Tracing Decay of Fine Root Carbon into Aggregate Fractions of Forest Soils

Honors Thesis

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Abstract

Soil carbon (C) makes up 50 % of terrestrial and atmospheric carbon pools globally.

We wanted to determine the fate of decomposing root C in forest soils. The three objectives of the experiment were (1) to determine the effect of different soil characteristics on root decay rates and incorporation of root C into aggregate fractions, (2) to compare decay of different root order classes, and (3) to model decay of root material over three years of decomposition.

Root fragments of *Acer saccharum* were enriched with ^{13}C isotope and incubated in PVC soil cores to measure decay and incorporation of traceable C atoms into soil aggregate structures. Five forest soils were selected from the experiment site at Cornell's Turkey Hill Tree Plantation and from the Adirondacks of northern New York. Soil samples were separated by means of physical fractionation into seven aggregate fractions, and analyzed for isotopic signals after two years of root decay.

The study suggested that higher-order roots decomposed more quickly than low-order roots, contrary to common understanding of root litter decay rates. Significant effects of soil type on decay rate or aggregate formation were not detected after three years of incubation. A comparison of these results with a study of *in situ* decomposing labeled roots indicated that incorporation of decaying root C into stable soil microaggregates was strongly suppressed when root fragments were added to soil in comparison with roots decaying *in situ*. This result has important implications for the use of root litter bags in the study of root decomposition. This research also has important implications for the understanding of global carbon dynamics, and for methodological decision making in future root decomposition studies.

Introduction

While fine-root turnover may represent one-third to one-half of total plant tissue turnover (Caldwell 1987), inadequate knowledge has been obtained about the fate of soil organic matter (SOM) (Fahey et al. 2011, Sollins et al. 1996, Heimann & Reichstein 2008). Roots contribute more to SOM than aboveground litter (Xia et al. 2015), and knowledge about the transformation of their constituent parts in the soil matrix is therefore necessary to better understand soil carbon sequestration (Mambelli et al. 2011). The rates and drivers that control whether SOM is adsorbed onto mineral particles or trapped within aggregates remain imperfectly understood (Sollins et al. 1996). The further study and subsequent modeling of root decay in forest soils is imperative given the large impact of soil-sequestered carbon on global carbon dynamics (Schmidt et al. 2011, Heimann & Reichstein 2008), and the role that soil carbon (C) modeling influences soil management practices (Dungait et al. 2012).

Forest soils are composed in part of soil aggregates, in which are a mixture of decomposing organic matter (OM), microorganisms and their excretions, and mineral particles bound together and incorporated into aggregate structures of various sizes (Tisdall & Oades 1982). Extensive research has been conducted with the goal of understanding the processes that incorporate OM into soil aggregates, many of which are discussed below (Six et al. 2004, Sollins et al. 1996, Tisdall & Oades 1982).

The current hypothesis is that carbon bound within aggregate structures is more stable than free OM in between soil aggregates. The reason for the stability could be the physical protection of OM from microbial communities, limited oxygen diffusion, regulated water flow, altered erosion scheme, and altered nutrient interactions within the soil matrix (Six et al. 2004). Oades (1984) suggests an aggregate hierarchy in which more stable microaggregates form within macroaggregates, the latter of

which are associated with higher turnover rates (Golchin et al. 1994). Likewise, free microaggregates are less stable in the soil matrix when compared to microaggregates held within macroaggregate structures (Six & Paustian 2014).

The known drivers of aggregate formation include biological activity, soil characteristics, and OM molecular composition (Sollins et al. 1996). The speed and extent of OM decomposition controls the rate of its incorporation into aggregate structures (Alago & Yilmaz 2008). In turn, the rate of root matter decay is highly reliant on the chemical properties of root structures (Goebel et al. 2011). A large proportion of fine root litter at one point passes through the microbial C pool (Mambelli et al. 2011). As soil microbes are a major participant in OM decay, biophysical inaccessibility of OM to soil biota is of the utmost importance to aggregate formation and stabilization (Dungait et al. 2012, Six et al. 1998, Sollins 1996, Fonte et al. 2006).

Organic binding agents, such as root exudates and extracellular mucilages, contribute to the superior stability of microaggregate structures. Fungal hyphae slow the rate of macroaggregate turnover via enhancement of constituent microaggregate formation (Li et al. 2015, Six 2004). The presence of living roots influences fine root decay via excretion of binding mucilage, root altered soil hydration, root penetration and root compaction of the soil (Six et al. 2004, Sollins et al. 1996). The excretion of glomalin by arbuscular mycorrhizal fungi is a significant binding agent in forest soils, though relatively little is known of glomalin structure or dynamics (Wright & Upadhyaya 1998).

Soil properties such as mineral composition, cation exchange capacity (CEC), pH, and climate also influence the binding of soil particles to a significant degree (Sollins et al. 1996, Six et al. 2004). While the structure, relative stability, and drivers of soil aggregates have been extensively studied, a quantitative look at how carbon flows from the living carbon pool into aggregate structures is still needed.

The mineral composition, organic content, pH, structure, and biotic community structure of forest soils is extremely variable even within a single forest community type. Many studies have looked into the role that various key soil properties play in the turnover/stabilization of SOM, particularly through the process of aggregate formation (Sollins et al. 1996). The interactions between OM and different clay types, soil oxides and minerals through electrostatic binding are among the most influential forces that stabilize carbon in forest soils (Six et al. 2004). Clays also interact directly with soil microbes, modify the cation exchange capacity of the soil, and alter microbial enzyme activity, implying a strong correlation between clay and SOM stabilization (Sollins et al. 1996). Oxides, specifically iron oxides, are the dominant binding agents in oxide rich soils (Six et al. 2004), though the specific effects of oxide abundance on SOM residence time have not been quantified.

Possibly the most elusive driver of soil carbon stability is the process which suspends rapid SOM turnover in the presence of other, partially decayed organic matter. Indeed, protection of carbon from decay due to aggregation is most effective in soils or soil layers with large labile SOM pools (Goebel et al. 2009). Perhaps the forces that keep organic matter in the O-horizon also act upon decomposing plant debris, to stabilize that C in the SOM pool. For this reason, the comparison of highly organic soils to mineral based samples is worthy of extended research.

Roots in the soil have been divided into two parts based on their structure and function; fine roots (< 2 mm diameter) are nonwoody, ephemeral and absorptive while coarse roots (all roots > 2 mm diameter) function for transport, anchorage, and storage (Guo et al. 2008). Differing chemical properties

of root orders are linked to root decomposition, such as C:N, lignin:N, and lignin:P ratios (Goebel et al. 2011). Microbial preference for more labile organic carbon serves as a selective force for decay of more easily utilized organic compounds in fine roots and soil, possibly causing a discrepancy in the rate of decay of different root classes, and therefore a difference in allocation of root-derived carbon to various aggregate fractions (Six et al. 1998, Sollins et al. 1996).

I selected five soil types that would be expected to exhibit notable influence on SOM properties based on the current understanding of SOM dynamics. Using stable carbon isotopes we can trace C flow from roots into different aggregate fractions of forest soils as labeled root fragments decompose. This study aims to elucidate the stabilization of root carbon in five different forest soils, which will contribute to future forest management decisions and carbon modeling concepts.

Materials & Methods

Site Description

This study was conducted at Cornell's Turkey Hill Tree Plantation in Tompkins County, NY (42.45'N, -76.42'E). The area around the site has a temperate climate with an annual rainfall of 940 mm yr^{-1} (NRCC). The native soil type of the area is Lordstown channery silt loam (Coarse-loamy, mixed, active, mesic Typic Dystrudepts) (NRCS). The experiments was performed over a three-year period between the fall of 2012 and fall of 2015, within a monoculture forest plot of mature sugar maple (*Acer saccharum*) trees.

Experimental Design

Roots of Sugar maple (*Acer saccharum*) were labeled with 45% atom-enriched $^{13}CO_2$ via fumigation of sugar maple trees in a northern hardwood forest (Horowitz et al. 2009), sufficient to enrich sugar maple roots to between 120% $\delta^{13}C$ and 240% $\delta^{13}C$. This level of enrichment appears to be enough to adequately trace ^{13}C into the large soil C pool (Fahey et al. 2011).

Roots were separated by hand into two classes. The first class was composed of 'Fine' roots with primary development: first and second order roots. The second class consisted of 'Coarse' roots and are composed of third and higher order with secondary xylem development (Figure 1).

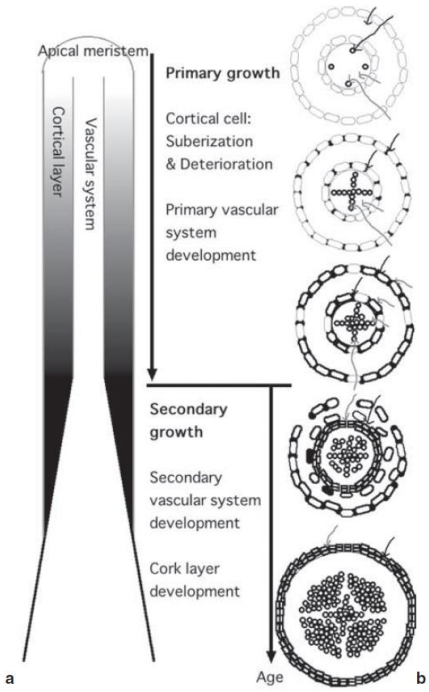

Figure 1a, b. Root development with age in *Chamaecyparis obtusa*. **a** Vascular and cortical tissues develop from the upper side to the lower side. In the longitudinal section through the root, *shading* in the cortex indicates decreasing physiological functions of cortical cells by their suberization or deterioration. *Black regions* in the cortex indicate dead tissues. **b** Tissue development in cross section and water and ion movements change with root age. *Small circles*, lignified tracheids; *large ovals*, cells of endodermis and exodermis; *small squares*, cork cells; *thick black cell walls*, lignified or suberized cell walls; *gray cell walls*, unsuberized cell walls; *black arrows*, apoplastic pathways; *gray arrows* (From Hishi 2007)

Five Soils were collected for comparison of OM decay process in this study. One soil was harvested from the actual incubation site (designated 'Turkey Hill'). The other four soils were harvested from within a ten mile radius of Old Forge NY in the Adirondack Mountains along the Big Moose River (43.71'N, -74.98'E). Two soil horizons were collected from a Typic Haplorthod, the surface organic (Oa) horizon (designated 'Organic') and the mineral Bh horizon located directly beneath the well-developed E horizon in this profile (designated 'Bhsir'). The Bhir horizon was collected from another Haplorthod developed on coarse sandy outwash supporting a mixed pine forest (designated 'FePod'). Finally, the surface mineral A horizon was collected from an Entisol whose profile was heavily mixed by invasive earthworms (designated 'HipH'); this soil developed in mixed till with inclusion of marble and hence a higher pH (5.5-6) than the other, more acidic soils (pH 4 -5).

Microcosms were constructed from segments of PVC pipe measuring approximately 25 cm in length and 5 cm diameter. Holes with a 2 cm diameter were drilled in the walls of the PVC core for the purpose of minimizing the soil moisture bias within the microcosm and to allow ingrowth of fungal

hyphae, then covered with 50μm mesh (Wallander et al. 2001). Cores were filled to a standard soil weight for each soil, and mixed with approximately 0.6 g of fine roots or 0.2 g of coarse roots, cut into at least 5mm long root segments. Each year for three years, a subset of each soil type and root type was harvested in November and refrigerated at 2 degrees Celsius, before further processing.

Soil Fractionation

The soil of each core was weighed and subjected to soil fraction and macroaggregate separation as described in Fonte et al. (2006). First, the soil samples were passed through a 2mm sieve to break up soil along natural planes of weakness. They were then submerged in deionized water and allowed to separate into light and heavy components, the float fraction and mineral component, respectively. The mineral component was then slaked through a 250μm and 53μm sieve using gentle oscillation to produce a macroaggregate (Macro) fraction (>250μm), free microaggregate (MicroA) fraction (53-250μm), and a silt and clay (called <53μm) fraction. The Macro fraction was then gently slaked with glass beads and the process repeated on the product to produce a coarse particulate organic matter (cPOM) fraction (>250μm), a microaggregate-within-macroaggregate (designated MicroB) fraction (53-250μm), and a silt and clay fraction (<53μm, called iPOM) within the macroaggregates.

Isotope Analysis

Sub-samples of each core from the 2013, 2014, and 2015 harvests were amalgamated to create a bulk sample set for isotope analysis. Sub-samples of each fraction from each core (2013 and 2014, only) were pooled by soil type and root diameter treatment. A subsample of each of these pooled samples was ground in a dental amalgamator for isotope analysis. An additional eleven individual sub-samples from the 2013 harvest make up the fraction sample set, with a total of seven fraction for each configuration.

The bulk and fractionated sample sets, as well as natural abundance (NA) samples of each soil type and fraction were analyzed for isotopic and element concentration with a Thermo Delta V isotope ratio mass spectrometer (TRMS) interfaced to a NC2500 elemental analyzer in March of 2016 by the Cornell Isotope Laboratory (COIL).

The percent of 13C isotopic label, which was added to sample cores in the labeled roots, that was remaining after incubation was calculated for the bulk sample set as

$$\%13C\ Remaining = \frac{Mass\ of\ 13C\ in\ roots - Mass\ of\ 13C\ lost}{Mass\ of\ 13C\ in\ roots} \times 100$$

where

$$Mass\ of\ 13C\ in\ substrate\ [g] = Mass\ of\ substrate\ [g] \times \%C\ of\ substrate\ \times\ Atom\%13C\ of\ substrate$$

The percent 13C recovered was calculated for each aggregate fraction (pooled and individual) as

$$\%13C\ recovered = 1 - \frac{Mass\ of\ 13C\ in\ roots\ [g] - Mass\ of\ 13C\ in\ fraction\ [g] - Mass\ of\ NA\ 13C\ in\ fraction\ [g]}{Mass\ of\ 13C\ in\ roots\ [g]} \times 100$$

where NA refers to natural abundance samples.

Statistical Analysis

Differences in %13C remaining were compared across all soil types and root treatments using a mixed model ANOVA. The model included soil type, root treatment and soil type by root treatment interactions. Differences in %13C recovery were compared independently for each soil fraction and root treatment across all soil types using one-way ANOVA. Differences in root treatment were compared independently by way of a T-test.

Additionally, average proportions of total 13C recovery were calculated for each fraction in the two-year incubation group (2014 harvest) and compared to the 13C recovery data from *in situ* fine root decomposition study conducted by Yavitt et al. (2015). This study provided soil fraction isotope data prepared using identical fractionation and isotope analysis methods, and *in situ* labeled roots from the same Acer saccharum enrichment chambers, such that the data (both having undergone two years of decomposition) are comparable, though not compatible with statistical analysis.

Results

Root order had a clear impact on the amount of carbon isotope that remained in the bulk soil samples after decomposition (Figure 2), with fine root carbon retention averaging 11.3% and coarse root carbon averaging 6.3%, indicating that coarse roots apparently decayed more quickly than fine roots. Soil type showed no significant difference on the percent of 13C that remained in the soil sample, with the exception of organic soils, which showed a significant difference from the iron podzol (Fepod) soil (Figure 3), though this is most probably a methodological artifact resulting from the high C concentration in the organic soils and its influence on the calculation of % 13C remaining.

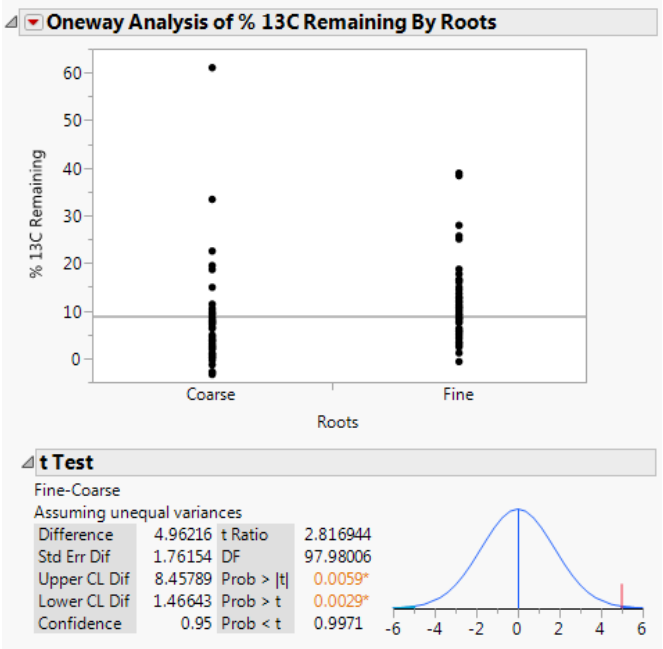

Figure 2. T test results comparing coarse and fine root type effects on bulk sample %13C remaining

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	2182.4884	242.499	3.2424
Error	99	7404.2355	74.790	Prob > F
C. Total	108	9586.7238		0.0017*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Roots	1	1	568.7880	7.6051	0.0069*
Soil	4	4	1212.6563	4.0535	0.0044*
Roots*Soil	4	4	306.3699	1.0241	0.3987

LSMeans Differences Tukey HSD

Level			Least Sq Mean
Adirondack Organic	A		13.496535
Adirondack Bhsir	A	B	11.856338
Turkey Hill	A	B	7.126787
Adirondack Hi pH	A	B	6.797784
Adirondack Fe pod		B	4.709303

Figure 3. Analysis of Variance (ANOVA) model results comparing bulk sample %13C remaining for soil type, root type, and soil*root type interaction. LSM Tukey HSD letter assignment results comparing soil type effect on bulk sample %13C loss

No significant effect of collection date was found for the three years of decomposition in the bulk samples or for the two years associated with the fractionated samples. That is, high variation among samples obscured the development of a decay curve. The amount of carbon isotope recovered in each fraction of soils harvested after one and two years of decomposition are presented in Figure 4.

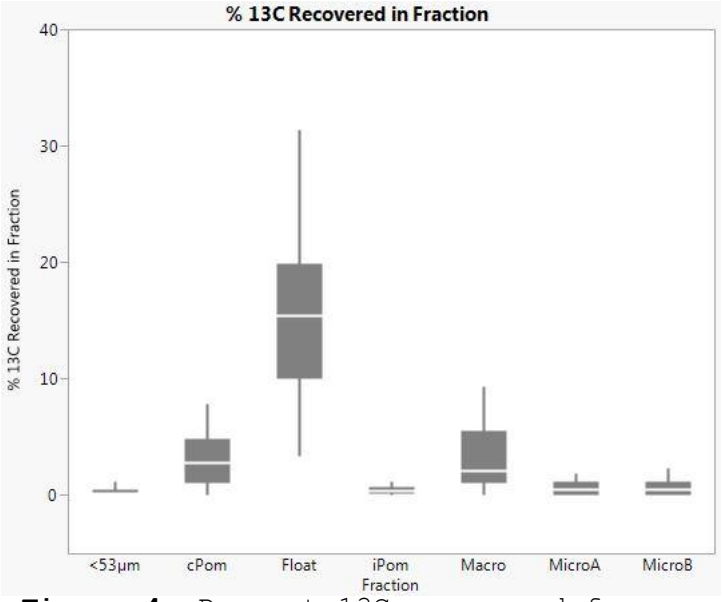

Figure 4. Percent 13C recovered from enriched root addition allocated to each aggregate fraction

As expected, the majority of isotope was retained in the light float fraction to a significant degree. It is also clear that a substantial portion of the root carbon found its way into the macroaggregate fraction, particularly in its constituent cPOM sub-fraction. The appearance of soil C in aggregate fractions (Macro, MicroA, MicroB) is represented in the mean 13C retention rates, showing that root carbon was incorporated into soil aggregate structures.

An analysis of variance (ANOVA) for each individual fraction against soil type signaled a substantially higher retention rate in the float fraction for organic soils and in the cPOM fraction for HipH soils (Figure 5). The organic soil effects were disregarded as an artifact of the proportion of total organic soil weight attributed to the float fraction, which was much higher than in any other soil.

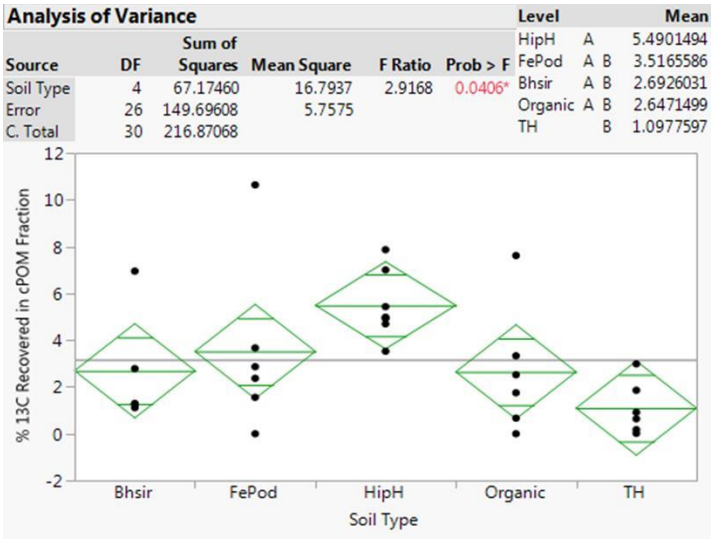

Figure 5. Analysis of Variance (ANOVA) and LSM Tukey HSD letter test results comparing soil type to %13C recovered in coarse particulate organic matter (cPOM) fraction

Discussion

Analysis of Decay Using Bulk Soil Samples

The bulk sample set recorded a significant effect of root type on retention of root derived 13C (Figure 2). In general, fine roots have higher nutrient concentrations than coarser roots, with higher N contents, and less C and lignified tissue (cellulose and hemi-cellulose) (Melillo et al. 1982). Low lignin/N and C:N ratios increase rate of earliest decomposition phase in fine roots (Hishi 2007). However, the results of bulk soil analysis found that the soils incubating fine roots retained a significantly higher percentage of 13C than coarse roots. This could be due to a common misunderstanding about the nature of fine root decomposition, notably that lignin/N ratio effects might not be fully applicable to root decomposition studies (Hishi 2007). The presence of other resistant compounds besides lignin associated with fine roots (i.e. fungal chitin, miscellaneous acid-insolubles) could inhibit decomposition (Fan & Guo 2010, Goebel et al. 2011). Meanwhile, greater carbohydrate storage in coarser roots could stimulate the decomposer community feeding there (Goebel et al. 2011). It is also possible that the

effects of laboratory root preparation and cutting prematurely exposed internal structures of coarse roots, thus escaping the limiting factor of accessibility during the decay of root tissues (Dungait et al. 2012).

There was a significant difference in the %13C remaining between the Organic soils compared to FePod and Bhsir soils. This is supported in the case of the organic soils due to the apparent stabilization of OM by the abundant presence of other OM (Melillo et al. 1982). However, the calculation of %13C remaining is highly reliant on soil weight. The organic soil is by far the lightest, while Bhsir and FePod are on average the second heaviest and heaviest soils respectively. The FePod soil was expected to be capable of stabilizing the most organic carbon, due to the abundance of highly charged iron oxides which form highly stable aggregate structures through strong mineral bonds to clay and OM (Borggaard 1983, Sollins et al. 1996, Tisdall & Oades 1982). Therefore, I believe that the findings are purely methodological.

No significant time effects were found so far, and it is possible that only the most labile root C has yet been respired or integrated into the mineral soil fraction. The decomposition measured in this study takes place between one and two years of decomposition, which accounts only for the initial burst, and structural root compounds most likely only began to break down. More incubation time will be needed for significant effects between years to become apparent.

The bulk sample root effects are suggestive but not conclusive. So far the differences are minimal but many very high values exist in the float fraction of coarse root material, suggesting that large pieces of slowly decomposed (structural) material from the coarse roots are still mostly intact, due to the presence of lignified root material associated with $3^{rd}/4^{th}$ order xylem tissues (Hishi 2007).

A likely explanation however is that the lack of decomposition of coarse root material created a bias in the subsampling technique used to analyze bulk samples. The processing and repeated subsampling involved in this analysis makes the recovery of a homogenized sample that accurately reflects the proportion of 13C in the soil volume very difficult. It is very likely that large sections of undisturbed coarse roots remained in the samples, but evaded selection before the amalgamation and further subsampling that preceded isotope analysis. This source of error might account for the variability within soil types and probably limited our ability to construct decay curves based on the bulk sample data. The more comprehensive grinding and rigorous homogenization during subsampling, along with the inclusion of more treatment replications, would most likely remedy this problem in future studies.

Root Carbon Incorporation into Aggregate Fractions

Most of the root C was recovered in the float fraction, which was expected, as any organic material that has not been sufficiently decomposed and bound to minerals would be less dense than water, and separate into the float. This fraction was also the most variable in its percentage of 13C remaining (Figure 4). Again, this may be an artifact of inadequate mixing before subsampling, as most of the potentially biasing root fragments would be found in the float fraction. The macroaggregate fraction retained the second greatest proportion of root carbon, also aligning with expectations, as natural soils typically store large proportions of soil C in aggregate structures (Six et al. 2000). Previous studies fostered the expectation that relatively equal portions of carbon would be detected in microaggregates and macroaggregates (Six et al. 2000). We found however that a larger portion of carbon was recovered in the cPOM fraction (POM held within macroaggregates) rather than the microaggregate fractions, and

indeed more macroaggregate carbon was found in the cPOM instead of the MicroB(microaggregates held within macroaggregaets), though not to a significant degree. Regardless, this represents a major deviation from my hypotheses about allocation of C into aggregate fractions.

The coarse particulate organic matter (cPOM) fraction is composed of relatively large (<250 µm) fragments of decaying root and hyphae that have been encrusted with minerals on their surfaces (Lūtzlow et al. 2006). These fragments have not been decayed into more basic compounds, apparent in the large range of C:N ratios found in cPOM fractions across soils (Lūtzlow et al. 2006), and are comparable to the constituents of the float fraction, with the exception of the mineral coating. The cPOM accounts for the temporary bonding of macroaggregates, as their surface bonds can also attach to microaggregates or other SOM (Lūtzlow et al. 2006). The major source of cPOM in forest soils is the root OM pool, as surface litter only reaches the mineral horizons as DOC (Kaiser & Kalbitz 2012). An interpretation of the abnormally high cPOM content in these forest soils is included in the next section.

Inter-Experimental Comparison

Although this study did not show definitive proof of soil effects on SOM decay, the comparison of proportional incorporation of decaying root 13C mass into separate soil fractions between this experiment and the in situ decomposition of isotope enriched root and leaf matter from Yavitt et al. (2015) yielded highly suggestive results.

The results of the two year decomposition from this study was compared to the study of *in situ* decomposition of root and leaf litter performed by Yavitt et al. (2015). The proportion of total 13C measured for the light fraction, mineral fractions, and in-macroaggregate fractions are recorded in table 1 below.

	Leaf Litter	***In situ***	**Core Incubation**
Of Total:			
Recovered in Float Fractions	20.0%	70.5%	84.5%
Recovered in Mineral Fractions	80.0%	29.5%	15.5%
Of Mineral Fraction:			
Recovered in Macro Aggregate	62.3%	55.6%	69.2%
Recovered in Micro Aggregate	23.8%	38.6%	18.8%
Recovered in Silt & Clay	14.0%	5.4%	12.0%
Of Macroaggregates:			
Recovered in cPOM	10.5%	26.2%	86.6%
Recovered in Micro Aggregate	53.9%	60.0%	5.7%
Recovered in Silt & Clay	35.6%	13.9%	13.7%

Table 1. Proportional distribution of carbon isotope through aggregate fractions compared between core incubation method, *in situ* decomposition, and leaf litter decomposition (from Yavitt et al. 2015)

The proportional distribution of C through the various fractions is comparable between the two root studies with the exception of the macroaggregate C, which deviated between studies (Figure 6). Likewise, *in situ* leaf litter and root litter were comparable with the exception of the silt and clay

fractions, which were favored by leaf litter derived C,and the float fraction, which was a larger pool for root C than leaf C (Figure 6).

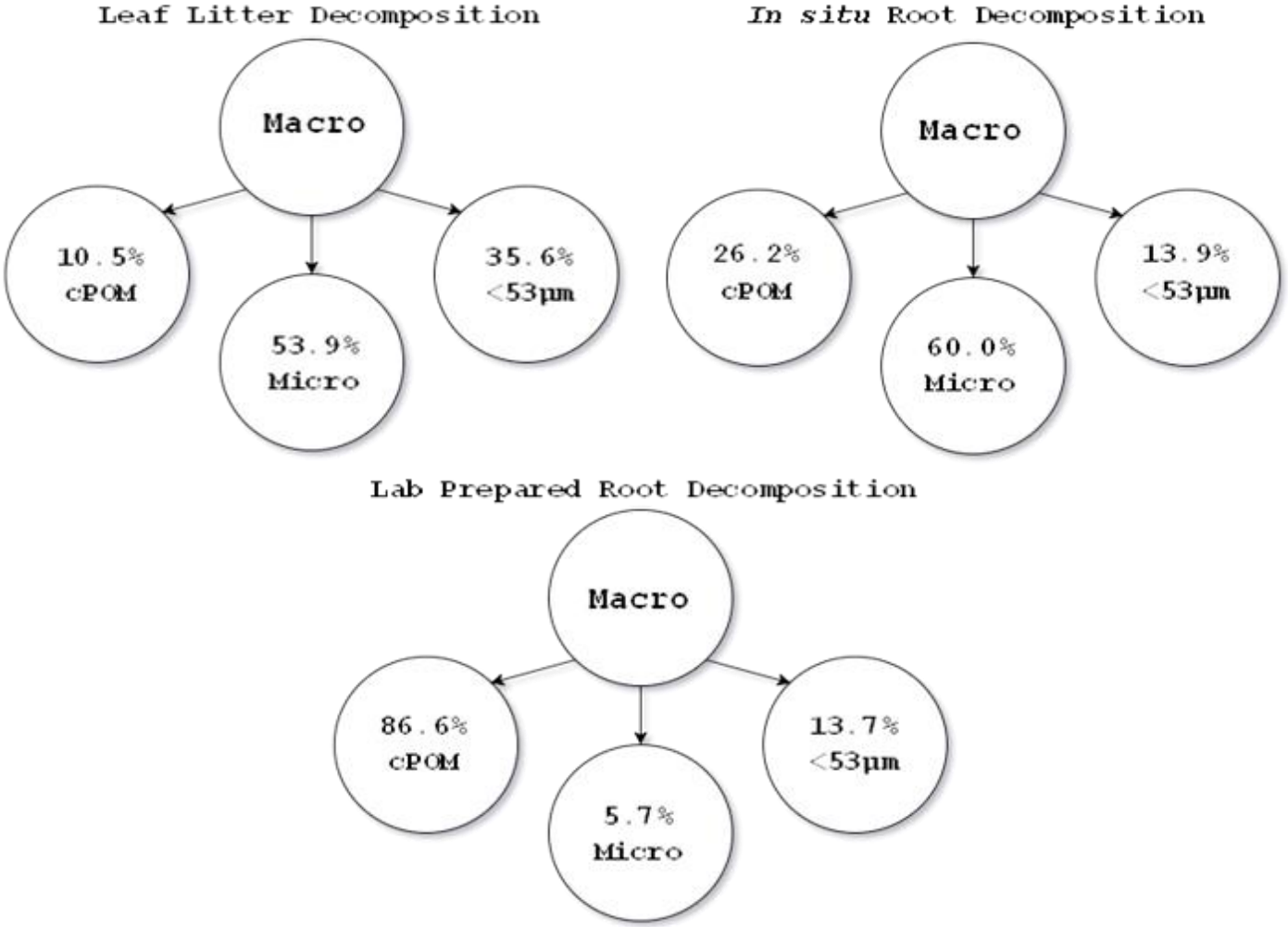

Figure 6. Proportional distribution of carbon isotope in components of macroaggregate fraction (cPOM, Microaggregates, <53µm silt & clay) compared between core root incubation method, *in situ* root decomposition, and leaf litter decomposition (from Yavitt & Fahey 2015)

Though the amount of carbon respired via microbial respiration cannot be estimated using this method, the assimilation of substrate carbon into the float fraction provides some indication of initial decomposition from low density plant matter to its organic constituent parts thereafter dissolved and transported into the mineral layer of the soil. This is apparent in the leaf litter decomposition, where only 20.0% of the recoverable 13C was found in the light fraction, compared to 70.5% and 84.5% for the incubation and *in situ* studies respectively (Table 1). This initial difference aligns with the expected relative decay rates between deciduous, labile leaf tissues and more permanent, structural root tissues (Silver & Miya 2001). The silt and clay fraction (<53µm) of the leaf litter soil held 35.6% of the samples'

aggregate carbon, compared to the average 13.8% distributed to this fraction during fine root decomposition. This result is explained by the fact that leaf litter can only penetrate into the mineral soil once dissolved in water as dissolved organic carbon (DOC), after initial decay at the surface into basic compounds (Kaiser & Kalbitz 2012, Xia et al. 2015). This is supported by the fact that most litter originated C associated with minerals has cycled through a microbial phase (Kaiser & Kalbitz 2012). DOC percolates downward to where it can be adsorbed onto clay and silt particles, as opposed to root matter, already present in the mineral soil, which can contribute larger fragments of OM to the formation of macroaggregates as cPOM (Smemo et al. 2006, Kaiser & Kalbitz 2012). In the mineral soil, most DOC is retained via mineral associations, and incorporated into aggregate structures as observed by Yavitt et al. (2015).

The most prominent effects of core incubation on fine-root decay occurred within the macroaggregate fraction, where the proportion of 13C incorporated into the coarse particulate organic matter (cPOM) and microaggregate-within-macroaggregate (MicroB) varied substantially from the *in situ* decomposition study. Most of the carbon from *in situ* decomposition of both litter and roots became stabilized in the microaggregate fraction, while in the present study almost none (5.7%) was retained in this fraction. In fact, the majority of aggregate carbon from in situ root decomposition was sequestered within microaggregates (60.0%).

This observation illustrates the effects of the soil biological community on the formation of stable microaggregate structures. The preparation of fine roots for field experimentation often involves removing them from the mycorrhizosphere environment, which includes separation from mycorrhizal fungi, excreted root exudates, and soil fauna such as isopods, nematodes, and mites (Dornbush et al. 2002, Sollins et al. 1996, Bardgett et al. 2014). The growth of fungi in the rhizosphere is stimulated by the growth of roots (Bardgett et al. 2014) and many are capable of root decomposition after root tissues have died (Trojanowski et al. 1984).

Soil aggregation and carbon stabilizing processes are driven in part by the excretion of extracellular root exudates containing polysaccharide rich mucilages, proteins, and glomalin that act as glue holding together OM and mineral particles (Sollins et al. 1996, Bardgett et al. 2014). This flocculation of SOM and mineral colloids is also influenced by physical enmeshment by fungal hyphae as well as binding agents in fauna derived fecal pellets (Bardgett et al. 2014, Sollins et al. 1996). Mucilage bound microaggregates contain some of the most stable sequestered C in soil (Tisdall & Oades 1982).

Many decay experiments involve live or dead and dried root segments, but omission of the often disregarded 'dying phase' of plant roots ignores important chemical and physical effects on tissues that occur before the they are fully part of the SOM pool (Dornbush et al. 2002). Previous experimentation has suggested that preexisting decomposer communities act immediately upon fine roots once the opportunity arises (Dornbush et al. 2002, Li et al. 2015).

The confounding effects of pretreatment root preparation on decomposition are further exacerbated by the disruption of root spatial structure in the rhizosphere. Root length, density, and depth contribute significantly soil carbon flows (Bardgett et al. 2014), but the distribution of fine roots in the soil can hardly be replicated artificially. Dornbush et al. (2002) also calls our attention to an "undeniable bias" towards larger than average roots when selecting samples based on root order or size class.

In situ decomposition could keep intact soil community associations, natural root structures, and rhizosphere chemistry removing the confounding effects of root pretreatment before decay (Dornbush et al. 2002, Fahey 1992).

The stabilizing effects of rhizosphere associations were diminished in the present core incubation study, as 86.6% of aggregate C was apportioned to the cPOM fraction. The cPOM, composed of relatively large intact root fragments encrusted with mineral particles, results in part from the comminution of organic matter by soil arthropods (Culliney 2013), rather than surface area limited decomposing microbes.

The stable microaggregate fraction of the *in situ* root decay however aligns more with the *in situ* leaf litter decay than with the core-incubated root soils. This illustrates the natural tendency of natural decomposition to form microaggregates from SOM independent of substrate, when allowed to decompose naturally *in situ*. It is also apparent that the primary source of cPOM in forest soils is root matter, which is stabilized in macroaggregates before it has the chance to be fully decomposed (Tisdall & Oades 1982).

With the cPOM fraction coming to light as one of the more vital pieces of this comparison, further speculation is warranted on the significant difference between the %13C cPOM recovery between the HipH and TH soils. The most defining characteristic of the HipH soil is its net negative charge common to more alkaline soils such as that used in this study, which originated in part from weathered marble. Past studies have attributed high soil pH to both the stabilization and destabilization of aggregates through alteration of the soil CEC and influence on soil microbe and enzyme activity (Sollins et al. 1996, Haynes & Naidu 1998)

In the case of the TH soils, which allocated significantly lower C to the cPOM fraction, an explanation may lie with the site effects of the research area. If the major differences between in situ and laboratory prepared root substrate lies with removal of microbe and hyphae associations (Dornbush et al. 2002), it stands to reason that these associations would be most adequately restored within soils that originated from the surrounding soil matrix during incubation. It is difficult for microbes to survive introduction into a soil habitat that differs in mineral and chemical composition from the initial soil matrix (Van Elsas & Heijnen 1990).

This theory warrants additional study into the effect of local soil environment on core incubation methods, preferably in a variety of soil types, as to fully investigate the prospect that this is not a characteristic of Turkey Hill soils in particular.

Conclusions

The findings of this study support the preexisting criticism that pretreatment lab preparation of root samples inherently skews the estimation of decay in an undisturbed setting (Dornbush et al. 2002, Fahey & Arthur 1994, Li et al. 2015). It is clear that a reassessment of the accuracy of root decay study methodology is called for. The results of this study confirm the emerging concept that environmental conditions play a more deciding role in litter decomposition than does substrate chemistry alone (Schmidt et al. 2011, Dungait et al. 2012, Santos et al. 2015). As carbon-driven processes such as global climate change become more of a central issue in the decision making process behind soil management practices, the clear and accurate understanding of SOM dynamics becomes of greater and greater importance. The comparison here between lab prepared and *in situ* root decay highlights the profound

effects that oversight during experimental design can have when attempting to estimate root decay rates and OM turnover.

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References

Alagöz, Zeki and Yilmaz E. "Effects of different sources of organic matter on soil aggregate formation and stability: A laboratory study on a Lithic Rhodoxeralf from Turkey." *Soil and Tillage Research* 103.2 (2009): 419-424.

Bardgett, Richard D., Mommer L., and De Vries F.T. "Going underground: root traits as drivers of ecosystem processes." *Trends in Ecology & Evolution* 29.12 (2014): 692-699.

Borggaard, Ole K. "Iron oxides in relation to aggregation of soil particles." *Trends in Ecology & Evolution* 33.3 (1983): 257-260.

Caldwell, M.M. "Plant architecture and resource competition." *Ecological Studies* 61 (1987): 164-179.

Culliney, Thomas W. "Role of arthropods in maintaining soil fertility." *Agriculture* 3.4 (2013): 629-659.

Dornbush, Mathew E., Isenhart T.M., and Rai J.W. "Quantifying fine-root decomposition: an alternative to buried litterbags." *Ecology* 83.11 (2002): 2985-2990.

Dungait, Jennifer A., Hopkins D.W., Gregory A.S., and Whitmore A.P. "Soil organic matter turnover is governed by accessibility not recalcitrance." *Global Change Biology* 18 (2012): 1781-1796.

Fan, Pingping and Guo D. "Slow decomposition of lower order roots: a key mechanism of root carbon and nutrient retention in the soil." *Oecologia* 163.2 (2010): 509-515.

Fahey, Timothy J. "Mycorrhizae and forest ecosystems." *Mycorrhiza* 1.2 (1992): 83-89.

Fahey, Timothy J. and Arthur M.A. "Further studies of root decomposition following harvest of a northern hardwoods forest." *Forest Science* 40.4 (1994): 618-629.

Fahey, Timothy J., Yavitt J.B., Sherman R.E., Groffman P.M., Fisk M.C., and Maerz J.C. "Transport of carbon and nitrogen between litter and soil organic matter in a northern hardwood forest." *Ecosystems* 14 (2011): 326-340.

Fonte, Steven J., Kong A.Y.Y., vanKessel C., Hendrix P.F., and Six J. "Influence of earthworm activity on aggregate-associated carbon and nitrogen dynamics differs with agroecosystem management." *Soil Biology and Biochemistry* 39 (2006): 1014-1022.

Goebel, Marc, Hobbie S.E., Bulaj B., Zadworny M., Archibald D.D., Oleksyn J., Reich P.B., and Eissenstat D.M. "Decomposition of the finest root branching orders: linking belowground dynamics to fine-root function and structure." *Ecological Monographs* 81.1 (2011): 89-102.

Goebel, Marc, Woche S., and Bachmann J. "Do soil aggregates really protect encapsulated organic matter against microbial decomposition? ." *Biologia* 64.3 (2009): 443-448.

Golchin, Amhed, Oades J.M., Skjemstad J.O., and Clark P. "Soil structure and carbon cycling." *Soil Research* 32.5 (1994): 1043-1068.

Guo, Dali, Xia M., Wei X., Chang W., Liu Y., and Wang Z. "Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species." *New Phytologist* 180.3 (2008): 673-683.

Haynes, Richard J. and Naidu R. "Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review." *Nutrient Cycling in Agroecosystems* 51.2 (1998): 123-137.

Heimann, Martin and Reichstein M. "Terrestrial ecosystem carbon dynamics and climate feedbacks." *Nature* 451.7176 (2008): 289-292.

Hishi, Takuo "Heterogeneity of individual roots within the fine root architecture: causal links between physiological and ecosystem functions." *Journal of Forest Research* 12.2 (2007): 126-133.

Horowitz, Michael E., Fahey T.J., Yavitt J.B., Feldpausch T.R., and Sherman R.E. "Patterns of late-season photosynthate movement in sugar maple (Acer saccharum Marsh) saplings." *Canadian Journal of Forest Research* 39 (2009): 2294-2298.

Kaiser, Klaus and Kalbitz, K. "Cycling downwards - dissolved organic matter in soils." *Soil Biology and Biochemistry* 52 (2012): 29-32.

Li, Ang, Fahey T.J., Pawlowska T.E., Fisk, M.C., and Burtis, J. "Fine root decomposition, nutrient mobilization and fungal communities in a pine forest ecosystem." *Soil Biology and Biochemistry* 83 (2015): 76-83.

Lūtzlow, Margit V., Kogel-Knabner I., Ekscmitt K., Matzner E., Guggenburger G., Marschner B., and Flessa H. "Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review." *European Journal of Soil Science* 57 (2006): 426-445.

Mambelli, Stefania, Bird J.A., Gleixner G., Dawson T.E., and Torn M.S. "Relative contribution of foliar and fine root pine litter to the molecular composition of soil organic matter after in situ degradation." *Organic Geochemistry* 42 (2011): 1099-1108.

Melillo, Jerry M., Aber J.D., and Muratore J.F. "Nitrogen and lignin control of hardwood leaf litter decomposition dynamics." *Ecology* 63.3 (1982): 621-626.

Oades, James M. "Soil organic matter and structural stability: mechanisms and implications for management." *Plant and soil* 76.1-3 (1984): 319-337.

Online Soil Survey, Natural Resources Conservation Service (NRCS), United States Department of Agriculture. Web Soil Survey. Available online at http://websoilsurvey.nrcs.usda.gov/. Accessed Mar 20, 2016.

Santos, Fernanda, Nadelhoffer K., and Bird J.A. "Rapid fine root C and N mineralization in a northern temperate forest soil." *Biogeochemistry,* Online (2015): 1-14.

Schmidt, Michael W.I., Torn M.S., Abiven S., Dittmar T., Guggenberger G., Jansens I.A., Kleber M., Kogel-Knabner I., Lehmann J., Manning D.A.C., Nannipieri P., Rasse D.P., Weiner S., and Trumbore S.E. "Persistence of soil organic matter as an ecosystem property." *Nature* 478 (2011): 49-56.

Silver, Whendee L. and Miya R.K. "Global patterns in root decomposition: comparisons of climate and litter quality effects." *Oecologia* 129.3 (2001): 407-419.

Six, Johan, Paustian K., and Doran J.W. "Aggregation and soil organic matter accumulation in cultivated and native grassland soils." *Soil Science Society of America Journal* 62.5 (1998): 1367-1377.

Six, Johan, Paustian K., Elliott E.T., and Combrink C. "Soil Structure and Organic Matter: I. Distribution of and Aggregate-Associated Carbon Aggregate-Size Classes." *Soil Science Society of America Journal* 64 (2000): 681-689.

Six, Johan, Bossuyt H., Degryze S., and Denef K. "A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics." *Soil and Tillage Research* 79.1 (2004): 7-31.

Six, Johan and Paustian K. "Aggregate-associated soil organic matter as an ecosystem property and a measurement tool." *Soil Biology and Biochemistry* 68 (2014): A4-A9.

Smemo, Kurt A., Zak D.R., and Pregitzer K.S. "Chronic experimental NO3 deposition reduces the retention of leaf litter DOC in a northern hardwood forest soil." *Soil Biology & Biochemistry* 38 (2006): 1340-1347.

Sollins, Phillip, Homann P., and Caldwell B.A. "Stabilization and destabilization of soil organic matter:

mechanisms and controls." *Geoderma* 74.1 (1996): 65-105.
The Ithaca Climate Page. Northeast Regional Climate Center (NRCC) at Cornell, 2016. Web. Mar. 2016.
Tisdall, Judith M. and Oades J.M. "Organic matter and water-stable aggregates in soils." *Journal of soil science* 33.2 (1982): 141-163.
Trojanowski, Jerzy, Haider K., and Hüttermann A. "Decomposition of 14C-labelled lignin, holocellulose and lignocellulose by mycorrhizal fungi." *Archives of Microbiology* 139 (1984): 2-3.
Van Elsas, Jan D. and Heijnen C. E. "Methods for the introduction of bacteria into soil: a review." *Biology and Fertility of Soils* 10.2 (1990): 127-133.
Wallander, Hakan, Nilsson L.A., Hagerberg D., and Bååth E. "Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field." *New Phytologist* 151 (2001): 753-760.
Wright, Sarah F. and Upadhyaya A. "A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi." *Plant and Soil* 198 (1998): 97-107.
Xia, Mengxue, Talhelm A.F., and Pregitzer K.S. "Fine roots are the dominant source of recalcitrant plant litter in sugar maple-dominated northern hardwood forests." *New Phytologist* 208.3 (2015): 715-726.
Yavitt, Joseph B., Fahey T.J., and Sherman R.E. "Lumbricid earthworm effects on incorporation of root and leaf litter into aggregates in a forest soil, New York State." *Biogeochemistry* 125.2 (2015): 261-273