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Host and habitat filtering in seedling root-associated fungal communities: taxonomic and functional diversity are altered in 'novel' soils

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Abstract Climatic and land use changes have significant consequences for the distribution of tree species, both through natural dispersal processes and following management prescriptions. Responses to these changes will be expressed most strongly in seedlings near current species range boundaries. In northern temperate forest ecosystems, where changes are already being observed, ectomycorrhizal fungi contribute significantly to successful tree establishment. We hypothesised that communities of fungal symbionts might therefore play a role in facilitating, or limiting, host seedling range expansion. To test this hypothesis, ectomycorrhizal communities of interior Douglas-fir and interior lodgepole pine seedlings were analysed in a common greenhouse environment following growth in five soils collected along an ecosystem gradient. Currently, Douglas-fir's natural distribution encompasses three of the five soils, whereas lodgepole pine's extends much further north. Host filtering was evident amongst the 29 fungal species encountered: 7 were shared, 9 exclusive to Douglas-fir and 13 exclusive to lodgepole pine. Seedlings of both host species formed symbioses with each soil fungal community, thus Douglas-fir did so even where those soils came from outside its current distribution. However, these latter communities displayed significant taxonomic and functional differences to those found within the host distribution, indicative of habitat filtering. In contrast, lodgepole pine fungal communities displayed high functional similarity across the soil gradient. Taxonomic and/or functional shifts in Douglas-fir fungal communities may prove ecologically significant during the predicted northward migration of this species; especially in combination with changes in climate and management operations, such as seed transfer across geographical regions for forestry purposes.

Keywords Assisted migration · Co-occurrence · Distribution boundary · Douglas-fir · Ectomycorrhizal · Lodgepole pine

Introduction

Climate change is expected to significantly influence the future ranges of plant species (IPCC 2013), with individual species experiencing expansion and/or contraction of regions that are suitable for establishment and growth (Iverson et al. 2004; Aitken et al. 2008; Coops and Waring 2011; Wang et al. 2012). Future distribution models are primarily based upon on temperature and moisture changes, and in northern hemisphere forests, rapid northward expansion of climatic suitability zones are predicted (Hamann and Wang 2006; Rehfeldt et al. 2014). Changes in climate are expected to alter interactions between plants and their fungal symbionts (Pickles et al. 2012), yet symbioses are rarely considered when modelling future species distributions (Dormann 2007). Recent paleoecological studies have indicated that historical range expansions did not simply follow climatic shifts, leading Elias (2013) to speculate that incompatibility with symbiont

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communities may have played a role in slowing host plant migrations in the Pacific Northwest. Thus, the contemporary distribution boundaries of host plants provide a useful ecological demarcation across which future range expansion can be examined with respect to host-symbiont interactions. Here, we assessed seedling interactions with symbionts across one such boundary to explore our hypothesis that mycorrhizal fungi could play a role in either limiting or facilitating host range expansion in response to climatic changes.

In western North America, *Pseudotsuga menziesii* var. *glauca* (interior Douglas-fir) is an ecologically and economically important constituent of forests, with a distribution that extends from isolated patches in Mexico around 19.0° N to approximately 54.5° N in western Canada. In British Columbia, interior lodgepole pine (*Pinus contorta* var. *latifolia*) and interior Douglas-fir co-occur within the same landscapes and across the same gradient of ecosystems (Pojar et al. 1987). Douglas-fir is thought to disappear from these ecosystems at its northern boundary because of high sensitivity to microsite conditions that favour the formation of frost pockets (Jull 1999; Griesbauer and Green 2010), though it is unlikely that this single factor controls the presence or absence of Douglas-fir. Thus, these two host species may display a degree of commonality in their regional and local symbiont species pools (for a discussion of the 'species pool' concept see Zobel 1997; Zobel et al. 1998, 2011) due to overlapping distributions, and the presence of lodgepole pine may facilitate the natural northward expansion of Douglas-fir through maintenance of symbiont reservoirs if environmental limitations to host distribution are removed through climatic changes (e.g. Reithmeier and Kernaghan 2013). Here, we refer to observed differences in the fungal communities of different hosts grown in the same soil as 'host filtering'.

Formation of symbioses with ectomycorrhizal fungi (EMF) are important for seedling establishment in North American Douglas-fir forests (Simard 2009), with community differences observed between different life stages of Douglas-fir (Twieg et al. 2007). In our examination of the literature, we have found that, where multi-host EMF systems have been examined, it appears common for approximately one third of EMF species to be shared (with the exception of *Alnus* and its more specialised/selective community; Bogar and Kennedy 2013; Roy et al. 2013), thus a reservoir of suitable fungal symbionts may be available outside a host's natural distribution through persistence on an alternate co-occurring host species (Ishida et al. 2007; Twieg et al. 2007; Timling et al. 2012; Lim and Berbee 2013). Indeed, the observed distribution of some EMF species covers temperate and boreal regions throughout the Northern hemisphere (e.g. *Cortinarius* spp.; Liimatainen et al. 2014), which suggests that many fungal symbionts are likely to be capable of utilising multiple hosts. Spore dispersal is also likely to provide hosts access to compatible symbionts, although this only appears relevant over short spatial scales (Peay et al. 2012), and the accumulation of potentially long-lived spores in soils (Bruns et al. 2009; Nara 2009; Nguyen et al. 2012) may increase the availability of symbionts in a manner analogous to the soil seed bank (Thompson 2000).

Alongside EMF, Douglas-fir and lodgepole pine are colonised by a variety of non-mycorrhizal root-associated fungi. Whereas the benefits of EMF are well established (Smith and Read 2008), the consequences of root colonisation by non-mycorrhizal fungi are less clear. In a meta-analysis of root endophytes, Mayerhofer et al. (2013) found that although beneficial effects of endophytes on plant biomass occurred, interactions were mostly neutral to negative; however, they also noted that plant responses are highly variable and specific to the environmental context. A meta-analysis of dark septate endophyte (DSE) fungi by Newsham (2011) reported increases in host root and shoot biomass when nitrogen was predominantly available in organic form and attributed this benefit to nutrient mineralisation. This latter hypothesis is especially compelling since beneficial effects of *Phialocephala fortinii* have been noted even in the absence of direct colonisation of the roots by the fungus (Ruotsalainen and Kytöviita 2004). Clearly, fungal root endophytes display a fundamentally different mode of action from EMF: whereas EMF generally aid the host via the direct uptake of nutrients by extramatrical hyphae, fungal endophytes are more variable in their effects upon growth but may improve nutrient uptake via mineralisation processes. For the purposes of this study, we considered the following to be fungal endophytes: (i) root endophytes, including DSE such as *Phialocephala* spp. (Addy et al. 2005), (ii) species for which there is limited evidence for definitive mycorrhizal status with the host e.g. Helotiales spp. (Tedersoo et al. 2009) and *Meliniomyces* sp. (Hambleton and Sigler 2005) and (iii) putative pathogenic fungi residing in roots (e.g. Nilsson et al. 2014).

Where a slowly dispersing host such as Douglas-fir is introduced into new regions, such as through management intervention (e.g. assisted migration; Pedlar et al. 2012), it may exhibit novel responses to the available symbiont communities in that region. As an extreme example, members of the Pinaceae have historically failed to establish in exotic forestry operations due to an absence of co-evolved symbionts (Mikola 1970; Nuñez et al. 2009). However, with range expansion or limited seed transfer, the relevant question is whether the formation of suboptimal symbioses outside of a tree's range could lead to reduced resilience during colonisation of a new habitat (Elmqvist et al. 2003), perhaps resulting from a loss of functional redundancy (Rineau and Courty 2011). Possible mechanisms for compromised resilience are as follows: (a) a legacy of host absence may lead to fewer compatible fungal species being available (especially those considered to be 'specialists'), (b) changes in the filters acting on the actual pool (environmental and biological) may lead to

shifts in symbiont function and/or (c) symbionts may readily interact with the host but provide less benefit. If available symbiont diversity is lowered, or key symbionts are absent, this could contribute to the observed range limits of Douglas-fir. Kranabetter et al. (2012) suggests this as a factor for consideration in seed transfer operations involving coastal Douglas-fir, although further examination is required. Here, we use the term 'habitat filtering' from the assembly rules literature (e.g. Diamond 1975; Weiher and Keddy 1999) to represent the combination of environmental variables influencing successful symbiont colonisation of a host at a site, such that only those species with suitable ecological or physiological adaptations will form symbioses.

We hypothesised that the degree of compatibility with fungal symbionts could facilitate or limit interior Douglas-fir migration northwards in response to climate change. To test this, we examined seedling root colonisation by EMF and fungal endophytes across a northern distribution boundary, using interior Douglas-fir as an experimental species and interior lodgepole pine as a background control due to its extensive natural distribution throughout British Columbia (Little 1978). Host genotypes show heritability for several EMF traits (Rosado et al. 1994; Peterson and Bradbury 1999; Karst et al. 2009). Plants also display evidence of local adaptation to mycorrhizal symbionts (Johnson et al. 2010; Hoeksema et al. 2012; Ji et al. 2013). Given these observations, testing the responses of multiple seed provenances (local populations of known origin) of each host species was warranted. Thus, seeds from eight provenances of each host were grown in forest soil taken from five sites through British Columbia selected along an ecosystem gradient of biogeoclimatic zones (BEC zones; Pojar et al. 1987). This gradient crossed the northern distribution boundary of Douglas-fir but was well within the distribution of lodgepole pine. Functional differences were approximated using the 'exploration type' concept, in which mycorrhizal morphology (e.g. extent of extramatrical hyphae, rhizomorphs, etc.) reflects differences in nutrient acquisition strategies (Agerer 2001). Fungal endophytes were added as an additional functional type in recognition of the fact that, while we cannot yet quantify their effect on host plants with certainty, the evidence points to differences in function from EMF. The contributions of host and/or habitat filtering to seedling root-associated fungal communities were assessed.

Materials and methods

Seed selection and site descriptions

Seed of interior Douglas-fir and interior lodgepole pine from eight provenances per species (five class A, selectively bred 'improved', provenances (A1–A5) typically used in forestry operations and three class B, seed orchard or 'wild', provenances (B1–B3)) was provided by the British Columbia Ministry of Forests, Lands and Natural Resource Operations (BC MoFLNRO). Class B seed lots were selected from the BC MoFLNRO seed bank based on their close geographic proximity and climatic similarity (Hamann and Wang 2005; Wang et al. 2006) to that of class A seed orchards (Online Resource 1).

Study sites were established at five 1-ha areas within British Columbia (BC) where interior Douglas-fir is either (a) currently the dominant tree species or (b) not currently present but projected to become climatically suitable within the next 45 years (Fig. 1). Each site was continental in climate (mean annual temperature ranged from 0.8 to 3.4 °C), experienced frost over more than half of the year and was located within an undisturbed forest with an estimated stand age of approximately 80 years. These sites are referred to in the text using a four-letter code based on location and host species; climatic and soil nutrient data are provided for each site (Online Resource 2). The mono-dominant host species at each site (>95 % of the stand) was either interior Douglas-fir (VN-DF, PG-DF, JP-DF) or interior lodgepole pine (MZ-LP, FS-LP), with no mixing of these host species within each site.

Greenhouse growth conditions

From mid-September to mid-October 2008, approximately 120 l of O-horizon soil (upper 15 cm, including small volumes of mineral horizon) was collected from five sub-locations per

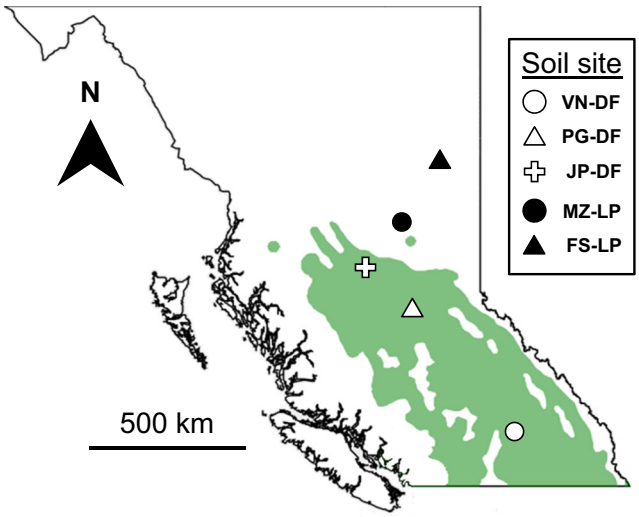

Fig. 1 Geographical distribution of study sites for soil collection and comparison with current natural range of interior Douglas-fir (*green shading*). *White symbols* were naturally regenerating interior Douglas-fir stands of harvestable age, with scattered *Betula papyrifera* (paper birch) also present. *Black symbols* were interior lodgepole pine stands of similar age structure, and scattered paper birch. Small numbers of *Picea glauca* (white spruce) were present in the FS-LP site. Each site had a ground layer of mosses and ericaceous plants, mostly *Vaccinium myrtilloides* (velvet leaf blueberry)

site, sieved to remove large debris (2 cm×2 cm mesh) and transported for homogenisation in clean conditions. Seed was pretreated for germination using cold stratification and surface sterilisation (with hydrogen peroxide) techniques (Kolotelo et al. 2001). Twenty seedlings per provenance per soil (800 seedlings per host species) were established (two seeds planted with subsequent thinning if both emerged) in individual 650 ml Deepot™ seedling containers (Stewe and Sons, Oregon, USA). Containers were filled to within 1 cm of the rim with soil then covered with a layer of fine gravel to reduce surface evaporation and 'damping off'. To stimulate mycorrhizal colonisation, no fertilisation was applied, but otherwise 'non-limiting' conditions (moisture, light and temperature) were maintained for approximately one growing season (8 months) to give time for mycorrhizal formation (initial examination at 3 months revealed little to no mantle formation). Soil moisture was maintained at a minimum of 80 % of saturated mass through irrigation only. Day length and temperature were standardised over the experimental period (16 h of daylight at 25 °C, 8 h of night at 20 °C, 15 min transition intervals of 22 °C) using 400-W high-pressure sodium lights (P.L. Light Systems, Beamsville, ON, Canada.).

Fungal identification

Following 8–9 months of growth, fungal community data was recorded for five randomly selected seedlings per provenance per soil (200 seedlings per host species; reduced to 195 Douglas-fir and 167 lodgepole pine due to seedling mortality). For each seedling individually, all fine roots were extracted and placed on a numbered grid in a container filled with deionised water. To avoid selection bias, 50 root tips were assessed from each root system using a random number generator to select grid squares. Excised fine roots were examined at ×40 magnification under a stereomicroscope and sorted into uncolonised (no discolouration or signs of hyphae or mantle formation) or individual fungal morphotypes. Five uncolonised tips and five tips of each putative morphotype were subsampled from each seedling and stored for later DNA analysis to ensure reliability of morphotyping. Where available, a minimum of 10 sequenced root tips per morphotype were used to confirm species identities. Root tips were homogenised using a micropestle, and fungal DNA was extracted using the DNeasy Plant Mini Kit according to the manufacturer's instructions (Qiagen, ON, Canada). The internal transcribed spacer region (ITS) was amplified for each DNA sample using ITS1 (White et al. 1990) and NLB4 primers (Martin and Rygiewicz 2005). Amplifications were performed on a PTC Dyad Thermal Cycler (MJ Research Inc., MA, USA) in 50 μl volumes containing: nuclease-free H_2O, 2 mM $MgCl_2$, 5.0 μl buffer (16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl (pH 8.8 at 25 °C), 0.01 % Tween-20)), 2 mM dNTP, 20 pmol each primer, 2.5 μl BSA, 1.15 units Platinum® Taq DNA polymerase (Invitrogen, ON, Canada) and 100 ng DNA. Thermocycling conditions: 3 min denaturation (95 °C), 35 cycles of denaturing, annealing and extension (95 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min, respectively) and 5 min final extension (72 °C). PCR products were purified using the Agencourt AMPure system (Invitrogen) then sequenced using ABI BigDye v3.1 Terminator chemistry and an ABI 3130xl Genetic Analyser (Applied Biosystems, ON, Canada). Raw sequence data were analysed with SEQUENCHER Version 4.7 (Gene Codes Corp., MI, USA) before comparison with NCBI and UNITE (Kõljalg et al. 2013) databases using the BLAST algorithm. Names were assigned to morphotypes based on the combination of morphological characteristics and minimum 97 % sequence matches corresponding to the indicated species. These sequence data have been submitted to the GenBank database under accession numbers JF792502–JF792516 (Douglas-fir sequences) and KM008614-KM008632 (lodgepole pine sequences).

Based on the morphotyping-sequencing technique described above, root tip communities were divided into EMF, fungal endophytes and uncolonised categories then into the different exploration types. 'Uncolonised' root tips displayed no discernible mantle and an insignificant quantity of fungal DNA following extraction with the DNEasy kit. Further examination of subsampled tips with microscopy found no evidence of Hartig net formation. Occasionally, a root tip contained an EMF sequence and a 'fungal endophyte' sequence. In these cases, the EMF was considered to take functional priority due to the presence of hyphae and/or a mantle indicating structural changes to the root tip. Tips that only contained fungal endophyte DNA and showed no evidence of EMF colonisation were assigned to the 'fungal endophyte' category. Finally, to confirm that fungal species encountered in the greenhouse were present in the field, rather than 'weedy' species (e.g. Marx et al. 1982; Smith and Read 2008), fungal sequences were compared to those detected on mature host roots (see Online Resource 3) at each of the five field sites in a separate study (Pickles et al. unpublished data).

Statistical analyses

We note that for all analyses in which multiple testing took place, the Benjamini and Hochberg false discovery rate (FDR) correction was applied to *P* values (Verhoeven et al. 2005). Normality and variance of data was examined prior to selection of an appropriate parametric (GLM ANOVA) or nonparametric (K-W) statistical test using Minitab® 16.

Fungal community interactions were assessed using *C*-score analysis (Stone and Roberts 1990) in EcoSim v 7.72 (Gotelli and Entsminger 2009), which was used here to determine the extent to which species co-occurred between hosts, as compared to randomised data. A negative *C*-score indicates that species occur together on a host more often than would be

expected by chance (interpreted as 'facilitation'), whereas a positive *C*-score indicates species are less likely to occupy the same host than would be expected if they were randomly distributed (interpreted as 'competition', although in the original conception of co-occurrence analysis (Stone and Roberts 1990) competition was between sites or habitats, whereas here, we ask whether available fungal species will colonise one host species preferentially over the other). Each seedling root system was considered to be a 'site' which fungi could colonise. Null model constraints were set to 'fixed-equiprobable', meaning that the occurrence of each fungal species in any null community was the same as in the observed data, and each seedling was considered equally suitable for each fungal species; this is the most stringent option for analysis (Gotelli and Entsminger 2009). Species present on fewer than three seedlings were removed from the data set due to a lack of interpretive value. Expected *C*-scores were obtained from 10,000 Monte Carlo randomisations of the data using a sequential swap algorithm. Observed *C*-scores were compared to the null distribution of expected values and considered ecologically relevant if statistically significant at $P<0.05$ and exhibiting a standardised effect size (SES) >2 or <−2 (a difference of two or more standard deviations from the expected mean). Null model assumptions were primarily applied to examine evidence for host filtering, which was expressed when competition was significant across all seedlings of both hosts and simultaneously nonsignificant when either host was considered separately. The overall presence or absence of habitat filtering could also be examined based on whether competition was significant across all sites for a single host and simultaneously nonsignificant within sites for that host (i.e. all site 'competitive' effects are purely due to differences in communities between sites, not actual competition between fungal species within sites). Where ecological relevance was detected, values in the upper (or lower) 97.5 % tail of the distribution of pairwise *C*-scores were considered to represent significant species interactions.

Patterns in fungal community data and exploration type data were assessed with nonparametric multi-dimensional scaling (NMS) using the Sørensen (Bray-Curtis) distance measure in PC-Ord 5 (MjM Software, OR, USA). Mean community data for each provenance was used in these analyses to account for variation in EMF communities within a site, resulting in eight data points per host per soil site. Each ordination began with a stress-minimising, resolution optimising step: 6-dimensional solution stepping down to 1-dimensional, instability criterion 0.0005, 200 iterations, 50 real data runs, 100 Monte Carlo simulations. Final ordinations used: 3-dimensional (fungal taxa) or 2-dimensional (exploration type) solution, stress-minimising starting configuration, no step-down in dimensionality, one real run, no randomised runs. Pearson's (r) linear and Kendall's (τ) rank correlations with ordination axes were assessed for fungal species data, soil chemistry and site-specific environmental factors. For factors correlated with each other at r^2 values≥0.90, only the factor producing the strongest correlations to ordination axes was presented.

Differences in community composition were examined for both fungal species and exploration types using permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) in PC-Ord 5 (MjM Software, OR, USA). Provenance mean community or exploration type data was converted into Sørensen (Bray-Curtis) distances and observed values from the model 'community dissimilarity ~ Host*Site' were compared to the expected values obtained from 5000 randomisations of the data. Pairwise site comparisons were determined by stratifying community permutations within each host species and significance was established after correcting for multiple testing. Since heterogeneity in group dispersion can lead to spurious PERMANOVA results, multivariate dispersion was examined in the R software environment (R Core Team 2014) using the Betadisper function in R-package 'vegan' (Oksanen et al. 2013).

Results

Seedling fungal communities and root colonisation

A total of 29 fungal species were encountered across both hosts and all soils, seven of which were shared between hosts (24.1 %), with nine exclusive to Douglas-fir (31.0 %) and 13 exclusive to lodgepole pine (44.8 %). Interior Douglas-fir seedlings were colonised by 14 EMF and three fungal endophyte species (Table 1) compared to 12 EMF and eight fungal endophytes on interior lodgepole pine seedlings (Table 2). The most frequently encountered fungal species on Douglas-fir seedlings were Pyronemataceae sp. (57.1 %) and *Rhizopogon* sp. 1 (48.2 %), which were the two most abundant ECM fungi on interior Douglas-fir at sites inside its distribution, yet had a negligible presence elsewhere (Fig. 2). Ectomycorrhizas of *Rhizopogon* spp. accounted for approximately 30–50 % of all Douglas-fir seedling root tips in soils from inside its range and only 5–10 % of root tips outside its range. The Douglas-fir associate *Suillus lakei*, considered a specialist symbiont of Douglas-fir, was only encountered on seedling roots in soils from *outside* the contemporary host distribution. For lodgepole pine, the fungal endophyte *Meliniomyces* sp. 1 was the most frequently encountered fungus overall (88.0 %), with *Rhizopogon* sp. 5 (49.7 %) and *Cenococcum* sp. (44.9 %) as the most frequent EMF (Fig. 2). Of these three fungi, the *Meliniomyces* sp. was abundant on the host at all five sites, the *Rhizopogon* sp. was only abundant at the three sites inside the distribution of Douglas-fir, and the *Cenococcum* sp. was present at low abundance across all five sites. Each fungal species observed on

Table 1 Identity and relative frequency of ectomycorrhizal and other root-associated fungi on *Pseudotsuga menziesii* var. *glauca* seedlings

Species ID	Accession	Total relative frequency (%)[a]	Best sequence match (NCBI accession)	Overlap (% similarity)
Uncolonised roots	N/A	70.2	–	–
Pyronemataceae sp.	JF792511	57.1	Uncultured Pyronemataceae (GU452518)	696/697 (99)
Rhizopogon sp. 1	JF792507	48.2	Uncultured *Rhizopogon* (GU452519)	761/770 (98)
Meliniomyces sp. 1	JF792512	34.6	Uncultured Meliniomyces (GU452532)	650/655 (99)
Cenococcum sp.	JF792502	34.6	Uncultured *Cenococcum* clone (GU452521)	668/669 (99)
Rhizopogon sp. 2	JF792508	17.8	Uncultured ectomycorrhiza (*Rhizopogon*) (EF218795)	752/756 (99)
Rhizopogon sp. 3	JF792509	15.2	Uncultured ectomycorrhiza (*Rhizopogon*) (EF218797)	766/766 (100)
Tuber sp.2	JF792505	6.8	*Tuber pacificum* (JQ712002)	736/736 (100)
Phialocephala sp.1	JF792514	4.7	*Phialocephala fortinii* (AY394921)	655/656 (99)
Suillus lakei (Seq 1)	JF792515	4.2	*Suillus lakei* (DQ367917)	745/750 (99)
Rhizopogon sp. 4	JF792510	3.7	Uncultured *Rhizopogon* (FJ786640)	624/627 (99)
Piloderma sp.	JF792503	3.1	*Piloderma olivaceum* (DQ469291)	586/591 (99)
Tuber sp.1	JF792504	2.6	Uncultured *Tuber* (EF218844)	726/738 (98)
Phialocephala sp. 2	JF792513	2.1	*Phialocephala* sp. RT-2012 isolate FFP1134 (JQ711934)	500/500 (100)
Suillus lakei (Seq 2)	JF792516	1.6	*Suillus lakei* (DQ367917)	759/766 (99)
Tuber sp. 3	JF792506	0.5	Uncultured *Tuber* (EF218844)	741/741 (100)
Unknown sp.1	No sequence	0.5	–	–
Unknown sp. 2	No sequence	0.5	–	–

[a] Percentage of 195 seedlings grown in five different soils that were colonised by the indicated species (or had one or more uncolonised roots)

Douglas-fir seedlings grown in a given soil was also detected at the corresponding field sites, as were 13 of the fungal species encountered on lodgepole pine (Online Resource 3). Species detected in the greenhouse expressed similar frequency and abundance profiles to those detected in the field (data not shown) with the exception of *Piloderma* sp. (common in the field, rare in the greenhouse) and *Cenococcum* sp. (rare in the field, common in the greenhouse).

Table 2 Identity and relative frequency of ectomycorrhizal and other root-associated fungi on *Pinus contorta* var. *latifolia* seedlings

Species ID	Accession	Total relative frequency (%)[a]	Best sequence match (NCBI accession)	Overlap (% similarity)
Meliniomyces sp.1	KM008614	88.0	Uncultured Meliniomyces BJP0855T (JF792512)	536/543 (99)
Uncolonised roots	N/A	80.8	–	–
Rhizopogon sp.5	KM008615	49.7	Uncultured fungus (*Rhizopogon*) (JF298208)	794/798 (99)
Cenococcum sp.	KM008616	44.9	Uncultured *Cenococcum* BJP0870T (JF792502)	643/643 (100)
Phialocephala sp.1	KM008617	35.9	*Phialocephala fortinii* (AY394921)	650/650 (100)
Tuber sp.2	KM008618	22.8	*Tuber pacificum* (JQ711989)	736/740 (99)
Pyronemataceae sp.	KM008619	13.2	Uncultured Pyronemataceae BJP1379T (JF792511)	652/652 (100)
Thelephora terrestris	KM008620	12.0	*Thelephora terrestris* (JQ711981)	710/714 (99)
Suillus sp.3	KM008621	9.6	*Suillus luteus* (JQ711940)	703/706 (99)
Leptodontidium sp.	KM008622	7.2	Uncultured *Leptodontidium* clone (GU055637)	571/591 (97)
Inocybe jacobi	KM008623	6.0	*Inocybe jacobi* (HQ604811)	586/586 (100)
Piloderma sp.	KM008624	6.0	Uncultured *Piloderma* (JF792503)	588/588 (100)
Meliniomyces sp.2	KM008625	4.8	Uncultured Meliniomyces clone PP46 H6 (EU726297)	567/584 (97)
Unknown sp.4	No sequence	4.2	–	–
Phialocephala sp.2	KM008626	3.6	*Phialocephala* sp. RT-2012 isolate FFP1134 (JQ711934)	678/678 (100)
Suillus sp.1	KM008627	3.6	*Suillus* sp. RT-2012 (JQ711950)	778/778 (100)
Laccaria sp.	KM008628	3.0	*Cortinarius callisteus* (DQ097876) [*Laccaria* sp. (UNITE database)]	794/795 (99)
Suillus sp.2	KM008629	1.8	*Suillus flavidus* (FJ845439)	748/749 (99)
Helotiales sp.	KM008630	0.6	Uncultured soil fungus clone TC_fungal-H7-ITSFL (GU083137)	596/600 (99)
Meliniomyces sp.3	KM008631	0.6	Uncultured Helotiales isolate B105_4 (FJ378858)	288/293 (98)
Unknown sp.3	KM008632	0.6	Uncultured soil fungus clone TC_fungal-H9-ITSFL (GU083139)	693/700 (99)

[a] Percentage of 167 seedlings grown in five different soils that were colonised by the indicated species (or had uncolonised roots)

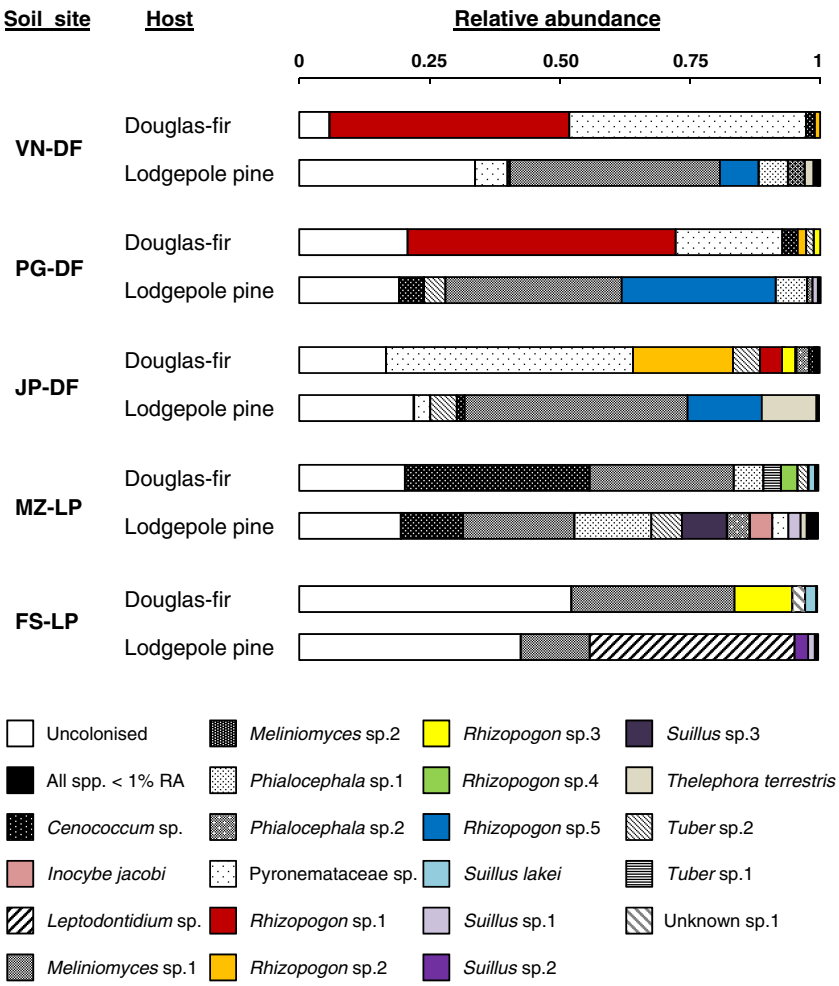

Fig. 2 Mean relative abundance of seedling ectomycorrhizal root tip communities by soil site and host species. Only species comprising ≥1 % of a community are indicated. Basidiomycetes presented in *colour*; ascomycetes use *pattern fill*

Examination of overall seedling colonisation patterns using three categories (uncolonised roots, EMF and fungal endophytes) indicated that each host species interacted differently with these groups (Table 3). Douglas-fir roots were significantly less colonised in the boreal soil (FS-LP) and significantly more colonised in the Douglas-fir BEC zone soil (VN-DF). One class A seed provenance showed consistently low root colonisation, and a further three of the 'improved' seed provenances had lower colonisation than the 'wild' seed provenances across all soils. No effect of seed provenance on root colonisation was noted for lodgepole pine seedlings, which were significantly less colonised in the boreal and Douglas-fir BEC zone soils. EMF colonisation of Douglas-fir was greatest in the soils inside its contemporary distribution. Lodgepole pine EMF colonisation was highest in the three sub-boreal soils and lowest in the boreal soil, where colonised root tips were primarily associated with a *Leptodontidium* sp. (considered a root endophyte of the DSE group and classified as a fungal endophyte; Fernando and Currah 1996) and in the interior Douglas-fir BEC zone soil where lodgepole pine is less common. Fungal endophyte species showed no significant differences in distribution across sites or provenances for lodgepole pine seedlings. Fungi of this category were not detected on Douglas-fir seedlings in the VN-DF or PG-DF soils but were abundant in soils outside its distribution (present on only 10.5 % of JP-DF seedlings, compared to 89.6 % of seedlings grown in MZ-LP and FS-LP soils).

Co-occurrence analysis

C-score analyses were performed across all seedlings of each species, and for each species separately, across all soils taken together and within each soil separately (Table 4). Removal of species with <3 occurrences (Helotiales sp., *Meliniomyces* sp. 3, *Tuber* sp. 3, Unknown 1, Unknown 2, Unknown 3) resulted in a maximum dataset of 362 samples (seedlings) in which 23 species occurred across both species and all soils. Large standardised effect sizes were primarily driven by significant fungal community differences between hosts (i.e. host filtering), as evidenced by their presence when seedlings of both species were considered together (all seedlings column, Table 4) and their absence when only a single host was considered (host only columns, Table 4). These tended to decrease in strength moving northwards along the BEC zone gradient,

Table 3 Differences in seedling % root colonisation by host species, site and provenance

Data[a] (test[b])	Factor	Stat $_{(d.f.)}$	Specific differences
Douglas-fir			
Uncolonised[a]	Site	$F_{(194)}$=35.24***	FS-LP>PG-DF/JP-DF/MZ-LP>VN-DF
(GLM)	Provenance	F=7.37***	A1>A2/A4/A5/B2>B1/B3/A3
	Interaction	F=2.11**	
EMF	Site	$H_{(4)}$=122.09***	VN-DF/PG-DF/JP-DF>MZ-LP>FS-LP
(K-W)	Provenance	ns	–
	Interaction	ns	–
Fungal endophyte	Site	Only detected in MZ-LP and FS-LP	
	Provenance	–	–
	Interaction	–	–
Lodgepole pine			
Uncolonised[a]	Site	$H_{(4)}$=14.95**	VN-DF/FS-LP>PG-DF/JP-DF/MZ-LP
(K-W)	Provenance	ns	–
	Interaction	–	–
EMF[a]	Site	$F_{(166)}$=25.21***	PG-DF/JP-DF/MZ-LP>VN-DF>FS-LP
(GLM)	Provenance	ns	–
	Interaction	ns	–
Fungal endophyte	Site	ns	–
(GLM)	Provenance	ns	–
	Interaction	ns	–

*P<0.05; **P<0.01; ***P<0.001; ns=P≥0.05

[a] Hellinger transformed

[b] GLM = GLM ANOVA; K-W=Kruskal-Wallis

with only one soil indicating no significant difference between the fungal communities of the two host species (MZ-LP). The strong standardised effect size (SES) between all Douglas-fir fungal communities indicated significant 'competition' between sites coupled with nonsignificant SES 'random assembly' within sites; taken together, these observations were suggestive of habitat filtering. Conversely, lodgepole pine fungal communities showed no evidence of habitat filtering between sites (nonsignificant all-site SES) and two cases of strong within-site SES. These latter data points indicated facilitative interactions between fungal species in the JP-DF soil (negative SES) and competitive interactions in the MZ-LP soil (positive SES). Inspection of pairwise species combinations revealed that the JP-DF pattern was driven by the presence of *Meliniomyces* sp.1 on all but one seedling, and the MZ-LP pattern was caused by minimal co-occurrence of *Suillus* sp. 3 and *Tuber* sp. 2.

Multivariate community analysis

Ordination of seedling provenance fungal community data illustrated striking clustering and separation patterns in three

Table 4 Co-occurrence analysis of Douglas-fir and lodgepole pine fungal community incidence data using *C*-score

Site	Samples[a]	Fungal species	C_{obs}	C_{exp}[b]	All seedlings (SES)	Douglas-fir only[c] (SES)	Lodgepole pine only[c] (SES)
All sites	362	23	927.71	834.40	6.68***	8.24***	ns
VN-DF	80	12	145.45	98.21	8.34***	ns	ns
PG-DF	80	10	224.6	159.12	7.94***	ns	ns
JP-DF	75	11	232.6	198.23	4.10***	ns	−3.90***
MZ-LP	71	13	61.22	56.99	1.35	ns	2.57**
FS-LP	56	8	45.81	34.53	2.38**	ns	ns

*P<0.05; **P<0.01; ***P<0.001; ns=P≥0.05

[a] All seedlings of either host species with 1 or more fungal species of frequency 3+

[b] Estimated *C*-score following 10,000 Monte Carlo randomisations where all species were equally likely to appear in any sample

[c] *C*-score analysis using all seedlings of this host only

dimensions (Fig. 3 and Online Resource 4), with two axes representing 75.2 % of the variance. For interior Douglas-fir, communities within the contemporary host distribution clustered closely together and were separated from communities outside the host distribution along the main axis of the ordination (axis 1, 61.9 % of the variance). Simultaneously, the Douglas-fir fungal communities formed in soils from outside of the host distribution were clustered together with all of the lodgepole pine communities, indicating that host filtering was taking place within the natural range of Douglas-fir but not outside of it. For interior lodgepole pine seedlings, there was no distinct separation of fungal communities between sites along axis 1, indicating that lodgepole pine fungal communities were not affected by the transition zone demarcating the range of Douglas-fir. None of the BEC zone climatic or edaphic factors were strongly correlated with this ordination axis. The second axis of the ordination (axis 2, 13.3 % of the variance) was strongly correlated with BEC zone temperature, length of frost-free period, soil cation exchange capacity and with biologically available soil nitrogen (mineral N; soluble nitrogen). Symbiont species and abiotic factors that were strongly correlated with ordination axes are provided for each host (Online Resource 5). Use of exploration types as functional proxies to categorise fungal species yielded significant separation of Douglas-fir provenance communities, but not lodgepole pine communities (Fig. 4a). Long-range and contact exploration types were the primary root colonists of Douglas-fir provenances inside its contemporary range, with short-range exploration types, uncolonised roots and fungal endophytes defining all lodgepole pine provenances and the Douglas-fir provenances grown in the more northerly BEC zone soils (Fig. 4b). Temperature-related factors were strongly correlated with axis 2 of the exploration type ordination (Online Resource 5).

Multivariate analysis of species and exploration type dissimilarity indices confirmed that the observed differences between fungal communities of each host and site were significant (Table 5). Host was the strongest factor in determining community composition, with the significant interaction effect supporting the observed differences in host by site interactions (Figs. 3 and 4). This indicated that both host and habitat filtering was taking place amongst the fungal communities.

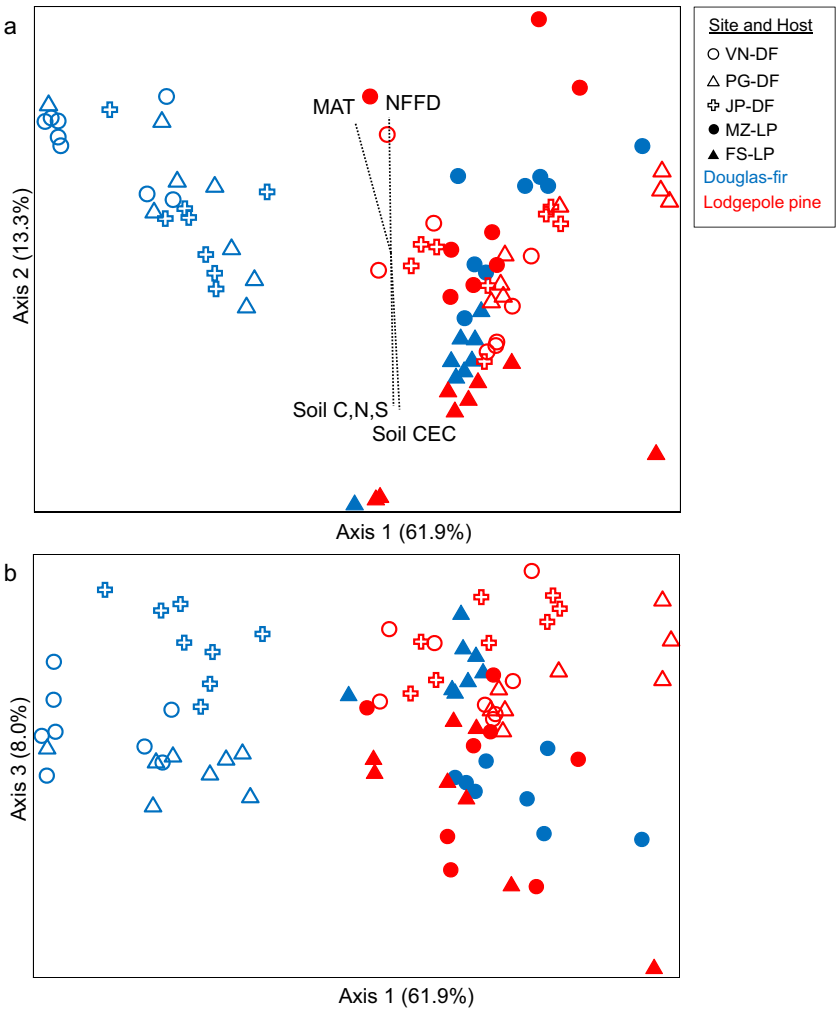

Fig. 3 NMS ordination of seedling root tip fungal species data illustrating community differences between hosts and sites, with *bi-plots* of selected environmental and soil properties correlated with axes at $r^2>0.40$ (*dotted lines* indicate direction of increase). Each data point is the mean community composition of a seedling provenance. Full solution is 3-dimensional (Stress=16.49, $P<0.05$): **a** Axes 1 and 2; **b** axes 1 and 3

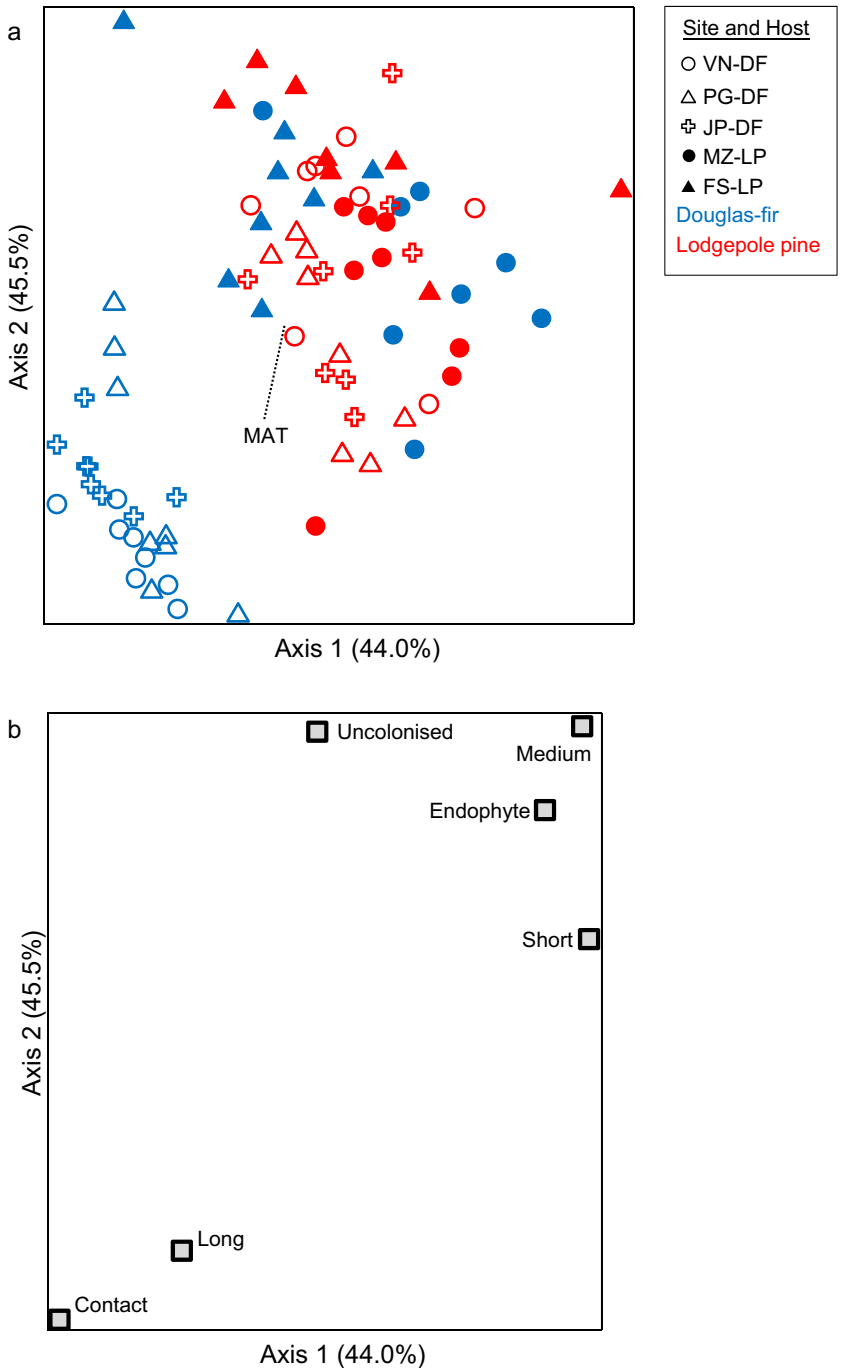

Fig. 4 NMS ordination of seedling root tip exploration type data illustrating: **a** differences between hosts and sites (data points are mean exploration type composition of a seedling provenance) and **b** differences between exploration types. *Bi-plot* indicates direction of increase of mean annual temperature (correlated with axis 2, $r=-0.545$). Full solution is 2-dimensional (Stress=18.09, $P<0.05$)

Pairwise analysis of community composition by site indicated different trends in each host (Table 5). Douglas-fir seedling communities were most similar in the two southernmost sites (VN-DF and PG-DF) with all other pairs of sites showing significant differences in composition. In terms of resource acquisition strategies, there were no significant differences in exploration type composition within the contemporary Douglas-fir range. All pairwise comparisons with and between sites outside of this range indicated that functional composition was significantly different. Two lodgepole pine seedling communities (VN-DF and PG-DF) displayed compositional similarity to that of the transitional JP-DF site; all other pairs of sites indicated significant differences in composition. However, resource acquisition strategies tended to be more similar between sites than those of Douglas-fir, and the differences were unrelated to the Douglas-fir range boundary. Analysis of the homogeneity of multivariate dispersions revealed no significant difference in pairwise dispersion between groups (for species or exploration type data), providing support for the significance of the PERMANOVA analyses.

Table 5 PerMANOVA analysis of main and interactive effects of host and site on seedling fungal community (mean of each provenance), as species or exploration types, using the Sørenson (Bray-Curtis) dissimilarity index

		Species		Exploration types	
Factor	*d.f.*	Pseudo *F*	*P* value	Pseudo *F*	*P* value
Host	1	48.99	<0.001	53.13	<0.001
Site	4	18.70	<0.001	20.64	<0.001
Interaction	4	16.76	<0.001	9.63	<0.001
Residual	70				
Total	79				
Site comparison		Douglas-fir	Lodgepole pine	Douglas-fir	Lodgepole pine
VN-DF:PG-DF		ns	*	ns	**
VN-DF:JP-DF		*	ns	ns	ns
PG-DF:JP-DF		**	ns	ns	ns
VN-DF:MZ-LP		***	***	***	*
VN-DF:FS-LP		***	***	***	ns
PG-DF:MZ-LP		***	***	***	ns
PG-DF:FS-LP		***	***	***	**
JP-DF:MZ-LP		***	***	***	ns
JP-DF:FS-LP		***	***	***	*
MZ-LP:FS-LP		***	***	***	**

ns no significant difference between sites

*$P<0.05$; **$P<0.01$; ***$P<0.001$

Discussion

We used a common-garden greenhouse approach to examine the hypothesis that the degree of compatibility with fungal symbionts could facilitate or limit interior Douglas-fir migration northwards in response to climate change. This approach enabled assessment of the fungal communities formed by two host species, from multiple seed provenances, in soils taken from an ecosystem gradient that crossed the distribution boundary of interior Douglas-fir. Both host and habitat filtering of fungal communities were observed.

Soil inoculum potential

This experiment focused on the potential of the soil to form root-associated fungal communities. We determined that soil from different habitats can influence colonisation, and we found evidence that selection takes place between host seedlings and the available pool of soil-residing symbiont propagules (with selection potentially driven by either partner). Our seedlings were grown from seed in a pooled and homogenised mixture of natural forest organic horizon, and thus the available symbiont species pool was derived from spores plus vegetative colonisation via surviving fungi attached to mature root tips excised during soil coring and collection (e.g. Alexander et al. 1992). Some of the species detected here are known to form long-lived resistant spores (e.g. *Rhizopogon* spp.; Bruns et al. 2009; Nara 2009; Nguyen et al. 2012) or sclerotia (e.g. *Cenococcum* spp.; Massicotte et al. 1992). The fungi derived from these sources are likely to be the most important source of the root-associated fungal community during seedling establishment as tree migration proceeds. Other potential sources excluded from this analysis are the living mycorrhizal networks present in forests (e.g. Simard et al. 2012). Our study focused exclusively on soil-derived fungal symbionts, and we found significant differences based on host and habitat filtering that we expect will play an important role in the field during seedling establishment.

Host filtering

Lodgepole pine and Douglas-fir root-associated fungal communities were significantly different in all soils except one: located 50 km north of the currently recognised distribution boundary of Douglas-fir (MZ-LP) which may be considered an ecological transition zone for this host. In every other soil, only a small fraction of the symbiont species were shared, and observed community differences between hosts were 2–8 standard deviations from the expected mean (where all fungal species colonise each host equally; as per the co-occurrence analysis). The magnitude of the differences between host communities was greater in soils that were collected within the distribution of both hosts (i.e. inside the current distribution of Douglas-fir). This indicates that each host was associating with different fungal species from the same species pool present in the soil, indicating selection by hosts for symbionts or vice versa. Multivariate community analyses strongly

supported the observation of host filtering within the current Douglas-fir distribution. Because the experiment was performed in greenhouse containers over a single growing season, preferential selection either by host or fungus, as well as priority effects (e.g. Kennedy et al. 2009) appear the most likely processes responsible for the observed communities. Lodgepole pine provenances showed similar levels of root colonisation in each soil, whereas Douglas-fir provenances varied in the proportion of their roots that were colonised. Importantly, for assisted migration operations with Douglas-fir, one of the 'improved' provenances showed low colonisation in all soils and a further three were generally less colonised by EMF than the 'wild' provenances. Thus, host genetics appear to be a factor in the overall level of mycorrhizal colonisation, suggesting that 'improved' Douglas-fir provenances may generally be less likely to form mycorrhizas during establishment than 'wild' provenances.

EMF species were more abundant on Douglas-fir seedlings. The division of the fungal community between Douglas-fir and lodgepole pine (31.0 and 44.8 %, respectively, 24.1 % shared) was similar to that observed between ectomycorrhizal hosts in other two-host studies (Twieg et al. 2007; Timling et al. 2012), but only half as many species were shared here as between Douglas-fir and ponderosa pine in an earlier greenhouse experiment (Massicotte et al. 1999). Interestingly, *Suillus lakei* was not observed frequently despite that: (a) it is regarded as a specialist of Douglas-fir, and (b) its sporocarps were frequently observed throughout the host range (specifically at each of the VN-DF, PG-DF and JP-DF sites). Yet, *S. lakei* only occurred as a seedling root symbiont in soils where Douglas-fir was not native. This was counter to our expectations, suggesting that *S. lakei* must have dispersed to these sites in the past and is able to persist in these soils, or it formed associations with an, as yet unknown, alternate host. In a study of Douglas-fir EMF communities along a chronosequence, Twieg et al. (2007) did not encounter *S. lakei* in seedling (≤5 year old) communities, whereas it made up ~10 % of the community for 26-year-old trees. Thus, the Douglas-fir/*S. lakei* association seems to prefer mature hosts or be less competitive on seedlings. Those Douglas-fir/*S. lakei* associations encountered in our study were likely formed due to a lack of alternative options for the host (in terms of symbionts available in the species pool) or symbiont (in terms of host age or species), particularly in the furthest soil outside its current range where overall percent colonisation of Douglas-fir roots was lowest.

Habitat filtering

Significant habitat filtering was observed in the fungal communities of interior Douglas-fir seedlings, with strong compositional differences primarily between soils collected from inside and outside the contemporary host distribution. This was particularly evident when the Douglas-fir communities were examined as exploration types. Communities of interior lodgepole pine also displayed habitat filtering along the ecosystem gradient, although this was much less pronounced amongst their exploration types and did not show any relationship to the range of Douglas-fir. Factors related to BEC zone temperature regime, frost-free period and soil nutrient status were strongly correlated with compositional divisions in seedling fungal communities, implying that they may be drivers of habitat filtering in soil fungal communities. However, the primary difference in community composition was between Douglas-fir communities from seedlings grown in Douglas-fir soil vs. all other communities. Thus, historical host distribution (i.e. the current availability of Douglas-fir compatible fungal symbionts may be influenced by the historical distribution of the host) appears to be the most significant factor in seedling EMF community composition. Douglas-fir exhibited less root colonisation in the FS-LP site soil, and lodgepole pine root colonisation was lowest in both the FS-LP and VN-DF site soils, indicating lower compatibility between hosts and the symbiont assemblage of these soils. The extensive distribution of lodgepole pine beyond the study sites suggests that it is not affected by reduced compatibility with symbiont assemblages. Whereas for interior Douglas-fir, variation in the ability to form fungal associations could be functionally relevant to survival, especially under the intense competitive interactions in non-managed stands and in areas where environmental conditions fluctuate widely and/or are at the extremes of host tolerance.

Where symbionts show a strong degree of host preference, it is likely that their distribution will closely follow the current distribution(s) of the host(s), unless they are able to disperse widely or else form long-lived resistant propagules (e.g. *Rhizopogon* and *Suillus* spp.). This latter ability appears an important one, given the presence of the host specialist *Suillus lakei* in soils collected tens and hundreds of km outside of the host's current distribution (see above). For symbionts that display a wide host tolerance, those considered to be generalists rather than specialists (Bruns et al. 2002; Smith et al. 2009), it is less likely that their distribution will reflect the distribution of any single host species and may instead represent the legacy of their evolutionary history, dispersal ability and the biogeography of all possible hosts.

Taxonomy vs. function

Fungal community composition varied between BEC zone soils for both taxonomic diversity (TD) and functional diversity (FD) assessments but to different extents. The major pairwise TD and FD compositional differences were between BEC zone soils within Douglas-fir's current distribution and those beyond. Roots of Douglas-fir seedlings grown in soils inside its natural distribution generally displayed a higher

abundance of *Rhizopogon* spp. (ectomycorrhizal), whereas soils beyond the natural distribution produced a higher abundance of *Meliniomyces* sp. (fungal endophyte: mycorrhizal status unknown) and uncolonised roots. The long-range exploration types expressed by *Rhizopogon* spp. are associated with increased translocation of water and N to the host (e.g. Bingham and Simard 2011), via thick rhizomorphs that can extend for 10s of centimetre through the soil (Agerer 2001). Reduced availability of fungi utilizing this resource acquisition strategy could potentially lead to increased drought stress following seed dispersal. Although Douglas-fir seedlings did form symbioses with fungi in soils from outside their current distribution, their exploration types shifted from long-range and contact types to short-range and fungal endophyte types. While there may be functional benefits in having roots colonised by fungal endophytes such as DSE (Newsham 2011), on balance, these associations appear more likely to be neutral to detrimental (Mayerhofer et al. 2013). Regardless, fungal endophyte symbionts are widely distributed associates of ectomycorrhizal and ericoid mycorrhizal hosts (Mandyam and Jumpponen 2005; Newsham et al. 2009; Upson et al. 2009; Timling et al. 2012) so further investigation into their ecological importance is warranted. Importantly, this points to functional differences in the available pool of symbionts that will associate with Douglas-fir outside of its current distribution, leading to initial symbioses that may be of little benefit to the host. For lodgepole pine seedlings compositional differences in TD were observed between pairs of sites, whereas differences in FD were less common and unrelated to the distribution boundary of Douglas-fir. Since all lodgepole pine communities were dominated by uncolonised roots, short-range exploration types and fungal endophytes, it may be that *Pinus* symbiont community assembly is inherently different to that of *Pseudotsuga*. An interesting follow-up experiment would be to test whether lodgepole pine seedlings display similar patterns when grown outside of their current southern boundary where Douglas-fir thrives.

It should be noted that the ecological challenges faced by a migrating host species during periods of rapid climatic change are expected to be different from those encountered following translocation to an entirely different region (e.g. exotic species forestry; Nuñez et al. 2009; Gundale et al. 2014). Migration across a host boundary within the same geographical region does not negate the impacts of historical biogeographic processes (Zobel et al. 2011) and coevolution (Johnson and Stinchcombe 2007) faced during natural range expansion, even if performed as a management operation. As a consequence, experiments that mimic short migratory distances are more realistic explorations of the initial dispersal processes leading to natural changes in the distribution of host-symbiont systems, rather than deliberate long-distance anthropogenic changes. The use of a control host species taken from within the same family appears to be a viable method for assessing dispersal effects across a host boundary and, although more soils and more host species are warranted, forms a solid basis for future investigations.

Conclusions

Here, we examined the response of organic horizon fungal symbionts to seedlings of two common western North American ectomycorrhizal host species (interior Douglas-fir and interior lodgepole pine) across an ecosystem gradient. Seedling root-symbiont community composition along the ecosystem gradient indicated significant host filtering (different species composition associated with each host) at all but one site, and significant habitat filtering (different species composition associated with each site) was observed for both hosts. Host species shared 24 % of fungi, which corresponded well with other multi-host studies. Host and habitat filtering of symbiont communities was indicated by co-occurrence and multivariate ordination techniques for both host species. Beyond the northern distribution boundary of Douglas-fir, fewer symbioses were formed, which involved different fungal species and fungal resource acquisition strategies compared to seedlings grown in soils from the contemporary host distribution. The benefit of these associations to newly dispersed seedlings requires further investigation, because even if seedlings are capable of growing with a novel community of EMF, it is possible that their success, in terms of growth potential and long-term survival, may be negatively affected. The lodgepole pine fungal communities did not display such strong patterns in the distribution of fungal exploration types. In this system, the regional-scale processes affecting host biogeography appeared to have an important impact on symbiont community structure. We suggest that a reduction in symbiont colonisation, coupled with a switch in the identity of symbionts, may have an important filtering effect on successful seedling dispersal outside of current distributions for interior Douglas-fir. This is an important consideration for future forest management under rapidly changing climatic conditions.

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Conflict of interest The authors declare that they have no conflict of interest.

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