AN ABSTRACT OF THE DISSERTATION OF

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Title: Detrital Influences on Soil Carbon Stabilization in Wet Temperate Forest Soils

Abstract approved: _____

Kate Lajtha

Soils, with their potential to store and stabilize carbon (C), are an essential resource for sustaining forest productivity, as well as for efforts to reduce atmospheric C concentrations. Protecting existing soil C and harnessing the sequestration potential of our soils require an improved understanding of the processes through which soil organic matter accumulates in natural systems. Currently, competing hypotheses exist regarding the dominant mechanisms for soil C stabilization. Many of our long-standing hypotheses revolve around an assumed positive relationship between the quantity of organic inputs and soil C accumulation, while more recent hypotheses have shifted attention towards the more complex controls of microbial processing and organo-mineral complexations. The Detrital Input and Removal Treatment (DIRT) experiment allows us to test these competing hypotheses through direct field manipulations of above and belowground detrital inputs. The following dissertation presents findings of soil C response to twenty years of detrital manipulations in the H.J. Andrews Experimental Forest, located in the Cascade Mountains of the Pacific Northwest U.S. Annual additions of low quality (high C:N) wood litter to the soil surface led to an apparent positive, yet highly variable change

in accumulation of soil C, while the separate, equal magnitude addition of higher quality (low C:N) needle litter had no significant effect on soil C over the twenty year study period. Additions of low quality (high C:N) wood litter led to a substantial accumulation of light fraction soil C. The observed contrast in soil C response between these two addition treatments demonstrates the role of litter quality in regards to soil C accumulation and suggests quality may be of greater relevance than litter quantity in certain forest environments. The detrital input reduction treatments, including the removal of belowground root inputs and the aboveground removal of surface litter, had no effect on bulk soil C concentrations over the twenty year study period. Further, the combined removal of both above and belowground organic matter inputs also had no effect on soil C, yet did show greater potential for driving soil C loss than the individual reduction treatments. Following the removal of live roots, concentrations of heavy fraction soil C increased, potentially due to reduced priming activity associated with the decline of the rhizosphere community. Above and belowground detrital reduction treatments caused a decline in soil solution DOC at 30 cm. The addition of wood debris led to a sharp increase in 30 cm soil DOC, while in contrast, the addition of needle litter led to a decline in DOC at the same soil depth, likely due to microbial priming. These diverse findings strongly support modern soil C stabilization hypotheses which emphasize the strength of control microbial activity and mineral stabilization have on soil C accumulation. The insights gained from these studies provide important avenues for improved land management practices to promote soil C accumulation, as well as an improved mechanistic understanding required for advances in earth system models.

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Detrital Influences on Soil Carbon Stabilization in Wet Temperate Forest Soils

by
Derek N. Pierson

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APPROVED:

Major Professor, representing Soil Science

Head of the Department of Crop and Soil Science

Dean of the Graduate School

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

Derek N. Pierson, Author

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CONTRIBUTION OF AUTHORS

Lucas Evans, Hayley Peter-Contesse and Kamron Kayhani contributed to data collection in the field and laboratory for Chapters 1-3. Hayley Peter-Contesse contributed the respiration data analysis for Chapter 1. Dr. Lajtha advised on the outline for Chapters 1-2. Lucas Evans and Dr. Lajtha assisted with the data analysis and writing of Chapter 3.

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DEDICATION

Above all, I dedicate this dissertation to my wife, Danielle. Her unwavering support kept me going when I was filled with doubt, kept me calm when student life was stressful, and most of all kept me focused on achieving my dreams. I also dedicate this dissertation to my parents, Fred and Sandra Pierson, for instilling in a me a passion for learning and exploration. For my two boys, Taylor and Thomas, thank you for your patience as I worked long hours and late nights. You inspire me each and every day. I hope this work sets an example for you that you can do anything you set your mind to.

INTRODUCTION

Soil organic matter (SOM) is a critical component of life on earth. SOM regulates terrestrial and aquatic ecosystem productivity, global crop production, clean water supplies, erosion and the global carbon (C) balance dictating earth's climate (Coleman et al. 2017). While the wide-ranging importance of SOM has been known for millennia, we have yet to fully understand the fundamental drivers and controls which lead to SOM formation. Organic material enters the soil matrix primarily as detritus in the form of either surface litter, such as leaves and woody debris, or as sub-surface organic constituents originating from plant roots. Decomposition of this detritus and the subsequent formation of SOM is carried out by the microbial community within the soil (Paul 2016). Depending on the structure and elemental composition ("quality") of the organic substrate, as well as the processing capabilities of the decomposer community, corresponding rates of SOM formation and stabilized C may differ substantially (Bird et al. 2011; Mambelli et al. 2011). The biogeochemical pathways that determine the proportions of carbon and nutrients that are retained in the soil, as opposed to being lost through respiration or leaching, remain poorly understood and are the subject of ongoing debate. Improving our knowledge of the mechanisms and relationships between detrital inputs, microbial communities and soil C stabilization remains of paramount importance as we look towards soils to supply future generations with food and clean water, as well as to develop viable approaches for mitigating further soil C emissions and associated effects on earth's climate (Jackson et al. 2017).

The biogeochemical pathways between organic matter inputs and soil organic matter turnover and stabilization are poorly understood. Improving our knowledge of these complex processes remains fundamental for the long-term management of soil carbon and nutrients, as well as for furthering important process-based models of global carbon dynamics. The multi-decadal Detrital Input and Removal Treatment (DIRT) experiment at the H.J. Andrews Experimental Forest provides a unique opportunity for detailed analysis to examine the long-term, in situ biogeochemical response to altered detrital input quantity and quality. Recent studies have highlighted the importance of such research to further elucidate microbial processes and the specific molecular signatures associated with SOM decomposition dynamics (Cotrufo et al. 2013). Induced changes to the biomolecular nature of soil are directly relevant to the regulation of soil carbon stocks, as microbial processes and products have direct interrelationships with the status of carbon stabilizing organo-mineral complexations (Newcomb et al. 2017). By furthering our understanding of the biomolecular response to shifting detrital input dynamics across forest systems, we will gain insights to better manage and predict effects on soil C resulting from disturbance, climate or management led changes in forest environments.

The Detrital Input and Removal Treatment (DIRT) project is a long-term experiment that manipulates litter inputs to large plots in forested areas (Lajtha et al. 2018). These manipulations include replicated treatment plots with removal and doubling of aboveground litter, removal of fine roots, removal of all organic inputs, addition of

woody detritus, and removal of the uppermost mineral soil horizon. This experiment is designed to address such questions as: what controls the long-term storage of C in soil organic matter (SOM)? Does the chemical quality of detrital inputs affect SOM stability? What chemical and physical fractions of SOM are the most stable C pools? Can added inputs of detritus increase soil C storage, or is maximum soil C storage determined exclusively by climate and soil mineralogy? What is the role of priming (both positive and negative) in controlling C stabilization in soils? What is the relative role of shoot vs. root inputs to SOM formation? Clearly the answers to these questions will be dynamic through time, taking decades to reach stable states, if ever. By following changes in C sequestration into different soil C pools across the DIRT experiment, we will follow the time course and mechanisms of C stabilization and C loss in forested ecosystems.

CHAPTER 1: Competing processes drive the resistance of soil carbon to alterations in organic inputs

Introduction

Soils represent the most significant long-term organic carbon (C) reservoir in terrestrial ecosystems, and forest soils account for almost 40% of soil C stored globally (Janzen et al., 2004). As we face potentially rapid changes in forest environments from increasing disturbances and shifting climates (Kirilenko and Sedjo, 2007; Morris, 2010), the response of forest soil C stocks remains a critical unknown in our efforts to predict future effects on atmospheric C concentrations, forest ecosystems and associated natural resources (Ziegler et al., 2017). In recent years, considerable knowledge gains have been made regarding how specific, individual environmental conditions and soil properties may affect soil C stabilization processes. Yet, we continue to lack a robust understanding of how these unique biogeochemical influences may interact in natural environments to regulate the turnover and stabilization of soil C (e.g. Campos et al., 2017; Chen et al., 2017; Chen et al., 2018; Harden et al., 2018). Numerous questions remain regarding how combinations of organic matter input quantity and quality, climate, soil mineralogy and microbial community properties coalesce to control soil C stocks (Davidson et al., 2000; Crow et al., 2009; Cotrufo et al., 2013; Fekete et al., 2014; Lajtha et al., 2014a). Further, we have yet to broadly determine where common relationships in soil C processing factors exist across unique forest types, versus areas where greater knowledge of site-specific factors and relationships will be necessary to unravel and predict soil C

responses. To answer these questions and improve our ecosystem scale understanding of soil C dynamics, we require further direct studies of soil C stabilization and sensitivities in diverse natural forest environments (Davidson and Janssens, 2006).

Forest environments provide opportunity to mitigate rising atmospheric C concentrations through altered land use and management practices that promote C sequestration (Griscom et al. 2017). Studies aimed at increasing forest C sequestration have largely focused on vegetation, delving into the potential avenues for increasing forest productivity and subsequently storing greater amounts of C in plant biomass (McKinley et al., 2011; Pan et al., 2011; Post et al., 2012). However, the associated effects on soil C stocks have not been as well characterized. As increases in forest productivity will drive subsequent increases in detrital inputs to forest soils, resulting changes in soil C may serve to either aid or potentially hinder total C sequestration gains. Assumptions of a positive linear relationship between detrital input quantity and soil C have long been prevalent and remain commonplace in popular ecosystem models of C cycling (Liski et al., 2002; Gottschalk et al., 2012). However, recent studies have demonstrated such a relationship may not be universal across forest ecosystems (Lajtha et al., 2018). Priming effects, where the addition of fresh organic material promotes microbial activity resulting in the destabilization of previously stored soil C, offers a potential explanation for a reduced or negative soil C response to increases in detrital input quantity (Fontaine et al., 2004; Kuzyakov, 2010; de Graaff et al., 2014; Sulman et al., 2014; Cardinael et al., 2015; Georgiou et al., 2015; Keiluweit et al., 2015; Kuzyakov and Blagodatskaya, 2015; Finley

et al., 2018; Jackson et al., 2019). However, the longevity and the potential magnitude of priming effects on forest soil C stocks is less well characterized. Priming effects are likely to have disproportionate impacts on soil C stabilization across disparate environments, as litter quantity, SOM decomposition rates and soil C stocks vary substantially across forest soils. Increases in soil C may also be limited by the capacity of a given soil matrix to retain and stabilize SOC, as differing soil types may provide a greater abundance of reactive mineral surfaces allowing for organo-mineral complexations (Stewart et al., 2008; Chung et al., 2010; Beare et al., 2014; Mayzelle et al., 2014; Castellano et al., 2015). In our efforts to identify future environments and practices that provide opportunity to improve C sequestration in forest systems, it remains essential to enhance our understanding of both generalizable and site-specific factors that regulate soil C stabilization.

Recent studies of soil C stabilization have placed renewed emphasis on the role of detrital input quality, which may be defined by the elemental ratio between C and limiting nutrients in an organic compound and the molecular complexity of the organic material. The quality of organic matter inputs has been widely hypothesized to influence soil C accumulation and stabilization by altering microbial activity, processing pathways and carbon use efficiencies (Cotrufo et al., 2013). Increases in carbon use efficiency and microbial products may increase as substrate quality approaches the elemental composition of microbial biomass due to reduced limitation on the nutrients required for microbial processes are more readily available. Analyses of the molecular nature of

stabilized SOC shows a close resemblance between microbial products and the organic material bound to mineral matrices (Amelung et al., 2008; Miltner et al., 2012; Gleixner, 2013; Kallenbach et al., 2016). As leaf or needle litter is often of a much higher quality (low C:N) than sources of woody debris (high C:N and higher lignin:N), we would expect a greater proportion of needle litter C to be stabilized in the soil relative to the C from woody debris. While root material and exudates are often of high molecular quality, their location in the mineral soil provides further advantage for subsequent stabilization of SOC. Linkages between root mass and SOC have been broadly reported across forest and agricultural soils (Rasse et al., 2005; Kätterer et al., 2011; Melillo et al. 2011). However, few studies have examined the potential difference in the relationship between roots and soil C accumulation versus soil C stabilization. Further understanding of how differences in the source and quality of detrital material influence microbial decomposition pathways and the potential for soil C stabilization is critical as we seek to resolve how changes in forest environments may alter soil C stocks.

The pathways and controls dictating processes for soil C stabilization may differ from those that govern soil C destabilization (Bailey et al., 2019). Consequently, reversing or limiting the processes and conditions found to give rise to gains in soil C, may not inherently lead to a loss of soil C. This concept is of paramount importance when studying processes of mineral stabilization, as the conditions that give rise to an organo-mineral interaction may be vastly different from those required to allow for cleavage of organic molecules from mineral surfaces. Similar to our uncertainty regarding how

detrital additions may increase soil C, we also continue to lack a clear understanding of the timescale and potential influences detrital quantity and quality may have on soil C destabilization (Sollins et al., 1996)

The Detrital Input and Removal Treatment (DIRT) Network was established to study long-term effects of altered above and belowground detrital inputs on soil C in natural forest environments (Nadelhoffer et al., 2004; Lajtha et al., 2018). This study presents findings following twenty years of a DIRT experiment in the wet temperate forest of the H.J. Andrews Experimental Forest (HJAF). The HJAF DIRT experiment is unique among sites in the DIRT network, as it is the first conifer forest site to study detrital effects on soil C over two decades. The HJAF DIRT is also the most productive forest type in the network, with the greatest stocks of soil C. As such, we expect soil C responses to the detrital treatments at HJAF to differ from those previously observed at other sites in the network. Further, the HJAF DIRT experiment is the first in the DIRT network to study the effects of detrital surface additions with differing quality, using two separate treatments that increase either the surface input of needle litter, or wood debris. Previous studies of detrital material at the HJAF DIRT study site have shown that the C:N content of the needle litter is ~8 times lower than the Douglas-fir wood material, both when fresh and after many years of decomposition (Yano et al. 2005). Molecular composition differences are also abundant between the two litter materials, as Douglas-fir wood contains ~3 times more lignin and cellulose than needle litter, while the needle litter has a greater concentration of tannins that may slow protein degradation (Horner et

al. 1987; Means and Cromack Jr., 1992; Valachovic et al. 2004). Phenolic and high molecular complexity compounds such as lignin and cellulose have been widely recognized for longer decomposition timescales relative to more simple and nutrient rich litter material (Talbot et al. 2012; Talbot and Treseder, 2012; Preston et al. 2009). Our intent in using different forms of litter for the addition treatments in the HJAF DIRT experiment is to observe how the substantial contrasting qualities of these two common detrital materials affect soil C. Such novel information will help to inform further studies of quality influences on soil C stabilization and improve modelling of detrital input effects on SOM decomposition pathways and timescales.

The objectives of the following study were to determine the effect of sustained detrital additions of differing quality on soil C accumulation, as well as the sensitivity of soil C to reductions in organic substrate inputs from above and belowground sources. We hypothesized that: 1) High quality needle litter additions would lead to greater increases in mineral soil C relative to the low quality wood debris additions. However, the additions of wood debris would result in larger increases of organic material in the organic soil horizon due to slow decomposition rates; 2)Root exclusions would cause greater declines in soil C than the reduction in aboveground inputs; 3) Exclusion of both above and belowground detrital inputs to soils would result in a far greater loss of soil C relative to soils subject to either root or aboveground litter exclusion.

Methods

The detrital input and removal treatment (DIRT) experiment was established at the H.J. Andrews Experimental Forest in the western Cascade Mountain region of Oregon, USA (44º15' N, 12º10' W). The climate is quasi-Mediterranean, with warm, dry summers and cool, wet winters, and a majority of annual precipitation as a rain-snow mix between the months of December - April. Mean annual precipitation at the site is 2080 mm yr-1 and the mean annual temperatures is 9.4 C (average from years 1999-2014). According to the USDA soil classification system (Soil Survey Staff, 1999) the underlying soils at the DIRT experiment site are a mix of coarse loamy mixed mesic Typic Hapludands (Lajtha et al. 2005) and Andic Dystrudepts. The study site lies at an elevation of 726 m with a south facing aspect. Slopes < 5% are consistent across the site. Erosion and overland flow are minimal, largely impeded by the gentle slopes and a thick (4-8 cm) organic soil horizon. Dominant overstory is mixed old-growth Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*), with a smaller mix of western redcedar (*Thuja plicata*), vine maple (*Acer circinatum*) and bigleaf maple (*Acer macrophyllum*). Large amounts of woody debris, overturned stumps and fallen logs are strewn across the forest floor due to the mature stand age and propensity for the shallow rooting Douglas-fir to topple from wind and snow burden.

The DIRT experiment in the H.J. Andrews Forest was initially established in 1997 and includes six detrital treatments (Table 1). All study plots were installed in a single area with uniform topography and forest vegetation characteristics. Each treatment was applied to individual plots and there were 3 plot replicates per treatment, which were

randomly distributed across the study site to account for any underlying variability in soil or vegetation properties. Plot sizes for all treatments other than the NR and NI plots are approximately 150 m2, while the NR and NI plots range from 55-75 m2. For the No Litter (NL) treatment, mesh screens were placed along the soil surface and used to collect and remove all aboveground litter from the plots on an annual basis. The 1 mm screen mesh allows for water and gas exchange, while preventing the majority of litterfall from interacting with the soil surface. The Double Litter (DL) treatment was performed by adding the litter removed from the NL plots to the surface of plots receiving natural litterfall. The additional litter was manually spread across the plot surface to achieve a total annual litterfall rate equal to twice the litter mass per area measured during the NL plot litter removal. The double wood (DW) treatment was similarly applied by manually spreading a thin layer of Douglas-fir wood chips across otherwise natural plots at a rate equivalent to the mass of annual litterfall. The No Root (NR) treatment was performed by lining a 1 m deep trench around each plot with a thick plastic material and back filling in a manner as to direct incoming roots down and away from the plot. Live trees within the NR plot border were girdled when the experiment was initiated to prevent root growth and activity. The No Input (NI) treatment involved the combination of litter screens and trenching as performed for the NL and NR treatments. Finally, the No Organic and A-horizon treatment (NOA) was performed by mechanically removing the topsoil to a depth of 30 cm, then backfilling with B-horizon material sourced from a hillslope immediately adjacent to the experimental area. Undisturbed control (CTL) plots were defined when the experiment was initiated. All treatment applications for the DL, DW, NL, and NI

treatments were applied annually in the late summer months. Natural litterfall input to plot surfaces, as was determined from the average mass of all litter material litter (excluding woody debris with diameter > 4 cm) removed from the NL and NI plots each year, were remarkably consistent, measuring 293 ± 29 g m-2 yr-1.

Soils were sampled in July 2017 after twenty years of sustained treatment applications. Samples for soil bulk density were collected first to allow us to determine how the soil surface may have expanded or compacted from the applied experimental treatments. At three random locations in each plot, a 5.4 cm diameter by 15 cm tall polyvinyl chloride (PVC) tube was gently inserted into the mineral soil surface. After installation, we measured the difference in distance from the top of the installed PVC tube to the outer soil surface and the inner soil surface. Differences were consistently less than 1 cm, allowing us to confirm minimal compaction occurred during the PVC tube installations. Due to the conducive soil texture and moisture, we were then able to remove the PVC tubes along with intact soil cores down to 15 cm depth.

From each plot, mineral soil samples were collected at 6 randomly chosen locations. The soil organic layer was collected by removing a 150 cm2 area of the forest floor down to the mineral soil surface. No evident amount of soil organic horizon remained on the No Litter and No Input plots. Starting at the mineral soil surface (e.g, below the organic layer when present) at each sampling location, mineral soil samples were subsequently collected at specific depth increments, including 0-10, 10-20, 20-40, 40-60 cm. Soil

samples from 60-100 cm depth were also collected at 2 of the 6 sampling locations per plot, as sampling to such depth was labor intensive and we expected variability in soil properties to be far less at depth. Samples from 0-10 and 10-20 cm were collected using a 5.8 cm diameter Oakfield style soil core sampler. A 5.1 cm diameter soil auger with a 12 cm soil collection bucket was used to collect samples at depths beyond 20 cm.

In the laboratory, the bulk density soil cores, still shrouded in the PVC tube casing, were cut with a fine-cut hacksaw to isolate the core section representing exactly 0-10 cm soil depth. All soil material was then removed from the core, dried at 105 °C and weighed to determine the dry mass per volume of the soil. For the few samples that contained large rocks, the bulk density was corrected by subtraction of both the rock mass and volume from the bulk density calculation. Rock volume was determined by water displacement. Soils from the bulk density analysis were not included in any other analyses performed in the study.

Soil organic horizon material was dried at 60 °C and weighed to determine the organic horizon mass per area for each sample. These values were then combined to determine the average organic horizon mass per area for each plot. After compositing and grinding the organic horizon samples from each plot, C and N content of the material was determined using an Elementar Vario Macro Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). During treatment application in fall of 2017, 6 individual samples of needle litter were collected from the surface of the removal plot screens to

determine the C and N content of the litter material applied to the DL plots. Similarly, six replicate samples of the wood chip material were collected for analysis. Respectively, the C concentration for the needle litter and wood chips were 459 ± 3 and 472 ± 1 mg C g-1, with C:N ratios (by mass) of 46.6 ± 2.2 and 117 ± 8.7.

Mineral soil samples from each plot and depth increment were separately sieved to 2 mm and allowed to air-dry for six-weeks. Roots removed by the sieve, as well as those manually removed after sieving, were grouped by size greater or less than 2 mm diameter, gently cleaned, dried and weighed. We did not expect the core sampling method to provide for an accurate estimation of large roots in the plots as the core samplers were unable to easily cut through roots larger than ~10 mm. Thus, we have limited our reports of root mass to only the fine roots < 2 mm in diameter. Composite mineral soil samples were made for each depth increment by combining the total sampled mass from each of the replicate samples taken within each plot. The composite soil samples by plot and depth were used for all chemical analyses performed in the study. The composite mineral soil and organic horizon samples were finely ground before analysis of total percent C by dry combustion using an Elementar Vario Macro Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). Accuracy of the C analysis was confirmed by >90% accuracy of included standard reference samples and >90% consistency in the analysis results between sample replicates. Soil pH was determined on mineral sample composites from only the 0-10 cm depth in a 1:2 soil/solution using 0.01 M CaCl2 slurry. Soil C stocks were calculated for each plot by

multiplying the composite soil percent C by the corresponding average soil bulk density measured at each depth increment. Depth increments for the mineral soil surface were adjusted to account for equivalent soil mass since changes in organic material incorporation, not mineral mass loss, were attributed with causing observed changes in bulk density.

From October, 2017 through November, 2018, soil CO2 efflux was measured roughly every week in May-July and once per month during the remainder of the year using a portable infrared gas analyzer (LI-8100A; LI-COR Inc., Lincoln, NE) attached to a closed dynamic respiration chamber (LI-8100-102; LI-COR Inc.) placed over a 10-cm diameter polyvinyl chloride (PVC) collar. Each PVC collar measures 5 cm in height and was inserted 2 cm into the mineral soil. Three PVC collars are permanently installed in each plot and collar volumes are measured frequently, with updated volumes of collars + chamber headspace used for flux calculations at each collar location. The portable infrared gas analyzer (IRGA) measures buildup of CO2 in the collar + chamber headspace over 90 s and the IRGA purges gas after each measurement. Measurements were typically taken randomly between 0900 h and 1300 h to minimize temporal effects on CO2 efflux. Seasonal mean soil respiration for each treatment group were computed for spring (n = 36), summer (n = 99) and fall (n = 63) and treatment group mean respiration values were compared with control group mean respiration by season (Table 2).

For the statistical analysis of differences in soil C concentrations and stocks, we performed a one-way analysis of variance (ANOVA) using the R statistical software with detrital manipulation treatment as the explanatory variable. Post-hoc Tukey honestly significant difference (HSD) tests were then performed to find significant differences among pairwise combinations of treatments with a standard significance level of $p < 0.05$. ANOVA and post-hoc Tukey HSD tests were performed separately by soil depth increment (0–10, 10–20 cm, etc.), as well as for the whole soil profile (0-100 cm). The statistical significance of mean seasonal respiration differences between treatment and the control soils were similarly tested using a one-way ANOVA and Post-hoc Tukey HSD for each seasonal comparison.

Results

Doubling needle litter (DL) inputs had no significant effect on the soil C concentration of the mineral soil after twenty years of sustained additions ($p < 0.99$, Figure 1A, Table 2). Throughout the 0-100 cm soil profile, the 10-20 cm layer was the only soil depth increment at which any indication of a potential treatment driven increase in soil C concentration was observed, with a mean difference in soil C concentration of 6.7 g C kg-1 soil (29% relative increase). In contrast, the addition of Douglas-fir wood chips (DW) to the soil surface showed much greater potential for increasing soil C concentrations (Figure 1A, Table 2). Mean soil C concentrations following the DW treatment were greater than control soil (CTL) throughout the 0-100 cm soil profile, with mean soil C increases of 24% and 54% observed at 0-10 cm and 10-20 cm respectively. While

substantial, the mean differences between the DW and CTL were not found to be significant ($p < 0.91$), given the relatively large variability in soil C response we observed. Replacing the organic and 0-30 cm mineral soil horizons with C poor mineral soil (NOA) had the greatest effect on soil properties at the soil surface (0-10 cm) relative to the control, with a mean difference in soil C concentration of 26.9 g C kg-1 soil (54% relative decline). Soil C concentrations did not recover at the soil surface following the NOA treatment and remained significantly lower than the control ($p < 0.01$). Also, no significant difference between the NOA and CTL soil C concentrations was observed at lower depths ($p < 0.99$).

Depriving soils of surface litter inputs (NL) led to small or negligible declines in mean soil C concentration across the depth increments sampled from 0-100 cm ($p < 0.98$, Figure 1B). Near the soil surface, NL treatment soil C concentrations remained strongly similar to the CTL, with mean C concentrations of 47.2 and 49.7 g C kg-1 soil respectively. Greater declines in mean soil C concentration (11-22%) from the NL treatment were observed at depths beyond 20 cm. However, these observed changes were also not sufficient in magnitude to reach statistical significance ($p < 0.98$). Similar to the NL treatment, cutting off the growth and activity of live roots in the mineral soil (NR) had no significant effect on soil C concentrations in the upper 0-10 cm of mineral soil ($p < 0.96$). However, at 10-20 cm, the NR treatment led to an interesting, yet non-significant ($p < 0.98$) 21% increase in mean soil C concentration relative to the CTL. We found this trend reversed at greater depths, where the NR treatment mean soil C concentrations

subsequently declined by a non-significant extent by 30% at 40-60 cm and 27% at 60-100 cm ($p < 0.99$). The no input (NI) treatment, for which both surface litter inputs and live roots were excluded from the soil, was consistently found to have the lowest mean soil C concentration across the three detrital reduction treatments (NL, NR, NI). Similar to the positive response observed from the NR treatment at 10-20 cm, the NI soil C concentration in the same layer also increased (+9%), yet the change was not found to be significant ($p < 0.99$). At depths other than 10-20 cm, declines in mean soil C concentration from the NI treatment were consistently between 17-44% relative to the CTL soil C ($p < 0.98$).

In the surface soils (0-10 cm), observed changes in soil bulk density by treatment type coincide precisely with the separate determinations of changes in mean soil C concentration, as we would expect from the inverse relationship between bulk density and soil organic matter content (Figure 2, Table 2). At a lower depth of 10-15 cm, differences in bulk density across all treatment types were not significant ($p < 0.99$). The observed increase of 0.05 g cm-3 in the 0-10 cm DL soil bulk density was not significantly different from the CTL, a finding in agreement with the minimal change observed in the DL soil C concentration at the same depth. In contrast, the mean soil bulk density in the DW treatment was 0.50 ± 0.08, compared to 0.61 ± 0.08 in the control, yet high variability in the bulk density measurements resulted in a non-significant statistical difference. Such a decline in bulk density is indicative of a large gain of soil organic matter in the top 10 cm of mineral soil, as was observed, yet not statistically confirmed.

Mean differences in bulk density of the 0-10 cm soils from the removal treatments were not significantly different ($p < 0.37$) from the CTL, but showed a consistent pattern of greater soil bulk density, with an average relative increase of 27 ± 2.1 % by comparison to the CTL soil.

Observed changes in the mean carbon to nitrogen ratio (C:N) of the study soils were reflective of the treatment manipulations, yet no changes were found to be statistically significant ($p < 0.92$). Reflective of the increased N available from the needle litter relative to wood debris, the mean 0-10 cm DL soil C:N declined by 22%. Such effect was limited to the surface of the DL soil, as minimal differences in C:N content were observed relative to CTL at lower depths. Despite the additional wood debris input, the DW soil C:N remained quite similar to the control at the soil surface. However, at 10-20 cm in the DW soil, a 24% increase in mean soil C:N content was observed. No further differences in soil C:N were notable between the DW and CTL at lower depths. The removal of surface litter (NL) led to no evident effects on soil C:N throughout the 0-100 cm profile. In contrast, soils without root activity (NR and NI treatments) had consistently lower mean C:N ratios than found in CTL from 0-100 cm, with slightly greater declines observed near the surface (Table 2). The C:N content of the 0-10 cm soil in the NOA was far more similar to the C:N content found at 10-20 cm in the CTL, reflecting the B-horizon origin of the added treatment soil.

Mean soil respiration rates were significantly lower for all of the detrital reduction treatments (NL, NR, NI) during spring and summer (Table 2, $p < 0.02$), but not during fall. The decline in mean respiration from treatments that removed live roots were the greatest, with an observed decline of 57% and 50% for the NR treatment in spring and summer, respectively. For the NI treatment, there was an observed decrease of 58% in spring and 64% in summer. Mean respiration from the NL treatment soils declined by 40% in the spring and 45% in summer. Increases in respiration from the detrital addition treatments were not significant in any season. We observed only a 5% and 6% increase in mean soil respiration from the DL treatment in spring and summer, respectively, and a 3% increase in spring and 23% increase in summer from the DW treatment. Increases in respiration were greater in the fall for DL and DW treatments, with a 40% increase in DL and 50% increase in DW, yet these differences were also not significant.

The mass of surface litter (organic soil horizon) increased by 61% from the DW treatment relative to control (Figure 3, Table 2; $p < 0.04$). Surface litter accumulation from the DL treatment was relatively minimal, with a non-significant mean difference of +15% ($p < 0.92$). The NR treatment was the only removal treatment to receive surface litter inputs. The removal of live roots led to an observed decline in surface litter by 31%, yet this change was not found to be statistically significant ($p < 0.62$). The NL and NI treatments resulted in the total loss of surface litter from the organic soil horizon.

Fine root mass in the 0-10 cm soil increased by 146% from the DW treatment ($p < 0.04$, Figure 4, Table 2). The DL treatment, for which we observed minimal effects on soil bulk density and surface litter mass, also had a non-significant effect on fine root mass ($p < 0.99$). Differences in fine roots between the CTL and the removal treatments were not statistically significant, yet large mean differences were observed across all removal treatments. The NR and NI treatment applications were successful in reducing root activity in the soils, as we found a >74% reduction in fine root mass across soils in both treatments ($p < 0.01$).The NL treatment may have also affected plant roots in the mineral soil, leading to an observed a decline in fine root mass of 51% relative to CTL ($p < 0.09$).

Treatment effects on soil C stocks were not directly proportionate with effects on soil C concentrations due to associated changes in soil bulk density (Figure 5, Table 2). To properly account for the effects of changing bulk density, soil C content was calculated using equivalent soil mass. Differences between stocks were not found statistically significant in comparisons between the CTL and the separate manipulation treatments ($p < 0.99$), yet some substantial changes in mean C stocks were observed near the soil surface. Following the twenty-year study period, the observed 0-10 cm DL treatment soil C stocks changed by +13% relative to CTL, while the 0-10 cm DW treatment soil C stocks changed by +24%. Effects on surface mineral 0-10 cm soil C stocks from the NL and NR treatments were small, with observed difference of < 5% relative to the CTL. The 0-10 cm NI treatment soil C stocks declined by 17%, while the 0-10 cm NOA treatment soil C stocks declined by 37%. Soil C stocks below 10 cm depth are reported with the

assumption of equal bulk density across treatment types as no significant change in bulk density was found from 10-15 cm soil depth across all treatment plots.

Discussion

Our findings provide a multi-decadal, field-based exploration of how changes in above and belowground detrital inputs may alter soil C stocks in the temperate conifer forests of the Pacific Northwest U.S. These wet temperate forests are highly productive and C rich, containing more C in aboveground biomass and soil than nearly any other forest system on earth (Smithwick et al., 2002). After twenty years of sustained detrital addition and removal treatments, we were surprised to find few significant changes in soil C and related soil properties. Field experiments remain as our most direct avenue towards improving our mechanistic knowledge of soil C stabilization dynamics, as the complexities of biogeochemical interactions in natural systems are not well-replicable in a laboratory environment (Malhotra et al., 2019; Smith et al., 2019). While this complexity presents challenges in isolating treatment effects and determining statistical differences, broader assessment of the common trends across separate, yet related analyses provides valuable insight into the potential effects from shifting organic matter inputs to soils.

In our analyses of the detrital treatment effects on soil carbon concentrations, the double litter (DL) treatment was consistently similar to the untreated control (CTL) soil. This was an unexpected result, as it appears very little of the additional surface litter C was

transferred to the mineral soil. The lack of a decline in bulk density further corroborates this finding. The strong evidence that the DL treatment had minimal effect on soil C invites the question, where did the additional litter C go over the 20-year study period? Addressing the possibility that the additional litter may have built up on the soil surface, we found that gains in surface litter mass of the organic horizon on the DL plots were also not significantly different from CTL, though confidence intervals from our statistical analysis allow that litter stocks may have increased slightly. However, we certainly did not observe an increase in standing surface litter mass of the magnitude necessary to account for any significant portion of the additional litter inputs. Substantial transfer of litter material off-plot due to erosion and wind is highly unlikely, as the study area is close to flat, winds are generally calm, and the additional litter quickly nestles in the forest floor, well protected by the dense understory vegetation. . Thus, it appears most likely that much of the litter material was decomposed by the microbial community, and the associated C was subsequently lost through respiration or leaching of dissolved organic carbon (DOC). A recent study of DOC production and transport from the HJAF DIRT plots estimated that DOC losses from the DL soils were generally equivalent to those from the CTL soil, and thus unlikely to be responsible for an increased portion of the litter C loss (Evans et al., 2020). Further, losses of C to leaching consistently account for only a small percentage of C losses relative to those from respiration in the H.J. Andrews DIRT plots (Lajtha et al. 2005). Thus, the elevated summer and fall soil respiration rates observed from the DL soils provide the greatest observed driver for the loss of the additional litter C input.

Increasing aboveground litter in forest systems has rarely led to an increase in soil C content, strongly suggesting the processes for transforming surface litter to soil organic matter (SOM) are more complex than a simple positive linear relationship between litterfall quantity and soil C accumulation. Similar DIRT experiments at the Bousson and Harvard Experimental Forests, both located in deciduous hardwood forests in the northeastern U.S., found no change in soil C after doubling surface litter inputs over a twenty year period (Bowden et al., 2014; Lajtha et al., 2014a). A recent study of detrital additions in the tropical forest of Panama also reported no significant change in soil C after thirteen years of doubling aboveground litter inputs (Sayer et al. 2019). Results from the DIRT experiment performed in this study provide further scope for the pervasiveness of these trends. The observed absence of soil C change from the litter additions at HJAF suggests highly productive forests with dense ecosystem carbon stocks are also not likely to gain soil C from increases in litterfall. Soils at the HJAF are unique from the other DIRT network sites, with their andic soil properties (reactive clay minerals) offering substantial reactive capacity for mineral stabilization of organic matter (Sollins et al., 2006; Matus et al., 2014). We expected these unique site properties at HJAF to provide greater opportunity for the detrital litter additions to increase soil C. Yet our results, taken along with those from other previous studies, suggest that additional aboveground litter inputs are not likely to alter soil C concentrations in most forest soils, and that the role of forest vegetation type, soil mineralogy, and climate require further study and attention as moderators of the relationship between litter inputs and soil C sequestration.

The double wood (DW) treatment, for which our experimental plots received annual additions of wood chips in an equal proportion to litterfall, led to a starkly different response in soil C related properties relative to the DL treatment. Among the two addition treatments, the DW clearly provided a greater likelihood for increasing soil C over the twenty-year study. The DW soils were found to have substantial increases in mean organic horizon mass, mean mineral soil C concentrations and stocks, as well as mean annual respiration rates relative to the CTL. At this time, when viewed as a combined set of treatment effects, a consistent trend is observed that suggests the woody debris is promoting soil C accumulation both above and belowground. The slow decomposition rate of woody debris is likely the mechanism for such C gains, as slower rates of decomposition provide greater time for the accumulation and distribution of organic matter in the soil profile. Differences in decomposition rates between wood and needle litter have long been recognized (Melillo et al., 1982; McClaugherty et al., 1985) and may be attributed primarily to the low quality (high C:N) of wood debris relative to the more nutrient rich needle litter (Bradford et al., 2016). Other studies have also shown that factors such as the presence of complex molecular structures (e.g. lignin) may also slow decomposition (Talbot et al., 2012; Rahman et al., 2013). Combining this information together with our results, we see strong evidence that a sustained increase in woody debris has greater potential than additions of needle litter to increase soil C stocks over short decadal timescales. However, based on the associated decline in DW soil bulk density, we suspect these apparent increases in soil C stocks are derived directly from

increases in free particulate, undecomposed woody debris rather than increases in mineral-stabilized C pools. How these gains in particulate SOM will contribute to the amount of stabilized soil organic carbon (SOC) in the mineral soil remains unknown and an important avenue for future research.

The observed differences in litter decomposition between the DL and DW treatments offer insight into the validity of current hypotheses pertaining to the stabilization of soil C. Additional organic inputs to soils have been recognized for the potential to cause priming effects (Kuzyakov, 2010), where the increase in microbial activity from a substrate addition leads to the respiration of previously stabilized SOC. Priming effects are more likely from high quality (low C:N) material, such as the needle litter in this study, which provides a greater abundance of limiting nutrients along with necessary C energy source to stimulate microbial activity (Wang et al., 2015). As priming effects have largely been studied in laboratory experiments, the magnitude of influence these phenomena may have on soil C stocks in natural systems is less well known (Wutzler and Reichstein, 2013; Cardinael et al.; 2015). Priming effects may explain the lack of an observed increase in soil C from the DL treatment by serving to offset any new gains in soil C that may have occurred in response to the additional litter. To provide further perspective on the role of priming effects on soils in natural forest environments, future analysis of age differences in SOC between treatment soils may provide opportunity to further understand the interplay between litter quality, priming and soil C accumulation.

In the absence of overwhelming priming effects, we had expected the microbial response to additional substrate inputs to drive an increase in mineral soil C. Further, we had expected a greater increase in mineral soil C from the higher quality needle litter, as opposed to the wood debris additions, due to the higher quality substrate allowing for greater rates of microbial processing and increased carbon use efficiency (Winsome et al., 2017; Bradford et al., 2013; Córdova et al., 2018). Previous studies have widely shown that SOC stabilized by organo-mineral interactions often resembles microbially processed material in structure and molecular composition, rather than the raw molecular components directly derived from the degradation of plant material (Sollins et al., 2009; Mambelli et al., 2011). These findings have led hypotheses postulating that the microbial processing of organic inputs facilitates subsequent SOC complexation with mineral surfaces (Cotrufo et al., 2013). The lack of an increase in DL soil C following twenty years of elevated microbial decomposition rates suggests additional controls may regulate the potential for mineral soil C gains from enhanced microbial processing. Alternatively, the timescale required for SOC accumulation may exceed our study period, as twenty years is a relatively short time relative to forest soil development.

Broadly, results from the removal treatments suggest mineral soil C concentrations in these temperate forest soils are quite resistant to decline over two decades of reductions in above or below ground detrital organic matter inputs. Surprisingly, soils with no detrital contributions from surface litter (NL) or live roots (NR) maintained soil C concentrations nearly identical to the otherwise natural soils (CTL). A lack of soil C loss

from the NL treatment without an observed increase in fine root growth, may suggest that forest soil C concentrations are supported largely by root activity. Further, the observed decline in organic horizon mass above the NR treatment suggests that in the absence of root activity, the surface litter may be more actively decomposed to sustain microbial activity and support soil C concentrations. As expected, the greatest potential for loss of soil C occurred when both aboveground and belowground detrital input sources were reduced (NI). Observed rates of respiration in the NI soils, along with the lack of a severe decline in soil C, shows that the soils continued to receive some amount of fresh organic inputs through the surface litter screens and possibly a few remaining live roots. Potential explanations for the persistence of soil C concentrations in the NI soils include: 1) a substantial portion of the mineral soil C is stabilized or otherwise well protected and unavailable for microbial decomposition (Castellanos et al., 2015); 2) microbial activity and community composition strongly regulate changes in soil C concentration (Georgiou et al., 2017; Liang et al., 2017); 3) relatively small amounts of detrital organic matter inputs to the soil may be sufficient over the course of the study period to prevent greater losses of soil C. Further study of the mineral matrix capacity for organo-mineral complexations, as well as detrital influences on microbial community and processing pathways, will be necessary to better elucidate the relative level of contribution for these biogeochemical controls over soil C stabilization.

Losses of soil C from the treatment reductions in above and belowground detrital inputs at HJAF were less substantial than soil C losses observed after twenty years at similar

DIRT sites in other forest environments (Bowden et al., 2014; Lajtha et al., 2014a; Lajtha et al. 2014b). However, declining soil C trends from the NR and NI treatments were similar. Our findings from HJAF DIRT expand the scope of these trends and provide further evidence that a reduction in live root activity leads to minimal effects on forest soil C over short decadal timescales, largely irrelevant to differences in soils, climate and vegetation. In contrast, the NL treatment at HJAF had far less effect on soil C concentration than observed across the other DIRT sites. A greater abundance of roots in the surface soils at HJAF may explain this disparity (Bowden et al., 2014; Lajtha et al., 2014a; Lajtha et al. 2014b).

The NOA treatment accumulated a minimal amount of soil carbon in the upper 0-20 cm of mineral soil, despite twenty years of direct litter inputs to the soil surface and a large return of belowground root activity. Similar to the DL treatment, this finding shows a remarkable resistance to increases in soil C from detrital additions. This lack of increase in soil C suggests that the timescale for soil C accumulation and development is far greater than our 20 year study period, which was unexpected given the high reactivity of the andic B horizon fill material.

Observed effects on soil C from the detrital manipulation treatments support further investigation of the mechanisms that connect litter quantity and quality to soil C stocks. The stark differences in soil response to additions of needle litter versus wood debris suggest quality may be the foremost determinant factor governing how changes in

temperate forest detrital input quantity influence soil C accumulation. Further, it is apparent that belowground detrital inputs have a greater supporting role for soil C than aboveground litter inputs, suggesting long-term disturbances or management actions that reduce root activity may deplete soil C stocks. Moving forward and building from this preliminary 20-year assessment of soil C change in the HJAF DIRT experiment, we expect further opportunities to investigate changes in specific mineral soil C pools, N cycling, and microbial decomposition dynamics to further refine our knowledge of the linkages between forest soil C stocks and detrital input quantity and quality.

Tables & Figures

Table 1.1

Description of detrital manipulation treatments.

Treatment	Abbreviation	Description
Control	CTL	Natural above- and belowground detrital inputs
Double litter	DL	Aboveground needle and leaf litter inputs doubled annually*
Double wood	DW	Double wood debris applied every other year as wood chips**
No litter	NL	Aboveground inputs removed annually in late fall season
No roots	NR	Live roots excluded via 0-140 cm tarp lined trenches around plots
No inputs	NI	Aboveground inputs excluded as in no-litter plots, belowground inputs are prevented as in no-roots plots
OA-less	NOA	Top 30 cm of soil (O and A Horizons) was replaced with mineral soil

* Additional litter supplied from the litter exclusion plots and allocated proportionally

** Wood addition mass estimated to equal falling wood debris in the control plots.

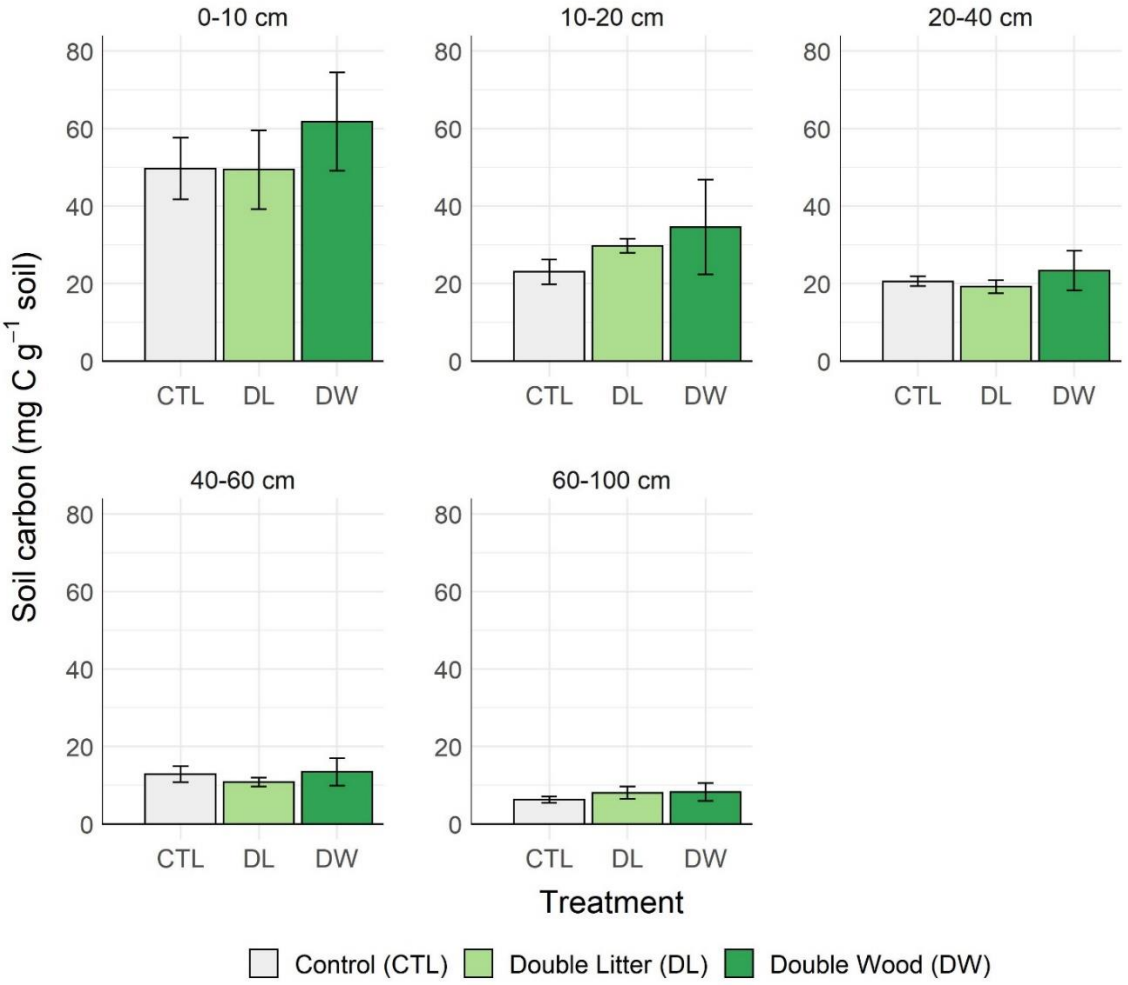
Table 1.2

Soil carbon (C) concentration, content, root mass, respiration, pH, bulk density and above ground litter mass by detrital manipulation treatment type following twenty years of treatment.

Depth (cm) [Season]	Control [CTL]	Double Litter [DL]	Double Wood [DW]	No Litter [NL]	No Roots [NR]	No Inputs [NI]	OA-less [NOA]
			Organic C concentration, g C kg^{-1} soil				
0 - 10	49.7 ± 8.0a†	49.4 ± 10.1a	61.8 ± 12.6a	47.2 ± 5.1a	50.6 ± 5.2a	41.4 ± 3.1a	22.8 ± 4.5b
10 - 20	23.0 ± 3.2	29.7 ± 1.8	34.6 ± 12.2	23.9 ± 2.7	27.9 ± 1.1	25.1 ± 4.4	21.7 ± 6.2
20 - 40	20.6 ± 1.2	19.2 ± 1.7	23.4 ± 5.1	18.0 ± 2.4	20.9 ± 1.7	15.1 ± 2.9	17.4 ± 3.2
40 - 60	12.9 ± 2.1	10.9 ± 1.2	13.5 ± 3.6	11.5 ± 3.0	9.1 ± 0.6	7.2 ± 0.6	12.0 ± 1.2
60 - 100	6.3 ± 0.9	8.1 ± 1.6	8.3 ± 2.3	4.9 ± 1.2	4.6 ± 0.6	4.1 ± 1.0	5.6 ± 1.0
			C:N				
0 - 10	28.7 ± 1.8	22.6 ± 1.4	27.6 ± 2.1	24.5 ± 1.5	22.2 ± 1.4	23.9 ± 0.8	20.3 ± 1.3
10 - 20	19.2 ± 1.2	19.1 ± 1.4	23.9 ± 3.8	19.0 ± 1.2	17.4 ± 0.8	16.3 ± 2.6	21.2 ± 0.7
20 - 40	17.0 ± 0.7	17.1 ± 0.3	18.0 ± 1.0	16.3 ± 1.2	15.7 ± 5.2	16.4 ± 1.7	18.7 ± 2.5
40 - 60	15.4 ± 0.5	16.5 ± 1.2	17.3 ± 1.8	15.8 ± 0.3	14.2 ± 4.7	15.3 ± 1.1	17.4 ± 1.2
60 - 100	14.2 ± 0.6	15.5 ± 1.2	14.4 ± 0.4	14.3 ± 1.5	14.7 ± 4.9	11.0 ± 0.7	15.5 ± 1.8
			Bulk density, g cm^{-3}				
0 - 10	0.61 ± 0.08	0.66 ± 0.07	0.50 ± 0.08	0.75 ± 0.06	0.79 ± 0.09	0.79 ± 0.05	0.91 ± 0.10
			Organic C content, kg C m^{-2}				
0 - 10	3.01 ± 0.48	3.20 ± 0.66	3.06 ± 0.63	3.50 ± 0.38	3.93 ± 0.41	3.29 ± 0.24	1.90 ± 0.38
10-20	2.85 ± 1.01	2.07 ± 0.36	1.90 ± 0.26	2.30 ± 0.09	1.79 ± 0.51	2.45 ± 0.15	1.97 ± 0.22
20 - 40	4.35 ± 0.95	3.57 ± 0.32	3.89 ± 0.32	3.83 ± 0.23	3.35 ± 0.45	2.81 ± 0.53	3.24 ± 0.59
40 - 60	2.27 ± 0.25	2.81 ± 0.74	2.70 ± 0.44	1.91 ± 0.12	2.40 ± 0.62	2.50 ± 0.25	1.50 ± 0.13
60 - 100	2.94 ± 0.40	3.77 ± 0.73	3.87 ± 1.06	2.30 ± 0.55	2.16 ± 0.27	1.93 ± 0.49	2.59 ± 0.46
			Root mass, g m^{-2}				
0 - 10	189 ± 44a	208 ± 97ac	466 ± 91c	92 ± 3ab	35 ± 17b	49 ± 36b	127 ± 40ab
			Soil Respiration, g C m^{-2} d^{-1}				
[Spring]	12.1 ± 0.24a	12.7 ± 0.29a	12.5 ± 0.40a	7.23 ± 0.12b	5.15 ± 0.35b	5.04 ± 0.05b	NA
[Summer]	14.1 ± 0.38a	15.0 ± 0.27a	17.3 ± 0.31b	7.70 ± 0.13b	6.99 ± 0.19b	5.04 ± 0.09b	NA
[Fall]	7.21 ± 0.25a	10.1 ± 0.44a	10.8 ± 0.47a	5.62 ± 0.18a	5.81 ± 0.29a	4.30 ± 0.14a	NA
			Surface litter mass, kg m^{-2}				
Organic horizon	115 ± 32a	133 ± 53a	185 ± 52b	NA	79 ± 16a	NA	NA
			Soil pH				
0-10	4.87 ± 0.05	4.44 ± 0.16	4.14 ± 0.26	4.28 ± 0.10	4.61 ± 0.08	4.35 ± 0.16	4.47 ± 0.06

† Means ± SE. Means followed by different letters are significantly different according to Tukey's HSD ($\alpha = 0.05$).

Figure 1.1A. Soil C concentration by depth and treatment type after twenty years of detrital manipulations



* Differences between the control soil and the addition treatment soils were not statistically significant (α = 0.05).

Figure 1.1.B

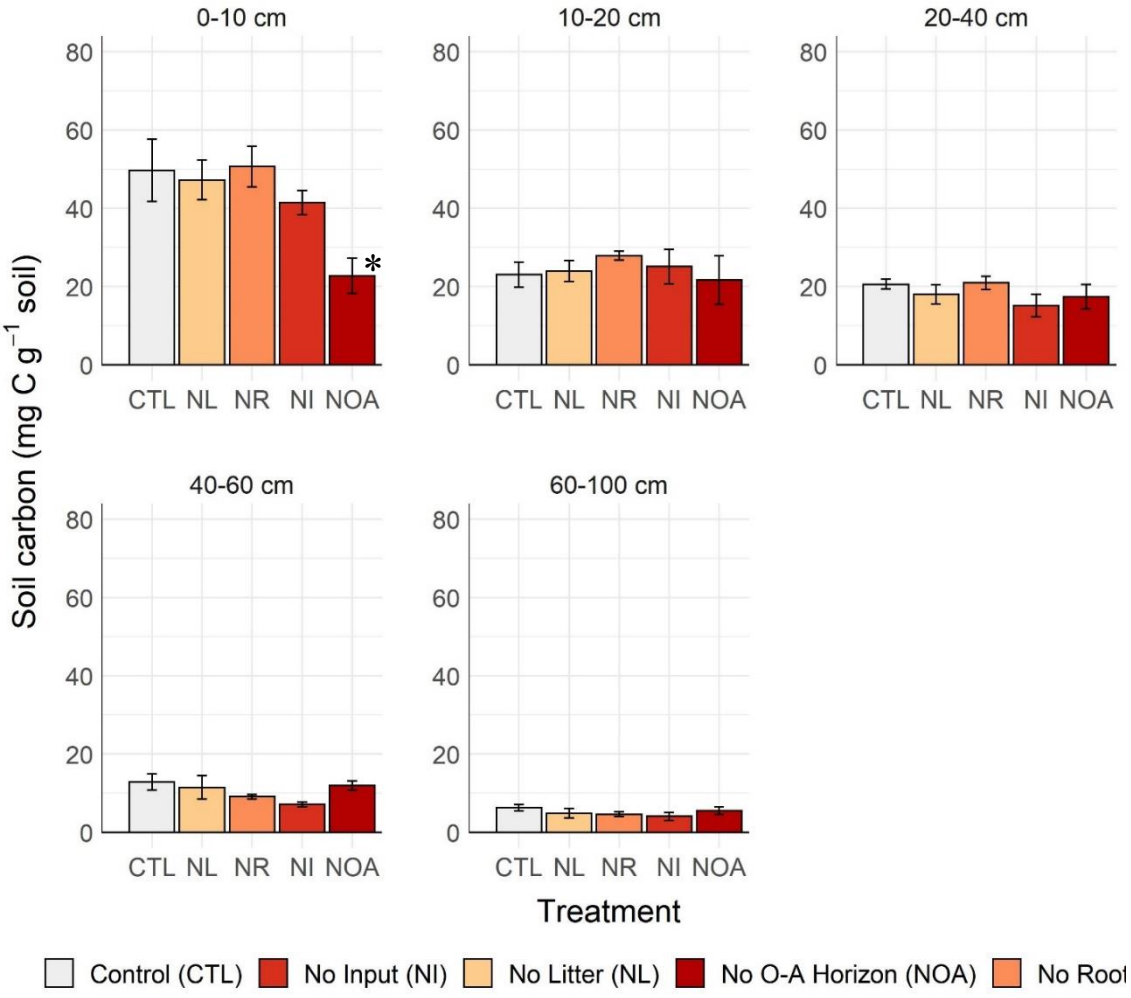
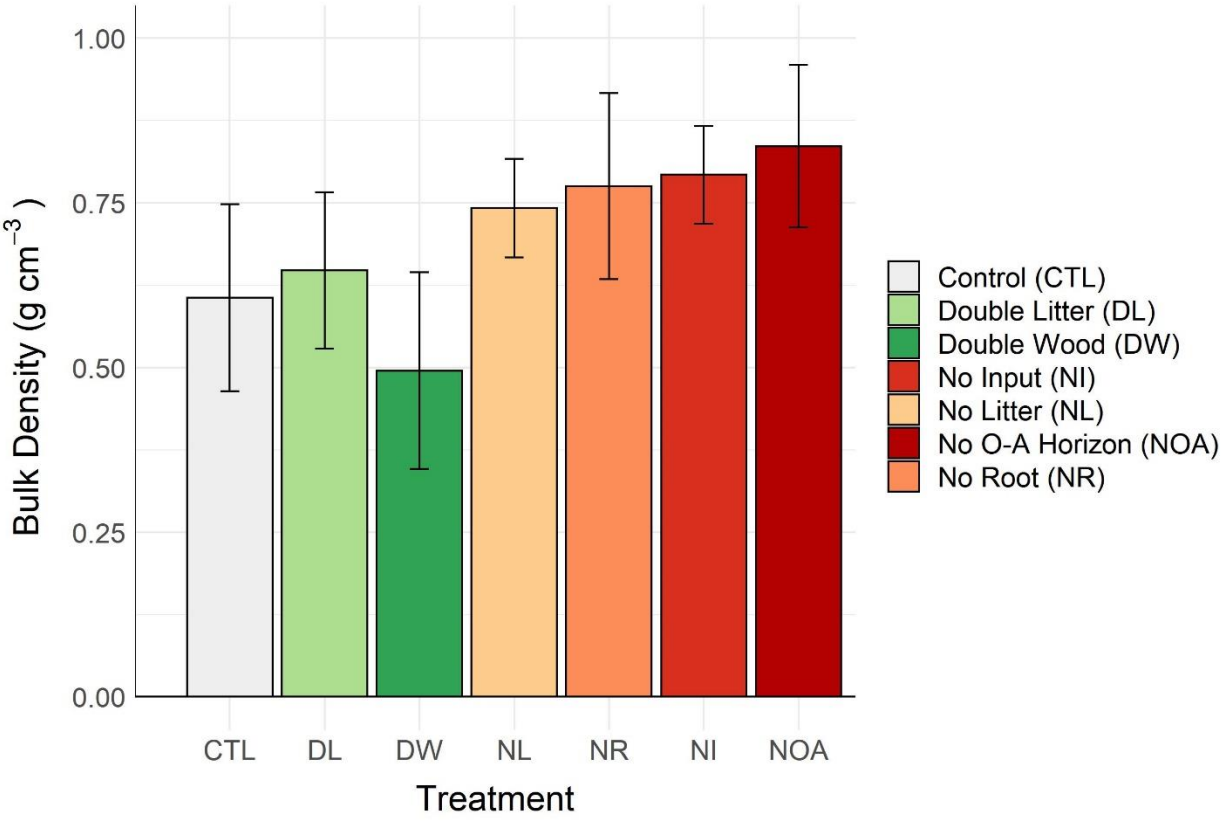

0-10 cm
10-20 cm
20-40 cm
40-60 cm
60-100 cm
Soil carbon (mg C g^{-1} soil)
0
20
40
60
80
CTL NL NR NI NOA
*
Treatment
Control (CTL)
No Input (NI)
No Litter (NL)
No O-A Horizon (NOA)
No Root

Figure 1.2. 0-10 cm soil bulk density by detrital treatment type after twenty years of manipulation



* Differences in 0-10 cm soil bulk density between the control soil and the treatment soils were not statistically significant ($\alpha = 0.05$).

Figure 1.3. Soil surface litter mass by detrital treatment type after twenty years of manipulation

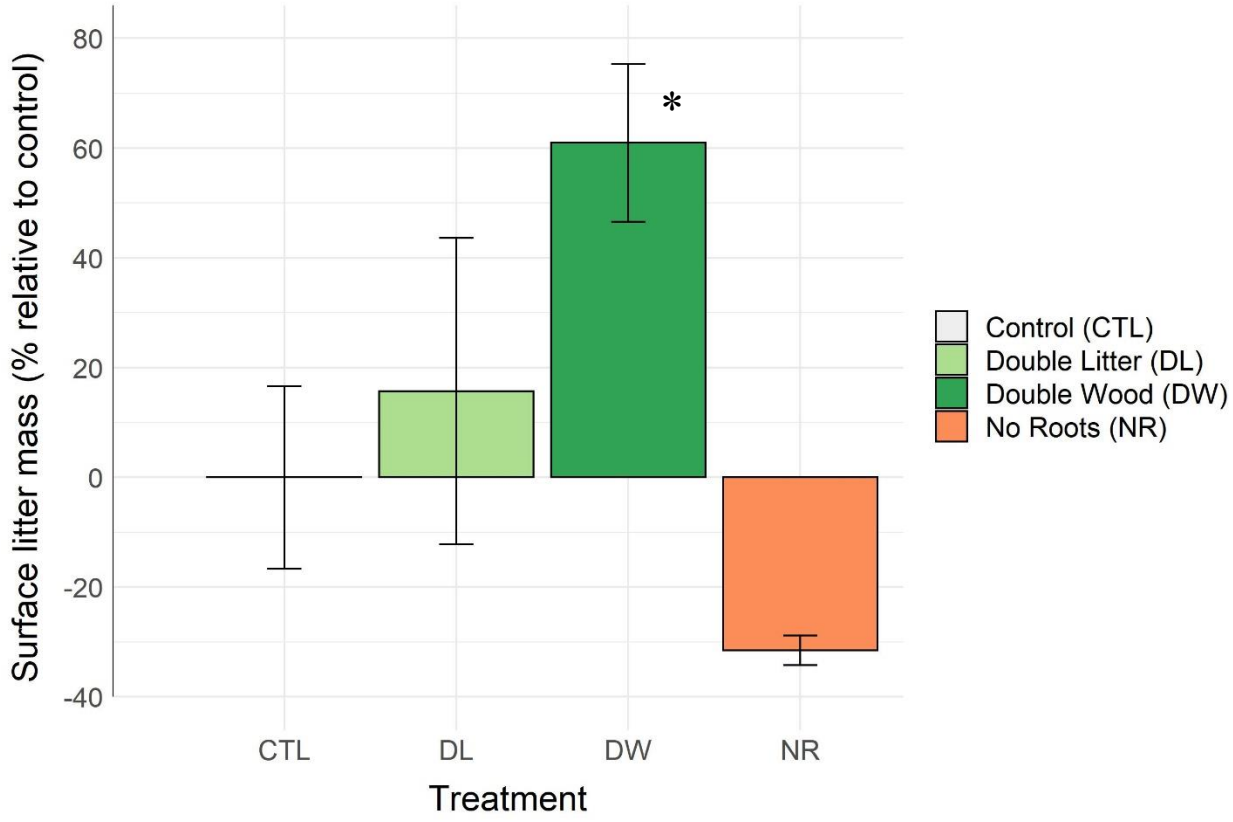


Figure 1.4. 0-10 cm soil fine (<2 mm) root mass by detrital treatment type after twenty years of manipulation.

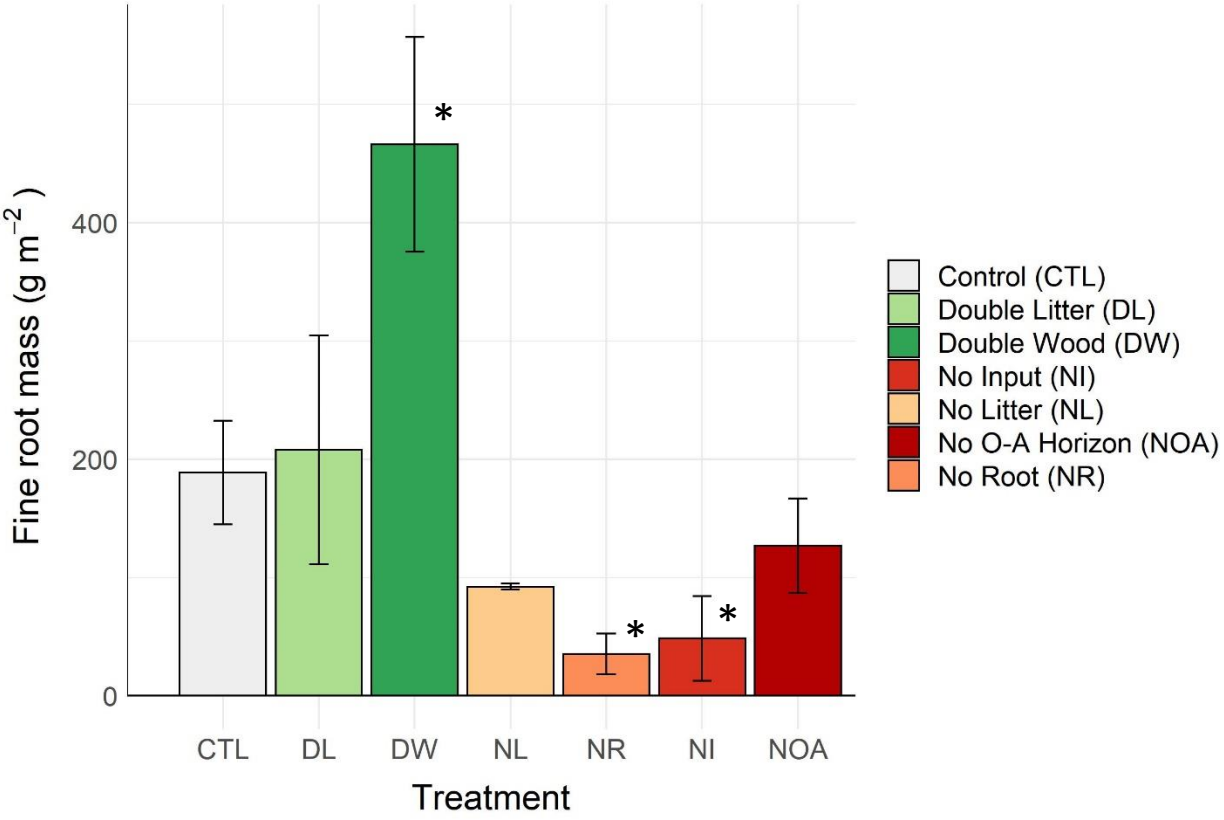
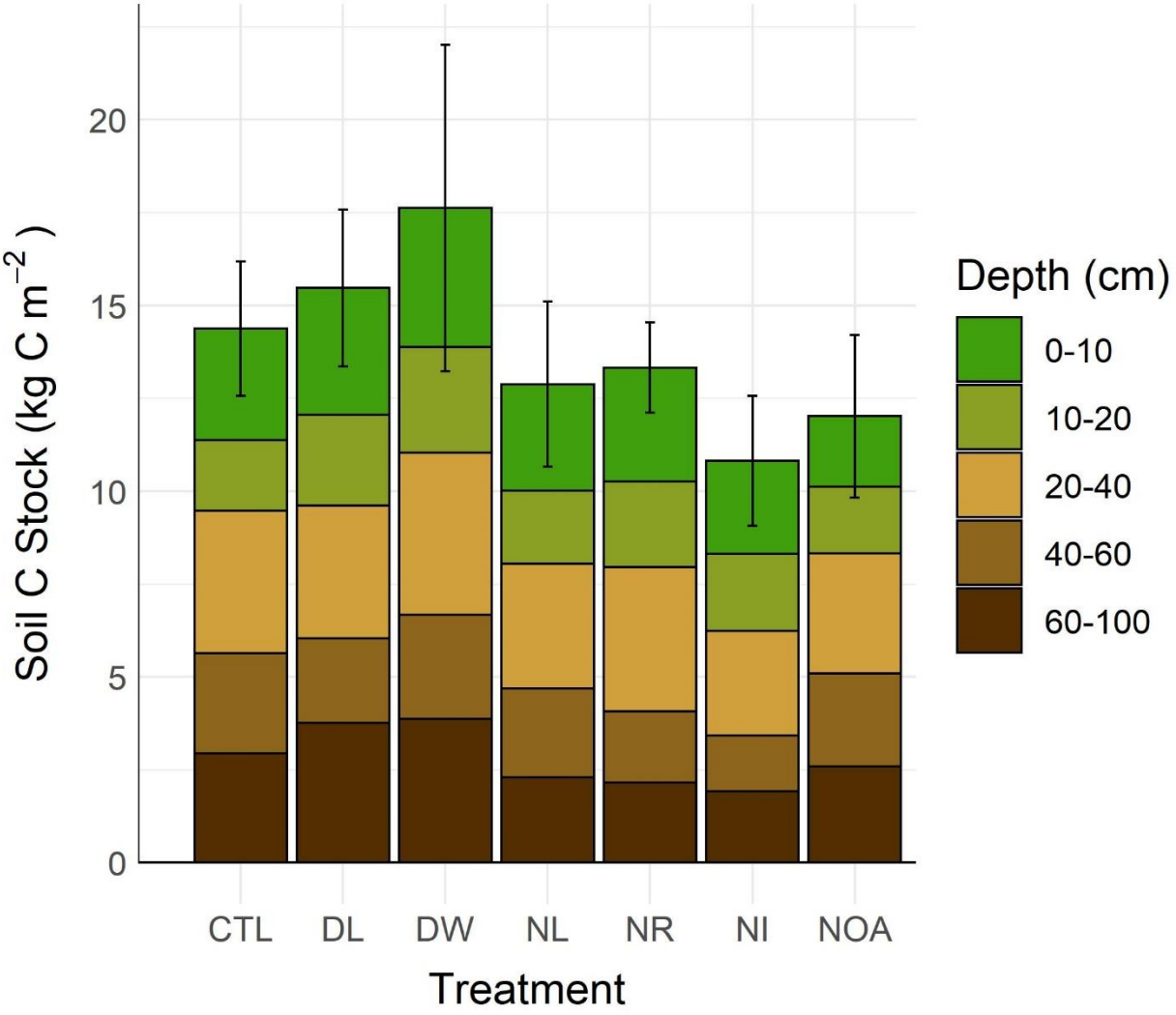


Figure 1.5. Soil C stocks by detrital treatment type after twenty years of manipulation



(CTL = Control, DL = Double litter, DW = Double wood, NL = No Litter, NR = No roots, NI = No inputs, NOA = No O-A horizons)

* Differences in soil C stocks were not statistically significant ($\alpha = 0.05$).

CHAPTER 2: Roots limit the mineral stabilization of soil carbon, outweighing controls from aboveground litter quality and quantity

Introduction

As large changes in climate, land use and natural disturbances continue globally, the associated effects on plant carbon (C) inputs to soil are likely to drive changes in soil C stocks. A wide range of climate, soil and biogeochemical factors are also known to have influences on soil C processing and accumulation. Yet, relative to human lifespans, most of the factors that contribute to governing soil C are slow to respond to changing environmental conditions, such as mineralogy, or are inherently difficult to control or manipulate. Changes in climate, while also slow and difficult to manipulate, will produce more rapid effects on soil biological activity as well as on vegetation, which includes both above and belowground litter inputs. Knowledge remains limited regarding how sensitive soil C stocks are to such changes in plant C inputs, as the pathways and mechanisms connecting plant C inputs with soil C accumulation and stabilization remain poorly understood. Without such knowledge, it remains difficult to predict the extent to which soil C stocks may decline following reductions in plant C inputs, or conversely, how much potential exists for soil C stocks to increase in response to additional C inputs. Further, beyond the unknown effects of altered plant C input quantity, numerous other questions also exist regarding the more complex linkages between plant inputs and soil C, including: 1) How do plant litter chemistry and nutrient content (i.e. quality) influence soil C accumulation and stabilization?; 2) Do stabilized soil C stocks have greater dependence on C inputs from roots or surface litter?; 3) How relevant are plant C input-

driven changes in microbial processing, such as priming, to long-term soil C storage?; 4) What processes regulate the formation and capacity for mineral stabilization of soil C?

Soil C exists as a heterogeneous mixture of decomposing and stabilized soil organic matter (SOM), with varied chemical properties and turnover rates. Yet for the purposes of studying controls on soil C stabilization, SOM must be separated into unique pools based on protection from microbial decomposition and associated timescales for turnover in the soil. Soil density fractionation, due to the low density of plant matter, allows for separation of particulate organic matter (POM, or Light Fraction SOM) from more mineral associated organic matter (MAOM, or Heavy Fraction SOM) (Sollins et al. 2006; Lavallee et al. 2020). The POM pool closely resembles initial plant C inputs, such as the plant litter and root detritus that enters the soil. In general, the POM pool is readily available for microbial decomposition and thus often responds quickly to changing conditions and management (Dorodnikov et al. 2011; Song et al. 2012). Induced changes in POM are often short-lived, as the microbial processing response to available substrate may quickly return POM pools to pre-existing levels following recovery from disturbance or the cessation of management activities. In contrast to POM, soil organic matter that becomes occluded in micro-aggregates or stabilized through complexation with mineral surfaces has far greater protection from microbial decomposition. Soil C in MAOM typically has much longer residence times in soil relative to POM (Lavallee et al. 2020). MAOM is less responsive to changing conditions than POM, yet losses of MAOM have commonly been observed following long-term detrimental land use practices such

as over-grazing and conventional tillage (Santos et al. 2020; Teixeira et al. 2020). In contrast to the observed processes that lead to losses of MAOM, processes that promote accumulation of MAOM, especially those that may lead to accumulation beyond naturally existing stocks, remain poorly understood. Reactive mineral surfaces may be a limiting factor for MAOM formation, and the extent to which natural soils may already be saturated with MAOM is widely uncertain (Cotrufo et al. 2019). Improving insight into the processes controlling MAOM formation, destabilization, and accumulation is clearly required to gain a better understanding of the pathways leading to soil C sequestration.

The Detrital Input and Removal Treatment (DIRT) experimental network (Lajtha et al. 2018) was designed to investigate the long-term effects of altered organic input source, quantity and quality on SOM processing and stabilization in a natural forest environment. For two decades the DIRT network experiment at the H.J. Andrews Experimental forest has been ongoing, including manipulations of soil C inputs that either reduce aboveground, belowground or both above- and belowground forms of litter and root inputs. The experiment also includes contrasting litter addition manipulations, which increase the amount of either needle litter or woody debris inputs to the soil surface. The addition treatment comparison allows for the examination of soil C effects in response to naturally occurring differences in surface litter quality. The chemistry and molecular composition differences between needle litter and woody debris are substantial, as the C to nitrogen ratio of woody debris in the H.J. Andrews temperate forest is approximately eight times lower than for the needle litter, and the lignin and cellulose contents are

approximately three times greater (Means et al. 1992; Valachovix et al. 2004, Yano et al. 2005). The detrital input effects on soil C throughout the long history of the DIRT experiment have rarely aligned with initial hypotheses and continue to provide unique insights into soil C turnover and stabilization.

Although we initially predicted that the DIRT litter reductions would result in soil C losses, after twenty years of reduced surface and root C inputs, soil C concentrations remain similar to the untreated (control) soil, despite sharp increases in bulk density across all detrital reduction treatments (Pierson et al. 2020, in press). The increase in bulk density is indicative of a loss of POM, suggesting that an increase in MAOM C content must have also occurred in tandem to offset the expected losses of POM. Soil C also did not increase with increased needle litter input, although soil C did increase with added wood debris. A decrease in bulk density in the wood debris addition soils suggests that the wood addition soils are gaining a substantial amount of POM. We hypothesized that these gains in high C:N POM would promote microbial processing and access to low C:N stabilized C, and thus we expected the gains in POM would correspond with losses of MAOM. While the C concentration in the needle litter addition soil remains surprisingly similar to untreated soil, we also hypothesized, based on current theory for the factors promoting MAOM formation (Cotrufo et al. 2013), that the improved quality of the C inputs would drive increases in MAOM. Based on these emerging hypotheses, the objectives of this study were to (1) quantify changes in particulate (POM) and mineral (MAOM) soil C pools following twenty years of the DIRT manipulations and (2) assess whether observed effects on soil C pools resulted from a change in the relative size of

POM and MAOM pools, or from a direct change in the C concentration of the pools. By examining the nature of change in the soil C pools, we hope to better identify the pathways through which the observed soil C changes have occurred, specifically whether C appears to be transferring between pools from POM to MAOM, or if pools appear to be gaining or losing C irrespective of changes in the other soil C pools.

Methods

The long-term Detrital Input and Removal Treatment (DIRT) experiment was established in the H.J. Andrews Experimental Forest in 1997. The H.J. Andrews Experimental Forest is located within the Willamette National Forest along the central Cascade Mountains of Oregon (44º15’ N, 12º10’ W). Mean annual precipitation is 2080 mm yr-1, with mean annual temperatures of 9.4 °C (averages from 1999-2014). Approximately 70% of the annual precipitation occurs between November and March (Sollins et al. 1980). The study site is located in an undisturbed, old-growth stand of predominantly Douglas-fir (*Pseudotsuga menziesii*), with intermixed growth of Western hemlock (*Tsuga heterophylla*), western red cedar (*Thuja plicata*), big-leaf maple (*Acer macrophyllum*) and vine maple (*Acer circinatum*). The DIRT experimental plots are arranged together at an elevation of 720 m, situated along a low-lying foot slope terrace with a uniform low slope across the study area (<5%). The soil surface is uniformly covered by an abundant mix of fungi, moss and understory vegetation, with large amounts of woody debris and fallen logs intermixed. The organic soil horizon is 4-8 cm thick in most areas. Soils at the

site are derived from volcanic parent material and are classified as coarse loamy mixed mesic Typic Hapludands (Lajtha et al. 2005).

The DIRT manipulations have been performed annually since the beginning of the experiment in 1997. Detrital manipulations in the DIRT experiment include 6 unique combinations of leaf litter or woody debris additions, or the exclusion of surface litter and roots (Table 1). Each treatment type is replicated across three large, separate plots (n=3), that were chosen randomly across the study site. Plot sizes are approximately 150 m2 for all treatment plots, except those with root exclusions. The root exclusion plots are approximately 75 m2, as the no root (NR) and no input (NI) plots are located adjacent to each other within a ~150 m2 root free zone. Root growth in this area has been restricted by a 1-m deep lining of thick, yet permeable plastic around the plot with an outward curved bottom edge to help divert incoming roots. Trees within root exclusion plots were girdled at the beginning of the experiment to terminate all root activity. Plots with litter exclusion treatments were initially cleared of large wood debris and covered with 1-mm nylon mesh screens to separate litter fall from the soil surface. During treatment application, the litter removed from the exclusion plots is used as the litter source for the litter addition plots. The added litter is spread evenly across the addition plots on a mass per area basis equal to the litterfall rate measured in the litter removal plots, which thus achieves a total annual litter input approximately twice the natural rate (DL). The wood debris addition plots (DW) receive an additional input of shredded Douglas-fir wood chips (5–20 cm in length) every other year in addition to natural litterfall. Wood chips

additions are distributed evenly at a rate (by mass) estimated to equal falling wood debris (Lajtha et al. 2005). The control plots (CTL) have not been disturbed other than low frequency foot traffic during treatment manipulations and occasional small core diameter soil sampling.

Soil for this study was collected in July 2017, twenty years after the DIRT experiment was initiated. Within each plot, mineral soil samples were collected in six random locations at depths below the O-horizon of approximately 0-10 cm and 10-20 cm, using a 5.8 cm diameter Oakfield style soil core sampler. The exact core sampling depths were determined by the treatment soil differences in bulk density compared to the untreated control soil to ensure that the soil samples were collected on an equivalent soil mass basis (Billings et al., in press). No evident O-horizon layer remained in the NL and NI plots, thus sampling commenced immediately at the existing soil surface. The soil bulk density and fine root content for each treatment plot was determined through separate sampling during the same week, as previously described and reported in Pierson et al., in press. In the laboratory, the soil core samples from each plot were composited by depth increment, homogenized and allowed to air dry for eight weeks. The individual samples were then passed through a 2-mm mesh sieve prior to further analysis.

To separate SOM into distinct soil C pools, we sequentially fractionated the study soils by density via disbursement in solutions of sodium polytungstate (SPT) as described in Sollins et al. (2006). To suit the study objectives to determine how detrital effects may proceed through soil C pools, we separated the soil into three distinct fractions: light,

intermediate, and heavy. When discussing study results in regards to pools of POM and MAOM, we associated the light fraction (LF) with POM, and the sum of the intermediate fraction (IF) and heavy fraction (HF) with MAOM. The intermediate fraction often represents a mixture of heavy fraction material and other organic materials associated into aggregates (Hatton et al. 2012) that is intermediate between the light and heavy fraction in terms of turnover time and resistance to microbial decay (Sollins et al. 2009). In brief, for the fractionation procedure we used a 50 g subsample of the <2 mm, composite soil from each field plot and depth increment. We initially shook the individual subsamples in a SPT solution with a density of 1.85 g cm-3 for two hours. The resulting slurry was then centrifuged to separate the light fraction (<1.85 g cm-3) from the rest of the soil material. The process was then repeated with the >1.85 g cm-3 soil to ensure full separation and recovery of the light fraction. Next, the >1.85 g cm-3 was put through the process again using a SPT solution with a density of 2.40 g cm-3, effectively separating the soil material into two further fractions with densities 1.85-2.40 g cm-3 (intermediate fraction) and >2.40 g cm-3 (heavy fraction). Fractionated soil material was rinsed with deionized water to remove the SPT and dried for 72 hours at 60 °C. Dry fraction mass of each fraction was recorded and sub-samples of the fraction material were ground and analyzed for total carbon and nitrogen using using an Elementar Vario Macro Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany).

Statistical differences in soil C concentration, content and fraction mass were determined using a one-way analysis of variance (ANOVA) with detrital manipulation treatment as

the explanatory variable. Post-hoc Tukey honestly significant difference (HSD) tests were then used to determine significant differences between pairwise combinations of each treatment type versus the control. The ANOVA and post-hoc Tukey HSD tests were performed separately for each fraction (light, intermediate, heavy) and soil depth increment (0–10, 10–20 cm) combination. Statistical differences were defined as significant at $\alpha = 0.05$. All data and statistical analyses were performed using R version 3.5.2 (Team 2019).

Results

Although twenty years of wood debris additions (DW) to the soil surface led to large apparent increases in the mean light fraction soil C content at 0-10 cm and 10-20 cm depths, the observed changes were highly variable and thereby non-significant ($p < 0.20$, Figure 1). The DW treatment approximately doubled the mean amount of light fraction soil C in the bulk soil (i.e. light fraction soil C content), increasing it by +99% at 0-10 cm and by 119% at 10-20 cm relative to the control soil. This increase in light fraction material from the DW treatment corresponded to a substantial decline in soil bulk density, with values reported previously of 0.50 ± 0.08 and 0.61 ± 0.08 g cm-3 for the DW and control 0-10 cm soils respectively. The observed increase in mean light fraction C content from the DW treatment is due to an increase in the proportion of light fraction material in the bulk soil, rather than from an increase in the C concentration of the fraction (Figure 2). Differences between the intermediate density fraction C contents in DW and control soils were relatively small, as both the proportional mass and C

concentration of the intermediate fraction remained similar between the soils at 0-10 and 10-20 cm. The mean heavy fraction C content in the 0-10 cm DW soil was 35% lower than in the control soil, yet high variability in the heavy fraction C concentrations also led this observed mean difference to be non-significant. The 0-10 cm decline in mean heavy fraction C content in the DW soil resulted from a combined and closely proportionate decrease in both the C concentration and the proportional mass of the heavy fraction pool (Figure 2). Heavy fraction C content at 10-20 cm in the DW soil remained closely similar to the control.

Doubling needle litter inputs (DL) to the soil surface did not affect the light fraction C content at 0-10 cm and 10-20 cm. In contrast to results from the DW treatment, the DL mean soil C content increased in the intermediate and heavy fraction soil C pools across both the 0-10 and 10-20 cm depth increments (Figure 1). Although the observed increases in mean intermediate and heavy fraction C content appeared substantial in magnitude, our statistical analysis of the change in individual fractions by depth increment did not confirm a significant change from the control soil ($p < 0.62$) due primarily to the variability in the fraction C concentrations (Figure 2). Across the soil C effects observed from the DL treatment, the largest increase in fraction C content was in the 0-10 cm intermediate fraction, which was 35% greater than the control. The increase in intermediate fraction C content derived primarily from an increase in the C concentration of the intermediate fraction pool, rather than a change in the proportional mass of the pool (Figure 2). DL treatment effects on the intermediate fraction were substantially

reduced at 10-20 cm relative to the larger change observed at 0-10 cm. In contrast, increases in the heavy fraction C content of the DL soil remained consistent across both sampling depths, with observed gains of 25% at both 0-10 and 10-20 cm. The increases in the DL heavy fraction C content were driven by a combined increase in both the proportional mass and C concentration of the heavy fraction pool.

There were no significant changes in C content, C concentration or fraction mass in the surface litter reduction treatment (NL) soils at either sampling depth (Figures 1 & 2). Further, the intermediate and heavy fraction C mass and concentration remained similar to the control soil, despite twenty years of sustained reduction in surface litter inputs. Termination of live root activity combined with the removal of surface litter inputs (NI treatment) led to a significant gain in heavy fraction C content in the 0-10 cm soil ($p < 0.01$), but no change at 10-20 cm ($p < 0.99$, Figure 1). The mean 0-10 cm heavy fraction C content in the NI soils was approximately double that of the control soil, with respective C contents of 10.40 and 5.37 mg C g-1 bulk soil. The 0-10 cm NI heavy fraction C content gains resulted from both an increase in the C concentration of the heavy fraction, as well as an increase in the proportion of bulk soil mass in the heavy fraction (Figure 2). No significant changes in C content were observed in the intermediate and light fractions ($p > 0.84$), although the mean NR intermediate fraction C content was well above control at both 0-10 and 10-20 cm depth increments. At 0-10 cm, the mean NI intermediate fraction mass was significantly less than the control ($p < 0.03$), while the mean C concentration increased, but not to a significant extent ($p < 0.22$). The same trend

of intermediate fraction mass loss in tandem with an increase in C concentration was observed at 10-20 cm, but the changes were less pronounced. Indication of a loss in intermediate fraction mass was unique to the NI treatment, as we observed minimal deviation in intermediate fraction mass across all other treatments (Figure 2). The mean mass of the 0-10 cm NI light fraction was slightly less than control, with a corresponding increase in C concentration, but these changes were not significant ($p < 0.95$, $p< 0.37$ respectively).

Termination of root activity combined with the continuation of natural, aboveground litter inputs (NR treatment) led to a significant 54% and 42% increase in mean heavy fraction C content at 0-10 and 10-20 cm respectively ($p < 0.05$, Figure 1). Increases in C content were also observed in the intermediate fraction, although to a slightly lesser and non-significant ($p < 0.82$) extent compared to the change in heavy fraction C. At 0-10 cm, the increase in heavy C content from the NR treatment was driven by non-significant increases in C concentration ($p < 0.30$) and mass of the heavy fraction ($p < 0.84$). At the lower 10-20 cm depth increment, the increase in heavy C content was predominantly driven by an increase in C concentration. A similar non-significant increase in mean C concentration with a much smaller adjacent increase in mean fraction mass was observed in the NR intermediate fractions at 0-10 and 10-20 cm. The NR treatment led to a non-significant change of -5% in the mean light fraction C content at 0-10 cm. However, the non-significant change in light fraction C content was not straightforward, as the light fraction mass declined by 31%, while the C concentration increased by 41%, though

these changes were also not found to be significant. Previous analysis of the NR soil bulk density found an increase from 0.61 ± 0.08 to 0.79 ± 0.09 g cm-3 respectively, which corresponds with the decline in light fraction material in the soil (Pierson et al., in press). Thus, it appears likely that the observed increases in the C concentration of the NR light fraction at both 0-10 and 10-20 cm effectively negated greater C content losses from the decline in light fraction mass.

Ten years prior to this study, a limited fractionation procedure was performed on the 0-10 cm soil from the H.J. Andrews DIRT experiment plots (previously unpublished) that isolated the heavy fraction (>2.40 g cm-3) soil and analyzed the associated soil C concentration (Figure 3). At year 10 of the experiment, no significant differences in heavy fraction C were observed between the control and detrital manipulation treatments. However, by treatment type, the detrital manipulation effects on heavy fraction C are remarkably similar at year 10 and year 20, with no significant changes observed over such time.

Across treatments, the comparison of 0-10 cm heavy fraction C content versus the mass of fine roots reveals a direct inverse relationship (Figure 4). Root mass decreased substantially in the NR and NI treatment soil where heavy fraction C contents increased. Conversely, the DW treatment led to substantial increase in fine root mass along with a substantial decline in heavy fraction C.

The greatest increase in 0-20 cm soil C stocks came from the DW and NR treatments (Figure 5), although the changes were not significant ($p<0.98$). However, the observed increases differed substantially in the nature of change to soil C, with the DW treatment C stock gains confined to the light fraction C pool, while the NR soil C stock increases derived from gains in the intermediate and heavy fraction C pools. Despite vastly reduced above and belowground inputs, the NI treatment C stock was not significantly different from control due to the significant increase in heavy fraction C that offset losses of light fraction soil C. NL and DL soil C stocks were not significantly different from the control.

Discussion

Increases in stabilized soil C following root death

We initially hypothesized that root exclusion would lead to decreases in both POM and MAOM, as microbes continued to respire organic matter, but had little new inputs to form the building blocks for new MAOM sequestration. Roots have been shown to be critical detrital inputs for SOM stabilization (Rasse et al. 2005), and thus root reduction was expected to result in a sharp decrease in SOM in all fractions. In stark contrast, our findings show that the stability of bulk soil C stocks persisted despite root exclusion, with declines in the POM C pool offset by the accumulation in MAOM C pools. Increases in MAOM after 20 years of root exclusion indicate that a sustained reduction in both new root inputs and rhizosphere activity led to further accumulation of mineral stabilized soil C in these andic, temperate forest soils. These results directly support that MAOM accumulation is not saturated in these soils and that if limiting controls are removed,

substantial potential exists to increase the amount of stabilized soil C in the top 20 cm of soil.

Two potential pathways may have led to the observed increases in MAOM. First, an addition of dead root material to the subsurface soil may have increased high-quality substrates available to microbes, promoting increased soil C stabilization through greater production of microbial products (Cotrufo et al. 2013). A broad array of data suggests that most MAOM is comprised of microbial rather than plant residues (Kögel-Knabner 2002; Sollins et al. 2009; Miltner et al. 2012). Further, recent conceptual studies advocate that the formation of MAOM is primarily achieved in soil environments where high quality plant C inputs support high rates of microbial activity, such as in the rhizosphere, where greater carbon use efficiency increases the proliferation of microbial products, thus providing greater opportunity for MAOM formation (Cotrufo et al. 2013; Sokol et al. 2019). Based on these broad findings and proposed dynamics, we concur with the possibility that following the termination of live roots in the DIRT treatment plots, the abrupt increase in root detritus provided an abundant, high quality C source to drive the observed increase in MAOM. However, these soils have received a great amount of sustained root C input for millennia, suggesting that either the transfer of root C to MAOM may be limited by factors other than root C input quantity or that the rate of C loss from soils is more critical to determining soil C levels.

Alternatively, the lack of live root and rhizosphere activity may have led to a reduction in rhizodeposition-induced microbial priming of existing C stores, including MAOM stores, suggesting that that cessation of rhizosphere activity is playing an outsized role in both MAOM maintenance and MAOM accumulation. Root exudation is known to stimulate SOM decomposition, especially when soil N availability is low (Drake et al. 2013), and enzymatic stoichiometry and considerable empirical evidence suggests that this destabilization of the low C:N ratio MAOM pool occurs (Guenet et al. 2012; Drake et al. 2013; Murphy et al. 2015). Root exudation may also promote carbon loss by liberating organic compounds from protective associations with minerals (Keiluweit et al. 2015). There is other empirical evidence for this second pathway to explain the increase in MAOM in soils without root activity; Hopkins et al. (2014) found that increased root production from elevated CO2 did not result in increased MAOM due to increased priming of mineral-associated C.

We propose the following conceptual pathway for how the loss of rhizosphere C inputs and activity would lead to the observed increases in MAOM. With a loss of root inputs and rhizosphere activity, we expect that over the timescale of the twenty-year study, the eventual lack of live root exudation effectively shut down the microbe-rhizosphere priming effect throughout the soil profile, shifting the dominant subsoil microbial C processing pathways towards greater dependence on the more limited C inputs from POM and dissolved organic carbon (DOC) originating from the soil surface. However, with the combination of existing soil POM, the dead roots that remained available for

microbial processing for years to decades post-treatment, and aboveground inputs, microbial products could still accumulate on unsaturated mineral surfaces and existing MAOM. The observed differences in MAOM accumulation between the root exclusion (NR) and total input exclusion (NI) soils offer circumstantial support for this proposed pathway, where MAOM accumulation is actively limited by plant roots and associated rhizosphere activity. Both the NR and NI treatment soils experienced the same input of dead roots and reduction in root activity, yet greater amounts of MAOM accumulation were found in the NR soil, that had far greater surficial litter inputs relative to the NI soil. Because surface litter inputs in the NR plots provide more DOC to subsoil (Evans et al. 2020), either direct sorption of this DOC is occurring (Kramer et al. 2012) or else byproducts of microbial transformation of this DOC are being stabilized as MAOM (Sollins et al. 2006). While further study is required to determine the nature of the underlying mechanisms for the significant accumulations of MAOM we observed following root exclusion, our findings strongly support that roots are, in one capacity or another, strongly linked with MAOM accumulation in these forest soils, and that priming is a long-term phenomenon affecting soil C sequestration potential.

At an ecosystem level, the proposed conceptual pathway for the limitation of soil C stabilization due to rhizosphere priming matches soil responses observed in studies of forest harvest effects on soil C. In a global assessment of the consequences of different management practices on soil organic carbon (SOC) storage in forests, Achat et al. (2015) found that conventional harvests caused a decrease in C storage in the forest floor,

but this loss was compensated for by an accumulation of SOC in deeper soil layers. In contrast, they found that in intensive harvests where all logging residue and detrital material was removed, SOC was lost in all soil layers. Our data suggest that the removal of rhizosphere priming, when other detrital material remains, can stabilize soil C losses after harvest or wildfire, and can offer an explanation for the observation of C increase in deep soil horizons. As forests regrow, new priming losses coupled with increases in root and aboveground litter can stabilize soil C pools over succession.

Although MAOM has been reported to have a mean age, based on 14C dating, of 100-500+ years (Crow et al. 2009), our results demonstrate that extreme disturbance can cause a shift in MAOM pools quite rapidly. With the cessation of root activity, the NR and NI soils gained significant amounts of MAOM after twenty years of manipulation, but the appearance of this trend in year 10 of the experiment suggests that a substantial amount of this accumulation, and possibly even greater amounts of accumulation, occurred within the initial decade of the experiment. The mechanisms responsible for the old 14C age of MAOM, relative to POM, are not well understood (Trumbore 2009). MAOM C may persist in soil due to stabilization, however the old 14C age of MAOM may also derive from the long-term recycling of soil C between microbial and mineral pools (Gleixner et al. 2002; Rumpel and Kögel-Knabner 2011). Further study time for the DIRT experiment will be required to determine if continued respiration and loss of existing LF and dead root pools in the root removal treatments causes a reversal of the observed trend of increasing MAOM stocks with the removal of priming, and how long it

will take for this reversal to occur. At this time point in the experiment, our findings clearly show that MAOM is responsive to environmental disturbances and stocks of MAOM are likely more dynamic over annual to decadal timescales than often expected.

Buffering of root and detrital effects on soil C

Our findings suggest that soil C pools and stocks in the temperate forest study soils are well buffered from reductions in plant C inputs. Across all of the detrital manipulations in the DIRT experiment, soil C pools were the least affected by the surface litter exclusion treatment (NL) and remained closely similar to the untreated soil. While not initially intended as a treatment outcome, the NL treatment also led to the loss of approximately half of the fine roots in the soil (Pierson et al., in press). Despite this substantial reduction in the amount of both above and belowground plant C inputs to the soil, the subsequent effects on soil C processing were not substantial enough to significantly alter soil C stocks. These findings indicate that common forest disturbances such as infestation, drought, and low severity wildfire, which often lead to large, yet not complete reductions in soil C inputs, are not likely to drive considerable changes in forest soil C pools.

Surface litter quality and soil C pools

The quality of surface litter additions led to stark differences in POM accumulation. Surprisingly, additions of needle litter did not lead to discernible increases in POM in the DL soil. Yet, the large increase in POM in the DW soil suggests low quality material has greater opportunity to incorporate in the soil matrix, either due to slower rates of

decomposition increasing turnover time, or the physical size of the wood material serving to aid burial. As a side effect of the DW treatment and the associated change in bulk density, as well as N availability from the addition of low-N material, root mass was previously found to have approximately doubled in the DW 0-10 cm treatment soil (Pierson et al., in press). Similar to indications in the NR and NI treatment soils that live roots might inhibit MAOM accumulation, the increase in root mass in the DW soil coincided with a substantial decline in MAOM. Greater microbial mining of SOC from the heavy fraction was likely responsible for the decline in MAOM since no changes in the IF fraction pool size or concentration were indicative of POM inputs binding with MAOM and transferring soil C towards lighter density fractions.

Detrital effects on soil C pool stocks

Low quality wood additions greatly increased soil C stocks. With a sustained rate of input, wood debris additions may be viable for increasing soil C, but the LF soil C is not well sequestered, and the transference of the increased LF to stabilized HF remains uncertain. Further, these findings warrant that analyses of bulk soil C stocks may be misleading relative to underlying changes in soil C pools. For example, in this study we observed great soil C gains from low quality wood additions, but from the standpoint of observing change in soil C stabilization, the DW treatment resulted in soils with the least amount of stable MAOM. Specific processes and rates at which POM may transfer to MAOM are not well characterized, though it is commonly proposed that changes in POM will to some extent, propagate through to more stable soil C pools. However, this may not

be accurate, as the changes in soil C pools we observed following the detrital manipulations mostly occurred without coincident or in any way similar effects occurring across other soil C pools.

The higher quality needle litter additions increased soil C stocks due to gains in the IF and HF, but to a much lesser extent than observed from the wood debris gains in LF. We suspect these IF and HF gains from the added needle litter may be more persistent, yet further study is required to determine the exact timescale for the turnover of the additional sequestered C. Root exclusion also led to a net effect of increasing soil C stocks, despite losses of POM. While the observed effects on soil C stocks from cutting off root activity improve understanding of root derived control on soil C processing and stabilization, such a response provides limited use for management activities to promote soil C sequestration, as vastly reducing root activity is not conducive with maintaining a healthy forest environment.

Conclusion

The findings of this long-term study should help inform models that link roots and SOM, including soil C models of forest harvest and wildfire effects. We also suggest that models need to revise currently used relationships between litter inputs and soil C stocks. Over the next few decades, as the H.J. Andrews DIRT experiment continues, we look forward to following how the observed changes in soil C pools from the varied detrital manipulations proceed. Without roots, increases in MAOM may proceed to grow further

with adequate surficial inputs remaining available to drive greater accumulations, and ultimately, MAOM may then become limited by another environmental factor such as the saturation of reactive mineral surfaces. Alternatively, MAOM stocks in the root restricted soils may decline slowly as the declining availability of dead root material proceeds to provide less and less support for microbial activity and the production of the biomolecular precursors for MAOM. While we expect these future investigations will be greatly insightful for improving knowledge of root, microbial and mineral controls over soil C, future experiment effects on soil C may align less with natural processes because the experiment is now proceeding over a timescale greater than typically required for plants and associated root activity to recover from common disturbances. Further study time remains important to better determine how improved litter quality litter contributes to soil C stabilization and the timescale for associated influences on soil C dynamics. The increases in POM observed from the wood additions soils will also be interesting to follow over time. Knowledge remains limited for predicting how such a large change in POM may promote or prevent stabilization of soil C over longer timescales, as well as where soil C stocks will find equilibrium with sustained additions of woody material.

Tables and Figures

Table 2.1

Detrital manipulation treatment types..

Treatment	Abbreviation	Description
Control	CTL	Natural above- and belowground detrital inputs
Double litter	DL	Aboveground needle and leaf litter inputs doubled annually*
Double wood	DW	Double wood debris applied every other year as wood chips**
No litter	NL	Aboveground inputs removed annually in late fall season
No roots	NR	Live roots excluded via 0-140 cm tarp lined trenches around plots
No inputs	NI	Aboveground inputs excluded as in no-litter plots, belowground inputs are prevented as in no-roots plots

* Additional litter supplied from the litter exclusion plots and allocated proportionally

** Wood addition mass estimated to equal falling wood debris in the control plots.

Figure 2.1. Amount of carbon stored in specific density fractions (Light: <1.85 g cm^{-3}; Intermediate: 1.85-2.40 g cm^{-3}; Heavy: >2.40 g cm^{-3}) of bulk soil following 20 years of the DIRT experiment manipulations. (*) denotes significant difference from control (α = 0.05). Error bars represent standard error

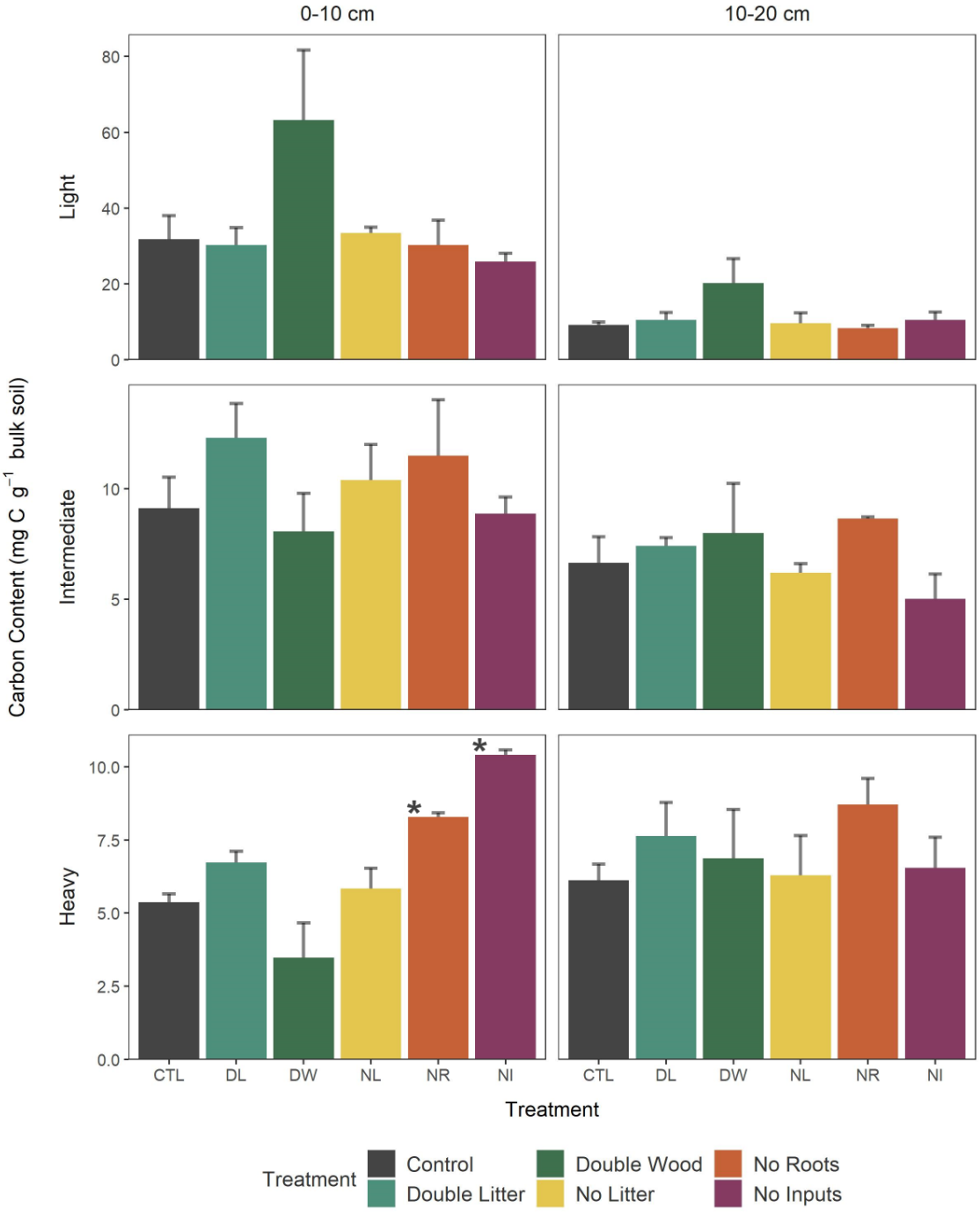


Figure 2.2. Carbon concentration and the proportion of bulk soil mass associated with the Light (<1.85 g cm^{-3}), Intermediate (1.85-2.40 g cm^{-3}) and Heavy (>2.40 g cm^{-3}) soil density fractions following 20 years of DIRT manipulations. Error bars represent standard error

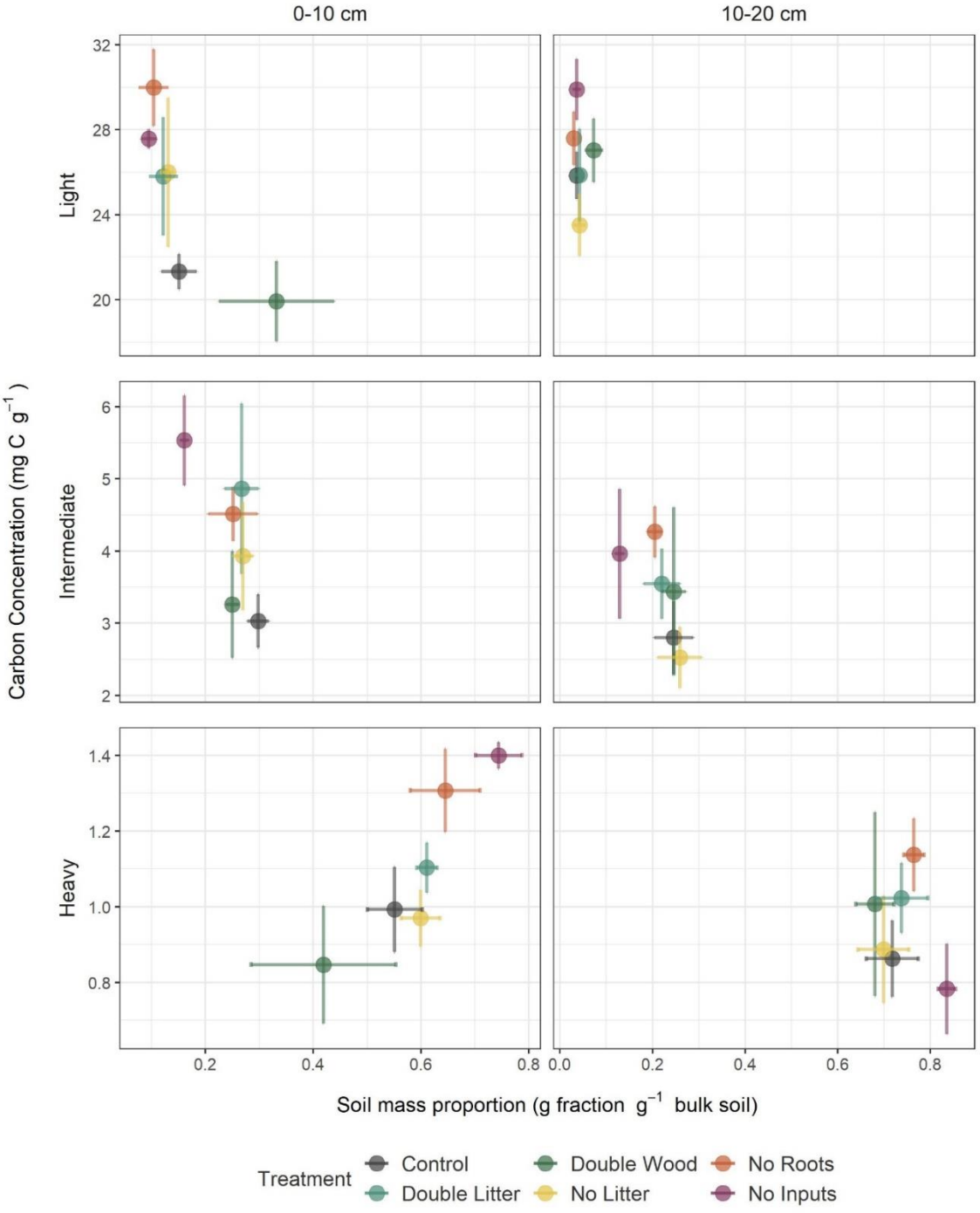


Figure 2.3. Carbon content of the heavy soil fraction (particles with density >2.40 g cm^{-3}) at a depth of 0-10 cm following 10 years of detrital manipulations. Differences between treatments are not significant (α = 0.05). Error bars represent standard error

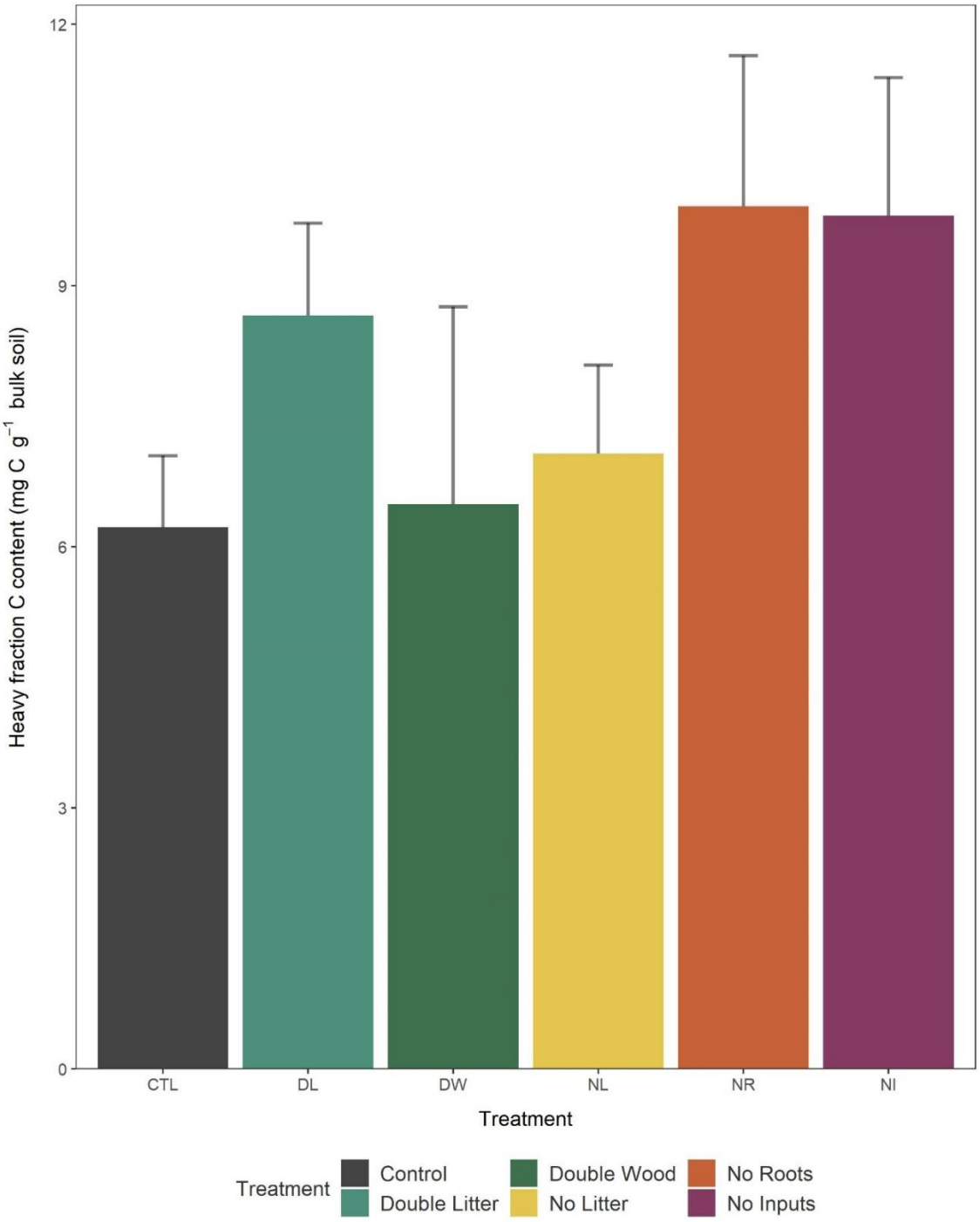


Figure 2.4. Relationship between the C content of soil particles with density > 1.85 g cm^{-3}, commonly referred to as the mineral associated organic matter (MAOM) soil fraction, and the mass of fine roots across the DIRT soils after 20 years of detrital manipulations. Error bars represent standard error

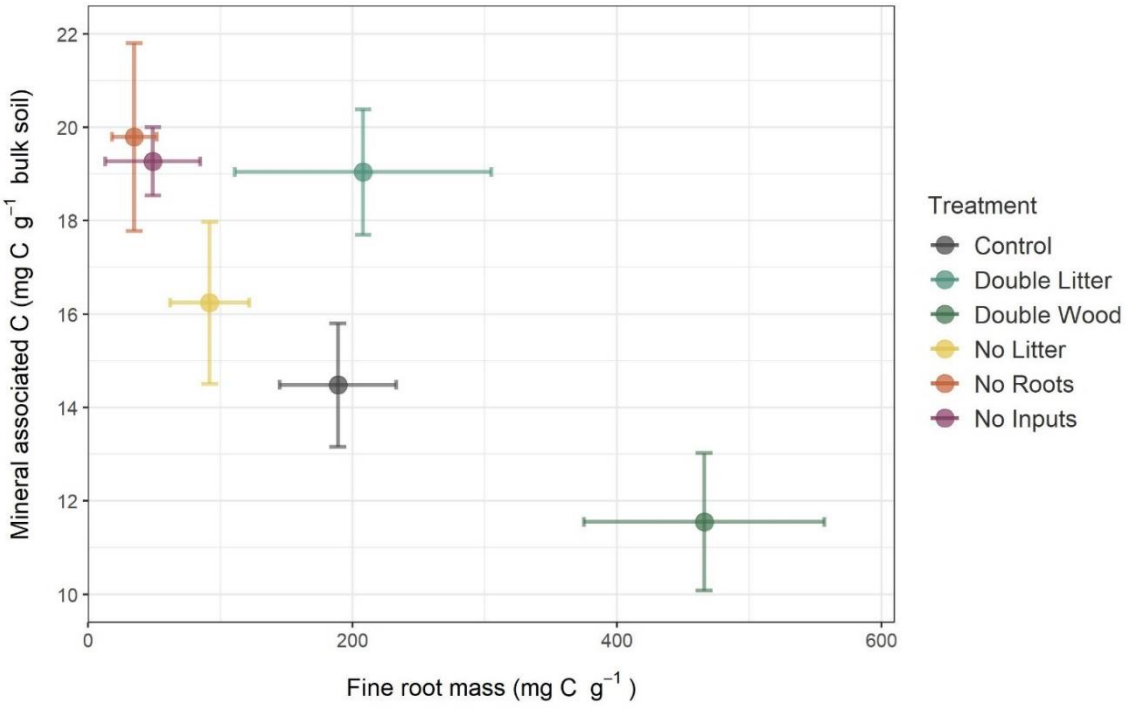
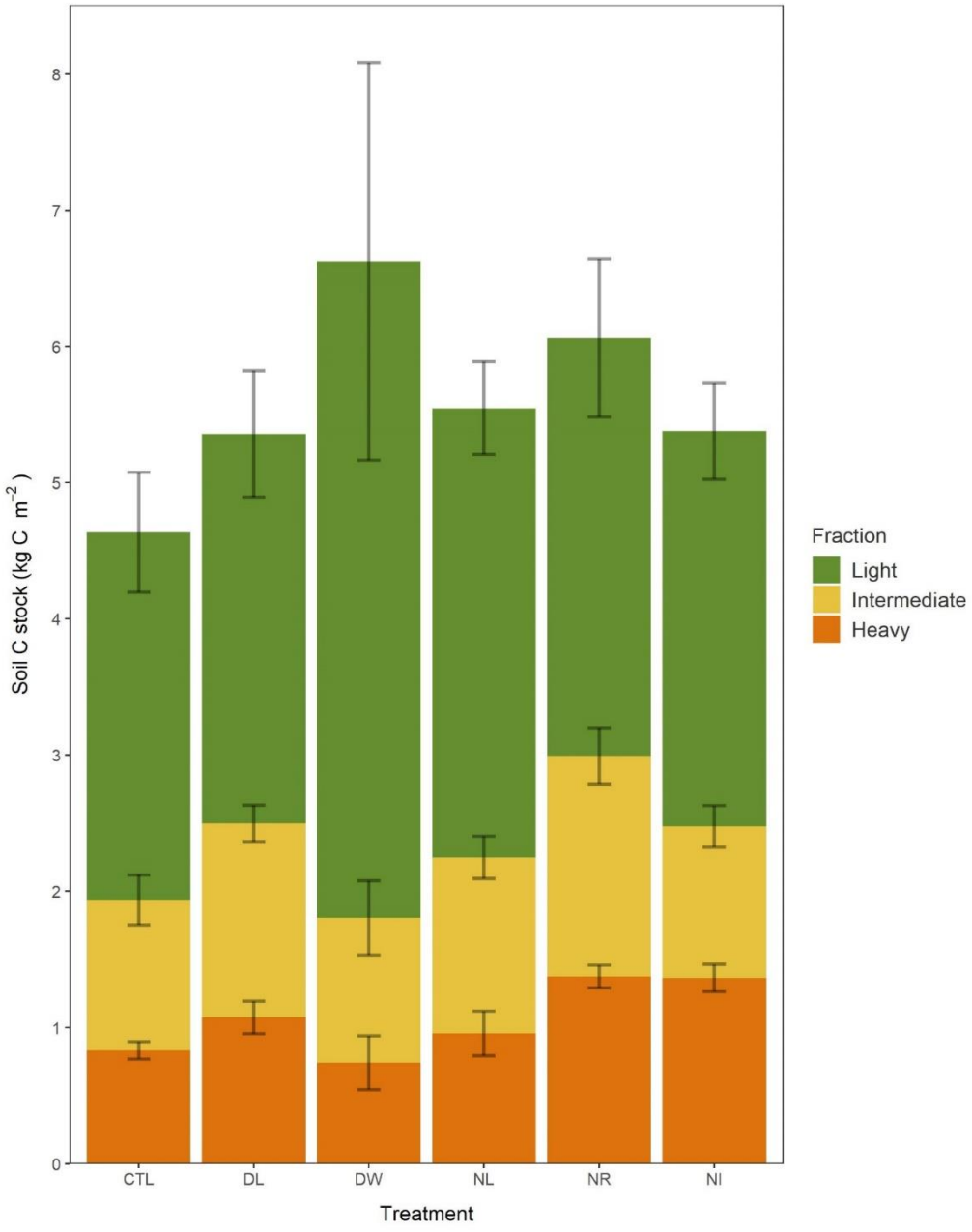


Figure 2.5. Total C stocks and the proportion of C stock in C pools separated by density fraction (Light: <1.85 g cm^{-3}; Intermediate: 1.85-2.40 g cm^{-3}; Heavy: >2.40 g cm^{-3}) from 0-20 cm in the DIRT soils after 20 years of detrital manipulations (CTL=Control, DL=Double Litter, DW=Double Wood, NL=No Litter, NR=No Root, NI=No Inputs). Error bars represent standard error



CHAPTER 3: Litter type and quantity controls on dissolved organic carbon production in a Pacific Northwest old-growth forest

Introduction

The storage and exchange of carbon (C) from soil are important processes that contributes to regulating the global C cycle and the productivity of terrestrial and aquatic ecosystems. Globally, soils store a massive amount of C, containing more C than the atmosphere and earth's vegetation combined (Crowther et al. 2016, Schuur et. al 2015). While soils serve as one of the most dominant stores of C on earth, they also exchange C with the atmosphere at annual rates far exceeding those from burning fossil fuels (Schimel et al. 2000). However, the processes and mechanisms through which climate and biogeochemical factors govern these exchange rates of C across natural soils remain poorly understood (Jackson et al. 2017). The lack of detailed knowledge regarding how soil C is formed and regulated continues to inhibit efforts to protect and promote soil C sequestration in the face of increasing natural disturbances and shifting climates.

Soil organic carbon (SOC) primarily originates from plant material, which enters the soil as either surface litter or wood debris, or via the subsurface soil in the form of dead roots, root exudates or dissolved organic C (DOC). The decomposition of particulate matter largely occurs near the soil surface or in close proximity to root detritus, while much of the remaining, deeper soil profile relies on soil C inputs from DOC, which is transported throughout the soil profile via leaching and diffusion. While less impactful on soil physical properties than particulate organic matter, dissolved organic carbon (DOC) is

widely recognized as an important C source supporting soil C accumulation (Kalbitz et al. 2008). Yet, the production rates of DOC from specific litter sources and the potential for DOC from these different sources to be either respired or stored as soil C has not been well characterized. In forest environments, where climate change is projected to increase productivity and alter litter inputs to soils (Melillo et al.
1993), it is important to determine how DOC from different litter sources is conveyed to soil C and which organic matter sources serve as dominant producers of DOC.

Plant materials in forest environments differ substantially in chemical composition and potential rates of DOC production (Joly et al. 2016, Lee et al. 2018). Deciduous leaves have much lower C:N ratios (i.e. high quality) than needle litter and wood material (high C:N, low quality) and typically have much faster rates of microbial decomposition (Pei et al. 2019, Remy et al. 2018). Further, disparate litter types also differ in their chemical structures, which may also govern rates of microbial decomposition and subsequent potential for either further processing or stabilization of DOC (Chen et al. 2020, Margida et al. 2020). Deciduous leaf litters are mostly composed of relatively simple organic compounds and thus are decomposed rapidly, typically over the course of weeks to months (Krishna and Mohan 2017). Needle litter from most conifer species is slower to decompose, often requiring a year or longer to fully breakdown, due to the presence of waxy coatings and internal tannins and other complex compounds, such as lignin and cellulose, which slow microbial decomposition rates (Gao et al. 2019, Zhang et al. 2008). Wood debris is by far the slowest decomposing form of common plant materials in forest

environments, with turnover times measured in decades to centuries due to a high C:N content and a chemical composition abundant in lignin and cellulose (Harmon et al. 2004, Zhou et al. 2007). Given the direct connection between decomposition and DOC production, the slow decomposition of wood material limits rates of DOC production relative to leaf and needle litter. However, the large volume of wood debris in certain forest environments, coupled with the long-term potential for sustained production of DOC, has led to recent hypotheses that wood material may be the dominant driver of streamwater DOC concentrations in some temperate forest ecosystems (Lajtha and Jones 2018). However, it remains uncertain whether the DOC produced from wood is more abundant in soil than DOC from other sources, or whether the chemical composition of wood DOC is simply more complex and thus more likely to avoid microbial decomposition as it passes through the soil profile.

The fate of DOC in soil is dependent on whether DOC is processed by microbes, stabilized by minerals, or passed through the profile to downstream waterways. Microbial processing of DOC is not uniform and the amount of C from DOC that is transferred to microbial biomass or respired to the atmosphere (e.g. carbon use efficiency, CUE) is dependent on the microbial community, nutrient availability in the surrounding soil environment, and the molecular and chemical composition of the specific DOC compounds. Recent studies and novel hypotheses pertaining to soil C stabilization suggest that more labile plant constituents are the primary precursors for stabilized SOC since they are more easily processed into microbial products, which dominate stabilized

SOC pools (Cotrufo et al. 2015, Robertson et al. 2019). However, other studies have shown that high quality soil C inputs may cause microbial priming effects (Crow et al. 2009a, b; Cordova et al. 2018; Sulzman et al. 2005). Priming refers to the microbial response to an addition of SOC that increases mineralization rates of previously stabilized SOC. These discrepancies reveal that there is not yet a clear consensus on the role of litter quality in stable SOM formation. However, it is apparent that DOC must play a key role in the formation of soil C stocks since the conversion of litter C to DOC is dependent on microbial decomposition, which has been widely associated with facilitating SOC accumulation in forest soils. Unraveling these connections between DOC and soil C is required to more accurately gauge how disturbance and climate effects on vegetation and associated litterfall quantity and quality may alter soil C stocks.

Numerous questions regarding the linkages between DOC and soil C accumulation led the following study. How does DOC production change in natural environments when a specific type of litter input increases or if litter inputs decrease? Does a specific type of litter (e.g wood debris, needle litter, deciduous leaves) support the lionshare of DOC transferred to the soil profile? Do soil microbes more quickly or efficiently process DOC from different litter sources? How sensitive are DOC production rates to changes in temperature that may arise from differences in where the litter resides along the soil profile, or from potential changes in climate? Answering these important pathway and mechanistic questions is vital to improve understanding of C transference from plant material to soil organic matter and for larger efforts to better balance C budgets across

forested regions. Based on these pervasive questions, the following study used both field and laboratory experiments to 1) quantify DOC production from dominant litter and wood debris sources over time, 2) determine how changes in litter quantity and quality alter DOC production in natural environments, 3) quantify the amount of DOC that is quickly respired by soil microbes across different litter types, and finally to 4) determine how the observed DOC production rates are influenced by changes in temperature. Prior to the study, we hypothesized that long-term increases in litter inputs to the soil surface would increase DOC concentrations in the soil water, with greater increases observed from additions of low quality wood material relative to additions of higher quality leaf and needle litter due to slower rates of microbial processing and less microbial priming response. We further hypothesized that aboveground litter is a greater source of DOC in soil than root activity and detritus, and thus a reduction in surface litter inputs would deplete surface organic matter sources for DOC production and lead to greater declines in soil water DOC concentrations relative to a reduction in root inputs. Finally, we proposed that DOC production is dependent on microbial activity and thus lower temperatures will reduce DOC production.

Methods

The detrital input and removal treatment (DIRT) experiment was established at the H.J. Andrews Experimental Forest, located in the western Cascade Mountain region of Oregon, USA (44º15’ N, 12º10’ W). Climate in the region is quasi-Mediterranean, with warm, dry summers and cool, wet winters, which yield the majority of annual

precipitation as a rain-snow mix between the months of December - April. Mean annual precipitation at the DIRT study site is 2080 mm yr-1 and the mean annual temperature is 9.4 C (average from years 1999-2014). Soils at the site are a mix of coarse loamy mixed mesic Typic Hapludands (Lajtha et al. 2005) and Andic Dystrudepts. The study site lies at an elevation of 726 m along with a south facing aspect. Slopes of < 5% steepness are consistent across the site. Erosion and overland flow are minimal, largely impeded by the gentle slopes and a thick (4-8 cm) organic soil horizon. Dominant overstory is mixed old-growth Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*), with a smaller mix of western redcedar (*Thuja plicata*), vine maple (*Acer circinatum*) and bigleaf maple (*Acer macrophyllum*). Large amounts of woody debris, overturned stumps and fallen logs are strewn across the forest floor due to the mature stand age and propensity for the shallow rooting Douglas-fir to topple from wind and snow burden.

The DIRT experimental design consists of six litter treatments that were initially established in 1997. The specific treatments include the addition of coarse woody debris, addition of natural leaf-needle litter mix, and the separate and combined exclusion of litter and roots (Table 1). Each detrital manipulation treatment is replicated across 3 individual, large plots (n = 3) and plot locations were assigned randomly to all treatments. Individual plots measure approximately 10 x 15 m and include natural features. Trees and understory vegetation were removed from the no input (NI) and no root (NR) treatments when the study began. Litter was excluded from the NL and NI treatments using 1 mm mesh screen cover over the plot surface to collect all falling debris. All litter material was

initially removed when the plots were first established and have since been kept bare in response to the screen and removal of litterfall. Litterfall from the NL plots has been systematically collected and transferred to the DL plots throughout the study period. In early stages of the study, litter was collected 4–5 times per year: at the end of the dry season, twice or more during the wet season (November to March), and at the beginning of the dry season (Lajtha et al. 2005). Since 2007, litter has been collected and transferred on an annual basis during the dry season. The annual basis for litter removal does allow some DOC flux into the NL and NI plots given that the organic debris is not immediately removed from the screens.

A mix of decomposed woody debris and shredded chips (5–20 cm in length) of Douglas-fir wood with a ratio of decomposed woody debris to intact woody debris of 4:1 have been added to the wood addition plots surface every other year since the study was initiated (Lajtha et al. 2005). The mass of this addition was estimated to be equal to falling fine litter mass in the control plots. Logs used for the wood chips were sourced from the surrounding forest area.. Roots were excluded from the NR and NI treatments using an impermeable barrier around the plot border that extends to 1 m deep in the soil and curves outward to redirect incoming roots away from the interior of the plot (Lajtha et al. 2005).

To allow for the extraction of water samples from the treatment soil matrix, 3 Prenart Superquartz tension lysimeters were installed at a 30 degree angle in each plot in 1997.

The lysimeters were installed at a depth of 30 cm in each plot according to the method described by Lajtha et al. (1999). The lysimeters were sampled on a monthly basis during the first three years of the DIRT study and were subsequently sampled multiple times per year until 2008. The consistency of sampling events decreased as the study progressed and no samples were obtained in 2009–2013. Lysimeter sampling resumed from 2014 to 2017 but was limited to two sampling events in 2014 and a single sampling event in the years 2015–2017. Not all lysimeters or plots were sampled during every sampling event because soil water content was too low for sampling or the lysimeter was not able to maintain suction. Samples were predominantly collected during the wet season (October to May) when sufficient soil moisture was available for water extraction. All samples were collected within 72 h of tension (- 50 kPa) being applied to the lysimeters. After extraction, samples were stored on ice and transferred to Oregon State University where they were frozen until analysis. Water samples were analyzed for DOC through Pt catalyzed high-temperature combustion using a Shimadzu TOC analyzer.

A laboratory incubation and leaching experiment was performed to directly observe DOC production rates from different litter sources present at the H.J. Andrews DIRT site. Litter types in the laboratory experiment included bigleaf maple *(Acer macrophyllum*) leaves, fresh needle litter and small diameter (<3 cm) wood debris from Douglas-fir (*Pseudotsuga menziesii*), 1-2 cm diameter chunks of approximately 20 year old Douglas-fir wood material, standing O-horizon material from the forest floor. All litter types were incubated individually, as well as combined with mineral soil from 0-10 cm depth

collected at the DIRT site. Each litter type was incubated and leached using a dry mass of 2.7 g and stored in a 50 ml plastic sample tube. A 1 cm diameter hole was drilled into the bottom of each sample table, along with a small layer of glass fiber material, which served to allow water to pass through the sample easily while preventing the loss of litter and soil material. For the litter + soil columns, 13.5 g of soil was mixed with the litter material to reach a litter to soil mass ratio of 1:5. The sample columns were incubated at temperatures of 4 C and 20 C to observe the effects of temperature on DOC production and respiration. Three replicate samples were used for each litter, soil and temperature sample combination. The C content of each litter type and the mineral soil was determined by combustion on an Elementar Solid CN analyzer and the C content of each litter and litter+soil combination was calculated in order to report DOC production in units of mg DOC per g litter C or soil + litter C.

The dilute cation and nitrogen solution was used for leaching the litter-soil columns to simulate the effects of natural rainfall. The sample columns received 30 ml of the rainfall solution every three days for 150 days. Samples were allowed to equilibrate over the first three leaching events (9 days) prior to analysis of DOC production and samples from this period were discarded. In the hour following the addition of the leachate solution, approximately 27 ml of the 30 ml of solution added to the columns was typically collected across all column sample types. At the end of the hour following the solution addition, the leachate samples were filtered using a pre-rinsed glass fiber filters (0.45 μm,

Whatman), then capped and frozen prior to DOC analysis on the Shimadzu TOC analyzer.

During the day following the addition of simulated rain solution to the sample columns, each column was placed in a sealed container for 1 hour and the rate of CO2 respiration was determined using a Picarro G1301 Gas Concentration Analyzer. Respiration measurements were completed at regular 3 day intervals, always the day following leaching, through day 75 of the incubation experiment.

Before analysis, the lysimeter DOC concentration data from the soil depth of 30 cm were averaged by plot, providing a total of three replicates per depth and treatment type for each sampling date. Seasonal trends in DOC concentration for each treatment and depth were assessed using data from the first 10 years of the study (1997–2006), when sampling dates and the number of samples recovered from each plot were most frequent. We tested for seasonal effects using both monthly and seasonally averaged DOC concentrations from each plot. For the seasonal averages, Fall included months October to December, Winter included months January to March, and Spring included months April to May. Summer was not included since no samples were collected in the dry season from June to September. Seasonal differences in DOC concentrations were determined using a linear mixed effects model with either month

or season as the fixed effect. Plot and treatment type were included in the model as random effects to account for treatment effects and repeated measures from the same plots over time.

Litter treatment effects on DOC concentrations were analyzed over the full, 20-year study period using mean plot DOC concentrations from 30 and 100 cm. Initially, we tested for treatment effects on DOC concentrations over time at both depth increments using a linear mixed effects model with year, treatment and the interaction between year and treatment included as fixed effects. Plot was also included in the model as a random effect to account for repeated measures. Based on an analysis of variance (ANOVA), time was not found to have a significant effect on DOC in this model, indicating the magnitude of treatment effects did not significantly change over the study period. Thus, we revised our statistical analysis to test for treatment effects on DOC concentrations irrespective of study time by adjusting our linear mixed effects model to include treatment as the only fixed effect, while retaining plot as a random effect. Post hoc Tukey honest significant difference (HSD) tests were then performed to ascertain significant differences among pairwise combinations of treatments.

Results

The DIRT litter manipulations resulted in significant effects on DOC concentrations at 30 cm soil depth when analyzed over the full 20-year period of the study ($p < 0.001$, Fig. 1). Effects on DOC concentrations from each of the detrital manipulation treatments

occurred rapidly and remained consistent over time, with no significant temporal trends in DOC concentration across monthly, seasonal and annual timescales. A clear difference in the effects of surface litter quality was evident throughout the study based on the difference in DOC at 30 cm between the double litter (DL) and double wood (DW) treatments. Additions of coarse woody debris increased DOC concentrations by 2.5 ppm (+58%, $p < 0.001$), while the additional input of aboveground leaf litter resulted in a decrease of 1.0 ppm (-30%, $p < 0.001$) in DOC concentration relative to the control soil DOC concentration. As expected, the root and litter exclusion treatments led to lower DOC concentrations relative to the control soil. The removal of surface litter (NL), root inputs (NR) and the combined removal of both above and belowground litter inputs (NI) all had similar effects on DOC concentrations, leading to approximately 2.5 ppm lower DOC concentration at 30 CM relative to the CTL treatment ($p < 0.001$ for all three treatments). Differences in DOC concentration between the exclusion treatments were not significant ($p > 0.10$).

Laboratory production rates of DOC by litter type, temperature and soil mixture are provided in Table 2. At a temperature of 20 ℃, the initial production of DOC from the Big Leaf Maple leaves was far greater than for any other litter type, with a mean DOC production of 2.9 mg DOC-C g-1 leaf C in the first week of decomposition and leaching (Figure 2). DOC production rates at 20 ℃ for all of the other litter and wood debris types were more consistent (Table 2), ranging between 0.12-0.67 mg DOC-C g-1 material C. The old wood debris had the lowest initial DOC production rate.

Of the litter types observed that had not decomposed substantially prior to the experiment (maple leaves needles, fresh wood debris), only the maple leaves and fresh wood debris showed substantial declines in DOC production rates over time, while the fresh needle litter DOC production remained relatively constant throughout the 150 day experiment. At the conclusion of the leaching experiment, maple leaves remained the highest producer of DOC yielding 0.46 mg DOC-C g-1 leaf C. However, sustained DOC production from the organic horizon material was only slightly less at the end of the study period, yielding 0.39 mg DOC-C g-1 organic horizon C. Fresh wood debris consistently outproduced the DOC production rate of the old wood by approximately double throughout the study period.

The difference in incubation temperature between 20 ℃ and 4 ℃ had varied effects on DOC production across the different litter types. Temperature had no effect on the DOC production from Maple leaves and the Douglas-fir needle litter. However, the lower 4 ℃ temperature had a strong negative effect on DOC production from fresh wood debris and the organic horizon material, with declines ranging from 30-51%. The effects of the lower 4 ℃ temperature on old wood debris and the bare mineral soil were inconsistent throughout the study period.

The effect of mixing litter and wood debris with mineral soil was mostly consistent in causing a decline in DOC production. The Maple leaves, fresh wood debris and organic

horizon DOC production rates all declined substantially when mixed with soil, with declines measured at the end of the study period ranging from 18-36%. Old wood debris, needle litter and mineral soil were less responsive to the cold temperature, with minimal differences in DOC production observed between temperature treatment groups throughout the study.

Respiration rates were not consistently proportional with rates of DOC production across the litter and wood debris decomposition experiment. The Maple leaf litter respired the greatest amount of CO2 at the beginning of the incubation, across all temperature and soil treatments (Figure 3). Yet, the disparity between respiration rates from the Maple leaf and Douglas-fir needle litter was consistently less than observed for DOC production rates (Figure 4). After the initial 3 weeks of incubation, the warm (20 ℃) needle litter consistently respired more CO2 than the maple leaf litter for the remainder of the 150-day period. Across all of the organic material incubation types, respiration rates were consistently reduced by colder temperature (4 vs. 20 ℃) . Respiration rates for the leaf, needle and organic horizon litter types were the most responsive to the temperature treatment, with greater rates of respiration observed in the warm treatment throughout the entire study period. Respiration rates for the fresh wood debris were substantially greater than for the old wood debris at 20 ℃, but the difference between the two wood types was less pronounced at 4 ℃. The organic horizon material respiration rates were reduced by ~50% when mixed with mineral soil and remained strongly similar to respiration rates from the needle litter throughout the entire incubation period. The ratio of organic

material C lost as either CO2 or DOC varied substantially by litter type (Figure 4), with the leaf and needle litters transferring more C to CO2 relative to the amount transferred to DOC by a margin of ~2:1 relative to ~1:1 margin of CO2 to DOC loss for the wood debris.

Discussion

Temporal trends

In field DIRT plots at 30 cm soil depth, DOC concentrations responded rapidly to the litter treatments, quickly reaching a new steady state for DOC production instead of gradually changing over annual timescales prior reaching a new equilibrium. The rapid onset of change was contrary to our initial hypotheses that litter treatment effects would be cumulative over time as either more litter was added (DW and DL treatments), or as the size of SOC pools declined due to reduced amounts of detrital input (NR, NL, NI treatments). These findings suggest that DOC production is strongly linked to fresh detrital sources of organic matter and will respond quickly to disturbances that alter detrital input quantity and quality.

The lack of significant observed trends in soil solution DOC concentrations across the DIRT soils between fall, winter and spring was unexpected, however numerous other studies have also found no evidence of seasonal DOC trends in soil solution at seasonal timescales (Solinger et al. 2001; Froberg et al. 2006). Elevated rates of litter decomposition in spring and fall seasons where temperatures and soil moisture contents

are conducive to microbial activity typically result in increased DOC concentrations relative to the cooler or drier months (Fellman et al. 2009; Laudon et al. 2004). Further, and specific to the H.J. Andrews Forest DIRT experiment, Lajtha et al. (2005) previously found different DOC concentrations at the soil surface between seasons for certain treatment types. We suggest three potential mechanisms to explain why we did not detect seasonal DOC trends from the 30 cm soil water: (1) the sampling frequency was insufficient to detect seasonal differences, (2) litter sources were able to sustain a steady DOC production rate in winter despite low temperatures, and/or (3) seasonal trends did occur, but were experienced almost entirely in preferential flow paths that were not sampled by the tension lysimeters. The transport of DOC through preferential flow paths provides a direct explanation for the inconsistent observations of seasonal DOC trends between studies focusing on streamwater versus soil solution (Solinger et al. 2001; Froberg et al. 2006). Preferential flow paths can be a significant pathway for infiltration in forest soils, especially for sandy soils under sustained unsaturated conditions similar to those at the DIRT study site (Kung 1990; Ritsema et al. 1993; Hagedorn and Bundt 2002).

Detrital treatment effects

Over the full twenty year study period, mean annual DOC concentrations at 30 cm were clearly different among treatments (Fig. 1). We hypothesized that DOC concentrations would be greater in the litter addition treatments (DW and DL) despite the potential effects of microbial priming from the additional inputs. Our findings show that priming

phenomena are a substantial control on DOC production, as the mean DOC concentration for the DL treatment at 30 cm decreased by 30% relative to the CTL (3.7 versus 5.3 mg C/L) despite approximately double the amount of annual litter C inputs. Previously, Lajtha et al. (2005) reported that DOC concentrations in zero-tension lysimeters placed immediately below the O-horizon in the DL treatment plots were approximately 15 mg C/L greater than the CTL treatment after 6 years of litter manipulation. The discrepancy in DOC concentration between the mineral soil surface and 30 cm depth suggests that the rate of DOC consumption was approximately 1.5 times (56%) greater between the surface and 30 cm under the DL treatment relative to the CTL. This accelerated consumption is greater than the 11.5–34% increase in C loss to respiration from priming reported previously (Sulzman et al. 2005; Crow et al. 2009a, b), although similar in magnitude. Previous studies (Cordova et al. 2018; Sulzman et al. 2005; Crow et al. 2009a, b) have shown that respiration can accelerate more rapidly than carbon assimilation rates when priming occurs, and indeed, SOM stabilization and SOM concentrations have not increased in DL plots compared to control plots (Pierson et al. 2020) . Therefore, the observed priming effect in our study provides evidence that higher input rates of labile C (leaf litter) may not result in higher rates of stable SOC formation.

The DW treatment had a mean DOC concentration more than 2 times greater than the DL treatment at 30 cm (8.4 mg C/L vs. 3.7 mg C/L), which supports our hypothesis that litter comprised of partially decomposed woody debris is more effective at transporting DOC to deep soil horizons than detritus composed of high-quality leaf and needle litter. DW

DOC concentrations at the soil surface were previously measured as ~100 mg C/L (Lajtha et al. 2005) which is 65 mg C/L greater than the CTL treatment (Table 3). Like the DL treatment, increased litter inputs accelerated C consumption rates. The rate of DOC consumption was approximately 3-fold greater between the surface and 30 cm under the DW treatment relative to the CTL. Therefore, wood derived DOC was subjected to a greater increase in consumption than the DL treatment. However, DOC concentrations in this treatment were still ultimately greater at 30 cm than the CTL and DL treatments. We believe that the DW treatment led to the highest observed DOC concentration because (1) the wood material is slower to decompose, thus building up in the soil over time and (2) wood derived DOC has a greater proportion of structurally complex and nutrient poor C molecular products which may slow microbial decomposition rates, allowing greater time for DOC to reside in the soil profile (Spears and Lajtha 2004; Yano et al. 2005).

The litter exclusion treatments (NL, NR, NI) had an average concentration that decreased by 46% relative to the CTL at 30 cm, yet we observed minimal differences in DOC concentration between the exclusion treatments. These findings suggest that the linkages between DOC production and litter inputs are not simple and that other controls, such as microbial processing and mineral desorption of DOC may exhibit greater control over DOC production as detrital inputs become more limited. The influence of these other controls is further evident by the non-additive effect on DOC observed from reducing both above and belowground detrital inputs in the NI treatment soils, where we observed

DOC concentrations starkly similar to those in the NR and NL soils. Sorption–desorption reactions have been proposed as the dominant mechanism to control soil solution chemistry in subsurface soil horizons previously (Yano et al. 2005; Froberg et al. 2006) and thus we suspect that under the induced, low detrital input conditions, the desorption of already stored C products from the soil matrix beame a greater source for of DOC. However, it remains uncertain whether these mineral sources will become eventually become exhausted with over longer timescales, leading to more severe declines in DOC production.

DOC production and respiration rates

Our laboratory findings are consistent with previous studies showing that deciduous leaf litter is a potent, yet short-lived contributor to DOC production (Lee at al. 2018). The production of DOC from the Big Leaf Maple leaves was approximately 5 times greater than any other dominant form of litter or wood type during the early stage of decomposition (<50 days). However, the maple leaf DOC production rates declined more rapidly than for the other litter types tested. Incorporation of the maple leaves in mineral soil served to further speed decomposition rates, a phenomena that was not observed for needle litter. Fresh wood debris also had initial DOC production rates that were much higher than the baseline observed after 150 days of incubation, suggesting that fresh wood inputs may increase DOC to a greater extent than observed from older woody debris. The elevated DOC production from fresh wood may be in part responsible for

short-term increases in streamwater DOC concentrations often observed following forest harvest and low severity wildfire.

Changing temperature had greater effects on DOC production rates for the wood debris than for the leaf and needle litter. These findings reflect the active limitations on DOC production for different litter materials. The decrease in DOC production at lower temperatures for wood debris indicates that microbial activity is predominantly governing the rate of DOC production. Conversely, for the leaf and needle litter, DOC production may be limited by other factors such as physical access, and thus DOC production from those litter types may be less responsive to changes in temperature. DOC production from the organic horizon material was also greatly affected by a change in temperature, suggesting that DOC production in the forest floor is dependent on rates of microbial activity and not strictly confined by nutrient availability or recalcitrance of the organic material. Overall, findings from the litter incubation experiment directly convey that the dominant control on decomposition rates for specific organic materials must be accounted for when predicting effects of changing temperatures on DOC production. Extrapolating our findings to a larger scale, the observed effects of temperature on DOC production rates suggest that temperate forest ecosystems with large stocks of woody debris, such as our experimental site at the H.J. Andrews Experimental Forest, may experience greater effects on DOC production under changing temperatures.

The observed ratio for litter and wood C losses from respiration versus leaching of DOC indicate that a greater proportion of wood-C may be transferred to waterways relative to the amount of DOC-C loss from leaf and needle litters. Over the course of the leaching experiment, the leaf and needle litters lost more C to respiration by a factor of ~2:1 relative to the C losses from the wood debris. These findings offer a potential mechanism for how wood debris may have greater control over streamwater DOC concentrations relative to leaf and needle litter inputs to soil (Lajtha and Jones 2018). While initial decomposition rates of DOC production are higher for leaf and needle litters relative to wood debris, these rates are short-lived compared to the sustained DOC production from wood debris over decadal to century timescales. The greater potential for wood-C to be conveyed to soil as DOC rather than lost via respiration fits with our observed results from the DIRT lysimeters, where we found a substantial increase in soil solution DOC at 30 cm soil depth from the DW treatment. Conversely, the DL treatment led to a decline in DOC at the same soil depth. Together, the field and laboratory findings strongly indicate that wood debris is the dominant source of DOC in the temperate forest study soils. Further, increases in leaf and needle litter inputs are not likely to promote soil C accumulation via microbial processing pathways dependent on DOC inputs due to the potential for priming effects and generally higher rates of respiration relative to DOC production.

Tables and Figures

Table 3.1

Description of detrital manipulation treatments.

Treatment	Abbreviation	Description
Control	CTL	Natural above- and belowground detrital inputs
Double litter	DL	Aboveground needle and leaf litter inputs doubled annually*
Double wood	DW	Double wood debris applied every other year as wood chips**
No litter	NL	Aboveground inputs removed annually in late fall season
No roots	NR	Live roots excluded via 0-140 cm tarp lined trenches around plots
No inputs	NI	Aboveground inputs excluded as in no-litter plots, belowground inputs are prevented as in no-roots plots

* Additional litter supplied from the litter exclusion plots and allocated proportionally

** Wood addition mass estimated to equal falling wood debris in the control plots.

Table 3.2

DOC production rates for by litter type, temperature, soil mixture and incubation time.

Temperature	4 °C			20 °C		
Incubation Day	12	50	140	12	50	140
DOC Production with litter only (mg DOC-C g litter-C^{-1} d^{-1})						
Maple leaves *(Acer macrophyllum)*	2.94 ± 0.59	1.10 ± 0.14	0.37 ± 0.02	3.16 ± 0.51	1.07 ± 0.08	0.44 ± 0.04
Needles *(Pseudotsuga menziesii)*	0.47 ± 0.09	0.33 ± 0.02	0.15 ± 0.01	0.59 ± 0.07	0.39 ± 0.02	0.40 ± 0.06
Fresh Wood Debris *(Pseudotsuga menziesii)*	0.29 ± 0.05	0.10 ± 0.01	0.08 ± 0.01	0.44 ± 0.20	0.20 ± 0.09	0.21 ± 0.07
Old Wood Debris *(Pseudotsuga menziesii)*	0.17 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	0.11 ± 0.01	0.13 ± 0.02	0.11 ± 0.01
O-Horizon Layer	0.34 ± 0.03	0.26 ± 0.02	0.22 ± 0.02	0.55 ± 0.03	0.36 ± 0.02	0.36 ± 0.06
Litter-Mineral soil mix (mg DOC-C g soil mix-C^{-1} d^{-1})						
Maple leaves *(Acer macrophyllum)*	2.13 ± 0.11	0.24 ± 0.03	0.13 ± 0.01	1.86 ± 0.26	0.23 ± 0.02	0.15 ± 0.03
Needles *(Pseudotsuga menziesii)*	0.36 ± 0.13	0.08 ± 0.01	0.06 ± 0.01	0.31 ± 0.05	0.10 ± 0.01	0.26 ± 0.09
Fresh Wood Debris *(Pseudotsuga menziesii)*	0.54 ± 0.23	0.11 ± 0.02	0.07 ± 0.02	0.35 ± 0.06	0.21 ± 0.07	0.12 ± 0.02
Old Wood Debris *(Pseudotsuga menziesii)*	0.18 ± 0.02	0.10 ± 0.01	0.06 ± 0.01	0.23 ± 0.02	0.11 ± 0.01	0.07 ± 0.02
O-Horizon Layer	0.17 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.30 ± 0.01	0.21 ± 0.01	0.31 ± 0.09
Mineral Soil	0.15 ± 0.01	0.12 ± 0.02	0.08 ± 0.01	0.17 ± 0.01	0.13 ± 0.01	0.12 ± 0.01

Table 3.3
CO_2 respiration rates for by litter type, temperature, soil mixture and incubation time.

Temperature	4 °C			20 °C		
Incubation Day	9	30	75	9	30	75
CO_2 Respiration with litter only (mg CO_2-C g litter-C^{-1} d^{-1})						
Maple leaves *(Acer macrophyllum)*	4.46 ± 0.25	2.65 ± 0.18	1.57 ± 0.13	3.59 ± 0.02	1.61 ± 0.1	1.12 ± 0.08
Needles *(Pseudotsuga menziesii)*	2.34 ± 0.08	2.05 ± 0.05	2.42 ± 0.01	0.75 ± 0.01	1.04 ± 0.12	0.9 ± 0.04
Fresh Wood Debris *(Pseudotsuga menziesii)*	1.14 ± 0.26	0.74 ± 0.06	0.77 ± 0.06	0.51 ± 0.08	0.37 ± 0.09	0.32 ± 0.03
Old Wood Debris *(Pseudotsuga menziesii)*	0.36 ± 0.04	0.26 ± 0.01	0.28 ± 0.04	0.11 ± 0.01	0.09 ± 0.01	0.16 ± 0.04
O-Horizon Layer	2.29 ± 0.29	1.95 ± 0.26	1.62 ± 0.15	0.51 ± 0.04	0.72 ± 0.08	0.72 ± 0.04
Litter-Mineral soil mix (mg CO_2-C g soil mix-C^{-1} d^{-1})						
Maple leaves *(Acer macrophyllum)*	4.86 ± 0.32	2.99 ± 0.07	1.78 ± 0.02	2.91 ± 0.45	1.42 ± 0.13	1.02 ± 0.04
Needles *(Pseudotsuga menziesii)*	3.48 ± 0.12	2.68 ± 0.11	1.93 ± 0.06	1.09 ± 0.23	1.19 ± 0.19	0.81 ± 0.1
Fresh Wood Debris *(Pseudotsuga menziesii)*	1.66 ± 0.11	1.11 ± 0.05	0.54 ± 0.19	0.64 ± 0.12	0.34 ± 0.09	0.23 ± 0.03
Old Wood Debris *(Pseudotsuga menziesii)*	0.77 ± 0.08	0.46 ± 0.02	0.52 ± 0.05	0.19 ± 0.04	0.19 ± 0.02	0.18 ± 0.04
O-Horizon Layer	2.17 ± 0.05	1.89 ± 0.13	1.78 ± 0.07	0.64 ± 0.07	0.72 ± 0.04	0.53 ± 0.03
Mineral Soil	0.19 ± 0.04	0.3 ± 0.05	0.14 ± 0.01	0.13 ± 0.03	0.07 ± 0.02	0.06 ± 0.01

Figure 3.1. Dissolved organic carbon (DOC) concentrations for soil solution collected at 30 cm soil depth for each of the detrital manipulation treatments. [A,B] Mean annual DOC concentrations for the addition and removal treatments relative to the control soil. [C] Mean DOC concentration by treatment type over the 20 year study period.

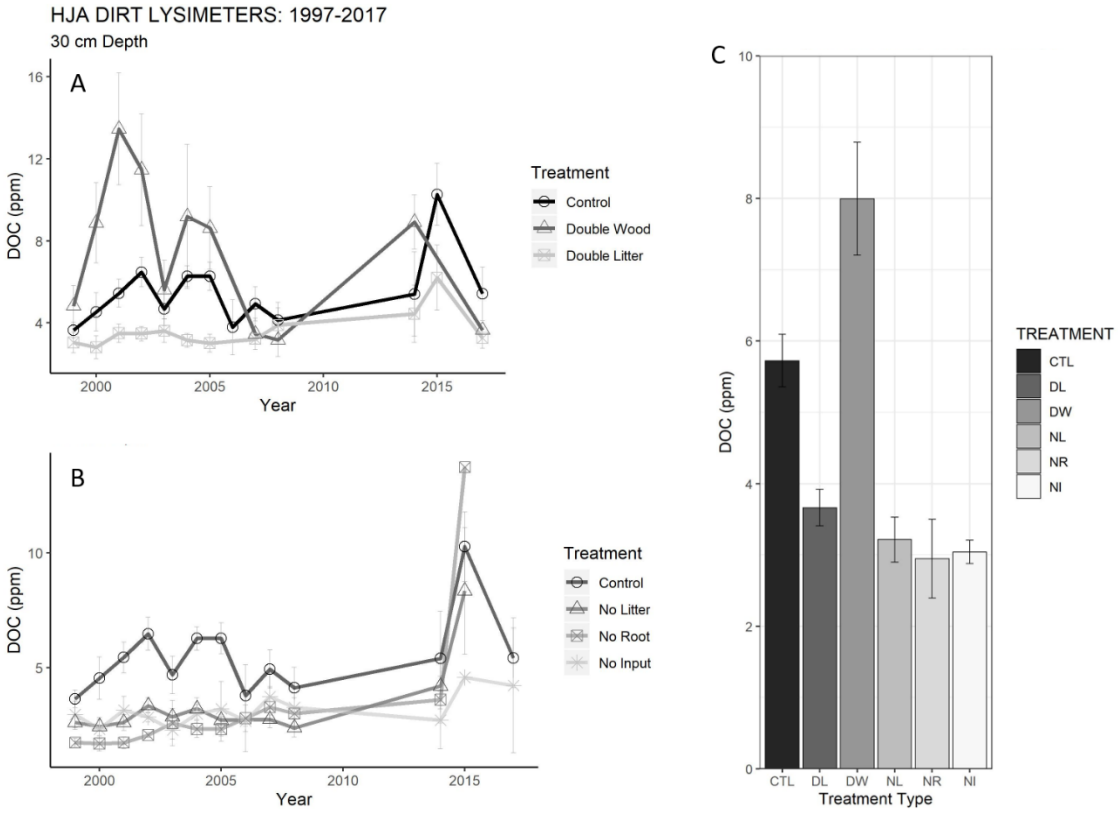


Figure 3.2. Dissolved organic carbon (DOC) production rates over time by organic material type, soil mixture, and incubation temperature.

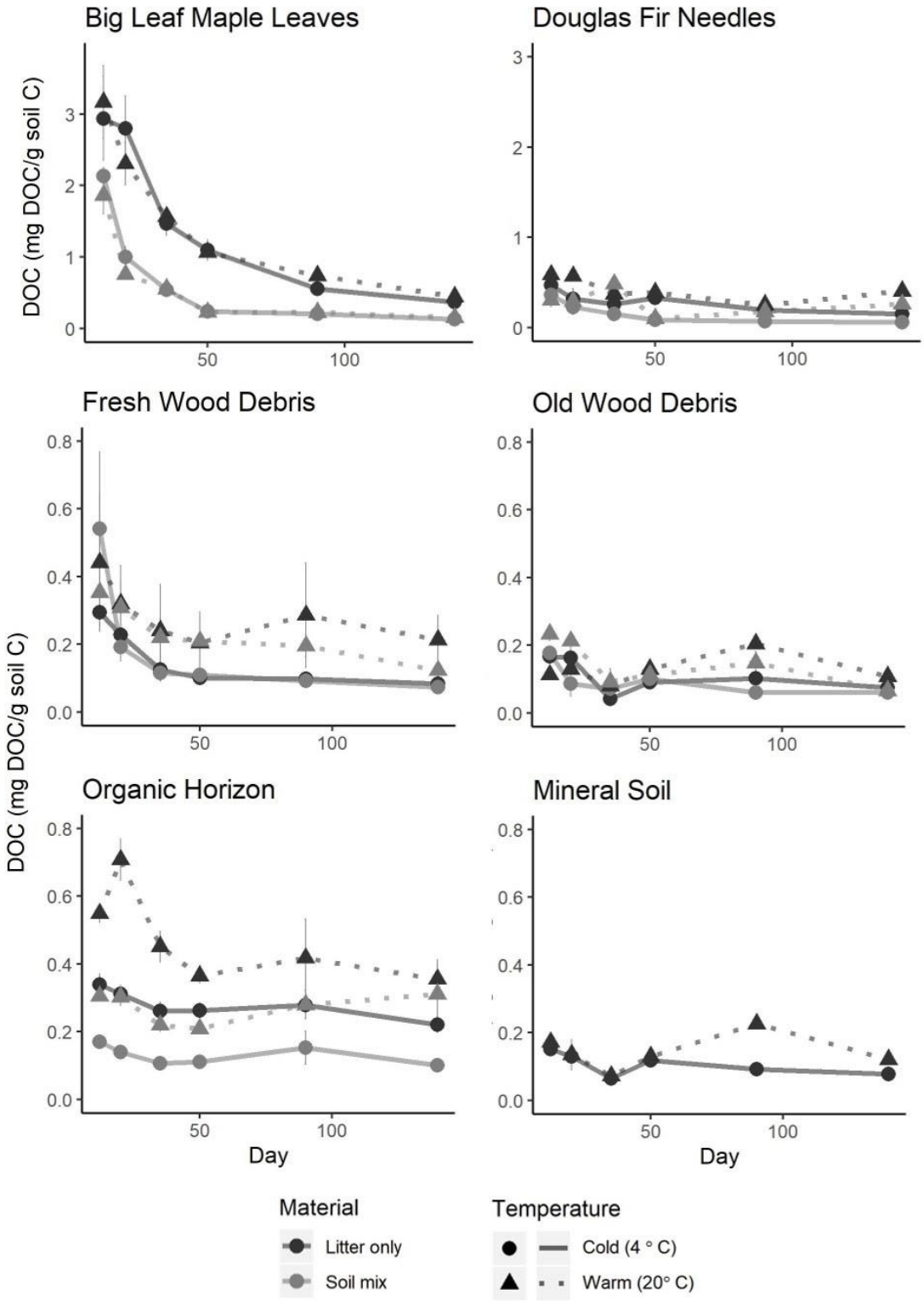


Figure 3.3. CO_2 respiration rates over time by organic material type, soil mixture, and incubation temperature.

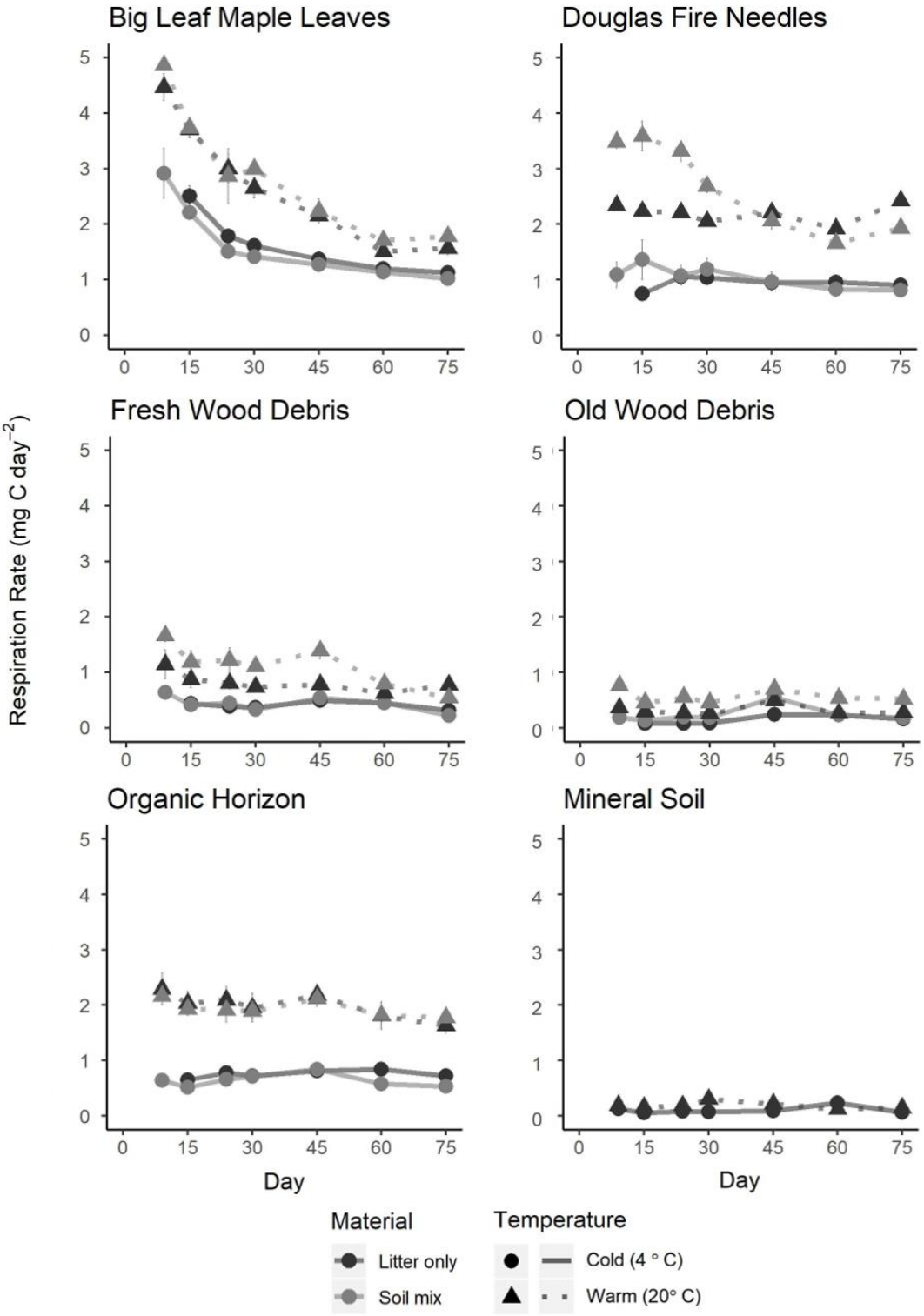
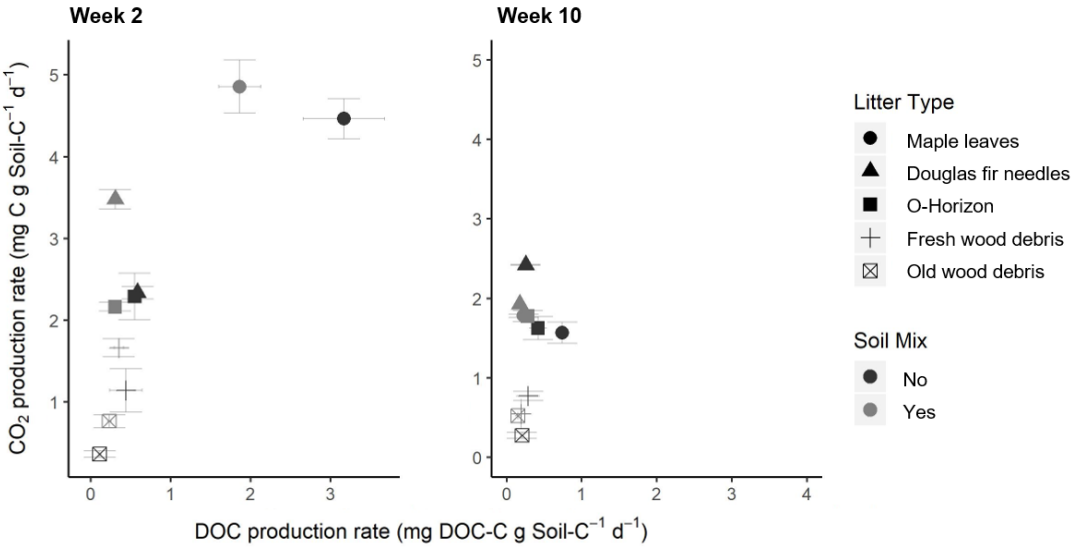


Figure 3.4. Production of CO_2 relative to DOC from different organic material types when allowed to decompose separately or with the inclusion of mineral soil.



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