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ORIGINAL PAPER

Mycorrhizal networks affect ectomycorrhizal fungal community similarity between conspecific trees and seedlings

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Abstract Ectomycorrhizal (EM) networks (MN) are thought to be an important mode of EM fungal colonization of coniferous seedlings. How MNs affect EM communities on seedlings, and how this varies with biotic and abiotic factors, is integral to understanding their importance in seedling establishment. We examined EM fungal community similarity between mature trees and conspecific interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) seedlings in two experiments where seed and nursery-grown seedlings originating from different locations were planted at various distances from trees along a climatic gradient. At harvest, trees shared 60% of their fungal taxa in common with outplanted seedlings and 77% with germinants, indicating potential for seedlings to join the network of residual trees. In both experiments, community similarity between trees and seedlings increased with drought. However, community similarity was lower among nursery seedlings growing at 2.5 m from trees when they were able to form an MN, suggesting MNs reduced seedling EM fungal richness. For field germinants, MNs resulted in lower community similarity in the driest climates. Distance from trees affected community similarity of nursery seedlings to trees, but there was no interaction of provenance with MNs in their effect on similarity in either nursery seedlings or field germinants as hypothesized. We conclude that MNs of trees influence EM colonization patterns of seedlings, and the strength of these effects increases with climatic drought.

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Introduction

Ectomycorrhizal (EM) networks (MN) are known to facilitate seedling establishment (Dickie et al. 2002, 2005; McGuire 2007; Teste et al. 2009; Booth and Hoeksema 2010; Bingham and Simard 2011). They appear to be most beneficial when the seedling is establishing in soils that are deficient of EM propagules or soil resources (Dickie et al. 2002, 2005; McGuire 2007). Interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) experiences resource limitations over most of its range, including growing season soil water deficits and nitrogen (N) limitations (Rehfeldt 1989). Its ability to form an MN during seedling establishment is likely influenced by its proximity to existing EM plants (Teste et al. 2009), relatedness to those same plants (Hoeksema 2010), and its own life history (Eissenstat and Volder 2005). It is assumed that the EM community of seedlings will be more similar to that of nearby trees where the seedlings are colonized by MNs.

The stress-gradient hypothesis predicts that plant-to-plant facilitation should be most prevalent when an establishing plant is limited by an abiotic stress, such as water deficit (Greenlee and Callaway 1996; Callaway et al. 2002; Liancourt et al. 2005). If MNs are a mechanism for facilitation, then seedlings in dry environments should be more reliant on EM colonization through networks than those in wetter environments. Networks might also form more readily between trees and seedlings

where the seedling provenance is best matched to its environment. Moreover, distance and plant age should be important to MN formation. Thus, EM fungal communities might be more similar to those of nearby trees where seedlings are older rather than younger, and where they are well adapted to the site, due to phenological and phenotypic effects.

The characteristics of a tree MN that is colonizing nearby seedlings can be controlled using mesh barriers with pores of different sizes. Here, increasing pore size of the mesh barrier corresponds to access of networking fungi of increasing hyphal diameter. Teste et al. (2006) found that mesh with a pore size of 0.5 μm inhibited development of MNs while still allowing soil water to pass through, whereas mesh with 35-μm pores allowed MNs comprised of fine hyphae to form between established trees and new seedlings. Using these pore sizes in a field experiment, Teste et al. (2009) found that the EM communities of mature trees and seedlings in interior Douglas-fir forests shared 61% of their taxa, indicating high networking potential, particularly where seedlings were planted in mesh with a large pore size. Here, we assess the effects of these mesh treatments on the similarity between EM colonization of trees and seedlings as a proxy for MN effects on colonization.

The objective of this study was to determine whether similarity between EM communities of establishing Douglas-fir seedlings and conspecific trees differed when seedlings were colonized directly by the tree's MN versus when they were colonized only by native soil propagules, and whether this effect varied by regional climate, distance from a conspecific tree, seed provenance, and seedling life history. To predict climatic effects, nine field sites were established along a climatic moisture gradient. The following hypotheses were tested: (1) EM fungal communities of seedlings will be most similar to those of conspecific trees when they have the opportunity to link into the full MN of these trees; (2) EM fungal community similarity will be highest when nursery seedlings from the driest provenance are planted in the driest environment, since seedlings should be more reliant on MNs in this scenario; (3) EM fungal community similarity between seedlings and trees should decrease with seedling distance from the tree; and (4) EM fungal communities of seedlings should be more similar to trees where seedlings belong to older than younger cohorts. We tested these hypotheses in interior Douglas-fir forests of south-central BC, along a strong precipitation gradient. These forests are expected to experience dramatic shifts in climate over the next century (Spittlehouse 2008). The experimental design includes replication within stands and biogeoclimatic units, and provides basic information on EM colonization dynamics.

Methods

Experimental design

The field study was conducted at nine sites located across a climatic gradient that varied according to drought stress (i.e., the ratio of mean annual potential evapotranspiration to precipitation). Interior Douglas-fir was the dominant tree species at all sites, and we attempted to establish three sites each in the Very Dry Hot Interior Douglas-fir (IDFxh) subzone; Dry Cool Interior Douglas-fir (IDFdk) subzone; and Moist Warm Interior Cedar Hemlock (ICHmw) subzone; however, the sites ultimately chosen only approximated these three biogeoclimatic units (Lloyd et al. 1990). Each site had been "clearcut with reserves" (i.e., clearcut leaving mature residual trees scattered throughout the opening) within 10 years of the study initiation in 2006. At each site, a minimum of 14 of the largest solitary residual Douglas-fir trees were selected for study. The three sites within each subzone were at least 2 km apart. Climatic, environmental, and stand history characteristics for each site are summarized in Bingham (2011).

Three experiments (distance experiment and two provenance experiments) were conducted simultaneously and were spatially integrated. The distance experiment was planned as a nested 3×5 factorial design, where distance between residual trees and regenerating seedlings (five levels) was nested within regional climate (three levels). Each treatment combination was replicated 21 times (9 sites×5 distances×21 replications=945 seedlings; 50% surviving). One-year-old nursery seedlings were planted over a period of 6 weeks in May and June of 2006 at five distances from the residual trees: 0.5, 2.5, 5, 10, and 15 m from the mature tree bole. The 0.5-m treatment was immediately under the tree canopy, the 2.5-m treatment was usually at the canopy dripline, and the remaining distances were outside the mature tree canopy (i.e., under open sky). Transects were randomly assigned to one of nine bearings from the tree (20°, 60°, 100°, 140°, 180°, 220°, 260°, or 340°; Fig. 1).

The two provenance experiments, one examining nursery-origin seedlings (cohort 1) and the other examining seed-origin seedlings (cohort 2), were each planned as a nested 3×3×3 factorial design, where MN access (three levels) and seed provenance (three levels) were nested within regional climate (three levels). For these provenance experiments, three interior Douglas-fir provenances were planted at 2.5 m from the residual Douglas-fir trees. The three seed provenances used for the provenance experiments were from seed planning zones Thompson Okanagan Arid (TOA: seedlot No. FDI 45272 2005 DKA0150), Thompson Okanagan Dry (TOD: seedlot No. FDI 30461 2005 DKA0150), and Shuswap-Adams (SA: seedlot No.

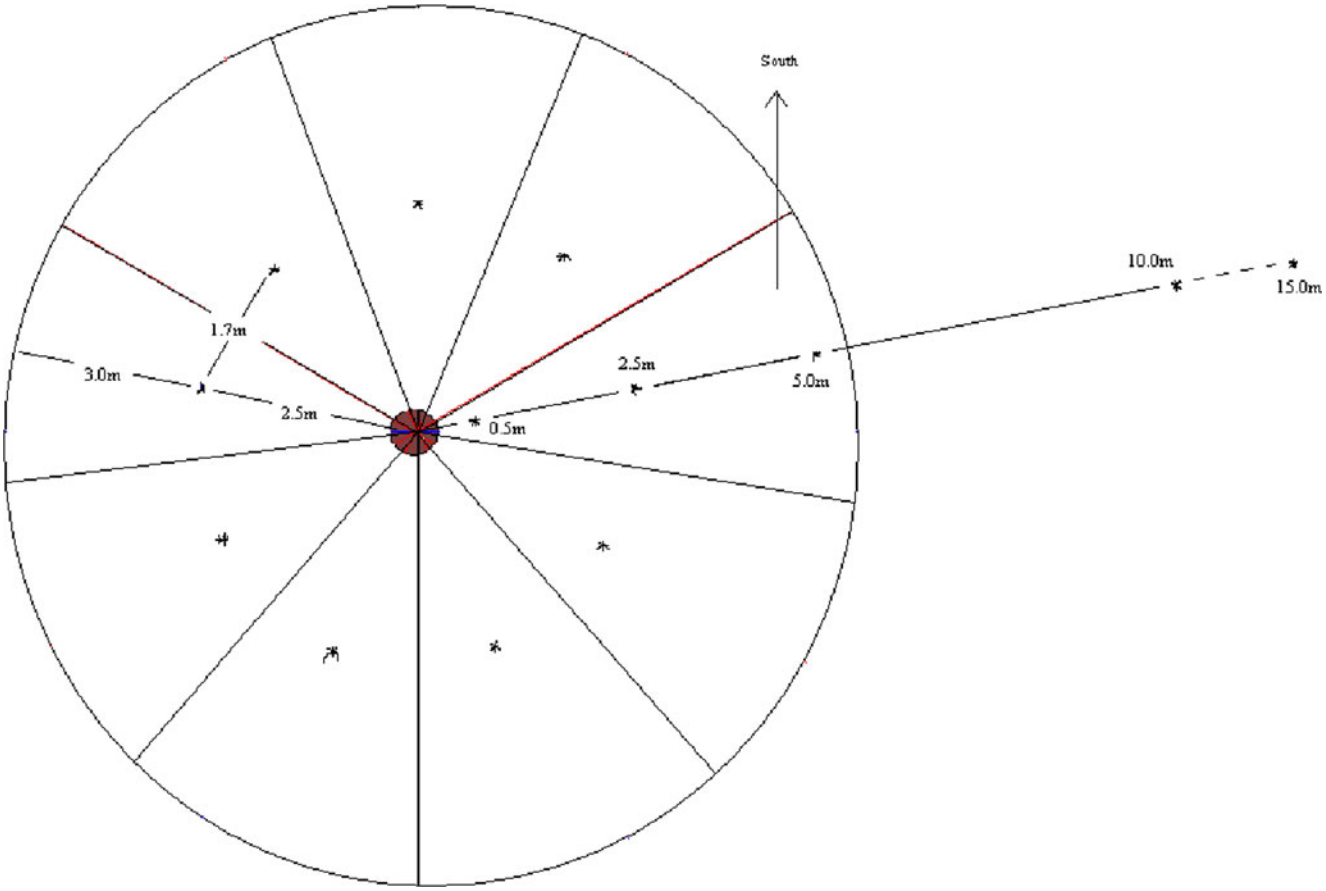

Fig. 1 Location of seedling positions (represented by *stars* at 2.5 m) around a residual tree (*brown circle*), with an example of a transect at 260° (*stars* along transect representing seedling positions on transect). Each position represents an experimental unit (from Bingham 2011)

FDI 42192 2005 DKA0150), while all seedlings used in the distance experiment belonged to TOD. The geographic origins of the three provenances decreased in regional precipitation in the order SA (wet)<TOD (medium)<TOA (dry). The two provenance experiments used seedlings (cohort 1) and seeds (cohort 2), where (1) 1-year-old nursery seedlings (cohort 1) were planted over a period of 6 weeks in May and June of 2006, and (2) unstratified seeds (cohort 2) were planted in September of 2006, and thus germinated the following growing season of 2007. Mesh and provenance treatments were randomly assigned to each 2.5-m planting position. Each treatment combination was replicated seven times (9 sites×3 MN treatments×3 provenances×7 replications=567 seedlings; 567 seedlings of cohort 1 (40% surviving) and 567 seed groups representing cohort 2 (15% germinating and surviving to harvest).

Access to an MN in the provenance experiments was controlled by planting the 1-year-old nursery stock seedlings or unstratified seeds into soil or mesh bags made of sturdy plain-weave nylon (Plastok, Birkenhead, UK). The three "mesh treatments," representing increasing access to a mycorrhizal network, were (1) 0.5-μm mesh, where hyphae, roots, and invertebrates were restricted from accessing seedlings; (2) 35-μm mesh, which allowed hyphae to access seedlings but restricted access by rhizomorphs, roots, and invertebrates; and (3) no mesh, where seedlings were planted directly into soil and thus could form hyphal and rhizomorph MNs, and their roots were free to intermingle with tree roots (after Teste and Simard (2008) and Johnson et al. (2001)). For the two mesh bag treatments, a cylinder of soil was removed from the ground, placed into the mesh bag, and seedlings were planted into this soil. For the no-mesh treatment, a cylinder of soil was disturbed in the same manner as that of the mesh treatments. This method allowed us to separate soil water, MN, and root/invertebrate pathways of water and nutrient flow without creating treatment differences in the degree of soil disturbance. Effects on soil invertebrates were not specifically investigated; however, Teste et al. (2006) found that moisture equilibrated rapidly between soil inside and outside of mesh bags of both pore sizes. Competition from understory plants was eliminated by applying an herbicide containing glyphosate and surfactant (Nufarm Credit® at a rate of 17 mL/m^2) in a 3-m radius around each planted seedling early every growing season.

Measurements

Seedlings

EM fungal communities on seedling and conspecific tree root tips were sampled and characterized following Teste et al. (2006) and Twieg et al. (2009). After completion of harvest (September 2007 for distance experiment and cohort 1; September 2008 for cohort 2), seedlings were morphotyped for EM fungi on 25 root tips, if 25 root tips were present (many seedlings from cohort 2 had fewer than 25 root tips). In addition, a soil core 13×13×32 cm deep was taken 2.5 m distance from three randomly selected trees at each site, and then 25 Douglas-fir tree roots were morphotyped, provided 25 were available. Roots from trees and seedlings were rinsed in cold water, cut into 1-cm fragments, placed in a baking dish with distilled water, and randomly subsampled from the baking dish. Morphotypes were characterized using Ingleby et al. (1990), Agerer (1987), and Goodman et al. (1996). The morphotypes were identified via sequencing of the rDNA internal transcribed spacer region (ITS) by University of British Columbia Okanagan Fragment Analysis & DNA Sequencing Services using the methods of Twieg et al. (2007) and Teste et al. (2010). Most morphotypes sequenced to multiple species.

EM fungal community similarity between trees and seedlings was calculated as:

$$S = 1 - \left(\sum_{i=1}^{K} |p_i - q_i| / K \right), \quad (1)$$

where S is community similarity, K is the number of morphotypes, p_i is the proportion root tips colonized by fungal morphotype i on the seedling, and q_i is the proportion root tips colonized by fungal morphotype i on the tree. Since only three tree cores were sampled per site, the values were averaged per site, thus a single q_i represents each site.

Climatic conditions

Weather and climate data for the period of 1950 to 2008 were obtained from the Global Historical Climatology Network (Vose et al. 1992) for the climate stations that were closest to the nine sites. Individual tree latitude, longitude, and elevation were used to generate climate data from the web-based tool ClimateBC, which interpolates weather parameters for the period 1950–2002 (Hamann and Wang 2005; Wang et al. 2006). We developed regression equations to estimate monthly precipitation and mean monthly maximum temperature for each tree for the duration of the experiments. We then predicted EM fungal community similarity values of seedlings from the distance experiment, and of cohorts 1 and 2 from the two provenance experiments, in response to variations in the summer (May-Sept.) heat:moisture index. For predicting EM fungal community similarity between conspecific trees and seedlings from the distance experiment and cohort 1 of the provenance experiment, the heat:moisture index was

$$\text{SH:M} = (\text{mean maximum 2006-2007 July temperature}) / ((\text{mean 2006-2007 summer precipitation})/1{,}000). \quad (2)$$

For cohort 2, the index was

$$\text{SH:M} = (\text{mean maximum 2007-2008 July temperature}) / ((\text{mean 2007-2008 summer precipitation})/1{,}000). \quad (3)$$

Soils

At each site, three soil pits were excavated to the bottom of the B horizon, if present, to characterize the humus form and soil order (according to the Canadian System of Soil Classification 1998). At the beginning of the experiment, percent cover of coarse woody debris (CWD) was assessed in a 1×1-m quadrat located in a random direction at the five seedling distances from each tree. These values were averaged by distance for each site.

Residual trees

Slope, slope aspect, latitude, longitude, and elevation were recorded for each residual tree. All residual trees were alive and healthy when selected in May 2006. During the course of the study, a number of trees died at various sites due to windthrow, accidental harvest, or undetermined causes. None of these trees, or their associated seedlings, were used in this analysis.

Data analysis

Effects of the treatments on community similarity were analyzed with least likelihood methods using the SAS System for Windows, V9.2 (2009). Climate factors were treated as continuous variables in all statistical models because the sites did not fit neatly into the predetermined biogeoclimatic units. The two cohorts from the provenance experiment were analyzed separately because variation between them was so great that it obscured variation of other factors.

Community similarity analyses were performed as an analysis of covariance (ANCOVA) for a factorial set of treatments using climatic and site variables (variables that are intrinsic to the site) as covariates in a completely randomized design using SAS PROC MIXED (Milliken

and Johnson 2002). Because fungal communities were assessed on only a subsample of 209 seedlings, replication was low enough that we tested hypotheses within the distance experiment seedlings, cohort 1, and cohort 2 beginning with two-factor ANCOVAs rather than three-factor ANCOVAs. The general form of the model was

$$Y_{ij} = \mu + \delta_i + \tau_j + (\delta\tau)_{ij} + \beta_1\left(X_{1ij} - \overline{X}_{1..}\right) + \ldots\beta_n\left(X_{nij} - \overline{X}_{n..}\right) + \varepsilon_{ij}, \quad (4)$$

where Y_{ij} is the dependent variable; μ is a general mean; δ_i, τ_j, and $(\delta\tau)_{ij}$ are the fixed effects parameters for the mesh treatment, climatic, seed provenance, or distance factors, and their interactions; $\beta_1 \ldots \beta_n$ are estimated coefficients; $X_1 \ldots X_k$ are climatic, site, or planting date covariates; and ε_{ijk} is the residual (Steel and Torrie 1980). However, if treatment factors and their interactions did not meet a threshold of $P \leq 0.1$ after covariate fitting, they were removed from the model. A threshold of $P \leq 0.1$ was used as the criterion for significance of treatment factors and their interactions because our power to detect differences was reduced by tree EM communities averaged across site, low seedling replication, numerous confounding factors, and the inherently low precision of morphotyping. Covariates were allowed to enter the model if they improved the overall fit but were ultimately removed from the model if they did not meet the criterion of $P \leq 0.1$. Akaike's information criterion (AIC) is presented for each model as a measure of goodness of fit but was not used in the process of model selection.

Results

Sixteen EM morphotypes comprised of 20 fungal taxa were identified in tree and seedling root systems (Table 1). Trees, outplanted nursery seedlings, and field-

Table 1 Ectomycorrhizal (EM) morphotypes on interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) trees and seedlings (outplanted nursery and field-germinated) at the nine sites located across a climatic moisture gradient (biogeoclimatic zones PP, IDF, and ICH) in southern interior British Columbia, Canada

Morphotype	Closest BLAST matches	Database	Accession number	Total base pairs aligned	NCBI% similarity	Morphotype host
Agaricus albolutescens	*Agaricus albolutescens*	NCBI	AY484675.1	713	99.3	Seedling
Amphinema byssoides	*Amphinema byssoides*	NCBI	AY838271.1	571	98.6	Both
CDE8	nd	nd	nd	nd	nd	Seedling
Cenococcum geophilum	*Cenococcum geophilum*	NCBI	DQ179119.1	915	97.05	Both
	Phialocephala fortinii	NCBI	AY394915.1	593	99.83	
Cortinarius sp.	*Cortinarius* sp.	NCBI	EU326169.1	805	97.52	Both
	Rhizoctonia sp.	NCBI	AJ419931.1	599	97.83	
DSE	*Tomentella* sp.	NCBI	GQ398250.1	641	97.04	Seedling
Lactarius rubrilacteus	nd	nd	nd	nd	nd	Both
Russula acrifolia	*Russula acrifolia*	NCBI	DQ421998.1	638	98.75	Tree
Russula brevipes	*Russula brevipes*	NCBI	AF349714.1	593	99.33	Both
Rhizopogon/*Suillus*	*Boletales*	NCBI	FJ554146.1	660	99.55	Both
	Inocybe sororia	NCBI	EU525947.1	672	99.4	
	Meliniomyces variabilis	NCBI	EF093172.1	562	99.47	
	Rhizopogon vinicolor	NCBI	AF263931	91	100	
	Tomentella sp.	NCBI	EF655697.1	601	98.84	
	Tricholoma myomyces	NCBI	FJ845443.1	656	99.7	
Russula sp.	*Russula* sp.	NCBI	EF218808.1	637	100	Both
Russula xerampelina	*Russula xerampelina*	NCBI	AY061734.1	706	99.86	Seedling
Sebacina sp.	*Sebacina* sp.	NCBI	EU668272.1	594	98.99	Both
Thelephora terrestris	*Thelephora terrestris*	NCBI	GQ267490.1	684	99.42	Both
Unknown	nd	nd	nd	nd	Nd	Both
Wilcoxina rehmii	*Geopora* sp.	NCBI	EU668289.1	600	98.33	Both
	Thelephora terrestris	NCBI	GQ267490.1	687	99.27	
	Wilcoxina rehmii	NCBI	AF266708.1	611	99.35	

Sampling was conducted in September 2007 (nursery seedlings) and 2008 (germinants)

NCBI National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/), *nd* not determined

germinated seedlings hosted 12, 12, and 11 EM morphotypes, respectively. Outplanted nursery seedlings had nine morphotypes in common with trees (60% shared), and field-germinated seedlings had ten morphotypes in common with trees (77% shared). More unique taxa were found on trees and outplanted nursery seedlings than on field-germinated seedlings. The five most abundant EM morphotypes were *Rhizopogon/Suillus*, *Wilcoxina rehmii*,

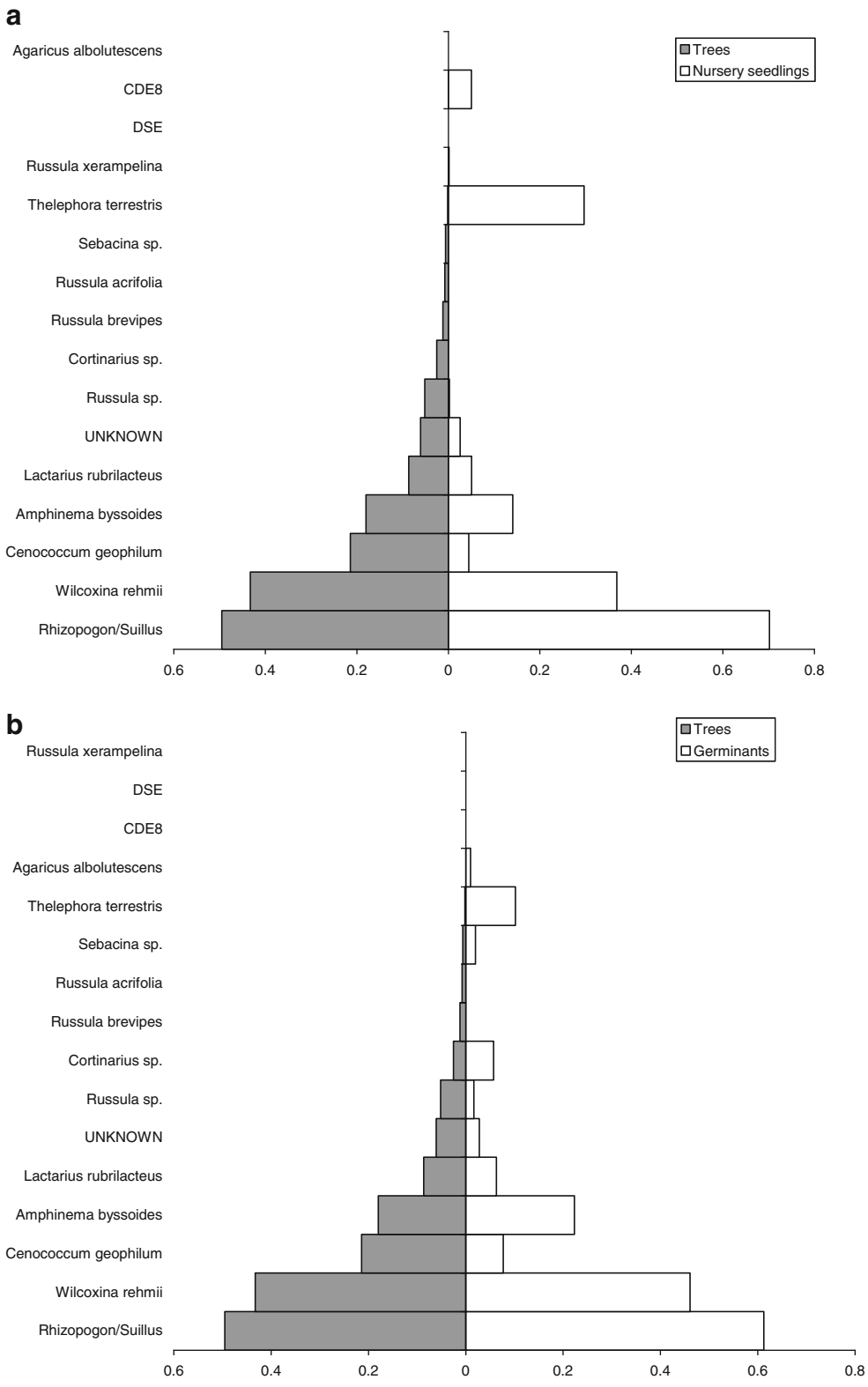

Fig. 2 Relative abundance of EM morphotype colonization of trees and seedlings for outplanted nursery seedlings (**a**) and field-germinated seedlings (**b**)

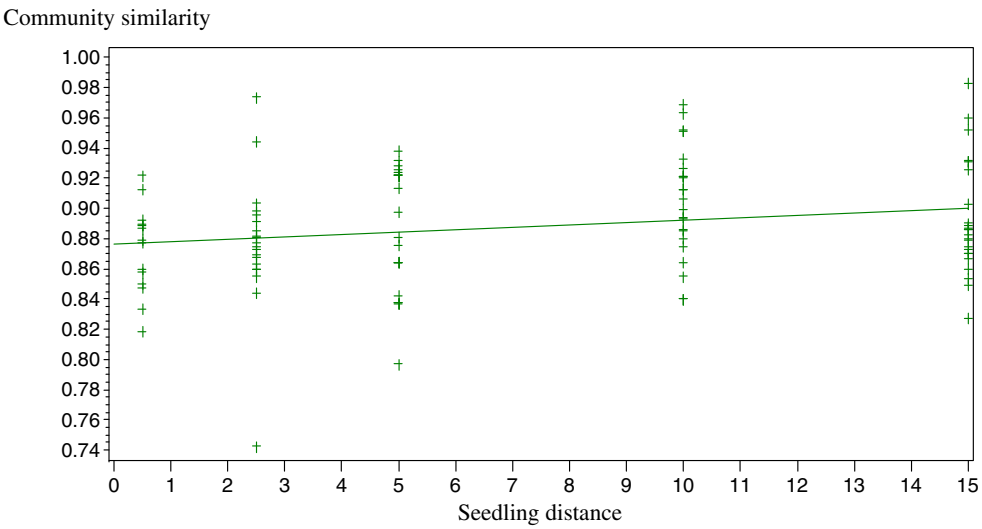

Fig. 3 Relationship of distance from tree with EM fungal community similarity between conspecific trees and nursery seedlings (p=0.0341) from the TOD provenance

Cenococcum geophilum, *Amphinema byssoides*, and *Lactarius rubrilacteus* (Fig. 2a, b). Sixty-seven percent of shared morphotypes on outplanted nursery seedlings and 60% of shared morphotypes on field-germinated seedlings had a relative abundance of >5% on root tips. Overall, community similarity between trees and seedlings was 0.90, and outplanted nursery seedlings did not differ from field-germinated seedlings, based on a t test.

EM fungal community similarity between outplanted nursery seedling and conspecific trees increased with distance (P=0.0279; Fig. 3).

It was also affected by an interaction between SH:M and seedling provenance (P=0.0604) when CWD and the interaction of CWD with SH:M were included as covariates (Table 2). Overall, community similarity increased with SH:M, but the magnitude of this increase was greatest for SA seedlings, and zero for TOA seedlings. Mesh treatment also affected community similarity in outplanted nursery seedlings after adjustment for whether the humus form was a mor (Table 3). Community similarity was lowest when seedlings grew in no mesh and greatest when seedlings grew in 0.5-μm mesh.

EM fungal community similarity between field-germinated seedlings and conspecific trees was affected by mesh treatment (P=0.0187; Table 4). Community similarity increased with SH:M, but the magnitude of this increase was lowest for seedlings growing in no mesh and highest for seedlings growing in 0.5-μm mesh (Fig. 4). Seed provenance was unimportant in influencing EM fungal community distance for cohort 2

Table 2 Analysis of covariance testing for response of the EM fungal community similarity between outplanted nursery seedlings and conspecific trees to seed provenance and 2006–2007 summer heat: moisture index (SH:M), after adjustment for percent cover of coarse woody debris (CWD) and its interaction with SH:M (provenance× cohort experiment)

ANCOVA: AIC=−70.1			
Effect	Coefficient	F value	$P>F$
SH:M	0.5820	4.86	0.0373
Provenance	N/A	3.16	0.0604
SH:M×provenance	N/A	3.16	0.0604
Covariates			
CWD	3.2339	4.34	0.0479
CWD×SH:M	−0.02624	4.33	0.0482

The coefficient values are only given for continuous variables

Table 3 Analysis of covariance testing for response of the EM fungal community similarity between outplanted nursery seedlings and conspecific trees to mesh treatment, after adjustment for whether the humus form was a mor

ANCOVA: AIC=−51.5			
Effect	Coefficient	F value	$P>F$
Mesh	N/A	3.68	0.0484
Covariates			
Mor	−0.04581	6.59	0.0207

The coefficient values are only given for continuous variables

Discussion

Effects of MNs on community similarity

The EM fungal community similarity between residual trees and TOD nursery seedlings planted 2.5 m from the bole of the residual tree (cohort 1) was lower where they had access to the MN of residual trees (lowest in the no-mesh treatment and greatest in the 0.5-μm mesh treatment). Community similarity between residual trees

Table 4 Analysis of covariance testing for response of the EM fungal community similarity between field-germinated seedlings and conspecific trees to mesh treatment and 2007–2008 summer heat:moisture index (SH:M), after adjustment for whether the tree was growing on a SSE slope aspect

ANCOVA: AIC=−72.5

Effect	Coefficient	*F* value	*P*>*F*
SH:M	0.000011	11.62	0.0024
Mesh	N/A	3.91	0.0345
SH:M×mesh	N/A	4.75	0.0187

The coefficient values are only given for continuous variables

and field-germinated seedlings (cohort 2) was affected by an interaction between mesh treatment and SH:M, where community similarity increased with drought index, and this effect decreased with network potential. The results from cohort 1 contradict our hypothesis that EM communities would be most similar when seedlings had the opportunity to join the network of conspecific trees. We found that seedlings able to form an MN were initially colonized by medium- to long-distance exploration type EM fungi (*Rhizopogon vinicolor*, *Tricholoma myomyces*, *A. byssoides*, *Cortinarius* sp., *Tomentella* sp., and *Boletales*) (Agerer 2001). It is plausible that these fungi were able to grow long distances and rapidly dominate the seedling EM community before other EM fungal species were able to establish. By contrast, seedlings that could be colonized only by free soil propagules were more likely to have a diverse community, analogous to that of the tree, and one explanation for this is that these fungi did not have the C source of the tree, and thus exhibited less dominance. The EM fungal community of cohort 2 exhibited the same pattern as cohort 1 in response to mesh treatment, at the driest sites. This apparent increase in strength of network effects under water-stressed conditions is buttressed by data we have for seedling growth and hydraulic redistribution of water (Bingham and Simard 2011).

Climate effects

The EM fungal community similarity between residual trees and seedlings increased with regional drought, and this was true across all cohorts, distances, provenances, and mesh treatments. This partially supports our second hypothesis, that is, EM fungal communities of seedlings would be most similar to neighboring trees in the driest climates. This may be explained by slower growth rates of seedlings in the driest environments, which would have lower ability to associate with the more C-demanding, medium- to long-distance exploration type EM fungi, thus contributing to the convergence in EM fungal diversity between the trees and seedlings. By contrast, seedlings growing in the wettest climate would experience rapid colonization by these types, resulting in greater differences with the community of residual trees. Alternatively, seedlings may have associated with a more diverse EM fungal community similar to neighboring trees in arid climates because of increased niche packing and access to scarce resources.

Provenance effects

Climatic drought stress and provenance interacted to affect community similarity in cohort 1. The TOA seedlings were most similar to conspecific trees in the wettest climate, while TOD and SA seedlings were most similar in the driest climate. Thus, we reject a portion of our second hypothesis, that EM fungal community similarity should be highest where seedling provenance is best matched to the climatic conditions of the site. However, the significance of the interaction between provenance and climatic drought stress does suggest that there is some phenotypic interaction

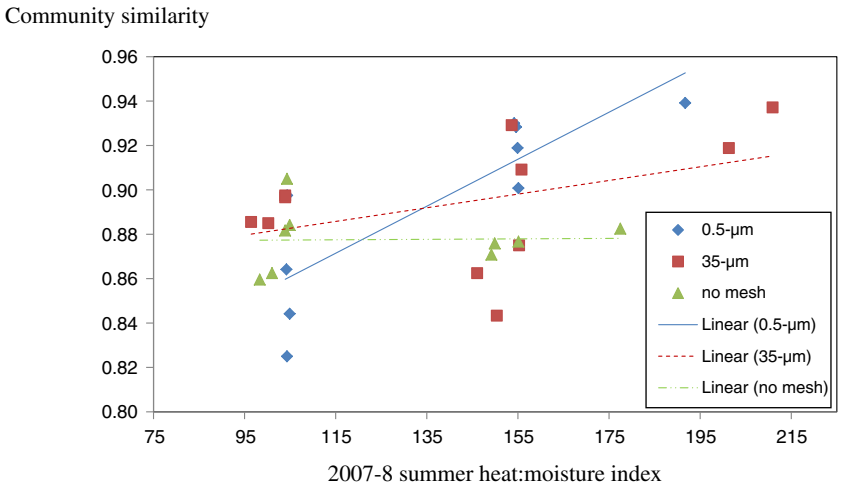

Fig. 4 Relationship of 2006–2007 summer heat:moisture index with EM fungal community similarity between conspecific trees and field-germinated seedlings from the TOD provenance growing in different mesh treatments. The slope of the regression for 0.5-µm mesh is statistically different from that of the 35-µm and no-mesh treatments ($P<0.05$)

between individual trees and EM fungi. The lack of interaction between provenance and network potential suggests that provenance effects on EM community similarity were driven more by ruderal rather than medium- to long-distance exploration type fungi.

Distance effects

The EM fungal community similarity between residual trees and nursery seedlings belonging to the TOD provenance decreased with proximity to the tree, regardless of mesh treatment. These results contradict our third hypothesis, that EM communities would be most similar when seedlings are growing in close proximity to conspecific trees. However, this result, that there is increasing disparity between seedling and trees with proximity, is consistent with our inference that the most rapidly colonizing fungi in the MN have an overriding influence on the EM fungal community of the seedlings. If these fungi increase MN prevalence with proximity to the tree, and they tend to dominate the seedling when colonizing via MNs, they should tend to skew the species distribution in their favor during early colonization of seedlings. We were surprised that distance did not interact with networking potential (mesh), where we expected community similarity to vary differently with distance depending on mesh size (e.g., no variation with distance in 0.5-μm mesh bags). This may be a consequence of our low level of replication.

Cohort effects

While drought index interacted with provenance in cohort 1, provenance was unimportant in cohort 2. It is not surprising that provenance effects were not observed in cohort 2, as many EM fungal species likely do not colonize germinants, an adaptation that would preclude them from investing in plants that have a low probability of survival. Provided rapid colonization by medium- to long-distance EM fungi indeed determined differences in community similarity among seedlings, little difference would be expected among provenances in cohort 2, due to lack of colonization by these same fungi.

Conclusions

The EM fungal community similarity between conspecific trees and seedlings was affected by MNs, climate, distance from conspecific trees, and seed provenance, but these effects differed by life history of the seedling. The major conclusions from this study are (1) EM fungal community similarity decreased with networking potential between residual trees and outplanted nursery seedlings regenerating nearby; (2) MNs interacted with climate such that EM community similarity of field germinants at 2.5 m from conspecific trees decreased with network potential at the driest sites; (3) EM fungal community similarity between seedlings and conspecific trees increased with summer drought index; (4) EM fungal community similarity increased with distance from the tree; and (5) provenance interacted with climate such that EM fungal community similarity was lowest among provenances best adapted to that particular climate for nursery grown seedlings, but provenance had no effect on field germinants. Taken together, our results suggest that environmental conditions conducive to better seedling performance (e.g., better phenotypic matches and wetter environmental conditions) favored long-distance exploration types, thus fostering an EM fungal community composition that, paradoxically, was quite different from that of the residual trees. Nevertheless, our results show that mycorrhizal networks of residual trees have important influences on EM colonization patterns of interior Douglas-fir seedlings, and the strength of these effects is greater in arid environments. This suggests that conservation of mycorrhizal networks is most important where regional aridity is high.

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References

Agerer R (ed) (1987–1993) Colour atlas of ectomycorrhizae. Einhorn-Verlag Eduard Dietenberger, Schwäbisch Gmiind

Agerer R (2001) Exploration types of ectomycorrhizae. Mycorrhiza 11:107–114

Bingham MA (2011) The role of ectomycorrhizal networks in plant-to-plant facilitation across climatic moisture gradients. Dissertation, University of British Columbia, Vancouver

Bingham MA, Simard SW (2011) Do mycorrhizal network benefits to survival and growth of interior Douglas-fir seedlings increase with soil moisture stress? Ecol Evol (in press)

Booth MG, Hoeksema JD (2010) Ectomycorrhizal networks counteract competitive effects of canopy trees on seedling survival. Ecology 91:2294–2302

Callaway RM, Brooker RW, Choler P, Kikvldze Z, Lortie CJ, Michalet R, Paolini L, Pugnaire FI, Newingham B, Aschehoug ET, Armas C, Kikodze D, Cook BJ (2002) Positive interactions among alpine plants increase with stress. Nature (London) 417:844–848

Dickie IA, Koide RT, Steiner KC (2002) Influences of established trees on mycorrhizas, nutrition, and growth of *Quercus rubra* seedlings. Ecol Monogr 72:505–521

Dickie IA, Schnitzer SA, Reich PB, Hobbie SE (2005) Spatially disjunct effects of co-occurring competition and facilitation. Ecol Lett 8:1191–1200

Eissenstat DM, Volder A (2005) The efficiency of nutrient acquisition over the life of a root. In: BassiriRad H (ed) Nutrient acquisition by plants: an ecological perspective, vol 181, Ecological studies. Springer Verlag, Berlin, pp 185–220

Greenlee JT, Callaway RM (1996) Abiotic stress and the relative importance of interference and facilitation in montane bunchgrass communities in western Montana. Am Nat 148:386–396

Goodman DM, Durall DM, Trofymow JA, Berch SM (1996) Concise descriptions of North American ectomycorrhizae. Mycologue Publications, Sidney

Hamann A, Wang TL (2005) Models of climatic normals for genecology and climate change studies in British Columbia. Agric For Meteorol 128:211–221

Hoeksema JD (2010) Ongoing coevolution in mycorrhizal interactions. New Phytol 187:286–300

Ingleby K, Mason PA, Last FT, Fleming LV (1990) Identification of ectomycorrhizas. ITE research publication no. 5. HMSO, London

Johnson D, Leake JR, Read DJ (2001) Novel in-growth core system enables functional studies of grassland mycorrhizal mycelial networks. New Phytol 152:555–562

Liancourt P, Callaway RM, Michalet R (2005) Stress tolerance and competitive-response ability determine the outcome of biotic interactions. Ecology 86:1611–1618

Lloyd D, Angove K, Hope G, Thompson C (1990) A guide to site identification and interpretation for the Kamloops Forest Region BC Ministry of Forests, Victoria Land Manag Handb 23

McGuire KL (2007) Common MNs may maintain monodominance in a tropical rain forest. Ecology 88:567–574

Milliken GA, Johnson DE (2002) Analysis of messy data, vol III, Analysis of covariance. Chapman and Hall, Boca Raton

Rehfeldt GE (1989) Ecological adaptations in Douglas-Fir (*Pseudotsuga menziesii* var. *glauca*): a synthesis. For Ecol Manag 28:203–215

Spittlehouse DL (2008) Climate change, impacts, and adaptation scenarios: climate change and forest and range management in British Columbia. Technical Report 045. B.C. Min. For. Range, Res. Br., Victoria. Available at http://www.for.gov.bc.ca/hfd/pubs/Docs/Tr/Tr045.htm

Steel RGD, Torrie JH (1980) Principles and procedures of statistics: a biometrical approach, 2nd edn. McGraw-Hill, New York

Teste FP, Karst J, Jones MD, Simard SW, Durall DM (2006) Methods to control ectomycorrhizal colonization: effectiveness of chemical and physical barriers. Mycorrhiza 17:51–65

Teste FP, Simard SW (2008) MNs and distance from mature trees alter patterns of competition and facilitation in dry Douglas-fir forests. Oecologia 158:193–203

Teste FP, Simard SW, Durall DM (2009) Role of mycorrhizal networks and tree proximity in ectomycorrhizal colonization of planted seedlings. Fungal Ecol 2:21–30

Teste FP, Simard SW, Durall DM, Guy RD, Berch SM (2010) Net C transfer between *Pseudotsuga menziesii* var. *glauca* seedlings in the field is influenced by soil disturbance. J Ecol 98:429–439

Twieg BD, Durall DM, Simard SW (2007) Ectomycorrhizal fungal succession in mixed temperate forests. New Phytol 176:437–447

Twieg BD, Durall DM, Simard SW, Jones MD (2009) Influence of soil nutrients on ectomycorrhizal communities in a chronosequence of mixed temperate forests. Mycorrhiza 19(5):305–316

Vose RS, Schmoyer RL, Steurer PM, Peterson TC, Heim R, Karl TR, Eischeid J (1992) The global historical climatology network: long-term monthly temperature, precipitation, sea level pressure, and station pressure data. ORNL/CDIAC-53, NDP-041. C Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge, p 325

Wang T, Hamann A, Spittlehouse D, Aitken SN (2006) Development of scale-free climate data for western Canada for use in resource management. Int J Climatol 26(3):383–397