Light availability and soil source influence ectomycorrhizal fungal communities on oak seedlings grown in oak- and hemlock-associated soils[1]

Gregory D. Turner, J.D. Lewis, J.T. Mates-Muchin, William F. Schuster, and Laura Watt

Abstract: Forests exhibit spatial heterogeneity in plant composition and light, which may influence ectomycorrhizal fungal (ECM) communities. We investigated whether light and soil source affect ECM colonization and community properties on red oak (*Quercus rubra* L.) seedlings. Seedlings were grown under 10%, 45%, and full sunlight in soils removed beneath red oak and eastern hemlock (*Tsuga canadensis* (L.) Carr.) trees. Between soils, colonization and diversity were significantly greater in intermediate–high versus low light. Across light levels, colonization, richness, and diversity were greater on seedlings grown in oak versus hemlock soils. The frequency of seedlings colonized by three of the four most common morphotypes was more responsive to light in oak versus hemlock soil. Colonization differences between soil sources were associated with differences in richness, which may in turn reflect host specificity and fine root length differences. Increasing colonization with increasing light was associated with increased richness, which in turn may reflect increased carbon allocation to roots. Results suggest that differences in responses of individual ECM morphotypes coupled with host responses to light and soil source may influence ECM colonization and diversity. Changes in ECM colonization and diversity could in turn affect seedling recruitment, especially for seedlings encountering variable light regimes and host species.

Résumé : Dans les forêts, l'hétérogénéité spatiale de la composition végétale et de la luminosité peut influencer les communautés de champignons ectomycorhiziens (ECM). Nous avons étudié si la luminosité et la source du sol influencent les propriétés de la communauté et la colonisation des ECM sur les semis de chêne rouge (*Quercus rubra* L.). Les semis ont été cultivés dans différentes conditions de luminosité (10 %, 45 % et 100 % du plein soleil) dans des sols prélevés sous des chênes rouges et des pruches du Canada (*Tsuga canadensis* (L.) Carr.). Dans les différents sols, la colonisation et la diversité étaient significativement plus élevées dans des conditions de luminosité intermédiaire à élevée plutôt que faible. Parmi les différents niveaux de luminosité, la colonisation, la richesse et la diversité étaient plus élevées sur les semis cultivés dans le sol du chêne que dans celui de la pruche. La fréquence des semis colonisés par trois des quatre morphotypes les plus communs réagissait davantage à la luminosité dans le sol du chêne que dans celui de la pruche. Les différences de colonisation entre les sources de sol étaient associées à des différences de richesse qui peuvent à leur tour refléter des différences liées à la spécificité de l'hôte et à la longueur des racines fines. L'augmentation de la colonisation avec l'accroissement de la luminosité était associée à l'augmentation de la richesse qui peut à son tour refléter une allocation accrue de carbone aux racines. Les résultats indiquent que des différences de réaction des morphotypes d'ECM individuels couplées à la réaction de l'hôte à la luminosité et à la source de sol peuvent influencer la diversité et la colonisation des ECM. Les changements dans la diversité et la colonisation des ECM pourraient à leur tour avoir un effet sur le recrutement des semis, particulièrement dans le cas des semis confrontés à des espèces hôtes et à des régimes de luminosité qui varient.

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G.D. Turner,[2,3] J.D. Lewis, and J.T. Mates-Muchin. Louis Calder Center – Biological Station, Fordham University, 53 Whippoorwill Road, Armonk, NY 10504, USA.
W.F. Schuster. Black Rock Forest Consortium, 129 Continental Road, Cornwall, NY 12518, USA.
L. Watt. Northland College, 1411 Ellis Avenue, Ashland, WI 54806, USA.

[1]Contribution No. 225 of the Louis Calder Center and Biological Station, Fordham University, Armonk, New York.
[2]Corresponding author (e-mail: gturner@wcupa.edu).
[3]Present address: West Chester University of Pennsylvania, Department of Biology, 750 S. Church Street, West Chester, PA 19383, USA.

Introduction

Spatial variation in light availability is a key factor driving differential seedling growth in heterogeneous habitats, such as forest understories (Catovsky and Bazzaz 2000). In addition to direct effects on growth, light availability may indirectly affect growth through effects on colonization by mycorrhizal fungi (Graham et al. 1982). Because mycorrhizal fungi play a central role in nutrient uptake and other processes in most plants, colonization changes generally lead to corresponding changes in plant growth (Smith and Read 2008). Plant growth is also influenced by mycorrhizal fungal community composition (Smith and Read 2008), which may vary with light availability if the effects of light availability on colonization vary among fungal species. Despite potential effects of fungal community composition on plant growth, relatively little is known about the effects of light availability on fungal communities or about the variation among fungal species in the sensitivity of mycorrhizal formation to host light environment.

Host light environment influences root colonization by both ectomycorrhizal fungi (ECM) and arbuscular mycorrhizal fungi primarily through effects on host carbohydrate production (Graham et al. 1982; Harley and Smith 1983; Son and Smith 1988). Soluble carbohydrate concentrations in plants generally increase with increasing light (Dixon et al. 1981). Accordingly, root carbohydrate concentrations tend to be greater in plants grown under high light (Björkman 1970; Hacskaylo 1973). Greater carbohydrate availability in host roots has been shown to stimulate mycorrhizal colonization (Björkman 1970; Marx et al. 1977), and can lead to greater mycorrhizal percent colonization (Lewis et al. 1994). However, because mycorrhizal fungal species may differ in their responsiveness to changes in carbohydrate supply (Parrent et al. 2006), increasing carbohydrate availability may also affect fungal diversity on hosts. Thus, increasing light may be expected to increase mycorrhizal fungal colonization and species diversity.

Studies examining effects of host light environment on mycorrhizal fungal colonization, however, have yielded mixed results. Increasing light availability has shown positive (Ekwebelam and Reid 1983; Harley and Smith 1983) or no (Mcinnes and Chilvers 1994) effects on numbers of root tip colonized by ECM. Studies examining light effects on root colonization by arbuscular mycorrhizal fungi also have yielded mixed results, with increasing light availability leading to increased colonization in some studies (Ferguson and Menge 1982; Tester et al. 1986) but resulting in no significant effects on colonization in other studies (Smith and Gianinazzi-Pearson 1990). One explanation for these differences may be that host plants and associated fungal communities vary in their sensitivity to changes in light availability.

Diverse plant assemblages are common in temperate forests (Pastor and Broschart 1990). Plants in these forests exhibit a broad range of shade tolerances (Davis et al. 1996), and differential host responses to light may influence ECM fungal properties (Zhou et al. 1997). Shade-tolerant hosts, such as eastern hemlock (*Tsuga canadensis* (L.) Carr.), generally have lower photosynthetic rates, growth rates, and growth responses to increased light than less shade-tolerant hosts, such as northern red oak (*Quercus rubra* L.) (Kelty 1986; Canham 1989; Lewis et al. 2000). Shade-tolerant hosts also may allocate less biomass to roots compared with less shade-tolerant hosts (Loach 1970), suggesting that root carbohydrate availability may be lower in shade-tolerant hosts than in less shade-tolerant hosts. These differences in host physiology may result in differences in the efficiency of carbohydrate acquisition by ECM fungi associated with shade-tolerant and less shade-tolerant hosts (Molina et al. 1992; Gardes and Bruns 1996; Horton and Bruns 1998). In turn, differences in carbohydrate requirements may affect the relative responsiveness of ECM species to increases in light availability.

ECM specificity for particular host trees may also confound effects of increasing light availability on ECM community composition. ECM specificity refers to the degree of affinity between ECM symbionts, where ECM species with high affinities for specific host species are referred to as "specialists" (Molina et al. 1992). For example, certain fungal species are found primarily in association with hardwood trees, whereas others are found primarily, or only, in association with conifers (Kretzer and Bruns 1999; Dahlberg 2001). Increasing light availability may not increase diversity if the pool of potential colonists consists of fungal species specific for tree species other than the host. In eastern US forests, for example, where eastern hemlock and hardwood stands are intermixed, seedlings may encounter very different ECM communities over very short distances and exhibit markedly different recruitment in each location (Lewis et al. 2008). Although responses of ECM communities to differences in light availability and host specificity alone have been examined, responses to interactions between light availability and host specificity have not.

In this study, we examined effects of host light environment on ECM communities on red oak seedlings grown in soil cores removed from beneath oak and eastern hemlock trees in oak- and hemlock-dominated stands, respectively. Cores were taken from beneath red oak and eastern hemlock trees because ECM may exhibit differential specificity to these hosts and because these trees differ in shade tolerance and associated physiological characteristics that may influence root carbohydrate allocation and, thus, ECM fungal interactions with these hosts. Red oak was used as the bait seedling species because it occurs in both hardwood- and hemlock-dominated stands in the eastern United States (Braun 1950); although it is less shade-tolerant than eastern hemlock, its seedlings grow under a range of light levels (Loach 1967). Our objectives were to determine (*i*) whether ECM root tip percent colonization and ECM community properties vary among seedlings grown under different light environments, (*ii*) whether these measures vary between seedlings grown in soils from contrasting host species, and (*iii*) whether ECM communities associated with the contrasting host soil sources differ in response to host light environment. ECM root tip percent colonization was assessed as the percentage of the total number of root tips on a seedling that were colonized (i.e., percent colonization), and ECM community properties were compared using standard morphotyping techniques to differentiate morphotypes (Ingleby et al. 1990).

Methods

Study area and site descriptions

The sites used as sources for the soil cores in this study were located in Black Rock Forest, Orange County, New York, USA (41′N, 74′W). Black Rock Forest is a 1500 ha natural area operated as a research station within the New York – New Jersey Highlands Physiographic Province, which overlies Precambrian gneiss, schists, and limestones (Schuberth 1968). All sites were located in the Black Rock Brook watershed at elevations ranging from 160 to 340 m above mean sea level. Red oak, chestnut oak (*Quercus prinus* L.), and eastern hemlock dominate host tree composition in this watershed (J.T. Mates-Muchin and J.D. Lewis, Fordham University, unpublished data). Oak- and hemlock-dominated plots were randomly chosen from 33 plots established in 1985 (Friday and Friday 1985). Plots were delineated based on an index of importance, which was calculated as the mean of oak or hemlock basal area and density per plot in relation to that of all trees found on a plot (McEwan and Muller 2006; Weber et al. 2006). Three 40 m × 40 m (0.16 ha) oak-dominated (i.e., relative importance >50% oak) and three 40 m × 40 m hemlock-dominated (i.e., relative importance >50% hemlock) plots were designated for this study. No two plots were <500 m from one another.

Field coring and greenhouse setup

In April 2002, three cores (7.5 cm in diameter, 15 cm tall, 663 cm^3) were collected within 1–3 m of the base of each of four randomly selected red oak trees (>25 cm DBH) in each of the three oak-dominated plots, and each of four randomly selected eastern hemlock trees in each of the three hemlock-dominated plots (i.e., 3 cores × 4 host trees × 3 plots = 36 cores; 36 cores × 2 host soil sources = 72 total cores removed). No core was located <3 m from another core. Other studies suggest that fungal assemblages >3 m apart are compositionally distinct (Horton and Bruns 1998; A. Sirulnik, J.D. Lewis, A.R. Tuininga, and J. Johnson, Louis Calder Center and Biological Station, Fordham University, unpublished report). The cores were collected with a plunge corer lined with plastic inserts. Inserts were surface-sterilized by immersion in a 9:1 (*v*/*v*) mixture of deionized water and bleach (America's Choice Ultra Bleach, Montvale, New Jersey) for 15 min, followed by rinsing for 3 min in running deionized water. After soil core extraction, the inserts with the cores were wrapped in aluminum foil and taken to the Calder Center of Fordham University, in Armonk, New York.

Red oak acorns (Sheffield Seed Co., Locke, New York) were surface-sterilized and rinsed thoroughly in deionized water. One acorn was randomly assigned and planted 1–2 cm below the soil surface of each core. The cores were placed in surface-sterilized PVC pipes with surface-sterilized nylon screen (1.5 mm mesh) attached to each pipe bottom to prevent soil loss. After planting, acorns were randomly assigned to one of three light treatments: 10% (low light), 45% (intermediate light), or 100% (high light) transmittance, and grown in a greenhouse at the Calder Center. One core from each tree and each plot was assigned to each light treatment (i.e., 1 core × 4 trees × 3 plots; n = 12 assigned to each of three light treatments = 36 × 2 soil sources = 72 cores total). However, results from only 46 seedlings are reported because of mortality and failure to develop by some seedlings. The low and intermediate light treatments were created using shade huts, constructed with neutral-density shadecloth and PVC placed over the target seedlings. The associated maximum photosynthetic photon flux densities were ~200 (low light), ~900 (intermediate light), and ~2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (high light), based on measurements made on a clear day using a quantum sensor (LI-190SA; LI-COR Inc., Lincoln, Nebraska) at the core top after the shade treatments were imposed. Seedlings were watered every 2 days to throughflow with deionized water throughout the study. Air temperature was generally maintained between 20 and 28 °C throughout the study period. Seedlings were harvested 20 weeks after emergence, which represents the length of the growing season at Black Rock Forest (i.e., May–September).

Ectomycorrhizal description and quantification

ECM identification used the methods of Agerer (1987–2002) and Ingleby et al. (1990), and included both macro- and micro-scopic characters (Table 1). Macroscopic characters and root tip counts were determined using a Nikon SMZ-1B dissecting stereoscope (Nikon Instruments Inc., Melville, New York) at 10–40×, whereas microscopic characters were assessed using a Nikon YS2 compound microscope (Nikon Instruments Inc., Melville, New York) at 400–1000×. Macroscopic characters examined included mantle color range, shape, texture, and lustre; mycorrhiza branching pattern; presence, frequency, and width of emanating hyphae; presence, appearance, and width of rhizomorphs; and presence and shape of hyphal cystidia. Examination of microscopic characters included presence of hyphal clamp connections and describing both outer and inner mantle structure using the structural mantle types of Agerer (1987–2002). Described morphotypes (Table 1) were photographed and compared with descriptions of ECM fungi from published studies (e.g., Kernaghan et al. 1995; Baxter et al. 1999) to make taxonomic associations. However, no databases or published descriptions of ECM associated with red oaks in southern New York exist to our knowledge, so the taxonomic associations we made are speculative. All root tips were assigned to a corresponding morphotype, regardless of how infrequently they were encountered. Thus, less common types, as well as types that may not have fallen within easily described types, were not excluded.

Color differences among morphotypes were distinct, except for some white and yellow types. However, these types were easily distinguished after comparing the full range of characters identified including root tip branching pattern, shape, and mantle texture and lustre. Most morphotypes had emanating hyphae, although the degree varied from rare for most types to profuse in one type (EC19-sw). Cystidia and clamp connections were found in only three types. Rhizomorphs were found with two types (EC3-y.b and EC19.sw) and were quite distinct in their structure.

The percent colonization of ECM root tips (i.e., tips covered at least partially by an ECM mantle) was quantified using glass dishes etched with thirty-six 1 cm^2 squares following morphotype assignments in the dishes. Fine root length was quantified using Tennant's (1975) root intercept method. Colonized root tips were counted and divided by

Table 1. Descriptions of ectomycorrhizal fungal (ECM) morphotypes on oak seedlings grown under three light treatments in oak or hemlock soils.

ECM type (taxa)	Soil source*	Light level†	Mycorrhiza descriptions: color range; branching; tip shape; texture; lustre	Mantle type‡		Emanating hyphae (μm wide); clamps	Cystidia	Rhizomorphs (μm wide)
				Outer	Inner			
EC1-b *Cenococcum geophilum*	B	A	Black to gray; unbranched; straight; grainy; matte	NS	NS	Common, black (2–5); none	—	—
EC3-y.b	O	L, I	Light yellow; monopodial-pinnate; straight; smooth; matte	NP	NS	Common, white (2–4); rare	—	Well-defined strands (6–10)
EC4-rb	B	A	Red to rusty; irregular; straight; grainy; matte	NS	RS	Common, white-brown (2–6); rare	Rare, oval	—
EC8-w.b	B	A	White to beige; monopodial-pinnate; straight; smooth-velvety; matte	NP	NS	Rare (2–4); none	—	—
EC9-w.u	H	H	White; unbranched; bent; smooth to felty	NS	NS	—	—	—
EC10-yb *Lactarius*-like	O	L, I	Yellow-brown; unbranched; straight; smooth; matte	NP	NS	—	—	—
EC12-sw *Boletus*-like	H	I	Silver to white; monopodial-pinnate; straight and bent; smooth; shiny	NS	NS	Common (2–4); none	Rare, elongated	—
EC19-sw	B	A	Silver to white; irregular to monopodial-pyramidal; generally bent; cottony; matte when seen	FP	NS	Profuse (1–4); none	—	Thin strands (5–10)
EC20-og *Tomentella*-like	O	L, H	Olive-green to brown; monopodial-pyramidal; straight to bent; grainy to smooth; some areas matte	NS	NS	Rare (3–5); none	—	—
EC24-go	H	I	Gold to yellow; unbranched; bent; smooth to grainy; shiny	NS	NS	Rare (2–4); none	Few, oval	—
EC30-yg	O	L	Yellow-brown to olive; irregular; bent; smooth to slightly grainy; matte	NS	NS	Rare (2–4); none	—	—
EC31-br *Tomentella*-like	O	L, I	Light to dark brown; monopodial-pinnate; straight; smooth to grainy; matte	NP	NS	Rare, brown (2–4); very few	—	—
EC32-clk	O	I	Chalk white; unbranched; straight; smooth; matte	NS	NS	—	—	—

Note: —, Absent.

*B, both; H, hemlock; O, oak.

†A, all; L, 10% (low light); I, 45% (intermediate light); and H, 100% sunlight (high light).

‡FP, felt prosenchyma; NS, net synenchyma; NP, net prosenchyma; RS, regular synenchyma.

the total number of tips on a seedling to calculate ECM percent colonization. Percent contribution by each morphotype was calculated by dividing the number of colonized root tips per type by the total number of tips pooled across all types on a seedling. ECM community properties assessed included morphotype richness, Shannon diversity, and Shannon evenness (Magurran 1988). ECM community similarity among soil sources and light treatments was assessed using Sorensen's quantitative index (Magurran 1988). This index was used because it considers not just species richness, but also the percent colonization of each morphotype and because it is sensitive to rare species (Magurran 1988).

Soil chemistry

Soil chemistry was examined on soil cores collected adjacent to the cores described above. After collection, samples were sieved through a 4 mm screen to remove rocks and large roots. Three 10 g (±0.05) subsamples were used for measurement of (*i*) soil pH, (*ii*) water-extractable cations, and (*iii*) percent C and N. Soil pH was measured using a pH probe (model 9107; Orion Research Inc., Beverly, Massachusetts) in a 1:1 (*v*/*v*) mixture of soil and deionized water. To measure soil cations, soil subsamples were extracted using 50 mL of deionized water in a 125 mL high-density polyethylene bottle agitated at 300 r·min^{-1} for 1 h. The extract was filtered through a 2.5 cm diameter glass fiber syringe filter (Pall Life Sciences, Type A/E). When large particles prevented samples from being filtered through the syringe, samples were gravity filtered using a 7.6 cm diameter glass fiber filter (Pall Life Sciences, Type A/E). Extracted samples were analyzed with an atomic absorption spectrophotometer (Traacs 800 Autoanalyzer, Bran & Lubbe, Buffalo Grove, Illinois). Dry soil samples (50 °C for 24–48 h until constant mass) were analyzed for percent C and N using a CHN analyzer (Model 2400 II CHNS/O, Perkin Elmer, Wellesley, Massachusetts). There were no significant differences in soil chemistry between soil sources in any measured parameter based on single-factor analysis of variance (ANOVA) with soil source as the main effect ($P \geq 0.208$ in all cases).

Statistical analyses

This experiment was designed to examine the separate and interactive effects of light and soil source on ECM community characteristics. Light effects were examined using shade huts to create three light treatments. The effect of soil source was examined by collecting soil cores from beneath red oak trees growing in oak-dominated plots and from beneath eastern hemlock trees growing in hemlock-dominated plots. All soil cores were treated as independent samples. The design was a full factorial with 12 replicates per light by soil source combination. A power test using data from a pilot study found that a sample size of eight replicates per treatment was large enough to ensure a 90% level of certainty of not committing a type II error based on $\alpha = 0.05$. The ECM community characteristics examined were morphotype percent colonization, richness, diversity, and evenness.

The main and interactive effects of light and soil source on plant growth measures and ECM community characteristics were analyzed using two-way ANOVA within the general linear model procedure in SYSTAT (SPSS, version 9.0; SPSS Inc., Chicago, Illinois). Four morphotypes were found in all light treatments and both soil sources. χ^2 contingency tables were used to examine whether the effect of light treatment on the percentage of seedlings colonized by each morphotype differed between soil sources. Four other morphotypes were found in at least two light treatments in one soil source. The effect of light treatment on the percentage of seedlings colonized by these morphotypes was tested with χ^2 goodness-of-fit tests. The effects of soil source on soil chemistry were analyzed using one-way ANOVA. All data were examined for normality by assessing histograms, skewness, and kurtosis and for homogeneity of variance using F_{max} tests. All variables except percent colonization met the assumptions for ANOVA. Percent colonization values were log-transformed to meet normality assumptions. ECM percent colonization and community properties were tested against fine root length, with soil source incorporated as a fixed effect, using simple linear regression (SPSS version 14.0; SPSS Inc., Chicago, Illinois). Treatment effects were considered significant if $P \leq 0.05$.

Table 2. ANOVA results for the effects of light and soil source on ectomycorrhizal fungal (ECM) percent colonization and community properties per seedling.

Variable and effect	df	*F*	*P*
Percent colonization			
Light (L)	2	40.069	0.000
Soil source (S)	1	68.279	0.000
L × S	2	0.086	0.918
Richness			
L	2	12.580	<0.001
S	1	27.288	<0.001
L × S	2	0.771	0.469
Diversity			
L	2	8.057	0.001
S	1	21.979	<0.001
L × S	2	0.733	0.487
Evenness			
L	2	6.204	0.005
S	1	9.481	0.004
L × S	2	0.994	0.379

Note: The main effects were as follows: light treatments were 10% (low), 45% (intermediate), and 100% sunlight (high), and soil sources were oak or hemlock. Oak, $N = 20$; hemlock, $N = 26$; 10% sunlight, $N = 13$; 45% sunlight, $N = 18$; 100% sunlight, $N = 15$.

Results

Effects of light and soil source on ECM percent colonization

ECM percent colonization significantly varied among light treatments and between soil sources (Table 2). Percent colonization was about 138% greater on oak seedlings grown under intermediate and high light compared with those grown under low light but did not significantly differ between oak seedlings grown under intermediate and high light (Table 3). Across light treatments, ECM percent colonization was 33% greater on oak seedlings grown in oak compared with hemlock soils (Table 3). The effect of light

Table 3. Separate effects of light and soil source on ectomycorrhizal fungal (ECM) percent colonization, ECM community properties, and growth characteristics of oak seedlings.

	Soil source		Light treatment		
Variable	Oak	Hemlock	10%	45%	100%
Percent colonization	65.0±2.0a	49.0±2.0b	25.0±3.0a	69.0±3.0b	70.0±3.0b
Richness	3.15±0.26a	1.77±0.20b	1.36±0.27a	2.71±0.26b	3.30±0.31b
Diversity	0.88±0.10a	0.38±0.07b	0.28±0.11a	0.56±0.11b	0.80±0.10b
Evenness	0.70±0.17a	0.36±0.08b	0.42±0.16a	0.62±0.11b	0.71±0.11b
Total plant mass (g)	4.78±0.61	4.71±0.46	1.95±0.09a	5.18±0.42b	6.69±0.48b
Fine root length (cm)	7.31±1.10	8.53±1.28	2.91±0.11a	10.72±1.89b	10.56±0.95b
Root:shoot (g/g)	1.80±0.75	1.31±0.11	0.97±0.10a	1.19±0.11a	1.47±0.17b

Note: Values are means ± SEs. Light treatments were 10% (low), 45% (intermediate), and 100% sunlight (high). Values with different letters are significantly different among treatments at $P \leq 0.05$. Oak, $N = 20$; hemlock, $N = 26$; 10% sunlight, $N = 13$; 45% sunlight, $N = 18$; 100% sunlight, $N = 15$.

Table 4. Percent colonization of each morphotype in relation to total root tips colonized by all types pooled across, and for individual, light treatments.

			Oak light treatment			Hemlock light treatment		
ECM type	Oak pooled	Hemlock pooled	10%	45%	100%	10%	45%	100%
EC1-b	30.7	46.1	43.6	28.4	31.7	93.5	48.3	50.2
EC3-y.b	4.0		2.1	9.1				
EC4-rb	17.3	10.5	2.1	14.1	20.6		5.7	1.7
EC8-w.b	11.5	35.0	4.3	10.4	12.7	6.5	33.5	40.0
EC9-w.u		2.2						7.3
EC10-yb	4.6		5.3	10.6				
EC12-sw		5.1					10.8	
EC19-sw	22.5	0.2	31.4	14.3	28.5			0.8
EC20-og	2.7		2.7		4.8			
EC24-go		0.9					1.7	
EC30-yg	0.3		8.5					
EC31-br	3.0			5.0	1.7			
EC32-clk	3.4			8.1				
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Note: Light treatments were 10% (low), 45% (intermediate), and 100% full sunlight (high). Oak, morphotype colonization from seedlings grown in oak soils; hemlock, morphotype colonization from seedlings grown in hemlock soils. Oak, $N = 20$; hemlock, $N = 26$; 10% sunlight, $N = 13$; 45% sunlight, $N = 18$; 100% sunlight, $N = 15$.

availability on ECM percent colonization did not significantly differ between soil sources.

Thirteen unique ECM morphotypes were identified via laboratory morphotyping (Table 1). Six and three types were exclusive to seedlings grown in oak and hemlock soils, respectively. Four types were found on seedlings grown in both soils. *Cenococcum geophilum* Fr. (EC1-b) was the most common type from both soil sources, representing roughly one-third and one-half of colonized ECM root tips on seedlings from oak and hemlock soils, respectively (Table 4). After *C. geophilum*, the three most abundant types on seedlings grown in oak soils were EC19-sw, EC4-rb, and EC8-w.b. These three types accounted for one-half of all ECM colonization on seedlings from oak soils and 82% when considered with *C. geophilum*. On seedlings from hemlock soils, EC8-w.b and EC4-rb were the most abundant types after *C. geophilum*, and these three types accounted for 92% of all root tips colonized on seedlings from hemlock soils (Table 4). Across soil sources, morphotypes showed greater colonization in 45% and 100% light compared with 10% light with the exception of *C. geophilum*, which decreased in percent colonization from 10% to 45% light, and EC30-yg, which was found only in the 10% light treatment (Table 4). Five rare types (EC9-w.u, EC12-sw, EC24-go, EC31-br, and EC32-clk) occurred exclusively on seedlings grown under intermediate and high light.

The percentage of seedlings colonized by individual ECM morphotypes generally paralleled patterns shown for the percentage of root tips colonized, with the notable exception of *C. geophilum*. This morphotype colonized an increasing percentage of seedlings with increasing light, but the percentage of root tips colonized per seedling decreased (Tables 4 and 5). χ^2 analysis indicated that the effect of light on the percentage of seedlings colonized by EC4-rb, EC8-w.b, and EC19-sw varied between soil sources. The percentage of seedlings colonized by these morphotypes increased with increasing light more in oak soils versus hemlock soils. The percentage of seedlings colonized by EC20-og, which was found only in oak soils, significantly differed between light treatments. The frequency of seedlings colonized by EC3-y.b,

Table 5. Percentage of seedlings colonized by ectomycorrhizal fungal (ECM) morphotypes.

	Oak light treatment			Hemlock light treatment			
ECM	10%	45%	100%	10%	45%	100%	Significance
EC1-b	67	100	100	71	100	100	ns
EC3-y.b	17	14					ns
EC4-rb	17	71	71		36	25	S × L
EC8-w.b	17	57	100	29	54.5	62.5	S × L
EC9-w.u						12.5	
EC10-yb	17	14					ns
EC12-sw					9		
EC19-sw	17	43	71			12.5	S × L
EC20-og	17		43				L
EC24-go					9		
EC30-yg	17						
EC31-br		14	14				ns
EC32-clk		14					

Note: Differences among treatments were considered to be sginificant at $P \leq 0.05$. Light treatments were 10% (low), 45% (intermediate), and 100% sunlight (high). Oak, seedlings grown in oak soils (N = 6, 7, and 7 under 10%, 45%, and 100% sunlight, respectively); hemlock, seedlings grown in hemlock soils (N = 7, 11, and 8 under 10%, 45%, and 100% sunlight, respectively). S × L, significant difference between soil sources in the effect of light treatment on the frequency of seedlings colonized; L, significant difference between light treatments; ns, no significant difference.

EC10-yb, and EC31-br did not significantly differ among light treatments, and the effect of light treatment on the frequency of seedlings colonized by *C. geophilum* did not significantly differ between soil sources.

Effects of light and soil source on ECM community properties

Morphotype richness, diversity (based on the Shannon diversity index), and evenness per seedling were significantly greater on seedlings grown under intermediate and high light versus those grown under low light (Table 2). Mean richness, diversity, and evenness per seedling were 78%, 131%, and 94% greater, respectively, on seedlings grown in oak versus hemlock soils (Table 3). The effect of light availability on richness, diversity, or evenness measures per seedling did not significantly differ between soil sources.

ECM morphotype community similarity, calculated using Sorensen's quantitative index, was 0.57 for seedlings grown across light treatments and between soil sources. For a given light treatment, ECM morphotype communities were most similar between seedlings grown in oak and hemlock soils at intermediate and high light (0.64 for both). ECM morphotype communities were moderately similar between seedlings grown in oak and hemlock soils at low light (0.51). For a given soil source, ECM morphotype communities were highly similar between seedlings grown at intermediate and high light (0.74), whereas comparisons between seedlings grown at low and intermediate light and at low and high light were both low (0.11 and 0.13, respectively).

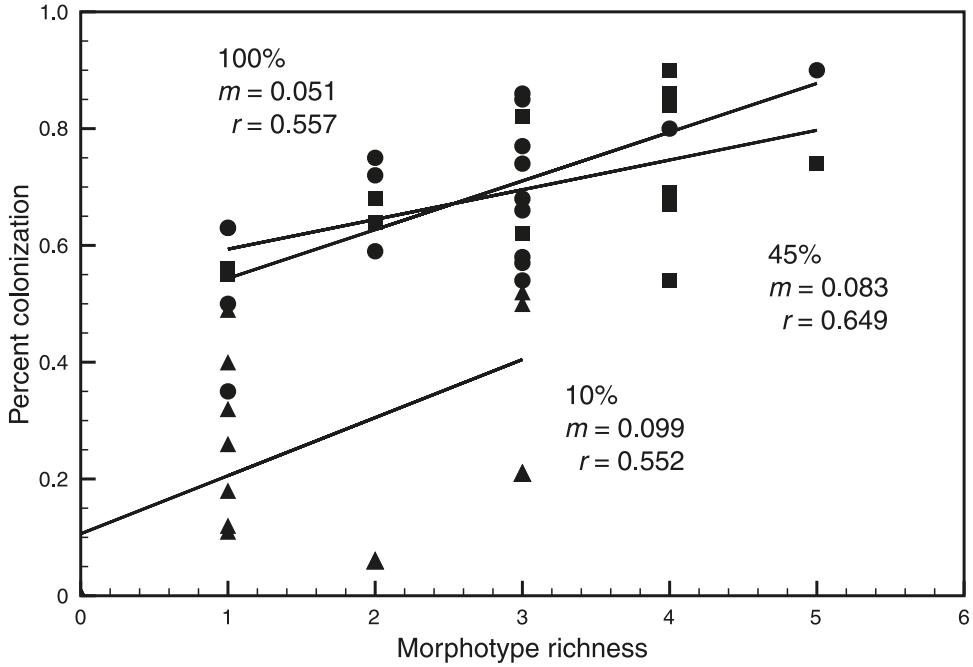
Effects of light and soil source on plant growth and allocation to roots

Seedling growth significantly varied with growth irradiance. Total plant dry mass (P = 0.036), fine root length (P < 0.001), and the ratio of root to shoot mass (P = 0.007) were significantly greater at intermediate relative to low light but did not significantly vary between intermediate and high light (Table 3). Effects of light treatment on leaf and stem mass paralleled effects on total plant growth (data not shown). There were no significant effects of soil source on these variables, and the effects of light treatment did not significantly differ between soil sources ($P \geq 0.080$ in all cases). Growth values for seedlings grown in oak and hemlock soils, respectively, were as follows: leaf mass, 1.34 ± 0.21 g (mean ± SE) and 1.26 ± 0.21 g; total root mass, 2.38 ± 0.36 g and 2.76 ± 0.12 g; stem mass, 0.99 ± 0.12 g and 0.85 ± 0.08 g; and stem height, 17.39 ± 1.0 cm and 16.67 ± (0.95) cm.

Regression analysis indicated that ECM percent colonization (P = 0.004, adjusted r^2 = 0.151) and richness (P = 0.005, adjusted r^2 = 0.145) significantly increased with increasing fine root length. In addition, ECM percent colonization significantly increased with increasing richness (P < 0.001, adjusted r^2 = 0.459). To estimate the relative effects of fine root length and ECM morphotype richness on ECM percent colonization, we used a regression model incorporating both fine root length and richness as independent variables. ECM percent colonization significantly increased with increasing richness (P < 0.001) but did not significantly vary with fine root length (P = 0.181). This model accounted for 47% of the variation in ECM percent colonization (adjusted r^2 = 0.469).

To examine whether the relationship between ECM percent colonization and morphotype richness could account for treatment effects on ECM percent colonization, we used analysis of covariance. Soil source did not have a significant effect (P = 0.914) on ECM percent colonization after taking into account differences in ECM morphotype richness. However, ECM percent colonization significantly differed among light treatments (P < 0.001) even after taking into account differences in morphotype richness (Fig. 1). Seedlings in the intermediate and high light treatments had significantly greater percent colonization than seedlings in the low light treatment. The model combining light treatment and morphotype richness accounted for 77% of the variation in

Fig. 1. Relationship between ectomycorrhizal fungal (ECM) total percent colonization and morphotype richness, on oak seedlings grown in 100% (high; ■), 45% (intermediate; ●), and 10% (low; ▲) light. ECM percent colonization on oak seedlings significantly increased with increasing morphotype richness ($P < 0.001$). ECM percent colonization was significantly greater in intermediate and high light than in low light, even after taking into account the effect of morphotype richness ($P < 0.001$). A model incorporating the main effects of morphotype richness and light treatment accounted for 77% of the variation in percent colonization. The slopes (m) and correlation coefficients (r) are shown for each regression (n = 15, 18, and 13 for the high, intermediate, and low light treatments, respectively).



ECM percent colonization (r^2 = 0.768). There were no significant interactions between morphotype richness and either soil source or light treatment, indicating that the slope of the relationship between percent colonization and morphotype richness did not significantly differ among treatments. We could not use analysis of covariance (ANCOVA) to examine whether the relationship between ECM morphotype richness and fine root length could account for treatment effects on ECM morphotype richness, because there were significant interactions between soil source and fine root length and between light treatment and fine root length when we tested the assumption of homogeneity of slopes.

Discussion

Light availability and host community composition are expected to play key roles in regulating ECM formation and community properties (Zhou and Sharik 1997; Dahlberg 2001). Consistent with these expectations, ECM formation and community properties on oak seedlings significantly varied with light availability and between soils from oak- and hemlock-dominated stands. ECM percent colonization was greater and the ECM morphotype community was more rich, diverse, and evenly distributed on seedlings grown under intermediate and high light compared with low light and on seedlings grown in oak versus hemlock soils. Further, light and soil source interactively affected the frequency of seedlings colonized by three of the four morphotypes found in both soil sources. Despite these clear effects of light and soil source, there were no significant interactions between light and soil source on overall ECM percent colonization or community properties. The lack of interactive effects on the overall ECM fungal community suggests that differences in responses of individual morphotypes to light and soil source may influence overall ECM colonization and diversity.

Our results further suggest that differences among seedlings in ECM morphotype richness were partly a function of differences in fine root length, whereas differences among seedlings in ECM percent colonization were a function of differences in ECM morphotype richness. ECM morphotype richness significantly increased with increasing fine root length, although the relationship differed among treatments. Other studies have observed similar relationships between fine root structure and ECM richness (Kranabetter and Friesen 2002; Korkama et al. 2006; Tedersoo et al. 2006), suggesting that fine root structure may play a key role in regulating EMC fungal richness in forest ecosystems. In turn, changes in ECM fungal richness may influence host trees both through direct effects (Richard et al. 2004; Lewis et al. 2008) and, indirectly, through effects on ECM fungal abundance (Lewis et al. 2008). In the present study, ECM percent colonization significantly increased with richness and fine root length when these variables were considered separately. However, when both richness and fine root length were included in the same regression model, ECM percent colonization significantly increased with richness, but there was no significant relationship between ECM percent colonization and fine root length. These results suggest that the increase in percent colonization with increased fine root length was attributable to increased ECM morphotype richness and suggest that ECM morphotype richness may play a key role in regulating ECM colonization (Lewis et al. 2008).

Our ANCOVA results suggest that the relationship between ECM percent colonization and morphotype richness could account for the greater ECM percent colonization on oak seedlings in oak as compared with hemlock soils. As expected, oak seedlings supported a richer ECM fungal community when grown in oak versus hemlock soils. It is well established that ECM fungi can form affinities with hosts ranging from general to specialist but that co-occurrence often results in tighter affinities between ECM fungi and hosts of specific taxonomic groups (Molina and Trappe 1982; Molina et al. 1992). For example, conifer-specific fungal species, which form associations primarily with conifers, have been observed (Gardes and Bruns 1996; Massicotte et al. 1999; Dahlberg 2001). If so, hemlock-associated ECM types may have been less responsive than oak-associated types to colonizing oak seedlings in this study. Accordingly, greater morphotype richness, and hence percent colonization, on oak seedlings in oak compared with hemlock soils may reflect a greater affinity by ECM fungi from oak soils than hemlock soils, as has been found in other studies (Lewis et al. 2008). In turn, spatial patterns in the distribution of ECM fungi may influence spatial patterns of tree recruitment into forests with a mix of host tree species, if seedlings encountering ECM types with greater affinity for them have higher growth and survival rates than conspecific seedlings encountering ECM types with less affinity for them.

Although our ANCOVA results suggest that soil source effects on ECM percent colonization were attributable to treatment differences in morphotype richness, the results also suggest that increasing light availability had a stimulatory effect on percent colonization beyond what could be accounted for by treatment differences in morphotype richness. Studies on the effect of increasing light availability on ECM colonization generally observe increased colonization with increased light availability (Harley and Smith 1983; Zhou and Sharik 1997) resulting from increased carbohydrate allocation to roots (Björkman 1970). Nonetheless, some studies suggest that ECM percent colonization increases with increasing light availability primarily at relatively lower irradiances (Björkman 1970; Zhou and Sharik 1997). Consistent with this pattern, ECM percent colonization increased from low to intermediate light in this study but did not significantly differ between the intermediate and high light treatments. The percent colonization levels found in our study are also consistent with those found in comparable studies (Beckjord et al. 1984; Godbold et al. 1997), suggesting that our results are not unusual. If so, then this pattern suggests that the differential effects of increasing light availability on ECM percent colonization at low and high irradiances may reflect differential effects of increasing light availability on carbohydrate production and allocation at these irradiances.

Relatively little is known about light effects on ECM richness, and our study is one of the first to report a stimulatory effect of increasing light availability on ECM morphotype richness. Overall, we found a broader range of types on seedlings grown under intermediate to high versus low light, because several types only colonized seedlings grown in the intermediate and high light treatments. For example, four of the five least common types were only found colonizing oak seedlings grown in the intermediate and high light treatments. In addition, increasing light was associated with increased frequency of seedlings colonized by several of the ECM morphotypes that were found in multiple light treatments. Because richness is affected by the presence or absence of a given morphotype, increasing the frequency of seedlings colonized by a given morphotype would increase the mean richness associated with that treatment. Although increased richness in response to increased light may partially reflect increased fine root length in response to increased light, the significant interaction between light and fine root length suggests that other mechanisms may also account for the effect of increasing light availability on ECM morphotype richness.

As with the effect of light availability on percent colonization, the effect on morphotype richness may also reflect increases in root carbon content. Whereas high resource availability can lead to competitive exclusion and reduce richness (Rosenzweig 1971), increasing availability of a limiting resource often increases richness in biological communities (Huston 1979). If so, greater root carbohydrate availability in oak seedlings grown under intermediate to high light may reduce competition among ECM morphotypes with differing carbohydrate costs and, thus, support a more speciose ECM community. In contrast, seedlings grown under very low light may not support speciose ECM communities because comparatively lower root carbohydrate availability of these seedlings may only support morphotypes with low carbohydrate costs. Consistent with this mechanism, evenness was greater on seedlings grown under intermediate to high light relative to those grown under low light, suggesting reduced dominance by one or a few morphotypes with increasing light availability. For example, *C. geophilum*, which is known to tolerate low light conditions commonly found in mature forests (Kranabetter and Wylie 1998), was most abundant on seedlings grown under low light, whereas the relative abundance and frequency of less-common morphotypes increased with increasing light (see Tables 4 and 5). It should be noted that the pattern of response of *C. geophilum* colonization to the light treatments may not reflect the response of a particular genotype. Other studies have observed significant genetic variability in *C. geophilum* at spatial scales similar to those used in our study (Jany et al. 2002; Avis et al. 2003; Dickie and Reich 2005). Such variability may account for why *C. geophilum* colonizes seedlings grown under a range of varying, and often adverse, environmental conditions (LoBuglio 1999), including drought (Jany et al. 2002), ozone, and high irradiance (Železnik et al. 2007). Accordingly, although we did not test for genetic variability in *C. geophilum* in this study, the presence of *C. geophilum* across the light treatments we used may at least in part reflect genetic variability within this species. Further, if discrete morphotypes in our study represented multiple genotypes, our results may underestimate total ECM fungal diversity on our study seedlings.

Light did not significantly alter the effect of soil source on overall ECM percent colonization or on community properties, because there were no significant interactions between light availability and soil source on ECM percent colonization, richness, diversity, or evenness. This indicates that, across light treatments, these variables were consistently and

significantly lower on seedlings grown in hemlock versus oak soils. Further, these results suggest that increasing light availability may counteract negative effects of host plant community composition on mycorrhizal colonization of oak seedlings growing in areas dominated by other plant species. These results are particularly pertinent to forests in the eastern United States that are experiencing defoliation in hemlock stands from hemlock woolly adelgid (*Adelges tsugae* (Annand)) infestation, because the changes in ECM colonization and richness we observed on oak seedlings in hemlock compared with oak soils may negatively affect oak recruitment into declining hemlock stands through negative effects on oak seedling growth rates (Lewis et al. 2008).

In summary, both host light environment and soil source influenced ECM community composition on red oak seedlings, with greater percent colonization and morphotype richness on seedlings grown under intermediate to high light and in soils associated with taxonomically related oak hosts. Differences between soil sources in percent colonization reflected differences in morphotype richness, which may have resulted from differences in fine root length and host specificity. Increasing percent colonization with increasing light availability may have resulted from differences in root carbohydrate concentrations and increased morphotype richness, which in turn may have resulted from differences in carbon allocation to roots. Although the ECM fungal communities on seedlings represented the inoculum potential of the soil cores rather than natural conditions and given the relatively low number of root systems that we assessed, the strong responses of ECM colonization and community properties to light and soil source, and the similarities between morphotype richness found in this study and in outplanted seedlings from another comparative study (Lewis et al. 2008), suggest that both light availability and host species may affect mycorrhizal colonization on red oak seedlings in situ. If so, then the changes in ECM colonization and richness we observed may affect oak recruitment through effects on oak seedling growth rates (Lewis et al. 2008). However, more research, including more extensive bioassay and outplanting assessments, is required to better understand how changes in the mycorrhizal fungal community in response to variation in light availability and plant community composition may affect oak seedling recruitment and how these changes are regulated by changes in environmental factors and other changes in the soil microbial community.

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