Received: 23 April 2024 | Accepted: 24 June 2024

DOI: 10.1002/ecm.1634

ECOLOGICAL MONOGRAPHS
ECOLOGICAL SOCIETY OF AMERICA

ARTICLE

Mycorrhizal fungi as critical biotic filters for tree seedling establishment during species range expansions

Jordon C. Tourville[1,2] | **Thomas R. Horton**[1] | **Martin Dovciak**[1]

[1]Department of Environmental Biology, State University of New York College of Environmental Science and Forestry (SUNY ESF), Syracuse, New York, USA

[2]Research Department, Appalachian Mountain Club, Gorham, New Hampshire, USA

Correspondence
Jordon C. Tourville
Email: jtourville@outdoors.org

Funding information
National Science Foundation, Grant/Award Number: 1759724; College of Environmental Science and Forestry, State University of New York; New York Flora Association; Botanical Society of America; Edwin H. Ketchledge Scholarship Fund

Handling Editor: Steve Kembel

Abstract

Global warming has been shifting climatic envelopes of many tree species to higher latitudes and elevations across the globe; however, unsuitable soil biota may inhibit tree migrations into these areas of suitable climate. Specifically, the role of mycorrhizal fungi in facilitating tree seedling establishment beyond natural species range limits has not been fully explored within forest ecosystems. We used three experiments to isolate and quantify the effects of mycorrhizal colonization and common mycorrhizal networks (CMN) on tree seedling survival and growth across (within and beyond) the elevational ranges of two dominant tree species in northeastern North America, which were associated with either arbuscular mycorrhiza (AMF, *Acer saccharum*) or ectomycorrhiza (EMF, *Fagus grandifolia*). In order to quantify the influence of mycorrhiza on seedling establishment independent of soil chemistry and climate, we grew seedlings in soils from within and beyond our study species ranges in a greenhouse experiment (GE) as well as in the field using a soil translocation experiment (STE) and another field experiment manipulating seedling connections to potential CMNs (CMNE). Root length colonized, seedling survival and growth, foliar nutrients, and the presence of potential root pathogens were examined as metrics influencing plant performance across species' ranges. Mycorrhizal inoculum from within species ranges, but not from outside, increased seedling survival and growth in a greenhouse setting; however, only seedling survival, and not growth, was significantly improved in field studies. Sustained potential connectivity to AMF networks increased seedling survival across the entire elevational range of *A. saccharum*. Although seedlings disconnected from a potential CMN did not suffer decreased foliar nutrient levels compared with connected seedlings, disconnected AM seedlings, but not EM seedlings, had significantly higher aluminum concentrations and more potential pathogens present. Our results indicate that mycorrhizal fungi may facilitate tree seedling establishment beyond species range boundaries in this forested ecosystem and that the magnitude of this effect is modulated by the dominant mycorrhizal type present (i.e., AM vs. EM). Thus, despite changing climate conditions beyond species ranges, a lack of suitable mutualists can still limit successful seedling establishment and stall adaptive climate-induced shifts in tree species distributions.

KEYWORDS
arbuscular mycorrhiza, climate change, ectomycorrhiza, establishment, forests, tree distributions, tree seedlings

INTRODUCTION

Many temperate tree species are expected to migrate to higher latitudes and elevations as a result of climate warming (Boisvert-Marsh et al., 2014; Harsch et al., 2009; Lenoir & Svenning, 2015). However, recent studies have found lags or delays in tree range expansion compared with the velocity of climatic change (Alexander et al., 2018; Liang et al., 2018; Sittaro et al., 2017; Zhu et al., 2012), at least in part because soil properties can limit tree species migrations (Ni & Vellend, 2023). Belowground biotic interactions in particular have the potential to create these observed migration lags, but they tend to be less well understood and generally underrepresented in tree species distribution models (Van Der Putten et al., 2010; Wisz et al., 2013), and especially so for mutualistic interactions with mycorrhizal fungi (Nuñez et al., 2009; Tedersoo et al., 2020). The study of the effects of belowground interactions such as mycorrhizal symbioses on tree migrations is complicated by the difficulty of separating the role of these interactions from the effects of climatic and edaphic factors that co-vary with soil biota at regional to global scales (e.g., Geml, 2017; Steidinger et al., 2019), which also influence aboveground plant community characteristics and tree seedling establishment (Tourville et al., 2022, 2023; Van Der Heijden & Horton, 2009; Zarfos et al., 2019).

The role of mycorrhizal inoculation in improving success of tree planting has long been known and used in forestry (Brundrett et al., 1996; Peterson et al., 1984), but the influence of mycorrhizal fungi on natural tree range expansions in forested ecosystems, particularly in the context of climate change, has received relatively little attention (Ibáñez & McCarthy-Neumann, 2015; Lankau et al., 2015; Nuñez et al., 2009). Mycorrhizal symbioses, including fungi representing two dominant mycorrhizal types, arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EMF), are present in ~94% of all plant taxa and are generally mutualistic, favoring plant growth and survival via increased nutrient and water uptake, heavy metal resistance, and pathogen defense (Harley & Smith, 1983; Johnson et al., 1997; Simard et al., 1997; Smith & Read, 2010; Tedersoo et al., 2020; Van Der Heijden & Horton, 2009). Consequently, mycorrhizal fungi can facilitate plant persistence in harsh environments; for example, mycorrhizal associations of North American trees improve their drought tolerance and allow them to occupy drier and otherwise unsuitable habitats (Lankau et al., 2015).

In addition to the benefits of local mycorrhizal colonization for host plants, common mycorrhizal (or mycelial) networks (CMN) form linkages between mycorrhizal fungi and at least two different plants (Selosse et al., 2006). The connection of a seedling growing beyond its species' range margin to such a network could potentially provide the necessary materials for the seedling to successfully establish, survive, and recruit into a larger size class (Beiler et al., 2010; Selosse et al., 2006; Simard et al., 2012). Field and greenhouse studies have posited that CMNs can potentially improve seedling growth and survival via increased nutrient acquisition rates in abiotically stressful environments (Nara, 2006a, 2006b; Pickles et al., 2017; Simard et al., 1997, 2012). However, there is an outstanding debate on the current evidence for the effects of CMNs on seedlings, questioning (1) whether ecologically meaningful resource transfer occurs between CMN-associated plants, (2) whether fungal hyphae are the primary conduit of belowground resource transfers, and (3) whether CMNs can help facilitate tree range expansions (see Henriksson et al., 2023; Karst et al., 2023).

Plant species population spread can depend heavily on mycorrhiza; EMF were found to facilitate non-native pine invasions (Hayward et al., 2015; Nuñez et al., 2009) and AMF have been implicated in the invasions of other non-native plants (Aslani et al., 2019; Urcelay et al., 2019). Mycorrhizal associations have also been suggested as an important factor in facilitating tree range expansions in North America following glacial retreat (Pither et al., 2018). Thus, given their broad distribution (e.g., Steidinger et al., 2019) and positive influence on plant growth and survival (e.g., Hayward et al., 2015; Van Der Heijden & Horton, 2009), mycorrhizal fungi may aid the establishment of trees beyond their range boundaries and thus facilitate climate-induced migrations of tree species.

Mycorrhizal fungi may be of special importance to low-elevation plant species migrating, in response to warming, to high-elevation forests, which are often characterized by poor soils, low nutrient availability, common root pathogens, and low pH with relatively high concentrations of plant-toxic metals (e.g., aluminum, iron; Brown & Vellend, 2014; Collin et al., 2017). While there is a long-held assumption that fungi are not dispersal-limited (De Wit & Bouvier, 2006), there is a growing understanding that dispersal limitation is common for mycorrhizal fungi (Chaudhary et al., 2020; Cortese et al., 2023; Galante et al., 2011; Peay & Bruns, 2014). While AMF are known to colonize many herbaceous understory plant species and tree seedlings and adults in temperate deciduous forests at low elevations, the number of AM plant species at high elevations and latitudes, which are dominated by functionally divergent EM coniferous boreal species, is comparatively small (cf. Evans & Brown, 2017; Tourville et al., 2023). Thus, even if AMF inoculum is present in these high-elevation forests, it may not be in sufficient quantity, or spatially homogenous enough to enable the long-term establishment of dispersing seeds of AM trees from lower elevation temperate deciduous forests.

Decreased performance (growth and survival) and AMF root colonization of tree seedlings have been observed when grown in soils from high-elevation conifer forests compared with low-elevation forest soils in a greenhouse setting (Carteron et al., 2020). Even if and when AMF inoculum is present in high-elevation soils, the AMF community may be less diverse or comprised of fungal species relatively less beneficial for range-expanding plant species (see De Bellis et al., 2022). On the other hand, EM trees expanding their ranges from lower elevations would likely encounter abundant EM inoculum in high-elevation conifer forests, suggesting that root colonization would not be meaningfully impacted, assuming the fungal species present are host generalists. Thus, while it seems likely that mycorrhizal fungi aid in seedling establishment beyond their species range, does it follow that the reverse is true: that the absence of compatible fungi, as may be the case of AMF at high elevations, inhibits successful establishment of AM trees? Could this ultimately influence plant community composition at high elevations?

To explore the link between mycorrhizal fungi and seedling establishment beyond plant species range limits, we used an integrated experimental approach to account for climate and soil abiotic properties changing along elevational gradients and across the temperate-boreal transition zone in well-preserved montane forests in the Northeastern United States. Specifically, we evaluated the degree to which an AM and an EM tree species occurring at low elevations could successfully establish upslope beyond their range limits through interactions with mycorrhizal partners. Deciduous trees of the northern hardwood forest are often expected to migrate upslope into high elevations currently occupied by conifer forests as the regional climate warms (Beckage et al., 2008; Lenoir & Svenning, 2015; Pucko et al., 2011), but other studies indicate the lack of such upslope movement of temperate deciduous tree species at least for now (e.g., Wason & Dovciak, 2017, Tourville et al., 2022). Given these inconsistencies, we address four related questions (cf. study questions, Table 1): (1) Can an AM tree seedling recruit under a warmer climate within an EMF-dominated forest at higher elevations beyond its current range limit? (2) Does an EM seedling perform better (relatively greater survival and growth) than an AM seedling in an EM-dominated forest beyond its range edge? (3) Does the potential connection to a CMN improve seedling establishment beyond range boundaries? (4) Is mycorrhizal benefit primarily due to nutrient supply to plants, or due to other mycorrhizal functions, such as pathogen defense or heavy metal protection?

MATERIALS AND METHODS

Study area

This study was conducted in montane forests of the Northeastern United States. Mountains in the region are characterized by strong zonation in forest communities typical of the broader area (cf. Beeles et al., 2022; Tourville et al., 2022; Wason & Dovciak, 2017), with northern hardwood forests at lower elevations heavily dominated by an AM tree species (sugar maple, *Acer saccharum*), and two EM species (American beech, *Fagus grandifolia*; and yellow birch, *Betula alleghaniensis*). While there are older reports of *Fagus* forming AM associations, and thus bringing into question the mycorrhizal status of

TABLE 1 Explanation of the study questions addressed.

Question	Experiment used	Description
1	1, 2	Determine whether tree seedling survival, growth, and mycorrhizal colonization is lower beyond a tree species elevational range than within the species range
2	1, 2	Assess whether an AM and EM tree species differ in survival and growth across elevational range boundaries
3	3	Examine the role of potential connectivity to a CMN on tree seedling survival and growth across range boundaries
4	1, 2, 3	Explore how mycorrhizal fungi benefit seedling survival and growth across range boundaries (e.g., nutrient exchange, heavy metal tolerance, pathogen defense)

Abbreviations: AM, arbuscular mycorrhiza; CMN, common mycorrhizal networks; EM, ectomycorrhiza.

this species (Bainard et al., 2011), Brundrett and Tedersoo (2020) suggest that these were more likely a result of endophytic activity (no AM structures were observed on any beech seedling roots during the course of this study). High-elevation spruce-fir forests are dominated by EM tree species (balsam fir, *Abies balsamea*; and red spruce, *Picea rubens*) and separated by a sharp ecotone (~800 m above sea level (asl)) from northern hardwoods below. Herbaceous understory plant species found in both major forest communities are commonly colonized by AMF (Carteron et al., 2020; De Bellis et al., 2022). The region is within the Adirondack-New England highlands, which is characterized by a highly variable terrain (generally ranging from 150 to 1220 m asl), rocky spodosol soils, and continental forest climate with warm summers and cold and snowy winters (mean annual temperatures between 3 and 11°C; mean length of the frost-free period ~100 days; mean annual snowfall >2550 mm) and evenly distributed precipitation (total annual mean precipitation of 890 mm; Janowiak et al., 2018). Additional information about the vegetation zonation and broader study region can be found in Beeles et al. (2022), Tourville et al. (2022), and Wason and Dovciak (2017).

Study design

In order to disentangle the effects of soil chemical properties and climate from the effects of soil biota on tree seedling germination, survival, and growth from both within and beyond the natural elevational range of two dominant tree species in northern hardwood forests of the Northeastern United States (sugar maple, American beech), we used three complementary experiments (see Table 2). All three experiments were conducted on, or used soils from, 12 field sites. These were established at every 200 m in elevation (600 m = northern hardwoods site, 800 m = ecotone site, 1000 m = spruce-fir site) along an elevational gradient (600–1000 m asl) on four mountains in the Northeastern United States: Mount Abraham, Mount Mansfield, Jay Peak, and Killington Peak (Figure 1, Appendix S1: Table S1). We address our four main study questions using a greenhouse experiment (Experiment 1, GE), a field soil translocation experiment (Experiment 2, STE), and a field CMN connectivity experiment (Experiment 3, CMNE, see below). Briefly, our GE separated abiotic and biotic soil components and their effects on seedling performance while controlling for climate. Our STE addressed the relative importance of both soils and climate on seedling performance in field settings, while our CMNE examined the potential role of CMNs on seedling performance (also in a field setting). All seeds that were used is these studies were purchased from Sheffield Seed Company (Locke, NY), surface-sterilized with 2% H_2O_2 for 2 h, and cold-stratified (at 4°C) for 3 months to break dormancy. The details of each experiment, how they complement one another, which questions they address, and variables measured can be found below and in Figure 2 and Tables 1–3 (also see Appendix S1: Figures S1–S4). A priori expectations for each study, which would demonstrate the importance of mycorrhiza (and soil biota) on seedling performance independent of climate and soils, are highlighted in Table 4.

Experiment 1: Greenhouse (GE) study design

We first conducted a GE to disentangle the effects of soil chemical properties from the effects of soil biota on tree seedling germination, survival, and growth across the natural elevational range of our study species (addressing Questions 1 and 2). We examined the responses (survival and early growth) of sugar maple and American beech to four different treatments of soils of three different origins. The experiment was carried out as a completely randomized 3 × 4 factorial for each species, with a replication of 30 independent seedlings per each of four soil treatments and three treatment levels ($n = 360$ per species). The four soil treatments included (1) homogenized live field soil

TABLE 2 Description of the experiments used to address each study question.

Experiment	Description	Purpose
1	Greenhouse (GE)	Can assess the relative importance of soil biotic and abiotic properties on seedling performance while controlling for variation in climate
2	Soil translocation (STE)	Can assess the relative importance of edaphic vs. climate factors on seedling performance
3	CMN connectivity (CMNE)	Can assess the role of potential CMN connectivity on seedling performance across species range boundaries

Abbreviations: CMN, common mycorrhizal networks; CMNE, common mycorrhizal network experiment.

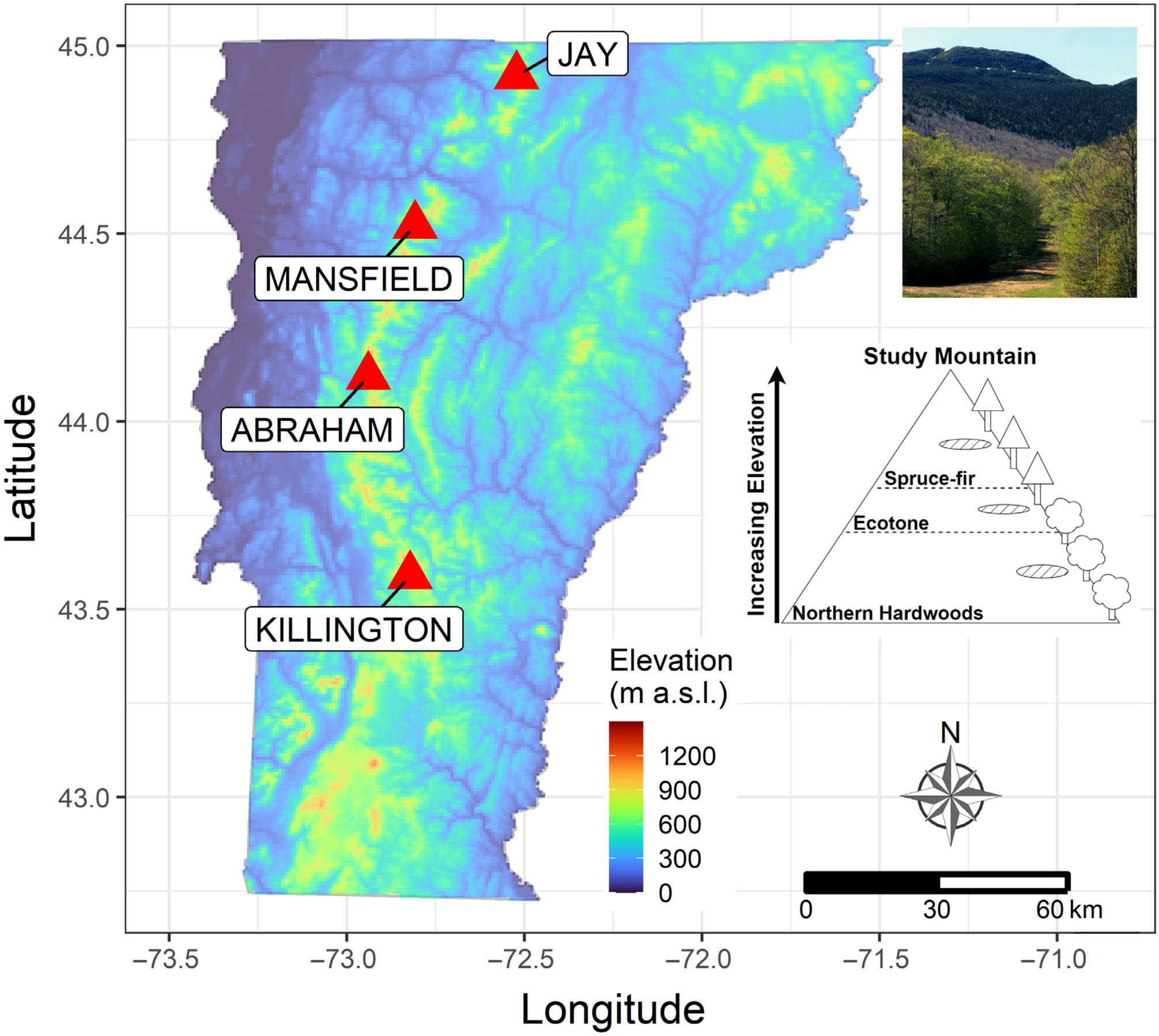

FIGURE 1 Map displaying the location of the four study mountains within the Green Mountains of Vermont. Lighter areas indicate areas of high elevation (>600 m). The lower inset depicts a simplified representation of how forest communities change across the elevation gradients, with hatched ovals indicating study site locations. The upper inset shows changing forest communities along an elevation gradient on Mt. Mansfield. Mountains include Mt. Abraham (Universal Transverse Mercator (UTM) 665126E 4887319 N Zone 18), Mt. Mansfield (UTM 673624E 4934600 N Zone 18), Jay Peak (UTM 695270E 4977503 N Zone 18), and Killington Peak (UTM 675924E 4830280 N Zone 18). Photo credit: Jordon Tourville.

(i.e., original soil with soil biota present; hereafter referred to as "Field"), (2) sterilized autoclaved field soil (i.e., original soil with soil biota absent; hereafter "Sterilized Field"), (3) potting mix inoculated with fresh field soils (i.e., standardized substrate with field soil biota added; hereafter "Inoculated"), and (4) an autoclaved potting mix control (standardized substrate without field inoculum, hereafter "Potting Mix"). The Sterilized Field treatment was accomplished by sterilizing field soil samples using two liquid autoclave cycles at 121°C for 20 min each with a day between each cycle. The Inoculated treatment was assembled from equal proportions of sterilized coconut coir and perlite (1/3), sand (1/3), and field inoculum mix (soils directly from the field, 1/3). The three levels of each treatment represented different soil origins based on elevation (and associated elevational vegetation bands): (1) low elevation (northern hardwood forest), (2) mid-elevation (ecotone with mixed forest composition), and (3) high elevation (spruce-fir forest). All greenhouse work was carried out at the SUNY-ESF greenhouse facilities.

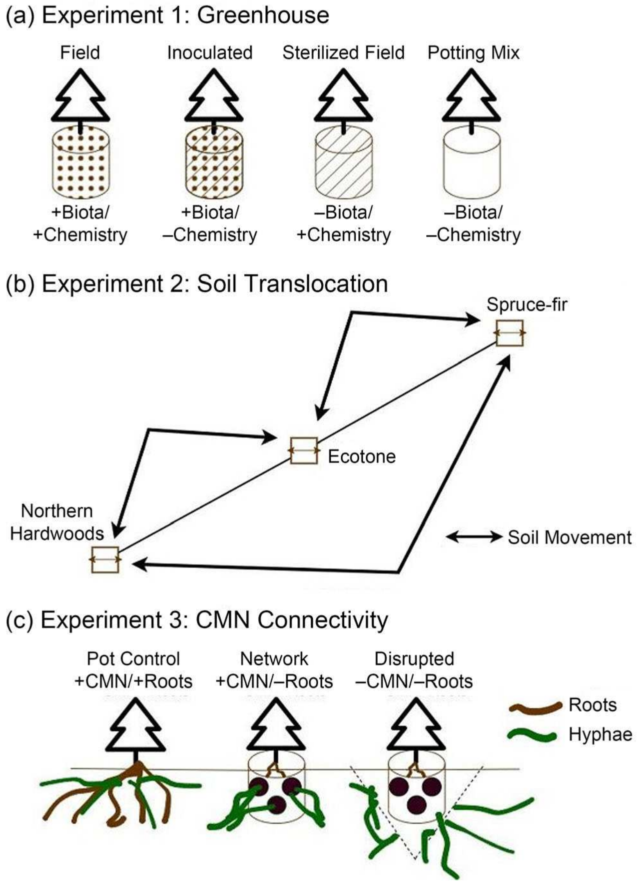

FIGURE 2 Conceptual diagram (including treatment descriptions and treatment levels) of the study design with the three experiments examining sugar maple (arbuscular mycorrhiza) and American beech (ectomycorrhiza) performance: (a) the greenhouse experiment includes four treatments (soil manipulation) with three levels (sites) and 30 replicates (seedlings) per treatment/level combination; (b) the soil translocation experiment includes four treatments (soil origin) within six blocks at each of three sites; and (c) the common mycorrhizal network experiment includes three treatments (common mycorrhizal networks [CMN] manipulations) within eight blocks at each of three sites.

Field soil samples were collected from Mt. Mansfield, Vermont, in fall 2019. Ten soil pits (4 L each) were dug to a depth of 30 cm at each of three sites and the soils were composited (O, E, B soil horizons) before transport to SUNY ESF, 1 week before experimental greenhouse planting. All experimental tree seedlings were grown from seeds sown at the beginning of the experiment. One seed was sown in individual 2 × 2 cm cells (level) within trays (treatment), with 30 seeds sown for each treatment and level combination. Individual emerged seedlings from each level and treatment were moved to larger (1/2 L) plastic containers (1 seedling per container). Seedlings were allowed to grow within each treatment for 5 months (approximately equal to the full length of the growing season at mid- to low elevations). Seedlings were watered twice a week, and greenhouse temperature was maintained at 20°C. The locations of the pots in the greenhouse were periodically switched at random to remove potential location effects on seedling performance. Seedling survival and height were recorded weekly. At the end of the experiment, all surviving seedlings were harvested for final aboveground biomass measurements. Seedling roots were separated from all aboveground material, washed, and stored in 70% ethanol. Aboveground biomass was dried at 60°C for 24 h and weighed for a final biomass measurement.

From stored seedling roots, mycorrhizal colonization was estimated using staining and light microscopy methods as follows. Root samples were cleared using a 10% KOH solution via a 30-min liquid autoclave cycle (at 121°C), stained with chlorazol black E in another 30-min liquid autoclave cycle, and stored in a 50% glycerin solution (Brundrett et al., 1996). The gridline intercept method was used to estimate AMF colonization for sugar maple, as described in McGonigle et al. (1990) and Brundrett et al. (1996). A simplified gridline intercept procedure was used to estimate EMF colonization (number of mycorrhizal root tips vs. non-mycorrhizal root tips) for unstained fungi on American beech roots (Brundrett et al., 1996). The same mycorrhizal colonization estimation methods were used for all subsequent harvested root samples in both greenhouse and field experiments (see below).

Experiment 2: Soil translocation (STE) study design

To assess the influence of soil origin on seedling survival and growth in the field while controlling for any climate effects, soil cores were extracted and translocated (STE) either (1) within the same site, (2 and 3) to one of the other two sites (elevations) on each study mountain, or (4) unmoved, resulting in four transplant treatments per site in three blocks (soils transplanted from the other two elevations, a within-site soil transplant treatment, and an unmoved treatment) at our previously established field sites (see *Study design*; addressing Questions 1 and 2). Six replicate soil cores (15 cm diameter × 30 cm depth) were extracted from each site (15 m apart) on each of the four study mountains. In order to control for climate (i.e., temperature) changing along the elevation gradient, cores were translocated (placed in the ground) to different sites with an emphasis on preserving the vertical structure of the cores. Soils were placed in the field inside 3-L plastic planters (Home Depot, Atlanta, GA, USA) with manually cut holes (accounting for a total of 50% planter surface area) to allow water transport with the inside and bottom

TABLE 3 Categorization and description of measured variables used in each study to compare seedling performance between experimental treatments.

Experiment	Category	Variable	Description	Units
(1) Greenhouse (GE)	Survival	Survival	Proportion seedlings surviving 5 months	%
	Growth	Height	Final seedling height	cm
	Growth	Total dry biomass	Final seedling dry total biomass	g
	Mycorrhiza	Colonization	AMF or EMF root length colonized	%
(2) Soil translocation (STE)	Survival	Germination	Proportion sown seeds germinating	%
	Survival	Survival	Proportion seedlings surviving 2 years	%
	Growth	RGHR	Seedling relative height growth rate	cm/day^{-1}
	Growth	Root-to-shoot	Ratio of belowground to aboveground dry biomass	NA
	Growth	Total dry biomass	Final seedling dry total biomass	g
	Mycorrhiza	Colonization	AMF or EMF root length colonized	%
(3) CMN (CMNE)	Survival	Survival	Proportion seedlings surviving 2 years	%
	Growth	RHGR	Seedling relative height growth rate	cm/day^{-1}
	Growth	Root-to-shoot	Ratio of belowground to aboveground dry biomass	NA
	Growth	Total dry biomass	Final seedling dry total biomass	g
	Growth	Diameter change	Change in seedling basal diameter over 2 years	mm/year^{-1}
	Growth	Leaf number	Final number of seedling leaves	NA
	Growth	Foliar nutrients	Conc. foliar nutrients (N, P, K, Ca, Mg, Al)	% dry weight
	Mycorrhiza	Colonization	AMF or EMF root length colonized	%

Abbreviations: AMF, arbuscular mycorrhiza fungi; CMN, common mycorrhizal networks; CMNE, common mycorrhizal network experiment; EMF, ectomycorrhiza fungi; RHGR, relative height growth rate.

of each planter lined with a 25-micron fine nylon mesh to prevent root infiltration and slow hyphal colonization from outside of the planter (Booth & Hoeksema, 2010).

Twenty seeds of either sugar maple or American beech were sown in each planter in October 2019, and we tracked their survival and growth in each treatment for two growing seasons. This seed density was selected to maximize the probability that at least one seed would successfully germinate, given the low germination rates observed for these species in the field, based on personal observations and Brown and Vellend (2014). The top of each planter was covered with a 0.5-cm plastic mesh (Home Depot, Atlanta, GA, USA) to protect against seed predation during the winter months (the mesh was removed after snowmelt the following year to allow seedling growth). Seedling height measurements and survival counts were taken twice a month starting in May 2020. Dead seedlings were removed at each survey and their roots were visually examined for the presence of any external fungi or damping off symptoms that might indirectly indicate a root pathogen; this was also done for live seedlings at the end of the experiment (see Brown & Vellend, 2014). The presence of browsing or foliar herbivory was also recorded during each site visit. Seedling height measurements were conducted in summer 2020, and again in summer 2021 for all surviving seedlings. Final seedling harvests were done in September 2021 following two full growing seasons. Roots were separated from the aboveground portions of each seedling and washed in the field. A 0.2-g sample of roots (weighed in the field using a digital scale [Anself, Atlanta, GA, USA]) was separated and placed in 70% ethanol for mycorrhizal colonization measurements. In order to collect all root material, all soil from experimental pots was washed through a 1-mm fine sieve. Seedling growth was assessed by measuring dry weight biomass (drying and measurements taken 1 week after collection), measuring biomass partitioned between aboveground and belowground components (root-to-shoot ratios), and quantifying mycorrhizal colonization, at the end of the experiment.

Experiment 3: CMN connectivity (CMNE) study design

To evaluate the influence of possible connection to a CMN on seedling growth and survival within and beyond

TABLE 4 Specific a priori findings (expectations) for each experiment, which would demonstrate the importance of mycorrhiza (biotic) in seedling performance independent of climate and soils (abiotic).

Experiment	Expectations
Greenhouse (GE)	(1) Higher mycorrhizal colonization of seedlings grown in soils within species ranges vs. beyond (2) Higher survival and growth (height and biomass) of seedlings grown in soils inoculated by within-range biota vs. beyond-range biota (3) Higher survival and growth (height and biomass) of seedlings grown in soils with biota present vs. in soils with biota absent
Soil translocation (STE)	(1) Higher mycorrhizal colonization of seedlings grown in soils within species ranges vs. beyond regardless of planting site (elevation) (2) Higher survival and growth of seedlings grown in soils within species ranges vs. beyond regardless of planting site (elevation)
CMN connectivity (CMNE)	(1) Higher survival and growth of seedlings grown in soils with a continuous connection to a potential CMN vs. disconnected regardless of planting site (elevation)

Abbreviations: CMN, common mycorrhizal networks; CMNE, common mycorrhizal network experiment.

our species elevational ranges, a separate field experiment was conducted (CMNE, addressing Question 3). At each of three sites (same as described in *Study design*), three distinct treatments were applied in order to test how seedling performance (survival and several metrics of seedling size; see below) was altered by the potential connection to a local CMN. Our treatments included: (1) potted seedlings with the opportunity to connect to a broader CMN (hereafter referred to as “network”), (2) potted seedlings with the connection to any CMN repeatedly broken (hereafter referred to as “disrupted”), and (3) a full control treatment of seedlings planted outside of pots in the field (hereafter referred to as “pot control”). The network treatment involved planting seedlings in 3-L plastic planters using the soil present at the spot it was planted. Holes were cut into the side of the pots (for a total of 50% open surface area) and a 44-micron nylon mesh was affixed to these holes, as well as to the drainage holes at the bottom of the pots, to allow passage of mycorrhizal hyphae but not plant roots (Booth, 2004). The disrupted treatment was designed in the same way except pots were placed into another pot of the same size and turned every 2 weeks in order to break the hyphal connection to the outside. The experimental pot control treatment involved planting seedlings directly in the ground without the pot (first disturbed by digging a hole and replacing the soil within) in order to assess the pot effect on the experiment, as well as interactions with plant roots.

This series of three treatments was replicated in four blocks at each site (same as above; hardwoods, ecotone, and spruce-fir) on each of four study mountains (for a total of 432 seedlings per species). In each block, three seedlings of both sugar maple and American beech were installed in each treatment (an individual 3-L pot) for a total of 9 seedlings. All seedlings were purchased as 2-year-old bareroot stock from Cold Stream Farm Nursery (Free Soil, MI). As we wanted to minimize introducing non-native mycorrhizal fungi via bareroot stock, we examined the roots of 4 randomly selected bareroot seedlings for each study species for the presence of EMF root tips or AMF colonization. Low colonization of either EMF or AMF (<5%) suggested that fungal introductions were likely limited.

Pots with planted seedlings were installed in spring 2020, and seedlings were surveyed twice a month throughout the growing season to determine survival, height, root collar diameter, number of leaves, and the presence of any foliar herbivory or browsing on each seedling. All dead seedlings were collected and assessed for the presence of any potential root pathogens. A final biomass measurement (partitioned between aboveground and belowground) was taken at the end of the experiment (fall 2021), and root samples were taken for quantification of mycorrhizal colonization (as above). Leaves from each surviving seedling were pooled by treatment and site following drying and biomass measurements, finely ground using a Wiley mill, and shipped to the Penn State Agricultural Analytical Services Laboratory for tissue analyses. Total nitrogen, phosphorus, potassium, calcium, and trace elements were extracted via acid digestion and ICP analysis (Huang & Schulte, 1985). All measured plant performance metrics are summarized in Table 3 for all experiments.

Statistical analyses

We used generalized linear models (GLMs) to analyze the response of sugar maple and American beech seedling survival, growth, and mycorrhizal root length colonization to soil origin and soil manipulation treatment in our GE (addressing Questions 1 and 2). Survival and mycorrhizal colonization assumed a binomial distribution of residuals. Growth, as measured by total seedling height and final dry biomass, assumed a normal distribution of residuals.

Generalized linear mixed effects models (GLMMs) were used to analyze the response of seedling germination (binomial), survival (binomial), growth (normal), and mycorrhizal colonization (binomial) to planting site (elevation) and soil source treatment in our STE, averaged for individuals within the same block and treatment, and using study mountain as a random effect (addressing Questions 1 and 2). Growth was measured as final total dry biomass, relative height growth rate (RHGR), and root-to-shoot ratio. Mean RHGR across the entire study period was used to avoid issues of temporal autocorrelation and was calculated as follows:

$$\mathrm{RHGR} = \left(\frac{\ln H_2 - \ln H_1}{t_2 - t_1} \right), \qquad (1)$$

where H_2 and H_1 are the two height measurements and t_2 and t_1 are the two different times where height measurements were taken (measurements taken twice a month).

GLMMs were also used to analyze the response of seedling growth and mycorrhizal colonization (binomial) to planting site and CMN manipulation treatment in our CMNE, averaging individuals within the same treatment and block, and using mountain as a random effect (addressing Question 3). Growth was measured as final total dry biomass (normal), root-to-shoot ratio (normal), RHGR (normal), basal diameter change (normal), and final number of leaves (Poisson). Survival curves and results of survival analyses performed via Kaplan–Meier estimators (see Appendix S1: Figure S5) were plotted from raw survival data averaged across study mountains for each species (Goel et al., 2010). We used linear mixed models (LMMs) to evaluate differences in foliar nutrient concentrations (N, P, K, Ca, Mg, Al) for CMNE sugar maple and American beech seedlings, both across planting sites and CMN treatments (Question 4). We also constructed GLMMs to analyze the response of observed seedling foliar herbivory, browse, and potential root pathogen presence (all assuming a binomial distribution of residuals) to planting site and experimental treatment for both Experiments 2 and 3, using study mountain as a random effect (addressing Question 4). Raw data were averaged across all seedlings within each block and treatment. Model assumptions and standard diagnostics were checked for each GLMM (see Appendix S1: Tables S2–S13 for all model results). Analysis of variance and post hoc Tukey's HSD tests were used to evaluate treatment and site differences and their interactions for each experiment.

The soil chemical gradient on all study mountains was previously characterized (see Tourville, 2022; Tourville et al., 2023). Briefly, one-way analysis of variance and post hoc Tukey's HSD tests were used to evaluate differences in soil chemical variables across the study elevation gradients used in our three experiments (pH, soil organic matter, cation exchange capacity, and concentrations of Al, P, K, Ca, and Mg; see Appendix S1: Table S14). All of the above analyses were conducted in R using the following packages: dplyr, reshape2, ggplot2, lme4, survival, and multcomp (R Core Team, 2022).

RESULTS

Experiment 1: GE

Addressing Questions 1 and 2 (1: assessing seedling performance across range boundaries; and 2: assessing differential performance of an AM vs. EM seedling species, see Table 4 for a priori expectations, Appendix S1: Tables S2–S4 for model results), we found that AMF root length colonization of sugar maple averaged across all treatments was much lower in soils of spruce-fir origin (~10%, <0.001) than in hardwood (~21%, $p < 0.001$) or ecotone soils (~16%, $p = 0.009$), while average EMF colonization of American beech did not differ between different soil origins ($p > 0.05$, Figure 3a,b). Additionally, mycorrhizal root colonization did not differ between inoculated soils and live field soils regardless of soil origin (elevation) for both AMF-associated sugar maple and EMF-associated American beech, suggesting successful inoculation of our Inoculated treatments (soil source × treatment: $p > 0.05$, Figure 3a,b). Root colonization was not significantly different from zero for sterilized field and potting mix soils, suggesting that our sterilization treatments were successful.

Survival of sugar maple seedlings grown in within-range soils of northern hardwood (~33%) and ecotone origins (~35%) was greater than that of those grown in beyond-range spruce-fir (~22%) soils averaged across all treatments ($p = 0.01$, Figure 3c). American beech seedling survival in hardwood soils (~28%) was greater than that in ecotone (~22%) and spruce-fir (~20%) soils but not significantly so averaged across all treatments (soil source × treatment: $p = 0.24$, Figure 3d). While biomass and plant height averaged across all experimental treatments did not differ between soils of different

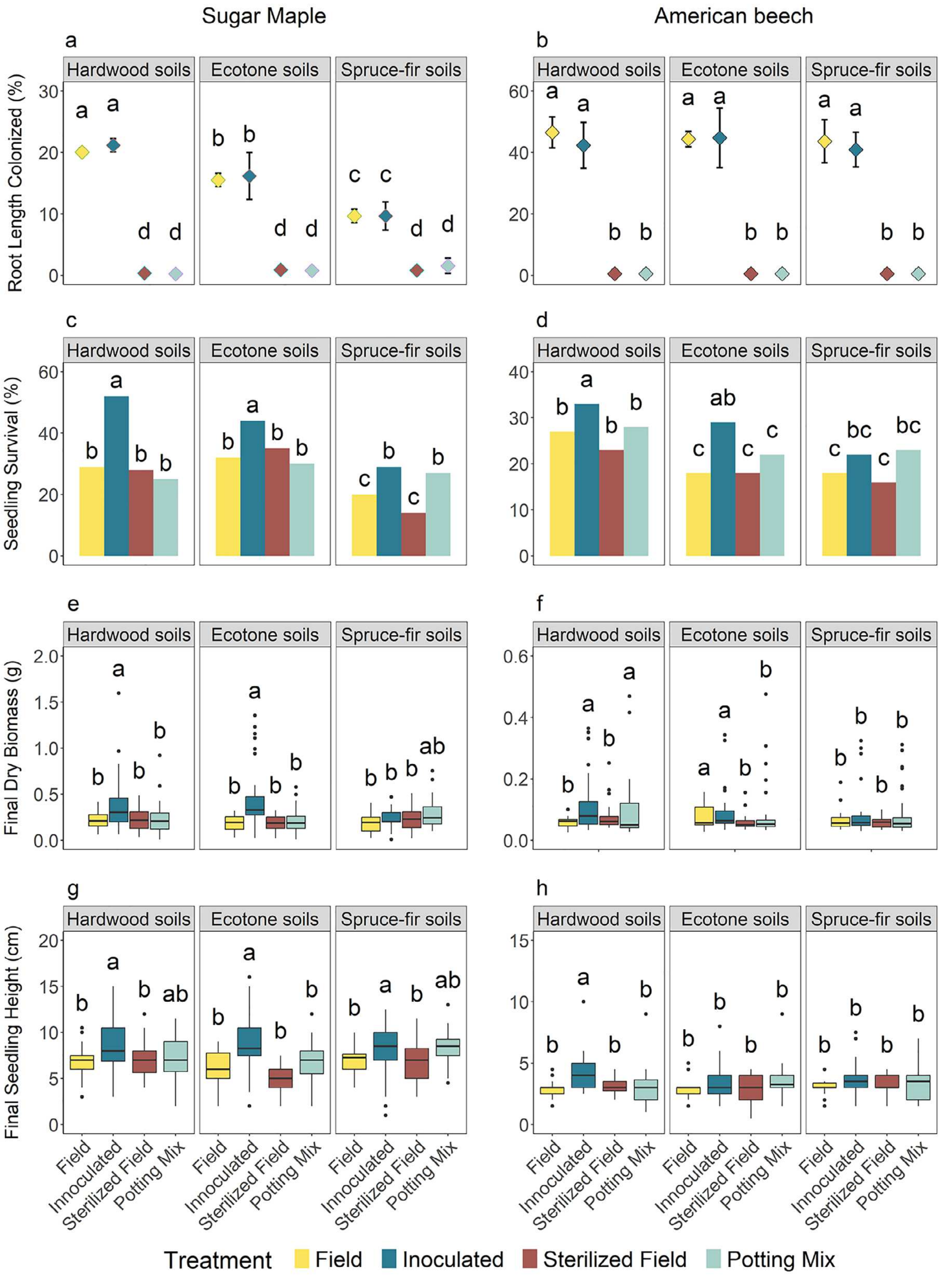

FIGURE 3 (a, b) Differences in mean (±SE) mycorrhizal colonization (root length colonized %) between the greenhouse study (GE) treatments for seedlings of arbuscular mycorrhiza sugar maple and ectomycorrhiza American beech. Treatments are soil manipulations and include Field (unmanipulated soil from field sites), Inoculated (field biota added to sterilized potting mix), Sterilized Field (autoclaved field soils), and Potting Mix (sterilized potting mix without live biota). Each treatment has three levels (three sites/elevations of soil origin: northern hardwoods, ecotone, and spruce-fir). (c, d) Bar plots of proportion of seedlings surviving between treatments and boxplots of final dry biomass (e, f) and final height (g, h) for sugar maple and American beech seedlings. Letters indicate significant differences between groups (α = 0.05). Boxes include mean values and are bounded by 25% and 75% quantiles.

origins for both sugar maple and beech (comparing between AM and EM tree, Question 2; Figure 3e–h), the inoculated potting mix supported significantly greater seedling survival than sterilized field soils, live field soils, or our potting mix treatments for both species ($p < 0.001$), indicating both a biotic and soil abiotic effect. We find this was true for soils of within-range hardwood and ecotone origin for both sugar maple and beech, but not for soils of spruce-fir origin (Question 1, Figure 3). Sugar maple seedling biomass and height were significantly greater in the inoculated treatment than in all other treatments in soils from within its range (soil source × treatment: $p < 0.05$), but not for soils outside of its range where seedling growth metrics were similar for the inoculated and potting mix treatments (Question 1, Figure 3e–g). Beech seedlings grown in hardwood soils in the Inoculated treatment had higher final height and biomass than seedlings grown in spruce-fir soils (Figure 3f,g).

Experiment 2: STE

Addressing Questions 1 and 2 in a field setting (STE, see Table 4 for a priori expectations, Appendix S1: Tables S5–S7 for model results), we find that mean AMF root colonization generally decreased for sugar maple when growing beyond its range and when growing in soils of spruce-fir origin (~39% colonization in spruce-fir soils averaged across all elevations compared with other soil origins, soil source × planting site: $p < 0.03$; Figure 4a). EMF root colonization of American beech did not differ across the elevation gradients (Figure 4b). Additionally, we find that rates of seed germination and overall observed seedling survival of both sugar maple and American beech decreased beyond their range margins, regardless of soil origin, indicating an abiotic climate effect (Figure 4c–f). Similarly, germination and survival were higher for both sugar maple and beech seedlings sown in within-range hardwood soils than in beyond-range spruce-fir soils ($p < 0.001$), with intermediate values for ecotone soils for all planting sites, consistent with the results of our GE and indicating a biotic effect (cf. Figure 3).

Unlike seedling survival, metrics of seedling growth, including RHGR, and final total dry biomass did not significantly differ between planting site and soil origin for either sugar maple or beech (Question 1, Appendix S1: Figure S6). Mean sugar maple root-to-shoot ratios were almost twice as high at spruce-fir sites for all soil origin treatments than for other sites (planting site: $p < 0.001$), but this was not the case for beech seedlings. Lastly, the observed proportion of experimental seedlings with potential root pathogens did not significantly differ across the elevation gradients for either sugar maple or beech (soil source × treatment: $p > 0.05$, see Appendix S1: Figure S7).

Experiment 3: CMNE

Addressing Question 3 (assessing potential role of CMN on seedling performance across range boundaries, see Table 4 for a priori expectations, Appendix S1: Tables S8–S10 for model results), we find that across both years of the CMNE, survival of network (N) and pot control (C) sugar maple seedlings remained significantly higher than that of seedlings in the disrupted (D) treatment, indicating a biotic effect (final survival: $N = 55\%$, $C = 54\%$, $D = 8\%$, Figure 5a). On the other hand, survival of American beech network seedlings tended to be higher than that of seedlings in the pot control and disrupted treatments for both years of the experiment but not significantly so at any time step (final survival: $N = 37\%$, $C = 24\%$, $D = 37\%$, Figure 5c). Overall, there were no significant differences in two-year seedling survival between planting sites for either species (planting site × CMN treatment: $p > 0.05$, see Figure 5b,d; Appendix S1: Figure S8).

Unlike seedling survival, there were no treatment differences across the elevation gradients for any seedling growth metric examined (mean basal diameter change, mean leaf number, RHGR, root-to-shoot, and total dry biomass, planting sites × CMN treatment: $p > 0.05$), with the exception of sugar maple root-to-shoot ratio and beech RHGR (Appendix S1: Figures S9 and S10), where both were significantly higher for seedlings grown beyond their range margin in spruce-fir forests than in within-range lower elevation hardwood forests (planting site: $p < 0.001$). While the proportion of sugar maple seedlings with potential root pathogens present did not change across the elevation gradients (planting site × CMN treatment: $p > 0.05$), there was a clear treatment effect, with our disturbed treatment seedlings hosting a significantly greater amount of potential root pathogens than network or pot control seedlings (Question 4; ~9% of pots, $p = 0.02$; Figure 6). This trend did not hold for American beech-disrupted treatment seedlings.

Exploring Question 4 (examining possible mycorrhizal benefits beyond seedling nutrition, Appendix S1: Tables S11–S13 for model results), we note that across the elevation gradients, sugar maple foliar N and base cation (K, Ca, Mg) concentrations from CMNE seedlings tended to decrease beyond their range margin, while foliar Al concentrations increased, and foliar P peaked within ecotone forests (Appendix S1: Tables S11–S13; Tourville et al., 2023). While most sugar maple seedling foliar nutrient concentrations did not differ between our

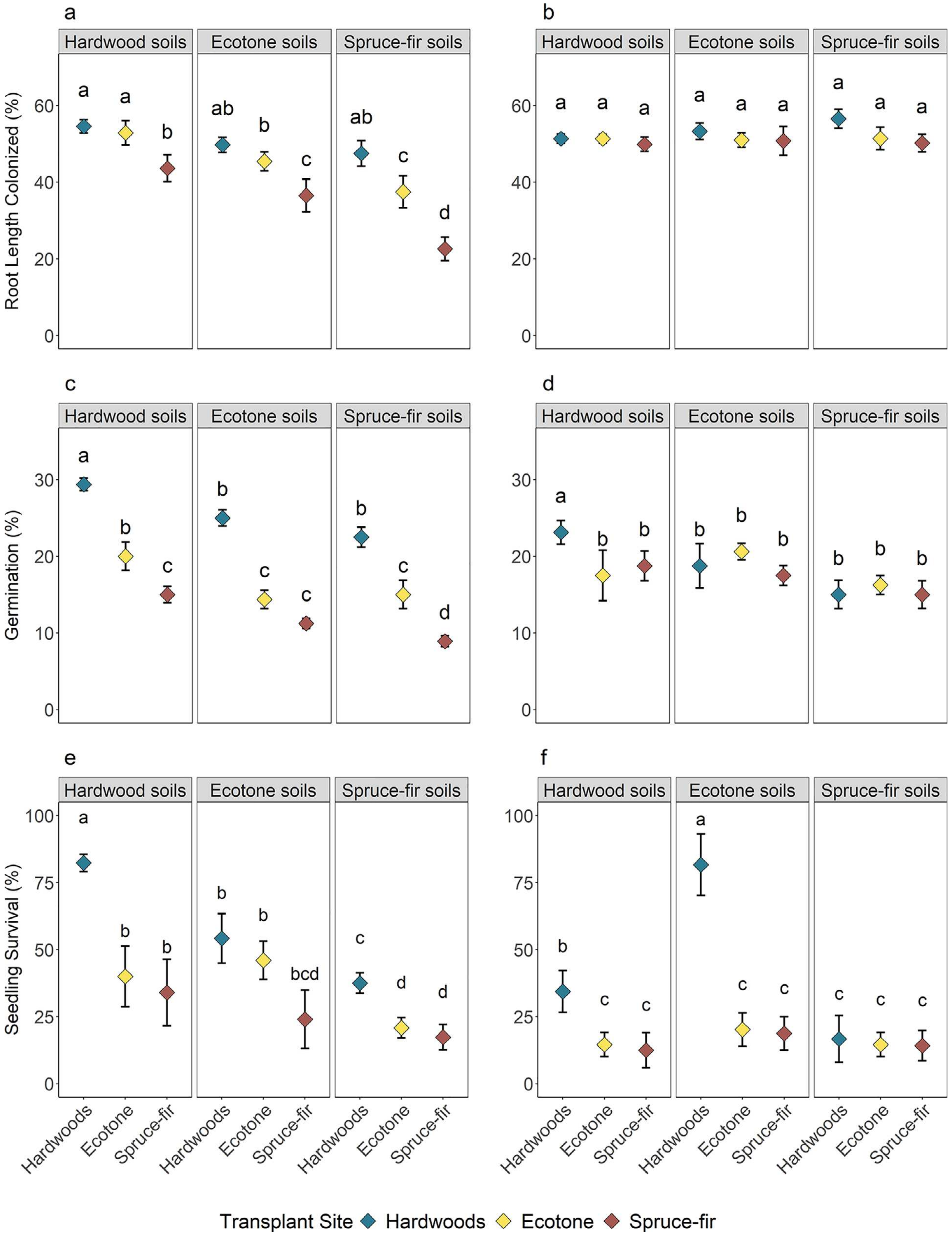

FIGURE 4 (a, b) Differences in mean (±SE) mycorrhizal colonization (root length colonized %) between treatments for our soil translocation experiment for arbuscular mycorrhiza sugar maple and ectomycorrhiza American beech seedlings. Mean (±SE) proportion of sugar maple and beech seeds germinating (c, d, 20 total seeds maximum) at each translocation site (elevation) and for each soil origin in our soil translocation experiment. Additionally, mean (±SE) proportion of sugar maple and beech seedlings surviving (e, f) for two growing seasons at each transplant site and for each soil origin. Letters indicate significant treatment differences from generalized linear mixed effects models (α = 0.05).

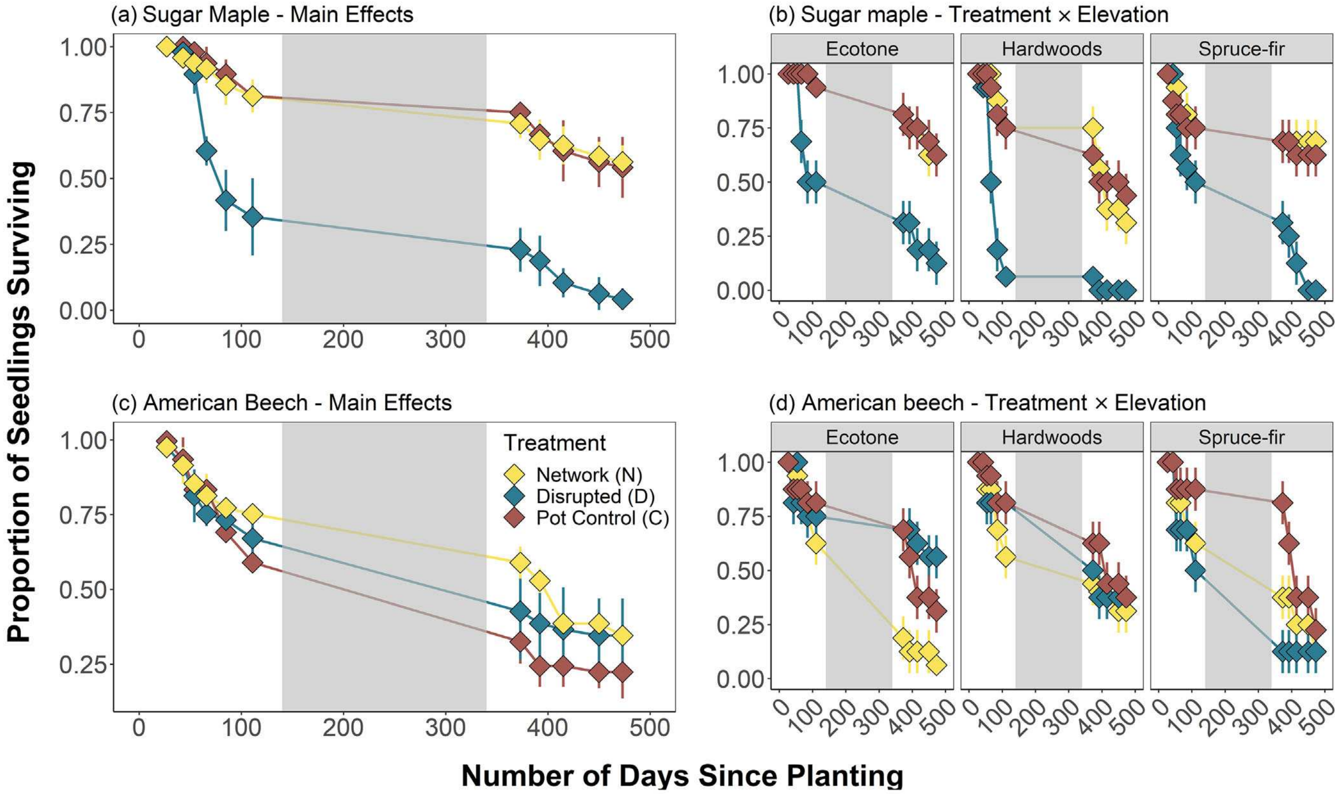

FIGURE 5 Survival curves of sugar maple (a) and American beech (c) seedlings for each treatment over the course of two growing seasons from our common mycorrhizal network experiment. Points indicate mean proportion (± SE) of treatment seedlings surviving at the time of each survey. The gray area indicates the non-growing (winter) season in between the first and second years of the experiment. (a, b) show main effects for treatments for each species while (b, d) show survival curves for each treatment and species partitioned by planting site (elevation).

three CMN treatments, foliar Al concentration was significantly greater for the disrupted treatment than for the network or pot control seedlings (CMN treatment: $p < 0.001$, Figure 7), and in spruce-fir planting sites compared with lower elevations (planting site × CMN treatment: $p < 0.001$, Figure 7). For American beech seedlings, we found a positive relationship between foliar P concentration and elevation, and a negative relationship between foliar N, K, Mg, and Al concentrations and elevation (Tourville et al., 2023). There were no CMN treatment differences in beech seedling foliar nutrient concentrations (Appendix S1: Table S12).

Similar to our GE and STE, we found that for our CMNE, AMF colonization was lower for sugar maple seedlings planted beyond their range in spruce-fir forests than for seedlings planted within their range (hardwood: $p < 0.001$; ecotone: $p < 0.001$; Appendix S1: Figure S11, Table S8). There were no site differences in EMF colonization among beech seedlings (Appendix S1: Figure S11, Table S8). Mycorrhizal root colonization did not differ between CMN manipulation treatments (network, disrupted, pot control) for either AMF- or EMF-associated seedlings (i.e., sugar maple and beech, respectively; Appendix S1: Figure S11, Table S8).

DISCUSSION

Our work demonstrates the importance of accounting for belowground biotic interactions in predicting future plant distributions (e.g., Iverson et al., 2008, 2019; Prasad et al., 2020). Our three complementary experiments suggest that mycorrhizal fungi can support tree seedling establishment via increased survival across species range boundaries, but only if the appropriate fungi are present (Question 1). The spatial dynamics of mycorrhizal types within a landscape also seems likely to determine the relative success of establishing seedlings (Question 2, AM vs. EM trees). Further, we posit that either potential CMN connectivity or increased foraging area has a positive influence on seedling performance across range boundaries, particularly in areas that could have limited inoculum (i.e., sugar maple AMF colonization beyond its range margin, Question 3). Lastly, in addition to the

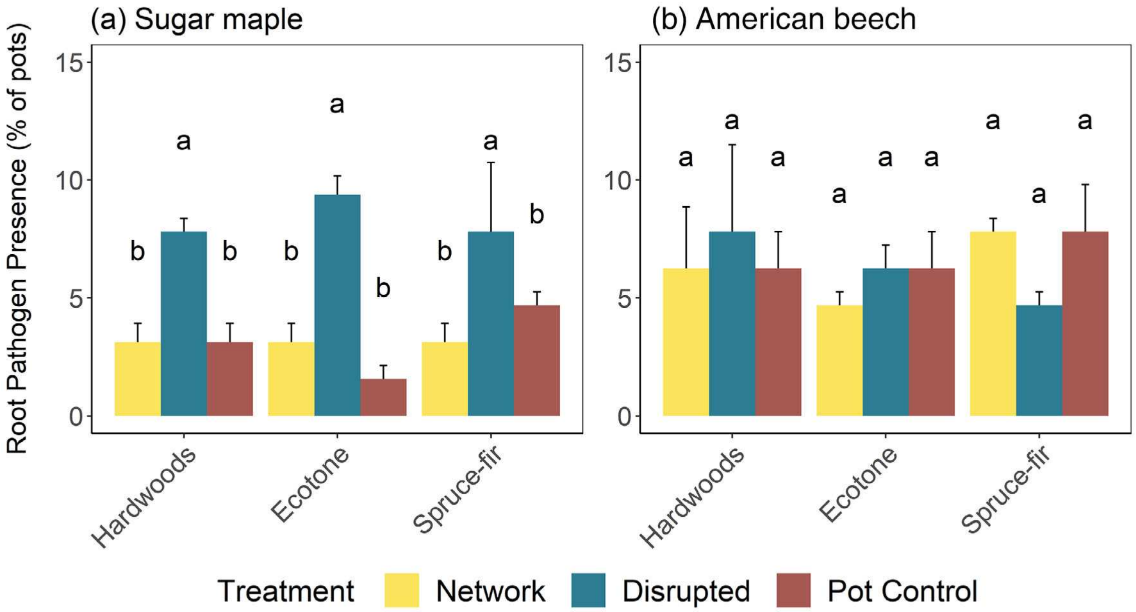

FIGURE 6 Common mycorrhizal networks treatment differences in mean (±SE) proportion of experimental pots across our study mountains with observed presence of potential root pathogens, for both sugar maple (a) and American beech (b). Letters indicate significant differences between groups (α = 0.05).

nutrient benefit derived from mycorrhizal associations, we find evidence that pathogen defense and metal protection could represent other important beneficial aspects of the mycorrhizal symbiosis in our study system, although the mechanisms behind these via CMN connection remains unclear (Smith & Read, 2010) (Question 4). Below, we expand on our findings from each question, place our work into a wider context, and highlight both some study limitations (e.g., the use of a single AM and EM tree species; challenges operating CMN field experiments) and implications for future research.

Tree seedling performance and mycorrhizal colonization beyond species range boundaries

While we found evidence that AMF inoculum potential in high-elevation conifer forests could be limited, AMF colonization is not completely absent among sugar maple seedlings, indicating at least some inoculum potential within these EMF-dominant forests; however, it may not be sufficient to promote successful establishment of sugar maple (Carteron et al., 2020). AMF colonization has been shown to be highly positively correlated with overstory basal area of AM trees (which decreases with elevation, $r = 0.85$) and pH ($r = 0.58$) and negatively correlated with elevation ($r = -0.92$; see Tourville et al., 2023). Conversely, EMF colonization of American beech remained consistent across the elevation gradients, despite the likelihood that EMF community composition in conifer forests is different than that found in low-elevation temperate hardwood forests (Kivlin et al., 2017; Lynn et al., 2019). Given the potential for high fungal community turnover and specificity between sugar maple and beech along the elevation gradient, our data indicate that incorporating molecular data to better resolve fungal community composition could be an exciting area of future research (Chaudhary et al., 2022; De Bellis et al., 2022; Kivlin et al., 2017; Urcelay et al., 2019).

Sugar maple survival was curtailed when growing in a beyond-range medium as seen in a recent greenhouse study (Carteron et al., 2020). Interestingly, our GE survival was not significantly different between fresh live field soils and sterilized field soils, either because soil chemistry had a more prominent role on survival than soil biota or possibly because removing both mutualistic fungi and pathogens had a net neutral effect on seedling survival (Brown & Vellend, 2014; Jiang et al., 2020; Liang et al., 2015, 2016). We stress that since we did not specifically remove any potential soil pathogens from our field soils, we cannot discount the influence of any saprotrophic fungi or other non-mycorrhizal soil microorganisms on seedling performance, aside from inferences made by examining mycorrhizal root colonization. However, because seedling survival in soils inoculated with hardwood and ecotone biota was much higher than in non-inoculated soils, we find an overall positive effect of soil biota.

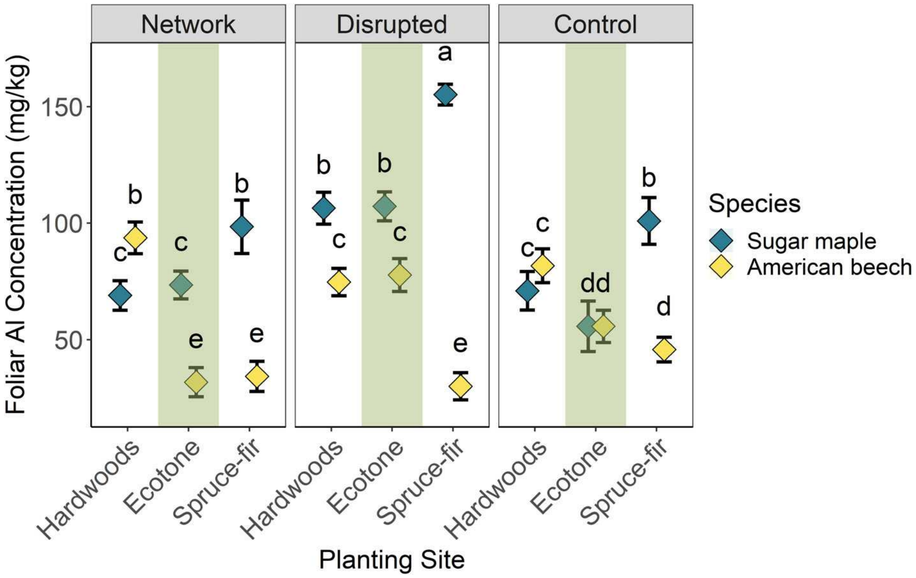

FIGURE 7 Mean (±SE) foliar Al concentration values for harvested sugar maple (blue) and American beech (yellow) seedlings from our common mycorrhizal network experiment partitioned across different common mycorrhizal network connectivity experiment treatments and planting sites (elevation). The shaded area highlights results from the disrupted treatment. Different letters indicate significantly different groups (α = 0.05).

Our field STE results implicate both soils (chemistry and biota) and climate as drivers of seedling establishment in addition to mycorrhizal colonization. However, the metrics of seedling growth (RHGR and biomass) did not differ either for tree species across the elevation gradients or for different soil origins, with the exception of significantly higher root-to-shoot ratios of sugar maple at high elevation indicative of increased carbon allocation to foraging in nutrient-poor soils (without the aid of sufficient mycorrhizal partnership) (Mašková & Herben, 2018; Paz, 2003; Weemstra et al., 2016). Brown and Vellend (2014) recorded a similar lack of response in growth for seedlings growing in soils of different origins along a similar elevation gradient. This seems to indicate that seedling survival is a more sensitive indicator of the influence of mycorrhizal fungi than seedling growth.

Performance of tree species of different mycorrhizal types across range boundaries

Our AM tree species (sugar maple) suffered from lower survival upslope of its range margin. Further, we find that our EM tree species (American beech) was less responsive to different sources of fungal inoculum, CMN connection, and soil chemical properties than our AM tree species. The differential performance of our AM and EM tree species could be attributed to the spatial distribution of trees with these different mycorrhizal types in our study system: Where sugar maple was faced with lower mycorrhizal colonization in EMF-dominated high-elevation conifer forests, beech did not face the same limitations. We cannot rule out experimental differences based on idiosyncrasies of the study species themselves in terms of stress tolerances or seed sizes (an indication of initial starting resource reserves), as the relatively smaller seeded sugar maple is known to be less tolerant of poor soils and colder temperatures than the larger seeded American beech (Cleavitt et al., 2018, 2021). Additionally, seed size has been shown to influence the degree of seedling mycorrhizal dependence, with smaller seeded species depending more on mycorrhizal associations for growth (Moyano et al., 2020, 2021). Despite the fact that our study species are dominant trees in the temperate forests of eastern North America, we caution that since we only examined one AM and one EM tree species, care should be taken when generalizing these results to other AM/EM associations in other systems. We also cannot exclude the possibility that future climate-induced changes in forest species composition across our elevation gradients can be influenced by the species' mycorrhizal type and the distribution of dominant mycorrhizal types in space (e.g., over elevational environmental gradients), assuming a lack of plant-fungal specificity. In other words, our expanding AM species may be filtered out in favor of an EM species at high elevations (Carteron et al., 2020, 2022).

Role of potential connectivity to a CMN on tree seedling performance across range boundaries

The potential connection to a CMN serves to effectively increase the foraging area and availability of nutrients available to root colonizing fungi (Selosse et al., 2006; Simard et al., 2015; Wallander & Ekblad, 2015). Plants also benefit from not having to allocate carbon to develop a CMN if it is already present, yet still gain access to the harvesting network (Newman, 1988). While seedling mycorrhizal fungi disconnected from a CMN must initially forage solely within their own hosts' rhizosphere, increased access to more distant nutrient pools by intact CMNs, particularly in a nutrient-poor and heterogeneous environment, may be of critical importance to seedlings in the early stages of development (Cortese et al., 2023; Cortese & Horton, 2023; Horton et al., 1999; Van Der Heijden, 2004; Wipf et al., 2019). Sugar maple is known to be more sensitive to low base cation and nutrient concentrations than American beech, and thus, less suitable to acidic high-elevation soils (Cleavitt et al., 2018; Sullivan et al., 2013). The CMN treatment effect seen for sugar maple, but not for beech, may be a direct result of this environmental sensitivity; however, we did not detect any treatment effects on seedling growth (Horton & van der Heijden, 2008). Another non-mutually exclusive possibility is that sugar maple seedlings use fewer resources to promote connection to an already established CMN than developing a local mycorrhizal network from AMF spores over a longer period of time, as seen with EMF CMNs (Horton et al., 1999; Nara, 2006a; Newman, 1988). The quick and less energy-intensive access to CMN-derived nutrients may prevent excess carbon loss and mortality in a stressful environment (see Van Der Heijden & Horton, 2009; but see Karst et al., 2023). However, we acknowledge that other research has found that the transfer of photosynthate from seedlings to fungi may be too costly in stressful environments (Ibáñez & McCarthy-Neumann, 2015; Jakobsen & Hammer, 2015; Van Der Heijden & Horton, 2009).

We stress that while some evidence exists for carbon and other resource transfer between interconnected AM plants (detected in plant roots and associated fungal hyphae, see Lerat et al., 2002) it is not clear whether ecologically meaningful amounts of nutrients or carbon are transferred, what pathway these resources take, and what the physiological mechanism behind such an exchange is (Fitter et al., 1998; Jakobsen & Hammer, 2015; Robinson & Fitter, 1999; Smith & Read, 2010). It is also not clear whether small seedlings or larger individuals benefit more from nutrient exchange as part of CMN, as studies have shown that nutrient reward from fungus to plant is proportional to the magnitude of carbon transfer from plant to fungus (i.e., reciprocal reward or nutrient market economy hypothesis; Fellbaum et al., 2014; Jakobsen & Hammer, 2015; Kiers et al., 2011; van't Padje et al., 2021). Thus, the lack of clear-cut data on the reality of ecologically meaningful resource transfer between CMN-associated plants, and the mechanisms involved, hinders our ability to make definitive claims about the true nature of CMNs (Henriksson et al., 2023; Karst et al., 2023).

Further, it is important to note several caveats that make interpretation of our CMN results challenging. First, the use of physical barriers and repeated disconnection of hyphal links in our disrupted treatment may have also served to reduce the potential foraging area of mycorrhizal fungi already present within our pots. This could potentially explain the lower survival of sugar maple seedlings in the disrupted treatment (also see Booth, 2004). However, we point out that lower seedling survival was not seen for our other species, American beech, and that our pots offered relatively greater soil volume (3 L) than in similar experiments using small mesh bags (Hoeksema, 2015; Karst et al., 2023). Second, we were not able to definitively show that CMN connections were formed in our network treatment pots, a common drawback of most CMN studies (e.g., Karst et al., 2023). While it seems evident that such studies are logistically challenging, we believe that integrating field studies of mycorrhizal interactions with more controlled greenhouse studies or mesocosms, and employing the use of molecular techniques, can enable hypotheses derived from idealized environments to be assessed in realistic settings (Hoeksema, 2015; Lekberg & Helgason, 2018).

Modes of mycorrhizal benefit on tree seedling performance

Mycorrhiza provide nutrient rewards to plants, which act to boost plant growth in stressful environments (Fellbaum et al., 2014; Kiers et al., 2011; Simard et al., 1997). However, given the decrease in AMF colonization and sugar maple survival with increasing elevation, coupled with a lack of any experimental treatment growth effects or CMN treatment effect on foliar base cation, N, or P status, nutrient exchange may not be the only relevant benefit of mycorrhizal colonization here. We suggest that phenomena such as pathogen defense or heavy metal protection may also be critical to consider in this system (Alotaibi et al., 2021; Kelly et al., 2005; Leyval et al., 1997; Sikes et al., 2009; Tedersoo et al., 2020).

We found increased foliar Al concentrations for sugar maple in our disrupted treatment, particularly at high elevations beyond the species range edge, despite the fact that soil Al concentrations did not significantly differ across our elevation gradients. Acute concentrations of Al are toxic to many plant species and could explain the decreased survival of sugar maple disconnected from any potential CMN (Halman et al., 2014; Schaberg et al., 2006; Sullivan et al., 2013). Indeed, AMF are known to protect plants from toxic levels of heavy metals in the soil (Alotaibi et al., 2021; Kelly et al., 2005). Even though our experimental sugar maple seedlings were colonized by AMF within treatment pots, we could conclude that either connection to a broader network may be required to better protect plants from Al toxicity (although the mechanism for this remains unknown in this study) or that the AMF species colonizing sugar maple in boreal forests were less able to protect seedlings from Al accumulation. We did not find this treatment effect for beech, possibly because of the high EMF root colonization levels found across the gradient, or because EMF provides relatively better protection to these plants than AMF (Branco et al., 2022).

Additionally, while we acknowledge that we did not directly measure root pathogen infection rates or identify pathogen taxa, we did find that observed potential pathogen damage was present more on sugar maple-disrupted treatment seedlings than on network or pot control seedlings, despite the lack of any elevation differences. Pathogen defense is an important service provided by the AMF mutualism, but receives relatively less attention than nutrient access services (Sikes et al., 2009; Tedersoo et al., 2020). Indeed, the role of an intact CMN to provide pathogen defense versus local mycorrhizal colonization is unexplored in the literature (but see Karban, 2021). Curiously, there was no evidence of an EMF CMN-specific role in pathogen protection for American beech seedlings despite the efficacy of EMF in providing this service (Dreischhoff et al., 2020; Luo et al., 2014). Potentially, EMF-associated species such as beech may benefit from the maintenance of mutualistic services offered by diverse communities whereas AMF-associated species like sugar maple may have access to a less diverse suite of relatively less helpful and functionally specialized fungi, although this hypothesis would require further testing using molecular techniques (De Bellis et al., 2022; Kivlin et al., 2017; Urcelay et al., 2019).

Conclusions

There is a growing understanding among ecologists that mycorrhizal interactions have a critical role to play in the functioning of terrestrial ecosystems vis-à-vis plant growth and controls on plant distribution and diversity (Bever et al., 2001; Delavaux et al., 2019; Tedersoo et al., 2020; Tourville et al., 2023; Zobel & Öpik, 2014). The mycorrhizal symbiosis is not only a factor influencing the advance of invasive species but also of natural range expansion across climatic gradients, especially in the context of global climate change (Lankau et al., 2015; Pither et al., 2018). Our study adopted a novel integrated approach to disentangle the effects of mycorrhiza, soil abiotic properties, and climate on tree seedling growth and survival across range boundaries. By leveraging experimental data collected from both greenhouse and field settings, we attempted to control for environmental heterogeneity while maintaining both study realism and generalizability (Booth, 2004; Hoeksema, 2015; Lekberg & Helgason, 2018). Our results reveal the importance of incorporating information on mycorrhiza and colonization status of trees into predictive models of future tree distributions. We also stress the importance of not only thinking about mycorrhizal associations solely in terms of nutrient exchange but also embracing the multifaceted role fungi play in providing benefits to plants.

AUTHOR CONTRIBUTIONS

Jordon C. Tourville conceived the research questions with input from Thomas R. Horton and Martin Dovciak. Jordon C. Tourville planned and designed the research and conducted all data analyses with input from Thomas R. Horton and Martin Dovciak. Jordon C. Tourville wrote the manuscript, and all authors contributed substantial revisions and edits.

ACKNOWLEDGMENTS

We thank field technicians Hailey Lynch, Heather Zimba, Ben VanderStouw, and Adam Busman for their help with experimental setup and data collection; local, state, and federal organizations in Vermont for providing research permits, access to sites, and logistic support; and the Forest Ecosystem Monitoring Cooperative and the Green Mountain Club for providing site access and logistic support. We also thank the Adair lab, Marie English, and the Aiken Forestry Sciences Laboratory at the University of Vermont for support and facilities for all mycorrhizal lab work. This research was supported partly by the US National Science Foundation research award to Martin Dovciak (NSF no. 1759724) and also partly by research awards from the Botanical Society of America, the New York Flora Association, the Edwin H. Ketchledge Scholarship Fund, and the Lowe-Wilcox Award from SUNY-ESF to Jordon C. Tourville.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and code (jtouriv, 2024) are available in Zenodo at https://doi.org/10.5281/zenodo.12689365.

ORCID

Jordon C. Tourville https://orcid.org/0000-0003-4005-5914
Thomas R. Horton https://orcid.org/0000-0002-2112-9618
Martin Dovciak https://orcid.org/0000-0002-9428-3122

REFERENCES

Alexander, J. M., L. Chalmandrier, J. Lenoir, T. I. Burgess, F. Essl, S. Haider, C. Kueffer, et al. 2018. "Lags in the Response of Mountain Plant Communities to Climate Change." *Global Change Biology* 24(2): 563–579. https://doi.org/10.1111/gcb.13976.

Alotaibi, M. O., A. M. Saleh, R. L. Sobrinho, M. S. Sheteiwy, A. M. El-sawah, A. E. Mohammed, and H. Abdelgawad. 2021. "Arbuscular Mycorrhizae Mitigate Aluminum Toxicity and Regulate Proline Metabolism in Plants Grown in Acidic Soil." *Journal of Fungi* 7(7): 531. https://doi.org/10.3390/jof7070531.

Aslani, F., A. S. Juraimi, M. S. Ahmad-Hamdani, M. A. Alam, M. M. Hasan, F. S. G. Hashemi, and M. Bahram. 2019. "The Role of Arbuscular Mycorrhizal Fungi in Plant Invasion Trajectory." *Plant and Soil* 441(1–2): 1–14. https://doi.org/10.1007/s11104-019-04127-5.

Bainard, L. D., J. N. Klironomos, and A. M. Gordon. 2011. "The Mycorrhizal Status and Colonization of 26 Tree Species Growing in Urban and Rural Environments." *Mycorrhiza* 21: 91–96.

Beckage, B., B. Osborne, D. G. Gavin, C. Pucko, T. Siccama, and T. Perkins. 2008. "A Rapid Upward Shift of a Forest Ecotone during 40 Years of Warming in the Green Mountains of Vermont." *Proceedings of the National Academy of Sciences* 105(11): 4197–4202. https://doi.org/10.1073/pnas.0708921105.

Beeles, K. L., J. C. Tourville, and M. Dovciak. 2021. "Characterizing Canopy Openness Across Large Forested Landscapes Using Spherical Densiometer and Smartphone Hemispherical Photography." *Journal of Forestry* 120(1): 37–50. https://doi.org/10.1093/jofore/fvab046.

Beiler, K. J., D. M. Durall, S. W. Simard, S. A. Maxwell, and A. M. Kretzer. 2010. "Architecture of the Wood-Wide Web: Rhizopogon Spp. Genets Link Multiple Douglas-Fir Cohorts." *New Phytologist* 185(2): 543–553. https://doi.org/10.1111/j.1469-8137.2009.03069.x.

Bever, J. D., P. A. Schultz, A. Pringle, and J. B. Morton. 2001. "Arbuscular Mycorrhizal Fungi: More Diverse than Meets the Eye, and the Ecological Tale of Why." *Bioscience* 51(11): 923–932. https://doi.org/10.1641/0006-3568.

Boisvert-Marsh, L., C. Périé, and S. de Blois. 2014. "Shifting With Climate? Evidence for Recent Changes in Tree Species Distribution at High Latitudes." *Ecosphere* 5(7): 1–33. https://doi.org/10.1890/es14-00111.1.

Booth, M. G. 2004. "Mycorrhizal Networks Mediate Overstorey-Understorey Competition in a Temperate Forest." *Ecology Letters* 7(7): 538–546.

Booth, M. G., and J. D. Hoeksema. 2010. "Mycorrhizal Networks Counteract Competitive Effects of Canopy Trees on Seedling Survival." *Ecology* 91(8): 2294–2302.

Branco, S., A. Schauster, H. L. Liao, and J. Ruytinx. 2022. "Mechanisms of Stress Tolerance and their Effects on the Ecology and Evolution of Mycorrhizal Fungi." *New Phytologist* 235(6): 2158–75.

Brown, C. D., and M. Vellend. 2014. "Non-climatic Constraints on Upper Elevational Plant Range Expansion under Climate Change." *Proceedings of the Royal Society B: Biological Sciences* 281(1794): 20141779. https://doi.org/10.1098/rspb.2014.1779.

Brundrett, M., N. Bougher, B. Dell, T. Grove, and N. Malajczuk. 1996. *Working with Mycorrhizas in Forestry and Agriculture*, Vol. 32, 374. Canberra: Australian Centre for International Agricultural Research.

Brundrett, M. C., and L. Tedersoo. 2020. "Resolving the Mycorrhizal Status of Important Northern Hemisphere Trees." *Plant and Soil* 454: 3–34.

Carteron, A., V. Parasquive, F. Blanchard, X. Guilbeault-Mayers, B. L. Turner, M. Vellend, and E. Laliberté. 2020. "Soil Abiotic and Biotic Properties Constrain the Establishment of a Dominant Temperate Tree into Boreal Forests." *Journal of Ecology* 108(3): 931–944. https://doi.org/10.1111/1365-2745.13326.

Carteron, A., M. Vellend, and E. Laliberté. 2022. "Mycorrhizal Dominance Reduces Local Tree Species Diversity across US Forests." *Nature Ecology and Evolution* 6(4): 370–74. https://doi.org/10.1038/s41559-021-01634-6.

Chaudhary, V. B., E. P. Holland, S. Charman-anderson, A. Guzman, L. Bell-dereske, T. E. Cheeke, A. Corrales, J. Duchicela, and C. Egan. 2022. "Trends in Ecology & Evolution What Are Mycorrhizal Traits?" *Trends in Ecology & Evolution* 37: 573–581. https://doi.org/10.1016/j.tree.2022.04.003.

Chaudhary, V. B., S. Nolimal, M. A. Sosa-Hernández, C. Egan, and J. Kastens. 2020. "Trait-Based Aerial Dispersal of Arbuscular Mycorrhizal Fungi." *New Phytologist* 228(1): 238–252. https://doi.org/10.1111/nph.16667.

Cleavitt, N. L., J. J. Battles, T. J. Fahey, and N. S. van Doorn. 2021. "Disruption of the Competitive Balance between Foundational Tree Species by Interacting Stressors in a Temperate Deciduous Forest." *Journal of Ecology* 109(7): 2754–68.

Cleavitt, N. L., J. J. Battles, C. E. Johnson, and T. J. Fahey. 2018. "Long-Term Decline of Sugar Maple Following Forest Harvest, Hubbard Brook Experimental Forest, New Hampshire." *Canadian Journal of Forest Research* 48(1): 23–31. https://doi.org/10.1139/cjfr-2017-0233.

Collin, A., C. Messier, and N. Bélanger. 2017. "Conifer Presence May Negatively Affect Sugar Maple's Ability to Migrate into the Boreal Forest through Reduced Foliar Nutritional Status." *Ecosystems* 20: 701–716. https://doi.org/10.1007/s10021-016-0045-4.

Cortese, A. M., J. E. Drake, M. Dovciak, J. B. Cohen, and T. R. Horton. 2023. "Proximity to an Old-Growth Forest Edge and Ectomycorrhizal Tree Islands Enhance Ectomycorrhizal Fungal Colonization of *Betula lenta* L. (Black Birch) Seedlings in Secondary Forest Soils." *Plant and Soil* 493: 391–405. https://doi.org/10.1007/s11104-023-06237-7.

Cortese, A. M., and T. R. Horton. 2023. "Islands in the Shade: Scattered Ectomycorrhizal Trees Influence Soil Inoculum and Heterospecific Seedling Response in a Northeastern Secondary

Forest." *Mycorrhiza* 33: 33–44. https://doi.org/10.1007/s00572-023-01104-w.

De Bellis, T., I. Laforest-Lapointe, K. A. Solarik, D. Gravel, and S. W. Kembel. 2022. "Regional Variation Drives Differences in Microbial Communities Associated with Sugar Maple across a Latitudinal Range." *Ecology* 2022: 1–13. https://doi.org/10.1002/ecy.3727.

De Wit, R., and T. Bouvier. 2006. "'Everything is Everywhere, but, the Environment Selects'; What Did Baas Becking and Beijerinck Really Say?" *Environmental Microbiology* 8(4): 755–58. https://doi.org/10.1111/j.1462-2920.2006.01017.x.

Delavaux, C. S., P. Weigelt, W. Dawson, J. Duchicela, F. Essl, M. van Kleunen, C. König, et al. 2019. "Mycorrhizal Fungi Influence Global Plant Biogeography." *Nature Ecology and Evolution* 3(3): 424–29. https://doi.org/10.1038/s41559-019-0823-4.

Dreischhoff, S., I. S. Das, M. Jakobi, K. Kasper, and A. Polle. 2020. "Local Responses and Systemic Induced Resistance Mediated by Ectomycorrhizal Fungi." *Frontiers in Plant Science* 11: 590063. https://doi.org/10.3389/fpls.2020.590063.

Evans, P., and C. D. Brown. 2017. "The Boreal-Temperate Forest Ecotone Response to Climate Change." *Environmental Reviews* 25(4): 423–431. https://doi.org/10.1139/er-2017-0009.

Fellbaum, C. R., J. A. Mensah, A. J. Cloos, G. E. Strahan, P. E. Pfeffer, E. T. Kiers, and H. Bücking. 2014. "Fungal Nutrient Allocation in Common Mycorrhizal Networks is Regulated by the Carbon Source Strength of Individual Host Plants." *New Phytologist* 203(2): 646–656. https://doi.org/10.1111/nph.12827.

Fitter, A. H., J. D. Graves, N. K. Watkins, D. Robinson, and C. Scrimgeour. 1998. "Carbon Transfer between Plants and its Control in Networks of Arbuscular Mycorrhizas." *Functional Ecology* 12(3): 406–412. https://doi.org/10.1046/j.1365-2435.1998.00206.x.

Galante, T. E., T. R. Horton, and D. P. Swaney. 2011. "95% of Basidiospores Fall within 1 m of the Cap: A Field-and Modeling-Based Study." *Mycologia* 103(6): 1175–83.

Geml, J. 2017. "Altitudinal Gradients in Mycorrhizal Symbioses." *Biogeography of Mycorrhizal Symbiosis* 230: 107–123.

Goel, M. K., P. Khanna, and J. Kishore. 2010. "Understanding Survival Analysis: Kaplan–Meier Estimate." *International Journal of Ayurveda Research* 1(4): 274–78.

Halman, J. M., P. G. Schaberg, G. J. Hawley, C. F. Hansen, and T. J. Fahey. 2014. "Differential Impacts of Calcium and Aluminum Treatments on Sugar Maple and American Beech Growth Dynamics." *Canadian Journal of Forest Research* 45(1): 52–59. https://doi.org/10.1139/cjfr-2014-0250.

Harley, J. L., and S. E. Smith. 1983. *Mycorrhizal Symbiosis* 483. London: Academic Press.

Harsch, M. A., P. E. Hulme, M. S. McGlone, and R. P. Duncan. 2009. "Are Treelines Advancing? A Global Meta-Analysis of Treeline Response to Climate Warming." *Ecology Letters* 12 (10): 1040–49. https://doi.org/10.1111/j.1461-0248.2009.01355.x.

Hayward, J., T. R. Horton, A. Pauchard, and M. A. Nuñez. 2015. "A Single Ectomycorrhizal Fungal Species Can Enable a Pinus Invasion." *Ecology* 96(5): 1438–44. https://doi.org/10.1890/14-1100.1.

Henriksson, N., J. Marshall, M. N. Högberg, P. Högberg, A. Polle, O. Franklin, and T. Näsholm. 2023. "Re-Examining the Evidence for the Mother Tree Hypothesis–Resource Sharing among Trees Via Ectomycorrhizal Networks." *New Phytologist* 239: 19–28.

Hoeksema, J. D. 2015. "Experimentally Testing Effects of Mycorrhizal Networks on Plant–Plant Interactions and Distinguishing among Mechanisms." In *Mycorrhizal Networks* 255–277. Dordrecht: Springer.

Horton, T. R., T. D. Bruns, and T. V. Parker. 1999. "Ectomycorrhizal Fungi Associated with *Arctostaphylos* Contribute to *Pseudotsuga menziesii* Establishment." *Canadian Journal of Botany* 77: 93–102.

Horton, T. R., and M. van der Heijden. 2008. "The Role of Symbioses in Seedling Establishment and Survival." In *Seedling Ecology and Evolution*, edited by M. Leck, V. T. Parker, and B. Simpson. Cambridge, UK: Cambridge University Press.

Huang, C. Y. L., and E. E. Schulte. 1985. "Digestion of Plant Tissue for Analysis by ICP Emission Spectroscopy." *Communications in Soil Science and Plant Analysis* 16(9): 943–958.

Ibáñez, I., and S. McCarthy-Neumann. 2015. "Effects of Mycorrhizal Fungi on Tree Seedling Growth: Quantifying the Parasitism–Mutualism Transition along a Light Gradient." *Canadian Journal of Forest Research* 46(1): 48–57. https://doi.org/10.1139/cjfr-2015-0327.

Iverson, L. R., A. M. Prasad, S. N. Matthews, and M. Peters. 2008. "Estimating Potential Habitat for 134 Eastern US Tree Species under Six Climate Scenarios." *Forest Ecology and Management* 254: 390–406. https://doi.org/10.1016/j.foreco.2007.07.023.

Iverson, L. R., A. M. Prasad, M. P. Peters, and S. N. Matthews. 2019. "Facilitating Adaptive Forest Management under Climate Change: A Spatially Specific Synthesis of 125 Species for Habitat Changes and Assisted Migration over the Eastern United States." *Forests* 10(11): 989. https://doi.org/10.3390/f10110989.

Jakobsen, I., and E. C. Hammer. 2015. "Nutrient Dynamics in Arbuscular Mycorrhizal Networks." In *Mycorrhizal Networks* 91–131. Dordrecht: Springer.

Janowiak, M. K., A. W. D'Amato, C. W. Swanston, L. Iverson, F. R. Thompson, W. D. Dijak, S. Matthews, et al. 2018. "New England and Northern New York Forest Ecosystem Vulnerability Assessment and Synthesis: A Report from the New England Climate Change Response Framework project." *U.S. Department of Agriculture*. Forest Service, Northern Research Station. https://doi.org/10.2737/nrs-gtr-173

Jiang, J., K. C. Abbott, M. Baudena, M. B. Eppinga, J. A. Umbanhowar, and J. D. Bever. 2020. "Pathogens and Mutualists as Joint Drivers of Host Species Coexistence and Turnover: Implications for Plant Competition and Succession." *American Naturalist* 195(4): 591–602. https://doi.org/10.1086/707355.

Johnson, N. C., J. H. Graham, and F. A. Smith. 1997. "Functioning of Mycorrhizal Associations along the Mutualism–Parasitism Continuum." *New Phytologist* 135(4): 575–585. https://doi.org/10.1046/j.1469-8137.1997.00729.x.

jtourvi. 2024. "jtourvi/NE_Myco: NE_myco (v1.0)." Zenodo. https://doi.org/10.5281/zenodo.12689365.

Karban, R. 2021. "Plant Communication." *Annual Review of Ecology, Evolution, and Systematics* 52: 1–24. https://doi.org/10.1146/annurev-ecolsys-010421-020045.

Karst, J., M. D. Jones, and J. D. Hoeksema. 2023. "Positive Citation Bias and Overinterpreted Results Lead to Misinformation on Common Mycorrhizal Networks in Forests." *Nature Ecology & Evolution* 7: 501–511.

Kelly, C. N., J. B. Morton, and J. R. Cumming. 2005. "Variation in Aluminum Resistance among Arbuscular Mycorrhizal Fungi." *Mycorrhiza* 15(3): 193–201. https://doi.org/10.1007/s00572-004-0321-6.

Kiers, E. T., M. Duhamel, Y. Beesetty, J. A. Mensah, O. Franken, E. Verbruggen, and H. Bücking. 2011. "Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis." *Science* 333(6044): 880–82.

Kivlin, S. N., J. S. Lynn, M. R. Kazenel, K. K. Beals, and J. A. Rudgers. 2017. "Biogeography of Plant-Associated Fungal Symbionts in Mountain Ecosystems: A Meta-Analysis." *Diversity and Distributions* 23(9): 1067–77. https://doi.org/10.1111/ddi.12595.

Lankau, R. A., K. Zhu, and A. Ordonez. 2015. "Mycorrhizal Strategies of Tree Species Correlate with Trailing Range Edge Responses to Current and Past Climate Change." *Ecology* 96(6): 1451–58.

Lekberg, Y., and T. Helgason. 2018. "In Situ Mycorrhizal Function – Knowledge Gaps and Future Directions." *New Phytologist* 220(4): 957–962. https://doi.org/10.1111/nph.15064.

Lenoir, J., and J. C. Svenning. 2015. "Climate-Related Range Shifts – a Global Multidimensional Synthesis and New Research Directions." *Ecography* 38(1): 15–28. https://doi.org/10.1111/ecog.00967.

Lerat, S., R. Gauci, J. G. Catford, H. Vierheilig, Y. Piché, and L. Lapointe. 2002. "^{14}C Transfer between the Spring Ephemeral Erythronium Americanum and Sugar Maple Saplings via Arbuscular Mycorrhizal Fungi in Natural Stands." *Oecologia* 132(2): 181–87. https://doi.org/10.1007/s00442-002-0958-9.

Leyval, C., K. Turnau, and K. Haselwandter. 1997. "Effect of Heavy Metal Pollution on Mycorrhizal Colonization and Function: Physiological, Ecological and Applied Aspects." *Mycorrhiza* 7: 139–153.

Liang, M., X. Liu, R. S. Etienne, F. Huang, Y. Wang, and S. Yu. 2015. "Arbuscular Mycorrhizal Fungi Counteract the Janzen-Connell Effect of Soil Pathogens." *Ecology* 96(2): 562–574. https://doi.org/10.1890/14-0871.1.

Liang, M., X. Liu, G. S. Gilbert, Y. Zheng, S. Luo, F. Huang, and S. Yu. 2016. "Adult Trees Cause Density-Dependent Mortality in Conspecific Seedlings by Regulating the Frequency of Pathogenic Soil Fungi." *Ecology Letters* 19(12): 1448–56. https://doi.org/10.1111/ele.12694.

Liang, Y., M. J. Duveneck, E. J. Gustafson, J. M. Serra-Diaz, and J. R. Thompson. 2018. "How Disturbance, Competition, and Dispersal Interact to Prevent Tree Range Boundaries from Keeping Pace with Climate Change." *Global Change Biology* 24(1): e335–e351. https://doi.org/10.1111/gcb.13847.

Luo, Z. B., C. Wu, C. Zhang, H. Li, U. Lipka, and A. Polle. 2014. "The Role of Ectomycorrhizas in Heavy Metal Stress Tolerance of Host Plants." *Environmental and Experimental Botany* 108: 47–62. https://doi.org/10.1016/j.envexpbot.2013.10.018.

Lynn, J. S., M. R. Kazenel, S. N. Kivlin, and J. A. Rudgers. 2019. "Context-Dependent Biotic Interactions Control Plant Abundance across Altitudinal Environmental Gradients." *Ecography* 42(9): 1600–1612. https://doi.org/10.1111/ecog.04421.

Mašková, T., and T. Herben. 2018. "Root-to-Shoot Ratio in Developing Seedlings: How Seedlings Change their Allocation in Response to Seed Mass and Ambient Nutrient Supply." *Ecology and Evolution* 8(14): 7143–50. https://doi.org/10.1002/ece3.4238.

McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. "A New Method which Gives an Objective Measure of Colonization of Roots by Vesicular—Arbuscular Mycorrhizal Fungi." *New Phytologist* 115(3): 495–501. https://doi.org/10.1111/j.1469-8137.1990.tb00476.x.

Moyano, J., M. A. Rodriguez-Cabal, and M. A. Nuñez. 2020. "Highly Invasive Tree Species Are more Dependent on Mutualisms." *Ecology* 101(5): e02997.

Moyano, J., M. A. Rodriguez-Cabal, and M. A. Nuñez. 2021. "Invasive Trees Rely more on Mycorrhizas, Countering the Ideal-Weed Hypothesis." *Ecology* 102(5): e03330.

Nara, K. 2006a. "Ectomycorrhizal Networks and Seedling Establishment during Early Primary Succession." *New Phytologist* 169(1): 169–178. https://doi.org/10.1111/j.1469-8137.2005.01545.x.

Nara, K. 2006b. "Pioneer Dwarf Willow May Facilitate Tree Succession by Providing Late Colonizers with Compatible Ectomycorrhizal Fungi in a Primary Successional Volcanic Desert." *New Phytologist* 171(1): 187–198. https://doi.org/10.1111/j.1469-8137.2006.01744.x.

Newman, E. I. 1988. "Mycorrhizal Links between Plants: Their Functioning and Ecological Significance." In *Advances in Ecological Research*, Vol. 18, 243–270. New York: Academic Press.

Ni, M., and M. Vellend. 2023. "Soil Properties Constrain Predicted Poleward Migration of Plants under Climate Change." *New Phytologist.* 241: 131–141. https://doi.org/10.1111/nph.19164.

Nuñez, M. A., T. R. Horton, and D. Simberloff. 2009. "Lack of Belowground Mutualisms Hinders Pinaceae Invasions." *Ecology* 90(9): 2352–59. https://doi.org/10.1890/08-2139.1.

Paz, H. 2003. "Root/Shoot Allocation and Root Architecture in Seedlings: Variation among Forest Sites, Microhabitats, and Ecological Groups." *Biotropica* 35(3): 318–332. https://doi.org/10.1111/j.1744-7429.2003.tb00586.x.

Peay, K. G., and T. D. Bruns. 2014. "Spore Dispersal of Basidiomycete Fungi at the Landscape Scale Is Driven by Stochastic and Deterministic Processes and Generates Variability in Plant-Fungal Interactions." *New Phytologist* 204(1): 180–191. https://doi.org/10.1111/nph.12906.

Peterson, R. L., Y. Piche, and C. Plenchette. 1984. "Mycorrhizae and their Potential Use in the Agricultural and Forestry Industries." *Biotechnology Advances* 2(1): 101-IN2.

Pickles, B. J., R. Wilhelm, A. K. Asay, A. S. Hahn, S. W. Simard, and W. W. Mohn. 2017. "Transfer of ^{13}C between Paired Douglas-Fir Seedlings Reveals Plant Kinship Effects and Uptake of Exudates by Ectomycorrhizas." *New Phytologist* 214(1): 400–411. https://doi.org/10.1111/nph.14325.

Pither, J., B. J. Pickles, S. W. Simard, A. Ordonez, and J. W. Williams. 2018. "Below-Ground Biotic Interactions Moderated the Postglacial Range Dynamics of Trees." *New Phytologist* 220(4): 1148–60. https://doi.org/10.1111/nph.15203.

Prasad, A., J. Pedlar, M. Peters, D. McKenney, L. Iverson, S. Matthews, and B. Adams. 2020. "Combining US and Canadian Forest Inventories to Assess Habitat Suitability and Migration Potential of 25 Tree Species under Climate Change." *Diversity and Distributions* 26: 1142–59. https://doi.org/10.1111/ddi.13078.

Pucko, C., B. Beckage, T. Perkins, and W. S. Keeton. 2011. "Species Shifts in Response to Climate Change: Individual or Shared Responses?" *Journal of the Torrey Botanical Society* 138(2): 156–176. https://doi.org/10.3159/TORREY-D-10-00011.1.

R Development Core Team. 2022. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing http://www.R-project.org.

Robinson, D., and A. Fitter. 1999. "The Magnitude and Control of Carbon Transfer between Plants Linked by a Common Mycorrhizal Network." *Journal of Experimental Botany* 50(330): 9–13. https://doi.org/10.1093/jxb/50.330.9.

Schaberg, P. G., J. W. Tilley, G. J. Hawley, D. H. DeHayes, and S. W. Bailey. 2006. "Associations of Calcium and Aluminum with the Growth and Health of Sugar Maple Trees in Vermont." *Forest Ecology and Management* 223(1–3): 159–169. https://doi.org/10.1016/j.foreco.2005.10.067.

Selosse, M. A., F. Richard, X. He, and S. W. Simard. 2006. "Mycorrhizal Networks: Des Liaisons Dangereuses?" *Trends in Ecology and Evolution* 21(11): 621–28. https://doi.org/10.1016/j.tree.2006.07.003.

Sikes, B. A., K. Cottenie, and J. N. Klironomos. 2009. "Plant and Fungal Identity Determines Pathogen Protection of Plant Roots by Arbuscular Mycorrhizas." *Journal of Ecology* 97(6): 1274–80. https://doi.org/10.1111/j.1365-2745.2009.01557.x.

Simard, S., A. Asay, K. Beiler, M. Bingham, J. Deslippe, X. He, and F. Teste. 2015. "Resource Transfer between Plants through Ectomycorrhizal Fungal Networks." In *Mycorrhizal Networks* 133–176. Dordrecht: Springer.

Simard, S. W., K. J. Beiler, M. A. Bingham, J. R. Deslippe, L. J. Philip, and F. P. Teste. 2012. "Mycorrhizal Networks: Mechanisms, Ecology and Modelling." *Fungal Biology Reviews* 26(1): 39–60. https://doi.org/10.1016/j.fbr.2012.01.001.

Simard, S. W., D. A. Perry, M. D. Jones, D. D. Myrold, D. M. Durall, and R. Molina. 1997. "Net Transfer of Carbon between Ectomycorrhizal Tree Species in the Field." *Nature* 388(6642): 579–582. https://doi.org/10.1038/41557.

Sittaro, F., A. Paquette, C. Messier, and C. A. Nock. 2017. "Tree Range Expansion in Eastern North America Fails to Keep Pace with Climate Warming at Northern Range Limits." *Global Change Biology* 23(8): 3292–3301. https://doi.org/10.1111/gcb.13622.

Smith, S. E., and D. J. Read. 2010. *Mycorrhizal Symbiosis*. New York: Academic press.

Steidinger, B. S., T. W. Crowther, J. Liang, M. E. Van Nuland, G. D. Werner, P. B. Reich, and K. G. Peay. 2019. "Climatic Controls of Decomposition Drive the Global Biogeography of Forest-Tree Symbioses." *Nature* 569(7756): 404–8.

Sullivan, T. J., G. B. Lawrence, S. W. Bailey, T. C. McDonnell, C. M. Beier, K. C. Weathers, G. T. McPherson, and D. A. Bishop. 2013. "Effects of Acidic Deposition and Soil Acidification on Sugar Maple Trees in the Adirondack Mountains, New York." *Environmental Science and Technology* 47(22): 12687–94. https://doi.org/10.1021/es401864w.

Tedersoo, L., M. Bahram, and M. Zobel. 2020. "How Mycorrhizal Associations Drive Plant Population and Community Biology." *Science* 367(6480): eaba1223. https://doi.org/10.1126/science.aba1223.

Tourville, J. 2022. "Spatial Dynamics of Climate Induced Tree Range Shifts and the Influence of Biotic Interactions on Tree Seedling Establishment." Doctoral diss., College of Environmental Science and Forestry.

Tourville, J. C., J. W. Wason, and M. Dovciak. 2022. "Canopy Gaps Facilitate Upslope Shifts in Montane Conifers but not in Temperate Deciduous Trees in the Northeastern United States." *Journal of Ecology* 110: 2870–82. https://doi.org/10.1111/1365-2745.13993.

Tourville, J. C., M. R. Zarfos, G. B. Lawrence, T. C. McDonnell, T. J. Sullivan, and M. Dovciak. 2023. "Soil Biotic and Abiotic Thresholds in Sugar Maple and American Beech Seedling Establishment in Forests of the Northeastern United States." *Plant and Soil* 491: 387–400. https://doi.org/10.1007/s11104-023-06123-2.

Urcelay, C., S. Longo, J. Geml, and P. A. Tecco. 2019. "Can Arbuscular Mycorrhizal Fungi from Non-invaded Montane Ecosystems Facilitate the Growth of Alien Trees?" *Mycorrhiza* 29(1): 39–49. https://doi.org/10.1007/s00572-018-0874-4.

Van Der Heijden, M. G. A. 2004. "Arbuscular Mycorrhizal Fungi as Support Systems for Seedling Establishment in Grassland." *Ecology Letters* 7(4): 293–303. https://doi.org/10.1111/j.1461-0248.2004.00577.x.

Van Der Heijden, M. G. A., and T. R. Horton. 2009. "Socialism in Soil? The Importance of Mycorrhizal Fungal Networks for Facilitation in Natural Ecosystems." *Journal of Ecology* 97(6): 1139–50. https://doi.org/10.1111/j.1365-2745.2009.01570.x.

Van Der Putten, W. H., M. Macel, and M. E. Visser. 2010. "Predicting Species Distribution and Abundance Responses to Climate Change: Why it is Essential to Include Biotic Interactions across Trophic Levels." *Philosophical Transactions of the Royal Society B: Biological Sciences* 365(1549): 2025–34. https://doi.org/10.1098/rstb.2010.0037.

van't Padje, A., G. D. A. Werner, and E. T. Kiers. 2021. "Mycorrhizal Fungi Control Phosphorus Value in Trade Symbiosis with Host Roots when Exposed to Abrupt 'Crashes' and 'Booms' of Resource Availability." *New Phytologist* 229(5): 2933–44. https://doi.org/10.1111/nph.17055.

Wallander, H., and A. Ekblad. 2015. "The Importance of Ectomycorrhizal Networks for Nutrient Retention and Carbon Sequestration in Forest Ecosystems." In *Mycorrhizal Networks* 69–90. Dordrecht: Springer.

Wason, J. W., and M. Dovciak. 2017. "Tree Demography Suggests Multiple Directions and Drivers for Species Range Shifts in Mountains of Northeastern United States." *Global Change Biology* 23(8): 3335–47. https://doi.org/10.1111/gcb.13584.

Weemstra, M., L. Mommer, E. J. W. Visser, J. van Ruijven, T. W. Kuyper, G. M. J. Mohren, and F. J. Sterck. 2016. "Towards a Multidimensional Root Trait Framework: A Tree Root Review." *The New Phytologist* 211(4): 1159–69. https://doi.org/10.1111/nph.14003.

Wipf, D., F. Krajinski, D. van Tuinen, G. Recorbet, and P. E. Courty. 2019. "Trading on the Arbuscular Mycorrhiza Market: From Arbuscules to Common Mycorrhizal Networks." *New Phytologist* 223(3): 1127–42. https://doi.org/10.1111/nph.15775.

Wisz, M. S., J. Pottier, W. D. Kissling, L. Pellissier, J. Lenoir, C. F. Damgaard, and J. C. Svenning. 2013. "The Role of Biotic Interactions in Shaping Distributions and Realised Assemblages of Species: Implications for Species Distribution Modelling." *Biological Reviews* 88(1): 15–30.

Zarfos, M. R., M. Dovciak, G. B. Lawrence, T. C. McDonnell, and T. J. Sullivan. 2019. "Plant Richness and Composition in Hardwood Forest Understories Vary along an Acidic Deposition and Soil-Chemical Gradient in the Northeastern United States." *Plant and Soil* 438(1): 461–477. https://doi.org/10.1007/s11104-019-04031-y.

Zhu, K., C. W. Woodall, and J. S. Clark. 2012. "Failure to Migrate: Lack of Tree Range Expansion in Response to Climate Change." *Global Change Biology* 18(3): 1042–52. https://doi.org/10.1111/j.1365-2486.2011.02571.x.

Zobel, M., and M. Öpik. 2014. "Plant and Arbuscular Mycorrhizal Fungal (AMF) Communities – which Drives which?" *Journal of Vegetation Science* 25(5): 1133–40.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Tourville, Jordon C., Thomas R. Horton, and Martin Dovciak. 2024. "Mycorrhizal Fungi as Critical Biotic Filters for Tree Seedling Establishment during Species Range Expansions." *Ecological Monographs* 94(4): e1634. https://doi.org/10.1002/ecm.1634