



# Mineral stabilization of soil carbon is suppressed by live roots, outweighing influences from litter quality or quantity

Derek Pierson · Lucas Evans · Kamron Kayhani · Richard D. Bowden ·  
Knut Nadelhoffer · Myrna Simpson · Kate Lajtha

Received: 30 September 2020 / Accepted: 29 April 2021 / Published online: 11 May 2021  
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**Abstract** Conserving soil carbon (C) and harnessing the potential for soil C sequestration requires an improved understanding of the processes through which organic material accumulates in soil. Currently, competing hypotheses exist regarding the dominant mechanisms that control soil C accumulation and transfers to mineral-associated pools. Long-standing hypotheses rely upon an assumed strong relationship between the quantity of organic inputs and soil C accumulation, while more recent hypotheses have

shifted the focus towards the more complex controls of root activity, microbial processing and priming, and organo-mineral complexation. The Detrital Input and Removal Treatment (DIRT) experiment can test these competing hypotheses through field manipulations of detrital inputs. After 20 years of detrital manipulations in the wet, temperate forest of the H.J. Andrews Experimental Station, we found that with the termination of live root activity, the significant influx of dead root material and absence of soil priming by roots led to decreases in particulate organic matter (POM), but increases in stable mineral associated organic matter (MAOM). This suggests that soil mineral particles in undisturbed soils are not saturated with C in the presence of live roots and that pools of MAOM are sensitive to the balance between microbial-induced stabilization and microbial-induced priming and destabilization. Twenty years of aboveground litter removal did not change bulk soil C stocks or pools. Soil C stabilization did not increase in response to increases in high quality litter inputs, in contrast to recent theory, but in accordance with other empirical results. In contrast, increases in low quality wood litter led to a large increase in bulk soil C, with gains over 20 years confined to increases in POM. These findings offer insight into the pathways controlling soil C contents and provide potential explanations for the often-limited potential to increase mineral associated soil C in many vegetated soils and observed buffered responses of soil C stocks to disturbances such as drought, fire, and timber harvest.

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Responsible Editor: Stuart Grandy

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D. Pierson · L. Evans · K. Kayhani · K. Lajtha  
Department of Crop and Soil Sciences, Oregon State  
University, Corvallis, OR 97331, USA

D. Pierson (✉)  
Department of Biological Sciences, Idaho State  
University, Pocatello, ID, USA  
e-mail: derekpierson@isu.edu

R. D. Bowden  
Department of Environmental Science, Allegheny  
College, Meadville, PA, USA

K. Nadelhoffer  
Department of Ecology and Evolutionary Biology,  
University of Michigan, Ann Arbor, MI, USA

M. Simpson  
Environmental NMR Centre and Department of Physical  
and Environmental Sciences, University of Toronto,  
Toronto, ON M1C1A4, Canada

**Keywords** Soil carbon · Roots · Stabilization · Fractionation · Litter manipulation · Long-term

## Introduction

As unprecedented 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 (Trumbore 1997; Pugnaire et al. 2019). Forecasted changes in atmospheric carbon dioxide, temperature and precipitation will impact plant community composition as well as net primary production, thereby serving to alter the chemistry and amounts of above and below ground detrital inputs to soil (Norby and Zak 2011; Fernández-Alonso et al. 2018; Heath et al. 2005). 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 (i.e., mineral-associated soil C formation) remain poorly understood. Further, how these changing inputs will interact with soil microclimate, soil microbes, and soil mineralogy to determine the response of diverse soil carbon stores is critical knowledge needed for earth system models (Sulman et al. 2018; Wieder et al. 2018), as well as to better determine whether soils will perform as net C sinks or sources for atmospheric C under future conditions (O'Rourke et al. 2015; Jackson et al. 2017; Malhotra et al. 2019).

Soil C exists as a heterogeneous mixture of decomposing and stabilized soil organic matter (SOM), with varied chemical properties and turnover rates. To investigate how these diverse chemical forms of SOM may respond differently to environmental change, SOM is often functionally separated into defined pools based on specific properties, such as mineral association (Sollins et al. 1999). By taking advantage of the large disparity in particle density between organic matter (light) and soil minerals (heavy), SOM can be separated into pools comprised mostly of particulate organic matter (POM, or light fraction SOM) and mineral associated organic matter (MAOM, or heavy fraction SOM) (Sollins et al. 2006; Lavalley et al. 2020). These two pools have substantial differences in chemical properties and turnover rates. POM 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; Huang et al. 2011). In contrast, soil organic matter that becomes occluded in micro-aggregates or complexed with mineral surfaces (i.e. MAOM) has far greater protection from microbial decomposition. Soil C in MAOM typically has much longer residency times in soil relative to POM. (Baisden et al. 2002; Lajtha et al. 2014). 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 dominant controls over soil C accumulation remain uncertain and competing hypotheses exist regarding the extent to which above and belowground plant C inputs contribute to both soil C stabilization and destabilization, and the pathways which promote the transfer of POM to MAOM. Roots have been widely associated with propelling the accumulation of SOM as root material and exudates provide organic inputs directly to the soil. Yet, these inputs, through priming, may also drive the decomposition of SOM (Bailey et al. 2019; Cardinael et al. 2018; Luo et al. 2015). Similarly, microbes and microbial residues support the formation of MAOM, but microbes are also the fundamental consumers of SOM. This balance between microbial mediated stabilization and destabilization of SOM is perhaps most critical for SOM accumulation in the rhizosphere. Unraveling these complex dynamics between plant C inputs, microbial function, and soil mineralogy that control the storage and release of soil C remains as perhaps the greatest barrier in improving predictions for how SOM stores will respond to environmental change.

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

which 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 higher than for the needle litter, and the lignin and cellulose contents are approximately three times greater (Means et al. 1992; Valachovic et al. 2004, Yano et al. 2005). The observed detrital input effects on soil C throughout the long history of the DIRT experiment have rarely aligned with initial hypotheses of a linear relationship between plant C inputs and soil C, and continuation of the experiment into decadal timescales continues to provide opportunity for unique insights into soil C turnover and stabilization (Lajtha et al. 2018).

Although we initially predicted that the DIRT litter reductions would result in soil C losses, after 20 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. 2021). 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 mineral associated 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 microbial carbon-use efficiency and retention of microbial residues as 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 20 years of the DIRT manipulations and (2) assess whether observed effects on soil C pools resulted from a change in the relative size of the different soil C pools, or from a direct change in the C concentration of the pool. 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 (44° 15' N, 12° 10' W). Mean annual precipitation is 2080 mm year<sup>-1</sup>, with mean annual temperatures of 9.4 °C (averages from 1999 to 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, with some small areas found to possess more andic soil properties (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 ( $n = 3$ ), which were chosen randomly across the study site. All plots are nested closely together within a topographically and vegetatively uniform area, situated approximately 2 m apart. Plot sizes are approximately 150 m<sup>2</sup> for all treatment plots, except those with root exclusions. The root exclusion plots are approximately 75 m<sup>2</sup>, as the no root (NR) and no input (NI) plots are located adjacent to each other within a  $\sim 150$  m<sup>2</sup> 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. Logs used for the wood chips were harvested locally from within the Willamette National Forest. Wood chips additions are distributed evenly at

a rate 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, 20 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. 2020). Sampling on such an equivalent soil mass basis was done to ensure that all soil samples represented an equivalent layer of soil, despite the treatment effects on bulk density. 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. 2021. In the laboratory, the soil core samples from each plot were composited by depth increment, homogenized and allowed to air dry for 8 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

**Table 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 1 m deep, 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

(SPT) as described in Sollins et al. (2006). Prior to fractionating soils, the SPT-0 solution was tested on a Shimadzu TOC-V CN analyzer to ensure negligible amounts of C and N contamination (Kramer et al. 2009). Post-fractionation recovery was determined by mass and deemed acceptable if > 90% mass was recovered. Dissolved losses of C were assumed negligible based on findings presented in Helbing et al. (2021). 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 with POM, and the sum of the intermediate fraction and heavy fraction 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  $1.85 \text{ g cm}^{-3}$  for two hours. The resulting slurry was then centrifuged to separate the light fraction ( $< 1.85 \text{ g cm}^{-3}$ ) from the rest of the soil material. The process was then repeated with the  $> 1.85 \text{ g cm}^{-3}$  soil to ensure full separation and recovery of the light fraction. Next, the  $> 1.85 \text{ g cm}^{-3}$  was put through the process again using a SPT solution with a density of  $2.40 \text{ g cm}^{-3}$ , effectively separating the soil material into two further fractions with densities  $1.85\text{--}2.40 \text{ g cm}^{-3}$  (intermediate fraction) and  $> 2.40 \text{ g cm}^{-3}$  (heavy fraction). Fractionated soil material was rinsed with deionized water to remove the SPT and dried for 72 h at  $60 \text{ }^\circ\text{C}$ . Dry fraction mass of each fraction was recorded and subsamples of the fraction material were ground and analyzed for total carbon and nitrogen using a Costech CHN elemental using an Elementar Vario Macro Cube (Elementar Analysensysteme GmbH, Langensfeld, Germany).

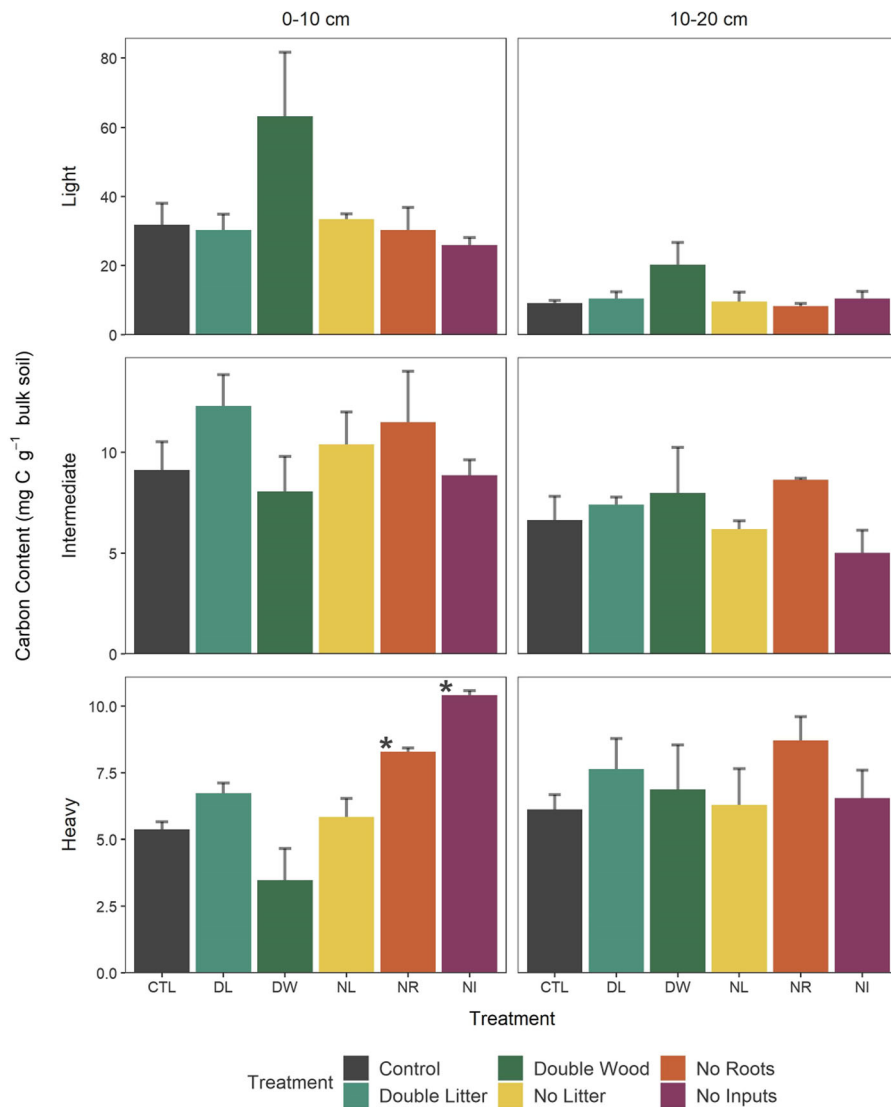
The effects on soil properties observed from the detrital manipulation treatments were determined relative to the control soil at the time of sampling. A comparison with initial, pre-treatment soil conditions was not possible given that no initial soil fractionation

data exists for the study. While we cannot completely rule out the potential for pre-existing differences to cause discrepancies in the soil analysis, the large, randomly distributed yet closely adjacent and visually unconfounded distribution of the study plots limits this potential issue. 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. The ANOVA assumption of data normality was analyzed by the Shapiro–Wilk test and visually verified with quantile–quantile (QQ) plots (Dodge 2008). The assumption of equal group variance was tested and confirmed using the Bartlett test for Homogeneity of Variance ( $\alpha = 0.05$ , Boos 2005). Post-hoc Tukey honestly significant difference (HSD) tests were 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 2013).

## Results

### Wood debris and needle litter addition treatments

There were no strong ( $\alpha = 0.05$ ) statistically significant differences between soil mean light fraction C contents at 0–10 cm depth ( $\text{g light fraction C g}^{-1}$  bulk soil) following 20 years of detrital treatment manipulations, as determined by one-way ANOVA ( $F(5,12) = 2.47$ ,  $p = 0.09$ ). However, across all of the detrital treatment types, the wood debris addition (DW) led to the largest observed mean difference in light fraction C content relative to the control, with the light fraction increasing by + 99% at 0–10 cm and by 119% at 10–20 cm. The change in the mean light fraction C content was not sufficiently consistent across the DW plots to yield a significant result (Tukey HSD,  $p < 0.20$ , Fig. 1). However, the increase in light fraction material is consistent with the previously reported (Pierson et al. 2021) decline in soil bulk density in the DW 0–10 cm soil sampled in same year as this study ( $0.50 \pm 0.08$  vs.  $0.61 \pm 0.08 \text{ g cm}^{-3}$  for



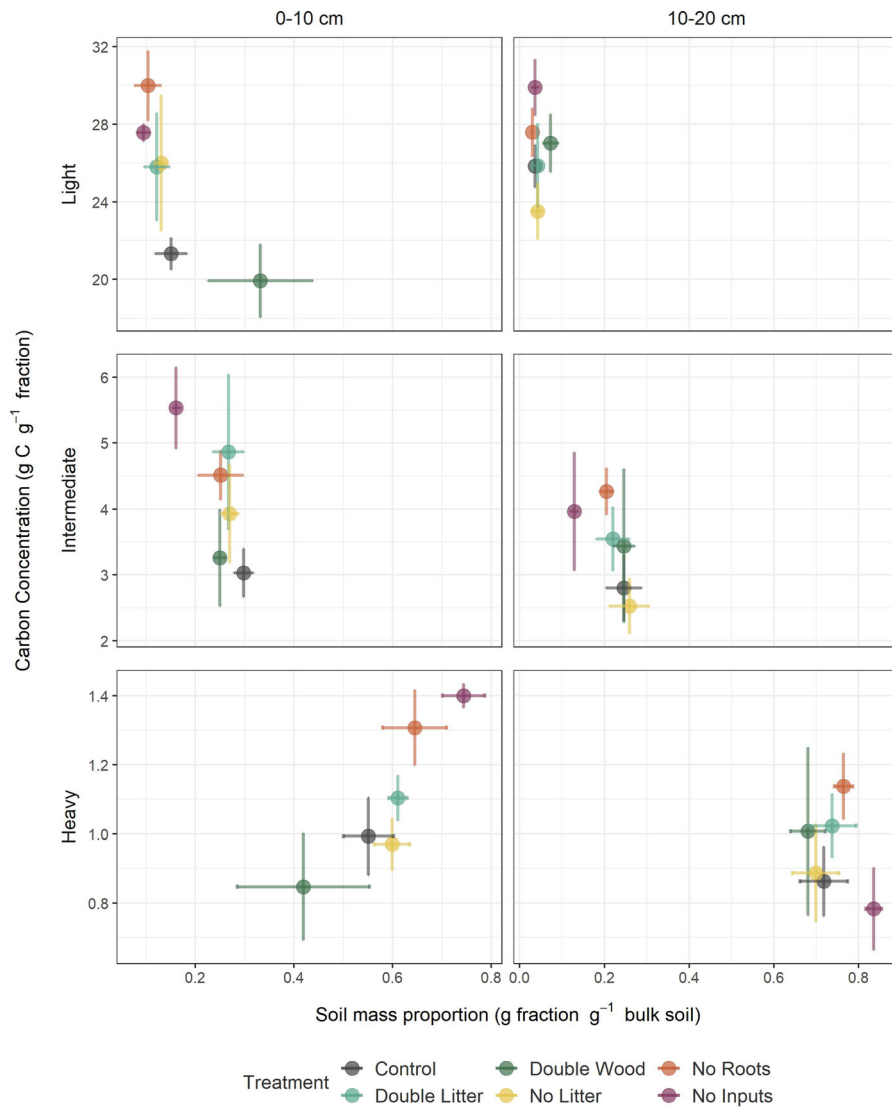
**Fig. 1** Amount of carbon stored in specific density fractions (Light:  $< 1.85 \text{ g cm}^{-3}$ ; Intermediate:  $1.85\text{--}2.40 \text{ g cm}^{-3}$ ; Heavy:  $> 2.40 \text{ g cm}^{-3}$ ) of bulk soil following 20 years of the

DIRT experiment manipulations. (\*) denotes significant difference from control ( $\alpha = 0.05$ ). Error bars represent standard error

the 0–10 cm DW and control soils respectively). The observed increase in mean light fraction C content from the DW treatment resulted primarily from 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 (Fig. 2). Differences between the intermediate density fraction C contents (ANOVA  $F(5,12) = 0.959$ ,  $p = 0.479$ ) in the DW and control soils were relatively small compared to the observed changes in mean C content for the DW light and heavy fractions, as both the proportional mass and

C concentration of the intermediate fraction remained similar to control at 0–10 and 10–20 cm. Differences in mean heavy fraction C content in the 0–10 cm soil (ANOVA  $F(5,12) = 16.02$ ,  $p < 0.001$ ) for the DW treatment relative to the control mirrored the observed change in light fraction C, as the mean 0–10 cm DW heavy fraction C content was 35% lower than the control. However, the observed decline in heavy fraction C content was also not statistically significant ( $p < 0.30$ ). Observed change in the 0–10 cm mean heavy fraction C content in the DW soil came from a





**Fig. 2** Carbon concentration and the proportion of bulk soil mass associated with the Light (<math>< 1.85 \text{ g cm}^{-3}</math>), Intermediate (<math>1.85\text{--}2.40 \text{ g cm}^{-3}</math>) and Heavy (> <math>2.40 \text{ g cm}^{-3}</math>) soil density fractions following 20 years of DIRT manipulations. Error bars represent standard error

combined and closely proportionate decrease in both the C concentration and the proportional mass of the heavy fraction pool (Fig. 2). Heavy fraction C content at 10–20 cm in the DW soil remained closely similar to the control (ANOVA  $F(5,12) = 0.713$ ,  $p = 0.625$ ).

In contrast to the DW treatment, doubling needle litter inputs (DL) to the soil surface did not lead to any evident change in light fraction C content at 0–10 cm or 10–20 cm. However, the mean soil C content of the DL soil intermediate and heavy fractions did increase at both the 0–10 and 10–20 cm depth increments (Fig. 1). Although the observed increases in mean

intermediate and heavy fraction C content were substantial in magnitude, our statistical analysis of the change in both fractions by depth increment did not confirm a significant change from the control soil ( $p < 0.62$ ), which we attribute primarily to the variability in the fraction C concentrations (Fig. 2, note the relative error bar length of the two axes). 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 (Fig. 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 mean 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.

#### Root and surface litter removal treatments

There were no significant changes detected in C content in the surface litter reduction treatment (NL) soils at either sampling depth (Fig. 1). The light, intermediate and heavy fraction C mass and concentration remained starkly similar to the control soil, despite 20 years of sustained reduction in surface litter inputs (Fig. 2).

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 ( $p < 0.99$ , Fig. 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<sup>-1</sup> 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 (Fig. 2). No significant changes were detected in C content 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 to a less 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 (Fig. 2). The mean mass of the 0–10 cm NI light

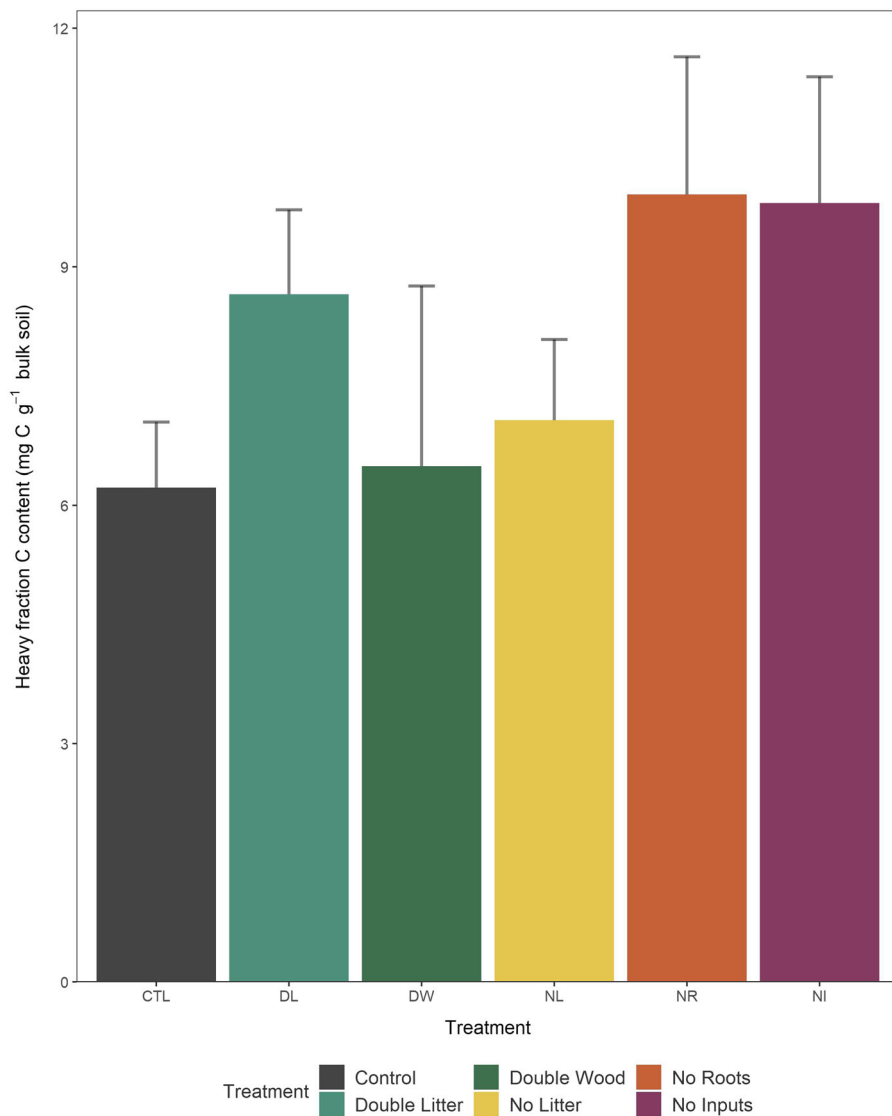
fraction remained similar to control ( $p < 0.95$ ), while the mean C concentration increased, yet not to a highly significant extent ( $p < 0.37$ ).

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 ( $p < 0.05$ , Fig. 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  $0.61 \pm 0.08$  to  $0.79 \pm 0.09$  g cm<sup>-3</sup> respectively, which corresponds with the decline in light fraction material in the soil (Pierson et al. 2021). 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.

#### Study year 10 soil C pools

Ten years prior to this study, a 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<sup>-3</sup>) soil and analyzed the associated soil C concentration (Fig. 3). While no significant differences in heavy fraction C were observed between the control and detrital manipulation treatments at year 10 of the experiment, the detrital manipulation effects on heavy fraction C are remarkably similar at year 10 and





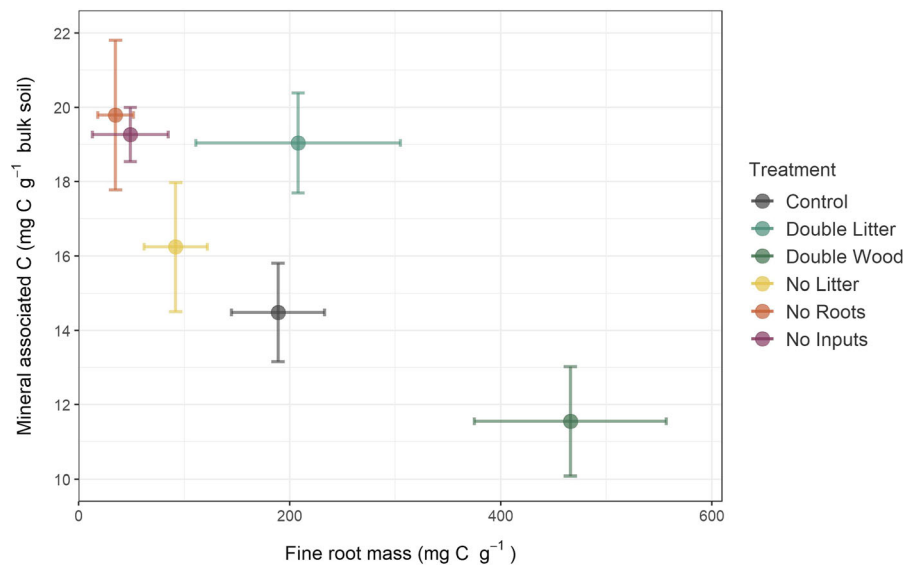
**Fig. 3** Carbon content of the heavy soil fraction (particles with density  $> 2.40 \text{ g cm}^{-3}$ ) at a depth of 0–10 cm following 10 years of detrital manipulations. Differences between treatments are not significant ( $\alpha = 0.05$ ). Error bars represent standard error

year 20. The DL, NR and NI 0–10 cm soil mean heavy fraction C contents were all substantially greater than the control at year 10, as we also found in year 20. We suspect the year 10 soil C pool results may not have gained wider attention due to non-significance. Differences in mean heavy fraction C content were small and non-significant between study years 10 and 20.

#### Fine root mass and heavy fraction C

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

**Fig. 4** Relationship between the C content of soil particles with density  $> 1.85 \text{ g cm}^{-3}$ , 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



#### Detrital treatment effects on soil C stocks

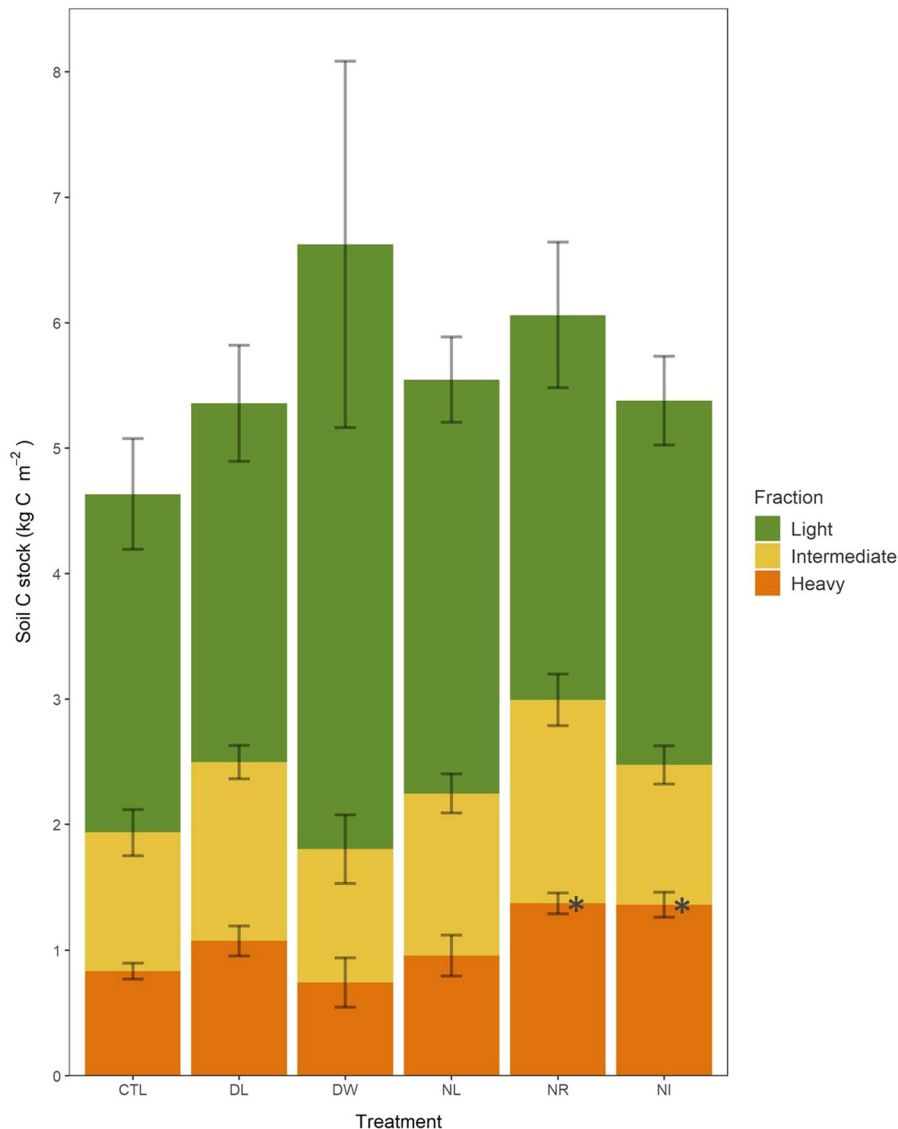
The detrital manipulations were not found to have a significant effect ( $F(5,12) = 0.659$ ,  $p = 0.66$ ) on the total 0–20 cm soil C stocks, nor were significant differences detected between 0–20 cm stocks of C in the light and intermediate fractions (Fig. 5,  $F(5,12) < 1.56$ ,  $p > 0.246$ ). A significant increase was found ( $F(5,12) = 5.78$ ,  $p = 0.006$ ) between the control and the NR ( $p = 0.042$ ) and NI ( $p = 0.048$ ) 0–20 cm heavy fraction soil C stocks. While most of 0–20 cm soil fraction C stocks changes were not statistically significant, for certain treatments, the observed mean differences were substantial, reflecting the changes observed in the light and heavy pools. While total increases were observed in both the DW (non-significant) and NR (significant) mean 0–20 cm soil C stocks, the nature of these changes differed, with the DW treatment C stock gains confined to the light fraction C pool, while the NR soil C stock increases were derived from gains in both the intermediate and heavy fraction C pools. The significant increase in heavy fraction C observed in the NI treatment soil had a limited effect on the 0–20 cm soil C stocks, as the heavy fraction C increase was partially offset by a loss of light fraction soil C. Mean 0–20 cm soil C stocks for the NL and DL treatment soils also increased over the study period, but were not significantly different from the control. For the DL soil, the increase in soil C stock is consistent with the observed increase in

intermediate and heavy fraction C content. However, the driver behind the soil C stock increase for the 0–20 cm NL soil derived primarily from an increase in bulk density in the 0–10 cm soil (Pierson et al. 2021).

#### Discussion

##### Increases in mineral associated 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 as the declines in the POM C pool were offset by the accumulation in MAOM C pools (Fig. 1). 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 associated soil C in these acidic, 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



**Fig. 5** Total C stocks and the proportion of C stock in C pools separated by density fraction (Light:  $< 1.85 \text{ g cm}^{-3}$ ; Intermediate:  $1.85\text{--}2.40 \text{ g cm}^{-3}$ ; Heavy:  $> 2.40 \text{ g cm}^{-3}$ ) from 0 to 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. Asterisks denote significant difference ( $\alpha = 0.05$ ) of fraction specific stocks from the control

amount of mineral associated 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, with the corresponding increase in microbial production serving to promote an increase in mineral associated soil C (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 large increase in root detritus could have provided an abundant, high quality C source which drove the observed increase in MAOM. However, these soils have received a great amount of sustained root C input for millennia. Further, the labile C input to these soils from both above-and below-ground litter greatly exceeds the rate of soil C accumulation, such that there must also be a limitation on MAOM accumulation that is not directly linked with the quantity of labile C inputs to the soil.

Alternatively, expected changes in microbial activity and processing following the cessation of root activity may also offer an explanation for the observed increases in MAOM. The lack of live root and rhizosphere activity may have led to a reduction in rhizodeposition-induced microbial consumption of existing C stores, including MAOM stores, thus allowing for greater amounts of C to accumulate on mineral surfaces. Previous studies of root exudate and rhizosphere effects on SOC and MAOM accumulation support this pathway. Root exudation is known to stimulate SOM decomposition, especially when soil N availability is low (Drake et al. 2013), and enzymatic stoichiometry, along with considerable empirical evidence, suggests that this destabilization of the low C:N ratio MAOM pool occurs across diverse ecosystems and soil types (Guenet et al. 2012; Drake et al. 2013; Murphy et al. 2015). Further, root exudation may also directly limit MAOM accumulation by promoting the liberation of organic compounds from protective associations with minerals (Jilling et al. 2018; Keiluweit et al. 2015). Also, Hopkins et al. (2014) found that increased root production from elevated CO<sub>2</sub> did not result in increased MAOM due to increased priming of mineral-associated C. While further research is necessary to fully determine the linkages between roots and MAOM, there is considerable evidence for this second pathway to explain the increase in MAOM in soils without root activity.

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 20-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 C inputs from POM and dissolved organic carbon (DOC) mostly 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 10–20 cm NR soil relative to the NI soil. Because surface litter inputs in the NR plots provide greater amounts of 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 retained 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.

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  $^{14}\text{C}$  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 20 years of manipulation, but the appearance of this trend in year 10 of the experiment (Fig. 3) 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  $^{14}\text{C}$  age of MAOM, relative to POM, are not well understood (Trumbore 2009). MAOM C may persist in soil due to stabilization, however the old  $^{14}\text{C}$  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 light fraction 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 (Fig. 1). 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 (Fig. 4, Pierson et al. 2021). Despite this substantial reduction in the amount of both above and below-ground 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 (Fig. 1). Surprisingly, additions of higher quality 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 (Fig. 4, Pierson et al. 2021). 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 intermediate 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

The low quality wood additions displayed the greatest potential to increase soil C stocks (Fig. 5) due to the incorporation of particulate organic matter in the soil. With a sustained rate of input, wood debris additions may be viable for increasing soil C, but the light fraction soil C is not well sequestered, and the transference of the increased light fraction to the more stable and mineral-associated heavy fraction 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 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 had a much lesser effect on 0–20 cm soil C stocks than that observed from the wood debris addition treatment. We suspect the small intermediate and heavy fraction stock gains from the added needle litter may be more persistent than the DW gains in light fraction material, yet further study is required to determine the exact timescale for the turnover of C in these pools. Root exclusion also showed potential for 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 productive forest environment. However, at the opposite end of the spectrum, our findings do call in to question the common assumption that more roots will lead to more soil C (Fig. 4), and present the possibility that, in certain environments, increasing vegetative productivity may be counteractive to promoting soil C sequestration.

## Conclusion

The findings of this long-term study should help inform models that seek to link surface and root C inputs with microbial activity and soil C stocks, including soil C models of forest harvest and wildfire effects. Our results suggest that surface litter quality plays a substantial role in the rate of soil C accumulation, as well as how surface C inputs are partitioned to different soil C pools. Further, we found that roots are not strongly linked with soil C stocks in these forest soils and that roots may be counteractive to further accumulation of MAOM. 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.

**Author contributions** DP, RDB, KN, MS and KL planned the research; DP, KK, and LE conducted the field and laboratory research; DP and KL analyzed results, and DP wrote the paper with KL.

**Funding** Support was provided by National Science Foundation Grants DEB-1257032 to K.L. and DEB-1440409 to the H. J. Andrews Long Term Ecological Research program.

**Availability of data and materials** The data that support the findings of this study are openly available in SOils DATA Harmonization database at <https://doi.org/10.6073/pasta/9733f6b6d2ffd12bf126dc36a763e0b4>, with an interactively viewable data platform at <https://cosima.nceas.ucsb.edu/Iter-som/>. Data pertaining to the H.J. Andrews LTER DIRT experiment may also be accessed at <https://andrewsforest.oregonstate.edu/data>.



## Declarations

**Conflict of interest** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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