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DIVERSITY OF MYCORRHIZAL FUNGI IN EASTERN HEMLOCK STANDS WILL SIGNIFICANTLY CHANGE WITH THE EFFECTS OF HEMLOCK WOOLLY ADELGID INFESTATION IN SOUTHWESTERN NOVA SCOTIA, CANADA

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ABSTRACT

A foundation tree species, eastern hemlock (*Tsuga canadensis*) is threatened by the invasive hemlock woolly adelgid (*Adelges tsugae*) (HWA) in southwestern Nova Scotia, Canada. The loss of this key species and its heavily shaded ecosystems may alter the diversity of important mycorrhizal fungi in hemlock forests. Mycorrhizal fungi share a vital mutualistic relationship with their host trees; consequentially, understanding if and how the predicted eastern hemlock decline will affect mycorrhizal diversity is paramount. Using available literature, we discuss three major consequences of HWA on eastern hemlock ecosystems – changing forest composition, loss of old-growth trees, and increased insect stress on host trees – and how they will likely influence mycorrhizal communities as adelgid infestations intensify. Environmental variables are also discussed as another major influence on fungal diversity. We conclude that the mycorrhizal community of eastern hemlock forests will likely change significantly as old-growth

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hemlock is replaced with mixed forest. We also suggest that mycorrhizal community composition will shift in favour of generalist mycorrhizae which form symbioses with multiple tree species. These conclusions illuminate the future of hemlock forest fungi in southwestern Nova Scotia and underline the importance of conserving old-growth eastern hemlock forests and their unique fungal communities.

Keywords: Eastern hemlock, Hemlock woolly adelgid, Mycorrhizal fungi

INTRODUCTION

Eastern hemlock (*Tsuga canadensis*) is a foundational tree species of Acadian or Wabanaki forest (Ellison *et al.* 2005, Ellison 2014, Parks Canada 2021). Widespread across eastern North America (Godman & Lancaster 1990, Ellison 2014), this species is often found growing in monoculture stands (Goerlich & Nyland 2000, Ellison *et al.* 2005) and matures slowly, sometimes achieving lifespans of 800 years or more (Godman & Lancaster 1990). Eastern hemlock creates distinctive environmental conditions – in particular, its thick, shady canopies reduce temperatures on the forest floor by restricting light levels (Canham *et al.* 1994, Hadley 2000). In addition, its fallen needles decompose slowly (Cobb *et al.* 2006) leading to low rates of nutrient cycling and calcium mineralization (Finzi *et al.* 1998a, b, Jenkins *et al.* 1999, Dijkstra 2003) and acidic soils which are rich in organic material (Mladenoff 1987, Finzi *et al.* 1998a, b, Fassler *et al.* 2019). Eastern hemlock stands host a unique ecosystem (Ellison 2014) which is dependent on the distinctive environmental conditions provided by this tree species. As such, eastern hemlock is considered a forest foundation species (Ellison *et al.* 2005) without which the associated ecosystem could not survive. Furthermore, recent declines of eastern hemlock due to an invasive pest are concerning ecologists throughout eastern North America.

The hemlock woolly adelgid (*Adelges tsugae*) (HWA) is a tiny, aphid-like insect which attaches to the base of hemlock needles and feeds on the xylem ray parenchyma, causing needle loss and eventual branch or tree mortality with large infestations (Orwig & Foster 1998). Native to East Asia and the Pacific Northwest of North America, HWA was first detected in the southeastern United States in the 1950s, likely introduced from a southern Japanese population (Stoetzel 2002, Havill *et al.* 2006, 2016, Crandall *et al.* 2022), and has been moving north ever since. HWA is particularly destructive for eastern hemlock because this pest has no natural insect predators in eastern North America, unlike in its native ranges in Asia and western North America (Sasaji & McClure 1997, Kohler 2007, Crandall *et al*. 2022). Further, eastern hemlock appears to have little to no natural immunity to HWA (Ingwell & Preisser 2011). A heavy HWA infestation can kill a mature eastern hemlock tree in 4-10 years (McClure 1991, Orwig *et al*. 2002) and can prevent stand regeneration by damaging saplings below infested canopies (Orwig & Foster 1998). Many of the organisms in eastern hemlock ecosystems may be affected by the loss of this foundation species, including one group which is often overlooked, yet crucially important to these ecosystems: fungi.

Forest ecosystems as we know them could not exist without forest fungi. These important decomposers and mutualists facilitate nutrient cycling in the ecosystem by liberating nutrients trapped in organic and inorganic material, which can then re-enter the food web (Hobbie *et al*. 1999, Fukasawa 2011, Shortle & Smith 2015). Many fungi form mutualistic associations with plants and trees (Egli 2011, Lindahl & Tunlid 2015, Sapsford *et al.* 2017). These are known as mycorrhizal fungi, or mycorrhizae, and nearly all land plants form associations with them (Wang & Qiu 2006, Willis *et al*. 2013, Jacquemyn & Merckx 2019). Evolved from saprobic fungi (Kohler *et al*. 2015, Lindahl & Tunlid 2015), which break down dead organic material, mycorrhizal fungi form mutualistic partnerships with plant roots to trade water and nutrients scavenged from the environment for photosynthetic sugars produced by the host plant. There are two main types of mycorrhizae that associate with trees: ectomycorrhizae (ECM) and arbuscular mycorrhizae (AM). Ectomycorrhizae form branching structures called Hartig nets around and between the cortical cells of the host tree's fine roots, which they use to interface with the tree and exchange resources (Nehls 2008). Many conifers, including eastern hemlock, and some hardwoods commonly associate with ECM (Molina *et al*. 1992). Arbuscular mycorrhizae also interface with the root cells of a host tree; however, rather than extracellular Hartig nets, these fungi form tree-like structures called arbuscules inside root cortical cells – this is why AM are also considered *endo*-mycorrhizae. Arbuscular mycorrhizae are much more common than ECM, colonizing hardwood trees such as maple and ash, and most herbaceous plants (Molina *et al.* 1992, Willis *et al*. 2013). They also show less preference for a particular host species, and are therefore considered generalists (Moora *et al.* 2011). Both types of mycorrhizae can be found in most forest ecosystems, including in those of southwestern Nova Scotia.

The landscape of southwestern Nova Scotia consists of rolling hills of Wabanaki mixed forest, frequently interspersed with lakes and rivers (Polach 1992). Much of this area is part of the Southwest Nova Biosphere Reserve – a UNESCO-designated reserve which is made up of crown lands, wilderness areas, nature reserves, national and provincial parks, and transition zones (Southwest Nova Biosphere 2023). This reserve includes the Tobeatic Wilderness Area and Kejimkujik National Park and National Historic Site, which contains some of the largest old-growth eastern hemlock stands in the province (Parks Canada, pers. comm. 2022). HWA was detected in southwestern Nova Scotia in 2017 and was likely present for about 10-15 years prior to its discovery (Parks Canada, 2021, Parks Canada, pers. comm. 2023). While conservation efforts are ongoing, unfortunately, researchers predict that up to 80% of eastern hemlock in southwestern Nova Scotia will be lost to this insect pest by 2030 (Parks Canada 2021). This may have profound consequences for the hemlock ecosystems in this region.

Mycorrhizal diversity in southwestern Nova Scotia is currently undocumented but is being studied by the authors of this essay. Since mycorrhizal fungi play important roles in sustaining long-term forest communities, any changes in fungal diversity and abundance can have profound effects on forest health and regeneration (O'Brien 2009, Talbot *et al.* 2014); therefore, it is crucial to understand if and how these mycorrhizal communities will be altered by forest disturbances. Mycorrhizal communities in eastern hemlock forests may be greatly impacted by HWA in several ways. Altered forest composition, loss of old-growth trees, and increased insect stress on host trees are all consequences of HWA which will likely affect fungal diversity. Environmental changes caused by pollution and by shifts in forest conditions may also have an important influence on mycorrhizal diversity and should be considered to gain a more complete understanding of mycorrhizal dynamics (Egli 2011, Sapsford *et al.* 2017). In this essay, we use the available literature to discuss the effects of three major factors related to HWA invasion – changing forest composition, loss of old-growth trees, and increased insect stress – on the mycorrhizal diversity of eastern hemlock stands. The effects of altered environmental variables on forest fungi are also considered. Finally, we discuss the significance of shifting mycorrhizal diversity and how changes in these key fungal communities may influence the forests of southwestern Nova Scotia as HWA infestations progress.

DISCUSSION

The increasing diversity of tree and plant species within historically hemlock-dominated stands may significantly alter mycorrhizal community diversity in the forests of southwestern Nova Scotia. Forest fungal communities are very sensitive to vegetation changes (Packham *et al*. 2002, Landi *et al*. 2015) and their diversity is mainly driven by forest succession and composition (Dighton *et al*. 1986, Last *et al*. 1987, Rineau *et al*. 2010, Spake *et al*. 2016, Tomao *et al*. 2020). Accordingly, the diversity of both ECM and AM are very likely to shift in southwestern Nova Scotian forests as eastern hemlock is replaced by other conifer and hardwood tree species. Some diversity may be lost because certain ECM can only associate with specific tree species (Molina *et al*. 1992, Dickie *et al*. 2009, Tomao *et al*. 2020). As hemlock stands decline, any ECM which rely solely on eastern hemlock will likely not survive; for example, stipitate hydnum fungi (toothed fungi) are generally hemlock-associated ECM, many members of which are in danger of regional extinction due to hemlock loss (Baird *et al*. 2013). Additionally, Fassler *et al*. (2019) suggest that ECM diversity becomes simplified and homogenized after eastern hemlock forests decline; however, the hemlocks in this study – conducted in western Massachusetts, USA – were replaced mainly by black birch (*Betula lenta*), which is absent in Nova Scotia. Instead, the hemlock stands in southwestern Nova Scotia will most likely be replaced by many different tree species; for example, in Kejimkujik National Park and National Historic site, eastern hemlock forests were replaced by white birch (*B. papyrifera*), red maple (*Acer rubrum*), Balsam fir (*Abies balsamea*), red and black spruce (*Picea rubens*, *P. mariana*), and white pine (*Pinus strobus*) after outbreaks of pale-winged grey moth (*Iridopsis ephyraria*) in 2002-2006 caused severe defoliation of eastern hemlocks (Hervieux 2013, Parks Canada, pers. comm. 2023). Tree species diversity and ECM diversity are positively related (Kernaghan *et al*. 2003, Cavard *et al*. 2011);

therefore, the ECM diversity of southwestern Nova Scotian forests may actually increase as hemlock-dominated stands are replaced with mixed forest, despite losing certain hemlock-dependent ECM species. Moreover, the abundance and diversity of AM are likely to increase as the forest canopy opens and more hardwood species and herbaceous plants move in with the increased light, outcompeting the slow-growing hemlock seedlings (Battles *et al.* 1999, Catovsky & Bazzaz 2000, Haskins & Gehring 2005, Weber *et al.* 2005). Overall, the diversity of ECM and the abundance of AM fungi in southwestern Nova Scotian forests are very likely to change due to eastern hemlock loss and may even increase due to the changing forest composition.

The loss of old-growth forest may also alter mycorrhizal diversity in southwestern Nova Scotia; however, this change is likely to decrease ECM diversity. It has been well established that old-growth forests can support a higher diversity of ECM, either because they accumulate fungal species over time, or because they attract mycorrhizal fungi which have adapted specifically to associate with older trees (Birch *et al.* 2023). A study by Kranabetter *et al.* (2018) found up to 238 ECM species in old-growth Douglas fir stands in British Columbia. Additionally, Kranabetter *et al.* (2005) determined that the ECM diversity of old-growth western hemlock stands was almost twice that of their younger counterparts. Canopy closure of maturing forests has also been associated with greater ECM species diversity (Dighton *et al.* 1986) and increased mushroom production (Wallander *et al.* 2010), perhaps because the shady canopies help to retain moisture on the forest floor. Twieg *et al.* (2007) suggest that the greatest increase in mycorrhizal diversity occurs between the ages of 5 and 26 years in mixed forest, which also coincides with canopy closure for many tree species. This not only implies that the loss of old-growth trees will significantly reduce mycorrhizal diversity, but also that the regenerating forest will not achieve canopy cover, and thus a more diverse mycorrhizal community, for decades afterward. This could include the loss of important ECM fruiting bodies such as truffles (Stephens *et al.* 2017) which support insect and small mammal food webs (Shaw 1992, Luoma *et al.* 2003). In summary, the loss of old-growth trees may cause a significant reduction of ECM diversity in southwestern Nova Scotian forests which would affect these ecosystems for years to come, if not permanently.

Stress to eastern hemlock caused by HWA infestation is another major factor which may affect mycorrhizal diversity in Nova Scotian forests. Many studies have noted that mycorrhizal diversity is altered by defoliation of host trees (Lewis *et al.* 2008, Baird *et al.* 2014), and insect-stress in eastern hemlock has been associated with reduced ECM abundance and diversity when compared with healthy stands (Baird *et al.* 2014, Caruso *et al.* 2021). In fact, one study found that mushroom production decreased by a third in defoliated conifer stands (Kuikka *et al.* 2003), while Schaeffer *et al.* (2017) observed a 14% decrease in mycorrhizal colonization of fine hemlock roots after only four years of HWA infestation. These results may occur because stressed trees with defoliated canopies may be unable to photosynthesize at the same rate as healthy trees. This would reduce the amount of sugar that mycorrhizal partners receive by either directly limiting their supply or by reducing the number of fine tree roots which they use to interface with a host (Lewis *et al.* 2008). Mycorrhizal fungi can be very demanding, sometimes taking up to 25% of a tree's photosynthetic sugars (Hobbie 2006, Nehls 2008), and would be impacted negatively by a stressed or dying host tree. Interestingly, recent research suggests that host trees can actively redirect the flow of sugars to mycorrhizal partners which are less demanding (Druebert *et al.* 2009, Egli 2011). This implies that mycorrhizae which require greater amounts of sugar from their host may disappear first when their supply is cut off. However, it should be noted that some ECM are less host specific and can associate with several different tree species (Horton & Bruns 2001, Izzo *et al.* 2005). Some ECM genera, such as *Russula*, may even be able to survive without a host plant by reverting to a saprotrophic lifestyle (Štursová *et al.* 2014). Because of these advantages, these species of ECM, as well as generalist AM, would likely persist despite eastern hemlock decline due to HWA infestation. This may help explain why one recent experimental study conducted in Rhode Island, USA, did not detect any significant differences in fungal diversity between healthy and infested hemlock stands (Schaeffer *et al.* 2017).

Changes in environmental variables caused by pollution and by the loss of eastern hemlock may provoke changes in fungal abundance and diversity which can significantly influence mycorrhizal dynamics. Human activities over the past century, such as the use of combustion engines and excessive fertilization in agriculture, have increased levels of nitrogen, ammonium, and carbon dioxide in the atmosphere. Although increased growth has been observed in certain fungi of the *Paxillus*, *Lactarius*, *Thelephora*, and *Cortinarius* genera (Egli 2011), excessive nitrogen and ammonium have been shown to decrease the growth, diversity, and mushroom production of most ECM in forest ecosystems (Arnebrant 1994, Nilsson & Wallander 2003). In fact, it has been suggested that a decline in European ECM in the 1980s was caused by increased nitrogen in the air and soil (Arnolds 1991, Rühling & Tyler 1991). Contrarily, heightened carbon dioxide levels increased mycorrhizal colonization of several conifer species in a study by Godbold *et al.* (1997), demonstrating that changes in atmospheric composition can have diverse effects on fungal communities. Aside from this, the loss of old-growth eastern hemlock and their shady canopies may alter the mushroom abundance in southwestern Nova Scotian forests by increasing light levels and temperature on the forest floor. Increasing photoperiod can positively affect mushroom production by increasing the photosynthetic capacity of the host tree (Fortin *et al.* 2008). However, mushroom production is highly dependent on water availability (Egli 2011), so reduced soil moisture caused by the increase in temperature may significantly reduce mushroom abundance. This effect may be further exacerbated by the warming effects of climate change (Garbary & Hill 2021). Finally, altered soil chemistry caused by the replacement of eastern hemlock with other plant species may also change mycorrhizal composition. Eastern hemlock stands create uniquely acidic soils which are rich in organic material (Mladenoff 1987, Finzi *et al.* 1998a, b, Fassler *et al.* 2019). The loss of eastern hemlock and its shady canopies may alter cation cycling (Finzi *et al.* 1998b), reduce acidity, and increase rates of decomposition and nutrient cycling in the soil by increasing temperature and reducing soil moisture content (Jenkins *et al.* 1999, Cobb *et al.* 2006). Since different mycorrhizal fungi tend to prefer specific soil chemistries (Frelich *et al.* 1993, Finzi *et al.* 1998 a, b), changing these conditions is likely to alter which mycorrhizal species can thrive in the new forest. In total, while the overall direction of these changes may be difficult to predict, mycorrhizal diversity and mushroom production in southwestern Nova Scotian forests will likely be altered by the loss of eastern hemlock.

New research is helping to illuminate the complex relationships between fungi and their forest ecosystems (Egli 2011, Sapsford *et al.* 2017). In this discussion, we have used available literature to predict the consequences of several influential factors – changing forest composition, loss of old-growth trees, increased insect stresses on trees, and altered environmental variables – on the mycorrhizal communities of southwestern Nova Scotian forests following HWA-caused eastern hemlock decline. Replacement of eastern hemlock stands with mixed forest may increase mycorrhizal diversity and AM abundance despite the loss of hemlock-associated ECM, as more varied forests can support more diverse fungal communities (Cavard *et al.* 2011). However, loss of old-growth eastern hemlock will likely reduce ECM diversity significantly for the long-term and may particularly affect fungi preferring to associate with older trees (Birch *et al.* 2023). Further, stress induced by HWA feeding on eastern hemlocks will restrict mycorrhizal access to photosynthetic sugars from the host tree, likely reducing the diversity and abundance of ECM in hemlock stands (Lewis *et al.* 2008, Birch *et al.* 2023), but also giving an advantage to adaptable generalist mycorrhizae. Finally, increased nitrogen and ammonia levels and reduced water access in forest ecosystems may reduce diversity and mushroom production of ECM (Egli 2011), but increased light and carbon dioxide levels may have the opposite effect (Godbold *et al.* 1997, Fortin *et al.* 2008). Given this evidence, the profound changes in forest composition, stand age, tree stress levels, and environmental conditions – all significant influences on fungal diversity (Kranabetter & Kroeger 2001, Rineau *et al.* 2010, Spake *et al.* 2016) – will likely reshape mycorrhizal communities in southwestern Nova Scotian forests as old-growth eastern hemlock declines due to HWA infestations. Current research suggests that the mycorrhizal community will shift in favour of AM and generalist ECM species since these fungi may adapt more easily to a rapidly changing forest. The overall effects of the discussed factors may even indicate a potential reduction in ECM diversity; however, fungal taxonomy and the relationships between mycorrhizae and their ecosystems are not yet fully understood (Birch *et al.* 2023) – because of this, the direction of mycorrhizal changes cannot be confidently predicted at this time. Indeed, more large-scale and long-term studies are needed to truly gauge the full scope of mycorrhizal community dynamics in a changing environment (Orrego 2018).

SIGNIFICANCE

Mycorrhizal fungi provide critical services for forest health, so changes in the mycorrhizal community may have profound implications for forest ecosystems in southwestern Nova Scotia. In exchange for photosynthetic sugars, mycorrhizae increase the access of their host plant to important resources such as water (Smith & Read 1997), nitrogen (Clemmensen *et al.* 2015) potassium (Dominguez-Nuñez *et al.* 2016), and phosphorus (Wallander *et al.* 1997) by breaking down organic and inorganic materials in the environment (Van Breemen *et al.* 1997, Hobbie *et al.* 1999, Landeweert *et al.* 2001,Wallander *et al.* 2001). They may also play a role in protecting host roots from harmful heavy metals in the soil (Adriaensen *et al.* 2004). While tree diversity is an important influence on mycorrhizal composition, mycorrhizal diversity can inversely influence forest community composition by promoting the growth of their preferred tree species (Booth 2004, Nara 2005). Furthermore, mycorrhizae may help to establish the next generation of their host tree species by transferring resources from the mature trees to their seedlings through common mycorrhizal networks (Orrego 2018), though it should be noted that this theory is contested in the scientific community and may not be sufficiently supported by evidence (Karst *et al.* 2023). Regardless, in these ways, forests and their mycorrhizal fungi are inextricably interdependent; changes in one partner may initiate a cascade of consequences that would affect the entire ecosystem, including forest regeneration. In the context of southwestern Nova Scotian forests, the decline of eastern hemlock due to HWA infestation and the subsequent changes in mycorrhizal diversity will likely result in the loss of rare hemlock-associated fungi such as stipitate hydnum species (Baird *et al.* 2013) and truffles (Stephens *et al.* 2017). This may also reduce the ability of the hemlock forests to establish new seedlings by limiting availability of compatible ECM and altering soil chemistry (Haskins & Gehring 2005). Soils which lose mycorrhizae have also been shown to lose the soil bacteria associated with those fungi (Hol *et al.* 2014), which would further alter nutrient availability and the suitability of the soil for hemlocks (Vendettuoli *et al.* 2015). Additionally, fungi are important food sources for small mammals such as flying squirrels, voles, and chipmunks (Luoma *et al.* 2003), and soil-dwelling organisms such as mites, collembola, and many insects (Newell 1984, Moore *et al.* 1988, Shaw 1992, Heděnec *et al.* 2013), so altering mycorrhizal diversity may have consequences for the larger food web. All this evidence suggests that the HWA disturbance will irreparably alter both the mycorrhizal and forest communities of southwestern Nova Scotia, unless immediate action is taken to protect old-growth eastern hemlock forests and their unique associated ecosystems.

CONCLUSIONS

The old-growth eastern hemlock ecosystems of southwestern Nova Scotia are expected to experience major disturbances in the coming years due to the recent arrival of invasive HWA. Based on literature regarding the influence of forest composition, tree age, insect-induced tree stress, and environmental variables on mycorrhizal fungi, we expect the diversity of mycorrhizae in southwestern Nova Scotian Wabanaki forest to change significantly with the loss of old-growth eastern hemlock. We also predict that the mycorrhizal community composition will shift toward more generalist ECM fungi and more AM fungi as mixed forest replaces previously hemlock-dominated stands. The profound consequences of these changes will permanently alter the forest ecosystems of southwestern Nova Scotia unless immediate action is taken to conserve old-growth eastern hemlock ecosystems.

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