Exploring the Role of Mycorrhizal Connections in Supporting Forest Regeneration and Diversity

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Introduction

The northeastern forests of the future will look different from the northern forests of today as invasive pests and climate change act to shift the distribution of tree species (Beckage et al., 2008; Fischer et al., 2013). While some species such as sugar maple (*Acer saccharum* Marsh.) have been observed in slow decline over decades (Cleavitt et al., 2018), the decline of ash (*Fraxinus spp.*) is expected to be more rapid following the arrival of the emerald ash borer (*Agrilus planipennis*), a pest projected to decimate virtually all ash trees upon its arrival (Morin et al., 2017). These changes in tree community composition will affect the ecosystem services forests provide such as soil carbon storage and nutrient availability and cycling (Groffman et al., 2012). Trees can affect these ecosystem services as well as the abundance of root pathogens in concert with two main types of symbiotic, mycorrhizal fungi, either arbuscular mycorrhizae (AM) or ectomycorrhizae (EcM) (Liang et al., 2016; Phillips et al., 2013).

Trees connect their root systems to mycorrhizal fungi to better access soil nutrients like nitrogen and phosphorus, that are often recycled through organic matter deposits (i.e. dead plant matter). Mycorrhizae are especially adept at accessing these soil nutrients through their hyphal networks, and in return they receive photosynthate (sugars and molecules fixed from CO_2) from their tree partners. While AM fungi penetrate the root with their hyphae to form nutrient exchange structures within root cells, EcM fungi exchange nutrients externally by covering the root tip with a woven sheath of hyphae, often a different color or texture than the root and visible without magnification. In northern New England, the main AM-associated trees are in the *Acer*, *Fraxinus*, and *Prunus* genera, with most other genera (e.g. *Pinus, Tsuga, Fagus, Quercus, Betula*) associated with EcM. While climate projections predict a northward shift of AM-associated species (Jo et al., 2019), the northeast region of the U.S. may simultaneously experience declines in AM-associating trees due to maple sugar decline (Cleavitt et al., 2018; Horsley et al., 2002) and to the emerald ash borer (Morin et al., 2017), giving way to more EcM-dominated forests.

EcM and AM-associated forests differ in several functions, including patterns of soil carbon storage: EcM forests store more carbon in organic horizons and AM forests store more carbon in mineral soil, where much of the carbon is connected to mineral surfaces (such as clay particles) and is protected from further microbial decomposition (Cotrufo et al., 2019). AM forests may also store more carbon overall (Craig et al. 2019). EcM and AM forests also differ in nutrient availability. More inorganic nitrogen is available to plants in AM forests than in EcM forests, where most of the nitrogen is held in organic matter that requires mycorrhizal-driven enzymatic decomposition (Phillips et al., 2013; Sinsabaugh et al., 2005). Lastly, EcM and AM forests differ in their root pathogen loads (Liang et al., 2020). AM-associated trees have been found to harbor more root pathogens especially when AM-associated seedlings are closer to AM-associated adult trees (Liang et al., 2021). A change in mycorrhizal fungal species following disturbances, such as forest harvesting, may lead to increased fungal root pathogens that could affect forest regeneration and diversity (Jones et al., 2003; Parladé et al., 2019). With the potential for complex climate and pest-driven changes in New England forests, understanding how AM and EcM forests transition after disturbance will contribute to strategies for promoting forest resilience, productivity, and carbon storage.

We established a series of experiments to better understand the role of mycorrhizae in northern New England forests and how they might affect disturbance responses. One of the main areas of focus with this research is on forest regeneration, and our overall goal is to understand how forests associated with different mycorrhizal types affect seedling survival and productivity following timber harvesting.

Project Approach

Our research takes place in a rich northern hardwood forest owned and managed by Dartmouth College in Corinth, VT in partnership with the “Adaptive Silviculture for EAB” project led by Anthony D’Amato of the University of Vermont and Dartmouth College Woodlands forester Kevin Evans. We established 16 quarter-acre plots, where half of the plots are dominated ($\geq$80% basal area) by EcM-associated trees (primarily eastern hemlock; “EcM-legacy”) and half are dominated by AM-associated trees (primarily sugar maple; “AM-legacy”). Four quarter-acre plots per mycorrhizal type (for a total of eight plots; half of the total 16 plots) were harvested in the winter of 2020-21 (Fig. 1). Four plots per mycorrhizal type were left as undisturbed controls. To compare seedling survival and growth between dominant mycorrhizal associations, we measured 1) soil nutrient availability (inorganic nitrogen and phosphorus), moisture, and temperature before and after harvest;

and 2) survival and growth of both naturally regenerating and planted AM and EcM-associated seedlings in harvested plots. To investigate the effects of logging on natural and assisted forest regeneration, we are continuing to monitor seedling survival and growth of naturally occurring seedlings (DBH <1 cm) in permanent 1x1 m subplots and planted seedlings that include potential climate change-adapted species. In May 2021, we planted 20 of eight different species per plot: *Acer saccharum* (sugar maple), *Acer rubrum* (red maple), *Nyssa sylvatica* (black gum), and *Prunus serotina* (black cherry) for AM species and *Quercus rubra* (red oak), *Carya cordiformis* (bitternut hickory), *Tilia americana* (American basswood), and *Betula lenta* (black birch) for EcM species.

Seedling Survival and Growth

In the first season after harvesting, AM-associating seedlings had the highest survival in AM-legacy plots — 8% higher than AM seedlings in EcM-legacy plots. EcM-associated seedlings also had the highest survival in EcM-legacy plots — 4% higher than EcM seedlings in AM-legacy plots (Fig 2). These results indicate that initial seedling survival is promoted in soils that were previously inhabited by trees of the same mycorrhizal type. This effect, also called the "home-field advantage," could be due to indirect mycorrhizal effects on soil characteristics such as pH, or directly facilitated by mycorrhizae if they were able to colonize seedling roots over the first growing season (Smith et al., 2012). If the latter is true, we predict that mycorrhizae promoted a survival advantage by protecting tree roots from pathogens. To address this prediction, we are currently processing soil fungal community data via DNA sequencing of soils collected prior to (October 2020) and following (June and October 2021) harvesting. This fungal community data will allow us to investigate whether mycorrhizal fungi initially decreased following harvesting (Borgmann-Winter et al., 2022) and were able to recover over the course of the growing season. It should also be noted that there was high variability among seedling species with the same mycorrhizal association, indicating that some species benefited more than others from being planted in soils of their respective mycorrhizal type. Specifically, black birch (EcM-associated) and black gum (AM-associated) seedlings had the lowest survival (35% and 55% on average, respectively). The lower survival of these two species could be due to the fact that they are experiencing different temperature and precipitation regimes relative to their native, more southern ranges (Sullivan, 1993). It could also indicate a higher susceptibility to soil pathogens, especially for black gum which has been noted for its ability to grow in a wide range of climates and soil types (Abrams, 2007).

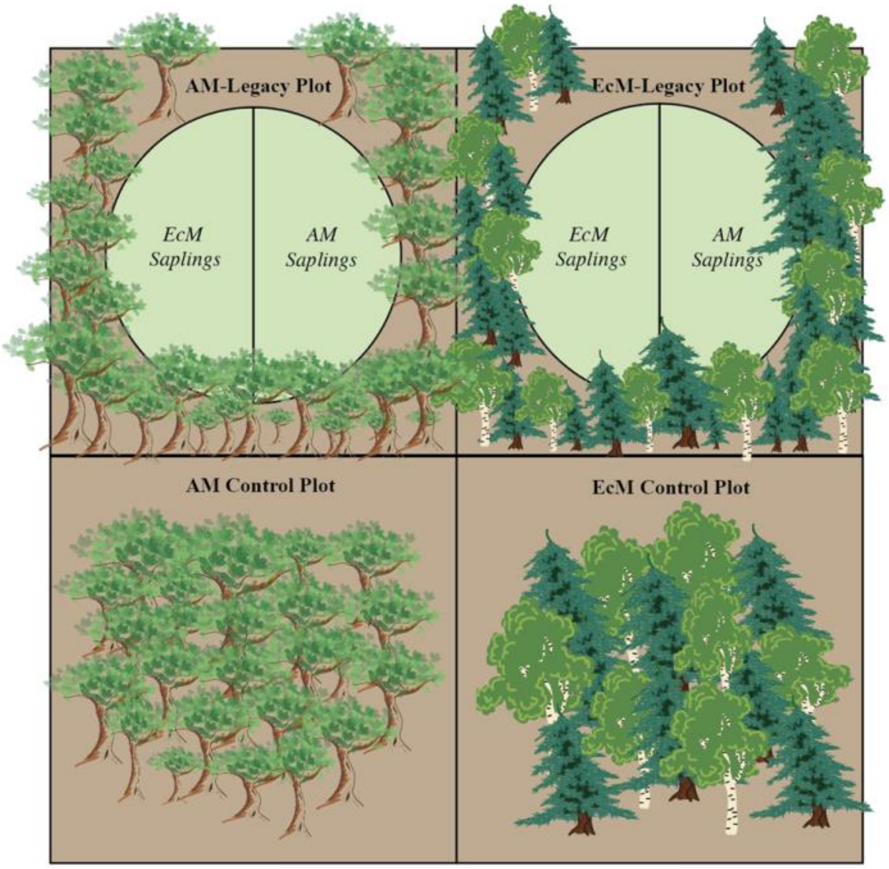

Figure 1. Eight quarter-acre gaps were established in the winter of 2021, four as arbuscular mycorrhizal (AM)-legacy plots and four as ectomycorrhizal (EcM)-legacy plots. These gaps were planted with AM and EcM-associated seedlings in May-June 2021, after which their survival and growth was routinely monitored. Eight quarter-acre control plots were also established: four in EcM-dominated and four in AM-dominated areas.

AM-associated seedlings had a 9% greater growth rate overall than EcM-associated seedlings during the first growing season. When AM seedlings were planted in AM-legacy plots they experienced a 6% increase in growth relative to AM seedlings planted in EcM-legacy plots. All seedlings, regardless of mycorrhizal association, had a positive growth response to increasing soil inorganic nitrogen and phosphorus availability, but only in previously AM-dominated soils (Fig. 3A, C). This difference in mycorrhizal plot effect suggests that established seedlings of both mycorrhizal types benefitted from either direct or indirect effects from AM forests. Given that soils beneath AM-associated forests generally have higher inorganic nitrogen content, AM-associated seedlings in AM-legacy plots were likely better able to capitalize on the increased soil nutrients through newly colonized mycorrhizae (DeForest & Snell, 2020). A lack of response by seedlings of both associations in EcM plots (Fig. 3B, D) suggests two potential mechanisms: 1) that AM-associated seedlings lacked access to mycorrhizal symbionts and 2) that EcM-associated seedlings may have been limited by their mycorrhizal symbionts at this stage in their development. Because EcM fungi have much more biomass than AM fungi, EcM-associated seedlings may have had to spend significantly more photosynthate carbon on newly established mycorrhizal symbionts (Frey, 2019). Seedling survival and nutrient data collected in August 2022 will provide further insight into how a full year of established mycorrhizal connections will interact with soil nutrient availability to affect seedling survival and growth.

Implications

In recent years, mycorrhizal fungi have come into the ecological and pop culture spotlight, though it is not yet clear how mycorrhizae are affected by forest management decisions in northeastern forests. We hope to add to the small, but growing body of resources on this subject matter by closing the knowledge gap on how legacy forests and associated mycorrhizae affect forest regeneration following harvesting. Our initial findings show that differences in seedling survival and growth differ between AM and EcM-legacy forests, even after just one season of growth. Both AM and EcM associated seedlings achieved higher survival when planted in soils formerly dominated by trees of their respective mycorrhizal type. However, high variability among survival of individual species indicates that some species may be more susceptible to differences in climate and potentially soil fungal pathogens. Seedling growth was likely affected differently by newly-established mycorrhizae. While AM-associated seedling growth appeared to benefit from soil nutrients when planted in AM-legacy plots, EcM seedlings experienced no effect. This difference indicates that EcM fungi may impose a greater carbon cost, outweighing the benefits to seedlings during the first growing season. We plan to monitor these seedlings and track soil nutrients and fungi over at least the next three growing seasons, allowing us to measure how re-establishing mycorrhizal communities and changing pathogenic communities will affect the trajectory of forest regeneration.

Within this experimental design, we plan to measure long-term soil carbon storage, given that other research findings show differences in soil carbon storage beneath trees associating with AM and EcM fungi. This information will allow us to predict how harvesting may contribute to soil carbon loss, and how forest soils recover over time. In parallel with this study, we are investigating how exclusion of AM fungal networks affects the survival, growth, and nutrient acquisition of AM-associated seedlings (sugar maple, black cherry, and black gum). This will serve as a more granular approach to the ecosystem-scale research described above and may shed light on the importance of maintaining seedling access to live adult AM-associated trees. We aim to make the results of all our experiments directly beneficial to the broader research mission for the Adaptive Silviculture for EAB project: to learn how we may best utilize adaptive silviculture to steward New England's forests into a more resilient future.

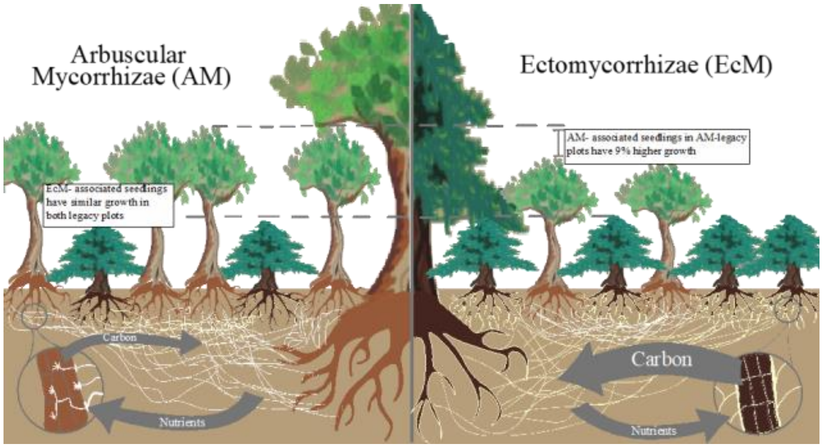

Figure 2. Conceptual representation of seedling survival and the "home-field advantage," where more AM-seedlings in AM-legacy plots represent an 8% increase in survival, and more EcM-seedlings in EcM-legacy plots represents a 4% increase in survival. Higher growth of AM seedlings in AM-legacy plots is represented by taller seedlings (9% higher growth).

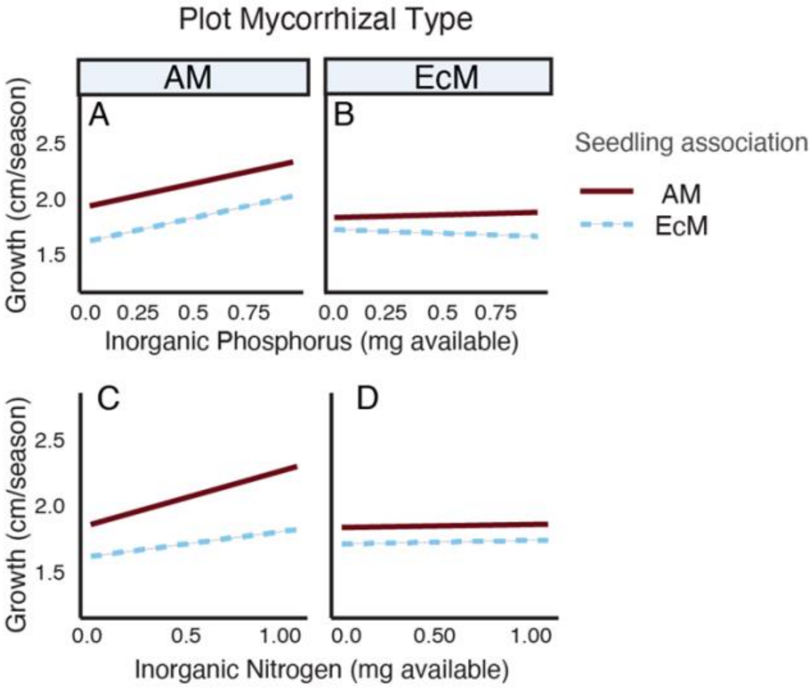

Figure 3. Panels show seedling growth measured as height accumulated over approx. three months in 2021 in response to inorganic phosphorus in (A) arbuscular (AM) plots and (B) ectomycorrhizal (EcM) plots, and inorganic nitrogen in (C) AM plots and (D) EcM plots.

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(*Article continues on page 18*)