Received: 11 February 2020 | Accepted: 24 August 2020

DOI: 10.1111/1365-2745.13507

RESEARCH ARTICLE

Journal of Ecology BRITISH ECOLOGICAL SOCIETY

Beyond seedlings: Ectomycorrhizal fungal networks and growth of mature *Pseudotsuga menziesii*

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Funding information
Natural Sciences and Engineering Research Council of Canada; University of Alberta

Handling Editor: Frank Gilliam

Abstract

1. Mycorrhizal networks are conduits for the transfer of resources between hosts. While ectomycorrhizal networks (EMN) are known to influence seedlings, their effect on adult tree growth remains unknown and may have important implications for forest responses to future climates.
2. We used annual basal area increment of trees and previously described *Rhizopogon vesiculosus* and *Rhizopogon vinicolor* EMNs to examine an association between the number of connections between trees through an EMN and the growth of adult interior Douglas-fir. We compared this relationship for the year the networks were mapped, in 2008, with 8 years previous and 8 years afterward. We also compared the variation in standardized growth (2000–2016) to examine the association between growth variability and EMN variables.
3. Greater growth was positively associated with (a) the number of connections to other trees via a *R. vinicolor* EMN and (b) the number of genets of *Rhizopogon vesiculosis* by which a tree was colonized. Variation of growth (2000–2016) was negatively associated with increasing number of connections to other trees via *R. vinicolor*.
4. *Synthesis*. These findings, for the first time, indicate that EMNs may positively influence the growth of adult trees. The difference in tree growth response between the sister fungal species highlights a novel avenue to identify interspecific and intraspecific differences between fungi occurring at different depths in the soil. Our study has important implications when considering the role of EMNs in influencing forest health and mitigating stress from environmental conditions.

KEYWORDS
dendroecology, fungal genets, interior Douglas-fir, multi-model inference, *Rhizopogon vesiculosus*, *Rhizopogon vinicolor*

1 | INTRODUCTION

When ectomycorrhizal fungal hyphae connect roots of neighbouring trees, an ectomycorrhizal network (EMN) is formed. Mycorrhizal networks can transfer carbon, essential nutrients, and water between connected trees and facilitate the sharing of resources from 'source' to 'sink' individuals (Lerat et al., 2002; Philip et al., 2010; Simard et al., 1997; Warren et al., 2008). Ectomycorrhizal fungal symbionts receive an average of 6% of net primary productivity, with higher proportions in nitrogen-limited, conifer-dominated systems (Hobbie, 2006; Ouimette et al., 2020; Schiestl-Aalto et al., 2019). In return, ectomycorrhizal fungi provide nutrients and water to their tree hosts, which can dramatically improve host growth and promote survival (Smith & Read, 2010). Carbon allocation and nutrient exchange have important implications for tree growth and survival through feedbacks with water transportation, defence against

biotic agents and carbon starvation (McDowell, 2011; Trugman et al., 2018). Networks provide an additional avenue for carbon transport and allocation (Simard et al., 2012) that may influence tree health. However, the costs and benefits of EMN membership may vary with individual tree characteristics, such as tree size or age (Teste et al., 2009).

Large, mature trees may act as 'source' trees in EMNs, with seedlings acting as 'sinks' (Booth & Hoeksema, 2010; Teste & Simard, 2008). In this source–sink dynamic, seedlings can receive growth-enhancing carbon (Teste et al., 2010) as well as experience lower drought stress (Teste & Simard, 2008) and higher survival when connected to an EMN (Booth & Hoeksema, 2010). Furthermore, the multiple benefits of EMNs increase with drought stress and promote seedling survival (Bingham & Simard, 2011). Interspecific and intraspecific fungal differences within EMNs may also influence the costs and benefits for connected trees (Kiers et al., 2011). Cumulatively, the benefits of EMNs indicate a potentially potent ameliorator of tree stress. However, how source–sink dynamics operate among a mature, even-sized forest is more difficult to predict. Klein et al. (2016) detected bidirectional carbon transfer without obvious source–sink dynamics. Despite the lack of size-driven source–sink dynamics, the transferred carbon accounted for 40% of fine root carbon among transferring trees (Klein et al., 2016).

Networks can be formed from single or multiple intraspecific fungal genets (Beiler et al., 2010). Each genet potentially provides a discrete network for the transport of resources among trees. One of the few mapped networks to date shows individuals of *Pseudotsuga menziesii* var. *glauca* (interior Douglas-fir) connected together by dense fungal networks formed by multiple genets of two sister fungal species, *Rhizopogon vinicolor* and *Rhizopogon vesiculosus*. The EMNs formed by these fungi were characterized by densely connected 'hub' trees that serve as central nodes, reducing the linkage distance between any two trees (Beiler et al., 2010, 2015). Both R*hizopogon* species can form hyphae that extend beyond 10 m (Beiler et al., 2010; Kretzer, et al., 2003), and are abundant and frequent colonizers of *P. menziesii* (Twieg et al., 2007). *Rhizopogon* spp. are likely to be particularly abundant in forests younger than 65 years and will gradually become a smaller portion of the fungal community as the forests mature (Twieg et al., 2007). The sister fungal species exist at differing depths within the soil profile, likely because of competitive avoidance (Beiler et al., 2012; Mujic et al., 2016). The unique location and topology of these fungal genets could conceivably result in differential abilities to shepherd or steal resources among or from trees. Trees with more connections to other trees could gain access to a wider array of sources from which to receive (or lose) growth-enhancing resources such as carbon, nitrogen or water. Trees with connections to more genets may possibly benefit from increased genetic and spatial heterogeneity of their ectomycorrhizal fungal partners. Networking with multiple genets stratified in the soil profile may be an important avenue for trees to access scarce water during droughts.

While much has been learned of EMNs in past experiments, methodological limitations have prevented comprehensive studies of EMNs in mature forests. First, previous studies identifying the benefits of EMNs have focused on seedlings (Booth & Hoeksema, 2010; Pickles et al., 2017; Teste et al., 2010). The significance of EMNs for mature trees remains unknown, and represents an important knowledge gap for predicting forest responses to future climates. Specifically, EMNs may promote forest resilience to recurrent droughts through the transfer of resources within the network. Second, EMNs have been treated as binary, with networked seedlings compared to non-networked controls (Bingham & Simard, 2011; Booth, 2004; Nara, 2006). This approach overlooks the continuous nature of mycorrhizal fungal connections observed in the field, and, importantly, a tree with multiple connections may experience a compound cost/benefit from network membership due to the existence of numerous pathways for the transport of resources. In light of these limitations, we used dendroecological analysis of a mature *P. menziessi* forest and leveraged a previously mapped EMN of sister *Rhizopogon* fungal species (Beiler et al., 2010, 2015) to identify the association between network connectivity and tree growth.

We predicted trees with many connections to other trees via the EMN would gain a radial-growth advantage over time through the exchange of limiting resources from a wider array of source trees. Multiple connections within the network should provide extra routes for the transport of resources as well as ensure security in case a tree is removed from the EMN. We also predicted increasing connections to unique fungal genets would result in greater tree growth due to differential nutrient and water acquisition between genets. Increasing connections with other trees through the EMN would likely result in lower variation of tree growth due to the loss of resources to other sink trees during favourable year and the gain of resources during unfavourable years. Finally, we expected the network to explain progressively less variance in tree growth with increasing time from the year the EMN was measured, due to changes in the network structure through time.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was in a lower montane forest dominated by *P. menziesii* var. *glauca* (Mayr) Franco (interior Douglas-fir), north of Kamloops, British Columba, Canada (50.853989, −120.522156). The forest has two cohorts of *P. menziesii* with younger *P. menziesii* in the mid-canopy and understory. The forest was last harvested in 1872 (Global Forest Watch Canada, 2008) with the oldest remnant tree dating to 1846. Post-harvest regeneration of the forest occurred naturally. Mean tree age among plots varied from 68 to 113 years old (Table 1). Scattered *Pinus ponderosa* (Ponderosa pine) were present within the forest but were killed in several *Dendroctonus* spp. outbreaks from 2005 to 2008 (Klenner & Arsenault, 2009). We did not encounter any living *P. ponderosa* within the study area.

TABLE 1 Summary statistics of the six plots in which ectomycorrhizal networks were measured. The plots were originally established by Beiler et al. (2015). Only the trees within the 10 × 10 m plots and ≥10.0 cm DBH (diameter at breast height) were used for summary statistics. Standard deviations are provided in parentheses. VEL = the mean number of trees connected via the *Rhizopogon vesiculosus* network; VIL = the mean number of trees connected via the *Rhizopogon vinicolor* network; VEG = the mean number of unique genets of *R. vesiculosus* and VIG = the mean number of unique genets of *R. vinicolor*

Plot	Trees sampled	DBH (*SD*)	Tree age (*SD*)	Clay/sand % (*SD*)	pH (*SD*)	Mean connections	VEG	VIG	VEL	VIL
1	6	20.3 (1.7)	87 (8.6)	20.9/30.7 (1.7/9.4)	5.8 (0.1)	22.1 (0.1)	1.3 (0.5)	0.0 (0.0)	22.1 (0.4)	0.0 (0.0)
2	5	21.4 (3.4)	102 (3.0)	16.7/42.7 (2.6/8.8)	5.7 (0.3)	11.4 (1.3)	1.2 (0.4)	1.4 (1.1)	9.0 (0.0)	7.4 (4.1)
3	4	36.6 (10.7)	115 (13.2)	18.2/37.2 (1.7/6.6)	5.6 (0.0)	24.75 (0.9)	1.2 (0.5)	2.0 (1.1)	21 (0.0)	13.2 (1.8)
4	5	24.1 (3.3)	64 (3.7)	11.0/48.5 (2.0/10.2)	6.3 (0.1)	18.2 (4.0)	2.2 (0.8)	0.6 (0.5)	18.2 (4.0)	0.6 (0.8)
5	6	21.9 (4.1)	64 (7.3)	12.1/50.7 (1.3/4.0)	5.7 (0.1)	24.5 (0.8)	1.3 (0.5)	1.3 (0.5)	18.5 (0.8)	15.3 (0.5)
6	9	22.5 (9.1)	65 (3.0)	16.5/31.9 (1.8/6.0)	5.4 (0.0)	24.8 (10.5)	1.5 (1.3)	1.6 (1.1)	17.4 (11.2)	13.3 (8.9)

Soils are Brunisols derived from glacial moraine parent material with variable soil texture (Table 1). Local climate is characterized by a mean annual temperature of 8.8 ± 9.1°C (*SD*), yearly precipitation of 268.5 ± 82.8 mm and a growing-season temperature of 17.8 ± 3.0°C (May–September) with growing-season precipitation of 134.8 ± 21.0 mm (Environment & Climate Change Canada, 2017). The trees within the forest are water-limited during the growing season and their growth positively correlates with precipitation during the current growing season (Supporting Information S1: Figure S1).

2.2 | Characterization of previously sampled ectomycorrhizal networks

Six plots were originally surveyed in 2008 by Beiler et al. (2015) to characterize the EMN associated with *P. menziesii*. The plots were placed at varied locations along a hillslope over a half-kilometre distance (Table 1). Each plot was at least 150 m away from the nearest plot. In each plot, a 10 × 10 m area was extensively surveyed for EM fungal connections, that is, mycorrhizal roots were genotyped to individual trees. Fungi colonizing roots were identified as one of two species, *R. vinicolor* Smith or *R. vesiculosus* Smith, and genotyped. Roots were sampled beneath the dripline, at four sides of every tree, and with dispersed samples between canopy cover (Beiler et al., 2015). Trees that were taller than their distance from the plot boundary were also sampled as reference material for roots sampled within the plots. A summary of the methods used and results of the Beiler et al. (2015) survey are available in Supporting Information S2. We assessed only the trees within the core 10 × 10 m plots for our analysis and excluded the surveyed trees outside of the plot boundaries to best capture the network topology (Supporting Information S1: Figure S2). Trees outside of the core 10 × 10 m plots were more likely to have undetected connections with outlying trees.

The previous survey described the topology of the EMN including a map detailing the mycorrhizal fungal connections between trees through each *Rhizopogon* spp. and the identity of the *R. vinicolor* and *R. vesiculosus* genets colonizing each genotyped tree. The average number of connections across all trees sampled by Beiler et al. (2015) was 16.2 ± 0.68 (*SD*) with each tree being connected to <1 (0.64 ± 0.6) *R. vinicolor* genets and 1.0 ± 0.6 *R. vesiculosus* genets. A minority of trees in each plot did not have any connections with genets of one or both *Rhizopogon* species. Within the 10 × 10 m plots, the average number of connections between trees was 20.9 ± 2.1, with each tree connected to 1.1 ± 0.7 *R. vinicolor* genets and 1.5 ± 0.1 *R. vesiculosus* genets (Table 1). For a full description of the network topology and features, see the original papers by Beiler et al. (2010, 2015).

2.3 | Sampling tree growth records and environmental variability

To construct continuous growth records, we sampled increment cores from *P. menziesii* within each plot (Table 1). In June 2017, we collected two increment cores per tree with a 4.3 mm increment borer (Haglöf Sweden; Mora, Sweden) from all trees ≥10 cm diameter at breast height (1.37 m). We selected a ≥10 cm DBH cut-off as coring could kill small trees. The unsampled, small trees accounted for 2% of the total tree basal area within the plots. The largest trees originally surveyed by Beiler et al. (2015) are exclusively outside of the core 10 × 10 m plots that we analysed for this study. Increment cores were sanded with increasingly finer grit sandpaper up to 600 grit. Cores were visually crossdated (Fritts, 1976; Speer, 2010; Stokes & Smiley, 1996), scanned at 1,200 DPI (Epson, 630 Pro Photo Scanner) and measured using the software CDendro 9.0 (Larsson, 2018). We verified crossdating using the COFECHA program (Holmes, 1983) and calculated basal area increment (BAI) and averaged by tree using the R package DPLR 1.7.0 (Bunn et al., 2019).

To characterize soil texture and pH as possible covariates of tree growth, we sampled soils adjacent to each cored tree in June 2017, 9 years post-EMN measurement. Four soil samples were collected at the dripline of each tree, separated by 90°, with one additional sample taken adjacent to the bole. Soil samples were collected to a depth of 20 cm using a soil knife (Zenport) and pooled by tree. Soil was analysed by hydrometer for particle size analysis (soil texture) and a pH/EC meter was used for pH (Fisher Scientific).

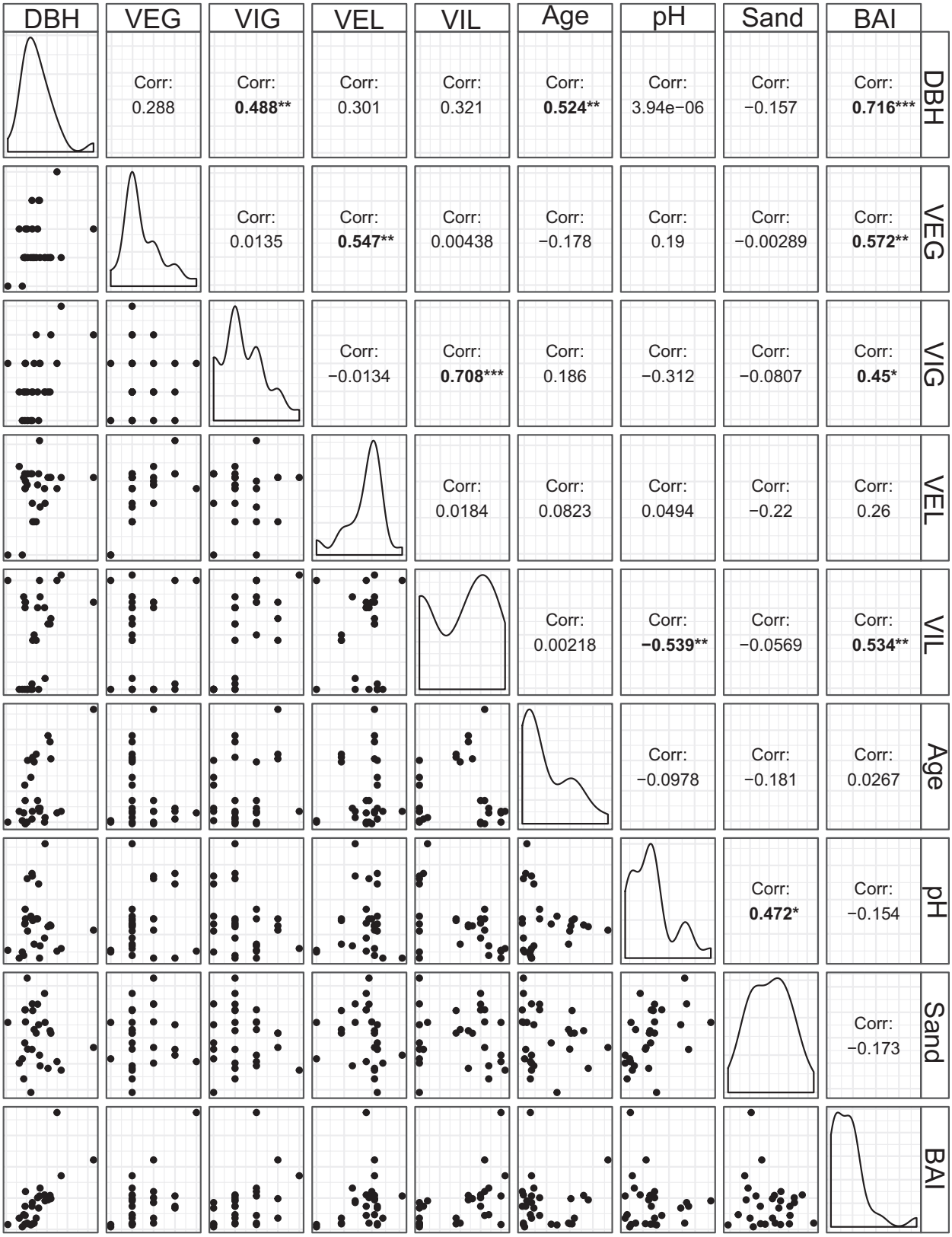

FIGURE 1 Pearson correlation values for each of the variables included in a global model testing the influence of ectomycorrhizal networks on basal area increment. The variables plotted are as follows: DBH = the diameter at breast height of the trees, VEG = the number of *Rhizopogon vesiculosus* genets colonizing a tree, VIG = the number of *Rhizopogon vinicolor* genets colonizing a tree, VEL = the number of connections to other trees through the *R. vesiculosus* network, VIL = the number of connections to other trees through the *R. vinicolor* network, Age = the age of the tree, pH = the pH of the soil, Sand = the sand content of the soil and BAI = basal area increment. Significant correlation coefficients are bolded and marked with asterisks (*$p < 0.5$; **$p < 0.01$; ****$p < 0.001$)

2.4 | Connectivity and growth analysis

To separate fungal species and genet influences on tree growth, we split the EMNs into species-specific categories for *R. vesiculosus* and *R. vinicolor*. Specifically, each tree located in the mapped EMN was characterized by (a) the number of other trees it was connected to through each *Rhizopogon* sister species' network and (b) the number of unique genets of a *Rhizopogon* spp. by which it was colonized. We only considered the effect of first-order network connections (tree 1—genet 1—tree 2) and excluded *n*th-order connections (tree 1—genet 1—tree 2—genet 2…) due to the small plot size and lack of replication.

2.5 | Statistical analysis

To identify the association between BAI and the EMN features, we used a multiple model inference and model averaging approach. Multiple model inference considers multiple competing models and weights them according to the scores of the Akaike information criterion with a correction for small sample size (AICc; Burnham & Anderson, 2002). We created an a priori subset of potential explanatory variables and a corresponding candidate set of models. We tested the model for multi-collinearity using the GGALLY 1.50 package (Schloerke et al., 2020) and found low-to-moderate collinearity between the variables (Figure 1). We used the 'geom_smooth' function to graph linear models of each pairwise combination of the dependent and independent variables (Wickham, 2016). We generated models for each hypothesis and ranked the models according to the lowest (best) AICc score and the difference in AICc scores (ΔAICc). Models scoring <7 ΔAICc are generally assumed to be plausible models and become increasingly implausible with increasing ΔAICc (Burnham & Anderson, 2002). We generated relative variable importance (RVI) scores, which represent the sum of model weights for all models including the variable of interest. We selected BAI (2008) and its variability across 2000–2016 as dependent variables. To calculate variability, we standardized each tree's growth by dividing its annual BAI with the average growth of the previous 2 years and following 2 years (Supporting Information S1: Figure S3). For the terminal rings, we averaged over the previous 2 years growth only. We then calculated the standard deviation of the standardized growth. We used the following variables as independent variables: the number of connections to other trees formed by each *Rhizopogon* sister species, the number of genets of each *Rhizopogon* sister species by which a tree was colonized, DBH, age of the tree, as well as soil texture (sand content) and pH. We tested a blocking-by-plot model (AIC = 408.04) against a non-blocked model (AIC = 410.04) and determined that blocking did not significantly improve AIC scores ($p = 0.99$) when soil pH and sand content were included. We used the MUMLN 1.43.6 package to test combinations of all variables and used a weighted-average of the top-performing models (ΔAICc < 10) to assemble a global model (Barton, 2020). We used the GGPLOT2 3.2.1 and GGPUBR 0.2.5 packages to graph the model outputs (Kassambara, 2020; Wickham, 2016).

2.6 | Model change through time

To test the temporal stability (change in RVI of model variables) of the EMNs association with tree ring width, we used multi-model inference for each of the years 2000–2016 and recorded the cumulative RVI value for each year's models. The 2000–2016 window included 8 years before and after the EMN measurement (i.e. the maximum balanced window). We used the BAI for each year as the dependent variable and the independent variables were those measured in 2008. We assumed that the independent variables would become increasingly inaccurate as time since measurement increased. For the global models, we report the full coefficient values with shrinkage. The full coefficient values treat variables as having a coefficient and variance of zero in models where they were not present for the purposes of averaging. The full-model averaging is useful in situations where the 'best' model does not have an overwhelming weight (Symonds & Moussalli, 2011). All statistical tests were conducted in the program R 3.6.2 (R Core Team, 2019) with the R Studio 1.3.1056 interface (R Studio Team, 2020). To identify any trends in model performance due to climate, we retrieved the annual Hargreaves climatic moisture deficit for 2000–2016 from ClimateNA v5.50 (Wang et al., 2016).

3 | RESULTS

Basal area increment BAI in 2008, the year the EMNs were mapped, was significantly, positively correlated with the number of *R. vesiculosus* and *R. vinicolor* genets colonizing a tree, the number of connections to other trees through the *R. vinicolor* network and DBH (Figure 2). The summed RVI for each variable across all models indicates that the strongest predictors of BAI (2008) were as follows: the tree DBH, number of *R. vesiculosus* genets colonizing the tree, the number of connections to other trees through the *R. vinicolor* network and the age of the tree (Figure 3a,b). The number of *R. vesiculosus* genets colonizing a tree and *R. vinicolor* connectivity variables were present in the top 10 models (cumulative weight 0.81; Table 2). Of lesser importance were the number of connections through the *R. vesiculosus* network (RVI = 0.65) and the number of colonizing genets of *R. vinicolor* (RVI = 0.45; Figure 3b). In the global model (2008), tree DBH had a positive association with BAI while tree age was negatively associated with BAI (Figure 3a). In comparison, the global model indicated a positive association between the number of colonizing *R. vesiculosus* genets, *R. vinicolor* connectivity and BAI (Figure 3b).

The summed RVI for each variable across all models indicates that the variation in tree growth (standard deviation of standardized BAI 2000–2016) was best explained by DBH (RVI = 0.96), the number of connections to other trees through *R. vinicolor* (RVI = 0.95) and sand content (RVI = 0.89). Of lesser importance were the number of connections to other trees through *R. vesiculosus* (RVI = 0.49), the number of *R. vesiculosus* genets colonizing a tree (RVI = 0.27), the soil pH (RVI = 0.22), the number of *R. vinicolor* genets colonizing a tree (RVI = 0.2) and tree age (RVI = 0.18). In the global model only DBH (coefficient = −4.813e-03; $p = 0.007$), the number of connections to

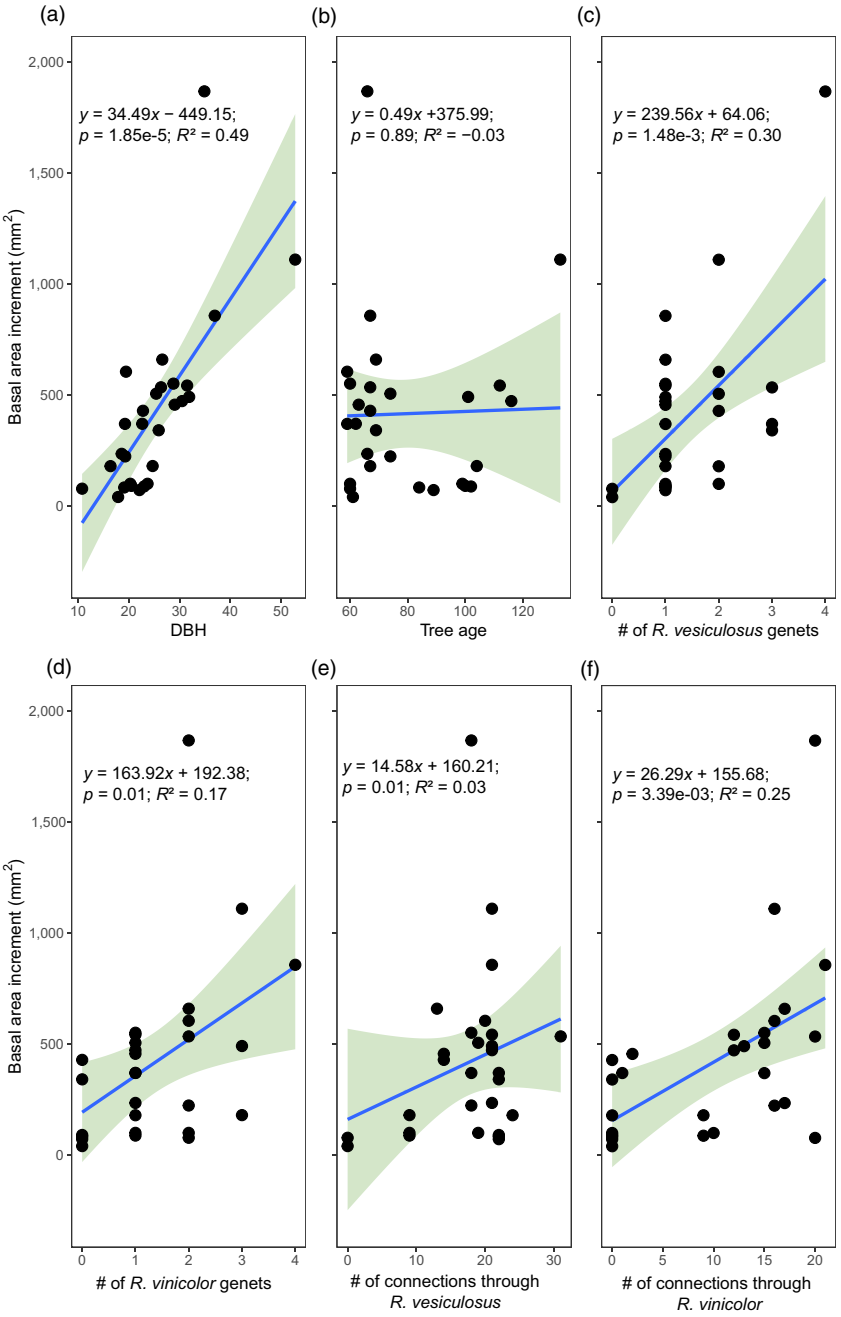

FIGURE 2 The linear relations between basal area increment (BAI) in 2008 and independent variables used in a global model testing the influence of ectomycorrhizal networks on basal area increment. (a) The linear relation between BAI and tree DBH (cm). (b) The linear relation between BAI and tree age. (c) The linear relation between BAI and the number of *Rhizopogon vesiculosus* genets colonizing a tree. (d) The linear relation between BAI and the number of *Rhizopogon vinicolor* genets colonizing a tree. (e) The linear relation between BAI and the number of trees connected to a tree through the *R. vesiculosus* network. (f) The linear relation between BAI and the number of trees connected to a tree through the *R. vinicolor* network

other trees through *R. vinicolor* (coefficient = −5.067e-03; $p = 0.012$) and sand content (coefficient = −2.4893e-03, $p = 0.052$) had RVI values above 0.5 and were all negatively correlated with the variation in tree growth (Supporting Information S3).

3.1 | Model change with time

In the best performing (ΔAICc = 0) non-averaged models from 2000 to 2016, the number of colonizing *R. vesiculosus* genets occurred in all models from 2000 to 2014 and the number of *R. vinicolor* connections occurred in all but the 2002 model (Supporting Information S3). Only DBH and tree age were more consistent and appeared in all the top models from 2000 to 2016 (Figure 3). From 2000 to 2009, the variable DBH had the highest RVI before declining slightly until 2013 (Figure 3a). From 2005 to 2013, the *R. vesiculosus* genet number had an RVI of ≥0.99. The RVI of the *R. vinicolor* network connectivity increased with time before peaking 2009–2013. The *Rhizopogon* spp. variables' RVI declined gradually in 2013, abruptly 2014–2015, and remained low in 2016 (Figure 3b).

The independent variables' coefficient values varied through time in each year's global model (Figure 4). The coefficients for DBH (Figure 4a) and the number of colonizing genets of *R. vesiculosis* (Figure 4b) were the most strongly positive of any of the variables. Similarly, the number of connections through the *R. vinicolor* network was slightly positive while tree age had a slightly negative coefficient. Each year's global model included all the independent variables.

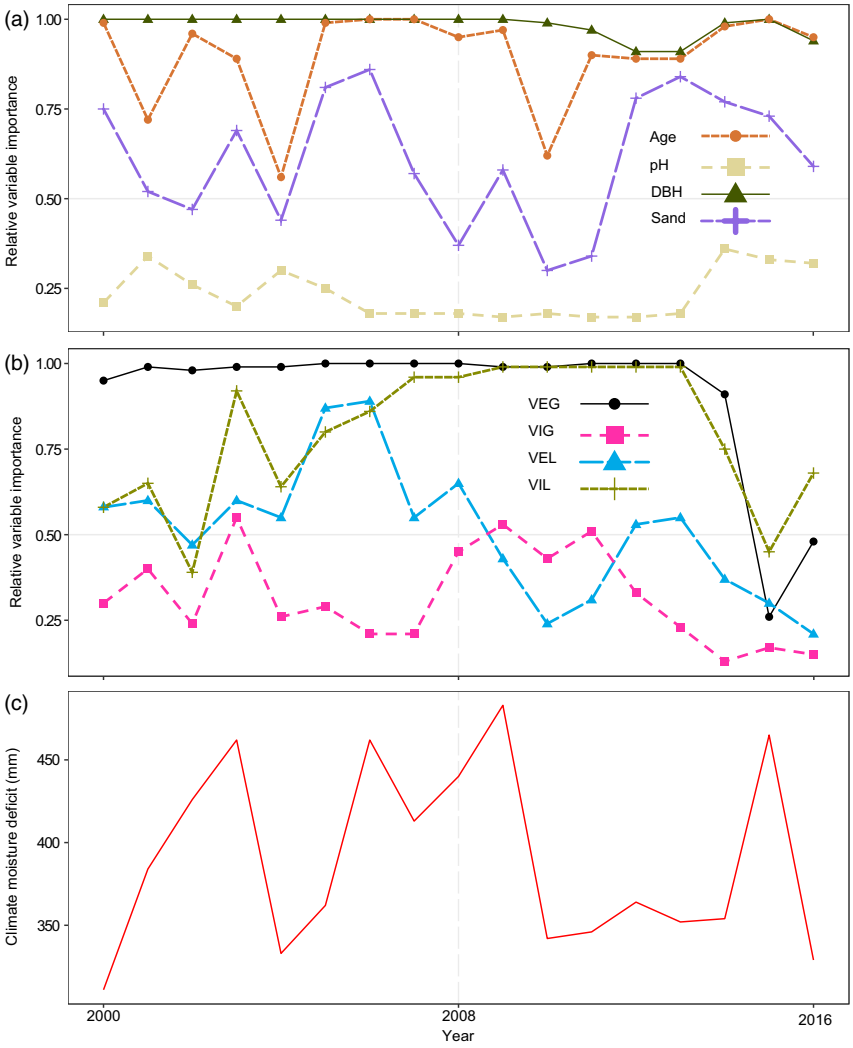

FIGURE 3 The relative variable importance scores (RVI, *y*-axis) for the variables used in a multi-model average for basal area increment of *Pseudotsuga menziesii* var. *glauca* trees. The *x*-axis plots the years over which models were generated. The RVI is the sum of the weights for models that contained the variable of interest. A high (low) RVI indicates the variable was present in models that were highly (poorly) weighted. For visual clarity, variables have been split into panels (a) and (b). (a) The RVI scores for DBH (medium-dashed line), age (solid line), sand content of the soil (mixed-dashed line) and soil pH (short-dashed line). (b) The RVI scores for the number of unique genets of *Rhizopogon vesiculosus* by which a tree was colonized (VEG, solid line), the number of unique genets of *Rhizopogon vinicolor* by which a tree was colonized (VIG, medium-dashed line), the number of connections to other trees via *R. vesiculosus* (VEL, long-dashed line) and the number of connections to other trees via *R. vinicolor* (VIL, short-dashed line). (c) The annual climatic moisture deficit (mm) for 2000–2016

TABLE 2 An abbreviated list (10/40) of the models with the lowest AIC and Δ*i* (ΔAICc) of <10.0 that were averaged to create a global model[a] to infer if ectomycorrhizal networks influence radial growth of interior Douglas-fir. Variables are as follows: BAI = basal area increment (2008), AGE = age of tree, DBH = diameter at breast height, pH = the soil pH, SAND = the sand content of the soil around a tree, VEG = the number of unique genets of *Rhizopogon vesiculosus* by which a tree is colonized, VEL = number of connections to other trees via *R. vesiculosus*, VIL = number of connections to other trees via *Rhizopogon vinicolor* and VIG = the number of unique genets of *R. vinicolor* by which a tree is colonized

Models	AICc	Δ*i*	LogLik[b]	Weight	R^2
BAI ~ AGE + DBH + VEG + VEL + VIL	470.553	0.000	−226.203	0.132	0.834
BAI ~ AGE + DBH + VEG + VEL + VIG + VIL	470.556	0.002	−224.509	0.132	0.849
BAI ~ AGE + DBH + SAND + VEG + VEL + VIG + VIL	470.756	0.202	−222.778	0.120	0.864
BAI ~ AGE + DBH + SAND + VEG + VEL + VIL	470.852	0.298	−224.657	0.114	0.848
BAI ~ AGE + DBH + VEG + VIL	470.884	0.330	−227.942	0.112	0.817
BAI ~ AGE + DBH + VEG + VIG + VIL	471.684	1.131	−226.768	0.075	0.829
BAI ~ AGE + DBH + SAND + VEG + VIL	472.794	2.240	−227.323	0.043	0.823
BAI ~ AGE + DBH + pH + VEG + VEL + VIL	473.508	2.955	−225.985	0.030	0.836
BAI ~ AGE + DBH + pH + VEG + VEL + VIG + VIL	473.728	3.174	−224.264	0.027	0.852
BAI ~ AGE + DBH + SAND + VEG + VIG + VIL	473.830	3.277	−226.146	0.025	0.835

[a]Global model: BAI ~ AGE + DBH + PH + SAND + VEG + VEL + VIG + VIL (full description of global model available in Supporting Information S3).
[b]LogLik: The log-likelihood of the model where larger (more positive) numbers indicate a better statistical fit of the model to the data.

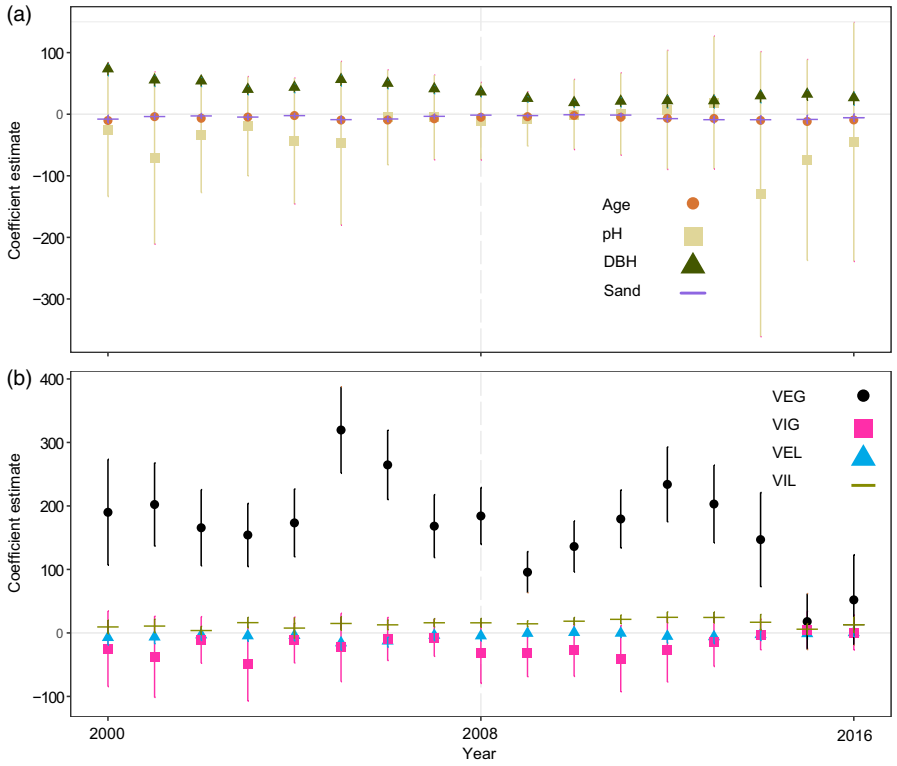

FIGURE 4 The coefficient values (±1 *SE*) for the ensemble global model variables. The *y*-axis plots the coefficient values of each variable. Coefficients represent how much a tree's BAI will change with a unit increase in each dependent variable. The *x*-axis plots the years over which ensemble global models were generated. The dependent variable for the global models was annual basal area increment of *Pseudotsuga menziesii* var. *glauca*. Independent variable values were constant from the year they were measured (2008). (a) The coefficient value and standard deviations for the independent variables of DBH (triangle), tree age (circle), sand content of the soil (line) and the soil pH (square). (b) The coefficient value and standard deviations for the independent variables of the number of unique genets of *Rhizopogon vesiculosus* by which a tree was colonized (VEG, circle), the number of unique genets of *Rhizopogon vinicolor* by which a tree was colonized (VIG, square), the number of connections to other trees via *R. vesiculosus* (VEL, triangle) and the number of connections to other trees via *R. vinicolor* (VIL, line)

4 | DISCUSSION

Our goal was to determine whether EMNs have a detectable influence on tree radial growth. We identified a positive association between fungal species-specific EMN connectivity and tree growth, lower growth variation with increasing connections through the *R. vinicolor* EMN and determined trees colonized by more genets of *R. vesiculosus* had greater growth. Importantly, the top 10 models for predicting BAI of Douglas-fir in the year that EMNs were mapped (ΔAICc < 3.5, cumulative weight 0.81) included *Rhizopogon* spp. variables (Table 2). These results highlight the potential for parsing fungal species-specific influences on mature tree hosts. While our results only identify an association and do not show causation, the novelty of the results and methods highlights an important new avenue for investigating the role of EMNs in mature forests.

4.1 | A tale of two networks: Connectivity and growth

Only one of the two *Rhizopogon* species surveyed, *R. vinicolor*, had a strong, persistent association between tree growth and the number of network connections to other trees. This is despite the fact that *R. vesiculosus* genets were more prevalent and larger than those of *R. vinicolor* (Beiler et al., 2010, 2012; Kretzer, et al., 2003). Though the sister species of *Rhizopogon* are closely related (Kretzer, et al., 2003), the difference in the association with connectivity to other trees and annual radial growth was considerable (*R. vesiculosus*, RVI = 0.65; *R. vinicolor* RVI = 0.96). Importantly, the coefficient estimate varied considerably more for *R. vesiculosus* connectivity than it did for *R. vinicolor* connectivity (Figure 2b). Furthermore, the number of connections through the *R. vinicolor* network was associated with lower variation in growth (RVI = 0.95) while the *R. vesiculosus* network did not significantly explain growth variability (RVI = 0.49). Our results suggest that EMNs made of *R. vinicolor* have a stronger influence on BAI and its variability than those made of *R. vesiculosus*. Additionally, the high variation in the RVI of *R. vesiculosus* network connectivity suggests that the relationship with BAI is not well defined by our dataset. We expect soil microfauna to disrupt fungal mycelium (Johnson et al., 2005) and that, all else being equal, greater hyphal surface area would be vulnerable to disruption. The smaller, shorter distances formed by *R. vinicolor* may be less prone to disruption than the larger *R. vesiculosus* genets. The connections of *R. vinicolor* are potentially more efficacious in the transfer of carbon or other resources between neighbouring trees.

The difference between the sister species may also be attributable to their frequented depths within the soil profile. The tree-derived benefits associated with connecting to multiple intraspecific genets indicates the potential for differential function at the intraspecific level. Our results suggest that *R. vesiculosus* genets are not functionally equivalent in the costs and benefits incurred upon their tree partner. Relatedly, our results indicate interspecific differences between the *Rhizopogon* spp. Work by Mujic et al. (2016) shows that differences in depth between the species are likely due to competitive avoidance rather than niche partitioning. The availability of soil nutrients changes with soil depth (Jobbágy & Jackson, 2001) and it is possible that *R. vesiculosus* was able to acquire nutrients or water that were more aggregated at depth or in strata not frequented by *R. vinicolor*. Beiler et al. (2012) reported *R. vinicolor* occurred at 4.95 ± 1.23 cm and *R. vesiculosus* 11.07 ± 4.42 cm depths within the soil profile. Soil depth not only separated these sister species but also fungal communities in other temperate forests (Toju et al., 2016). There was a significant negative correlation between soil pH and the number of connections through *R. vinicolor* ($r^2 = -0.539$; $p < 0.05$) as well as a non-significant correlation between pH and the number of unique *R. vinicolor* genets ($r^2 = -0.312$; $p = 0.106$; Figure 1). We speculate that the correlations may be a result of differences in pH with soil depth. Finally, the site is in a region that experiences frequent water limitations during the growing season (Supporting Information S1: Figure S1), and we expect soil moisture to be higher at greater depths within the soil profile. The greater depth of *R. vesiculosus* may provide for greater uptake of soil moisture and subsequent transfer to tree hosts. The greater tree growth associated with colonization by unique genets of *R. vesiculosus* indicates that niche differentiation may have a substantial influence on the efficacy of ectomycorrhizal fungal species in providing resources to their host.

4.2 | Who pays the price? The potential sources for EMN-delivered resources

We did not directly measure or identify the transfer of resources along the EMNs and can only speculate that growth-enhancing resources may flow through the EMN from one or more potentially unmeasured sources. Because resource transfer in EMNs is driven by source/sink dynamics (Booth & Hoeksema, 2010), we expect that there is a cost for source trees that are highly connected to sink trees. However, the current study design precluded the separation of trees into biologically meaningful size categories. We expect that variation in growth would increase for source trees that were highly connected and being drained of growth-limiting resources. However, the variation in growth decreased with increasing number of connections through the *R. vinicolor* EMN as well as with DBH. This result suggests that the *R. vinicolor* EMN is either an overall stabilizer of tree growth or does not drain large trees of a detectable amount of growth-limiting resources. Carbon transfer through the EMN is typically <10% of all photosynthetically derived carbon (Simard et al., 2015), which may indicate that other resources transferred through the networks are partially responsible for the greater tree growth. Multiple source–sink gradients for carbon, water and nitrogen could exist within the same EMN. Large trees could conceivably act as both a carbon source and water sink through the EMN.

Carbon and other limiting resources could be flowing from the forest outside of the plots and the scattered, large remnant trees that predate the post-logging cohort. Our current knowledge of EMNs would suggest that these large trees would be the most likely source trees within each EMN. Unfortunately, these trees had lower resolution of EMN topology and were excluded from our study. Another alternative source of carbon could be the now-deceased *P. ponderosa* that existed concurrent with the original EMN mapping in 2008. The *P. ponderosa* died from *Dendroctonus* spp. outbreaks in 2005–2008 (Klenner & Arsenault, 2009) and could conceivably been source trees for carbon and nutrient flux into the EMN. Song et al. (2015) showed an interspecific transfer of carbon and stress signalling enzymes through a shared EMN from defoliated *P. menziesii* to *P. ponderosa*. Similarly, the mortally wounded *P. ponderosa* at our sites may have exported resources through a shared EMN.

We expect that all trees will have a limiting growth factor (LGF) per Liebig's Law of the Minimum (Liebig, 1840) and that the same LGF need not be shared between all trees within a stand. Given that mycorrhizal networks can aid in the reallocation of carbon, water and nitrogen (Egerton-Warburton et al., 2007; He et al., 2009; Teste et al., 2009), we speculate that limiting resources could be transferred via EMN between trees experiencing different LGFs. A tree could lose a non-limiting nutrient to the EMN without incurring a growth loss and still benefit another connected tree. Greater access to a larger pool of trees with different LGFs could provide an avenue for an exchange of limiting resources between trees with LGFs.

4.3 | The rise and fall of *Rhizopogon* importance

The rise and subsequent fall of the *Rhizopogon* spp. RVI values could indicate that the topology of network connections changes with time. Our map of EMN topology was recorded once, in 2008, and is likely increasingly inaccurate with time from 2008. However, the presence of continuous tree-ring records provides an opportunity to indirectly investigate EMN stability through time. Fungal rhizomorphs, such as those produced by *Rhizopogon* spp., can live for an average of 11 months (Treseder et al., 2005) with colonized root tips averaging 1 year (Bledsoe et al., 2014). Twieg et al. (2007) reported 97% EM fungal colonization of *P. menziesii* root tips at a nearby forest in British Columbia. Due to the likelihood of high colonization by *Rhizopogon* and other EM fungal species, it is likely that the death of a single rhizomorph or root does not fully sever a tree's connection to a particular fungal genet.

The elevated (RVI > 0.5) values for *R. vesiculosus* genets (2000–2014) and *R. vinicolor* connectivity (2000–2002, 2004–2014) could indicate that EMNs have topology that is stable for multiple growing seasons but changes at decadal timespans. The decline in *Rhizopogon* spp. RVI values pre-2008 might be best explained by network topology

that did not exist by the time of surveying in 2008. We expect EMNs to fluctuate through time due to fine-root and fungal turnover and the potential for network disturbance. For example, EM fungal communities may shift, and connections severed, due to below-ground fungivores (Antunes & Koyama, 2017; Janoušková et al., 2018). Additionally, drought stress could also negatively impact the function and health of the *Rhizopogon* species. Other research has reported that EM fungal communities can shift with drought stress and warming temperatures (Cudlin et al., 2007; Li et al., 2015; Swaty et al., 2004). The plots experienced notable periods of drought in 2003, 2009 and 2015. The sharp decline of RVI values in 2015 is likely due to the severe drought in that year (Figure 3c). The proximity of the plots prevented us from testing the interaction of climate and EMN topology. Further research is needed to investigate EMNs through time and parse the potential factors influencing topology stability.

4.4 | Caveats and directions for future research

One alternative explanation of our results is the variables representing EMNs formed by *Rhizopogon* spp. are serving as proxy variables for the rooting extent and distribution of individual trees. Beiler et al. (2010) reported that older and larger DBH trees were associated with greater EMN connectivity. While our results may be partly attributable to EMNs mirroring rooting extent, we would expect tree DBH and age to also be strong predictors of rooting extent (Smith, 1964). Unfortunately, the largest trees were outside the core plots, established in Beiler et al.'s (2015) study, and had a low resolution of EMN topology. However, the distribution of tree ages within the plots was similar to that of the surrounding forest (Table 1; Supporting Information S1: Table S1) and would also be a proxy for rooting extent. The average rate of coarse lateral root growth has been reported at 7.4 cm/year in British Columbia and higher elsewhere (Richardson, 2000). Considering the average age of the trees within the core plots (Table 1), it is likely that even smaller trees have extensive distributions of roots. Oldest trees would conceivably have the greatest rooting extent and consequently have high colonization by fungal genets and growth if the EMN variables were simply a proxy for rooting extent. However, trees in the oldest plots had lower genet colonization and growth compared to those in the younger plots (Table 1; Supporting Information S1: Table S1). A nearby study of EM fungi in British Columbia found divergent foraging strategies between EM fungi and fine roots (Defrenne et al., 2019). This provides further evidence to suggest that the *Rhizopogon* spp. variables are poor proxies of rooting distribution. Similarly, the *Rhizopogon* spp. variables could act as proxies for increasing association with EM fungi and correspondingly higher levels of EM-sourced nutrients. However, Twieg et al. (2007) reported 97% EM colonization *of P. menziesii* root tips at a nearby forest in British Columbia. The high rate of colonization suggests that the *Rhizopogon* spp. variables are likely poor proxies for overall EM fungal colonization and that colonization does not vary enough to account for dramatic growth differences.

Another potential route for resource transfer between mature trees is through root grafts (Fraser et al., 2006). Neighbouring coarse roots from different trees can graft onto one another and provide numerous potential benefits (Lev-Yadun, 2011). While we expect that root grafting occurs throughout our sites, tree age or DBH would likely be the best proxy for root extent and likelihood of grafting with other trees. Finally, root grafts would be long-lasting and unlikely to cause changes in RVI at sub-decadal time scales. We excluded all trees that were <10 cm DBH from our study which may be one of the most sensitive cohorts to EMN-associated growth modulation. Small trees generally have less connections to other trees through the *R. vinicolor* EMN (Figure 1) and could conceivably have the most growth to gain from resources transferred through the EMN. However, juvenile trees frequently exhibit growth curves that differ markedly from mature trees (Speer, 2010) and many were not capable of being cored. We speculate that small trees likely show a stronger growth benefit from connections through the EMN. Different methods for measuring growth (i.e. annual measurements with calipers or dendrometers) would be needed to include these trees within our study.

Another potential caveat is that we investigated only two ectomycorrhizal fungal species associating with *P. menziesii* and have no data on the trove of other fungi that may have detectable influence on their tree hosts. However, *Rhizopogon* spp. are common fungi that account for a substantial portion of the ectomycorrhizal fungal community of *P. menziesii*. Specifically, *Rhizopogon* spp. colonized between 50% and 88% of all trees across the plots (Beiler et al., 2015). Additionally, Twieg et al. (2007) reported that *R. vinicolor*-type fungi were the most abundant colonizers of *P. menziesii* roots in separate interior Douglas-fir forests in a nearby region of British Columbia. While other EMNs may exist within the stand, the dual-*Rhizopogon* species likely occupy a substantial portion of the below-ground community. Unfortunately, the enormous effort of measuring EMNs in non-tuberculate fungal species makes it extremely challenging to identify the full range of networks that exist. Advances in field and laboratory methods are needed to map the full topologies of EMNs across different fungal species.

The topology of the *Rhizopogon* spp. EMNs is likely underrepresented in the core 10 × 10 m plots and particularly for the fringe trees that were mapped outside of the plots (Beiler et al., 2015). The difference between the average connectivity across all trees (16.2) and those in the exhaustively mapped 10 × 10 plots (20.9) highlights the potential of underreporting connectivity at the fringes of small plots (Beiler et al., 2015). While we restricted our sample size to the core 10 × 10 m plots, it is likely the topology of the EMN is still underrepresented. Larger plot sizes and the inclusion of more trees in EMN maps will enable for second- and *n*th-order connection efficacy to be tested. At present, the small sample size results in most trees having identical second-order connectivity. Surveying larger plots within smaller stands would improve our ability to map network topology and understand the influence of network connectivity.

With respect to future research, continued rises in atmospheric CO_2 could interact with EMNs in several ways and in confluence with

altered climate. With increased atmospheric CO_2, we expect that much of the range of *P. menziesii* will grow warmer and drier with associated climate change (Hamann & Wang, 2006). Increases in CO_2 can increase plant growth and EM fungal biomass (Alberton et al., 2005) and ectomycorrhizal trees show a greater biomass increase than do plants associated with arbuscular mycorrhizas (Terrer et al., 2016). Approximately 20% of global tree-ring series show a slight CO_2 fertilization effect during the latter half of the 20th century (Gedalof & Berg, 2010). With increased tree biomass and growth rates, we might expect an increased flux of carbon to EM fungi and the EMNs. However, much of the influence of EMNs will depend on how source trees respond to drier climates. Large trees have an outsized influence as reservoirs of above-ground biomass (Lutz et al., 2018) and frequently serve as network hubs within EMNs (Beiler et al., 2015). Globally, large trees have greater relative growth reductions due to drought than do small trees (Bennett et al., 2015). This suggests that drier conditions would lead to large trees providing less carbon to the EMN. However, this may be offset by increasing allocation of resources to root development at the expense of above-ground tissues, as a way of promoting water-uptake (Poorter et al., 2012). The redistribution of water via EMN may become increasingly important under drier conditions. The implications of rising CO_2 concentrations and associated climate change will likely vary substantially with forest community composition, stand structure, tree phenology, resource availability and interactions therein. More research is needed to disentangle the influence of climate, CO_2 and EMN efficacy in mature forests.

5 | CONCLUSIONS

This study provides evidence that EMN connectivity and connections to different individual mycorrhizal fungal genets can positively influence the growth of mature trees. Importantly, the capacity to form EMNs is not limited to *Rhizopogon* spp. or *P. menziesii* and our results may be generalizable to other forest systems. However, our results suggest that network efficacy may be highly variable at both the intraspecific and interspecific fungal species levels and EMNs may not always provide measurable advantages to mature trees. We expect our results to be mirrored in other forests across western North America, particularly where *Rhizopogon* species proliferate (Mujic et al., 2019). More research is needed to better isolate the mechanisms and strength of interactions between EMNs and tree growth. Current dendroecological models for tree growth may benefit from the inclusion of data on below-ground symbionts and their relations with neighbouring trees. Furthermore, the union of dendroecological tools and the study of ectomycorrhizas provides great utility in examining the impacts of ectomycorrhizal fungal species and networks in mature forests. Future research exploring EM-associated signals within tree rings may provide a fruitful ground for identifying species-specific impacts on tree hosts and changes through time and with climate. Our research provides further support for the influence of ectomycorrhizal fungal species and networks on forest health.

ACKNOWLEDGEMENTS

Lauche Fraser kindly provided storage space and logistical support for fieldwork. Alexia Constantinou assisted with fieldwork, and Colten Stephens volunteered for fieldwork. Jason Hoeksema provided advice on statistical analysis. Marcel van der Heijden and Ted H. Hogg provided helpful reviews to improve the manuscript. We would like to thank the anonymous reviewers for their thoughtful and helpful feedback. The Natural Science and Engineering Research Council of Canada provided funding to J.K. J.D.B. was generously supported by multiple awards from the University of Alberta. The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

J.D.B. conducted the survey; J.D.B. and J.K. designed and conducted the analysis. J.D.B., S.W.S., K.J.B. and J.K. contributed to synthesis and writing.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/1365-2745.13507.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.dncjsxkx7 (Birch et al., 2020). Raw tree ring measurements are accessible online as 'CANA611' at the International Tree Ring Data Bank at https://www.ncdc.noaa.gov/paleo-search/study/31053.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Birch JD, Simard SW, Beiler KJ, Karst J. Beyond seedlings: Ectomycorrhizal fungal networks and growth of mature *Pseudotsuga menziesii*. *J Ecol*. 2021;109:806–818. https://doi.org/10.1111/1365-2745.13507