measurements, microcosms were stored in the dark at room temperature.

*Sequential density fractionation (SDF).* We separated the bulk soils from the 0-10cm and 10-20cm depths by particle density by suspension in solutions of sodium polytungstate (SPT) at three densities (1.85 g/cm\textsuperscript{3}, 2.4 g/cm\textsuperscript{3}, 2.8 g/cm\textsuperscript{3}) and centrifugation following Sollins et al (2009). We placed one 20g sample of air-dried soil from each plot, each made up of 10g subsamples from each core, into 225ml clear polycarbonate centrifuge tubes with conical bottoms and five centimeter mouths and added 40ml 1.85 g/cm\textsuperscript{3} SPT (10-20cm samples) or 45ml 1.85 g/cm\textsuperscript{3} SPT (0-10cm samples). We shook the samples for two hours on a shaker table to disperse weakly bound aggregates. We then centrifuged the samples in a swinging bucket rotor for 20-40 minutes, until there was clear separation of sediment (particle density >1.85 g/cm\textsuperscript{3}) and floating supernatant (particle density <1.85 g/cm\textsuperscript{3}). We aspirated and rinsed the supernatant over a Whatman GF/C glass fiber filter with DDI water. We added >2.4 g/cm\textsuperscript{3} SPT to the sediment still in the centrifuge tubes, shook the samples for one hour on a shaker table, and repeated the centrifugation and aspiration a fraction with particle density 1.85-2.4 g/cm\textsuperscript{3}, which we rinsed by filling the centrifuge tube with, centrifuging, and discarding DDI water four times. We verified the density by pipetting out and weighing 10 ml of the SPT in each sample. We added >2.8 g/cm\textsuperscript{3}
SPT to the sediment, shook for one hour, and repeated the procedure as described above to isolate fractions with 2.4-2.8 g/cm$^3$ and >2.8 g/cm$^3$ particle densities. We rinsed all the samples into Fisherbrand pie tins and dried at 50°C and weighed them.

*Acid Hydrolysis.* We hydrolyzed 1 g of soil from each core after Paul et al (1997), with minor adjustments. Briefly, we assumed particulate organic matter had a density of <1.85 g/cm$^3$ and removed it as described in the first step of SDF. We then pulverized the samples without organic matter, and refluxed a 1 g subsample with 6M HCl at 95°C for 18 hours. After 18 hours we rinsed the remaining soil over a Whatman GF/C glass fiber filter with DDI water, transferred the soil to tins, and dried at 50°C and weighed. We calculated the percentage of non-hydrolyzable C and N (%NHC, %NHN) as: \[
\%NHC, \%NHN = \frac{(g \text{ C,N/kg sample})_{after} \times (mass_{after}/mass_{before})}{(g \text{ C,N/kg sample})_{before}}
\]

*Statistical analysis.* A linear mixed effects model (nlme) was used to test for differences between treatments, depths, and for an interaction between treatment and depth for %C, %N, and C:N in bulk soils, %NHC and %NHN, and for each density fraction. C and N contents in the each density fraction were analyzed as percentages, as g C g fraction$^{-1}$, as g C g C bulk soil$^{-1}$, and as g C kg soil$^{-1}$, as well as C:N. Prior to analysis, residuals were examined visually to ensure they met equal variance and normal distribution assumptions of the model. Analyses were done in R 2.14.1. All values were log-transformed for the analysis, and reported means have been back-transformed.

2.3 RESULTS
2.3.1 Carbon and Nitrogen of bulk soils.

%C decreased with depth by about 33% (p<0.001). There was large variability in bulk soil measurements, especially in the top 10cm, and treatment did not have a significant effect on %C at either depth (figure 2). Although not significant, it is interesting that 20 years of increased litter inputs resulted in lower C contents compared to the natural litter inputs at both depths, which may indicate priming. Also notable is the large C content of the No Input treatment at 0-10 cm.

![Figure 2: Mean %C and standard errors of bulk soil at 0-10 cm and 10-20 cm depths](image)

N concentration also decreased with depth (figure 3). Soils 10-20cm below the surface had about 57% less N than the soils in the top 10cm of soil (p<0.001). There was no statistically significant difference between N contents of different treatments. As with %C, increasing litter inputs resulted in a lower %N in both depths, although
not significantly, and eliminating all inputs resulted in unexpectedly high N concentrations in the top 10cm of the soil.

![Bar chart showing mean % N and standard errors of bulk soil at 0-10 cm and 10-20 cm depths](image)

**Figure 3: Mean % N and standard errors of bulk soil at 0-10 cm and 10-20 cm depths**

2.3.2 Microbial respiration measurements.

Respiration rates of the O-horizon material fluctuated greatly in the first three months, especially for the control treatment (figure 4a). Even large fluctuations were generally consistent between treatments, so while variability may be high, it did not affect the relative cumulative respiration of the treatments. The O-horizon material of DL plots had a lower respiration rate and lower cumulative respiration compared to the control and no root plots, both of which had normal litter inputs. Respiration rates of litter from NR and CO and had overlapping rates during the first three months. The plots without roots had consistently lower rates of respiration than the control plots after three months, and lower cumulative respired C (figure 5a).
In soil from the top 10 cm of soil, respiration rates were low for all treatments during the first sampling and increased to the maximum rate within two weeks (figure 4b). For the first two months respiration rates were erratic for soils from the control plots and plots with added litter compared to the other treatments. The respiration rate of the control and increased litter soils was higher than the other treatments at all samplings except one, resulting in a higher amount of C respired over the course of the incubation. Removing roots and removing both roots and litter inputs resulted in the lowest rates of respiration, except for a spike in the respiration rate of the soils with on days 3 and 7. Removing litter inputs resulted in reduced respiration compared to normal inputs and increased litter, but not as much as removal of roots. Cumulative respiration mirrored this pattern; removing roots, with or without litter removal, resulted in the lowest total C respired, and removal of litter resulted in only slightly higher C respired (figure 5b).

Respiration rates in soils from 10-20cm below the surface were initially high, but dropped off quickly (figure 4c). There was a slight increase in respiration rate for most treatments around day 60. Plots with normal inputs respired at the highest rate after this increase, and had the highest level of total C respired at the end of the incubation. The plots excluding or increasing litter inputs, without changing root inputs, respired less C over the course of the incubation than control, but more C than plots without root inputs. Eliminating both root and litter inputs resulted in the lowest amount of cumulative C respired (figure 5c).
Figure 4. Average respiration rates ($\mu$gC respired gC initial$^{-1}$ m$^{-2}$ day$^{-1}$) over the 370 day incubation period for soils sampled from a) the O-horizon, b)0-10 below the surface, and c) 10-20 cm below the surface
Figure 5. Average cumulative respiration ($\mu$gC resired gC initial$^{-1}$) over the 370 day incubation period for soils sampled from a) the O-horizon, b)0-10 below the surface, and c) 10-20 cm below the surface.
2.3.3 Acid hydrolysis

Neither treatment nor depth had an effect on %NHC or %NHN (figures 6 and 7). The soils from the no input plots had the highest %NHC and %NHN in the top 10 cm of soil, and the soils without root inputs had the lowest. At 10-20cm, the soils with increased litter inputs had the highest %NHC, and soils with no roots had the highest %NHN. There were very similar NHC:NHN ratios between treatments.

![Figure 6: Average % non-hydrolysable C (NHC) and standard errors for each treatment in the top 0-10 cm and 10-20cm of the mineral soil.](image-url)
There were no significant effects of treatment on the C or N content of any of the fractions at either depth. In the 1.85-2.4 g cm$^{-3}$ fraction, there may have been differences due to an interaction between treatment and depth in the C (p=0.08) and N (p=0.10) contents as well as in the C content of the 2.4-2.8 g cm$^{-3}$ fraction (p=0.09). However, there were no consistent patterns in these fractions, and these may be due to variances in measurement and standard errors of the measurements.

Overall, there were very large variances within and between the treatments (and a small sample size), potentially obfuscating trends. There were no consistent trends in any of the fractions, except in the fraction >2.8 g cm$^{-3}$, where soils from the no root treatments had the lowest N content in every fraction. Notable values found in the upper 10 cm, are exceptionally large C and N contents of the soils from the no
input and no root plots in the fraction $>2.8 \text{ g cm}^{-3}$, and exceptionally low C and N contents of no litter and no root plots in the fraction $<1.85 \text{ g cm}^{-3}$. The soils from the control plots had very high C and N contents in the fraction $<1.85 \text{ g cm}^{-3}$ of the deeper soil.
<table>
<thead>
<tr>
<th>Treatments</th>
<th>0-10 cm</th>
<th>NO SOIL</th>
<th>1.85-2.4 cm$^{-3}$</th>
<th>2.4-2.8 cm$^{-3}$</th>
<th>&gt;2.8 cm$^{-3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.17</td>
<td>3.80</td>
<td>1.01</td>
<td>0.26</td>
<td>27.20</td>
</tr>
<tr>
<td>Double Litter</td>
<td>14.76</td>
<td>5.74</td>
<td>1.03</td>
<td>0.24</td>
<td>26.24</td>
</tr>
<tr>
<td>No Inputs</td>
<td>19.31</td>
<td>3.44</td>
<td>0.99</td>
<td>0.25</td>
<td>25.80</td>
</tr>
<tr>
<td>No Litter</td>
<td>5.41</td>
<td>1.32</td>
<td>0.29</td>
<td>0.04</td>
<td>27.20</td>
</tr>
<tr>
<td>No Roots</td>
<td>6.62</td>
<td>3.03</td>
<td>0.25</td>
<td>0.16</td>
<td>29.55</td>
</tr>
<tr>
<td>10-20 cm</td>
<td>Control</td>
<td>19.84</td>
<td>8.80</td>
<td>1.07</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Double Litter</td>
<td>7.41</td>
<td>0.32</td>
<td>0.11</td>
<td>15.25</td>
</tr>
<tr>
<td></td>
<td>No Inputs</td>
<td>9.25</td>
<td>6.70</td>
<td>0.54</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>No Litter</td>
<td>6.33</td>
<td>1.11</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>No Roots</td>
<td>6.67</td>
<td>1.57</td>
<td>0.25</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 2: Mean C (g C kg$^{-1}$ soil) and mean N (g N kg$^{-1}$ soil) in each fraction separated by sequential density fractionation. Treatments are grouped by depth. Mean values of C or N are on the left side of each column and corresponding standard errors are on the right side of the column.
2.4 DISCUSSION

Our objectives were to determine how SOM dynamics were affected by different detrital input sources and amounts, and to compare the effects of above and below ground inputs on SOM dynamics. We hypothesized that 1) as input amount increased, there would be an increase in the labile SOM fractions, 2) that there would not be a corresponding increase of more stable SOM as a result of increased inputs, and 3) the manipulation of belowground inputs would have a greater effect on SOM stabilization compared to above-ground inputs. Trends in previous analyses of DIRT soils prompted us to consider the following alternative hypotheses: 4) if positive priming effects are large, there may be a decrease in labile SOM with increases in input amount, 5) complicated and interacting controls on SOM dynamics may override any effect of input rate or source, and 6) it may take longer than 20 years to for differences between treatment, especially within the more stable SOM fractions, to be measureable.

Our results were statistically inconclusive, but there were some interesting trends that were consistent between our analyses and with other DIRT studies that are worth discussing. Specifically, our data do not support our hypothesis that SOM would increase as litter inputs increased (1), but there was evidence of positive priming (4). Our data does support our hypothesis that the more stable SOM would be relatively unaffected by litter inputs (2), although it may be due to the mechanisms behind (5) and (6). Finally, there are indications that changes in root inputs have stronger effects on SOM stabilization than changes in litter inputs (3).
Although we took cores from multiple locations in each plot to account for spatial variability, the variability within treatments in most of our analyses was high. This likely accounted for some of the inconclusive results. Alternatively, lack of differences between treatments may be due to length of the study; 20 years for observable differences in SOM chemistry to occur. We will continue with our discussion of interesting trends as if the spatial variation within treatments was more responsible in most cases for large p values than similarities between means

2.4.2 Effects of Manipulating Litter: Evidence of Priming

We predicted an increase in the quantity of litter inputs to cause an increase in bulk soil percentages of C and N near the soil surface, as well as an increase in cumulative respiration and grams per soil of C and N in the lightest pool of soil isolated by SDF, which has density <1.85 g cm\(^{-3}\) (light-fraction C and light-fraction N) light-fraction, both of which are measures of available, easily decomposable organic matter. However, at all depths, doubling the amount of litter inputs soil resulted in decreased %C and %N of the bulk relative soils under natural conditions and lower rates of respiration, and thus cumulative C respired over the course of the incubation experiment. There was less light-fraction C at both depths for soils with increased litter inputs relative to control. These results potentially indicate positive priming. In this case, increased litter inputs result in increased rates of microbial respiration such that the amount of C lost as CO\(_2\) is greater than the amount of C incorporated into the soil (Fontaine et al 2003, 2004). The result is lower contents of labile (relatively easily accessible and degradable) SOC where inputs have increased.
These results are consistent with data from DIRT sites at the H. J. Andrews Experimental Forest in OR and from the Allegheny College Bousson Experimental Forest in PA which also indicate positive priming. Crow et al (2006, 2009) found a decrease in respired C of soil from double litter plots relative to control at both sites. However, those DIRT sites are younger than the Harvard Forest DIRT site, and priming is usually a short-lived phenomenon (Guenet et al 2010), so we did not expect to see effects on a multi-decadal timescale.

2.4.3 Contribution of roots to SOM dynamics

*Bulk soil and density fractionation measurements.* Several studies suggest roots may contribute to the more stable C pools more than above-ground residues (i.e. Oades 1988, Boone et al 1998, Six et al 2004, Rasse et al 2005). Therefore, we expected the removal of roots to decrease the C and N contents of bulk soil and of both the light fraction and intermediate fractions recovered from density fractionation. We also expected to see a decrease in cumulative respiration with the removal of roots. We did not expect root removal to have any effects on the more stable carbon pools after only 20 years. There were no differences in bulk soil C or N between any treatments. The soil from which both roots and litter were removed had unexpectedly high C and N contents between 0-10 cm that we cannot account for. We sampled in the field and prepared subsamples in lab to reduce the likelihood that outlying values would affect our analysis. Removing roots alone, removing litter alone, and increasing litter all resulted in similar contents of C and N which were lower than control soils at both depths, although not significantly lower. There were no
consistent patterns within the density fractions. For example, in the top 10 cm, soils with no roots had a very low mean C concentration in the lightest fraction (<1.85 g cm\(^{-3}\)) but the highest mean C concentration in the following fraction (1.85-2.4 g cm\(^{-3}\)), and soils which had both roots and litter removed had a very high mean C concentration in the lightest fraction but a moderate one in the following fraction. We observed during sampling that roots had recolonized the no root and no input plots at some point, and this likely explains the lack of consistency between plots with no roots. Otherwise, it could be due to a) high spatial variation of soils was not well-enough accounted for in sampling, b) high variability of density fraction recovery, and the chemistry of the fractions recovered, or c) the possibility that density fractionation is not sensitive enough that we can observe differences in OM content of the fractions after 20 years.

*Respiration measurements.* In the O-horizon, cumulative respiration of soil from the no-root plots was lower than from control soil but higher than soils with increased litter. This suggests the priming effect of litter additions is stronger than the decrease in microbial respiration due to root removal in the O-horizon. Since leaf litter contributes substantially more to the O-horizon than roots, this would not be surprising. However, in the top 10 cm of mineral soil, organic matter removal of any kind resulted in lower respiration measurements than soils from the control or double litter plots. 10-20 cm below the surface, all soils with manipulated inputs had lower respiration than control soils, and soils from soils from any plot without roots had lower respiration than soils from any plot with roots. Although some of the differences are small, especially in the deepest soil, the consistency of these trends through the depth profile indicates that roots contribute heavily to the pools of labile
carbon in the mineral soil, perhaps more than litter, and that the effect of root presence may even be seen in the organic horizon of soil, primarily composed of leaf litter. The roots at the in these plots tend to be high in the soil profile, so this may be less important in ecosystems where roots tend to be concentrated farther away from the surface. Furthermore, during sampling, there was evidence of root invasion in every plot, so these values may represent respiration the effect of decreasing roots rather than eliminating them.

**Acid hydrolysis and non-hydrolysable SOM.** We did not expect the amount of non-hydrolyzable C or non-hydrolyzable N (NHC and NHN, respectively) to change after 20 years of litter and root manipulations because NHC and NHN tend to be older than the bulk soil (Leavitt et al 1996, Paul et al 1997). Therefore, after 20 years of eliminating new organic inputs, we expected the proportions of NHC and NHN to be higher in soils where there was less labile material. Although bulk C and N measurements and respiration measurements indicated that there may have been lower amounts of labile OM in soils from which litter or root inputs had been removed, there were no indications of the corresponding increase of %NHC and %NHN we expected. In the top 10 cm, soils without inputs had the highest %NHC and %NHN of all the soils, but soils without roots had the lowest %NHC and %NHN. In soil from 10-20 cm, soils from plots with either no roots or increased litter inputs had the highest %NHC and %NHN. There are many reasons the data may not match predictions: a) high spatial variability of soil, b) high variability in %C and %N measurements of non-hydrolyzed fractions and bulk fraction, c) 20 years is not long enough to be able to measure a difference in non-hydrolyzable OM caused by litter and root
manipulation, d) root and litter manipulations are having an effect on the turnover of hydrolysable SOM such that the ratio of the non-hydrolyzable SOM pool to total SOM is constant, or e) acid hydrolysis is not an appropriate tool for measuring the most stable proportion of a SOM.

During acid hydrolysis, compounds such as proteins, nucleic acids, and polysaccharides are digested by 6M HCl, and a solid residue is left behind consisting of compounds that are resistant to digestion, including aromatic components and wax-derived long-chain aliphatics (Paul et al 2006). This residue is analyzed as the non-hydrolysable fraction of C and N (%NHC and %NHN, respectively). For the proportion of non-hydrolysable SOM to increase with decreasing amounts of labile SOM pool as hypothesized, NHC and NHN would have to directly correspond with the stable SOM pool. Then, a decrease in labile SOC, as is suggested by our cumulative respiration measurements, should correspond to an increase in the percentage of SOM that is non-hydrolyzable. However, although NHC and NHN are generally older than bulk soil C and N, it is not analogous to the resistant SOM pool, due in part to non-chemical factors that contribute to SOM stability (Bruun and Luxobi 2006, Plante 2006, Bruun et al 2008). Therefore, a combination of (d) and (e) above probably account for the lack of significant trends.

2.5 CONCLUSIONS

SOC and SON measurements from soils after 20 years of continuous input manipulation indicated the possibility of several trends, but none were statistically significant. Trends in bulk SOC and SON contents and long-term respiration
measurements suggest positive priming caused by increased litter input rates. In the 0-
horizon, manipulations of both roots and litter resulted in lower rates of C respired but
the litter seemed to have a stronger influence on the labile C fraction than roots. In the
mineral soil, however, this was not true; while the respiration rates of soils from all
combinations of litter and root manipulations were lower than the respiration rate of
the control soil, root manipulation seemed to have a stronger effect than litter. These
trends agree with trends reported from analyses of soils from other DIRT sites. The
DIRT study is unique in its application of experimental manipulations of organic
inputs on large, minimally disturbed (except for the study itself) forest plots. This
allows an analysis of different SOM fractions that would not be possible from the
observational studies which are often used to describe stand or landscape level
processes. Despite sampling designed to account for spatial variability there were
large variances within treatments which, along with the inherent complexity of the
system, which likely decreased the statistical significance of our results.
3 SYNTHESIS: REINTERPRETING FOREST MANAGEMENT WITH DIRT

To review, soils from the 20 year sampling of the Detritus Input and Removal Treatments (DIRT) site in Harvard Forest, MA, were analyzed for 1) the C and N concentrations of bulk soil, 2) respiration rates and cumulative respired C over the course of a year, 3) proportions of bulk C and N that are resistant to digestion in strong HCl, and 4) the C and N contents of four different pools of soil separated based on particle density. Although there were no statistically significant treatment effects for any analysis, we did see trends that are consistent with data from other DIRT sites, and which indicate interesting biogeochemical pathways.

The goal of this chapter is to illustrate the importance of trends such as those presented previously in the analysis of Harvard Forest DIRT soils. I will briefly review the trends we saw in the analyses, and the processes we hypothesize that they represent. Then I will discuss how accounting for these processes may affect ecosystem, or stand-level C budgets, and how that might eventually influence policy decision. This is not meant to be quantitative or prescriptive, but to demonstrate potential applications of DIRT results in a scenario in which a synthesis of biogeochemistry, forest ecology, and stand management would be useful.

3.1 EVIDENCE FOR POSITIVE PRIMING AND FOR ROOT-DOMINATED SOM

Despite 20 years of increased litter inputs, soils from Double Litter plots tended to have lower Bulk %C and %N than control soil at 0-10cm and 10-20cm. Respiration rates of DL soils were lower than control soils, and the cumulative amount C respired was also lower for DL soils than control soils. This is consistent with the effects of positive priming due to increased litter inputs.
Respiration rates of soils from either treatment without roots were lower than the respiration rates of soils from any treatment with normal root inputs. As previously stated, additional litter input also caused decreased respiration rates, but the negative effect of root removal on respiration rate was greater than that due to positive priming in the mineral soil.

These data suggest that in this soil, below-ground organic inputs have a more important role in the stabilization of SOM than above-ground litter inputs. If roots do have a large role in the stabilization of C in soils compared to litter inputs, then we would expect disproportionately large amounts of SOM to be root-like.

Neither response (priming or increased importance of roots relative to litter) is new. Fontaine et al (2003) reviewed priming literature and proposed a mechanism to explain priming based on the thermodynamics of microbial enzymes, chemistries of the new organic inputs and OM already present, and microbial community dynamics. A review by Rasse et al (2004) of evidence for a root-dominated pool of stable SOM found that the proportion of root-derived SOM compared to litter-derived SOM increases after incubations remove the most labile SOC. He suggests that roots promote OM interactions with metal oxides, and protection in microaggregates and in tiny spaces associated with mycorrhizae and root hairs, which protect it from microbial degradation.

3.2 Potential effects on C and N dynamics in managed systems

If these patterns indicate real potential responses to changes in above- and below-ground organic inputs in forest ecosystems, they would have to be acknowledged in considerations of C and N fluxes in the ecosystem. This may be
especially true in management decision-making. For example, forest management for profit often involves consistent removals of C and N in the form of live biomass, woody debris, and/or slash. These removals usually result in an environment conducive to the growth of seedlings, quickly incorporating new roots and litter into the soil as they grow (Mallik and Hu, 1997, Eriksson et al 2007).

In stands managed for harvest, the removal of organic matter is repeated periodically, and the amount removed may be more extreme than natural fluctuations in stand level C or N (as is certainly the case when harvesting). Furthermore, the selective removal of one type of detritus is possible, and sometimes desirable. While the DIRT site shares these characteristics, many observational studies follow dynamics over time after one event (i.e. clear cutting). Below, I describe some of the same silvicultural practices that I did before, focusing on how the results of this DIRT study could change my short- or long-term interpretation of harvest-related SOM dynamics.

*Harvest intensity.* Olsson et al (1996) measured C lost due to whole-tree, conventional stem, and stem only harvesting, and while they saw decreases in C with each type of harvesting, they did not find a difference in amount of C lost. However, if trends seen in the DIRT data are real, I would expect lower C stores would persist longer in plots where the whole tree was removed, due to the removal of an important promoter of C stabilization. The same study also saw increases in C in the upper 20 cm of the soil due to increases in organic depositions and migration of O-horizon materials. If this increase in SOM results in priming, as we have seen in several DIRT sites, then it would compound the loss of SOM from these sites.
**Rotation length.** The Kyoto Protocol will accept an increase in length of time between harvests (rotation length) towards mitigation of GHG emissions. Rotation length can determine many of the site properties and C fluxes. If rotation length is small, to keep growth rates near the maximum annual increment (MAI), harvests, with associated disturbances, are frequent but biomass production is high. Despite the high rates of biosynthesis with low rotation lengths, Eriksson et al (2007) found an increase in ecosystem C when rotation length was increased. Increased rotation length increased both C stored as biomass and detrital C. Incorporating C into biomass will increase both root and litter inputs. Data from the DIRT sites demonstrate the importance of roots for long-term C stability, so this practice may result in larger SOC pools in the long run. However, Eriksson et al relates increases in detrital C to increased respiration. Even if priming is short lived, it may be around for years (or decades, as in the HF DIRT plots) and represent a large loss of soil C. Rotation length was more important than harvesting intensity or slash management in determining C dynamics.

### 3.3 Conclusions

Results from the DIRT studies can be applied to a wide variety of situations, including silvicultural management. Because it is a replicated, experimental study, we are able to analyze our data for specific SOM pools, where in observational studies it is more difficult to tease out specific pools. After twenty years of manipulating root and litter inputs to the DIRT plots in Harvard Forest, MA, two trends were seen that indicated 1) positive priming may be taking place in response to doubled litter inputs, and 2) C content of at least some pools may be decreasing in response to root removal,
demonstrating the importance of roots in SOM stabilization. While these trends were not statistically significant, they were consistent with data from other DIRT plots.
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