Lack of forest tree seedling recruitment and enhanced tree and shrub growth characterizes post-*Tsuga canadensis* mortality forests in the southern Appalachians

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ABSTRACT

The loss of *Tsuga canadensis* from invasion by hemlock woolly adelgid (*Adelges tsugae*, HWA) has altered ecosystem structure and function in forests across the eastern United States. In southern Appalachian forests, *T. canadensis* co-occurred with hardwood species and an evergreen *Rhododendron maximum* shrub layer in riparian and cove positions. In this region, HWA infestation was detected in 2003, with mortality reaching 97% by 2014. In this study we examined responses of light, soil moisture, tree seedling density, and overstory and understory vegetation growth from 2004 to 2014 following HWA infestation and mortality of *T. canadensis*. We hypothesized that seedling recruitment and vegetation growth would continue to increase over time as observed with initial trends reported through 2009, and that species that associate with ectomycorrhizal (ECM) fungi may benefit more than those that do not due to the pulse of organic material in these stands from dead *T. canadensis* trees. Light transmission measured at 1 m above the ground increased from 2006 to 2009, but gradually decreased from 2015 to 2017. Basal area of overstory non-*Tsuga* trees increased only marginally, and there was no recruitment of tree seedlings to the overstory size class, even though seedling density of deciduous species increased initially. Increased basal area and stem density of *R. maximum* may explain the light and seedling responses, as this species can inhibit tree seedling recruitment by limiting light and nutrients. Overstory species with the highest basal area increment (BAI) in the post-*T. canadensis* stands were *Pinus rigida*, *Betula lenta* and *Quercus coccinea*, which all associate with ECM fungi. However, not all ECM tree species grew significantly more following *T. canadensis* mortality compared to pre-mortality growth rates—only those ECM species that had high growth rates prior to mortality did. After a decade, growth of both overstory trees and *R. maximum* has not compensated for the loss of *T. canadensis*. Active management of *R. maximum*, which may involve the removal of the evergreen shrub and soil organic layer, may be required to allow for diverse tree seedling recruitment; and subsequently, restore riparian forest structure, diversity, and function.

1. Introduction

The loss of a foundation species can change ecosystem structure and function (Tomback and Kendall, 2001; Ellison et al., 2005; Logan et al., 2010; Orwig et al., 2013; Ellison et al., 2015). For example, the loss of coniferous evergreen *Tsuga canadensis* (L.) Carrière to hemlock woolly adelgid (*Adelges tsugae* Annand., hereafter HWA) infestation throughout eastern forests of United States has resulted in the recruitment of deciduous species into canopy gaps (Orwig and Foster, 1998; Small et al., 2005; Stadler et al., 2005; Ford et al., 2012; Orwig et al., 2013; Ellison et al., 2015), indicating a potential shift towards the dominance of deciduous species (Case et al., 2017). Meanwhile, the effects of *T. canadensis* mortality on ecosystem function have also been well documented. Decreasing aboveground biomass, soil respiration and fine-root biomass (Nuckolls et al., 2009), and increasing soil organic carbon (Fraterrigo et al., 2018) have affected carbon cycling. Nutrient cycling has been altered by increasing throughfall nitrogen concentration (Stadler et al., 2005), foliar nutrient concentrations (Stadler et al., 2005; Gómez et al., 2012; Domec et al., 2013; Gonda-King et al., 2014; Williams et al., 2016; Lapham et al., 2018), and soil nitrogen (N) availability (Rubino et al., 2015). Changes in hydrologic cycling were observed from increasing peak storm flows (Brantley et al., 2015), water yield (Kim et al., 2017), and stream nitrate concentrations (Miniat et al., unpublished).

Changes in many ecological processes such as stream N cycling and soil respiration, may be short-term, lasting one or two years; but other

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processes may be altered for decades. Long-term changes of ecosystem function can be attributed to changes in vegetation composition following *T. canadensis* mortality rather than the direct effect of the loss of *T. canadensis* (Cobb, 2010). Thus, long-term monitoring of post-disturbance vegetation dynamics may help us understand which processes have enduring effects, and whether the ecosystem may return to a stable state.

In southern Appalachian forests, *T. canadensis* co-exists with deciduous tree species (Elliott and Vose, 2011), primarily in riparian and cove landscape positions (Narayanaraj et al., 2010). Tall-statured, mature forests typically with an evergreen shrub understory layer have persisted following *T. canadensis* decline (Ford et al., 2012). The initial seedling responses were documented with a mix of *Fagus, Betula, Acer,* and *Magnolia* recruiting into gaps (Ford et al., 2012). This initial seedling recruitment occurred along with an increase in spring maximum daily transmittance at 1 m above ground (from 10% to 15% after three years of infestation); but increases in transmittance were transient and no different in the fourth year after infestation, which coincided with dramatic increases in shrub growth. Two recent long-term studies of forest structure, both with comparable aged stands, in the southern Appalachians have shown that where evergreen shrubs are present in the understory, trees comprising the forest canopy are on average 6 m shorter than areas without it (Bolstad et al., 2018), and trees in areas with a prominent evergreen shrub understory are systematically older than the shrubs surrounding them (Elliott and Vose, 2011). Combined, these recent studies suggest that the evergreen shrub understory may act as a recruitment bottleneck for canopy tree species-despite tree seeds in the seedbank (Cofer et al., 2018) – by limiting water, nutrients, and/or light. As such, these shrubs may shape the long-term structure of these forests.

Nutrient acquisition strategies of co-occurring species may also shape the long-term structure and composition of the resulting forest. *Rhododendron maximum* leaves are resistant to decomposition (Hoover and Crossley, 1995), resulting in the development of a thick O-horizon under the rhododendron canopy where nitrogen and cations are bound in complex organic compounds (Wurzburger and Hendrick, 2007, 2009), and limit inorganic nitrogen availability to trees (Knoepp et al., 2011; Block et al., 2012). This combined with the pulse of organic matter from dead and dying hemlock trees may promote the growth response of co-occurring trees with mycorrhizal associations that can access organic nitrogen compared to those with mycorrhizal associations that rely on inorganic nitrogen acquisition. Several co-dominant species in *T. canadensis* stands, including *B. lenta* and *Quercus* spp., are associated with ectomycorrhizal (ECM) fungi. Other abundant tree species that are associated with arbuscular mycorrhizal (AM) fungi in *T. canadensis* stands include *A. rubrum,* and *Liriodendron tulipifera* (Ford et al., 2012). ECM fungi can mine nitrogen (N) from soil organic matter by excreting extracellular enzymes, whereas AM species rely on N uptake from inorganic pools (Read and Perez-Moreno, 2003). The pulse of organic matter from *T. canadensis* mortality in these forests (Fraterrigo et al., 2018) may increase availability of N supply to ECM species. Where ECM species are present, we might also expect increased growth from these trees, or rapid growth where they can recruit, particularly if other resources are not limiting (e.g., light and soil moisture).

Scientists at Coweeta Hydrologic Laboratory have been monitoring the effects of HWA, and the decline of *T. canadensis*, on functional attributes and vegetation dynamics of riparian forest plots since HWA was first noticed in 2003 (Elliott and Vose, 2011). In this study, we report on microclimate changes, growth of non-*Tsuga* tree species and tree seedling recruitment for over a decade. We used long-term measurements from several different studies, both current and past, and merge data streams across projects to make inferences on seedling recruitment, vegetation dynamics and microclimate. Permanent plots first established in 2004 quantified vegetation dynamics (Ford et al., 2012), carbon and nutrient pools and fluxes (Nuckolls et al., 2009; Knoepp et al., 2011), and soil solution chemistry (Knoepp et al., 2011). A

second study used some of these plots to measure microclimate; and a third study used a subset of these plots in 2014 and new plots to remeasure and continue vegetation and microclimate measurements. We hypothesized that the loss of *T. canadensis* would result in changes to microclimate and vegetation structure in the short-term (a single decade); with the interaction between those factors influencing long-term (over multiple decades) ecosystem structure and function. Although light incident on the *Rhododendron maximum* subcanopy increased 5–10% in the initial years of infestation (Ford et al., 2012), we expected that increased light transmission on the forest floor would be transient as overstory non-*Tsuga* trees responded to canopy openness by increasing growth and leaf area. While all non-*Tsuga* species would respond to increased resources initially; longer-term, some species may have differing growth rates based on their nutrient acquisition traits. We also hypothesized that ECM species may have responded more favorably than AM species. Our results have important implications and will highlight the difficulties in restoring these riparian forests that have substantial *T. canadensis* mortality and a dense evergreen understory. If land managers are aiming to restore function and structure, then information on the longer-term growth responses is needed.

2. Methods

2.1. Study location and site descriptions

Coweeta Hydrologic Laboratory (CWT, latitude 35°03′N, longitude 83°25′W), a USDA Forest Service Experimental Forest and NSF long-term ecological research (LTER) site, is within the Nantahala Mountain Range of western North Carolina, USA, within the Blue Ridge Physiographic Province, near the southern end of the Appalachian Mountain chain. Soils are deep sandy loams and are underlain by folded schist and gneiss. Two soil orders are found: immature Inceptisols and older developed Ultisols; soil types include the Cullasaja-Tuckasegee complex along the stream channel and the Edneyville-Chestnut complex and Plot fine sandy loams on the uplands (Thomas, 1996). Mean annual temperature is 12.6 °C; and seasonally ranges 3.3–21.6 °C. While annual rainfall is usually abundant in this region averaging *ca.* 1800 mm, dry years, such as the recorded droughts between 1985–1988 and 1998–2002, are increasingly common (Laseter et al., 2012). Climate stations across CWT have been recording as early as 1934.

2.2. Plot layout and sampling

In 2004, eight 20 m × 20 m plots were established within CWT to study the effects of HWA on forest dynamics (Ford et al., 2012). Measurements for light transmission, soil moisture, tree and seedling growth were conducted. All plots were located in mesic, riparian areas with low-to-moderate slopes (< 30%) and elevation ranges from 760 to 1060 m. Prior to mortality, *T. canadensis* comprised more than 40% of the basal area. Although half of the original plots were subjected to girdling to simulate infestation by HWA, or naturally infested with invasion of HWA, the girdling treatment effect on response variables was indistinguishable from HWA infested plots (Ford et al., 2012). Thus, for this study, we treat all eight original plots as statistical replicates.

A subset of the original plots (1–2, and 5–8) were re-measured in subsequent years as outlined in Table 1. In 2014, we established four additional plots with similar abundance of *R. maximum* and dead *T. canadensis* to the original plots. Light transmission and soil moisture were measured in seven original plots (Section 2.3); vegetation was measured in six of the original plots in survey years 2004–2009 (Ford et al., 2012), and again in 2010–2012, and 2014 (Section 2.4); and tree increment cores were collected in four original plots and four additional plots (Section 2.5). Mortality of *T. canadensis* was also assessed over time in the six original plots. All *T. canadensis* trees were number tagged in 2004 to follow mortality through 2014.

Table 1

HWA sample plots, measurement parameters and length of data record. Four additional plots (9–12) were added in 2014, where tree increment cores were extracted; these four plots were subsequently used for a new study (Elliott and Miniat, 2018).

HWA Plot	Overstory tree and shrub census	Seedling census	Tree and shrub basal area and density	Understory light and soil moisture	Overstory tree increment core
1	2004–2014	2004–2010, 2014	2004–2010, 2014	2015–2017	1985–2014
2	2004–2014	2004–2010, 2014	2004–2010, 2014	2015–2017	1985–2014
3	NA[§]	NA[§]	NA[§]	2006–2009[*]	NA[§]
4	NA[§]	NA[§]	NA[§]	2006–2009[*]	NA[§]
5	2004–2014	2004–2010, 2014	2004–2010, 2014	2015–2017	1985–2014
6	2004–2014	2004–2010, 2014	2004–2010, 2014	2006–2009[*], 2015–2017	1985–2014
7	2004–2014	2004–2010, 2014	2004–2010, 2014	2006–2009[*]	NA[§]
8	2004–2014	2004–2010, 2014	2004–2010, 2014	NA[§]	NA[§]
9	NA[§]	NA[§]	NA[§]	NA[§]	1985–2014
10	NA[§]	NA[§]	NA[§]	NA[§]	1985–2014
11	NA[§]	NA[§]	NA[§]	NA[§]	1985–2014
12	NA[§]	NA[§]	NA[§]	NA[§]	1985–2014

[*] Soil moisture data were only available from 2006 to 2007 growing seasons.

[§] NA indicates data were not collected in all subsequent years after 2004.

2.3. Light transmission and soil moisture

Light transmission and soil moisture were collected in four of the original plots in 2006–2009 (Ford et al., 2012), while measurements in 2015–2017 were obtained from one original plot and three other original plots (Table 1). Light measurements were used to characterize the light incident on the forest floor at 1 m height and were expected to be similar across all plots over time. Photosynthetically active photon flux density (Q_i, μmol m^{-2} s^{-1}) incident on tree seedlings (stems ≤ 0.5 m height) was estimated from GaAsP photodiodes (ModelG1118, Hamamatsu Corp., Pontailler 1990) calibrated against a commercial quantum sensor (LI190, Li-Cor Inc.). Photodiodes were arrayed in an alternating grid pattern throughout the plot at 1 m height ($n = 10$ during 2006–2009, and $n = 6$ during 2015–2017). Sensors were queried every 60 s and 15-minute averages were logged (CR10X and CR1000, Campbell Scientific, Inc., Logan, UT, USA). To characterize the change in incident light over time, we calculated light transmittance (Q_i/Q_o), where Q_o (μmol m^{-2} s^{-1}) was measured at an open-field climate station located approximately 2 km from the plots.

Soil moisture (θ) was measured using time domain reflectometry (TDR, CS615 and CS655, Campbell Scientific Inc., Logan, UT) in each plot ($n = 3$ during 2006–2007, and $n = 4$ during 2015–2017). The probes were installed vertically or at a slight angle in the mineral soil and sensed the average percent volumetric soil moisture (v/v %) in the 0–30 cm depth (during 2006–2007) and in the 7.5–20.5 cm depth (2015–2017). All lead wires were connected to data loggers with a multiplexer peripheral (AM416 or AM25T, Campbell Scientific Inc., Logan, UT). Sensors were queried every 15 min and 60-minute averages were logged. Due to the high organic matter in these soils, we calibrated TDR probes in the lab for each soil type.

2.4. Vegetation measurement

Six of the original eight plots first established in 2004 were remeasured for tree growth and seedling density in all subsequent years (Table 1). In all survey years, we tagged and measured diameter of overstorey non-*Tsuga* trees (diameter at breast height (dbh) ≥ 2.5 cm) and *R. maximum* to the nearest 0.1 cm within each 20 m × 20 m plot. In survey years 2004 to 2010, we counted tree seedlings (diameter < 2.5 cm, height ≤ 0.5 m) using the line intercept method (Clarke, 1986). One 20 m line-transect was placed within each 20 m × 20 m plot, the same line transect was resampled in each survey year. Presence of each tree seedling by species was recorded that occurred within 2.5 cm of either side of the line. In 2014, we placed four 1.0 m^2 quadrats in the corners of each plot and recorded the number of tree seedlings present in each quadrat. Species nomenclature follows Kirkman et al. (2007).

2.5. Basal area increment (BAI)

Tree increment cores were collected in four original plots and four additional plots (9–12, Table 1). During Sep–Nov 2016, we extracted increment cores from each tree at 1.37 m above ground using a 35 cm long increment borer (Haglöf Inc., Långsele, Sweden). Air-dried cores were mounted and sanded with 400 grit paper until ring boundaries were clearly visible. Cores were measured to the nearest 0.001 mm under 40 x stereoscope (Olympus SZ-4045, Japan) magnification with a linear-encoded measurement stage using the Velmex system (Bloomfield, NY), and measurements were digitally recorded. We used the COFECHA program (Holmes, 1983) to cross-date and ensure that all cores were correctly dated. Annual basal area increment (BAI, cm^2) for each tree was calculated from the ring widths and stem diameter, assuming stems were circular.

2.6. Statistical analysis

Linear mixed effect models with repeated measures were applied to test for changes over time in microclimate (R, version 3.4.2, The R Foundation for Statistical Computing, Vienna, Austria), and vegetation growth (PROC MIXED, version 9.4, SAS Institute Inc., Cary, North Carolina). In the repeated statement, for all parameters, the experimental unit (subject) was the plot and year was the 'within-subject' repeated measure. We used the covariance option in the repeated statement that produced the smallest value for the Akaike's information criterion (AIC) and Schwarz' Bayesian criterion (SBC) (Littell et al., 2004). We evaluated the main effect of time (year of survey as fixed effect). If overall *F*-tests were significant then least squares means (LS-means, Tukey-Kramer adjusted *t*-statistic) tests were used to evaluate significance among years. We considered parameters with *p* values ≤ 0.05 significant, and parameters with *p* values ≤ 0.10 marginally significant (sensu Hulbert and Lombardi (2009)). Even though two statistical packages were used for our analyses, the procedures were the same.

Changes in microclimate were examined with Q_i/Q_o and θ measurements. To compare the mean daily Q_i/Q_o among years, we split the data into two periods: 2006–2009 and 2015–2017, because the same plots were not consistently measured over time. A similar approach was applied to compare θ among years, with the data split into 2007–2008 and 2015–2017 periods.

Changes in vegetation growth were assessed with tree seedling density, overstory basal area and density, and *R. maximum* understory basal area and density measurements. To examine the direct influence of *R. maximum* on tree seedlings, we included *R. maximum* density and basal area in the repeated measures model for tree seedling density. However, neither density ($F_{1,29} = 0.17$, $p = 0.6872$) nor basal area

($F_{1,29} = 0.90$, $p = 0.3509$) were significant. Even though *R. maximum* density and basal area increased over time, its abundance was high in all plots across years (density range: 1125–2725 stems ha^{-1}; basal area range: 2.13–7.87 m^2 ha^{-1}).

Mean BAI of each tree was estimated for before and after HWA infestation: pre-disturbance (1985–2003) and post-disturbance (2004–2014). Species-based average BAI and average BAI for all trees were also calculated. Each species was assigned a mycorrhizal association: ectomycorrhizal (ECM) or arbuscular mycorrhizal (AM). We excluded *Robinia pseudoacacia* and *Oxydendrum arboreum* samples from the analysis because the former is a tree that fixes atmospheric di-nitrogen and the latter is ericoid mycorrhizal (Gorman and Starrett, 2003). To test the effect of mycorrhizal association on growth, we applied linear mixed effect models on growth ratio of pre-disturbance to post-disturbance period. Mycorrhizal association was the fixed effect, while species was the random effect (R, version 3.4.2, The R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Mortality of Tsuga canadensis

Mortality of *T. canadensis* increased over time (Fig. 1). All trees, regardless of size, were infested with HWA by 2005, and mortality occurred rapidly. By 2010, 50% of the *T. canadensis* trees were dead; with nearly complete mortality (97%) by 2014. While trees had not yet completely fallen, bark sloughed off and crown branches fell, leaving standing snags (personal observations).

3.2. Understory light transmission and soil moisture

The fraction of light transmitted onto tree seedlings was low in these forests, but increased over time as *T. canadensis* declined (Fig. 2a). From 2006 to 2009, mean daily Q_i/Q_o during the growing season increased from 4.3% to 8.4% ($F_{4, 9} = 25.5$, $p < 0.01$). In contrast, in more recent years from 2015 to 2017, Q_i/Q_o has declined from 10.8% to 7.6% ($F_{3, 6} = 16.9$, $p < 0.01$).

Mean daily θ in these riparian forests was generally high, and variable among years. Across all years, θ averaged 25.1%. The range of θ in both periods suggests these plots were not water-limited. Mean daily θ increased by 2.7% from 2006 to 2007 ($F_{2, 3} = 45.5$, $p < 0.01$; Fig. 2b). Although the variation in θ was significant from 2015 to 2017 (year effect: $F_{3, 6} = 75.8$, $p < 0.01$), no continuing trend was observed.
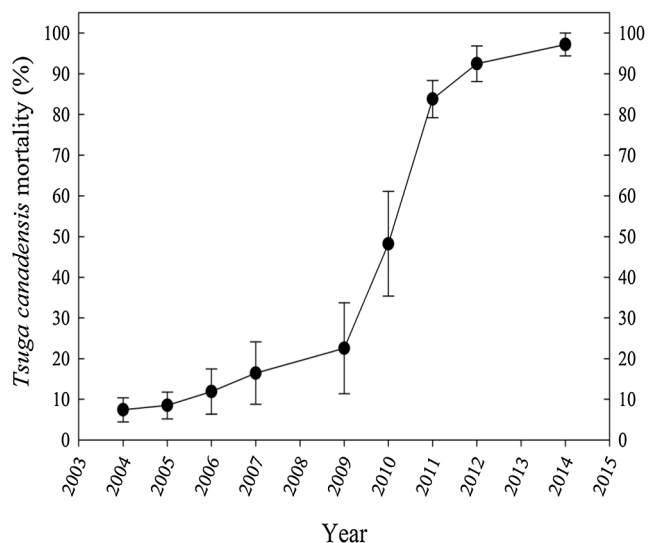

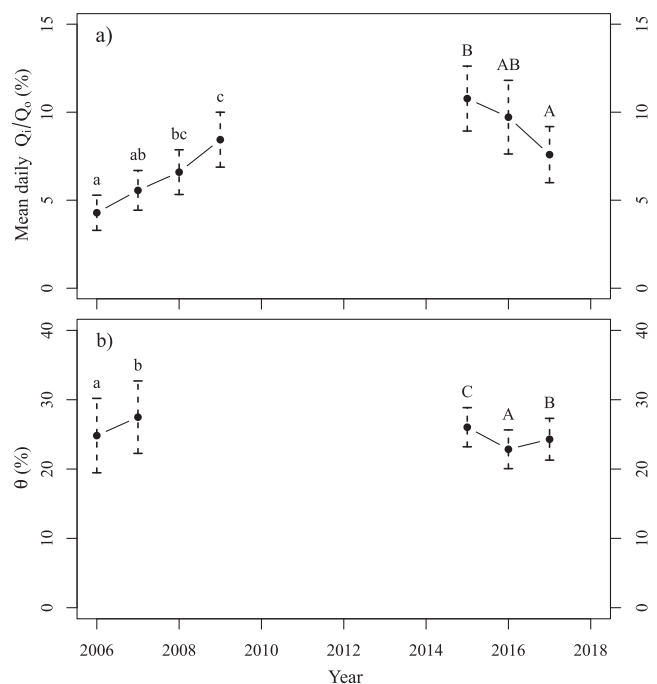**Fig 1.** Mortality of *T. canadensis* over time.

Fig 2. Mean daily sub-canopy light transmission (Q_i/Q_o) at 1 m above the ground (a) and mean daily soil moisture (θ) content (b) during the growing season. Error bars denote standard errors. Different letters indicate significant differences ($p < 0.05$) among years: small letters refer to data from 2006 to 2009, capital letters refer to data from 2015 to 2017.

3.3. Tree, shrub and seedling responses

Overstory basal area of live non-*Tsuga* trees increased 12% over time from 16.67 m^2 ha^{-1} in 2004 to 18.69 m^2 ha^{-1} by 2014 ($F_{8, 40} = 1.97$, $p = 0.076$) (Fig. 3a). The increase in basal area was not associated with an increase in the number of stems, however, as density did not change over time ($F_{8, 40} = 1.40$, $p = 0.226$). By 2014, 10% of the standing non-*Tsuga* trees were dead, and there was little tree recruitment into the overstory size class over time (Fig. 3b). Of the 14 non-*Tsuga* tree species found in the study plots, seven of these experienced mortality and most of these were relatively small trees (< 20 cm dbh). Ranked by highest to lowest percent mortality, *Cornus florida* > *Acer pensylvanicum* > *Pinus rigida* > *Hamamelis virginiana* > *Betula lenta* > *Quercus alba* > *Acer rubrum*. Likely the mortality of this smaller size class was due to competitive exclusion, however, no discernable cause was detected

Basal area of *R. maximum* also increased over time ($F_{8, 39.9} = 2.17$, $p = 0.052$) to a maximum of 5.84 m^2 ha^{-1} in 2014 (Fig. 4). Although density of established *R. maximum* declined ($F_{8, 40} = 13.91$, $p = 0.002$), recruitment of new stems into the overstory size class beginning in 2010 greatly increased stem density by 2014.

While initially tree seedlings responded to the loss of *T. canadensis*, over time these seedlings experienced mortality and could not recruit into the next size class (Fig. 5a). Seedling density increased in 2006, 2008 and 2010, but declined to pre-infestation levels by 2014 ($F_{5, 30} = 2.32$, $p = 0.068$). The initial seedling response was species-specific and not related to mycorrhizal association (Fig. 5b). In 2006, *O. arboreum*, *A. rubrum*, and *Quercus* spp. were the most abundant seedlings; however, *O. arboreum* died back by 2008. *Amelanchier arborea* recruited in 2008, then died back in 2010, while *Acer rubrum* and *B. lenta* increased (Fig. 5b). By 2014, seedlings that initially recruited had declined and total seedling density was similar to that in 2004 (Fig. 5a). In addition, very few tree saplings were found in these plots, 0.14 ± 0.04 saplings m^{-2}, in 2014. This suggests that tree seedlings died rather than grew into the sapling size class, however, we did not tag individual
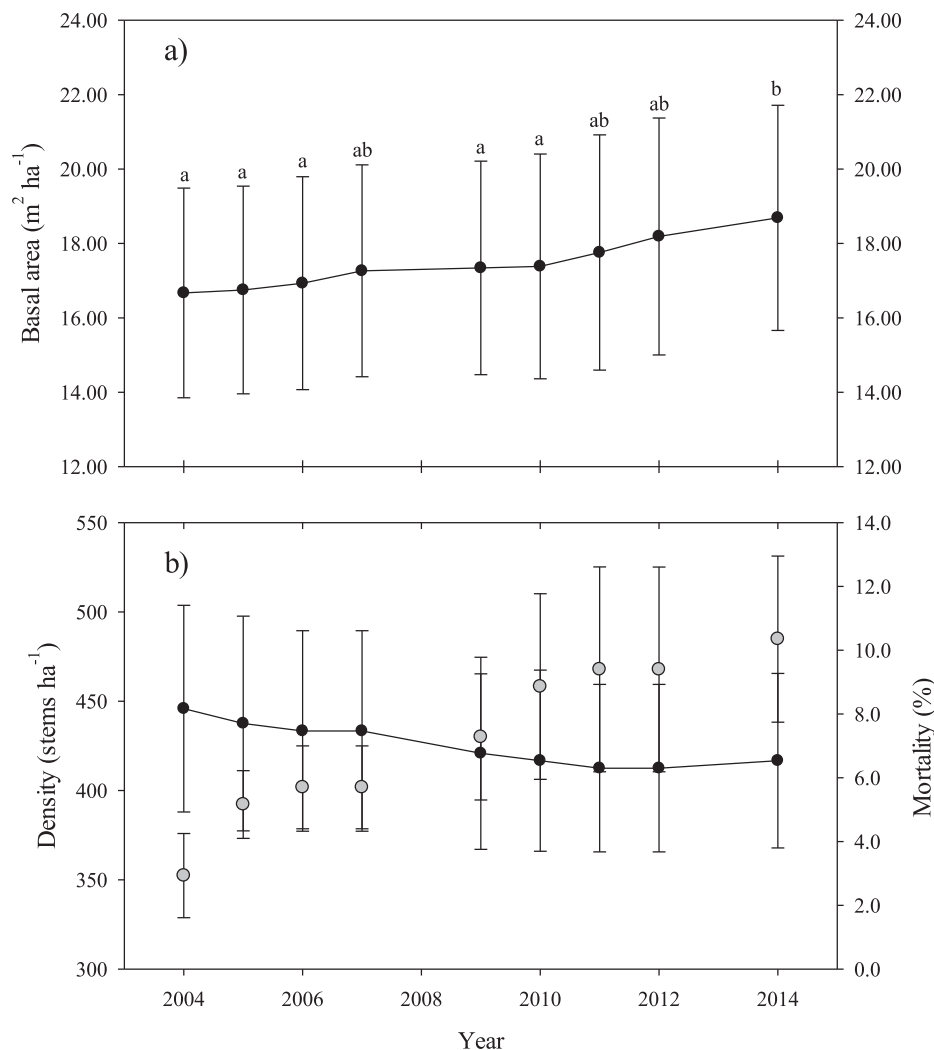
Fig 3. Mean basal area (a) and density (b) of live pine and deciduous trees (dbh ≥ 2.5 cm) over time since HWA infestation. Error bars denote standard errors. Different letters indicate significant differences (p < 0.05) among years (a). Gray circles denote cumulative tree mortality (b).

seedlings when they germinated to follow their population dynamics.

3.4. Basal area increment (BAI) changes

On average, trees showed a greater growth rate after HWA infestation and *T. canadensis* mortality compared to the years prior to infestation (Fig. 6). Combined, trees across all diameters sampled had 18% greater growth rate following infestation and mortality compared to before mortality. The change in growth rate between pre- and post-disturbance periods varied amongst species, with *P. rigida*, *B. lenta* and *Q. coccinea* showing the greatest increase in growth rate (58%, 37% and 34% respectively). Although these three species are associated with ECM fungi, not all ECM tree species exhibited a large increase in growth. Meanwhile, the growth of most species that are associated with AM fungi did not increase in the post-disturbance period, except for *A. rubrum* and *Magnolia fraseri* that grew 20% and 28% more following HWA infestation than prior to its arrival.

ECM species had a greater growth ratio of post-disturbance to pre-disturbance period than AM species, 1.38 compared to 1.20 respectively. However, mycorrhizal association did not have a significant effect ($F_{1,9} = 0.987$, $p = 0.35$) on growth ratio.

4. Discussion

We hypothesized that the decline and mortality of *T. canadensis*

would result in changes in microclimate, forest structure and species recruitment. We examined these responses to the invasion-related disturbance at the plot and individual species levels. We found that both light transmittance and tree seedling responses were transitory. Overstory basal area increased only marginally (ca. 2 m² ha⁻¹ over 10 years), deciduous tree mortality had a cumulative increase of 10% (ca. 1% per year), and there was no evidence of recruitment of tree seedlings into the sapling or overstory size classes. In contrast, basal area and density of *R. maximum* increased over time. Overstory BAI growth rate on average was higher in the post-disturbance period compared to pre-disturbance, a trend that was primarily driven by three ECM species, *P. rigida*, *B. lenta* and *Q. coccinea*.

The increase of *R. maximum* following *T. canadensis* mortality confirmed earlier predictions and observations (Ellison et al., 2005; Ford and Vose, 2007; Spaulding and Rieske, 2010; Elliott and Vose, 2011; Ford et al., 2012; Brantley et al., 2015). *Rhododendron maximum* was likely able to take advantage of the initial increase in light transmission and possibly legacy soil characteristics (*i.e.*, acidic, organic, low nutrient availability) of *T. canadensis* stands (Pfennigwerth et al., 2018). Although we did not find a direct correlation between *R. maximum* density and tree seedling recruitment, *R. maximum* density was high across all plots and could have contributed to the low tree seedling density. Others have found that *R. maximum* thickets strongly restrict tree seedling recruitment and survival (Beier et al., 2005; Hille Ris Lambers and Clark, 2013); by limiting light (Nilsen et al., 2000; Lei
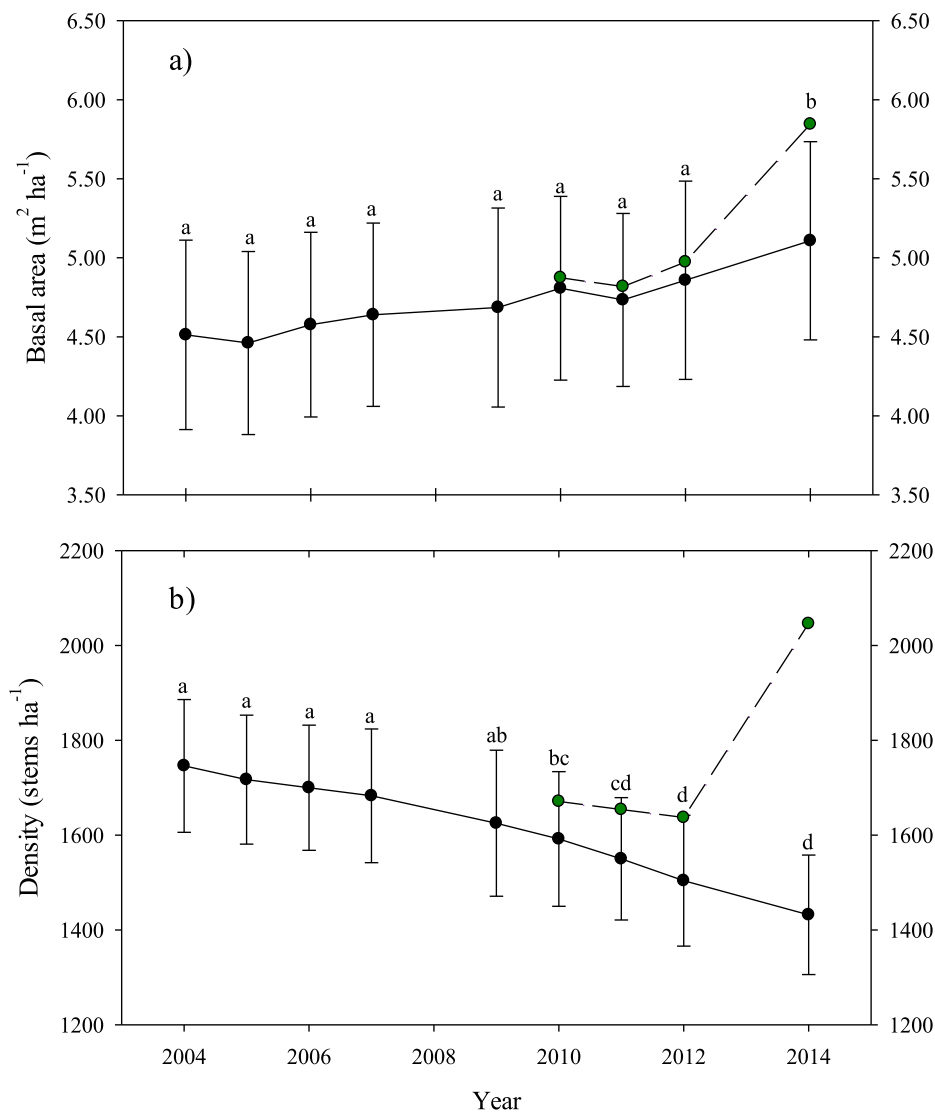
Fig 4. Mean basal area (a) and density (b) of live *Rhododendron maximum* (dbh ≥ 2.5 cm) over time since HWA infestation (black symbols). Error bars denote standard errors. Different letters indicate significant differences ($p < 0.05$) among years. Green symbols (b) denote contribution due to stem recruitment from small individuals (diameter < 2.5 cm) growing into the overstory size class (dbh ≥ 2.5 cm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2006) and nutrients (Nilsen et al., 2000; Wurzburger and Hendrick, 2007; Horton et al., 2009), and establishing an intricate soil N feedback through its litter chemistry, mycorrhizal roots and fungi (Wurzburger and Hendrick, 2009). Cofer et al. (2018) found that seed bank richness and herbaceous seed bank density were lower under a *R. maximum* understory, which would limit diversity in the recruitment of overstory trees and herbaceous species. In contrast, a significant recruitment of *B. lenta* seedlings into the sapling and overstory size classes has been observed in response to the mortality of *T. canadensis* in the northeastern U.S. (Orwig et al., 2013), where forests do not have a dense *R. maximum* understory. *Betula lenta* is relatively shade-intolerant and early-successional, traits that combined with its ECM association may explain its proliferation following disturbance in these forests (Orwig and Foster, 1998; Cobb, 2010).

Comparison amongst overstory pines and deciduous species has shown that several species had positive growth responses to *T. canadensis* mortality. Species with the highest post-disturbance growth rate compared to pre-disturbance, such as *P. rigida, B. lenta, Q. rubra* and *Q. coccinea*, are associated with ECM fungi. The growth release of those ECM species may have occurred due to the loss of *T. canadensis*, also an ECM species, as they are competitors for the same N source, while AM

species relied on organic N transformation rates to inorganic N (NO_3, NH_4) for uptake (Read and Perez-Moreno, 2003). Dominance of ECM species has been associated with a high soil carbon (C) to N ratio (C:N) (Zhu et al., 2018). Decreases in O-horizon mass, increases in organic soil C concentration, and increases in the fraction of mineral-associated organic matter C compared to particulate organic matter over time were observed in the same study plots following *T. canadensis* infestation and mortality (Fraterrigo et al., 2018). These trends in soil carbon all point to increased rates of decomposition and processing of organic matter, and likely greater soil organic N content. Interestingly, all ECM species did not respond with a greater growth rate compared to all AM species. Because this disturbance did not promote a greater growth rate in all ECM species following *T. canadensis* mortality, perhaps only trees that can take advantage of higher light *and* organic N with a rapid growth rate benefitted. Indeed, the trees with the highest growth rate, the upper right quadrat of Fig. 6, seemed to have the greatest difference in post- compared to pre-disturbance BAI. Both *P. rigida* and *B. lenta* are known to have rapid-to-moderate growth rates, and red oaks (*Q. rubra, Q. coccinea*) are known to have more rapid growth rates than white oaks (*Q. montana, Q. alba*) in high light (Kobe et al., 1995). While consistent patterns in AM *vs.* ECM dominated communities or vice versa through
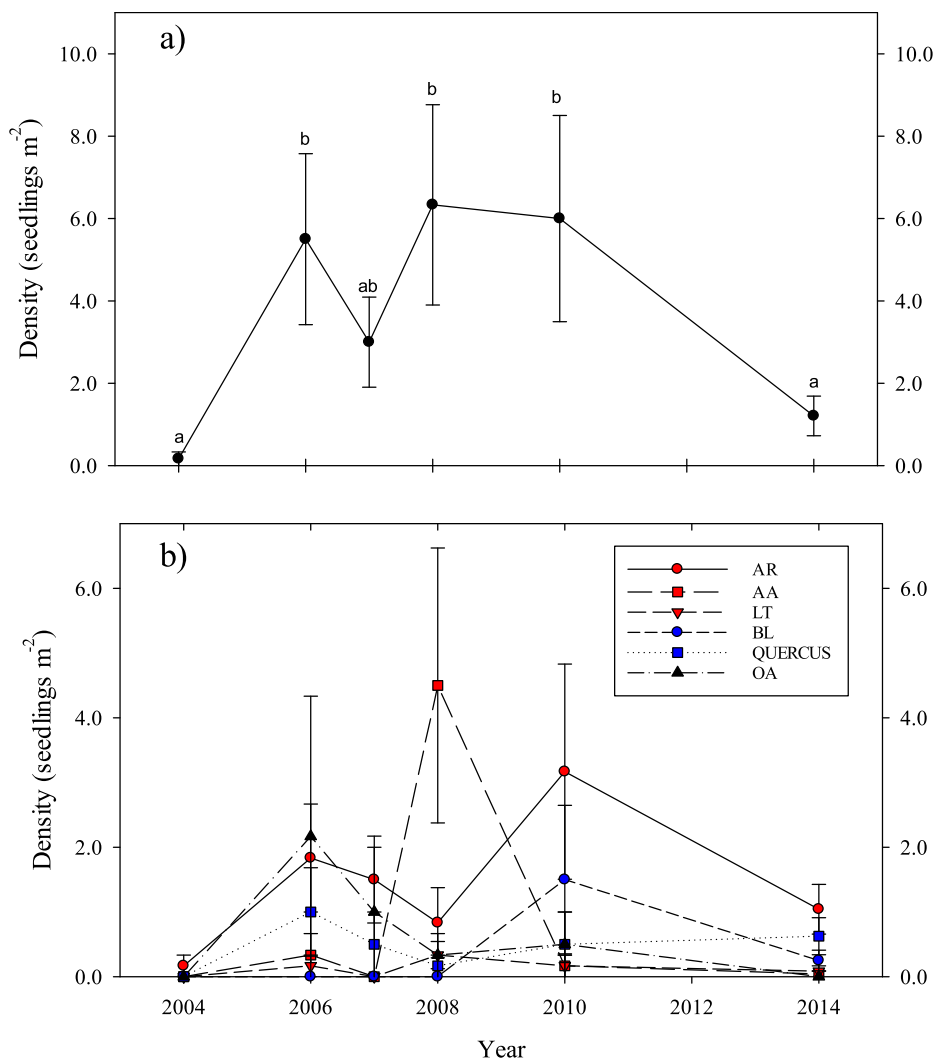
Fig 5. Mean total tree seedling density over time since HWA infestation (a) and mean density of the six most abundant species (b): AR, *Acer rubrum*; AA, *Amelanchier arborea*; LT, *Liriodendron tulipifera*; BL, *Betula lenta*; QUERCUS, all *Quercus* spp. combined (*Q. rubra*, *Q. coccinea*, *Q. montana*, *Q. alba*); and OA, *Oxydendrum arboreum*. Red symbols represent arbuscular mycorrhizal (AM) species, blue symbols represent ectomycorrhizal (ECM) species, and black symbol represents ericoid mycorrhizal species. Error bars denote standard errors. Different letters denote significant differences ($p \leq 0.05$) among survey years (a). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ecosystem development have not been found (Dickie et al., 2013), our results suggest that post- *T. canadensis* mortality forests in the southern Appalachians will be dominated by trees that are already in the canopy layer, rather than by seedling recruitment, particularly in forests with a shrub layer.

Simulation models based on data from the central Appalachians (Spaulding and Rieske, 2010) and northeastern U.S. (Case et al., 2017), where *R. maximum* is absent or much less abundant, have predicted seedling recruitment and an eventual shift towards stands that are dominated with pines and deciduous overstorey species following the *T. canadensis* mortality. Where *R. maximum* is a dominant species in the forest, as in our study, stand dynamics may be much different. Despite the initial increase in seedling density, we found no recruitment of deciduous species into the overstory canopy over the ten-year period of our study. The total increase in stand basal area of deciduous overstory trees and *R. maximum* from 2004 to 2014 was approximately 4.5 m^2 ha^{-1} (Figs. 3 and 4), while basal area of dead *T. canadensis* at CWT was 23 m^2 ha^{-1} by 2014 (Elliott and Miniat, 2018). Supporting our results, Abella (2018) found little change in basal area of deciduous trees and a decrease in stand density 15 years after the initial HWA infestation in a forest in South Carolina. In addition to recruitment suppression by understory evergreen shrubs, the decrease in stand density was attributed to wind events during the study period, self-thinning of deciduous trees and pests attacking some deciduous species (Abella, 2018).

Removal of *R. maximum* and the soil O horizon may increase light transmission to the forest floor (Elliott and Miniat, 2018) and disrupt the plant-soil feedback (Osburn et al., 2018) that has enabled the expansion of *R. maximum*. Additional data, such as a gradient of *R. maximum* density across plots, are needed to confirm the relationship between *R. maximum* and poor tree species recruitment in our study. However, these active management actions may help increase diversity of species recruitment in both understory and overstory layers in post-*T. canadensis* stands with a dense *R. maximum* shrub understory. Repeated interventions may be needed to achieve this goal (Elliott and Miniat, 2018). Seed introductions may also be required to establish a diverse and rich understory community due to the few numbers of tree and herbaceous species in seedbanks under *R. maximum* subcanopies (Cofer et al., 2018).

In addition to affecting forest structure and composition, removal of *R. maximum* may also affect ecosystem function. Removal of the *R. maximum* understory and forest floor (O-horizon) increased soil C and N availability (inorganic N), stimulating microbial activity (Osburn et al., 2018). Stand-level transpiration may also decrease initially with *R. maximum* removal, as leaf-level transpiration rates are similar between *R. maximum* and *T. canadensis* (Brantley et al., 2015). However, in these stands with high soil moisture and potentially rapid recruitment and growth of other species, transpiration and growth of other hardwood trees could result in transient changes in evapotranspiration (ET) amounts, but persistent changes in the seasonality of ET and streamflow (Brantley et al., 2015).
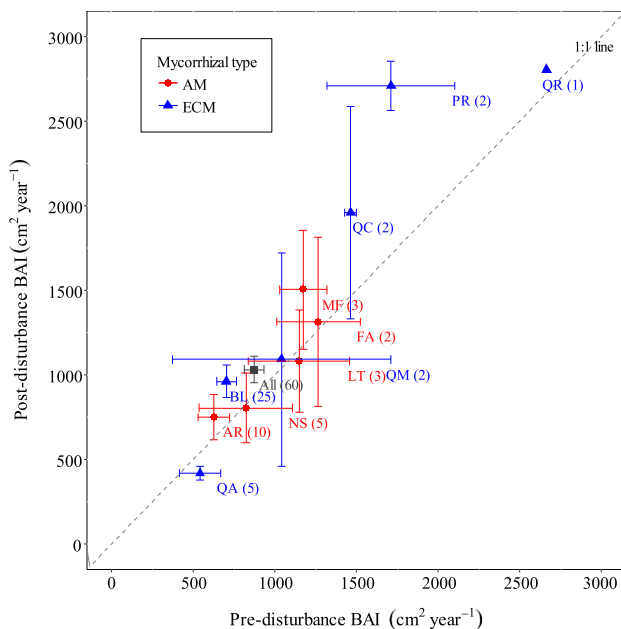
Fig. 6. Mean basal area increment (BAI) of pre- and post-*T. canadensis* mortality periods (1985–2003 and 2004–2016, respectively) for each non-*Tsuga* species. Number of sample trees is in parentheses. Red symbols represent arbuscular mycorrhizal (AM) species, blue symbols represent ectomycorrhizal (ECM) species, and black represents the mean for all species. Species acronyms are: AR, *Acer rubrum*; BL, *Betula lenta*; FA, *Fraxinus americana*; LT, *Liriodendron tulipifera*; MF, *Magnolia fraseri*; NS, *Nyssa sylvatica*; PR, *Pinus rigida*; QA, *Quercus alba*; QC, *Quercus coccinea*; QM, *Quercus montana*; and QR, *Quercus rubra*. Error bars denote standard errors. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5. Conclusions

HWA infestation of *T. canadensis* in southern Appalachian forests has resulted in widespread mortality. Our study examined the effects of this disturbance on microclimate, growth and recruitment of overstory and understory vegetation species. Light transmission to tree seedlings increased initially following infestation, but light and tree seedling density have gradually decreased to the pre-infestation level in the last few years. Growth rates have increased in *P. rigida* and selected overstory hardwood species, with ECM species exhibiting greater average growth rates than AM species. However, in our study mycorrhizal association was not a significant predictor of growth ratio, suggesting greater competition for other resources (e.g., light) between ECM and AM species to dominate post-*Tsuga canadensis* stands. There was no recruitment of tree seedlings to the overstory size class. After a decade, growth of both overstory hardwood trees and *R. maximum* shrubs has not offset the loss of *T. canadensis* basal area. Both light and tree seedling declining responses may be linked to increases in *R. maximum* density and basal area as suggested by other studies. Additional data are needed to confirm this relationship in our study areas. Meanwhile, active management, such as the removal of *R. maximum* and the recalcitrant soil O horizon, may help enhance and diversify seedling recruitment

Acknowledgements

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2019.03.024.

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