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Assessing The Effect Of Eastern Hemlock Decline From Hemlock Woolly Adelgid Infestation On Ectomycorrhizal Fungus Communities

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Abstract

Eastern hemlock (*Tsuga canadensis* (L.) Carrière) is a foundation species in eastern North American forests, providing critical habitats for a number of species. Hemlocks are experiencing widespread decline due to the spread of hemlock woolly adelgid (HWA: *Adelges tsugae* Order Hemiptera) into their range, potentially resulting in the functional disappearance of hemlocks from eastern forests. Hemlock dieback can lead to cascading effects on associated ecosystems, including below-ground, mycorrhizal fungal communities. Ectomycorrhizal fungi (EM), which are mutualistic with many tree species and provide nutrients to plant hosts, are known to colonize hemlock and neighboring tree species at lower rates following HWA infection. This study investigated the effect of hemlock decline from HWA infestation on mycorrhizal communities. Hemlock health surveys were conducted in healthy (Carl Sandburg Home National Historic Site – CARL) and declining (Warren Wilson College – WWC) stands in western North Carolina, and trees were paired between stands based on diameter. In each stand, northern red oak (*Quercus rubra* L.) "bait" seedlings were planted within a meter of "host" hemlock trees in early summer and allowed to grow for eight weeks, when they were harvested. Seedling growth and dry biomass were recorded at harvest and roots were sampled for mycorrhizal colonization rates. Different mycorrhizal morphotypes were collected from seedling roots for DNA extraction to compare mycorrhizal community assemblages between the two stands. Mycorrhizal inoculation rate (percentage of total number of root tips on a seedling that were colonized by EM) and growth in seedling height were significantly greater in the healthy hemlock stand (CARL) relative to the declining stand (WWC), suggesting that healthy hemlock stands are more favorable for oak seedling growth than declining stands. DNA barcoding determined that a greater proportion of seedlings grown in a healthy stand were inoculated with EM taxa, indicating that EM assemblages differ between a healthy and declining hemlock stand. Root:shoot ratio decreased significantly with increasing mycorrhizal colonization driven by significant decreases in root biomass. In declining stands, mycorrhizal inoculation was lower and the mycorrhizal community was different, resulting in differential growth in the declining stand relative to the healthy stand. We conclude EM communities differ between a healthy and declining stand and that changes in EM communities following hemlock dieback may affect the growth of replacement species.

1. Introduction

Eastern hemlock (*Tsuga canadensis* (L.) Carrière), referred to hereafter as hemlock, is a slow-growing, long-lived—up to 900 years[1]—late-successional conifer responsible for creating unique climax forest ecosystems throughout eastern North America[2]. Hemlock ranges from southern Canada, where it forms contiguous stands, to the southern Appalachian Mountains, where it forms mixed hardwood stands that are restricted to riparian valleys, cool moist coves, and escarpments, particularly north or east-facing slopes[1]. Although hemlock constitutes, at most, five percent of the total volume of trees present in Southern Appalachia[3,4], it is a foundation species[2] that contributes to highly diverse forest communities by constructing habitats and regulating fundamental ecosystem processes[5].

Hemlock stands persist for thousands of years[6,7] by forming distinct, self-perpetuating communities where conspecific seedling recruitment is promoted relative to heterospecifics[8] due to conditions naturally found in hemlock stands, such as low light availability and low nitrogen levels[9]. In these climax forests, hemlock's high leaf area index, which increases year-round shading, minimizes daily variation in temperature in terrestrial and aquatic ecosystems[10], creating cool, moist microclimates in the forest understory that are of particular importance to fish[11,12] and salamander species[13]. The structural characteristics of hemlock canopies also support diverse arboreal arthropod communities due to greater vegetative structural variation in branch architecture relative to deciduous canopies[14,15] and avian communities through year-round provision of habitat for feeding and nesting[16]. No other native evergreens are capable of filling the ecological role that hemlocks serve in Southern Appalachia[17].

In the past two decades, hemlock has been declining due to infestation by the exotic hemlock woolly adelgid (*Adelges tsugae* Annand, Order Hemiptera; HWA) that feeds on phloem at the base of hemlock needles. Chemical and biological treatments are available to control HWA in Southern Appalachia, where management is focused on controlling populations rather than eradication[4]. Imidacloprid, a systematic neonicotinoid insecticide and one of the most common forms of HWA chemical control[4], lasts for over two years and causes, on average, 98.5% HWA mortality when administered via soil injection[18]. High pressures sprays of insecticidal soap on hemlock foliage is have been shown to be 95-99% effective at controlling HWA[19] but only for HWA present on foliage at the time of application and on foliage lower than 45 feet[17]. Biological control of HWA using introduced predator species such as *Laricobius nigrinus* Fender., which is native to the Pacific Northwest, is also effective at reducing HWA populations[20]. Without treatment, hemlocks generally die within 4-15 years of initial infestation[21,22] and show little capacity to reestablish[2], which may lead to the extirpation of hemlock within decades. This decline has cascading effects throughout ecosystems, including to below-ground, mycorrhizal fungus communities associated with hemlock stands[23-26].

Mycorrhizal fungi form symbioses (mycorrhizae) with plants[27]. These fungi gain carbohydrates from their plant symbiont in exchange for nutrients that fungi harvest from the soil[28]; tree species allocate as much as 21%[29] of net photosynthate to mycorrhizal mutualists[30]. Most tree species form ectomycorrhizae[30] (EM), and tree species in the Pinaceae, which includes hemlocks, are almost exclusively ectomycorrhizal[31]. Ectomycorrhizal fungi mediate trees' water and nutrient uptake and are characterized by having a sheath of fungal hyphae that encloses a plant root, a network of hyphae between root epidermal and cortical cells, and hyphal elements that grow outward from the plant root to form connections to the soil and to the fruiting bodies of fungi forming the mycorrhizae[31]. Many mycorrhizal fungi are capable of colonizing the roots of two or more plants via a single mycelium[32] and, in doing so, form a mycorrhizal network that directly connects the roots of the colonized plants[33]. When individuals of multiple fungus species colonize the roots of multiple plant species, they form a common mycorrhizal network (CMN)[34], which has been described as a "wood-wide web"[35] due to its ability to transfer nutrients among plants[36-39]. Ectomycorrhizal networks also facilitate seedling establishment[40] as seedlings growing near mature trees join the in existing ectomycorrhizal networks[41].

Hemlocks are known to associate with over 100 species of mycorrhizal fungi, a majority of which—75 out of 113—are EM, and host one of the richest and most diverse fungal communities in temperate forests[42]. Declines in hemlock health from HWA infestation have been shown to reduce EM colonization by as much as 67% because of reduced transport of photosynthate belowground[24]. Decreased belowground photosynthate transfer combined with reduced photosynthetic rates, which are as much as 36% lower in trees infested with HWA[43], may alter soil EM community composition.

Changes in EM community composition are known to impact the success and of conspecific[44] and heterospecific seedlings[23,45]. The EM colonization and root tip density of neighboring northern red oak (*Quercus rubra* L.), referred to hereafter as red oak, a common hemlock replacement species colonized by EM[42,46,47] are lower in declining hemlock stands than in oak-dominated stands[23]. Differences in EM communities between secondary hardwood forests and old-growth hemlock stands lead to differential growth among hemlock seedlings, likely due to increased potential of CMNs to develop with seedlings under conspecifics[44]. These differences in seedling growth due to changes in EM communities could affect reforestation following hemlock dieback by lowering EM inoculation potential in declining hemlock stands, which could alter the recruitment and productivity of conspecific and heterospecific seedlings alike.

In this study, we compared the EM communities between a declining hemlock stand (heavy impacts from HWA) and a healthy stand (relatively little impact from HWA) to determine how above-ground HWA herbivory alters the composition of below-ground fungal assemblages and their effects on oak seedling growth. We predicted lower EM inoculation and slower growth in oak seedlings planted in the declining stand compared to those planted in the healthy stand. Additionally, we expected that the EM community composition would differ between these stands.

2. Methods

2.1. Study Area and Hemlock Health Assessments

The study was conducted in the Blue Ridge Mountain physiographic province in western North Carolina at Carl Sandburg Home National Historic Site (CARL) in Flat Rock, NC and the Berea Grove at Warren Wilson College (WWC) in Swannanoa, NC. Both sites are mixed hardwood forests with a substantial hemlock component. CARL has a relatively healthy hemlock stand that received regular imidacloprid chemical treatments, which began in 2005 and continued until 2016 on an every-other-year treatment schedule, with half of the hemlocks treated one year and the other half treated the following year (Van Hoff, Irene *pers. comm*.). Foliar chemical treatments were also administered on hemlocks at CARL with a diameter of less than four inches at breast height (dbh) using insecticidal soaps and *L. nigrinus* was released at CARL in 2017 and 2018 (Van Hoff, Irene *pers. comm*.). Hemlocks at WWC have a greater health impact from HWA infestation due to less recent and frequent treatment of HWA. Hemlocks growing at WWC were treated with imidacloprid soil injections in 2004 and 2005 and with release of *L. nigrinus* in 2015 (Swartz, Shawn *pers. comm*.).

Twenty "host" hemlock trees were selected at each site. These were selected so that trees at each site were paired based on dbh. The health of selected trees was compared based on trees' uncompacted live crown ratio, crown density, and recent crown dieback[48]. To determine the influence of particular tree species on forest floor properties surrounding each seedling, and likely EM communities associated, we calculated the Individual Tree Influence Index (ITII), which combines tree diameter and distance, and combined ITII by species to determine the Tree Species Influence Index[49] (TSII) for each seedling location.

2.2. Bait Seedling Propagation

Red oak seedlings were germinated from acorns collected from Bent Creek Experimental Forest near Asheville, NC. Acorns were treated with Captan fungicide when collected to prevent fungal illness during germination. Acorns were cold stratified at 4ºC for four months then germinated in 3.8 L pots of Fafard 3B Metro-Mix 380 in a solarium at the University of North Carolina Asheville. After one month's growth, these seedlings were planted at field sites during the first two weeks of May 2019 within 1 meter of previously selected host hemlock trees to act as "bait" for EM fungi. Seedling height, basal diameter, number of leaves, and percent leaf damage were recorded at initial planting and subsequently monitored biweekly. Seedlings that died within 5 weeks of initial planting were replaced.

After three months, 19 surviving seedlings were harvested from each site for a total of 38 seedlings. At the time of harvest, seedlings were removed from the soil with roots intact, bagged, and stored at approximately 4℃ until processing, which was completed within two weeks of harvest. Seedling shoots were separated into leaves, stems, and roots and all organs (including roots after EM processing) were dried at 65℃ for 48 hours and weighed to measure dry biomass.

2.3. EM Root Tip Sampling

Soil was removed from seedlings' root systems manually by washing under water. After cleaning, each seedling's root system was examined under a dissecting microscope, and a total of 10 cm of root length was subsampled from the top, middle, and bottom of the secondary root system. EM colonization was assessed as the proportion of the total root tips that were colonized by EM along the 10 cm length of root sampled (i.e. inoculation rate) and as differences in EM taxa among seedlings (i.e. community assemblages). Root tips colonized by *Cenococcum geophilum* Fr. were identified by morphology and counted separately from other EM species due to their higher frequency and abundance among seedlings[50] and their varied ability to promote seedling growth[51].

Excluding *C. geophilum*, 165 EM root tip samples were collected from all 28 red oak seedlings that had non-*C. geophilum* root tips and stored frozen until DNA extractions were conducted. DNA was extracted from EM root tips using the Qiagen DNeasy Plant Minikit and amplified by polymerase chain reaction (PCR) with primers ITS1F and ITS4[52,53]. PCR reactions contained 11 µL of Master Mix, 1 µL of ITS1F, 1 µL of ITS4, and 6 µL of DNA, for a total reaction volume of 19 µL. Gradient PCR was used to determine an optimal annealing temperature of 54.4℃ and amplification of DNA was performed for 45 cycles. PCR conditions were as follows: initial 10 min at 94℃ followed

by 30 sec at 94℃, 30 sec at 54.4℃, 2 min at 72℃, and a final 10 min at 72℃. Amplification success was verified by running PCR products on a 2% agarose gel stained with ethidium bromide.

Successfully amplified samples were sent to the North Carolina State University Genomics Laboratory (GSL) for Sanger sequencing. Sequences returned from the GSL were filtered for quality and trimmed in Geneious Primer (version 2019.2.3). DNA barcoding was used to identify fungal species collected from the seedling roots to assess differences in EM community composition. Unique ITS types were compared to sequences in GenBank with BLAST searching for identification and only sequences that returned with ≥96% similarity were used[54].

2.4. Analytical Methods

Hemlock health (live crown ratio, crown density, and recent crown dieback), red oak seedling growth (stem and leaf biomass and height increase), and EM colonization were compared between sites using a nonparametric Wilcoxon signed-rank test because data did not fit the assumption of normality associated with a parametric analysis of variance (ANOVA). Mean total seedling biomass, root biomass, diameter growth, and root:shoot mass were compared between sites using a one-way ANOVA. Seedling parameters were pooled between sites and analyzed against inoculation rate using linear regression. All analyses were conducted in SAS v9.4[55].

3. Results

3.1. Host Hemlock Assessments

Trees at both sites did not differ in dbh (Fig 1A), but host hemlocks at CARL were significantly healthier than their counterparts at WWC, with higher live crown ratios (Fig 1B; $Z = -5.1596$, $p < 0.0001$), lower foliar transparency (Fig 1C; $Z = 2.9089$, $p = 0.0018$), and lower variable dieback (Fig 1D; $Z = -4.3982$, $p < 0.0001$). According to calculated TSII values, hemlocks had the greatest influence on forest floor properties surrounding a majority (26 out of 38) seedlings, the second greatest influence on 10 seedlings, and the third greatest influence on two seedlings (Table 1).

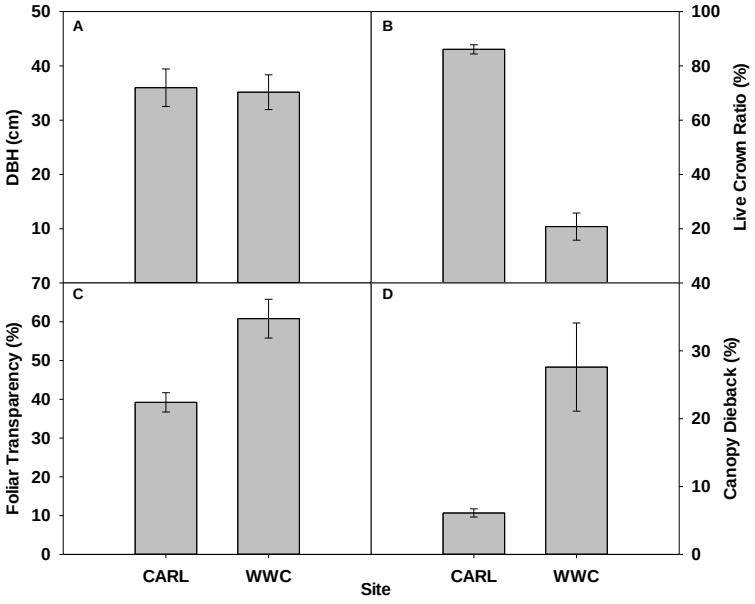

Figure 1. Host hemlock health.

Figure 1. Pairing of host hemlocks based on DBH (A) and differences in host hemlock health between sites based on variable live crown ratio (B), crown density (C), and variable dieback (D).

Table 1. Tree species listed in descending order of influence on oak seedling based on species' TSII (indicated in parenthesis). When two TSII values are listed for hemlocks, the first value includes the combined value of living and dead hemlocks, whereas the second value includes only living hemlocks. *Indicates that, when dead hemlocks were excluded, the hemlocks had a lower TSII value than the species with the next greatest TSII.

Stand	***Dominant Species (TSII)***	***Second Species (TSII)***	***Third Species (TSII)***
CARL	Hemlock (10.20)		
CARL	Sourwood (10.19)	Hemlock (9.57)	
CARL	Eastern white pine (18.98)	Hemlock (8.39)	
CARL	Tulip poplar (13.27)	Eastern white pine (12.00)	Hemlock (9.63)
CARL	Hemlock (18.53)		
CARL	Hemlock (13.52)		
CARL	Hemlock (6.49)		
CARL	Hemlock (12.38, 11.14)		
CARL	Hemlock (11.47, 11.04)		
CARL	Hemlock (9.67)		
CARL	Eastern white pine (16.25)	Hemlock (10.49)	
CARL	Hemlock (17.45)		
CARL	Eastern white pine (8.64)	Hemlock (8.16)	
CARL	Hemlock (14.61)		
CARL	Hemlock (11.20)		
CARL	Eastern white pine (10.68)	Hemlock (8.15)	
CARL	Eastern white pine (18.08)	Chestnut oak (4.05)	Hemlock (3.55)
CARL	Eastern white pine (12.15)	Hemlock (6.54)	
CARL	Chestnut oak (10.44)	Hemlock (10.44)	
WWC	Eastern white pine (16.64)	Hemlock (13.60, 12.53)	
WWC	Hemlock (18.84)		
WWC	Hemlock (17.66)		
WWC	Hemlock (21.68, 20.36)		
WWC	Hemlock (26.77, 19.02)		
WWC	Hemlock* (12.68, 9.62)	Red maple (11.93)	
WWC	Hemlock (26.98, 25.73)		
WWC	Tulip poplar (10.97)	Hemlock (10.68, 6.57)	
WWC	Hemlock (14.23, 11.99)		
WWC	Hemlock (17.37, 9.47)		
WWC	Hemlock (11.06, 10.30)		
WWC	Hemlock* (9.66, 6.16)	Eastern white pine (6.64)	
WWC	Hemlock* (12.25, 9.69)	Eastern white pine (10.88)	
WWC	Eastern white pine (22.59)	Hemlock (7.41, 5.61)	
WWC	Hemlock (10.74, 9.93)		
WWC	Hemlock (19.19, 12.67)		
WWC	Hemlock (10.60, 10.12)		
WWC	Hemlock* (8.18, 6.07)	Eastern white pine (7.79)	
WWC	Hemlock (21.93, 16.92)		

3.2. Seedling Growth Parameters and EM Inoculation

Total seedling biomass was not significantly different between seedlings grown at CARL and seedlings grown at WWC ($F = 1.66$, $p = 0.2064$). However, seedlings' biomass allocation differed between the two sites. CARL seedlings had smaller root biomass (Fig 2A; $F = 5.88$, $p = 0.020$) and smaller diameter growth (Fig 2C; $F = 38.46$, $p < 0.0001$) but greater increase in stem height (Fig 2B; $Z = 2.183$, $p = 0.015$) than WWC seedlings (Fig. 2). Percent inoculation of root tips was significantly greater among CARL seedlings when *C. geophilum* inoculation was included (Fig. 2D; $Z = 4.0581$, $p < 0.0001$) but only marginally higher when *C. geophilum* was excluded (Fig. 2D; $Z = 1.519$, $p = 0.0644$).

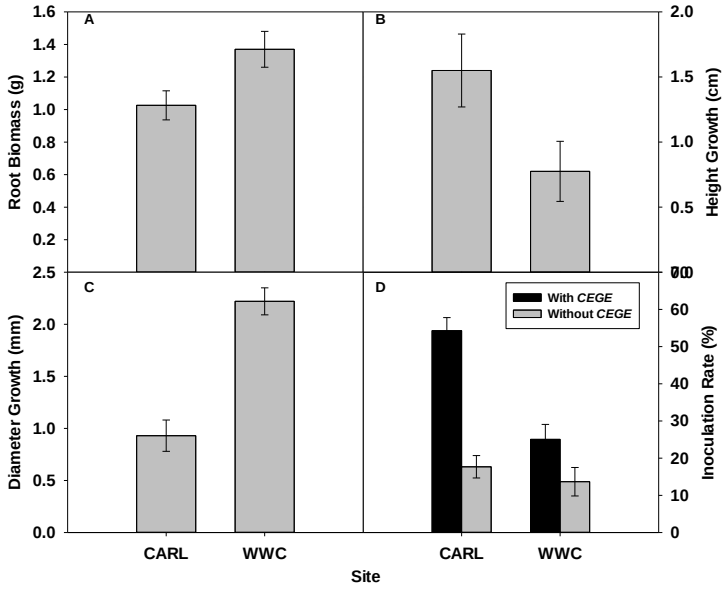

Figure 2. Seedling growth and inoculation.

Figure 2. Differential growth (root biomass (A), stem growth (B), basal diameter growth (C), and inoculation rate (D)) between CARL and WWC seedlings.

When seedlings were combined between sites, the ratio of root to shoot mass (root:shoot ratio) significantly decreased with increasing total inoculation rate (Fig 3A; $p = 0.005$), driven by significant decreases in stem diameter growth (Fig 3B). This relationship between stem diameter growth was significant with total inoculation including *C. geophilum* (Fig 3B; $p = 0.003$) and was marginally significant when *C. geophilum* was excluded (Fig 3B; $p = 0.045$).

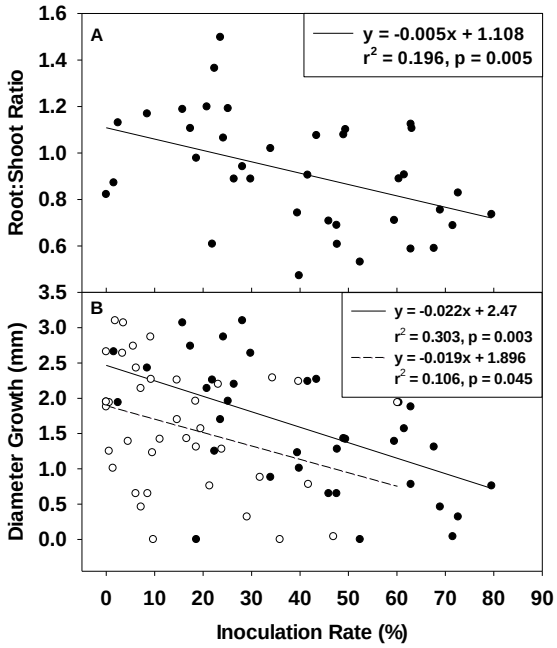

Figure 3. Root:shoot mass and diameter growth with increasing inoculation.

Figure 3. Decreasing root:shoot mass ratio (A) and diameter growth (B) with increasing inoculation rate. At CARL, root:shoot ratio was 0.78 ± 0.05 on average compared to 1.05 ± 0.05 at WWC. In diameter growth versus inoculation rate, inoculation rates including *C. geophilum* are indicated by closed circles and a solid line and inoculation rates that exclude *C. geophilum* are indicated by open circles and a dashed line.

3.3. Assemblages of Mycorrhizal Species

Among non-CEGE EM root tips collected from seedlings at CARL and WWC, 81 amplified with EM taxa: 54 at CARL and 27 at WWC. The proportion of seedlings with EM fungal taxa was significantly greater ($\chi^2_1 = 0.07$, p = 0.044) at CARL, where 15 of 19 seedlings had non-CEGE EM taxa, than at WWC, where only 9 of 19 did (Fig. 3). Although species richness was the same at both sites, taxa differed between the sites, indicating that assemblages of EM differ between healthy and declining hemlock stands.

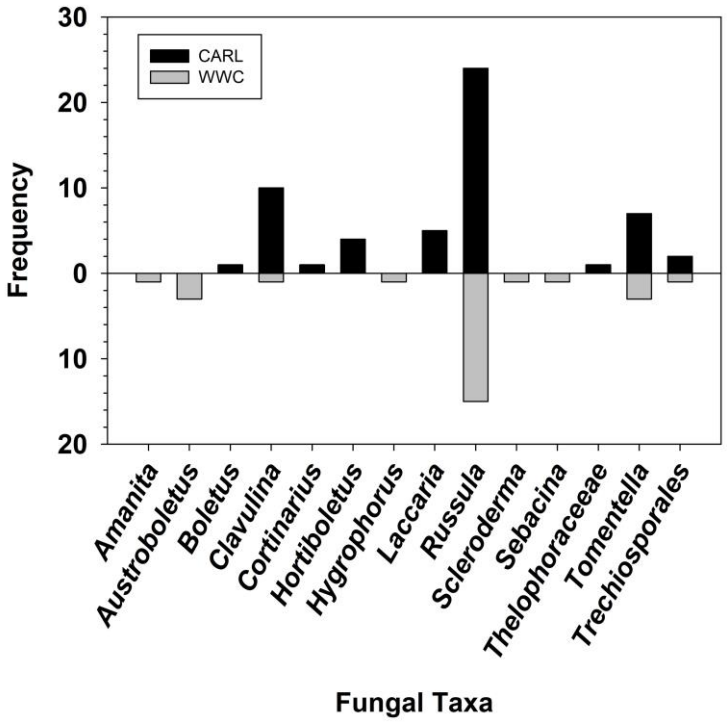

Figure 4. Frequency of fungal taxa.

Figure 4. Frequency of root tips that amplified at each site by fungal taxon at CARL and WWC, which was significantly greater among CARL seedlings. Richness did not differ between sites (n = 9).

4. Discussion

In this study, we found that EM inoculation rate of red oak seedlings was higher (54%) in a healthy hemlock stand than in a declining stand (25%), which supports our hypothesis that healthy hemlock stands have greater EM inoculation than declining stands. Lower EM inoculation in declining stands was predicted because HWA infestation reduces photosynthetic rates[43], thereby lowering the amount of photosynthate available to EM. This result is consistent with other studies, which found that EM inoculation is significantly lower among hemlocks infected with HWA[24-26] and among red oak seedlings grown in declining hemlock stands compared to seedlings grown in healthy oak stands[23]. However, no study, to our knowledge, has analyzed *in situ* EM inoculation potential of a healthy and declining hemlock stand by comparing EM inoculation of heterospecific seedlings grown in both environments.

Relative to a declining stand, red oak seedlings grown in a healthy hemlock stand were more frequently colonized by fungal taxa. The positive relationship of greater hemlock health with higher EM inoculation and more frequent colonization by EM taxa indicates that healthy hemlock stands support different assemblages of EM than declining stands. Seedlings grown in a healthy stand may be more frequently colonized by fungal tax due to greater EM inoculum in the healthy stand. Hemlock health has the greatest influence on fungal communities in hemlock stands, with trees that have greater canopy density and live-crown ratio associating with richer, more diverse fungal communities[42]. As hemlock canopy defoliation reduces the richness, diversity, and evenness of fungal communities[42], EM inoculation of neighboring seedlings may also decline due to reductions in the availability of EM to form mycorrhizae.

Variation in hemlock health between stands were due, in part, to differential treatment history of hemlocks with neonicotinoid imidacloprid insecticide to control HWA infestation. Imidacloprid is known to alter soil microbial communities[56,57] as well as non-target soil arthropods associated with hemlocks[58], which regulate below-ground detrital food webs[59]. Therefore, differential imidacloprid application between healthy and unhealthy sites may have altered the soil microbial communities, including EM, in hemlock stands we studied. However, imidacloprid has been

observed to have no adverse effect on the structure, measured as species diversity and richness, of fungal communities associated with hemlocks[42]. Rather, imidacloprid use may preferentially support dominant hemlock-associated taxa, thereby enhancing the stress tolerance and stability of hemlock-associated fungal communities[42]. Thus, imidacloprid application was not likely responsible for differences in EM communities between stands in this study.

Biomass did not differ among red oak seedlings grown in healthy and unhealthy hemlock stands but biomass allocation was different, with seedlings grown in a healthy stand having smaller root biomass and greater increase in stem height than their counterparts in a declining stand. Greater EM inoculation of red oak seedlings grown in a healthy hemlock stand may account for seedlings' overall smaller root biomass and greater stem height growth. In an *ex situ* environment, hemlock seedlings' height growth is greater when seedlings are grown in soil with greater EM soil inoculum, indicating that EM composition influences seedling growth and success[44]. Root weight is also correlated with EM inoculation, with reductions in root weight due to EM colonization observed in Pinaceae species other than hemlock including Sitka spruce[60], Douglas-fir[61], jack pine[62], and black spruce[63].

The negative relationship between root biomass and EM inoculation indicates that portions of trees' roots can be replaced by an EM component[63], allowing seedlings to preferentially allocate carbon to shoots. This is consistent with our observation of decreased root:shoot ratio with increasing EM inoculation. The negative relationship between root:shoot biomass and inoculation rate among all seedlings was likely due to significantly decreasing stem diameter growth and marginally significant increasing leaf mass with inoculation rate. Sim and Eom (2006)[64] also observed lower root:shoot ratio among seedlings treated with multiple EM species, which also lead to greater overall colonization rates, than seedlings treated with a single EM species. If altered EM assemblages in declining hemlock stands lead to differential seedling growth, different EM assemblages may impact the growth and productivity of replacement species and the recovery of forests following hemlock dieback.

Differences in biotic and abiotic characters between sites is a further limitation of this study. Variability in attributes such as neighboring vegetation, land-use history, light availability, temperature, and soil moisture and pH may have influenced EM communities and red oak seedling growth irrespective of the influence of variation in hemlock health on EM. Dense populations of garlic mustard (*Alliaria* petiolate (M.Bieb.) Cavara & Grande), an invasive species of the non-mycorrhizal Brassicaceae, for example, lower EM inoculation of red oak seedlings[65]. Thus, variation in vegetation and, particularly, the presence of non-native vegetation, between sites may have influenced EM communities. Differing levels of light availability between sites may also have influenced both EM communities and seedling growth; red oak seedlings grown under intermediate and high light have 138% greater EM inoculation, greater biomass, and greater root:shoot mass ratio compared to seedlings grown under low light[45]. Observations of differences in EM communities and seedling growth between a healthy and unhealthy stand in this study may, therefore, have been compounded by differences in attributes between sites.

However, many of the biotic and abiotic characters of hemlock stands that influence EM and seedling growth shift as hemlocks decline from chronic HWA herbivory. Light availability in the lower canopy and forest floor[66,67] and invasion by nonnative plants[66,68] which are associated with shifts in EM richness and community composition[45,65], increase under HWA-infested trees. Thus, biotic and abiotic attributes are expected to differ between a healthy and declining hemlock stand. Further study, particularly of seedling growth and EM colonization in an *ex situ* environment where seedlings are grown in soil collected from a healthy and declining stand, is required to better understand how differences in hemlock health affect EM inoculum and, in turn, seedling growth.

Following dieback, hemlocks are predicted to be replaced by a mix of maple (*Acer*), birch (*Betula*), beech (*Fagus*), and oak (*Quercus*) species[67]. However, when great laurel (*Rhododendron maximum* L.)—referred to hereafter as great laurel—is present, it will likely limit the establishment of other hardwood species[67]. Great laurel may further suppress the growth of hardwood replacement species because, in great laurel thickets, EM inoculation of seedlings is reduced[50], likely because a feature of the environment in these thickets inhibits the process of mycorrhization itself[47]. When hardwood growth is not limited, changes in EM inoculation potential in declining hemlock stands are predicted to shift the competitive balance of tree replacement species; the growth of species that host predominantly EM, including members of the Fagaceae family such as oak species[31], may decline relative to species that host predominantly arbuscular mycorrhizal fungi such as maple species[40]. The differences in EM communities that we observed between a healthy and declining hemlock stand may therefore be due to reductions in EM inoculum following hemlock decline and subsequent shifts the competitive balance at the expense of EM tree species.

The loss of hemlock as a foundation species in climax forests throughout eastern North America and subsequent changes in EM communities have implications for the management of forests following hemlock dieback. Differential EM colonization of heterospecific seedlings, which we observed between a healthy and unhealthy hemlock stand, suggests that hemlocks decline due to HWA alters EM communities. However, mature trees maintain diverse EM communities following disturbance and can serve as sources of EM inocula until conditions become favorable for

their spread[69]. Mature hemlock trees likely support different EM assemblages than juveniles[70] and thus have potential to support unique EM communities following disturbance.

Given the potential of mature trees to serve as repositories of EM communities, the preservation of even a limited number of mature hemlock trees via chemical or biological treatment of HWA could support the conservation of associated EM communities. The conservation of these EM communities, in turn, could impact forest recovery if they are capable of increasing the EM inoculation potential of forest communities following hemlock dieback. Given that almost all EM growing with hemlocks are not associated specifically with hemlock but are instead found in association with other tree species within or bordering hemlock stands[70], EM from mature hemlock trees are capable of associating with and supporting the growth of conspecific and heterospecifics alike. In turn, shared EM are capable of connecting hemlocks with other neighboring species in a CMN, which facilitate the exchange of nutrients between conifers and hardwoods[36]. Thus, preservation of mature hemlock trees and associated EM could continue to support CMN connecting hemlocks to neighboring species and the sharing of nutrients through those networks. Preservation of mature hemlocks has the potential to maintain EM taxa following widespread hemlock dieback and, in so doing, could mitigate changes observed in this study in EM communities following hemlock decline and support reforestation by promoting the recruitment and productivity of EM replacement seedlings.

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