Journal of Geophysical Research: Biogeosciences

RESEARCH ARTICLE
10.1029/2018JG004431

Tree Mortality From Insect Infestation Enhances Carbon Stabilization in Southern Appalachian Forest Soils

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Key Points:
- Eight years postdisturbance, hemlock mortality plots accumulated carbon in mineral soils at higher rates than reference plots, whereas forest floor mass declined
- Soil carbon accumulated predominantly in the mineral-associated fraction of the soil organic matter
- Soil carbon accumulation was attributed to the transformation and translocation of carbon from detritus and the particulate soil organic matter fraction

Supporting Information:
- Supporting Information S1

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Citation:
Fraterrigo, J. M., Ream, K., & Knoepp, J. D. (2018). Tree mortality from insect infestation enhances carbon stabilization in southern Appalachian forest soils. *Journal of Geophysical Research: Biogeosciences, 123*, 2121–2134. https://doi.org/10.1029/2018JG004431

Received 5 FEB 2018
Accepted 18 JUN 2018
Accepted article online 25 JUN 2018
Published online 14 JUL 2018

Abstract Forest insect and pathogen outbreaks may exacerbate anthropogenic climate change if they accelerate soil carbon loss to the atmosphere. We quantified soil respiration and carbon content for nearly a decade after girdling or natural infestation of hemlock (*Tsuga canadensis* L. Carr., a codominant species in southern Appalachian forests) by hemlock woolly adelgid (*Adelges tsugae*) to improve understanding of soil carbon response to disturbance from forest insect and pathogens. From 2005 to 2013, net soil respiration was similar among hemlock mortality (~50% basal area reduction) and reference hardwood plots, but both girdled and hemlock woolly adelgid-infested plots showed greater activities of β-glucosidase (a cellulose-hydrolyzing extracellular enzyme), decreased O-horizon, and decreased fine root biomass. During this period, mineral soil carbon accumulated at a higher rate in hemlock mortality plots than in reference plots in both surface (0–10 cm) and subsurface (10–30 cm) soils, driven by increases in the mineral-associated fraction of the soil organic matter. In contrast, particulate organic matter (POM) carbon accrued slowly in surface soils and declined in the subsurface of girdled plots. $\delta^{13}C$ values of the POM fraction demonstrate increased microbial processing of surface soil organic matter over time, suggesting enhanced decomposition of organic matter in this pool. These findings indicate that hemlock mortality in this system has led to enhanced soil carbon stabilization through the transformation and translocation of carbon from detrital and POM pools to the mineral-associated organic matter pool. Accelerated responses in the girdled versus naturally infested treatments highlight limitations associated with using girdling to simulate natural mortality.

1. Introduction

Forests have undergone pronounced changes in recent decades in response to increased outbreaks of forest insects and pathogens (FIPs; Hicke et al., 2012; Lovett et al., 2006). Between 2003 and 2012, 98 million ha of forest were substantially affected by FIPs outbreaks (van Lierop et al., 2015), mostly in temperate North America where outbreaks of native bark beetles have intensified as a result of climate change (Kurz et al., 2008; Raffa et al., 2013). Invasive FIPs outbreaks are also rising as a result of growing international trade and travel (Aukema et al., 2010; Lovett et al., 2016; Santini et al., 2013), with an estimated 334 million ha of forestland in the United States currently at risk for basal area mortality of host tree species from well-established forest pests (Krist et al., 2014).

FIPs outbreaks can alter forest carbon (C) cycling with research showing they may shift forest C balance, switching forests from being net C sinks to C sources (Kurz et al., 2008). However, other studies show that FIPs have no effect on C balance (Gough et al., 2013; Moore et al., 2013; Morehouse et al., 2008; Raymer et al., 2013), highlighting the uncertainty in predicting how FIPs will influence global atmospheric carbon dioxide (CO_2) concentrations and feedback to climate change.

Approximately two thirds of forest ecosystem C is stored in the soil (Dixon et al., 1994), and soil respiration is the second largest and least well constrained terrestrial C flux (Bond-Lamberty & Thomson, 2010; Trumbore, 2006). Understanding the response of soil C cycling to FIPs is thus critical for anticipating FIPs effects on forest C balance. Despite recent conceptual advances in predicting the impacts of FIPs on forest C cycling (Dietze & Matthes, 2014; Hicke et al., 2012; Peltzer et al., 2010), there are few long-term, experimental studies that track soil C dynamics during the period from initial disturbance to community reassembly, making it difficult to test model predictions and determine governing processes (Bond-Lamberty et al., 2015). The lack of knowledge is particularly acute for FIPs that target codominant or subdominant species. These types of outbreaks are becoming more common globally (Lovett et al., 2016) and can have markedly different consequences for forest C cycling compared to severe, stand-replacing disturbances (Amiro et al., 2010; Nave et al., 2011).

Empirical and modeling studies of forests experiencing moderate disturbances from FIP infestations have revealed resilience in net ecosystem production (Albani et al., 2010; Amiro et al., 2010; Bond-Lamberty et al., 2015) and nutrient cycling (Block et al., 2013; Knoepp et al., 2011; Nave et al., 2011). The primary mechanism facilitating such resilience is a compensatory increase in photosynthesis and growth of nonhost species (Block et al., 2012; Gough et al., 2013). Similar mechanisms may promote resilience in soil respiration. In a study that simulated oak (*Quercus* spp.) mortality by girdling, soil respiration initially declined due to root mortality but rebounded to pregirdling levels 2 years after treatment, suggesting that nonhost trees responded rapidly to and compensated for oak loss (Levy-Varon et al., 2014).

The vulnerability of soil C stores to moderate disturbance is more difficult to anticipate because our understanding of what controls the balance between plant-C inputs and C outputs from microbial metabolism has changed considerably over the past two decades. Soil microorganisms are now recognized as dominant agents of soil C formation that promote soil C accumulation by converting a fraction of plant-C inputs into more decomposition-resistant forms (Lehmann & Kleber, 2015; Miltner et al., 2012). This microbially controlled flow of C from plant inputs to stable soil organic matter (SOM) is thought to be regulated by factors that affect the efficiency with which soil microbes use substrate-C for growth versus mineralization or respiration (i.e., microbial carbon-use efficiency, CUE; Manzoni et al., 2012; Sinsabaugh et al., 2013) and also the allocation of plant-derived C into different microbial products (Cotrufo et al., 2013; Schmidt et al., 2011). Factors leading to higher CUE and/or greater C allocation to persistent microbial products are expected to favor C stabilization, which can be further enhanced through mineral-matrix interactions that result in the physical protection of microbial residues (Dungait et al., 2012; Six et al., 2002). As a result, a larger amount of stable soil C is expected to be formed from high-quality litter (i.e., with a low lignin and high nitrogen [N] concentration) than low-quality litter because the former can be used by soil microbes more efficiently, resulting in greater production of microbial residues (Manzoni et al., 2012). Similarly, more soil C should be formed from fine root litter than aboveground litter because of its higher nitrogen concentration and close proximity to the soil matrix (Denef & Six, 2006). Because tree mortality from FIPs infestations should increase root litter inputs and may also be accompanied by increases in the quantity and quality of litterfall (e.g., because of increases in the productivity of nonhost species or species turnover), it could ultimately increase soil C. Alternatively, priming theory predicts that the shift from low- to high-quality litter would stimulate the soil microbial community, leading to higher rates of soil respiration and decreased soil C because of enhanced SOM decomposition (Fontaine et al., 2007).

Studies to date provide mixed support for these hypotheses. In the Catskill Mountains of New York, beech bark disease (BBD) is shifting stands codominated by American beech (*Fagus grandifolia* Ehrh.) and sugar maple (*Acer saccharum* Marsh.) to sugar maple dominance. In stands where BBD has been present for several decades, growing season soil respiration decreased with increasing disease severity whereas annual aboveground net primary productivity (ANPP) and soil C stocks (0- to 12-cm depth) were not statistically related to BBD severity, possibly due to altered belowground C allocation (Hancock et al., 2008). In New England where black birch (*Betula lenta* L.) is replacing eastern hemlock (*Tsuga canadensis* L. Carr.) infested by hemlock woolly adelgid (*Adelges tsugae* Annand; herein HWA), annual ANPP was higher but soil respiration did not differ between 18-year-old aggrading birch stands and 132-year-old uninfested eastern hemlock stands (Finzi et al., 2014). Moreover, although C in the organic horizon was lower, C in the mineral soil was higher, resulting in no net difference in total soil C stocks between the aggrading black birch and uninfested hemlock stands (Raymer et al., 2013). These patterns were attributed to the higher litterfall quality of birch along with increased rates of soil N uptake and N-use efficiency by the rapidly growing black birch (Finzi et al., 2014).

To evaluate the effects of moderate disturbance from a FIP infestation on soil C cycling, we quantified changes in soil respiration and soil C content over 8 years following either girdling to simulate hemlock mortality or natural infestation by HWA. HWA is an invasive forest pest that was first reported in the eastern United States in the early 1950s and has caused significant mortality of eastern hemlock since the mid-1980s (McClure, 1990). A small, aphid-like insect of the order *Hemiptera*, HWA colonizes hemlock trees of all ages and sizes, reducing tree vigor by feeding on the xylem ray parenchyma cells (Young et al., 1995). Because soil organic carbon (SOC) changes on decadal time scales (Smith, 2004), resampling over a longer timeframe is needed to investigate the processes that contribute to shifts in soil C cycling. In addition to revealing the longer-term fate of soil C following hemlock mortality, examining girdling and natural infestation treatments in tandem allowed us to determine how well our experimental manipulation reproduced the

effects of a FIPs infestation. An earlier study showed that soil respiration declined by approximately 20% in the year after hemlock girdling and natural infestation, corresponding with a reduction in fine root biomass (Nuckolls et al., 2009). In the second year after disturbance, however, co-occurring deciduous tree and evergreen shrub species showed increased growth in response to girdling and infestation, which was sustained for the next 3 years (Block et al., 2012; Ford et al., 2012). We thus hypothesized that nearly a decade after hemlock mortality soil respiration would be similar to pretreatment levels because of the recovery of root biomass associated with the compensatory growth of nonhost woody species. Regarding postdisturbance changes in soil C content, we hypothesized that soil C would (1) increase because of enhanced soil C stabilization driven by higher inputs of root litter and high-quality litter from co-occurring deciduous trees. Furthermore, (2) if mineral-matrix interactions are important for stabilizing C, then the magnitude of soil C increase would be greater in the mineral-associated than the particulate SOM fraction. Alternatively, we hypothesized that soil C content would (3) decrease because of increased soil C decomposition resulting from priming.

2. Methods

2.1. Site Description and Experimental Design

This study was conducted in the Coweeta Hydrologic Laboratory, a U.S. Forest Service Experimental Forest, embedded in the Coweeta basin in the Nantahala Mountain Range of western North Carolina, USA. Climate in the basin is classified as marine humid temperate with cool summers and mild winters (Swift et al., 1988). The growing season lasts from early May to early October, and the area experiences its highest temperatures from June–August (~20°C) and lowest from December–January (~5°C). Mean annual temperature is 12.6°C and MAP is 179 cm. Soils within the study area are Cullasaja-Tuckasegee series Inceptisols (mesic Typic Humudepts) and are characterized by high organic matter content in the A horizon, a B horizon (designated Bt) with some clay accumulation, and a depth to saprolite of 80–100 cm (Soil Survey, 2014), making them well suited for testing hypotheses about the role of mineral-matrix interactions in soil C stabilization.

In the summer of 2004, twelve 20 m × 20 m (0.04 ha) study plots were established in low elevation (<890 m) cove hardwood forests (Knoepp et al., 2011; Nuckolls et al., 2009). Plots were adjacent to the two main streams located on the southern and northern sides of the Coweeta basin. Eight plots had >50% basal area of hemlock (Table S1 in the supporting information); half of these were randomly assigned to a girdling treatment to accelerate hemlock defoliation and mortality (hereafter, GDL plots, $n = 4$). All hemlock trees in the GDL plots (and within 5 m of the plot boundaries) were girdled by handsaw or chainsaw at breast height (1.37 m) in summer 2004 and regirdled in summer 2005 and 2006 if they still appeared alive. By the fall of 2006, >90% of the girdled trees were dead. The other half of these plots were naturally infested by HWA by December 2004 (hereafter, HWA-infested plots, $n = 4$). Infestation levels were initially low, and hemlock crowns were full and healthy. By fall 2005, trees in the HWA-infested plots were beginning to drop their needles and show reduced crown vigor. Hemlock mortality in these plots was about 10% by 2006 and 20% by 2008. By 2010, 50% of hemlock trees had succumbed in the HWA-infested plots. The remaining four plots contained <3% hemlock were used as reference (hereafter, REF plots). They were similar in soils, aspect, slope, and elevation, and had similar hardwood composition as the hemlock plots (Table S1); thus, they represented the potential future characteristics of hemlock stands following hemlock mortality. Plots with hemlock unaffected by HWA would have been an ideal reference, but there were no stands containing hemlock unaffected by HWA within the Coweeta basin. We thus relied on temporal trends of hemlock plots to detect the effects of FIPs infestation on soil C cycling, while comparison with trends of REF plots allowed us to control for potential shifts in baseline conditions. The dominant tree species present across all plots were rhododendron (*Rhododendron maximum* L.), black birch (*B. lenta* L.), tulip poplar (*Liriodendron tulipifera* L.), white oak (*Quercus alba* L.), blackgum (*Nyssa sylvatica* Marsh.), and red maple (*Acer rubrum* L.; Ford et al., 2012; Nuckolls et al., 2009). In 2013 and 2014 measurements, one REF plot was excluded because of human disturbance.

2.2. Soil and Vegetation Sampling

Table S2 summarizes the measurements made and the years they were performed. Mineral soils were initially sampled by Knoepp et al. (2011) in January 2005, prior to hemlock mortality. To examine how soil C content changed nearly a decade after hemlock mortality, we resampled soils in 2013. In each plot, we

collected mineral soil in the same manner as Knoepp et al. (2011), using a 2.2-cm-diameter soil probe to sample from 0- to 10- and 10- to 30-cm depths. Each sample represented a composite of ~20 individual soil samples taken randomly across the plot. Soils were air dried, mixed thoroughly, and sieved (<2 mm) prior to subsampling for determination of pH, and C and N by combustion for both whole soil and the fractionated organic matter (see section 2.4). Bulk density was determined for soils in 2009 (Table S1). Because soil density can change due to variations in SOC, we report changes in soil C concentrations rather than stocks. In 2014, we collected additional samples using the same methods as described above, but sieved samples in the field immediately after collection (<2 mm), refrigerated them at −4 °C for 3 days, and then transferred an aliquot of each sample to a −80 °C freezer until it could be analyzed for extracellular enzyme activities (see section 2.4).

Fungi are important for soil aggregate formation, and fungal hyphae are also more resistant to biodegradation than bacterial cell walls, making it likely that fungal abundance affects soil C content (Clemmensen et al., 2013; Six et al., 2006). To investigate whether fungal abundance could explain potential treatment effects on soil C dynamics, we quantified fungal mycelial biomass in 2014 using in-growth bags (Wallander et al., 2013). Bags (10-cm long and 3-cm diameter) were constructed of nylon mesh (53 μm; Global Gilson Inc., Lewis Center, OH, USA) and filled with #30–50 size sand that had been pretreated by placing it in a muffle furnace at 500°C for ~6 hr to burn off any organic matter. Six bags were placed in each plot: three bags at a depth of 0–10 cm and three bags at a depth of 10–20 cm. Bags were deployed in May 2014 and recovered in October 2014, at which time they were frozen at −4°C until processing. Prior to processing, we pooled the bags by depth and plot to create one sample per depth per plot. Each pooled sample was rinsed at least three times with deionized water and poured through a 53-μm mesh filter to collect the mycelia. This process was repeated until there were no more visible clumps of mycelia on the filter following a rinse. Mycelia were then freeze dried for ~48 hr and weighed.

The organic soil horizon (O-horizon) was sampled initially in winter 2005 by Nuckolls et al. (2009) and again by Knoepp et al. (2011) in winter 2008 and 2009. We resampled the O-horizon in all plots using identical methods in winter 2013. We collected four replicate 0.09 m^2 samples from each plot and divided each into two components: O_i horizon and O_a + O_e horizon; samples were dried at 60 °C to a constant weight an weighed.

Nuckolls et al. (2009) sampled roots in June 2004 and 2006 to determine total standing root biomass, and we resampled them in June 2014 using identical methods. From each plot, we collected three replicate 20-cm deep soil cores (5-cm diameter), which were frozen until roots could be processed. Roots samples were washed with deionized water over a 53-μm sieve, sorted into size classes, dried at 55 °C, weighed, and ground in preparation for combustion analysis. Size classes that were manually sorted from each plot sample included, coarse roots (CR, >2-mm diameter), medium roots (MR, 1- to 2-mm diameter), and fine roots (FR, 0.5- to 1-mm diameter). Very fine root biomass (<0.5-mm diameter) was determined from the remainder of each sample using the line intercept method (Hendrick & Pregitzer, 1993), where the length of roots was measured and root biomass was estimated using specific root length (cm/g). To determine the rate and extent of hemlock loss, the overstory composition and diameter at breast height of all live trees >2.54 cm of each plot was surveyed during June–July of 2004, 2005, 2006, and 2014 and used to calculate plot-level basal area (see Ford et al., 2012, for details).

2.3. Soil Respiration and Environmental Parameters

Five 10-cm-diameter PVC collars were installed in each plot, and soil respiration (R_{soil}, $\mu mol \cdot m^{-2} \cdot s^{-1}$) was measured in July and August 2004, 2005, and 2006 with a LI-COR 6400 portable photosynthesis system operated in closed-path mode and attached to a LI-COR 6009 soil CO_2 flux chamber (LI-COR Inc., Lincoln, NE; Nuckolls et al., 2009). During each measurement, soil temperature (T_{soil}, °C, LI-COR 6400–09, LI-COR Inc., Lincoln, NE, USA) and volumetric soil moisture (θ,% *v*/v, Field Scout TDR 100, Spectrum Technologies, Aurora, IL, USA) were also measured at 0- to 15-cm depth, directly adjacent to each collar. In July and August 2014, we remeasured soil respiration in each plot with a LI-COR 8100A closed-path infrared gas analyzer system coupled to a LI-COR 8100-102 soil CO_2 flux chamber (LI-COR Inc., Lincoln, NE, USA). Measurements were made on three 10-cm PVC collars installed in each plot 4 weeks prior to sampling. Concurrently, soil temperature and moisture were measured adjacent to each collar at 10-cm depth.

2.4. Laboratory Analyses

Each soil sample was analyzed for pH (2:1 ml H_2O: g air-dried soil) using a bench-top pH meter (Accumet AB15, Fisher Scientific), total C and N, and $\delta^{13}C$ on a Costech 4010 CHNSO Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) interfaced with an isotope ratio mass spectrometer (Thermo Fisher Delta V Advantage, Fisher Scientific) at the University of Illinois. Analytical error was <10% for total C and N and ±0.2‰ for $\delta^{13}C$. Stable isotopic compositions are expressed in δ notation (‰):

$$\delta^{13}C = \left[\left(R_{sample}/(R_{standard} - 1)\right)\right] \times 1,000,$$

where R_{sample} is the ratio of the heavy to light isotope ($^{13}C/^{12}C$) of a sample and $R_{standard}$ is the ratio of a standard (VPDB; Fry, 2006).

To determine effects of hemlock loss on SOC pools of differing stability, we fractionated archived soil samples from 2005 and samples collected in 2013 using a modified physical fractionation technique (Marriott & Wander, 2006). We separated the SOM into two fractions: particulate organic matter (POM; >53 μm in size) and mineral-associated organic matter (MAOM; <53 μm in size). The POM fraction is composed of younger, less processed, plant-derived organic matter with a relatively fast rate of turnover, whereas the MAOM fraction is composed of older organic matter that has undergone more microbial processing and turns over relatively slowly (Bradford et al., 2008). To separate these fractions, we placed 10 g of soil into a 30-ml-plastic bottle with a 53-μm mesh lid. These small containers were placed in a larger plastic container filled with a 5% sodium hexametaphosphate solution and shaken for 1 hr, after which the larger container was emptied, filled with deionized water, and shaken for ten 10-min intervals, changing out the water each time. After washing, the POM fraction remaining in each small container was dried at 60 °C, ground, and analyzed for total C and $\delta^{13}C$ as described above. We calculated the total C mass in the MAOM fraction as the difference between the mass of C in the unfractionated sample and C in the POM fraction. Following Fry (2006), we estimated the $\delta^{13}C$ in the MAOM fraction using the following mixing model:

$$\delta^{13}C_{MAOM-C} = \left\{\delta^{13}C_{Whole} - \left[\delta^{13}C_{POM-C} \times (1 - f_{MAOM-C})\right]\right\}/f_{MAOM-C}$$

where $f_{MAOM\ -\ C}$ is the proportion of C that is in the mineral-associated fraction.

To determine an approximate value for the C isotopic signature of several potential sources of SOC, we also randomly selected eight study plots and conducted $\delta^{13}C$ analysis of coarse and fine roots, leaf litter from the O_i horizon, and fungal mycelia as described above.

We measured the activities of four extracellular enzymes involved in the breakdown of SOM constituents of varying turnover times. β-glucosidase (BG) and β-1,4-N-acetylglucosaminidase (NAG) are hydrolytic enzymes associated with the breakdown of fast- to moderate-cycling SOM pools. NAG is also associated with N acquisition. Phenol oxidase (PPO) and peroxidase (PER) are lignolytic enzymes associated with the breakdown of SOM with slow rates of organic matter turnover (Weintraub et al., 2007). Enzyme assays were done on each composited sample from each plot, for each sampling depth (0–10 and 10–30 cm), and are described in Finzi et al. (2006). Briefly, soil slurries consisting of 1 g of fresh soil suspended in 125 ml of 50 mM, pH 5.0 acetate buffer were dispensed in 24 replicate, 200-μl aliquots to 96-well microplates. For the hydrolytic enzyme assays, eight replicate subsamples received 50 μl of 200 μM 4-methylumbelliferone, and eight replicates received a 4-methylumbelliferone substrate so that there were eight replicate wells for each blank, quench control, standard, and substrate assay. For the lignolytic enzyme assays, L-DOPA + 10 μl of 0.3% hydrogen peroxide were added to each soil/buffer well. Microplates were incubated in the dark at 20°C for 2 hr (BG and NAG) or 4 hr (PPO and PER). For BG and NAG microplates, we added 10 μl of 1 M NaOH to each well to stop the reaction and read the microplates at 360-nm excitation and 460-nm emission using a Bio Tek Synergy HTX Multimode Plate Reader (Winooski, VT, USA). PPO and PER microplates were read at 460-nm absorbance after transferring the supernatant to a fresh microplate to avoid absorbance interference by soil particles. Enzyme activity was calculated as the amount of substrate cleaved during the incubation. Because PPO and PER break down similar types of SOM, we summed their activities and hereafter refer to that number as total oxidase activity (Craig et al., 2015).

2.5. Data Analysis

We evaluated the effects of treatment and year on R_{soil}, T_{soil}, and soil moisture with linear mixed effects models (*lme4* package, Bates et al., 2015) in R (R Core Development Team, 2017). Respiration (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), temperature, and moisture data were aggregated prior to analysis by averaging measurements on each collar by sampling date. Year and treatment were included as main effects and collar nested within plot, and year was specified as a random intercept (1|collar: plot: year) to account for potential temporal and spatial nonindependence of the residuals. To enable direct comparisons of effect size, we standardized all numeric variables to dimensionless units (z-scores) by subtracting the mean from each observation and dividing by the standard deviation. We also log-transformed soil respiration values to meet normality assumptions. To evaluate the main effects of treatment within each year, we performed planned contrasts using the *lsmeans* package (Lenth, 2016) applying Tukey's correction for multiple comparisons.

To evaluate changes in soil C and N, soil C isotopic ratio, root biomass, and basal area, we used mixed effects models, including treatment, year, and year *x* treatment interaction as main effects and specifying a random intercept (1|plot) to account for repeated measures within a plot. The main effects of treatment in each year were analyzed as described above, with planned contrasts used to compare treatment types within years. Variables not measured over multiple years (soil enzyme activities and mycelial biomass) were analyzed for significant differences between treatments using linear models. Response variables were log-transformed as needed to meet normality assumptions. The data set is publicly available on the EDI Data Portal (Fraterrigo, 2018).

3. Results

3.1. Basal Area

From 2004 (pretreatment) to 2014, stand basal area declined by 31 ± 18% (SE) in GDL ($P < 0.01$) and 60 ± 4% in HWA-infested plots ($P < 0.001$) but did not change in REF plots (Figure S1). Hemlock basal area decreased rapidly in GDL plots, losing 99 ± 2.3% by 2006 ($P < 0.001$). Basal area of hemlock in HWA-infested plots did not begin to decrease until after 2006, losing 98 ± 2.2% of hemlock basal area by 2014. In 2006, hemlock basal area in GDL plots was not significantly different from that in REF plots (i.e., less than 0.6% of stand basal area), and by 2014 hemlock basal area was <1.3% in all plots (GDL, HWA, and REF) and did not differ among treatments (Figure S1).

3.2. Soil Respiration

During both the 2004–2006 and 2014 study periods, soils in former hemlock plots (GDL and HWA infested) were cooler (0.6–0.8°C; $P < 0.01$) and wetter (6.3–7.4% *v*/v, $P < 0.01$) compared to REF plots (Figure 1). The deviation in soil moisture between REF and former hemlock plots was greatest in 2014 when above-average precipitation resulted in higher soil moisture in GDL and HWA-infested plots compared to REF plots ($P < 0.05$).

Soil temperature and soil moisture had small but significant effects on soil respiration. Averaging over all years, R_{soil} increased with soil temperature (effect size = 0.25, $P < 0.05$) and decreased with soil moisture (effect size = −0.08, $P < 0.001$). R_{soil} differed significantly among sampling years. Compared to the pretreatment year of 2004, R_{soil} was lower in 2005 ($P < 0.001$) and higher in 2014 ($P < 0.001$). R_{soil} did not vary with treatment in any years and showed a similar relationship to soil temperature within years (Figure S2).

3.3. Mineral Soil C and C:N

From 2005 to 2013, surface (0–10 cm) soil C concentration increased in all treatments ($P < 0.001$, Figure S3). The average increase in C concentration was 114 ± 42% ($P < 0.001$) in GDL plots, 80 ± 31% ($P < 0.01$) in HWA-infested plots, and 68 ± 4% ($P < 0.05$) in REF plots. Surface soil C concentration did not differ among treatments in 2005. By 2013 it was 52% higher in GDL ($P = 0.01$) and 42% higher in HWA-infested plots ($P = 0.05$) compared to REF plots.

In treatments containing dead hemlock, surface soil C increases were predominantly in the MAOM fraction (Figure 2). Surface MAOM-C concentration increased 79 ± 24% in GDL (year: $P < 0.05$) and 89 ± 13% in HWA-infested plots from 2005 to 2013 (year: $P < 0.05$); REF plots showed no change. This translated to average MAOM-C accrual rates of 2.72 g C · kg-dry-soil^{-1} · year^{-1} in GDL and 2.75 g C · kg-dry-soil^{-1} · year^{-1} in

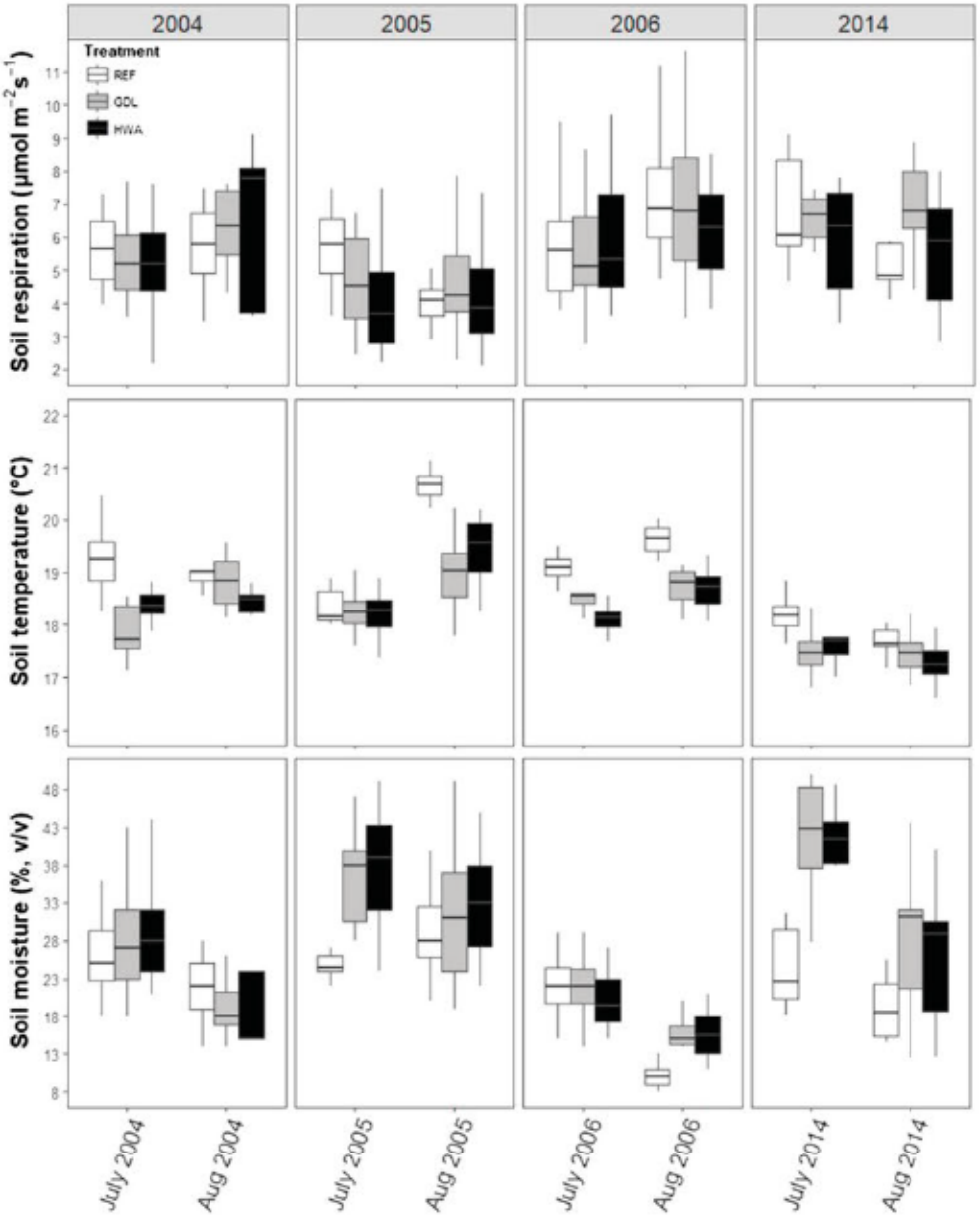

Figure 1. Monthly net soil respiration ($\mu mol \cdot m^{-2} \cdot s^{-1}$), temperature (°C), and soil moisture (%, *v*/v) from 2004 to 2014. Data from 2004 are pretreatment; eastern hemlock trees were girdled in GDL plots and hemlock woolly adelgid arrived in hemlock woolly adelgid (HWA) plots in late 2004. Reference plots (REF) had <3% hemlock trees. All plots were sampled at the same time each month; points are jittered to improve visibility.

HWA plots. Over the same period, surface soil POM-C concentration increased 235% in GDL plots ($P < 0.05$); however, there was no change in POM-C concentration in either HWA-infested or REF plots.

For subsurface (10–30 cm) soils, GDL and HWA-infested treatments increased in total C concentration by 87 ± 24% ($P < 0.001$) and 34 ± 16% ($P = 0.07$), respectively, between 2005 and 2013; REF plots showed no change (Figure S3). This translated to accrual rates of 1.82 g C · kg-dry-soil^{-1} · year^{-1} in GDL and 0.73 g C · kg-dry-soil^{-1} · year^{-1} in HWA-infested plots. Subsurface soil C gains occurred exclusively in the MAOM fraction (Figure 2), with MAOM-C concentration increasing 190% in GDL ($P < 0.001$) and 66% in HWA-infested plots ($P = 0.07$). By 2013, GDL plots had 77% more MAOM-C in subsurface soils than REF plots ($P = 0.03$) and 54% more than HWA-infested plots ($P = 0.06$). In contrast, GDL plots lost C in the POM fraction, which declined 50% on average ($P = 0.02$).

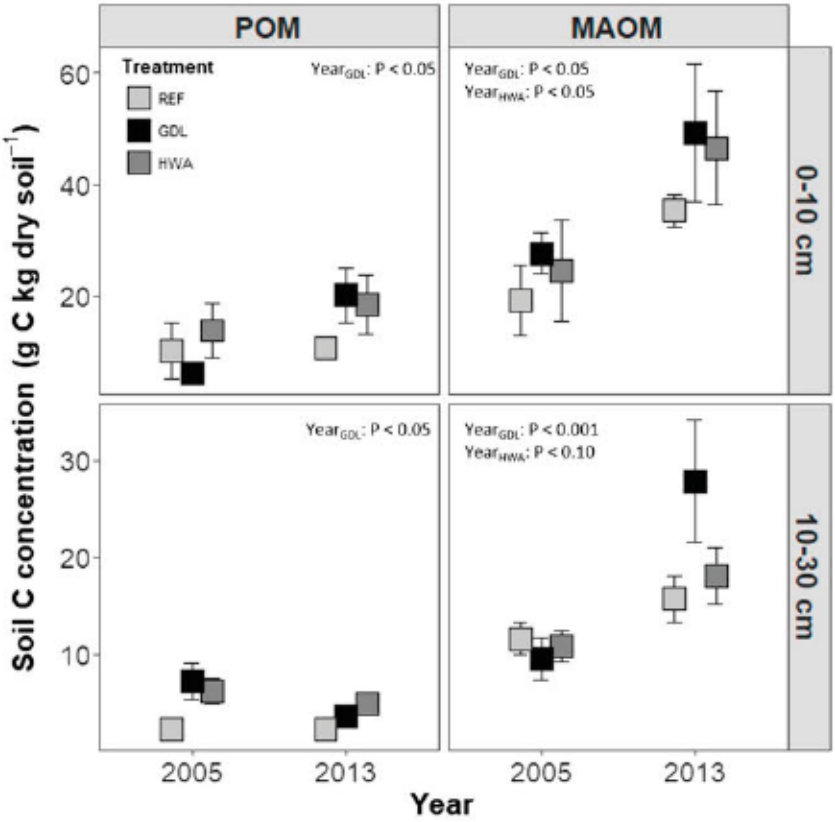

Figure 2. Total carbon concentration in particulate (POM; left) and mineral-associated soil organic matter (MAOM; right) fractions in 2005 and 2013 in surface (top, 0–10 cm) and subsurface (bottom, 10–30 cm) mineral soils. Data (mean ± 1 SE) from 2005 were collected just after treatment; eastern hemlock trees were girdled in GDL plots and hemlock woolly adelgid arrived in HWA plots in late 2004. Reference plots (REF) had <3% hemlock trees. All plots were sampled at the same time each year; points are jittered to improve visibility. Treatments showing a significant change over time are indicated.

Surface soil C:N ratio also changed over the study period (Figure 3). Prior to hemlock mortality, GDL and HWA-infested plots had significantly higher soil C:N ratios compared to REF plots (all comparisons: $P < 0.001$). By 2013, however, soil C:N did not differ among treatments (all comparisons: $P > 0.25$). Soil C:N decreased in GDL plots ($P = 0.02$) and increased in REF plots ($P = 0.03$) from 2005 to 2013, converging at a value of approximately 22 (Figure 3). Subsurface soils had significantly greater C:N in GDL and HWA-infested plots compared to REF plots. Only GDL plots showed a change in C:N ratio, which decreased by an average of 15% ($P = 0.01$).

3.4. Mineral Soil $\delta^{13}C$

Surface soil C isotopic composition of the POM fraction was enriched over time in GDL plots (0.5 ± 0.3‰ increase; $P = 0.08$), depleted in REF plots (1.1 ± 0.2‰ decrease; $P < 0.01$), and remained unchanged in HWA-infested plots from 2005 to 2013 (Figure 4). The 2013 POM $\delta^{13}C$ values were significantly higher in GDL ($P < 0.01$) and HWA-infested ($P < 0.01$) plots compared to REF plots. Prior to hemlock mortality in 2005, POM $\delta^{13}C$ values did not differ among treatment types. In subsurface soils, the POM fraction became more depleted in ^{13}C in REF plots (0.38 ± 0.1‰ decrease, $P < 0.01$) but did not change in plots that experienced hemlock mortality (Figure 4). Values of $\delta^{13}C$ estimated for the MAOM fraction from surface or subsurface soils collected in 2013 did not vary by treatment (data not shown).

3.5. O-Horizon Mass

The mass of the O-horizon declined in all treatments between 2005 and 2013 (year: $P < 0.001$); however, the trajectory of losses differed among treatments (treatment × year: $P < 0.05$, Figure S4). In REF plots, a 61% decrease in O-horizon mass occurred between 2005 and 2008 ($P < 0.001$), but mass remained unchanged from 2008 to 2013. In GDL and HWA-infested plots, mass declined throughout the study period. As a

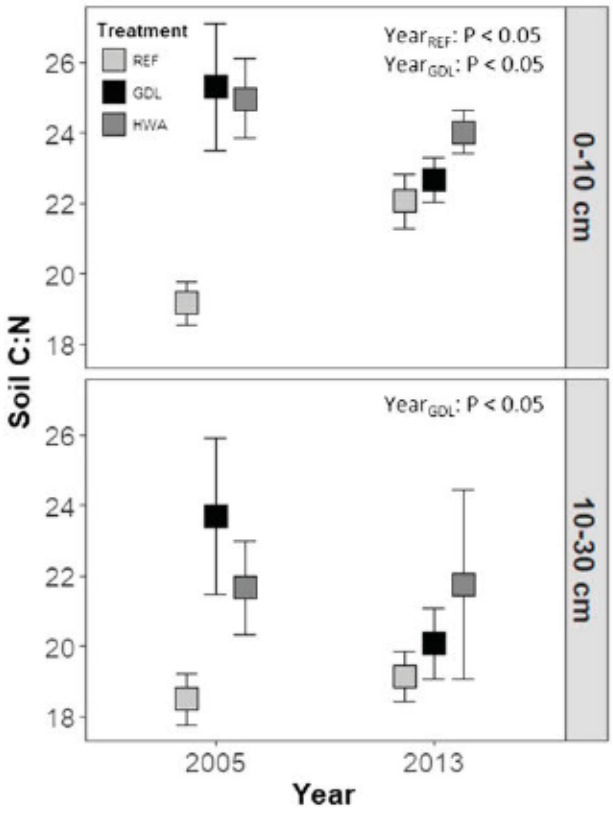

Figure 3. Mineral soil C:N in 2005 and 2013 in surface (top, 0–10 cm) and subsurface (bottom, 10–30 cm) soils. Data (mean ± 1 SE) from 2005 were collected just after treatment; hemlock trees were girdled in GDL plots and hemlock woolly adelgid arrived in HWA plots in late 2004. Reference plots (REF) had <3% hemlock trees. All plots were sampled at the same time each year; points are jittered to improve visibility. Treatments showing a significant change over time are indicated.

result, there were no significant differences among REF, GDL, and HWA-infested treatments in either the initial or final sample collection; whereas in the intermediate sample collection years, 2008 and 2009, O-horizon mass in GDL and HWA-infested treatments was 42–52% higher compared to REF. The overall estimated loss rates differed among treatments between 2008 and 2013 (P = 0.04) and were 2.64 Mg · ha^{-1} · year^{-1} in GDL (P = 0.003) and 2.26 Mg · ha^{-1} · year^{-1} in HWA-infested plots (P < 0.001), versus 0.68 Mg · ha^{-1} · year^{-1} in REF plots (P = 0.08).

3.6. Root and Fungal Mycelial Biomass

Total root biomass varied significantly with treatment and year; the treatment by year interaction was also significant (P < 0.01, Figure S5). There was no detectable change in total root biomass from 2004 to 2006 in any of the treatments. From 2006 to 2014, however, total root biomass decreased by 52 ± 12% in HWA-infested plots (P < 0.001) showed no change in GDL plots, and a marginal decrease in REF plots (28 ± 46%, P = 0.07). The decrease in HWA-infested plots was attributable to a decline in medium, fine, and very fine root size classes; coarse root biomass did not change (Table S3). In contrast, in GDL plots coarse root biomass increased 109 ± 83%, there was no change in the medium size roots, and both fine and very fine size roots decreased (Table S3). REF plots showed no change in root biomass in coarse or medium size classes and significant decreases in the fine and very fine size classes. Fungal mycelial biomass was highly variable and did not differ with treatment at either depth (Figure S6).

3.7. Extracellular Enzyme Activities

In 2014, activities of the cellulose-hydrolyzing enzyme BG were elevated in the surface soils of HWA-infested relative to REF plots (Figure 5). Compared to REF plots, BG activities in surface soils were 328% higher in HWA-infested plots (P = 0.04). Although GDL plots showed a trend for higher activities of BG, activities were highly variable and differences were not statistically significant (P = 0.10). Likewise, NAG showed a nonsignificant trend for higher activities in HWA-infested plots (P = 0.08) than in REF plots. Neither BG nor NAG activities differed among treatments in subsurface soils, and total oxidase activities did not vary with treatment in surface or subsurface soils (Figure S7).

4. Discussion

We find that soil respiration in southern Appalachians forests is resilient to moderate disturbance by FIPs infestation of codominant tree species over an intermediate timeframe. Eight years after disturbance, soil respiration did not differ among girdled, HWA-infested, and reference hardwood plots. In contrast, girdled and HWA-infested plots gained more C in the mineral-associated SOM fraction of both the surface and subsurface soil compared to reference plots. Observed decreases in O-horizon mass and fine root biomass (Figures S4 and S5), and elevated cellulose-degrading enzyme activities in surface soils (Figure 5) in plots with hemlock mortality indicate enhanced C stabilization through the transformation of C from biomass to the more stable, mineral-associated SOM pool.

Observed, depth-dependent changes in the particulate SOM pool of girdled plots provide evidence that enhanced organic matter transformation promoted C stabilization. The girdled plot surface POM fraction gained C, but these gains were relatively small compared to the C gains observed in the mineral-associated fraction. Moreover, the surface POM fraction became enriched in ^{13}C (Figure 4), consistent with enhanced decomposition and microbial transformation, loss of the lighter C isotope, and retention of the heavier isotope in the remaining organic C pool (Bostrom et al., 2007; Natelhoffer & Fry, 1988). By contrast, girdled

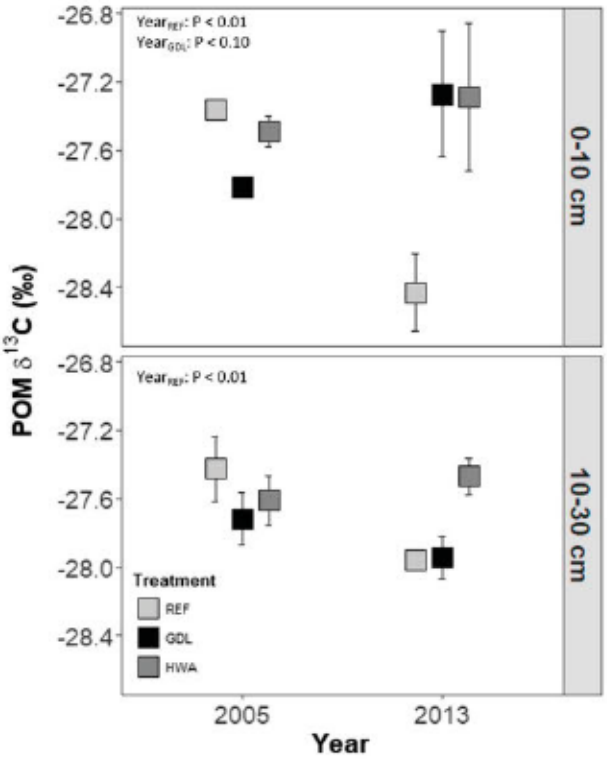

Figure 4. $\delta^{13}C$ in the particulate organic matter fraction (POM; mean ± 1 SE) in 2005 and 2013 in the surface (top, 0–10 cm) and subsurface (bottom, 10–30 cm) mineral soil. Data from 2005 were collected just after treatment; eastern hemlock trees were girdled in GDL plots and hemlock woolly adelgid arrived in HWA plots in late 2004. Reference plots (REF) had <3% hemlock trees. Points are jittered to improve visibility. For comparison, $\delta^{13}C$ was −26.9 ± 0.03 in fungal mycelia, −28.9 ± 0.17 in roots, and − 29.5 ± 0.09 in the forest floor O_i horizon. Treatments showing a significant change over time are indicated.

plots lost subsurface soil POM C. These findings point to preferential decomposition of the POM in the surface and subsurface soils. We hypothesize that substantial portions of this decomposed organic matter were stabilized in the mineral-associated SOM pool as this pool increased over time. This hypothesis is consistent with theoretical and experimental evidence indicating that the microbial byproducts of decomposition have a high affinity for soil minerals that favors the formation of mineral-stabilized organic matter (Grandy & Neff, 2008; Miltner et al., 2012).

Changes in the C concentration and isotopic composition of the POM fraction further indicate that disturbance effects on the balance between rates of input and loss from the POM pool are depth dependent. The small but significant increase in surface POM-C of girdled plots together with the nonsignificant increase in POM $\delta^{13}C$ suggests that plant-C input rates outpaced C loss rates from the POM fraction in the top 10 cm of soil, despite enhanced SOM mineralization. Following timber harvest, surface soil C concentration can decline while $\delta^{13}C$ increases because of high mineralization rates (Diochon & Kellman, 2008). In our study, surface C likely increased in the girdled plots over the study period because of the incorporation of hemlock residues from the organic horizon. Notably, there was no change in the C concentration of the surface POM fraction in reference plots, even though $\delta^{13}C$ in the POM fraction decreased. This suggests that incorporation of plant residues approximately balances losses from the surface POM fraction in the absence of disturbance. In contrast, the subsurface POM fraction of girdled plots lost C and showed no significant change in $\delta^{13}C$, suggesting that loss rates exceeded input rates to this pool. In temperate forests, inputs of plant-C decline with depth (Billings & Richter, 2006). Consequently, deep C losses can take longer to replenish than surficial C losses (Richter et al., 1999). This may explain why soil carbon recovery occurs more slowly in deep than surface soils (James & Harrison, 2016; Knoepp et al., 2014) and suggests that disturbance-driven SOC losses can be underestimated when SOC pools are measured at depths less than or equal to 10 cm.

Our findings do not support current paradigms predicting that overstory mortality from FIPs will result in a greater proportion of decomposition products partitioned to respiration than SOM (Hicke et al., 2012; Peltzer et al., 2010). R_{soil} declined relative to predicted values in the first 2 years after hemlock girdling due to root mortality (Nuckolls et al., 2009) and was comparable among treatments (including reference plots) nearly a decade later. These findings agree with those of related studies (Levy-Varon et al., 2014; Moore et al., 2013). The small difference in R_{soil} between girdled and HWA-infested plots observed in 2014 (Figure S2) was consistent with observed differences in root biomass (girdled > HWA infested; Figure S5 and Table S3) and the expected autotrophic response to treatment. Ekberg et al. (2007) found that heterotrophic respiration in forest plots 2-year postgirdling was equal to combined autotrophic and heterotrophic respiration in control plots due to increased substrate availability for microbial decomposition, particularly in the form of dead root matter. Similarly, Moore et al. (2013) suggested that persistent declines in R_{soil} following tree mortality from mountain pine beetle were associated with the loss of autotrophic substrate. Thus, the recovery of R_{soil} in the girdled plots 8-year posttreatment was likely a result of root biomass recovery and, to a lesser extent, microbial respiration, due to increased substrate availability. In agreement with other studies (Jenkins et al., 1999; Kizlinski et al., 2002; Raymer et al., 2013), we observed declines in O-horizon mass and fine root biomass over the study period in plots with hemlock mortality, providing strong evidence that these pools were the primary sources of substrate.

Our findings highlight a need to understand the mechanisms that led to enhanced C stabilization in the mineral-associated SOM fraction. Although we did not detect an increase in hardwood basal area, an

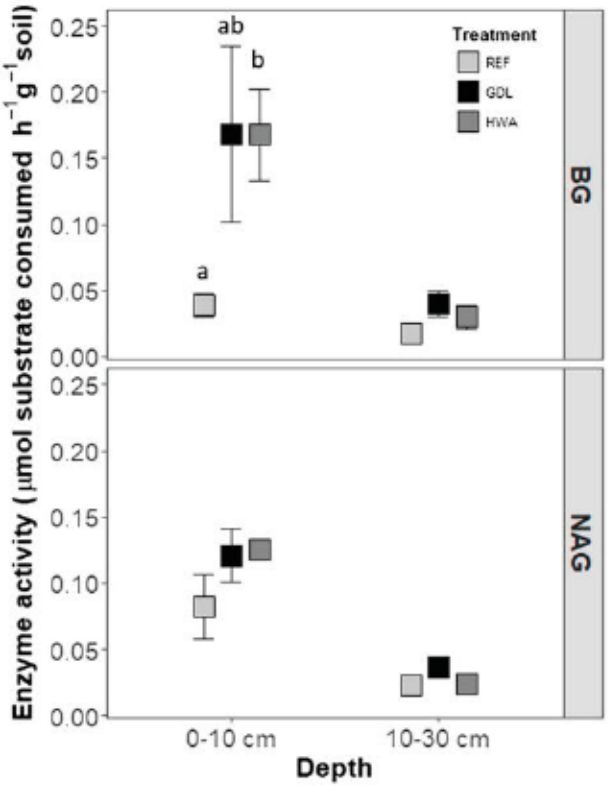

Figure 5. Activities (μmol substrate consumed · hr^{-1} g^{-1} soil; mean ± SE) of β-glucosidase (BG) and β-1,4-N-acetylglucosaminidase (NAG) in surface (0–10 cm) and subsurface (10–30 cm) mineral soils collected in August 2014 (8 years posttreatment). GDL = girdled plots, HWA = plots infested by hemlock woolly adelgid; REF = reference plots with <3% hemlock trees. Means with different letters indicate statistically significant differences at α = 0.05 (Tukey's HSD for multiple comparisons).

earlier study that controlled for interannual climate variability showed that hemlock decline resulted in an increase in the growth of existing hardwood species, primarily *A. rubrum*, *B. lenta*, and *L. tulipifera* (Ford et al., 2012). This compensatory response of the tree community and attendant shifts in substrate quality may have promoted C stabilization. Compared to hemlock, these hardwood species produce leaf litter with lower lignin and higher N concentrations (Knoepp et al., 2011). Accordingly in 2008, Knoepp et al. (2011) observed that leaf litterfall N flux in girdled plots was lower than in reference plots but higher than in HWA-infested plots, in line with the rate of hemlock decline in the treatments. Similarly, O-horizon C:N in girdled plots was higher than in reference plots but lower than in HWA-infested plots. Over the next 6 years as hardwoods continued to replace hemlock, leaf litterfall N flux should have continued to rise (and O-horizon C:N fall) in hemlock mortality plots, at rates commensurate with the rate of hemlock mortality in each treatment. These predictions agree with the surface and subsurface decreases in soil C:N that we observed (Figure 3). We hypothesize that such inputs favored the growth of fast-growing, soil microbes with high CUE (Chen et al., 2014). This is supported by our observations of higher activities of hydrolytic extracelluar enzymes in plots that experienced hemlock mortality. As existing labile substrate (i.e., dead biomass and POM) in hemlock plots was depleted, the turnover of these microbes would favor the physical stabilization of microbial biomass C on soil clay minerals (Kallenbach et al., 2016). This may explain why mineral-associated C content increased more rapidly in girdled and HWA-infested plots and why this response was greater in the 10- to 30-cm soil depth in the clay accumulating Bt horizon.

Finzi et al. (2014) and Raymer et al. (2013) measured C budgets in a chronosequence describing time since disturbance of hemlock forests, including secondary hemlock forests (>130 years since establishment) and disturbed stands (5-year postgirdling and 18-year post-HWA infestation) with regrowing black birch saplings. While they found that total soil C did not differ among stand types, C content in the surface mineral soil (0- to 15-cm depth) was significantly higher in stands 18 years post-HWA than in secondary hemlock and girdled stands, consistent with our findings. However, they found no differences among stand types with respect to labile C-degrading extracellular enzyme activities in mineral soils. This discrepancy could be a result of methodological differences, as Finzi et al. compared weighted-average activities based soil C concentration in 15-cm depth increments. We did not weight activities and found no effect of treatment in subsurface soils. Additionally, in their study plots, black birch (*B. lenta* L.) was the primary species replacing hemlock. Black birch has a high nutrient use efficiency rate and exhibited high rates of ANPP following hemlock loss, which they suggest compensated for C losses associated with increased decomposition (Finzi et al., 2014).

Although our girdled and HWA-infested plots showed similar C cycle responses to disturbance, they did not consistently mirror each other throughout the study period. Hemlock trees died more quickly in girdled than in HWA-infested plots, with 50% mortality in 2 and 6 years, respectively (Ford et al., 2012). The accelerated mortality of hemlocks in girdled plots suggests that these plots reflect what the HWA-infested plots will look like several years into the future. For instance, in 2014 R_{soil} in girdled plots was comparable to reference plots and slightly higher than HWA-infested plots. This suggests that R_{soil} in the HWA-infested plots is still recovering and microbial activity is not yet as high as in girdled plots. This could result from the more recent addition of hemlock woody debris in HWA-infested plots, organic material that is not readily available to soil microbes. In 2014, there was more woody debris in HWA-infested plots than in girdled plots (data not shown). Additionally, the root biomass may not have fully recovered in HWA-infested plots (Figure S5). Working at the same study site, Nuckolls et al. (2009) found that very fine root biomass declined more quickly in girdled than in HWA-infested plots from 2004 to 2006. Collectively, these findings suggest an accelerated response in

girdled plots. Thus, although girdling is a common technique used to simulate FIPs or other causes of tree mortality, this treatment may yield differences in rates and patterns of soil C cycle responses.

In our system, changes in soil C cycling following FIP infestation and host tree mortality were primarily due to increases in organic matter decomposition and soil microbial activity. Both organic and mineral soils in this formerly mixed hemlock forest show evidence of increased decomposition over time with declines in O-horizon mass and mineral soil POM, while accumulating soil C within the mineral-associated SOM pool. We conclude that changes in soil C cycling in this ecosystem in response to FIP disturbance resulted from accelerated rates of decomposition and C translocation into and between SOM pools. Our findings run counter to the results from simulation modeling of HWA-infested stands in northern states, which show a brief, small increase in heterotrophic respiration followed by two decades of decline, which may or may not be followed by a period of increasing respiration (Albani et al., 2010). A model developed by Crowley et al. (2016) suggested that in an 80% hemlock/20% yellow birch stand soil C pools would take ~145 years to recover from decreased inputs of recalcitrant hemlock litter following HWA-induced hemlock mortality. Our data show that SOC pools increase within a decade of hemlock mortality because a large portion of the added detritus and a fraction of the POM pool becomes part of the more stable, mineral-associated SOM pool and is not lost by soil respiration as predicted by simulation models. We speculate that in this mixed forest where hemlock ranged from 43% to 77% of total aboveground biomass (Table S1), compensatory growth of nonhost trees facilitated this transfer, thereby buffering the soil C losses predicted or observed elsewhere (Crowley et al., 2016; Dixon et al., 1994; Kurz et al., 2008).

In addition to improving our understanding of the mechanistic impacts of FIPs on soil C cycling processes, our study illustrates the importance of collecting and maintaining long-term data sets. Whereas mineral soil C did not change in the first 3–4 years following HWA infestation or girdling (Knoepp et al., 2011), we observed increases 5 years later that provide strong evidence of soil C redistribution as a result of FIPs infestation. Moreover, our study highlights that the direction and magnitude of responses can be variable depending on the type and extent of the disturbance and the rate at which remaining, nonhost trees respond to gaps left by FIPs. As Hicke et al. (2012) and Peltzer et al. (2010) note, we currently have a limited understanding of how the long-term trajectory of forest C cycling, and especially soil C cycling, will change following disturbance by FIPs. Our study lends valuable insight into the mechanisms through which decadal soil C cycling responds to forest disturbance.

Acknowledgments
Funding for this project was provided by the USDA or United States Department of Agriculture Forest Service (13-CA-11330140-119) and a grant from NSF to the Coweeta LTER Site (DEB-1440485). We thank for T. Cascia for lab assistance, and E. DeLucia and two anonymous reviewers for their helpful comments. Data are available on the LTER Network Data Portal at https://doi.org/10.6073/pasta/dfb19dbb5fcfee394721 1afa5884c9d8.

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