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REGULAR ARTICLE

The effect of forest soil and community composition on ectomycorrhizal colonization and seedling growth

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Abstract Plant–soil feedbacks have been observed in many forest communities, but the role of the mycorrhizal community in perpetuating feedback loops is still poorly understood. Mycorrhizal community composition is closely linked to soil properties and host plant composition, which highlights their potential importance in plant–soil–fungus loops. Eastern hemlock (hemlock; *Tsuga canadensis*) seedlings were grown in soil bioassays in growth chambers and transplanted under closed forest canopy to examine the effect of hardwood and hemlock forest soil on seedling growth, survival, and ectomycorrhizal fungi (EMF) colonization. Seedlings propagated in hemlock forest soil had greater height growth compared with sterile control soil and achieved greater mycorrhizal colonization than seedlings grown in hardwood forest soils after 9 months in a growth chamber. Outplanted seedlings grown in hemlock communities achieved significantly greater increment growth than those seedlings grown in hardwood communities (mean height difference (95% CI)=0.39 cm (0.14–0.63 cm)), although final survival and EMF colonization was similar between forest types. EMF diversity (Shannon-Wiener index (SE)=1.88 (0.28) and 1.23 (0.44) for hardwood and hemlock, respectively) and community assemblage (Jaccard index (SE)=19.0% (4%)) differed between the two forest communities. EMF community assemblage was associated with both the forest type (i.e. plant community/microsite effects) and initial soil type (i.e. soil characteristics/resistant inoculum). The results support previously observed positive feedbacks between conspecifics under hemlock forest communities and provides evidence for the role of the EMF community within this feedback loop. Alternatively, the reduced growth of hemlocks under hardwoods may be attributed to the different EMF community associated with that forest.

Keywords Community assemblage · Eastern hemlock · Ectomycorrhizal fungi · Feedback loops · Species interactions · *Tsuga canadensis*

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Introduction

The success of a tree species at acquiring soil resources is largely dependent on the symbiotic

mycorrhizal fungi associated with the root tips. The importance of mycorrhizal fungi in seedling survival and establishment has been well studied in primary and secondary successional communities (Perry et al. 1989; Horton et al. 1999; Nara and Hogetsu 2004; Nara 2005; Ashkannejhad and Horton 2006). In the mycorrhizal context, northeastern temperate forests in the USA provide a unique landscape of communities with dominant tree species that associate primarily with arbuscular-mycorrhizal fungi (AMF), primarily with ectomycorrhizal fungi (EMF), or with both mycorrhizal types (Molina et al. 1992; Phillips and Fahey 2006). These mycorrhizal associations correlate with within-community positive feedback loops (i.e. spatial autocorrelation where seedling establishment is associated with conspecific overstory trees) and between-community negative feedback loops (i.e. negative reciprocal autocorrelation) (Frelich et al. 1993). Differences in mycorrhizal types may play an influential role in determining tree species distributions across this landscape.

Eastern hemlock (hemlock; *Tsuga canadensis* (L.) Carr.), an ectomycorrhizal species in the northeastern temperate forests, often forms distinct communities within a matrix of hardwood forests dominated by both arbuscular and ectomycorrhizal species (Davis et al. 1998; Catovsky and Bazzaz 2000). Hemlock communities are commonly self-perpetuating with increased seedling abundance under conspecifics relative to heterospecifics (Frelich et al. 1993). Many factors promote the formation and persistence of hemlock communities including topographic position, nutrient availability, and biotic interactions (Godman and Lancaster 1990; Van Breemen et al. 1997; Finzi et al. 1998a, b; Schwarz et al. 2003), but the role of mycorrhizal associations in preferential establishment of conspecific seedlings within these communities has not been examined (Woods 1984; Frelich et al. 1993; Catovsky and Bazzaz 2000). Hemlock seedling establishment success may be associated with differences in inoculum potential (i.e. quantity and quality of spores, sclerotia, and hyphae) of the soil within a plant community, and plant communities dominated by species related to establishing seedlings may have increased abundance of compatible EMF soil inoculum (Molina et al. 1992; Dickie et al. 2002, 2005; Weber et al. 2005).

Studies in many ecosystems have shown the importance of mycorrhizal fungi in contributing to plant species compositions (van der Heijden et al. 1998). Weber et al. (2005) provided an example of mycorrhizal mediated negative feedback loops where western red cedar (*Thuja plicata*), an obligate arbuscular species, had reduced growth and fungal colonization when growing in ectomycorrhizal dominated forest soil. In the temperate northeastern forests, Booth (2004) showed that restricting access to common mycorrhizal networks (CMNs) alters competitive dynamics of seedlings growing in an ectomycorrhizal forest. Further, shifts in forest community composition and successional stage affect the mycorrhizal fungal community composition (Kennedy et al. 2003; Ashkannejhad and Horton 2006; Ishida et al. 2007).

We hypothesized that hemlock establishment in hardwood forests developing following agriculture abandonment would support a different ectomycorrhizal community from adjacent old-growth hemlock, and that this would result in increased seedling growth, survival, and mycorrhizal colonization under the intact hemlock forest. The objectives of this study were: 1) to assess the effects of hardwood and hemlock dominated forest soils on hemlock seedling success (i.e. growth and survival); 2) to examine inoculated seedling growth and survival under hardwood and hemlock forest types; and 3) to determine if hemlock and hardwood dominated forests alter EMF assemblage and colonization percent on hemlock seedlings.

Methods

Study site

The study site was located at the Mianus River Gorge Preserve (MRGP; Lat:41.18°, Long: 73.62°), a 303 ha property approximately 64 km northeast of Manhattan in Westchester County, NY (Weckel et al. 2006). The main portion of the property runs north–south along the Mianus River and was approximately 3 km long and at most 0.6 km wide. The property had hemlock forest on 82 ha of which 24 ha were classified as old-growth (Weckel et al. 2006). The rest of the site was classified as second growth hardwoods dominated by red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), black birch (*Betula lenta*), yellow birch (*B. alleghaniensis*), American beech (*Fagus grandifolia*), white ash (*Fraxinus americana*), and oak (*Quercus* spp.). Due to heavy deer browse, neither community had a significant shrub or herbaceous layer except in

areas near the urban buffer which contained Japanese barberry (*Berberis thunbergii*) and Japanese stilt grass (*Microstegium vimineum*) and along riparian zones which contained various fern species (Weckel et al. 2006).

Field sampling was restricted to a contiguous MRGP section approximately 2.5 km by 0.5 km in size. This area restriction was set for two reasons: (1) to limit disturbance in ecologically sensitive areas (e.g. steep slopes and wetlands) and (2) to avoid the urban surroundings. The study area was approximately 115 ha of which 79 ha fell within the hardwood community and 36 ha within the hemlock.

Overall study procedure

Overstorey forest sampling was initiated in order to determine forest structure and identify soil collection sites under hemlock and hardwood communities. To measure the effects of different forest soils and resistant inoculum (i.e. spores and sclerotia) on establishing hemlock seedlings, soil was collected from each forest community and used in a 9 month ex situ soil bioassay. Bioassay seedlings were then outplanted into mature hardwood and hemlock forest communities to identify the effect of forest community interactions on seedling success. DNA sequencing was used to identify fungal species colonizing hemlock seedlings, and EMF diversity was compared between establishing hemlock seedlings harvested from hardwood and hemlock communities.

Soil bioassay treatment

A basic stand assessment of 56 plots (0.05 ha) was completed in August 2007. Based on the stand assessment, ten plots were chosen as soil collection sites for the ex situ bioassay (Supplement 1). Five plots were predominately hemlock, and five plots were dominated by hardwood with no hemlock. The criteria for plot selection was that hemlock plots had at least 40% hemlock basal area and some understorey hemlock while the hardwood plots had no hemlock in the understorey or overstorey. Each plot had <30% slope and an easterly aspect (25–160°).

In order to assess the effects of forest soil and associated resistant fungal inoculum on establishing hemlock, nine soil cores (5 cm dia.×15 cm depth) were taken within each plot. Samples were taken during fruiting season in August 2007, which provided collection of freshly released and latent fungal propagules. Soil cores were cleaned of rocks, roots, and debris, pooled by plot, paper bagged, and left to dry for 3 weeks to ensure that only resistant propagules were present as EMF inoculum. Field soil from each plot was mixed with autoclaved sand (1:1 ratio) and distributed among 33 seedling cone-tainers (Ray Leach, SC10 UV stabilized). An additional 33 cone-tainers were filled with autoclaved soil and sand (1:1 ratio) to serve as controls (363 cone-tainers in total).

Hemlock seeds (Sheffield's Seed Co., Locke, NY Lot # 060823) were surface sterilized in 30% H_2O_2 for 10 min, rinsed and soaked in gently stirring distilled water overnight, placed in autoclaved peat moss, and cold stratified for 70 days. One seed was placed in each cone-tainer and assigned a random location in a controlled chamber at a temperature of 24°C with 20% full-sunlight and a 16:8 light:dark schedule. If the seed did not germinate or died within 4 months of germination, then that cone-tainer was replanted (creating two ages for outplanting 9 and 5 month old). Prior to outplanting 12 hemlock soil seedlings, seven hardwood soil seedlings, and two controls were harvested and investigated for mycorrhizal colonization (see "EMF identification" section). The number of surviving hemlock and the effort to balance outplanting replication restricted the number of within-treatment destructive samples prior to outplanting.

Forest community outplanting

To test the effects of the predominate forest community on hemlock seedling establishment and root tip colonization, 210 seedlings were outplanted into six blocks (three blocks from the original hemlock and three blocks from the original hardwood soil plots; Supplement 1) on May 12th, 2008. Thirty-five seedlings (18 hemlock soil, 15 hardwood soil, and two control soil grown seedlings, respectively) were planted in a 5×7 grid at 30 cm spacing. Seedlings were stratified by age (9 month old seedlings or 5 month old replanted seedlings) to ensure equal representation of ages in each forest type and then randomly assigned to a block and planting point. The entire block was fenced with 1.25 cm mesh to prevent browse. Blocks were chosen to reduce site

variation in factors such as slope, aspect, and basal area and to increase differences in local tree mycorrhizal types (Supplement 1; Fig. 1). The hemlock blocks were dominated by hemlock (>40% basal area and >60% of all stems) with a few individuals of birch and sugar maple. Two of the hardwood blocks were solely red and sugar maple. The third hardwood block was mostly tulip tree (*Liriodendron tulipifera*) with 30% of the basal area consisting of black birch.

Initial height was recorded followed by biweekly growth and mortality measurements. All seedlings were measured for final height on October 10th, 2008. Eleven seedlings from each block were harvested (one from each initial hemlock soil treatment, one from each initial hardwood soil treatment, and one control). Due to high mortality in one hemlock block, only eight seedlings were harvested (four hemlock, three hardwood, and one control). The largest seedling from each of the original soil bioassay treatments was selected for harvest. Soil was removed from the roots and entire seedlings were stored at 4°C for no more than 1 week until EMF identification (see "EMF identification" section).

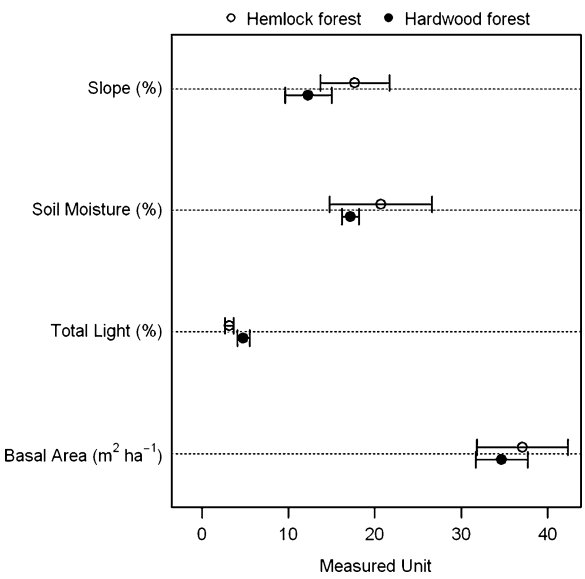

Fig. 1 Mean (SE) of total overstory basal area, percent total light as estimated from hemispherical photos, percent volumetric soil moisture, and percent slope at each outplant plot. There were no significant differences between the two field sites (N= 3 for hemlock and N=3 for hardwoods)

Soil and light conditions

Soil was measured for volumetric soil moisture content monthly at 12 locations within the planting grid at each block using a Hydrosense 620 soil moisture probe (Campbell Scientific, Logan, UT). These measurements were averaged to obtain a soil moisture value for each block per month. Hemispherical photographs were taken at the grid center with a Coolpix 4500 digital camera and FC-E8 fish-eye lens (Nikon Corp., Chiyoda-ku, Tokyo). All photos were taken at 50 cm above the ground and oriented north. Photos were taken with even cloud cover on August 7th, 2008. Following Zhang et al. (2005) proper F-stop settings were determined using an open field reference photo prior to each closed canopy photo. Photos were digitized using Sidelook 1.1 to set grey level thresholding (Nobis and Hunziker 2005). Total percent light transmittance was estimated using Gap Light Analyzer 2.0 (Frazer et al. 2000).

EMF identification

Ectomycorrhizal morphology based on color, mantle texture, and extramatrical hyphae was used to identify distinctive fungal types for each seedling. Root tips with inconspicuous external features (e.g. smooth brown mantles) were inspected under a compound microscope (400×) for presence of mantle and hartig net. Morphotypes were conservatively grouped for each seedling in order to decrease the possibility of combining unique species. The number of initial morphotypes ranged from one to nine per seedling.

A restricted fragment length polymorphism (RFLP) pattern was produced for one root tip of each unique morphotype per seedling in order to confirm conservative grouping. The internal transcribed spacer (ITS) region of nuclear DNA coding for ribosomal RNA (rDNA) was amplified by polymerase chain reaction (PCR) using ITS1-f and ITS4 primers (Gardes and Bruns 1996). The ITS region was digested using *Dpn*II and *Hinf*I restriction enzymes following manufacturer recommendations (New England BioLabs), and the RFLP was separated using gel electrophoresis in 3% agarose gels, stained with ethidium bromide, and digitally photographed (EpiChemi II Darkroom; UVP Laboratory Products, Upland, CA, USA). RFLP patterns across

all gels were visually grouped with one to four types per seedling.

The ITS from each unique RFLP type was sequenced at Cornell University's DNA Sequencing Facility using an Automated 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were manually checked and edited using Finch TV v 1.4.0 (Geospiza) and the basic local alignment search tool (BLAST; National Center for Biotechnology Information) was used to identify sequences. Closely related sequences were tested for similarity using Geneious basic v 4.5.4 and were considered identical species if >97% similar. Two unique RFLP types consistently failed to produce quality template necessary for sequencing and were therefore designated as 'Unknown RFLP Type'.

Statistical analyses

The effect of soil type on growth prior to seedling outplanting was assessed with an ANOVA and compared with least significant differences (lsd). Differences in seedling survival between each soil type prior to seedling outplanting were minor so no tests were done. Significant differences in outplanted seedling height between forest community types were tested to ensure no prior treatment effect. All analyses were done in the R statistical environment v 2.10.1 (R Development Core Team 2010).

The combined effects of treatments (soil bioassay treatment and outplanted community treatment as a 3×2 factorial design) on individual seedling growth, survival, and percent fungal root tip colonization following 5 months of in situ growth were assessed with linear-mixed models. This analysis was used due to the unbalanced and pseudo-replicated block design. Growth was analyzed using the lme function in the nlme library, and survival and percent colonization were analyzed using the lme4 library with *p*-value estimates generated with a Markov chain Monte Carlo sample using pvals.fnc and aovlmer.fnc functions in the languageR library (Pinheiro and Bates 2000; Pinheiro et al. 2008; Bates 2005; Baayen 2010; Supplement 2).

The random effects tested for all three response variables were initial soil plot, outplanted forest block, and initial soil type crossed in outplanted forest. Akaike information criterion was used for model comparison which resulted in growth being dependent on both soil bioassay type and forest community type as additive fixed effects with initial soil collection plot as the random effect; survival being dependent on forest community type as the sole fixed effect and initial soil collection plot crossed in outplant block as the random effect; and EMF colonization being dependent on soil bioassay type, forest community type and their interaction as fixed effects and initial soil collection plot crossed in block as the random effect (Pinheiro and Bates 2000; Pinheiro et al. 2008; Bates 2005; Supplement 2).

Fungal diversity within each site was quantified with Shannon-Wiener index, and the Jaccard index was used to compare similarity of EMF communities between hemlock and hardwood blocks. Sampling effectiveness was assessed using species pool estimates from species accumulation curves using the Chao index in the vegan package (Chao 1987; Colwell and Coddington 1994; Oksanen et al. 2010). EMF community similarity on seedlings following harvest was analyzed with nonmetric multidimensional scaling (NMDS) ordination in order to elucidate the effects of treatments on final EMF assemblage. EMF community composition on seedlings grouped by the initial soil type, the outplanted community type, and outplant block was normalized and assessed for dissimilarity using the metaMDS command in the vegan package. The Bray-Curtis dissimilarity function was used to calculate the distance matrix (Oksanen et al. 2010). All EMF species found on only one seedling were excluded from the analysis (as there was no replication) leaving 17 EMF species in the Jaccard calculation and the ordination.

Results

Seedling success after soil bioassay treatment

Seedling survival in the growth chamber was 77.8%, 84.0%, and 81.7% for the control, hemlock, and hardwood, respectively. Growth was significantly different between control soil grown seedlings and the hemlock soil, but not between any other treatment combination. The mean heights (lsd) were 2.08 cm (±1.66), 3.92 cm (±1.76), and 3.21 cm (±1.78) for control, hemlock, and hardwood soil treatments, respectively. Eleven of the 12 pre-outplant seedlings

from the hemlock soil were colonized with fungi (91.6%), one of the seven seedlings from the hardwood soil was colonized (14.2%), and neither of the controls were colonized. Five RFLP types were found on the seedlings grown in the growth chamber. Unknown RFLP Type I was specific to the hardwood soil treatment (Fig. 2).

Seedling success after community treatment

There was no statistical difference in the mean site characteristics between the three hemlock and three hardwood blocks (Basal Area $F_{1,5}=0.16$, $p=0.71$; Light $F_{1,5}=4.0$, $p=0.12$; Soil Moisture $F_{1,5}=0.35$, $p=0.57$; Slope $F_{1,5}=1.19$, $p=0.34$), but the hemlock blocks had more basal area and less light (Fig. 1). Initial seedling heights between forest communities were not statistically different ($F_{1,209}=6.35$, $P=0.40$). Mean (SE) for hemlock community seedlings and hardwood community seedlings were 1.65 cm (0.10) and 1.27 cm (0.10), 80.0% (13.5) and 92.4% (1.9), and 92.5% (2.1) and 94.3% (1.2) for growth, survival, and colonization, respectively.

Forest community type had a significant influence on outplanted seedling increment growth regardless of soil bioassay types ($F_{1,173}=9.97$, $p=0.002$), and seedlings initially grown in forest soil outperformed non-mycorrhizal controls at marginally significant levels($F_{2,8}=3.27$, $p=0.09$). The seedlings planted in hemlock communities had greater growth than seedlings in hardwood communities (mean height growth difference (95% C.I.)=0.39 cm (0.14–0.63)) across all initial soil treatments (Fig. 3a).

Forest community type did not significantly affect survival ($p=0.36$; Fig. 3b). There was relatively low variability between forest communities with mortality ranging between 0–14.3% and 5.7–11.4% for hemlock and hardwood blocks, respectively except in one hemlock block that had 45.7% mortality. The high mortality in this block was likely related to mammal disturbance from soil tunneling and browse (O'Brien, *personal observation*).

Initial soil type and forest community did not significantly affect EMF colonization (Soil type: $p=0.36$; Community type: $p=0.65$; Interaction: $p=0.92$; Fig. 3c). Seedlings grown in the hardwood commu-

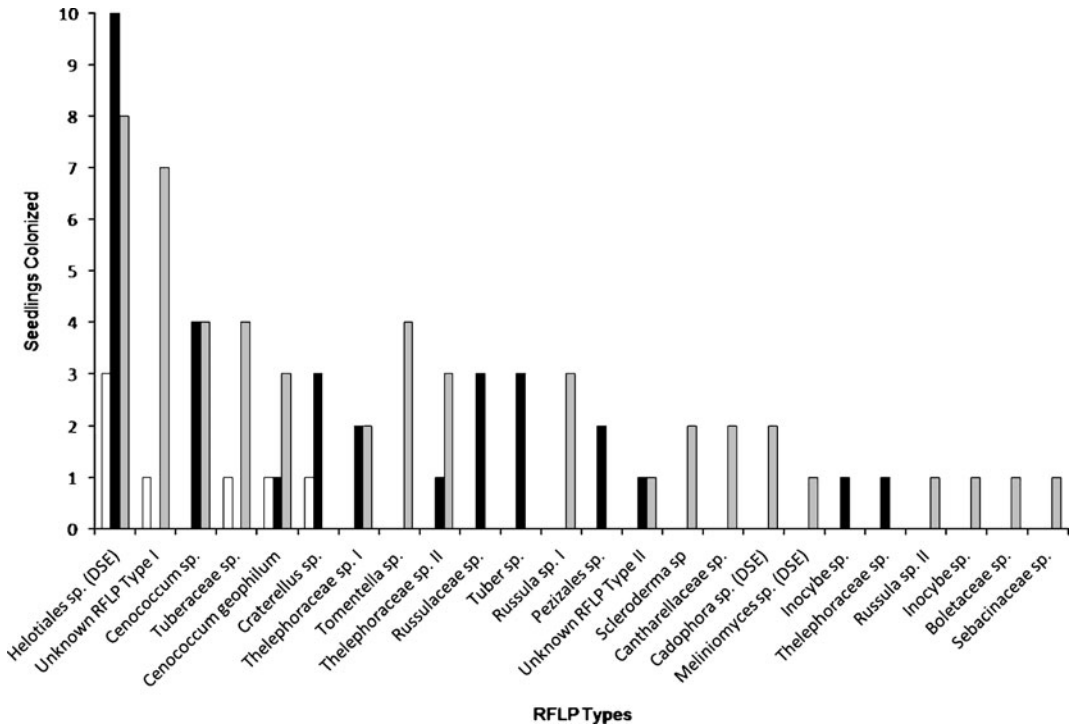

Fig. 2 The rank abundance of fungal species by the number of seedlings colonized. *White bars* are seedlings harvested from the soil bioassay (Unknown RFLP Type I is from hardwood soil treatments.), *gray bars* are seedlings harvested from the hardwood community, and *black bars* are seedlings harvested from the hemlock community

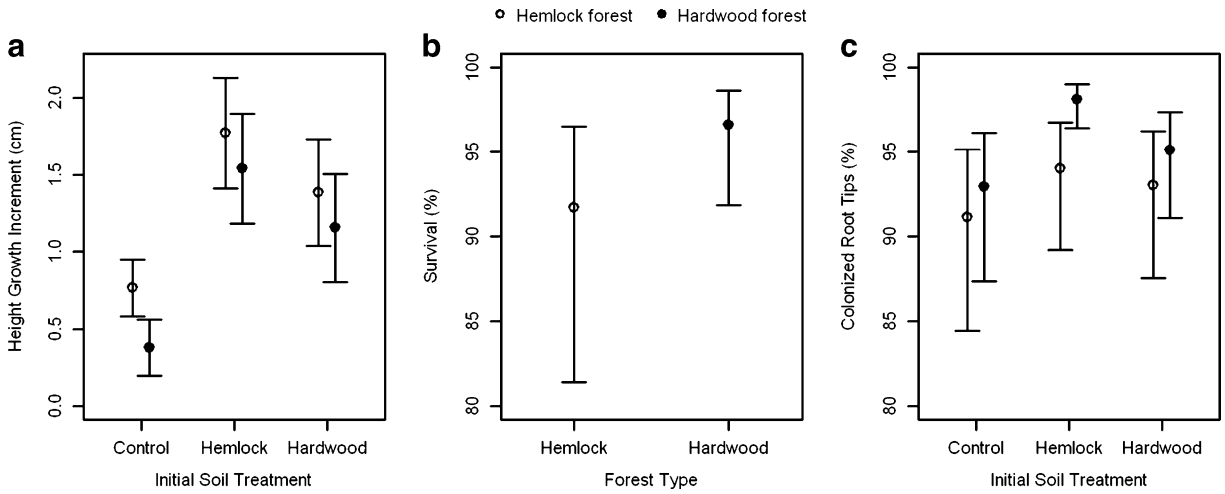

Fig. 3 Mean (SE) estimates of 5 month growth, survival and colonization following outplanting in the forest understory from the optimal models. **a** Height growth was affected by initial soil treatment (y-axis) and outplant forest type (*open circles*-hemlock and *closed circles*-hardwood). **b** Survival was affected solely by outplant forest type. **c** Colonization was affected by initial soil treatment (y-axis) and outplant forest type

nities had higher root tip colonization than seedlings grown in the hemlock community with those differences being greatest for the initial hemlock soil grown seedlings (Fig. 3c).

EMF community after final harvest

Twenty-four RFLP types were identified, 12 of which were found on the seedlings grown in the hemlock community and 18 on the seedlings grown in the hardwood community (Fig. 2). GenBank accession numbers are presented in Supplement 3. The hemlock fungal community on harvested seedlings shared two types with those identified on soil bioassay seedlings while the hardwood shared three types. Unknown RFLP Type I was found solely on seedlings grown in the hardwood soil bioassay inoculum and on seedlings harvested from the hardwood community. Six types were found in both communities, six were unique to the hemlock, and 12 were unique to the hardwood. The Jaccard index (SE) indicated only 19% (4%) community similarity. The hemlock community had lower Shannon-Wiener index compared to the hardwood indicating that the hemlock community had lower species diversity (Table 1). The majority of the diversity in the hardwood community came from the plot with a large proportion of birch. Eight species were unique from other hardwood plots and six species were unique from all other plots. The hemlock community treatment had a lower estimated fungal community size than the hardwood community (Table 1).

The ordination showed that EMF community composition was dependent on forest type (i.e. plant community/microsite characteristics (NMDS 1)) and initial soil type (i.e. soil characteristics/resistant inoculum (NMDS2)) (Fig. 4). Most seedlings from hemlock and control soil treatments grouped on the positive side of NMDS 2 and hardwood on the negative. While seedlings from the same planting blocks grouped together. The only exception to this was the third hardwood block, which had a similar ectomycorrhizal community composition as hemlock block 3 (Fig. 4). Hemlock forest blocks one and two clustered along NMDS axis 1 (Fig. 4) and hardwood forest blocks clustered in the middle along NMDS axis 1 (Fig. 4).

Discussion

Despite more than 70 years since agricultural abandonment and an established canopy of hardwoods, growth, survival and mycorrhizal infection of hemlock were all reduced in post-agricultural hardwood forest soil compared to intact old-growth hemlock soil. Additionally, seedling growth was significantly lower under hardwood forest canopy. Although our

Table 1 Mean (SE) of Shannon-Wiener diversity index of EMF for each treatment (N=1 and 3 for soil bioassay and community treatments). Observed species number and estimated population size (SE) of EMF on seedlings harvested from soil and outplanted forest community treatments (N=19 and 27 for hemlock and hardwood forests)

	Treatment	Shannon-Wiener diversity	Observed species #	Estimated species pool (SE)
Soil bioassay	Hardwood	0 (NA)	1	NA
	Hemlock	0.86 (NA)	4	NA
Community	Hardwood	1.88 (0.28)	18	23 (4.8)
	Hemlock	1.23 (0.44)	12	16 (4.9)

analyses cannot clearly distinguish between the effect of successional stage and forest composition, the long time-period since agricultural abandonment and the results from the ordination indicate that community composition is playing a strong role in EMF composition and in turn seedling success.

Growth and root tip colonization in forest soil treatments

In an ex situ environment, hemlock seedlings grew better in hemlock forest soil than in control soil and achieved greater EMF colonization than hardwood forest soils. The hardwood forest developed after agricultural fields were abandoned (Weckel et al. 2006), and the soil propagule bank would have been disturbed due to historical agricultural practices (i.e. physical soil disturbance and conversion to arbuscular-mycorrhizal dominated plants). Arbuscular-mycorrhizal soil propagules would facilitate establishment of arbuscular-mycorrhizal trees as evident by the dominance of maple and tuliptree which in turn has perpetuated a soil propagule bank dominated by AMF. Removing ectomycorrhizal forest vegetation would decrease EMF soil inoculum and hinder seedling establishment success (Perry et al. 1989). Conversely, the hemlock community has remained intact, and the development of the soil propagule bank has been relatively unimpeded. There were also larger quantities of ectomycorrhizal sporocarps observed under the hemlock overstorey, which would directly increase spore presence in the soil of that community (O'Brien, *personal observation*).

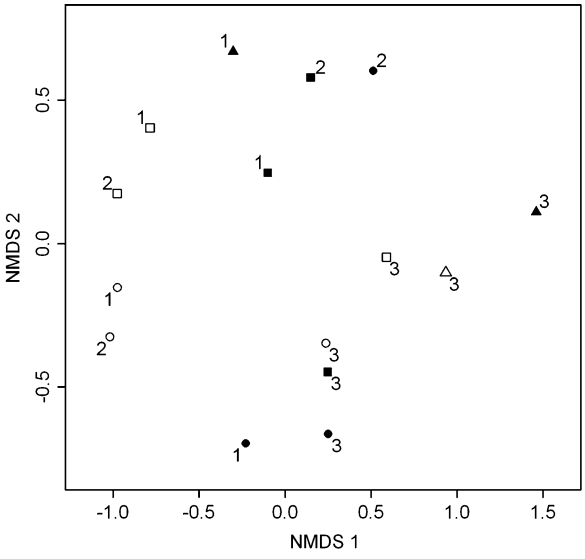

Fig. 4 NMDS ordination of EMF community composition on seedlings. *Shapes* represent different soil treatments (*circles*-hardwood soil; *squares*-hemlock soil; *triangles*-control soil), *fill* represents different outplant forest types (*solid*-hardwood forest; *open*-hemlock forest), and *numbers* represent different plots

The lack of ectomycorrhizal tree species in the hardwood overstorey was likely the greatest limitation on ectomycorrhizal soil inoculum in the hardwood stand. The dominance of arbuscular-mycorrhizal tree species equates to, fewer ectomycorrhizal root tips, and lower EMF soil inoculum (Haskins and Gehring 2005; Weber et al. 2005). Additionally, differences in fungal host specificity between plant families may be another factor influencing EMF colonization (Molina et al. 1992; Ishida et al. 2007). The ectomycorrhizal hardwood trees may associate with some EMF species that do not associate with hemlock which may be evident by the different species assemblages on seedlings (Fig. 2). Only generalist fungi would be shared between angiosperms and gymnosperms (Molina et al. 1992). Turner et al. (2009) found that oak seedlings had lower ectomycorrhizal colonization and diversity when planted in soil from hemlock stands which provides evidence that limited fungi may be shared between oak and hemlock. Although this reduction in inoculum was evident in the growth

chambers, inoculum was not limiting for the seedlings in the forest communities which implies that there may be few resistant spores in the hardwood soil but abundant hyphal inoculum.

Root tip colonization in forest communities

Seedlings in the hardwood community had more colonized root tips (Fig. 3c). The biological implications of this increased colonization were likely unimportant as seedlings in all treatments were highly colonized (i.e. large mean and small standard errors) by the end of the growing season. For example, on average seedlings in hardwood communities at most had only 4.1% more colonization than those from the hemlock community (98.1% vs. 94.0%) which likely had negligible biological impacts (Fig. 3c).

Growth rates in forest communities

Growth was greater for hemlock seedlings outplanted into the hemlock community (Fig. 1). Edaphic factors were not accounted for in this study, but in a companion study, hemlock communities had significantly lower soil nutrients than hardwood communities (mean (SE) pH 4.02 (0.04) versus 4.3 (0.03); C:N ratio 22.4 (0.41) versus 17.9 (0.37); P 20.2 (1.7) versus 30.4 (3.7) ppm) at Harvard, Hubbard Brook, and Bartlett forests which is consistent with other studies on the soil chemistry associated with these tree species (Van Breemen et al. 1997; Finzi et al. 1998a, b). Although the effects of these contrasting soils on seedling dynamics is not fully understood, it has been observed that the soil chemistry under hemlock inhibits establishment of other tree species such as sugar maple (Frelich et al. 1993; Kobe et al. 1995) while hemlock seedlings have a tolerance to the low pH and nutrient availability. There is little evidence that hemlock seedlings grow better in low nutrient soil conditions, and some evidence shows that hemlock does have improved growth in higher nitrogen soils such as the soil found under a sugar maple overstorey (Bigelow and Canham 2002). The soil derived from the hardwood community should improve hemlock seedling growth, but the results contradict this in both the competitive forest environment (Fig. 3a) and in the absence of the overstorey interaction within the growth chamber. Additionally, soil moisture is commonly observed as the limiting variable in hemlock seedling establishment (Lewin 1974; Rogers 1978; Godman and Lancaster 1990), but the 2008 growing season had continuously wet conditions which removed the importance of this variable by equilibrating moisture conditions between forest communities (Fig. 1). Given edaphic factors and site characteristics did not account for the differences in hemlock seedling growth between these two communities, below ground biotic interactions were likely important.

Differences in EMF with forest communities

The EMF community composition on hemlock seedlings was influenced by plant assemblage, outplanting location, and initial soil treatment (Fig. 4). Although the mechanisms driving the EMF community composition at each block were not distinguished, factors associated with the two forest communities were impacting the EMF assemblage. These factors likely included but were not limited to differences in edaphic factors, disturbance history, and surrounding forest matrix composition (Reynolds et al. 2003).

The one exception to the hemlock and hardwood community delineations in the ordination was the third hardwood block which grouped near a hemlock block (Fig. 4). This third hardwood block contained an ectomycorrhizal hardwood component which would alter the EMF soil inoculum from the other two hardwood blocks lacking ectomycorrhizal trees. Generalist species can be shared between plant species related beyond the level of family (Molina et al. 1992). Other studies have shown similar EMF taxa on unrelated plant species (Horton et al. 1999; Kennedy et al. 2003; Nara and Hogetsu 2004; Ishida et al. 2007). Therefore, the EMF community on establishing hemlock seedlings would likely vary with changes in tree species composition as well as the edaphic factors associated with a specific site (Johnson et al. 1997; Reynolds et al. 2003).

Conclusions

This research provides an example in which ectomycorrhizal seedling success is impacted by changes in the mycorrhizal community composition. Frelich et al. (1993) observed a negative reciprocal spatial autocorrelation between hemlock and sugar maple.

The contrasting EMF and AMF associations appear to have an influence on this spatial correlation through interactions between mycorrhizal types. In this process, the plants facilitate the propagation of the mycorrhizal symbiont which contributes to the establishment success of additional conspecific individuals (Jonsson et al. 1999). This feedback loop decreases the presence of the alternative fungal types and in doing so hinders the establishment success of opposite fungal plant hosts (Horton et al. 1999; Horton et al. 2005). The fungal-plant interaction works in conjunction with additional mechanisms such as soil nutrient alterations due to differences in nutrient acquisition, plant physiology, and leaf chemistry (Frelich et al. 1993; Van Breemen et al. 1997; Finzi et al. 1998a, b; Templer and Dawson 2004; Ehrenfeld et al. 2005).

Hemlock derived soil increased seedling success in growth chambers, and seedlings planted under a hemlock overstorey and initially grown in hemlock soil grew significantly better than seedlings planted under hardwood trees and grown from hardwood derived soil. Differences in EMF communities in the two forest soils and the increased likelihood of common mycorrhizal networks under conspecifics may offer additional mechanisms which may contribute to improved hemlock seedling establishment success under mature hemlocks. This research highlights the importance of examining the role of EMF in niche partitioning and species persistence across the temperate forest landscape.

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