

Local adaptation in migrated interior Douglas-fir seedlings is mediated by ectomycorrhizas and other soil factors

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Summary

- Separating edaphic impacts on tree distributions from those of climate and geography is notoriously difficult. Aboveground and belowground factors play important roles, and determining their relative contribution to tree success will greatly assist in refining predictive models and forestry strategies in a changing climate.
- In a common glasshouse, seedlings of interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) from multiple populations were grown in multiple forest soils. Fungicide was applied to half of the seedlings to separate soil fungal and nonfungal impacts on seedling performance. Soils of varying geographic and climatic distance from seed origin were compared, using a transfer function approach.
- Seedling height and biomass were optimized following seed transfer into drier soils, whereas survival was optimized when elevation transfer was minimised. Fungicide application reduced ectomycorrhizal root colonization by c. 50%, with treated seedlings exhibiting greater survival but reduced biomass.
- Local adaptation of Douglas-fir populations to soils was mediated by soil fungi to some extent in 56% of soil origin by response variable combinations. Mediation by edaphic factors in general occurred in 81% of combinations. Soil biota, hitherto unaccounted for in climate models, interacts with biogeography to influence plant ranges in a changing climate.

Introduction

Climatic and land-use changes are expected to be the major causes of shifting tree distributions in the next hundred years (IPCC, 2013, 2014). In plant populations, climate is usually identified as the primary driver of natural selection that generates local adaptation (e.g. Clausen *et al.*, 1940; Macel *et al.*, 2007), but the extent to which abiotic or biotic soil factors also contribute to local adaptation is largely unknown and may be obscured by the extreme fine-scale spatial heterogeneity of soils (Ettema & Wardle, 2002). Given the importance of soil processes to ecosystem function, further investigation of aboveground–belowground interactions is clearly warranted (e.g. Wardle *et al.*, 2004; Bardgett *et al.*, 2013; Gundale *et al.*, 2014). Soil organisms in particular are expected to be relevant to local adaptation, given the complex relationships between species and across trophic levels (van der Putten *et al.*, 2010). Plant–fungal symbioses may be especially significant during periods of climatic change due to the suite of benefits mycorrhizas can provide (e.g. improved uptake of water and nutrients, protection from pathogens, etc.; Smith & Read, 2008). Here we explored the impact of belowground factors on local adaptation of interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*), a dominant tree species in many forest ecosystems of

western North America (Coops *et al.*, 2011), with a range that is predicted to move significantly northwards in the next century (Hamann & Wang, 2006; Wang *et al.*, 2012; Rehfeldt *et al.*, 2014a).

Local adaptation can be considered as the fitness superiority of one population over others when grown together in a common environment, and may be measured as the contribution of individuals to the next generation, habitat-specific population growth rates, or adaptation traits (Kawecki & Ebert, 2004). For seedlings this may be demonstrated through increased survival, implying a stronger chance of reaching reproductive age (Leiss & Müller-Schärer, 2001), or enhanced growth traits, which may be a suitable proxy for the ability to reach reproductive age (Berglund *et al.*, 2003), although the latter is an indirect measurement (Kawecki & Ebert, 2004). By growing populations in a common, nonstressful glasshouse environment, climatic impacts on growth are minimized and most remaining local adaptation effects will be abiotic and biotic soil factors. For an obligate host of ectomycorrhizal fungi (EMF) such as Douglas-fir, soil fungi are expected to play a significant role in mediating any belowground adaptive responses (Simard, 2009). Mycorrhizas transfer water and nutrients effectively between plants (He *et al.*, 2003; Egerton-Warburton *et al.*, 2007), and in the specific case of

interior Douglas-fir, mycorrhizal networks mediate the transfer of nitrogen, water and carbon between multiple cohorts (Simard *et al.*, 1997a; Plamboeck *et al.*, 2007; Teste *et al.*, 2009b; Bingham & Simard, 2011). Evidence of local adaptation in host–symbiont communities has been found in both arbuscular mycorrhizal (Johnson *et al.*, 2010; Ji *et al.*, 2013) and ectomycorrhizal systems (Hoeksema *et al.*, 2012). Where local adaptation in these communities occurs, host populations are predicted to display local optimization when matched with local symbiont populations and perform worst with distant symbiont populations. Thus, generally negative effects on growth or survival are anticipated if host populations are moved considerable climatic or geographic distances from their origin.

The movement of tree populations is globally relevant due to current and historical forest management approaches. For example, Douglas-fir has a natural range of *c.* 92 million ha (Mathys *et al.*, 2014), spanning western North America from central British Columbia (55°N) into central Mexico (19°N) (Hermann & Lavender, 1990), yet it has also been planted across millions of hectares throughout Europe, Australasia and South America (Hermann & Lavender, 1999). Thus, research on tree population responses to climate change must also consider forest management practices. Here we focused on the ‘assisted migration’ or ‘facilitated migration’ (FM) approach, which has been presented as a tool to mitigate the impacts of climate change on biodiversity (McLachlan *et al.*, 2007). In the context of forestry FM (Pedlar *et al.*, 2012) tree seedling populations are traditionally planted in areas that are a short geographic and climatic distance from their point of origin (e.g. transferred no more than a few hundred kilometers geographic distance or a few hundred meters elevation). To date, forestry FM studies have focused on climatic effects on local adaptation in tree populations (Gray *et al.*, 2011; Kreyling *et al.*, 2011). However, the extent to which the combination of processes taking place in soil contributes to transfer effects, in a similar manner to those already observed for climate (Andalo *et al.*, 2005; O’Neill *et al.*, 2008; Wang *et al.*, 2010), has not yet been addressed. FM far beyond a host’s natural ability to disperse may show positive effects due to the same processes that enable species invasion, for example reduced pathogen loads or well-matched climatic variables (Shea & Chesson, 2002). Indeed a recent study on extreme seed transfer of lodgepole pine (*Pinus contorta*) – from western Canada to Sweden – found that nonlocal soils produced a positive effect on seedling growth, and suggested that this was a consequence of release from inhibitory plant–soil biotic interactions (Gundale *et al.*, 2014). The scale at which any such edaphic effects operate may be of assistance in refining seed transfer regulations and guidelines for specific host species.

Distinguishing climatic, soil abiotic and soil biotic (e.g. EMF) effects on host growth is further complicated in Douglas-fir for two main reasons: almost all Douglas-fir seedlings form associations with EMF in the field (primarily with *Rhizopogon* spp.; e.g. Twieg *et al.*, 2007); and *c.* 95% of Douglas-fir seedlings are non-mycorrhizal when grown under standard nursery conditions for use in forestry in British Columbia (Kazantseva *et al.*, 2009). Although a reasonable amount of information on EMF

associations with interior Douglas-fir exists (Jones *et al.*, 1997, 2010; Simard *et al.*, 1997b; Hagerman & Durall, 2004; Durall *et al.*, 2006; Twieg *et al.*, 2007; Teste *et al.*, 2009a), the geographic scope of those studies has been mostly limited to two ecosystem types in south-central British Columbia. This lack of information on host–symbiont interaction across varied ecosystems presents a major barrier to understanding how local adaptation to soil fungi might interact with tree migration. Because growing seedlings in forest soils promotes the development of symbiont communities that differ between soil origins (Massicotte *et al.*, 1999), combining this approach with a treatment preventing or reducing EMF colonization (such as fungicide application; Teste *et al.*, 2006) should assist in parsing out the contribution of EMF to local adaptation in host populations.

Using a common-garden experimental approach, we examined the responses of interior Douglas-fir populations from a range of climatic origins to soils collected from a range of climatic origins. The impacts of seed transfer were quantitatively assessed using ‘transfer functions’, which relate the responses of populations to climatic or geographic transfer distance when multiple populations are grown together in a common garden (Raymond & Lindgren, 1990; Schmidting, 1994). A fungicide treatment enabled comparison of fungal effects on seedling growth and survival, at natural and reduced levels of symbiont availability. To address the extent to which local adaptation occurs in host populations, and is driven by association with EMF, we asked the following questions: does matching the climatic and geographic origins of soils to that of seeds improve the success of seedlings (e.g. increased growth and/or survival) as compared with situations where there is a climatic and geographic mismatch? Is any growth or survival advantage conferred by local adaptation to soil associated with EMF colonization?

Materials and Methods

Materials

This work was conducted as a part of the ‘Assisted Migration Adaptation Trial’ (AMAT), in which populations from 15 tree species native to western Canada and the USA were planted at 48 sites located between the southern Yukon and northern California (O’Neill *et al.*, 2013). Six interior Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca* (Beissn.) Franco) seed sources (populations) were used in this experiment. Five of these were from Canadian interior Douglas-fir seed orchards – one in each of the five interior Douglas-fir breeding zones in British Columbia – and one was from a Douglas-fir seed orchard in Idaho. Seedlings used in both this experiment and the AMAT were grown together under standard containerized glasshouse nursery conditions (as per Kolotelo *et al.*, 2001). In November 2009, 8 months after sowing into peat moss medium, seedlings were removed and transferred to cold storage. In operational forestry the terms ‘provenance’, ‘seedlot’, and ‘seed source’ may be used interchangeably to denote a population from a specified geographic location. Here we consistently use the term ‘seed origin’ to assist in interpretation of the data.

Glasshouse techniques

Twelve seedlings of each of the six seed origins (Table 1b) were grown in each of the eight soils and each of two soil fungicide treatment levels (i.e. 12 seedlings $\times$ 6 seed origins $\times$ 8 soils $\times$ 2 treatments = 1152 seedlings). Following removal from storage, seedlings were planted singly in 4-l treepots (Stuewe and Sons, Corvallis, OR, USA) in May 2010 in the University of British Columbia (UBC) Horticulture glasshouse (Vancouver, Canada). Each pot contained a soil mixture with the following vertical characteristics (from the base up): folded paper towel; 400 ml 1 : 1 mix of peat : perlite; 1 : 1 mix of site mineral soil : perlite; 1 : 1 mix of site organic horizon soil : perlite; 200 ml 1 : 1 mix of peat : perlite. The total mineral and organic mixture volume (3.4 l) was achieved by adjusting the proportion of each mixture to reflect the ratio of soil and organic soil volumes found at the matching field site so as to maintain a similar soil profile for glasshouse seedlings.

Seedlings were planted with an additional 100 ml of forest soil surrounding the root plug. Half of the seedlings (576 = 12 seedlings $\times$ 6 seed origins $\times$ 8 soils) were treated biweekly with 400 ml of Topas 250E fungicide at a concentration of 0.5 g l^{-1} propiconazole, whereas the other half was left as an untreated control. Propiconazole is a systemic fungicide of the triazole chemical family that inhibits ergosterol synthesis, which is necessary for fungal cell wall formation, thus it has a broad effective range across the fungi (Marin, 2011). Growth of EMF (Manninen *et al.*, 1998; Zambonelli & Iotti, 2001; Walker *et al.*, 2010), arbuscular mycorrhizal fungi (Kjoller & Rosendahl, 2000) and fungal pathogens (Amiri *et al.*, 2008) are all suppressed by this fungicide. In a previous experiment using Douglas-fir seedlings (Teste *et al.*, 2006), colonization by EMF was reduced by *c.* 55% following propiconazole application at 0.5 g l^{-1} (no significant difference in EMF reduction over 6 months between three application levels: single, once per month, once per 2 months), whereas 100% suppression of EMF fungal development over 3 months was observed on Norway spruce seedlings following a single application of 75 ml at 0.5 g l^{-1} (Koele & Hildebrand, 2010). A reduction in soil respiration has been reported following application of this fungicide (Manninen *et al.*, 1998), suggesting cumulative effects on the entire community of soil biota. The authors found no evidence in the literature that propiconazole acts as a carbon substrate. Seedling positions were randomized every 3 wk to minimize effects of spatial differences in light or irrigation in the glasshouse. Summer maximum daily glasshouse temperatures were in the range 24–30°C, with soils kept at or near field capacity via flood bench watering until late July. Switching to hand-watering from above in late July was used to simulate mild drought, which is an important cue for the initiation of Douglas-fir dormancy in autumn (Larcher, 2003). Glasshouse seedlings were overwintered outside at the Kalamalka Research Station (Vernon, Canada) where the winter climate is similar to that of the seed origins. In March 2011, seedlings were returned to the UBC Horticulture glasshouse and widely spaced to prevent shading. Water availability was reduced to hand-watering from above once every 2 wk

from May 2011, to reflect the lower availability of water in the field. Moisture stress could not be precisely matched to field conditions due to the practical difficulties involved with accessing such widely distributed sites at regular intervals.

Collection of survival, biomass and fungal colonization data

In autumn 2011, two growing seasons after planting, seedlings were harvested and survival, root collar diameter, height, and root and shoot dry biomass were recorded. Roots were carefully extracted from the soil medium using a washing and sieving procedure to avoid loss of ectomycorrhizal root tips, moistened to prevent drying out, and stored in ziplock freezer bags. Before biomass assessment, 100 root tips from each of four seedlings from each of the 48 population $\times$ soil combinations were randomly selected for assessment of EMF colonization using a morphotyping approach (Goodman *et al.*, 1998). A subsample of each morphotype was retained for future molecular analysis (not discussed here); all remaining morphotyped root tips were then returned to their respective seedling root samples for biomass assessment.

Transfer distances

Soil geographic and climatic 'transfer distances' were calculated for each combination of seed origin and soil origin used in the experiment. By convention transfer distances are expressed as 'test environment' (soil origin) – 'population environment' (seed origin), and therefore represent the difference in a geographic or climatic property between a seed's origin and the location (or, in this case, soil) into which it was translocated.

Statistical analyses

Analyses were carried out in the R software environment (R Core Team, 2014), and measurements were examined for normality and equality of variance. Where necessary, the Benjamini & Hochberg false discovery rate (FDR) correction (Verhoeven *et al.*, 2005) was applied to *P*-values to account for multiple testing.

Seedling survival analysis was conducted using a binomial regression model with a logit link function (logistic regression) and a quasibinomial generalized linear model (GLM) with a logit link function (glm function with family set to binomial or quasibinomial). Deviance-based tests were used to examine the significance of regression and treatment levels using the 'anova' function with test set to Chisq (binomial) or F (quasibinomial).

Significant differences in the residual variance structure of height, shoot mass and root mass were detected between soils (Levene's test, $\alpha = 0.05$). Height data were amenable to transformation ($\sqrt{(x + 3/8)}$), whereas the residual variance of biomass measures remained unequal following log, square root, and power transformations. For EMF colonization, height and diameter, three-way ANOVA, with type III sums of squares, was conducted using the model: measurement = soil $\times$ treatment $\times$ seed origin. Tukey's HSD was applied *post hoc* to determine specific

differences at the $\alpha = 0.05$ level. Seedling EMF colonization data will be presented elsewhere (B. J. Pickles, unpublished data). For shoot mass and root mass, a three-way permutational ANOVA, with type III sums of squares, was conducted using the 'adonis' function in R-package 'vegan' and the model: measurement = soil $\times$ treatment $\times$ seed origin.

In order to assess the effect of transfer distances on local adaptation, polynomial regressions were developed for each combination of response variable and soil origin, using the model: measurement = $c_0 + c_1 \times \text{transfer distance} + c_2 \times \text{transfer distance}^2 + \varepsilon$ (c_i , regression coefficients; ε , residual error). In the forest genetics literature this regression approach is referred to as a 'transfer function' (TF) (Rehfeldt *et al.*, 1999; Leites *et al.*, 2012). The core of a standard TF is a scatter plot of the mean values of a specific response variable (e.g. survival, height, biomass, etc.) for each seed origin tested (y -axis), plotted against a transfer distance of interest (e.g. the difference in MAP between the soil origin and the seed origin), on which the polynomial regression is performed. Here, for the first time, we applied this technique to examine the responses of seed origins to growth in forest soils in a common-garden glasshouse environment, rather than in the field. This novel approach allowed us to assess how seed transfer into different soil origins affected seedling success, emphasizing

the contribution of soil properties by examining them within a single climate. In each soil, TFs were generated using the transfer distances for elevation, MAT, MAP and AH : M (see Fig. 2 for illustrated examples using MAP). A total of 384 TFs were developed: 2 treatments $\times$ 8 soil origins $\times$ 6 response variables $\times$ 4 transfer distance variables. Error rate control was performed within treatments for each combination of response variable $\times$ transfer distance variable (sets of eight tests) to reduce type II errors generated by multiple testing while minimizing type I errors due to application of error rate correction across the entire experiment. The strength (r^2) and significance (P -value) of the polynomial regression was used to determine whether local adaptation was evident in terms of survival or growth. Each TF meeting the FDR corrected significance threshold ($\alpha = 0.05$) was examined for local or nonlocal optimality (Fig. 2), for example whether average to maximum values of a response variable (the interval X_1 – X_2) contained transfer distance = 0 (Fig. 2a), or fell outside it (Fig. 2b), respectively. With our novel approach, TFs enabled an exploration of seedling responses to soils in the presence and absence of fungicide application. Thus, the significance and magnitude of seed origin responses to soil origin transfer under normal and reduced root colonization regimes could be directly compared (Fig. 2c).

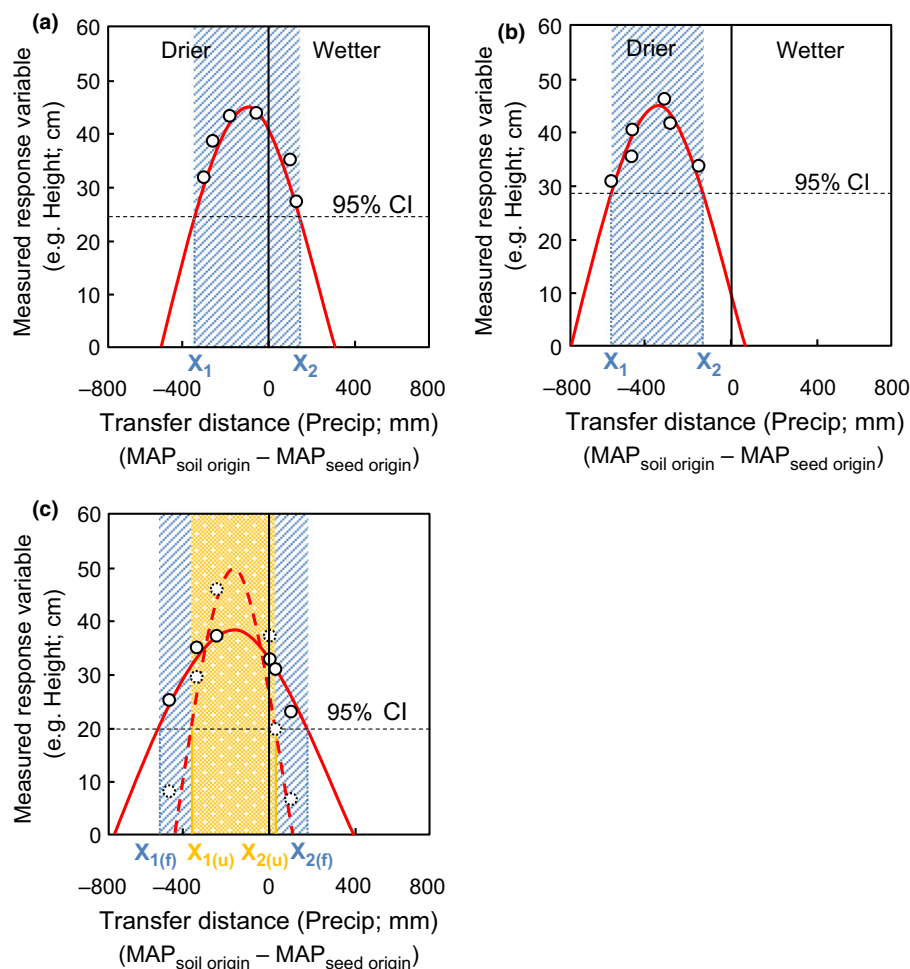


Fig. 2 Example quadratic transfer functions (i.e. polynomial regressions) for any measured variable (y -axis; e.g. height) plotted against any transfer distance variable (x -axis; e.g. difference in mean annual precipitation (MAP) of soil origin - MAP of seed origin). Circles indicate mean values of measured variable for each seed origin. The lower 95% confidence interval (95% CI) of the mean of the measured variable (y -axis) is calculated and used to define the interval, X_1 – X_2 , over which mean to maximum values of that variable occur (hatched area). (a) Example of local optimality: the interval X_1 – X_2 contains $x = 0$; minimal transfer distances result in maximized values of the measured variable. (b) Example of nonlocal optimality: the interval X_1 – X_2 does not contain $x = 0$; transfer away from the seed origin results in maximized values of the measured variable. (c) Example of a comparison between the control and fungicide treatment where both result in local optimality: the interval $X_{1(f)}$ – $X_{2(f)}$ (fungicide, solid circles, solid regression line, blue hatched area) covers a wider range of MAP than the interval $X_{1(c)}$ to $X_{2(c)}$ (control, dotted circles, dashed regression line, orange shading) and generates a lower maximum height; fungicide addition *increases* the climate range in which mean to maximum height can be achieved, but *reduces* the maximum height that seedlings can attain.

Results

Seedling survival and growth – responses to seed and soil origin

Regardless of treatment, the survival and growth properties of seedlings varied between soil origins and between seed origins, with significant interactions between them (Table 2); model r^2 values were typically in the range 0.30–0.40. Soil origin had a significant effect on all response variables (Fig. 3) and tended to interact with both seed origin and fungicide treatment (Table 2; Fig. S2). Seedling survival and growth was consistently high in the McLure soil and consistently low in the Strouse lake and Skelton soils. Seed origin also had a significant effect on all response variables (Fig. 4), was typically the strongest main effect (Table 2; Fig. 3) and did not interact with the fungicide treatment except in the case of seedling height (Table 2; Fig. S2). Seedlings from seed origin 105 showed consistently low survival and growth, seed origin 109 had high growth and low survival, whereas seed origin 106 had high survival and low growth. Interactive effects were consistently observed between treatment and soil origin, and between soil origin and seed origin. No three-way interactions were observed.

Seedlings grown in Deep Creek and McLure soil were taller than those grown in Glenmerry, Skelton and Strouse lake soil (Fig. 3b); seed origin 105, 106 and 107 seedlings were consistently shorter than those of seed origin 108, 109 and 141 (Fig. 4b). Significantly greater shoot biomass was detected in four of the eight soils, with untreated control seedlings always expressing greater mass (Fig. 3c); four seed origins produced significantly greater shoot biomass in the control, with seed origins 105 and 106 consistently producing the least shoot biomass (Fig. 4c). Although there was no significant difference in root biomass between fungicide treated and untreated control seedlings, fungicide application generated no significant differences in root biomass between soils, whereas control seedlings exhibited greater

root mass in McLure soil compared with Deep Creek, Skelton and Strouse lake soils (Fig. 3d). Seed origin 106 consistently produced the least root biomass, and seed origin 109 produced the greatest root biomass (Fig. 4d).

Seedling survival and growth – responses to transfer distances

Conversion of soil and seed origin data into geographic and climatic transfer distances enabled polynomial regression analyses of response variables (transfer functions; TFs) for each individual soil origin (see examples in Fig. 5), which typically accounted for 75–95% of the variance in seed origin response variable mean values (Table 3). Mean annual temperature did not generate any significant TFs for survival or growth (data not presented). Both climatic and elevation transfer distances generated TFs for seedling survival and/or growth measurements. The most consistent seedling survival response was to elevation transfer distance, and MAP transfer distance was also significant in two soils (Table 3). All of the significant seedling survival TFs contained the zero transfer distance between X_1 and X_2 (Figs 2a, 5a; Table 3), meaning that minimizing the difference in elevation or MAP between seed origin and soil origin was optimal for survival.

Seedling growth responses were consistently explained by their relationship to MAP and AH:M transfer distances (Table 3; Fig. 5b,c). In each soil, seedling height was significantly related to climatic transfer distance, with six soils indicating optimal height following transfer of seed into soil of drier origin (Figs 2b, 5b; Table 3) and two soils displaying optimal height with minimal seed transfer (Fig. 2a; Table 3). In terms of biomass, both shoot and root mass showed the same transfer responses as height. The two exceptions to the general pattern of increased seedling growth in soils from drier environments were: the Glenmerry soil origin, where all growth measurements were optimized at minimal AH:M transfer distances (Fig. 5c); and the Deep Creek soil

Table 2 Analysis of main and interactive effects of soil origin (So), fungicide treatment (T), and *Pseudotsuga menziesii* var. *glauca* seed origin (Se) on seedling survival and growth factors using binomial regression and parametric or permutational ANOVA, respectively

Factor	DF	Survival ¹ P-value	Height ^{2,4}		Diameter ²		Shoot mass ³		Root mass ³	
			F-value	P-value	F-value	P-value	Pseudo-F	P-value	Pseudo-F	P-value
Soil origin (So)	7	<0.001	3.37	0.001	2.67	0.010	16.12	0.001	3.99	0.001
Treatment (T)	1	<0.001	1.37	0.242	11.95	<0.001	32.74	0.001	3.20	0.067
Seed origin (Se)	5	<0.001	17.62	<0.001	4.17	<0.001	35.87	0.001	21.18	0.001
So × T	7	0.018	2.75	0.008	2.04	0.047	4.49	0.001	2.45	0.019
So × Se	35	0.396	3.37	<0.001	1.99	<0.001	3.30	0.001	1.92	0.001
T × Se	5	0.042	2.60	0.024	1.05	0.385	1.61	0.154	0.94	0.464
So × T × Se	35	0.069	1.30	0.116	1.02	0.444	1.38	0.070	0.78	0.812
Residuals	876									

Values in bold are significant at the $\alpha \leq 0.05$ level.

¹Binomial regression.

²Three-way ANOVA.

³Three-way permutational ANOVA.

⁴Transformed data ($\sqrt{(x+3/8)}$).

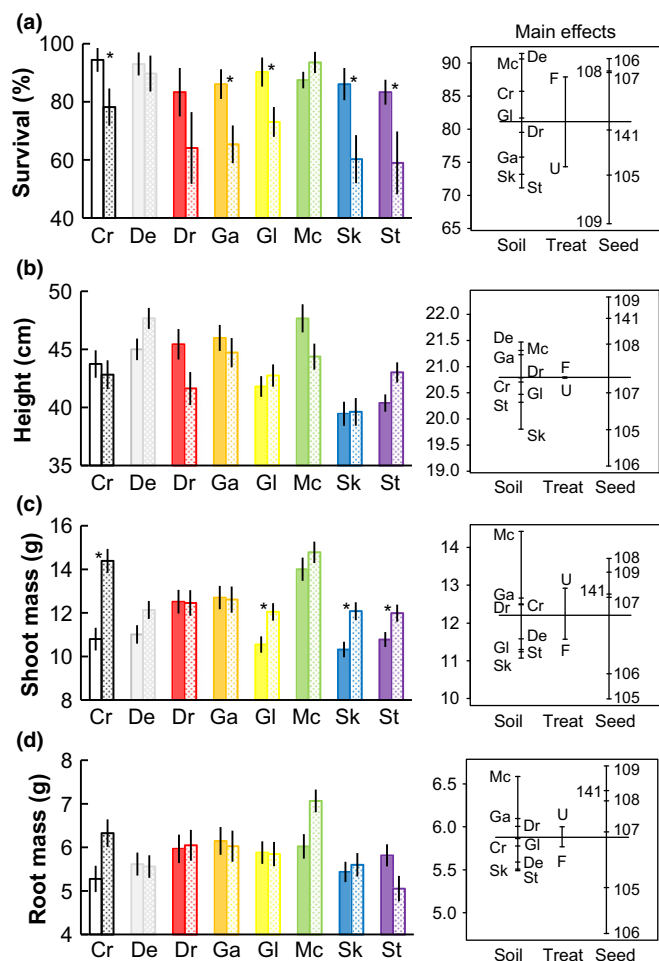


Fig. 3 The mean ($\pm$ SEM) of (a) survival, (b) height, (c) shoot mass and (d) root mass across all interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) seed origins grown in each soil origin with (closed bars) or without (hatched bars) the addition of fungicide. Main effects plots show mean values of the measured variable for each level of each factor. Asterisks indicate a significant difference (*, $\alpha = 0.05$) between fungicide treatment and control seedlings (see Table 2). Cr, Cranbrook; De, Deep Creek; Dr, Driftwood; Ga, Gavin Lake; Gl, Glenmerry; Mc, McLure; Sk, Skelton; St, Strouse lake.

origin, where height and shoot mass were optimized at minimal AH: M and MAP transfer distances, respectively.

Effectiveness of fungicide treatment on EMF colonization

Application of the fungicide treatment successfully reduced root colonization by EMF in 47 out of 48 seed origin $\times$ soil origin combinations (seed origin 105 in Deep Creek soil was the sole exception, displaying no difference). On average, treated seedlings displayed a level of EMF colonization equal to 47.3% ($\pm$ SEM 3.16%) of that observed on untreated seedlings (i.e. fungicide application reduced mean EMF colonization by 52.7%). The main effect of the fungicide treatment was reduced root colonization, with soil origin influencing the magnitude of colonization, and seed origin interacting with soil origin (such that each seed origin exhibited high % colonization in at least one soil).

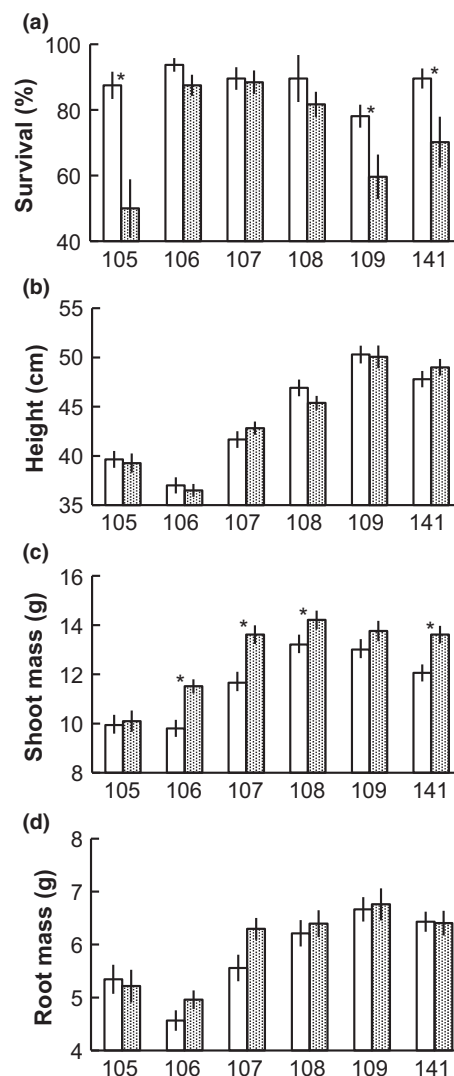


Fig. 4 The mean ($\pm$ SEM) of (a) survival, (b) height, (c) shoot mass and (d) root mass of each individual interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) seed origin (105–109 & 141) across all soil origins with (open bars) or without (hatched bars) the addition of fungicide. Main effects plots show mean values of the measured variable for each level of each factor. Asterisks indicate a significant difference (*, $\alpha = 0.05$) between fungicide treatment and control seedlings (see Table 2).

Overall percentage colonization was significantly greater in the Gavin Lake and Glenmerry soil origins, and significantly lowered in the Cranbrook, Skelton and Strouse lake soil origins (B. J. Pickles, unpublished data). Each ectomycorrhizal fungal species was reduced by a similar percentage following fungicide application (i.e. the entire community appeared to be equally suppressed).

Seedling responses to fungicide treatment

The main effects of fungicide application were that seedling survival was increased (Figs 3a, 4a) and both shoot biomass (Figs 3c, 4c) and basal diameter (Fig. S3) were decreased compared with the untreated controls (Table 2). Significant

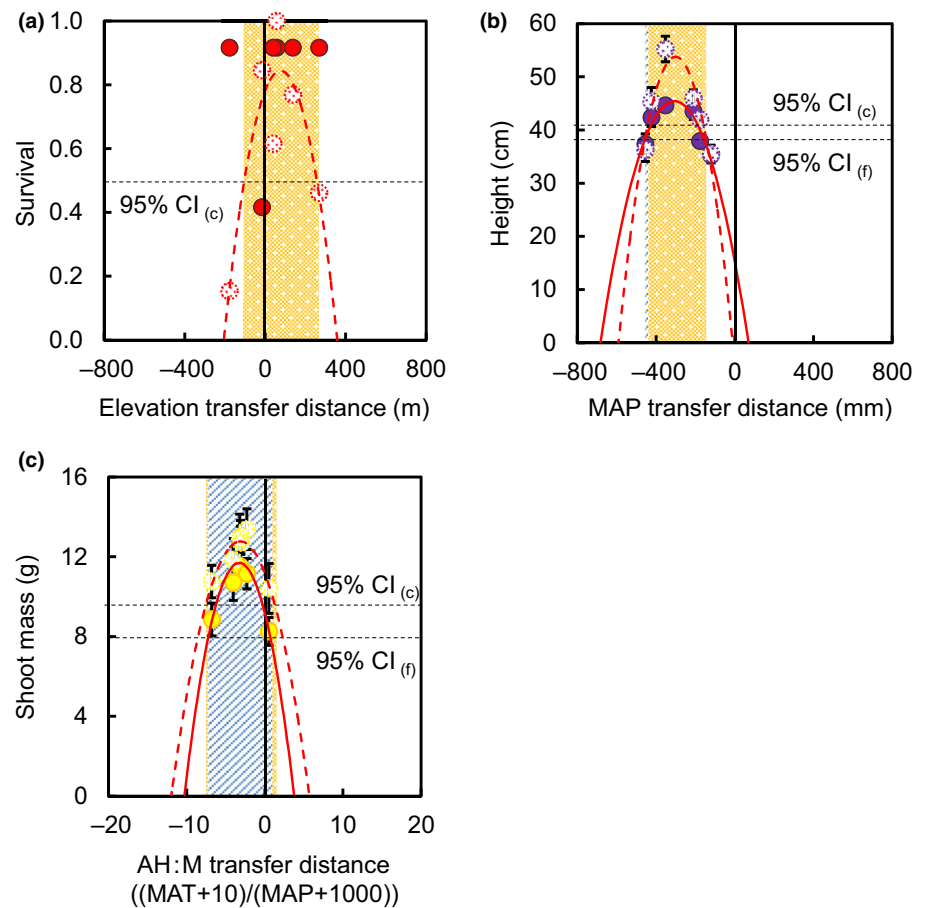


Fig. 5 Transfer functions illustrating differences in the survival or growth responses between the fungicide treatment and untreated controls for (a) survival in Driftwood soil vs elevation transfer, (b) height in Strouse lake soil vs mean annual precipitation transfer, and (c) shoot mass in Glenmerry soil vs annual heat: moisture index transfer (AH : M). Circles represent mean values ($\pm$ SEM) of the response variable for each seed origin. Fungicide treatment: solid circles, solid regression line, blue shading. Control treatment: dotted circles, dashed regression line, orange shading.

differences in survival were observed between treatments with a single treatment $\times$ soil origin interaction (greater survival in McLure soil for untreated controls; Fig. S2). Seedling death began following a reduction in watering to emulate conditions at the soil origin field sites. Dead and dying seedlings became infested with mites, and appeared to be suffering from water stress, parasitism or an attack by pathogens. Survival was greatest in the fungicide treatment for five of the eight soil origins (Fig. 3a). The highest survival occurred in Deep Creek and McLure soils, with the lowest survival in the Skelton and Strouse lake soils. Seed origins 105 and 109 exhibited the greatest mortality (Fig. 4a). In the fungicide treatment, survival between soil origins did not differ significantly (Fig. 3a), whereas seed origin 109 survival was lower than all other seed origins in the fungicide treatment (Fig. 4a). No significant overall effects of fungicide were observed for seedling height or root mass, although interactions occurred in both (Fig. S2). Height was greater in untreated Deep Creek and Strouse lake soils, and lower in untreated McLure and Driftwood soils. For root mass, treatment interacted with soil origin such that it was greater in untreated Cranbrook and McLure soil, and lower in untreated Strouse lake soil.

Parsing the effect of soil fungi on local adaptation was achieved by comparing TFs developed for fungicide-treated and untreated seedlings across four response variables and the eight soil origins (Table 4; examples in Fig. 5). Four outcomes were possible for

each pairing of response variable and soil origin: only untreated control seedlings expressed significant TFs; only fungicide-treated seedlings expressed significant TFs; both fungicide-treated and untreated control seedlings expressed significant TFs; or neither untreated control nor fungicide-treated seedlings expressed significant TFs. These corresponded to the following outcomes for a given transfer factor: local adaptation mediated by soil fungi alone (6/32 occurrences); local adaptation mediated by nonfungal soil properties (8/32 occurrences); both fungal and nonfungal mediation of local adaptation within the same soil (12/32 occurrences); and no evidence of local adaptation mediated by soil factors (6/32 occurrences). In total 18 of the 32 soil origin $\times$ seedling response combinations exhibited significant evidence of local adaptation mediated by soil fungi, and 20 of 32 exhibited mediation by nonfungal soil properties (Table 4). Each soil origin displayed a significant TF mediated by soil fungi for at least one response variable following correction for multiple testing.

Discussion

Here we have shown that, in a common-glasshouse environment, edaphic factors play a role in local adaptation of interior Douglas-fir seedlings. Matching the elevation of seed origins to soil origins generally optimized seedling survival, whereas transfer of

Table 3 Significant quadratic regression analyses (transfer functions), with and without fungicide addition, for *Pseudotsuga menziesii* var. *glauca* seed origin survival, height, shoot mass, and root mass (dependent variables) vs geographic or environmental transfer distances (independent variables)

Soil ¹	Treat ²	Survival			Height			Shoot mass			Root mass		
		TD ³	r^2	X ₁	X ₂	TD ³	r^2	X ₁	X ₂	TD ³	r^2	X ₁	X ₂
Cr	F	Elevation	0.979**	-218	226	MAP	0.990***	-538	-206	AH : M	0.935**	16.2	23.4
	U					MAP	0.843*	-547	-227	MAP	0.888*	-532	-198
De	F	Elevation	0.901*	-196	432					MAP	0.902*	-240	850
	U	Elevation	0.877*	-2	450	AH : M	0.941**	-2.2	5.0	MAP	0.957**	-382	-42
Dr	F					AH : M	0.803*	0.50	8.00				
	U	Elevation	0.822*	-144	298								
Ga	F	Elevation	0.916**	-104	304								
	U					MAP	0.772*	-424	-125				
Gl	F					AH : M	0.810*	-7.0	0.8	AH : M	0.931**	-7.4	0.9
	U					AH : M	0.790*	-6.75	1.75	AH : M	0.933**	-7.6	1.4
Mc	F					MAP	0.965**	-472	-138	AH : M	0.979**	8.8	15.7
	U	Elevation	0.909*	-180	466	AH : M	0.861*	8.5	12.0	AH : M	0.962**	7.4	15.0
Sk	F					MAP	0.820*	-465	-125				
	U	MAP	0.963**	-380	1060	AH : M	0.868*	5.2	12.0	MAP	0.764*	-460	-118
St	F					MAP	0.872*	-478	-136				
	U	MAP	0.980**	-432	468	MAP	0.870*	-468	-132	MAP	0.864*	-486	-140
										MAP	0.918**	-460	-130

Bold type, local optimality (X₁–X₂ includes transfer distance = 0). Underlined type, nonlocal optimality (X₁–X₂ does not include transfer distance = 0). Following FDR correction for multiple testing: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Where multiple transfer distances produced significant regressions for the same measurement in the same soil, only the strongest fit is presented.

¹Cr, Cranbrook; De, Deep Creek; Dr, Driftwood; Ga, Gavin Lake; Gl, Glenmerry; Mc, McLure; Sk, Skelton; St, Strouse lake.

²Treatment: F, fungicide treatment; U, untreated.

³Transfer distance: TD, (Soil origin_{variable} – Seed origin_{variable}); MAP, mean annual precipitation; AH : M, annual heat: moisture index.

Table 4 Combinations of geographic and climatic variables¹ generating significant transfer functions by soil origin

Soil origin	Response variable			
	Survival	Height	Shoot mass	Root mass
Cranbrook	<i>Elevation</i>	<u>MAP + AH : M</u>	<u>MAP + AH : M</u>	<i>MAP + AH : M</i>
Deep Creek	<i>Elevation</i>	AH : M	<i>MAP</i>	
Driftwood	Elevation	<i>AH : M</i>	<i>MAP</i>	
Gavin Lake	<i>Elevation</i>	<u>MAP</u>		
Glenmerry		<u>AH : M</u>	<u>AH : M</u>	<u>AH : M</u>
McLure	Elevation + AH : M	<u>MAP + AH : M</u>	<u>MAP + AH : M</u>	<u>MAP + AH : M</u>
Skelton	MAP + AH : M	<u>MAP + AH : M</u>		<i>MAP</i>
Strouse lake	MAP	<u>MAP</u>	<i>MAP</i>	<u>MAP</u>

Typeface indicates the soil factors associated with local adaptation in each response variable based on fungicide and control responses: soil fungi only (bold type), nonfungal soil factors only (italicized type), both soil fungi and nonfungal soil factors (underlined), or no local adaptation observed (no text).

¹MAP, mean annual precipitation; AH : M, annual heat : moisture index.

seed into a drier soil origin generally optimized seedling growth. Beyond the impacts of climate and weather, soil fungi and nonfungal belowground factors generated significant positive and negative outcomes on seedling growth and survival, which varied by soil and seed origin. We expected survival and growth to be greater in untreated controls than in treated seedlings, through increased access to growth-limiting nutrients provided by EMF (Smith & Read, 2008). Although aboveground growth was indeed greater for untreated control seedlings, survival was generally greater in the fungicide treatment. Although we did not directly assess how fungicide affected non-EMF soil fungi, a similar reduction in abundance to that expressed by EMF (c. 50% reduction of each species) appears a safe conclusion based on the known physiological impact of the fungicide (e.g. Teste *et al.*, 2006; Amiri *et al.*, 2008; Marin, 2011). The polynomial regression 'transfer function' approach assisted in identifying the underlying factors responsible for generating the different seedling responses and, when used in conjunction with the fungicide treatment, provided strong evidence for mediation of local adaptation in hosts by fungal symbiont populations and nonfungal soil factors.

Response to seed transfer

Several major trends in the interactions of response variables between treatment, soil and seed origin were observed. Elevation, MAP and AH : M transfer distances all displayed significant optimization for survival and/or growth, despite the seedlings being grown in a common-glasshouse environment. Matching the climatic and elevation origins of soils to that of seeds improved the survival of seedlings. This effect was clearly expressed in the transfer functions for elevation and MAP, where mean to maximum survival always occurred in conjunction with minimum seed transfer. Elevation is often assumed to be a direct proxy for MAT; however, here it is quite clear that they are not directly equivalent, with changes in elevation generating host responses that changes in MAT did not. The biogeoclimatic classification system for British Columbia shows significant changes in soil orders, plant communities and tree composition with elevation (Pojar

et al., 1987). Because soil genesis is a complex interactive process involving climate, organisms, relief, parent material, time and space (e.g. Jenny, 1961; McBratney *et al.*, 2003) several candidate factors could be driving local adaptation (Hoosbeek & Bryant, 1992; Ibanez *et al.*, 1998). The optimization of seedling survival to soil originating from a similar elevation suggests that soil-forming factors may be useful predictors of transfer success.

Matching the climatic origins of soils to that of seeds only improved seedling growth in two of the eight soil origins. In each of the other six soils a mismatch to soils of drier climatic origin led to growth optimization. These effects were clearly expressed in the transfer functions generated from MAP and AH:M transfer distances. Because interior Douglas-fir is adapted to the dry interior regions of western North America, the ability to colonize somewhat drier edaphic environments may be advantageous. However, our results indicate that individual soil origins can break this pattern and prove more advantageous to the growth of seed sourced from similar climatic origins. Although seedling height is commonly used as a measure of seedling success in operational forestry, we note that the seed origin which consistently expressed the greatest survival also tended to express the lowest growth (seed origin 106) and, conversely, the seed origin with the most consistently high growth also displayed lowest survival (seed origin 109). We anticipate that seed transfer based on optimal survival responses will prove more sustainable in the field than attempts to maximise growth. This is because in the glasshouse the general growth trend was for increased growth following seed transfer to annually drier soil origins, but in the field extreme drought events are expected to increase in frequency, and are likely sources of mortality for any seedlings transferred for growth in such environments.

Fungal and edaphic mediation

The majority of untreated control seedlings exhibited greater basal diameter and shoot or root biomass than those in the fungicide treatment, suggesting that EMF associations improved resource acquisition by seedlings to an extent that outweighed the cost of forming them. However, with mite infestation absent

from the fungicide treatment, there appears to have been a negative host response to soil fungi in some instances – either a result of dedicated soil pathogens or the antagonistic effects of EMF (Egger & Hibbett, 2004; Jones & Smith, 2004). Thus, the variable responses of seed origin to fungicide may reflect natural interpopulation variability in mycorrhizal dependence or resistance to fungal pathogens. In interior Douglas-fir, negative effects on survival from typically mutualistic partners should be ameliorated where seedlings can tap into existing mycorrhizal networks (Simard, 2009), thereby potentially avoiding the start-up costs of ectomycorrhizal association. For example, survival of interior Douglas-fir germinants in the field increased from *c.* 10% to 40% when they could access the mycorrhizal network of established trees (Teste *et al.*, 2009b). Hence, increased mortality may have been a consequence of long-term growth in a containerised environment, where total nutrient amounts are finite and EMF are unable to exploit more spatially distant resources.

It is important to note that if we had relied upon comparison of seedling survivorship between treatments alone we would likely have concluded that soil fungi simply produced an overall negative effect. What the transfer function approach revealed was that the reduced survival of control seedlings primarily took place in soil from increasingly distant origins (specifically an increasing difference in elevation or MAP between soil origins and seed origins). Therefore, the key effect of fungicide appears to have been suppression of negative effects on seedlings caused by increasingly distant transfer. This suggests that fungal communities from increasingly different elevations or precipitation regimes negatively affect seedlings, and that it is this mismatch between seed origins and soil origins that is the source of the negative response. Only by explicitly considering the climatic and geographic transfer distances was this local adaptation response revealed. Differences in the response to fungicide between soil origins may reflect differences in the inoculum potential of soil biota. Soil fungal communities are known to vary markedly in vegetation types that are linked to elevation gradients (Wagg *et al.*, 2011; Gorzelak *et al.*, 2012; Miyamoto *et al.*, 2014), and we note that the greatest overall seedling survival occurred in the two soil origins with the greatest regional abundance of mature interior Douglas-fir. Matching transfer populations to soils with similar historical biogeography may therefore help to improve the compatibility of seed to potential FM sites.

Comparison of survival and growth between treatments in each soil revealed that seedling success was optimized by fungal or nonfungal soil factors in 26/32 cases (i.e. edaphic factors mediated local adaptation in 81% of the combinations). Soil fungi at least partially mediated local adaptation, with or without significant seed transfer, in 56% of the combinations. Importantly for silvicultural operations, the different responses indicate that soil factors can be as significant as climate in terms of their effects on seedling success, but that this significance varies amongst specific soil and seed pairings. Generally, planting guidelines already attempt to minimise elevation transfer distances due to the observation of decreased seedling performance with increased elevation transfer (Snetsinger, 2010); previously this was assumed to reflect the impact of mean annual temperature on overall growth and

success. However, our results show that fungal symbionts also play a role in mediating the observed effect, with soil fungi generating a comparable host response to that thought to be due to climate (e.g. O'Neill *et al.*, 2008; Wang *et al.*, 2010), expressed over similar distances.

Implications for natural and FM

Varying impacts of climate change on tree distributions have been observed or predicted for different ectomycorrhizal host species (Aitken *et al.*, 2008) and their EMF symbionts (e.g. Kranabetter *et al.*, 2012). Species within the Pinaceae are generally expected to shift their ranges northward and upward in elevation in Western Canada, likely reducing the range of the threatened whitebark pine (*Pinus albicaulis*; McLane & Aitken, 2012) while increasing the range of species such as interior Douglas-fir and western larch (*Larix occidentalis*; Hamann & Wang, 2006). These predictions have all been based on climatic factors, because measuring the impacts of the soil environment on species ranges has proven challenging in practice (e.g. Monserud *et al.*, 1990). Of course, this does not equate to edaphic factors being unimportant, as we have illustrated here and Gundale *et al.* (2014) have indicated for extremes of species transfer. Recently it has even been hypothesised that symbiont availability may have played a role in tree migrations since the last glacial maximum, at least in the forests of western North America (Elias, 2013). Soil biogeography is clearly an important factor that needs to be incorporated into models projecting the future ranges of species under climate change scenarios.

The complex adaptive systems framework (Puettmann *et al.*, 2009) has been advocated as a means by which climate change impacts on forest composition (e.g. Hastings *et al.*, 1993; Haeussler, 2011) can ultimately be factored into management approaches (O'Neill *et al.*, 2008; Wang *et al.*, 2012; Rehfeldt *et al.*, 2014b). Although extremely useful for theoretical understanding of ecosystem responses, forest management requires practical applications. Optimization of seedling survival around minimum elevation transfer, and growth in annually drier soil, implies that forestry FM with interior Douglas-fir should source seed for plantations from similar elevations with wetter climates. Because the aridity of terrestrial ecosystems tends to increase with warming (Sherwood & Fu, 2014), particularly during the growing season in British Columbia (Hamann & Wang, 2006), seed transfers could potentially be expanded to drier locations as climate warms northward. However, the potential for extreme weather is also expected to increase with climatic warming (IPCC, 2013), so any growth benefits must be balanced against an increase in the severity of drought events, heat waves and the timing of frosts. Reduced survival with containerised growth suggests that reforestation strategies will also benefit from supplementing seed transfer with natural regeneration through green tree retention; thereby mitigating start-up costs through mycorrhizal association with existing EMF networks and providing a complement of locally adapted seed.

In conclusion, soil fungal and abiotic factors can generate local adaptation patterns in tree populations comparable to those previously assumed to be entirely climatic in origin, with important

consequences for future natural and managed distributions. Future work on additional host–symbiont systems is required because the overwhelming majority of plants rely on mutualistic soil fungi, and the degree of symbiont dependence is likely to vary between host species. Given practical difficulties in assessing the direct impacts of edaphic factors on seedling growth and survival, it is understandable that they are often overlooked in favour of purely climatic explanations. Examining fungicide application in field contexts may be advantageous, to explore whether growth and survival outcomes thought to be driven by climate are altered, as they were in the common glasshouse. Where seed transfer for forestry purposes is kept to relatively short distances from the seed origin, as is typically employed in British Columbia, negative impacts caused by disrupting local adaptation between seedlings and their edaphic environment should be minimised. However, it has been predicted that seed transfer distances will likely need to be increased to keep pace with predicted rates of future climate change (Rehfeldt *et al.*, 2014b). The potential for soil biogeography to impact the survival and growth of seedlings is clearly an important consideration for understanding natural changes in species distributions, and in making informed decisions about future forest management.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Regional abundance of interior Douglas-fir.

Fig. S2 Interaction plots for pairwise interactions.

Fig. S3 Basal diameter with main effects plot.

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