



Decline of a foundation tree species due to invasive insects will trigger net release of soil organic carbon

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Abstract. Eastern U.S. forests are witnessing an ecologically disruptive decline in one of the region's distinctive foundation tree species, the eastern hemlock (*Tsuga canadensis*). The exotic insect pests hemlock woolly adelgid (*Adelges tsugae*, HWA) and elongate hemlock scale (*Fiorinia externa*) have greatly altered many forest ecosystems previously dominated by this important evergreen conifer. The consequences for ecosystem processes are far reaching because hemlock is often replaced by deciduous tree species, such as black birch (*Betula lenta*), which have strongly divergent effects on forest floor microenvironments and nutrient cycling. We took advantage of an accidental experiment initiated by patch-level timber harvesting ~30 yr ago to investigate how the removal of hemlock, and its replacement by deciduous trees, has affected leaf litter characteristics, soil organic layer mass, C:N content, and soil respiration rates. We also contrasted these areas to nearby forest plots where deciduous *B. lenta* has been dominant for almost a century. The inclusion of healthy, intact hemlock stands in the design, and the close proximity of plots, allowed for a powerful space-for-time approach to detect ecosystem changes that are likely to occur across the broader landscape with HWA-induced hemlock loss in coming years. Three years of data collection from a series of plots in hemlock, young birch, and mature birch stands revealed dramatic differences in soil carbon pools and cycling. Between forests dominated by hemlock vs. mature birch, we saw a significant decrease in soil organic layer mass and in the C:N of the remaining organic material. Although hemlock and young birch stands showed no significant differences in soil respiration rates, mature birch stands had significantly higher soil respiration rates throughout the entire growing season, regardless of wet or dry years. Our results suggest that the carbon pool in the forest floor is likely to mobilize through greater decomposition with a 6.8× decline in soil organic layer C storage as hemlocks are replaced by deciduous trees, leading to a potential net release of ~4.5 tons C per hectare. We conclude that the ramifications of this change for carbon storage could be extensive, but may take decades to manifest.

Key words: Black birch; carbon sink; carbon source; eastern hemlock; hemlock woolly adelgid; invasion; soil organic carbon; soil respiration.

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INTRODUCTION

Biotic disturbances are having major effects on forest ecosystems across the United States and Canada (Ellison et al. 2005, Lovett et al. 2006, Hicke et al. 2012). The impacts of invasive pests have been especially disruptive when these invaders target dominant plant species that strongly

influence the structure and characteristics of their local environment. Eastern hemlock (*Tsuga canadensis*), a distinctive evergreen conifer, is considered a foundation species in the temperate deciduous forests of the eastern United States, as its presence structures avian communities (Tingley et al. 2002), influences levels of diversity in ant and benthic invertebrate stream communities,

and favors certain forest floor organisms such as bryophytes and soil mesofauna, like mites and collembolans (Snyder et al. 2002, Ellison et al. 2005, Zuskwert et al. 2014, Siddig et al. 2016). In addition to their influence on ecological communities and other species, foundation tree species like hemlock affect ecosystem processes such as decomposition, nutrient cycling, and carbon sequestration (Ellison et al. 2005). Compared to deciduous tree-dominated areas, hemlock stands have lower light levels due to the dense, evergreen canopy, and the forest floor tends to be moister due to cooler conditions (Ellison et al. 2005). Hemlock stands dramatically influence forest floor structure and edaphic conditions, and typically develop deep, acidic organic layers with slow rates of decomposition and nutrient cycling (Lovett et al. 2006).

Invasive insect pests are currently threatening hemlock with decline and extirpation across much of its range in the eastern United States, and the species' decline has the potential to produce profound changes in ecosystem function (Orwig et al. 2002, Ellison et al. 2005). The hemlock woolly adelgid (*Adelges tsugae*, HWA) is a xylem parenchyma cell-feeding insect introduced to eastern North America from southern Japan, where it had co-evolved with native Japanese hemlocks (Havill et al. 2006). Hemlock woolly adelgid was first reported in Richmond, Virginia, USA, in the 1950s, and has since expanded its range into the northeastern United States (McClure 1990, Orwig and Foster 1998), appearing in nearly 90% of stands in southern New England (Orwig et al. 2002). With HWA infestation, eastern hemlocks experience high levels of foliar loss and tree mortality in as little as 4–5 yr (Orwig and Foster 1998, Lovett et al. 2006). The HWA infestation may cause a systemic hypersensitive response in eastern hemlocks, resulting in elevated foliar hydrogen peroxide (H_2O_2) levels plant-wide and ultimately tissue death (Radville et al. 2011). HWA infestation also leads to a significant increase in the formation of false rings, areas of cells with thick cell walls that disrupt water flow throughout the plant (Gonda-King et al. 2012).

In addition to HWA, hemlocks are also being attacked by a second invasive insect, the elongate hemlock scale (*Fiorinia externa*, EHS). This insect co-occurs in many areas with HWA and is generally considered less of a threat to hemlock

(Gonda-King et al. 2012), although observations suggest that high-density infestations may also result in foliar loss (McClure 1980, Abell and Van Driesche 2012). It is currently debated whether EHS contributes to hemlock decline or, conversely, might mediate or lessen the effects of HWA through competitive interactions (Preisser and Elkinton 2008, Gomez et al. 2015). Regardless, the abundance and dominance of hemlock in many eastern U.S. forests have already declined substantially and the species is expected to be lost from many areas in coming decades.

In New England, hemlock decline typically leads to the conifer's replacement by black birch (*Betula lenta*), a deciduous tree species with substantially different effects on forest floor characteristics (Orwig and Foster 1998, Cobb 2010, Zuskwert et al. 2014). Black birch often occurs as an associate of hemlock in New England forests, and birch's abundant seed production and the strong response of its seedlings to higher-light environments, such as those generated by thinning or dying hemlock, lead to its rapid growth in declining hemlock stands (Orwig and Foster 1998, Catovsky and Bazzaz 2001). Hemlock-dominated ecosystems are generally characterized as having low and slow N cycling due to the tree's low foliar and litter N, which results in soils with low extractable N pools, and low rates of potential net mineralization and nitrification (Lovett et al. 2004, Cobb 2010). In contrast, net mineralization, nitrification, and N turnover increased in sites that experienced hemlock mortality (Jenkins et al. 1999). These changes relate in part to hemlock's replacement by black birch, as previous studies have found that birch litter decomposes more rapidly than that of hemlock, indicating marked differences in leaf litter quality (Cobb 2010, Raymer et al. 2013, Finzi et al. 2014). Finally, black birch biomass and soil have also been found to have a significantly higher N content than hemlock (Finzi et al. 2014). Overall, the decline of hemlock and associated shift in dominant tree species is likely to have broad consequences for ecosystem function through changes in nutrient cycling and edaphic conditions (Ellison et al. 2005, Raymer et al. 2013, Zuskwert et al. 2014). Extensive damage to hemlocks due to defoliation, combined with reductions in water use, can lead to dramatic reductions in gross primary productivity (Domec

et al. 2013, Gomez et al. 2015). However, despite major alterations in leaf litter and N cycling with hemlock decline (Jenkins et al. 1999, Lovett et al. 2004, Cobb 2010, Orwig et al. 2013, Finzi et al. 2014), there has been surprisingly little evidence to date of impacts on soil C efflux (Orwig et al. 2013, Raymer et al. 2013, Finzi et al. 2014).

In this study, we explore how soil C and N, and soil CO₂ fluxes, have been altered as a result of forest conversion from evergreen hemlock to deciduous black birch dominance. It has been well established that the transition from hemlock to birch stands results in short-term increases in N cycling and decomposition (Jenkins et al. 1999, Cobb 2010, Orwig et al. 2013, Finzi et al. 2014). However, previous research has not observed large or long-lasting effects on C fluxes. The absence of clear evidence for C pool changes might trace to the limited time spans of the ecosystem conversions studied to date (e.g., a few years during and after natural hemlock decline, or with recent experimental removal), or the lack of comparisons between hemlock and closely paired stands of mature deciduous trees. In the present study, we take advantage of a long-running accidental experiment in hemlock decline by contrasting intact areas of mature hemlock with embedded patches of young black birch generated by logging ~30 yr ago in the late 1980s (Zukswert et al. 2014). These two forest types are further compared to nearby deciduous stands dominated by mature black birch on similar terrain and soils, where birch has been dominant for almost a century. Our study differs from previous work in that all of our hemlock and black birch stands are in close proximity to each other and were sampled for soil respiration on the same day over multiple survey dates. We use these three forest types as a space-for-time substitution to understand how ecosystem processes are likely to be altered as hemlock forests are replaced by birch stands across the broader landscape in coming decades. With a shift from hemlock- to deciduous tree-dominated ecosystems, we predict that (1) mass of the soil organic layer will decrease, (2) organic layer C:N will decrease, and (3) soil respiration rates will increase, resulting in higher rates of C cycling and a substantial net mobilization of soil organic carbon from former hemlock stands into the atmosphere.

METHODS

Study site description

This study was conducted in a forested area at Smith College's Ada and Archibald MacLeish Field Station, located in Whately, Massachusetts, USA (42°27.32' N, 72°40.96' W), in central New England (Fig. 1). The field station consists of 105 ha of largely secondary growth northern hardwoods–hemlock–white pine forest (Zukswert et al. 2014). Hemlock woolly adelgid and EHS have been observed on hemlocks at the MacLeish field station since 2009–2010, although few trees have died due to the pests and most trees retain healthy foliage. The MacLeish Field Station is located near the northern edge of HWA's distribution, where cold winter conditions appear to cause occasional dieback of HWA populations in the region. The HWA infestation in this area appears to be lower level and chronic compared to the more severe outbreaks in warmer regions to the south. Finally, there is some evidence that the presence of EHS discourages HWA colonization of hemlock (Preisser and Elkinton 2008), possibly leading to hemlocks in central New England declining at a slower pace than observed elsewhere.

These forests developed on abandoned agricultural land (mostly upland pasture) in the late 19th and early 20th centuries. In the late 1980s, a commercial selective logging harvest was conducted on the property, in which over 1/3 of the total lumber removed was hemlock (Zukswert et al. 2014). Forest gaps created by this logging event led to the development of dense young stands of ~25- to 30-yr-old black birch embedded in a matrix of mature northern hardwoods–hemlock forest (Zukswert et al. 2014). The young birch stands provide an example of potential forest conditions following predicted hemlock decline, similar to the dense stands of young birch that have developed in the wake of HWA-induced hemlock mortality in southern New England (Orwig and Foster 1998). Although these young birch stands are not entirely free of hemlock leaf litter inputs from nearby hemlock stands, they do represent systems where hemlock inputs have declined substantially relative to the surrounding hemlock-dominated forest. This accidental experiment effectively allows a comparison of environmentally matched plots of pre-hemlock decline forest



Fig. 1. Study area in western Massachusetts at Smith College's MacLeish Field Station in the towns of Whately and Conway. The locations of the three plot pairs in mature hemlock forest and young birch patches are indicated by black circles, and the locations of three plots in mature birch forest are indicated by black diamonds.

and forests patches comparable to late-stage hemlock decline. In 2010, six research plots (10×15 m) were established in these adjacent young birch and mature hemlock forest patches. In 2015, three additional plots were established in nearby (~ 100 – 150 m east) mature birch forest where no hemlock trees are present. Trees in the two mature forest types (hemlock, black birch) are ~ 80 – 100 yr in age based on tree core samples, while those in the young birch plots are 25–30 yr old (J. Bellemare, *personal communication*).

Characterization of basal area and leaf litter inputs

Basal area was estimated for each 10×15 m plot by measuring diameter at breast height (dbh) of all trees and saplings >1.4 m in height. We characterized each of the three forest types by total basal area, %hemlock basal area, %black birch, and %other deciduous species. Leaf litter inputs were sampled in all plots to estimate mass and tree species composition. Litter deposition rate was based on collections from five mesh-lined laundry baskets (0.55×0.39 m) per 10×15 m plot, maintained and monitored for a

full year. Litter included deciduous leaves, conifer needles, twigs, bark, fruits, seeds, cones, insect droppings, and insect bodies. Litter samples were parsed out for total leaf litter (g/m^2) and %hemlock needles vs. %black birch leaves vs. other deciduous tree species. A subsample of the leaf litter sorted to species was dried and pulverized for testing %C, %N, and C:N analysis at the Cornell University Stable Isotope Laboratory (COIL, Ithaca, New York, USA) using a Thermo Finnigan Delta Plus mass spectrometer connected to a NC2500 elemental analyzer (Carlo Erba Instruments, Milan, Italy) for continuous flow analysis of carbon and nitrogen.

Characterization of soil organic layer

During 2013–2015, forest floor soil organic layer samples were collected to quantify differences between the three forest types in organic layer mass and nutrient composition. For each plot, a series of 10 randomly established 0.25×0.25 m subplots were sampled, from which the soil organic layer was collected, oven-dried, and weighed to determine forest floor dry mass per unit area. This material was then

homogenized and sieved to <2 mm, from which a subsample was drawn for testing %C, %N, and C:N analysis at the Cornell University Stable Isotope Laboratory (COIL).

Soil respiration

Using a closed-path infrared gas analyzer system (LI-6400; LI-COR Inc., Lincoln, Nebraska, USA), the rate of soil respiration was measured every ~3–4 weeks throughout the growing season from May to November in three consecutive years (2015–2017). Three 10 cm diameter PVC (polyvinyl chloride) soil collars per plot in each forest type were installed during the summer of 2014. In order to capture maximum CO₂ efflux rates, three measurements were made on each collar between 10 a.m. and 2 p.m. on sampling dates. Soil temperature was measured in the area adjacent to each soil collar. Additionally, volumetric water content (VWC) measurements were made with a soil moisture probe (HydroSense; Campbell Scientific, Logan, Utah, USA) next to each soil collar at a depth of 12 cm. We used mean soil respiration rates on a given sampling date to calculate % difference in soil respiration of each birch forest type relative to hemlock conditions.

Statistical analysis

A nested ANOVA was used to analyze differences in the mean organic layer mass, mean carbon and nitrogen of the organic layer, mean leaf litter characteristics, and mean carbon and nitrogen of the leaf litter between forest types. Additionally, Tukey's honestly significant difference (HSD) post hoc comparison and *t* tests were made in order to distinguish significant differences between the individual forest types. A nested repeated measures ANOVA was used to test for significant differences in mean soil respiration and VWC over time, forest type, and their interaction. The assumptions of normality and homogeneity of variance were met. Percent

differences in soil respiration rates over time were not statistically analyzed as they were calculated using mean soil respiration rates per forest type on a given date. All analyses and graphing were conducted in R version 3.3.3 (R Core Team 2017).

RESULTS

Basal area and leaf litter inputs

Among the three forest types sampled, basal area (Table 1) was highest in the hemlock plots (mean, 45.9 m²/ha ± 11.14 standard error [SE]) and mature black birch plots (34.52 m²/ha ± 1.85 SE); as expected, the young birch forest type had much lower basal area (14.06 m²/ha ± 1.80), reflecting loss of all mature trees to logging in the 1980s. These differences in basal area were not analyzed statistically, as replication was low (*n* = 3 plots per forest type). The hemlock forest type was indeed dominated by hemlock (58.9% of basal area; Appendix S1: Fig. S1), but also included deciduous species like black birch (33.6%) and other deciduous trees (7.6%). Mature birch plots were heavily dominated by black birch (78.7% of basal area) and other deciduous tree species (21.2%), with only a single stem of hemlock sampled (0.2%). The young birch plots were also dominated by sapling-stage black birch (83.4%) and other deciduous tree species (16.6%), with no hemlock present into the canopy, subcanopy, or ground layer.

We used a nested ANOVA to test for differences in total litter inputs between the three forest types (Table 1). Mean total leaf litter was significantly different by forest type (Appendix S1: Table S1; *P* < 0.0001), with mature birch plots having the highest mean total leaf litter inputs (412.2 g/m² ± 11.44 SE), followed by hemlock (320.0 g/m² ± 10.83 SE), then by young birch (275.5 g/m² ± 10.30 SE). A Tukey's HSD post hoc test revealed that mature birch litter inputs were

Table 1. Mean basal area (m²/ha), mean total leaf litter (g/m²), mean % hemlock leaf litter, and mean % birch leaf litter and ±1 standard error for each forest type.

Forest	Basal area	Total leaf litter	% Hemlock leaf litter	% Birch leaf litter
Hemlock	45.90 ± 11.14	320.0 ± 10.83	40.40 ± 1.78	29.85 ± 2.07
Young birch	14.06 ± 1.80	275.5 ± 10.30	12.78 ± 1.52	58.88 ± 3.04
Mature birch	34.52 ± 1.85	412.2 ± 11.44	0.07 ± 0.03	63.90 ± 2.16

significantly higher than hemlock ($P < 0.0001$) and young birch ($P < 0.0001$). Additionally, hemlock inputs were significantly higher than young birch ($P = 0.01$).

We characterized the leaf litter inputs by tallying the dry mass percentage attributed to hemlock needles vs. black birch leaves and other deciduous litter (Table 1). The hemlock forest type contained $40.4\% \pm 1.8$ SE hemlock needles and $29.9\% \pm 2.1$ SE birch leaves, with the remaining leaf litter represented by a mix of deciduous species (e.g., *Quercus rubra* and *Acer rubrum*). The mature birch forest type included only $0.1\% \pm 0.03$ SE hemlock needles (drifting in from outside-plot trees) and $63.9\% \pm 2.2$ SE black birch leaves; the remainder of litter was a mix of other deciduous tree species (e.g., *Fagus grandifolia* and *A. rubrum*). Finally, the young birch forest type included $12.8\% \pm 1.5$ SE hemlock needles, drifting in from hemlock trees adjacent to the plots, and $58.9\% \pm 3.0$ SE black birch leaves, with the remainder being other deciduous tree leaves.

We analyzed sorted leaf litter samples for C and N content to determine the quality of inputs to the soil organic layer from the two dominant tree species (hemlock, black birch). Specifically, we used a Welch two-sample t test to distinguish mean differences of %C (Fig. 2a), %N (Fig. 2b), and C:N (Fig. 2c) in samples of fallen hemlock needles compared to birch leaves. Hemlock needles had significantly ($t_{17.89} = -3.69$, $P = 0.002$) higher mean %C (54.26 ± 0.25 SE) compared to fallen birch leaves (52.88 ± 0.27 SE). Contrastingly, birch leaves had significantly ($t_{15.55} = 2.77$, $P = 0.01$) higher mean %N (0.87 ± 0.02 SE) compared to hemlock needles (0.79 ± 0.02 SE). These differences led to divergent C:N ratios between the two tree species: hemlock shed needles with significantly ($t_{17.47} = -3.49$, $P = 0.003$) higher mean C:N (80.11 ± 1.57) than seen in fallen birch leaves (71.55 ± 1.88 SE).

Soil organic layer characteristics

A nested ANOVA showed a significant difference in mean organic layer mass (Fig. 3; Appendix S1: Table S2) between the three forest types ($P < 0.0001$). Organic layer mass was highest in hemlock plots ($1089.89 \text{ g/m}^2 \pm 40.70$ SE), followed by young birch ($611.68 \text{ g/m}^2 \pm 59.48$

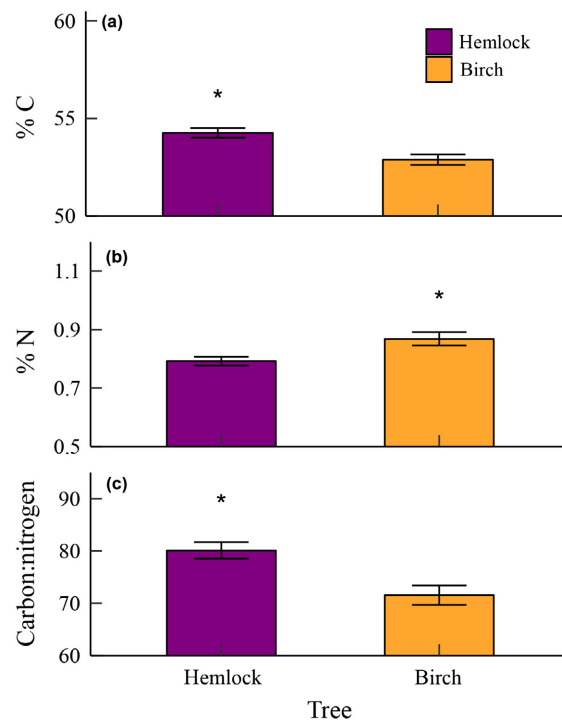


Fig. 2. Mean %C (a), %N (b), and C:N (c) in leaf litter of hemlock and black birch. Error bars are ± 1 standard error. An asterisk indicates significant differences between forest types.

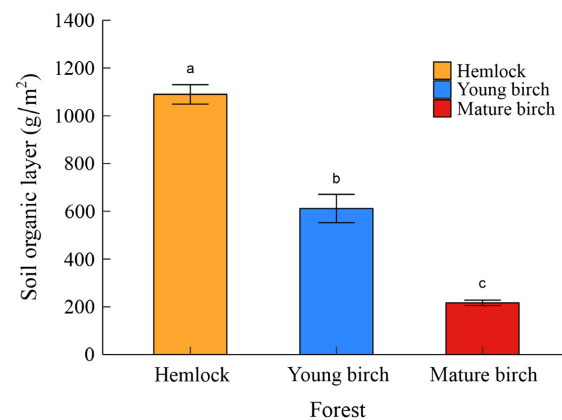


Fig. 3. Mean soil organic layer (g/m^2) for hemlock, young birch, and mature birch forest types. Error bars are ± 1 standard error. Letters designate forests that were significantly different via post hoc Tukey's honestly significant difference analysis.

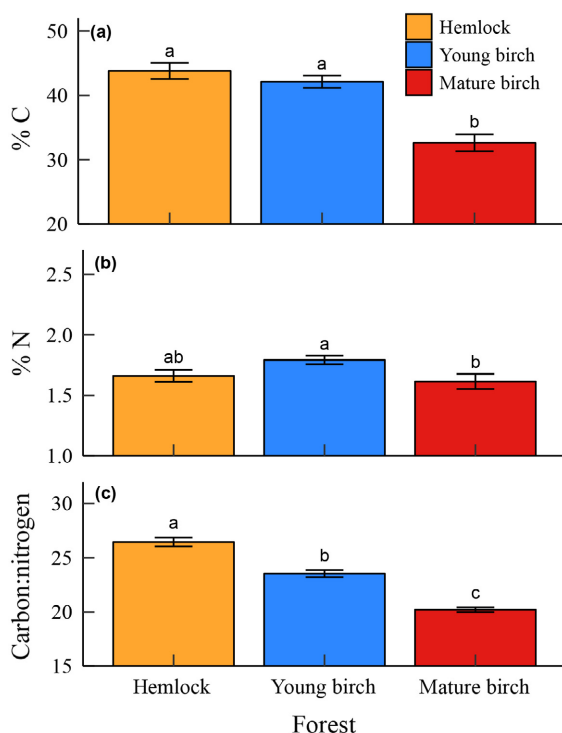


Fig. 4. Mean %C (a), %N (b), and C:N (c) in the soil organic layer of hemlock, young birch, and birch forest types. Error bars are ± 1 standard error. Letters designate forests that were significantly different via post hoc Tukey's honestly significant difference analysis.

SE), with the lowest organic layer mass seen in the mature birch plots ($216.53 \text{ g/m}^2 \pm 11.41 \text{ SE}$). A Tukey's HSD post hoc test revealed all sites were significantly different from one another with hemlock ($P < 0.0001$) > young birch ($P < 0.0001$) > mature birch ($P < 0.0001$).

We also analyzed %C, %N, and C:N of the soil organic layer for each forest type using a nested ANOVA (Appendix S1: Table S3–S5, respectively). Mean %C (Fig. 4a) was significantly different by forest type ($P < 0.0001$). Hemlock had the highest %C ($43.80 \pm 1.25 \text{ SE}$), followed by young birch ($42.13 \pm 0.95 \text{ SE}$), then by mature birch ($32.63 \pm 1.30 \text{ SE}$). A Tukey's HSD post hoc test revealed that hemlock and young birch were not significantly different, while mature birch had significantly lower %C in its organic layer compared to young birch ($P < 0.0001$) and hemlock ($P < 0.0001$). Mean %N (Fig. 4b) was also significantly different by forest type ($P = 0.03$). A Tukey's HSD post hoc test revealed that %N was significantly different only

between young birch and mature birch ($P = 0.03$). Analyses showed that mean organic layer C:N (Fig. 4c) was significantly different among the forest types ($P < 0.0001$). Hemlock had the highest %C (26.46 ± 0.40), followed by young birch (23.54 ± 0.33), then by mature birch (20.21 ± 0.22). A Tukey's post hoc comparison revealed that hemlock was significantly different from young birch ($P < 0.0001$) and mature birch ($P < 0.0001$), and young birch and mature birch were significantly different ($P < 0.0001$).

Volumetric water content

We measured VWC in conjunction with each soil respiration measurement (Appendix S1: Table S6, Fig. S2). A repeated measures ANOVA revealed that mean VWC was significantly different by forest type ($P < 0.0001$) during the 2015 growing season. Young birch consistently had the highest soil moisture at each sampling date. Mature birch plots were not added until 5 August 2015, but soil moisture values were similar to hemlock. Trends in the 2016 growing season were substantially different from those seen in 2015, and data from the 2016 season differed significantly by forest type ($P < 0.0001$) and date ($P < 0.0001$). Late spring and early summer had ample rainfall, and young birch had the highest soil moisture. Concurrently, mature birch and hemlock had very similar soil moisture content. However, a widespread drought developed during the 2016 growing season, which led to all forest types having exceptionally low soil moisture during the peak of the growing season. A slight increase in late summer precipitation increased soil moisture conditions and young birch resumed its trend of having the highest soil moisture. The 2017 growing season showed no significant differences.

Soil respiration rates and percentage of differences in soil respiration

A repeated measures ANOVA was used to analyze soil respiration for 2015, 2016, and 2017 (Appendix S1: Table S7). The analysis revealed that soil respiration was significantly different by a forest type and date interaction ($P < 0.0001$) during the 2015 growing season (Fig. 5a). Overall, soil respiration rates for all forest types were the lowest pre- and post-growing season. Hemlock and young birch had similar soil respiration rates throughout the most active periods of the

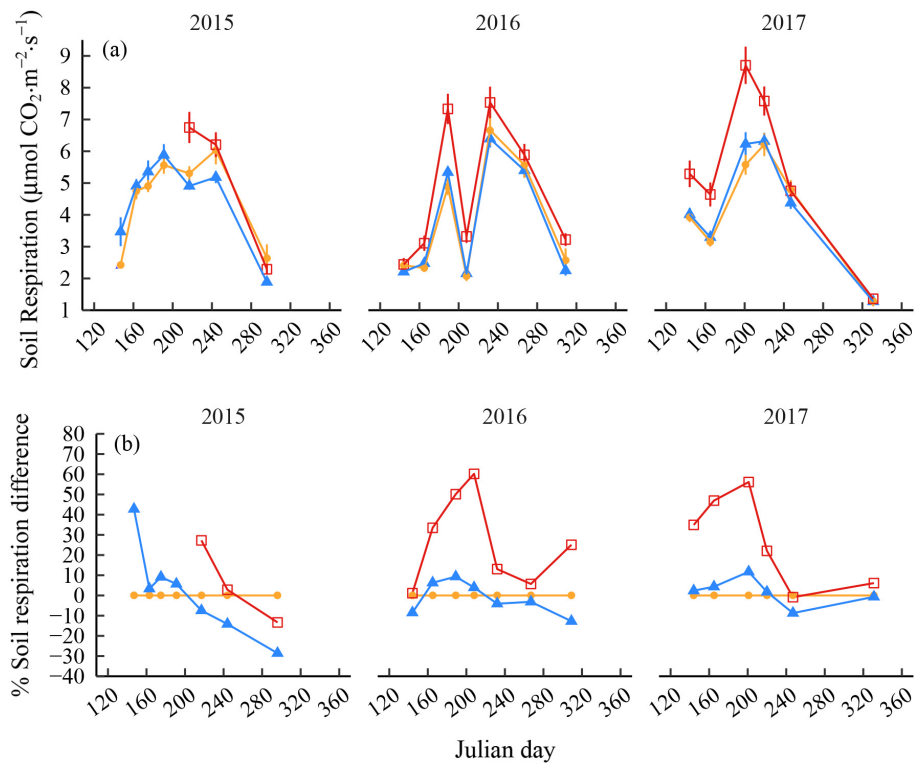


Fig. 5. Mean soil respiration rate (a; $\mu\text{mol CO}_2\text{-m}^{-2}\text{-s}^{-1}$) and % soil respiration difference (b) for each forest type over Julian day in 2015, 2016, and 2017. Error bars are ± 1 standard error. Hemlock has yellow circles. Young birch has blue triangles. Mature birch has red squares.

growing season. Relative to hemlock, young birch had at $>40\%$ higher soil respiration at the start of the growing season, but up to 30% lower soil respiration during the fall (Fig. 5b). Mature birch plots were not added until 5 August 2015, but exhibited much higher soil respiration rates at the peak of the growing season in 2016 and 2017. As the season progressed into the fall, young birch and mature had a steeper decline in respiration rates (-28% and -13% , respectively).

Soil respiration was significantly different only by date ($P < 0.0001$) in the 2016 growing season (Fig. 5a). During the 2016 growing season, soil respiration closely tracked available soil moisture, with the lowest rates observed during the mid-season drought. Young birch and hemlock forest types had very similar soil respiration rates on a given sampling date. In fact, young birch differed from hemlock within a 10% difference until November (Fig. 5b).

Soil respiration rates during the 2017 growing season were significantly different by date

($P < 0.0001$) and the forest and date interaction ($P < 0.0001$). Overall, the trends were similar for the 2016 and 2017 growing season, with the lowest rates for all forest types observed pre- and post-growing season (Fig. 5a). Mature birch always had the highest soil respiration rates, reaching differences of as much as 60% greater than hemlock rates during the middle of the growing season in all years (Fig. 5b).

DISCUSSION

Hemlock forests serve a crucial role in the eastern United States due to their strong effects on ecosystem characteristics, unique associations with other organisms, and their function as a sink for atmospheric CO_2 (Hadley and Schedlbauer 2002, Snyder et al. 2002, Tingley et al. 2002, Ellison et al. 2005, Lovett et al. 2006, Zuckswert et al. 2014, Siddig et al. 2016). Based on our comparisons of soil organic layer mass and %C content between hemlock forest and mature

birch stands, we estimate a $6.8\times$ difference in the C pool held in the forest floor (477.4 g C/m^2 vs. 70.6 g C/m^2), which suggests the potential for a ~ 4.5 ton C release per hectare if hemlock organic layers decomposed and transitioned to a new, lower equilibrium comparable to nearby mature birch stands. While some of this release might be offset initially by rapid C accumulation in the growing biomass of young black birch trees, the longer-term result appears likely to be a net release of C because mature birch forests do not maintain as large a pool of carbon in their soil organic layer, and tree basal area (\sim biomass) of mature stands between mature hemlock and birch forest areas appears comparable. This suggests the C lost from the hemlock soil organic layer will not be entirely offset by a future C sink in birch forest soils or above-ground biomass.

This novel finding is striking because previous work has demonstrated impacts of hemlock loss on decomposition and nitrogen cycling, but there has been little evidence to date of these forests becoming significant sources for atmospheric CO_2 (Orwig et al. 2013, Finzi et al. 2014). However, in this study, using a space-for-time substitution approach, we demonstrate that major changes in carbon cycling and soil carbon pools are likely to develop as hemlock declines and are replaced by deciduous tree species. These substantial differences emerge due to significant decreases in soil organic layer mass and its C:N between forests dominated by hemlock vs. deciduous tree species. These patterns were matched by elevated soil respiration rates in mature birch stands, consistent with higher decomposition and more rapid C cycling. Taken together, these results suggest great potential for shifting CO_2 sink vs. source dynamics as eastern U.S. forests lose this important foundation species.

Soil respiration

We found that soil respiration rates for mature birch plots were consistently higher than the hemlock or young birch forest type throughout the entire growing season for three consecutive years (Fig. 5), a difference not seen in earlier studies. This pattern was consistent during both an abnormally dry year (2016; Appendix S1: Fig. S2b; 459.5 mm of precipitation from April to October) and in years characterized by more typical levels of precipitation (2015, 2017; Appendix S1: Fig. S2a with

801.9 mm in April–October and Appendix S1: Fig. S2c with 470.152 mm in April–August). In contrast, Finzi et al. (2014) found similar soil respiration rates in black birch stands (~ 135 yr old) compared to second- and primary-growth hemlock stands (132 and >230 yr old, respectively). Likewise, Orwig et al. (2013) removed and girdled hemlock from experimental plots, and compared these to deciduous forest plots, but found that variation in soil respiration was higher within treatments and years than among treatments or years (Orwig et al. 2013). Thus, they concluded that soil respiration showed only modest and transient responses to hemlock loss, giving rise to only small impacts on ecosystem function.

Because of its effects on soil respiration, it is important to note that the hemlock and mature birch forest types in our study had similar VWC (VWC %). These forest types differed from the young birch stands, which maintained the highest VWC throughout the entire season for all three years (Appendix S1: Fig. S2). This is likely due to the young birch stands having the lowest basal area (Table 1) and least developed canopy, potentially yielding greater throughfall and lower evapotranspiration rates. Nevertheless, between hemlock and mature birch stands of similar age (~ 80 – 100 yr) on similar soils with comparable VWC, we found large differences (up to 60%) in soil respiration. Although predictable based on leaf litter characteristics (e.g., %N, C:N) and the observation that leaf litter inputs are almost completely degraded each year in mature birch forest (i.e., thin, low mass organic layer), these striking differences in soil respiration between forest types have not been well documented in the past. This may be due to previous studies sampling from independent field sites of hemlocks and black birch, while our study has the advantage of sampling all forest types in close proximity to each other on similar soils and topographic setting.

Overall, our results suggest more carbon being released to the atmosphere from mature birch forest soils, a trend that might be predicted to emerge in current hemlock stands if these forests lose hemlock and gain increased birch litter inputs. Such a scenario might also be exacerbated by the future mobilization of the large pool of soil organic layer C currently stored in these hemlock systems due to increased decomposition rates in the absence of hemlock.

Soil organic layer characteristics

Substantial differences were evident in organic layer characteristics between all forest types as organic layer C:N decreased in the predicted pattern (hemlock >young birch >mature birch; Fig. 3c). Our results showed that %C of the soil organic layer was the highest in hemlock soils, but did not significantly differ from young birch soil in %N. This is likely due to the close proximity of hemlock and young birch plots leading to some levels of litter mixing, slowing predicted shifts, while mature birch stands were almost entirely free of hemlock needle inputs. This possibility is supported by our leaf litter inputs results demonstrating ~30% birch contribution in the litter of hemlock plots, and ~13% of hemlock contribution of litter to nearby young birch plots (compared to ~40% needles in hemlock plots; Appendix S1: Fig. S1b). This finding underscores that the young birch plots should likely be viewed as an intermediate stage between hemlock vs. birch dominance, but not a system where hemlock influence has been completely eliminated. This situation might be most analogous to HWA-affected areas where hemlock has declined, but not been entirely lost, while birch importance has begun to increase.

In contrast, mature birch soil organic layers, where hemlock influence was almost entirely absent, differed significantly from the hemlock and young birch forest plots by having the lowest %C. These results contradict previous research conducted in central Massachusetts and south-central Connecticut that showed no significant difference in organic layer %C between hemlock and mature birch forest plots (~135 yr old; Raymer et al. 2013). Our values for secondary hemlock forest organic layer %C are slightly lower than values reported in Raymer et al. (2013) for primary hemlock forests and slightly higher than values reported for secondary hemlock (43.80 ± 1.25 , 44.7 ± 1.2 and 38.2 ± 3.0 , respectively), while our values for mature birch %C are considerably lower (32.63 ± 1.30 vs. 38.2 ± 3.0 ; Raymer et al. 2013).

Taken together, these results suggest an incremental C:N decrease as hemlock forests transition to mature birch forest (Fig. 4c). The higher C:N of hemlock soils likely traces to differences in the chemical composition of hemlock vs. birch litter entering the forest floor system. Our results indicate that hemlock needles exhibit

significantly higher %C, lower %N, ultimately yielding higher C:N ratios, and slower decomposition (Fig. 2). Decomposition rates can also be affected by secondary plant metabolites, like tannins, which bind proteins and form organic compounds resistant to further decay (Kraus et al. 2003). Tannins produced by hemlock have been found to decrease N mineralization in soils with elevated protein levels (Talbot and Finzi 2008). As with other conifers, the high aluminum content of hemlock leaf litter may also be a contributing factor, as high aluminum content is correlated with slow decomposition, possibly due to its influence on pH and stabilization of soil organic matter, or directly due to the toxic effects of aluminum (Al) on decomposers (Hobbie et al. 2007).

Consistent with differences in organic layer quality and decomposability, substantial differences were also evident in organic layer mass, which decreased in the predicted pattern (hemlock >young birch >mature birch; Fig. 3). Generally, the leaf litter deposited in black birch stands is expected to decompose significantly faster than that of hemlock-dominated stands due to its higher quality for decomposers (Cobb 2010). The lower C:N ratios of black birch leaves compared to hemlock needles, in combination with the fact that hemlock needles contain higher concentrations of lignin and polyphenolic compounds, are consistent with the finding that hemlock stands developed significantly greater organic layer mass. These results are also consistent with the findings of previous studies of soil organic layer C:N under hemlock canopies and organic layer depth (Finzi et al. 1998, Zuskwert et al. 2014).

A contributing factor to black birch's higher decomposition rates and lower organic layer mass may be birch species' high leaf litter nitrogen concentrations (Cobb 2010). The residual effects of hemlock on nutrient dynamics such as N cycling may also take time to dissipate in the young birch plots, or might be delayed by inputs from nearby hemlock trees, as was the case in our young birch plots (Zuskwert et al. 2014). Once these effects dissipate, the more dramatic nutrient differences seen between hemlock and mature birch may become even more evident in the forest floor. The significantly lower mean organic layer mass and C:N of mature birch forest compared to both the hemlock and young

birch forest types suggest that the ecosystem changes due to hemlock decline will increase and persist over time. Notably, we also do not see evidence that the decline in organic layer mass is due to a transfer in organic C to the lower, mineral soil layers: Exploratory tests in the hemlock and mature birch plots show no significant differences in organic matter content of the A horizon between forest types (20.2% vs. 18.7%, respectively; J. Bellemare, *unpublished data*).

More persistent soil organic carbon, such as that which likely comprises much of the deep organic layer of hemlock forests, is primarily recalcitrant humic compounds (formed by detritivores consuming other carbon compounds) and, to a lesser extent, plant structural and secondary compounds (Allison 2006). Soils with higher levels of fungal- relative to bacterial activity have been found to exhibit significantly higher total soil carbon content, and organic compounds derived from fungal decomposition play a large role in organic matter accumulation in other conifer-dominated ecosystems, such as boreal forests (Bailey et al. 2002, Clemmensen et al. 2013). This suggests that differing levels and types of microbial activity (e.g., fungal vs. bacterial decomposers) may play a role in driving differential organic layer accumulation in hemlock vs. birch forest types.

The dramatic differences in soil carbon pools that we observed between hemlock vs. birch forests may be partially offset in the initial decades of hemlock decline by uptake to new tree tissue (e.g., wood or other plant biomass). Some work has suggested that an initial decrease in carbon uptake by eastern U.S. forests is likely with the spread of HWA, but these effects were not predicted to be long-lasting or to significantly compromise regional uptake (Albani et al. 2010). Rather than permanently decreasing forest carbon storage, HWA-induced hemlock decline has been predicted to alter the distribution of carbon among the various forest pools (Raymer et al. 2013). For example, rapid growth of young black birch might result in an expansion of the live biomass carbon pool and an ecosystem C content equal to secondary growth hemlock in ~20 yr (Raymer et al. 2013). However, our results suggest that the role of soil organic carbon loss in these dynamics might be underestimated or overlooked. Although the above-ground C loss triggered by dying

hemlocks might eventually be offset by the birch trees that typically replace them, this dynamic does not account for the dramatic changes in the pool of soil organic layer C likely to be mobilized by decomposition as these forest ecosystems transition from hemlock to birch dominance. Consequently, the C release driven by increased decomposition in the forest floor is likely to be substantial, and it might never be fully offset by growth of above-ground biomass, resulting in a substantial net release of C from these ecosystems. In this regard, further work integrating measurements of soil and biomass C pools between hemlock and birch forests is needed.

CONCLUSIONS

The loss of eastern hemlock, an important foundation tree species, is likely to have broad impacts on overall ecosystem function due to its effects on processes such as decomposition, nutrient cycling, and carbon sequestration (Ellison et al. 2005). Additionally, these effects are likely to be extensive and long-lasting as these ecosystems are converted to deciduous forest following hemlock decline. While previous work has documented substantial changes in decomposition and nitrogen cycling with the loss of hemlock (Jenkins et al. 1999, Orwig et al. 2002, 2013, Cobb 2010, Finzi et al. 2014), a consensus has yet to emerge on the magnitude and longevity of carbon flux impacts. Our findings suggest these changes could be substantial and consequential, as we found dramatic increases in soil respiration and significant declines in soil carbon pools when comparing hemlock forests to mature birch forests. The full extent of these impacts on carbon sequestration may take several decades to manifest, but our results suggest that the loss of hemlocks will have substantial long-term impacts on forest ecosystem structure, function, and carbon pools.

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