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Differentiating spatial from environmental effects on foliar fungal communities of *Populus trichocarpa*

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Abstract

Aim: Foliar fungi – pathogens, endophytes, epiphytes – form taxonomically diverse communities that affect plant health and productivity. The composition of foliar fungal communities is variable at spatial scales both small (e.g. individual plants) and large (e.g. continents), yet few studies have attempted to tease apart spatial from climatic factors influencing these communities. Moreover, few studies have sampled in more than 1 year to gauge interannual variation in community structure.

Location: The Pacific Northwest of western North America.

Taxon: Foliar fungi associated with the deciduous tree *Populus trichocarpa*.

Methods: In two consecutive years, we used DNA metabarcoding to characterize foliar fungal communities of *Populus trichocarpa* across its geographic range, which encompasses a sharp climatic transition as it crosses the Cascade Mountain Range. We used multivariate analyses to (a) test for and differentiate spatial and environmental factors affecting community composition and (b) test for temporal variation in community composition across spatial and environmental gradients.

Results: In both study years, we found that foliar fungal community composition varied among sites and between regions (east vs. west of the Cascades). We found that climate explained more variation in community composition than geographic distance, although the majority of variation explained by each was shared. We also found that interannual variation in community composition depended on environmental context: communities located in the dry, eastern portion of the tree's geographic range varied more between study years than those located in the wet, western portion of the tree's range.

Main conclusions: Our results suggest that the environment plays a greater role in structuring foliar fungal communities than dispersal limitation.

KEYWORDS

Cascade Mountain Range, fungal leaf endophyte, microbial community structure, plant pathogen

1 | INTRODUCTION

Foliar fungi exist in and on living plant leaves. The most well-known members of these communities are pathogenic fungi, which cause plant disease and thereby affect the structure and composition of plant communities (Gilbert, 2002). Less well understood are the non-pathogenic epiphytic and endophytic fungi. These fungi are functionally diverse and can be mutualists (Arnold, 2007; Arnold et

al., 2003; Busby, Ridout, & Newcombe, 2016; Clay, 1988; Rodriguez, White, Arnold, & Redman, 2009), latent saprotrophs (Promputtha, Hyde, McKenzie, Peberdy, & Lumyong, 2010; Sun, Guo, & Hyde, 2010) or commensal symbionts (May, 2016). Because of their effects on plants, foliar fungi are consequential for ecosystems. Given the ecological roles played by foliar fungi, understanding how these communities vary through space and time may help to explain variation in plant and ecosystem function.

Many factors can result in spatial patterns in foliar fungal community composition. For example, foliar fungi display biogeographic patterns similar to animals and plants, such as distance decay (Meiser, Bálint, & Schmitt, 2014), negative associations between richness and latitude (Arnold & Lutzoni, 2007; Meiser et al., 2014) and variation over elevational and climatic gradients (Arnold & Lutzoni, 2007; Vacher, Cordier, & Vallance, 2016; Zimmerman & Vitousek, 2012). Additionally, foliar fungi can vary between host plant species (Wearn, Sutton, Morley, & Gange, 2012), and among individuals (Christian, Sullivan, Visser, & Clay, 2016) or genotypes (Bálint et al., 2013) of the same host plant. At smaller spatial scales, foliar fungi can even vary by the position (Harrison, Forister, Parchman, & Koch, 2016) or age (Arnold & Herre, 2003) of leaves on a plant.

While appreciation for the importance of spatial and environmental factors in structuring foliar fungal communities has deepened with recent studies, particularly those utilizing high-throughput DNA sequencing, few studies have attempted to tease apart spatial distance from abiotic environmental effects. Distinguishing between these two drivers is important for understanding whether communities respond more to dispersal limitation or environmental filters. U'Ren, Lutzoni, Miadlikowska, Laetsch, and Arnold (2012) found that climate was a better predictor than spatial distance for foliar endophyte communities at a continental scale. Further, they found that endophyte isolation frequency increased as a function of both growing season length and annual precipitation (U'Ren et al., 2012), suggesting that climate plays a strong role in structuring these communities. Focusing on a smaller, landscape level scale (elevational gradient on Mauna Loa, Hawaii) and a single host plant (*Metrosideros polymorpha*), Zimmerman and Vitousek (2012) also found that among-site variation in foliar endophyte community composition varied with temperature and precipitation, even after controlling for geographic distance. Giauque & Hawkes (2016) also found that endophyte communities associated with one grass species in Texas were controlled primarily by a climate gradient. All three of these studies suggest that climate may play a stronger role in structuring foliar fungal communities than spatial distance. However, U'Ren et al. (2012) and Giauque & Hawkes (2016) employed culture-based methods in their study, which do not capture as much diversity as culture-free methods, and thus may fail to detect certain taxa. Further, Zimmerman and Vitousek (2012) focused their study over a relatively small area, which may have failed to capture differences in community composition due to spatial distance and dispersal limitation.

Foliar fungal communities have also been shown to vary temporally, although less is known about the drivers of this variation. Seasonal variation in communities has been shown to track fungal life cycles, physical and chemical changes associated with leaf ageing, and seasonal changes in weather conditions (Fort, Robin, Capdevielle, Delière, & Vacher, 2016; Jumpponen & Jones, 2010; 2003, & Tokumasu, 2003; Osono, 2008; Suto, 1999). Year-to-year variability in community composition has been found in both tropical evergreen (Higgins, Arnold, Coley, & Kursar, 2014; Del Olmo-Ruiz & Arnold, 2014) and temperate deciduous systems (Giauque & Hawkes, 2016), although the drivers are poorly known. Year-to-year variability of foliar fungal communities, at least in deciduous plants, likely stems from the fact that foliar fungi are primarily horizontally transmitted (Rodriguez et al., 2009) and communities must reassemble each year on the newly emerging leaves of annual or deciduous plants. However, whether the magnitude of this temporal variation is uniform across spatial and environmental gradients is not known. For example, dry environments may provide fewer opportunities for leaf colonization, which generally requires moisture on the leaf surface, potentially resulting in more stochastic community assembly outcomes and thus higher temporal variability in community composition (Fukami, 2005, 2015). Alternatively, very harsh environments may impose a severe abiotic filter, restricting the set of potential colonists and resulting in more deterministic assembly outcomes.

To address these knowledge gaps in our understanding of spatial and temporal patterns in foliar fungi, we sampled leaves of the model deciduous tree species, *Populus trichocarpa*, in two consecutive years from 10 sites, spanning the trees natural range. *P. trichocarpa* occupies a broad geographic area in the Pacific Northwest of North America which is bisected by the Cascade Mountains (Figure 1). This physical barrier arose in the Pliocene, and it marks a sharp transition from a marine, wet and mild climate (USDA plant hardiness zones 8 and 9) to a dry and more continental climate (USDA plant hardiness zones 6 and 7) from west to east as well as acting as a potential barrier to dispersal of some plants and animals (Brunsfeld, Sullivan, Soltis, & Soltis, 2001; Daubenmire, 1975). The sharp climatic transition created by the Cascades thus sets up a good study system for exploring environmental effects on community structure as it partly decouples space and environment. We used DNA metabarcoding and multivariate analyses to (a) test for patterns of spatial structure in foliar fungal community composition arising from geographic distance or environmental conditions and (b) test whether the magnitude of temporal variation in community composition is uniform in contrasting environments.

2 | MATERIALS AND METHODS

2.1 | Study location and sample collection

In October 2013 and 2014, leaves were collected from 10 *P. trichocarpa* populations, five west and five east of the Cascade Range in the Pacific Northwest of North America (Figure 1). Each

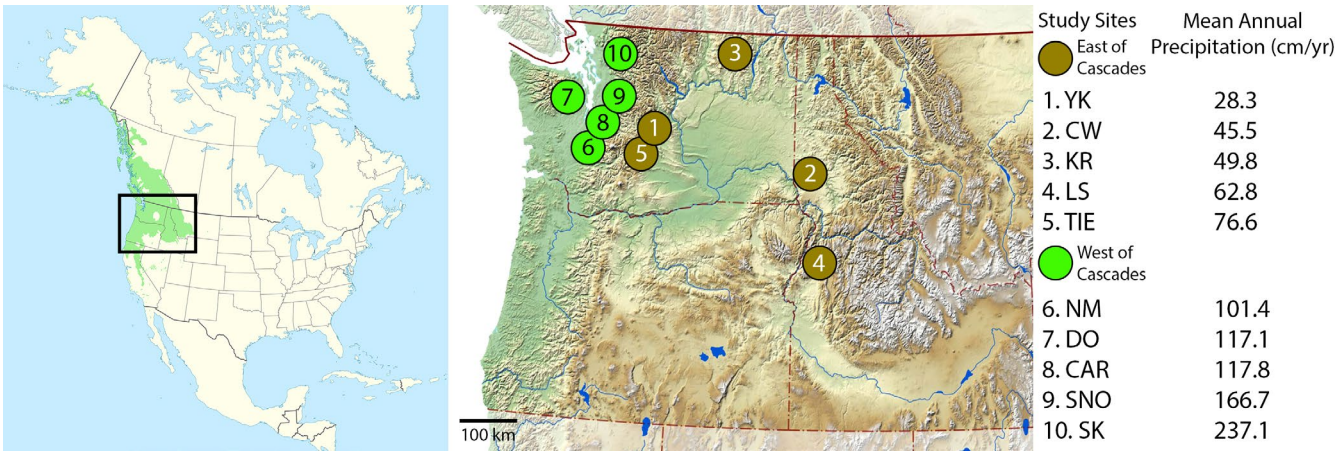

FIGURE 1 Map of study sites located across the core of the native range of *Populus trichocarpa* in the Pacific Northwest of North America. The study area spans a rainfall gradient from west (wet) to east (dry) of the Cascade Range. Sites west of the Cascades are coloured green, and sites east of the Cascades are coloured brown

population is associated with an independent river valley; western rivers drain into Puget Sound, and eastern rivers drain into the Columbia River. The weather patterns (monthly mean temperature and precipitation) were broadly similar between the two study years and similar to 30-year averages (Appendix S1). However, precipitation was generally lower both east and to a lesser extent west of the Cascades in the winter prior to 2013 sampling (Appendix S1). Three representative leaves per tree (i.e. not necessarily asymptomatic) were sampled from five trees per population, targeting lower canopy leaves of a standardized age (n = 2 years × 10 sites × 5 trees × 3 leaves = 300). Because our intention was to explore variability of communities between study years at the level of regions and tree populations, not at the level of individual trees, we did not sample the same trees between study years.

Leaves collected in the field were transported to the laboratory in coolers and processed within 24 hr. Leaves were surface sterilized in a laminar flow hood by soaking in a 1% sodium hypochlorite (NaClO) solution for 2 min followed by two rinses (1 min each) in sterile deionized water (Raghavendra & Newcombe, 2013) and then air-dried in a laminar flow hood and lyophilized.

2.2 | Molecular methods

We used the same protocols for DNA extraction and library preparation in both study years, with minor exceptions noted. We extracted DNA from approximately 10 mg of lyophilized tissue per leaf using Qiagen DNeasy 96 Plant Kits (Qiagen, Valencia, CA, USA), following the manufacturer's protocols. For the 2013 samples, we used an ethanol precipitation to remove impurities; DNA was eluted in 200 μl elution buffer. For the 2014 samples, DNA was eluted directly into 100 μl elution buffer with no ethanol precipitation.

We amplified and sequenced the fungal ITS1 region for each leaf sample using a modified version of the primers ITS1F and ITS2 (Smith & Peay, 2014) and the Illumina MiSeq platform (Illumina, San Diego, CA, USA). In 2013, PCRs consisted of 6 μl genomic DNA, 0.5 μl of each 10 μM primer, 5 μl of OneTaq Standard Reaction Buffer (New England Biolabs, Ipswich, MA, USA), 0.5 μl of 10 mM dNTPs (New England Biolabs), 0.63 units of OneTaq Hot Start DNA polymerase (New England Biolabs) and water up to 25 μl. In 2014, PCRs consisted of 1–2 μl genomic DNA, 0.5 μl of each 10 μM primer, 5 μl of OneTaq Standard Reaction Buffer (New England Biolabs, Ipswich, MA, USA), 0.5 μl of 10 mM dNTPs (New England Biolabs), 0.63 units of OneTaq Hot Start DNA polymerase (New England Biolabs) and water up to 25 μl. PCR conditions were the same in both years: initial denaturation at 94°C for 1 min; 35 cycles of 30 s at 94°C, 30 s at 52°C and 30 s at 68°C; followed by a 7 min final extension at 68°C. We visualized all PCR products using gel electrophoresis.

In 2013, PCRs were cleaned using the Agencourt Ampure XP kit (Beckman Coulter, Brea, CA, USA), and DNA yield was quantified for normalization using the Qubit hs-DS-DNA kit (Invitrogen, Carlsbad, CA, USA) on a Tecan Infinite F200 Pro plate reader (Tecan, Morrisville, NC, USA). In 2014, PCR products were cleaned and normalized in one step using Just-a-Plate™ 96 PCR Purification and Normalization Kits (Charm Biotech, San Diego, CA, USA). Both libraries were sequenced at the Stanford Functional Genomics Facility on Illumina MiSeq (250-bp paired-end in 2013, 300-bp paired-end in 2014). In 2013, three samples yielded no sequence data; raw reads for 147 samples are deposited in NCBI's Short Read Archive (accession no. SRP064132; Busby, Peay, & Newcombe, 2016). In 2014, all 150 samples yielded sequence data; raw reads for 150 samples are deposited in NCBI's Short Read Archive (accession no. SRP137181).

2.3 | Bioinformatics

Raw reads were processed first with cutadapt v1.4 (Martin, 2011) to trim low-quality 5' tails and 10 bp from the 3' end. Operational taxonomic units (OTUs) were identified using usearch v10.0.240 (Edgar, 2010) by merging paired-end reads, quality filtering (max expected

error <1) and denoising with unoise3 (Edgar, 2016a). Denoised reads were then abundance-sorted and clustered at 97% similarity. Spurious OTUs were removed by excluding those with <65% match to Kingdom Fungi in the UNITE database (Kõljalg et al., 2005). Predicted taxonomy was assigned first with SINTAX (Edgar, 2016b), querying the Warcup v2 fungal ITS region database (Deshpande et al., 2016), and then curated using manual blastn queries of GenBank (Clark, Karsch-Mizrachi, Lipman, Ostell, & Sayers, 2016). We used the R package metacoder (Foster, Sharpton, & Grünwald, 2017) to visualize taxonomic composition in both years for the 200 most abundant OTUs. To filter the data prior to conducting analyses, we removed OTUs making up <0.1% of total reads in each sample and removed samples with fewer than 1,000 reads (n = 29). We normalized variable sequencing depth by calculating the proportional abundance of each OTU in each sample (McMurdie & Holmes, 2014). To further ensure that variable sequencing depth among sites (Appendix S2) was not driving spatial patterns of variation in community composition, we also ran analyses on samples rarefied to 1,000 reads and found that patterns were the same as for non-rarefied data. Thus, we chose to present only the results of analyses focusing on non-rarefied data to avoid the loss of information that comes with rarefying (McMurdie & Holmes, 2014).

2.4 | Community composition

Community analyses were carried out using the R package vegan (Oksanen et al., 2007) unless noted otherwise. To visualize fungal community composition among sites, we used non-metric multidimensional scaling (NMDS) of Bray–Curtis dissimilarities among trees and displayed site-level means. To test whether community composition varied with sampling year, region (east or west of the Cascade Range) and among sites within regions, we used permutational analysis of variance (PERMANOVA) of Bray–Curtis dissimilarity among trees (leaf samples pooled to avoid pseudoreplication). Year was specified first in the model to account for year effects prior to testing for region and site effects. To visualize the relative effects of climate and spatial distance on community composition, we constructed a scatterplot of pairwise geographic distance between sites versus Bray–Curtis dissimilarity and colour-coded each point as a west–west, east–east or east–west comparison. Because the heterogeneity of group variances can influence PERMANOVA results, we calculated betadispersion both within sites and within each region using the function "betadisper". To test for a difference in within-site and within-region betadispersion between regions, we fit a linear mixed model for each response (within-site betadispersion, within-region betadispersion) using the R package lme4, function "lmer" (Bates, Maechler, Bolker, & Walker, 2015). Predictor variables in each model included the fixed effect region and the random effects year + site. Statistical significance of fixed predictors was assessed using Type III ANOVA in the R package lmerTest (Kuznetsova, 2016). Betadispersion did not significantly differ between regions (within-site p = 0.074; within-region p = 0.726) (Appendix S3, Appendix S4). However, the betadispersion results suggest that the PERMANOVA may have been influenced by differences in within-site betadisperion between regions.

To explore whether particular OTUs were more common or abundant east or west of the Cascades in each year, we conducted indicator species analysis of the 25 most abundant OTUs using PC-ORD (McCune & Mefford, 2015). p-values were corrected for multiple hypothesis testing using the method of Holm (1979) at alpha = 0.05. The relative abundances of the 10 most abundant OTUs overall were visualized for each study site using a bipartite network, linking sites and OTUs with the R package bipartite, function "plotweb" (Dormann, Fründ, Blüthgen, & Gruber, 2009).

In order to determine whether alpha diversity varied by year and region (east vs. west of the Cascades), we first estimated Shannon diversity and species richness (Chao1) for each sample using the R package phyloseq, function "estimate_richness" (McMurdie & Holmes, 2013). Diversity metrics were calculated using non-normalized sequence counts for all OTUs, including low abundance OTUs filtered for other analyses (Chao, 1984). To test whether alpha diversity varied by year, region and year × region, we fit a linear mixed-effects model using the R package lme4, function "lmer" (Bates et al., 2015). Log-transformed sequencing depth was included as a fixed effect to account for unequal sampling effort, and both tree and site were included as random effects to account for the non-independence of samples. Statistical significance of fixed effects was assessed using Type III ANOVA in the R package lmerTest (Kuznetsova, 2016).

2.5 | Partitioning spatial and environmental effects

We used variation partitioning to explore the relative effects of spatial versus climatic effects on community composition. To control for the differences between years, we first performed a distance-based redundancy analysis (function "dbrda") using Bray–Curtis dissimilarity among sites as the response variable and year as the predictor, and extracted the residuals for further analysis. We interpolated 30-year average climate variables for each site using ClimateNA (Wang, Hamann, Spittlehouse, & Carroll, 2016) (Appendix S5). The climate variables selected were mean annual precipitation (MAP), mean annual temperature (MAT), continentality (i.e. difference in mean temperatures of the warmest and coldest months; TD) and mean number of frost-free days (NFFD). Because climate variables were highly correlated, we performed principal component analysis (PCA), retaining the first two PC axes, representing 96% of the variation among sites and clearly separating the sites by region (east vs. west of Cascades) (Appendix S6). To assess spatial patterns of fungal community composition, we used trend surface analysis (Gittins, 1968), with second-degree orthogonal polynomials generated from site coordinates using the "poly" function in R. We then determined which spatial and climate axes most significantly explained variation in Bray–Curtis community dissimilarity among sites using distance-based redundancy analysis and forward model selection with functions "dbrda" and "ordiR2step". Forward model selection retained one spatial predictor (the linear east–west axis) and one principal

component of climate data (PC1) (Appendix S7). Finally, we partitioned the variation in community composition among sites by climate versus spatial factors using function "varpart". We then tested the significance of the unique fractions of variation in community composition explained by climate versus spatial factors using partial distance-based redundancy analysis and permutation tests.

2.6 | Between-year variation

To investigate variation in community composition between study years, we pooled samples at the tree level and conducted PERMANOVA analyses separately for each site, using Bray–Curtis dissimilarity among trees as the response variable and year as the predictor variable in each analysis. We used R^2 (proportion of variation explained by year) to compare the relative strength of the year effect among sites/regions.

3 | RESULTS

3.1 | Community composition

P. trichocarpa hosted a taxonomically diverse community of foliar fungi representing 50 identifiable orders and 1,216 OTUs (786 OTUs in 2013 and 849 OTUs in 2014). Communities were dominated in both years by Ascomycota (2013 = 92%, 2014 = 95%), followed by Basidiomycota (2013 = 3%, 2014 = 4%), and Mucoromycota (2013 = 5%, 2014 = 1%) (Figure 2). Dominant classes in both years included Dothideomycetes, Leotiomycetes and Sordariomycetes in the Ascomycota (Figure 2). The most abundant genus sampled in both years was *Sphaerulina* (Ascomycota, Capnodiales), which contains the known *P. trichocarpa* pathogen *S. populicola* (Figure 2). Other genera containing *Populus* pathogens that were dominant in both years included *Venturia* (Pleosporales) and *Marssonina* (Helotiales). Ascomycetes that are non-pathogenic on *P. trichocarpa*, and which were abundant in both years, included species of *Cladosporium* and *Ramularia* (Capnodiales), *Aureobasidium* (Dothideales), *Alternaria*, *Articulospora* and *Epicoccum* (Pleosporales). Other non-pathogens, such as *Trichoderma* (Hypocreales) and *Phialocephala* (Helotiales), both in the Ascomycota, and *Mortierella* (Mortierellales) in the Mucoromycota, were abundant in 2013 but nearly non-existent in 2014. Basidiomycota was represented primarily by the rust pathogen *Melampsora* (Pucciniales) in both years (Figure 2).

The community composition of fungi associated with *P. trichocarpa* leaves varied among sites and between regions (east vs. west of Cascades) in both years (Appendix S8, Figure 3a). Differences in community composition between east–west site pairs were large even at short spatial distances, suggesting a sharp turnover in community composition on opposite sides of the Cascades (Figure 3b). In both years, 13 of the 25 most abundant OTUs were associated with a geographic region: 10 west and 3 east (Appendix S9, Figure 4). Shannon diversity and estimated richness (Chao1) were higher in the region west of the Cascades in both years (Appendix S10, Figure 5).

3.2 | Partitioning spatial and environmental effects

We found that spatial distance (the linear east–west axis) and climate (PC1) explained 1.4% and 8.3% of unique variation in *P. trichocarpa* foliar fungal community composition, respectively, although the majority of overall variation explained by each was shared between the two sets of predictors (10.1%) (Figure 6). The unique variation explained by climate (PC1) was significant (F = 2.435, p = 0.033) but

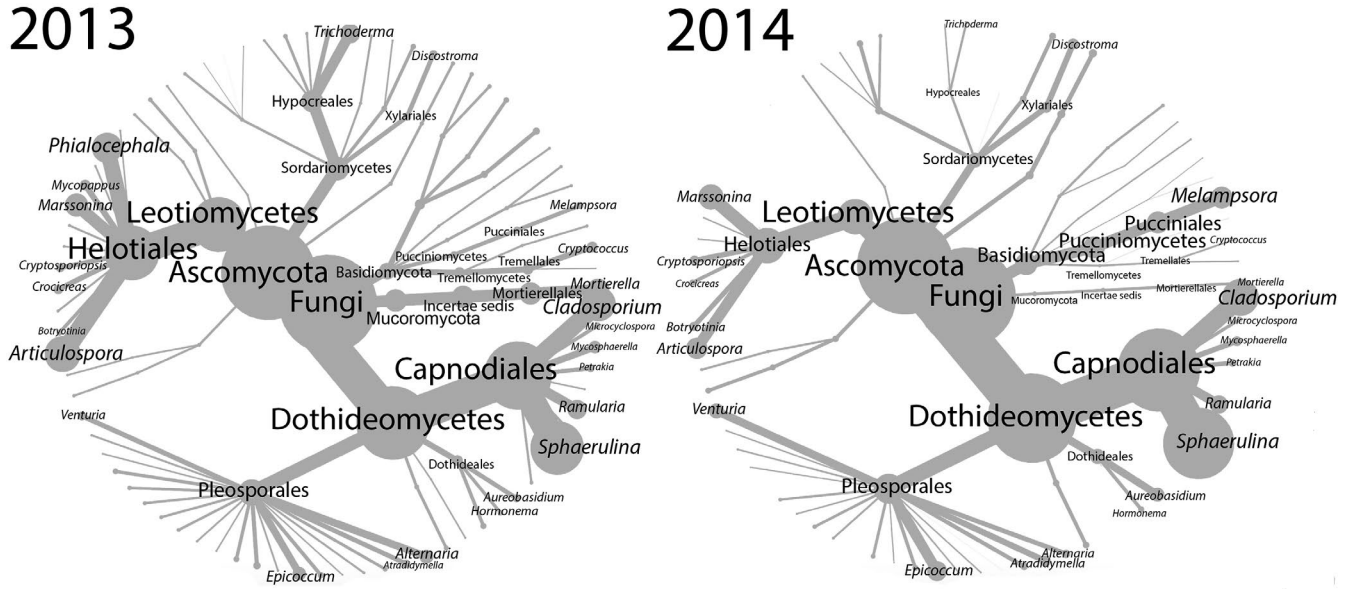

FIGURE 2 Taxonomic distribution of the 200 most abundant foliar fungal OTUs (displayed to genus) associated with *Populus trichocarpa* throughout its native range in the Pacific Northwest in 2013 and 2014. Node width indicates the proportional abundance of sequence reads assigned to a given taxonomic classification. Labels display the taxonomy of the 25 most abundant genera overall, and label size corresponds to relative abundance

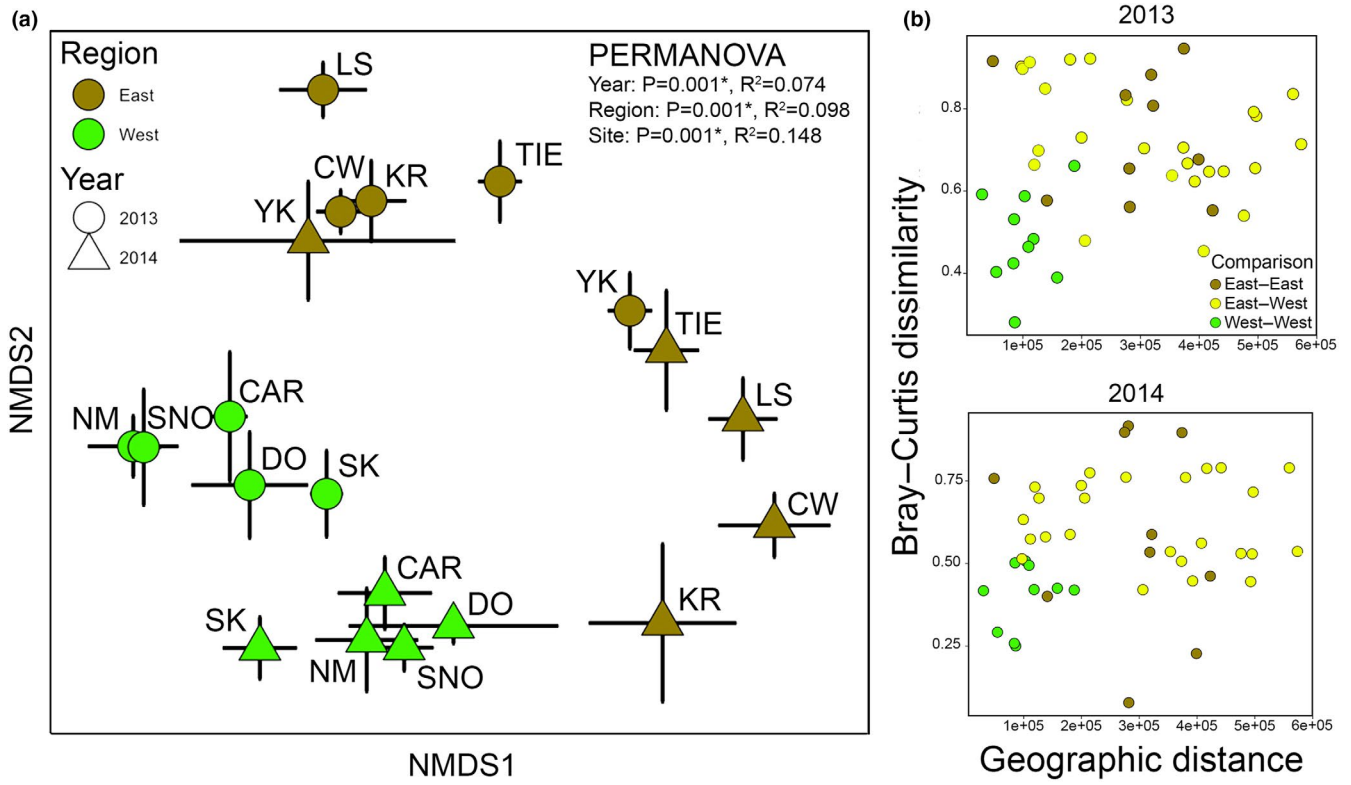

FIGURE 3 (a) Non-metric multidimensional scaling of foliar fungi associated with *Populus trichocarpa*, showing site-level means (±*SE*). Communities sampled west of the Cascades are coloured green, and communities sampled east of the Cascades are coloured brown. Communities sampled in 2013 are represented as circles, and communities sampled in 2014 are represented as triangles. Text adjacent to each point indicates the site name. Results of PERMANOVA testing temporal (year) and spatial/environmental (site, region) effects on community composition are also displayed. *significant at alpha = 0.05. (b) Scatterplot of Bray–Curtis dissimilarity (*y*-axis) versus geographic distance (*x*-axis) for all pairwise comparisons among plots, colour-coded by whether comparisons are west–west (green), east–east (brown) or east–west (yellow). East–west sites separated by small geographic distances are just as dissimilar as east–west sites separated by large geographic distances, suggesting that distance alone is not driving differences in community composition between eastern and western sites.

the fraction of unique variation explained by spatial distance was not (F = 1.099, p = 0.307).

3.3 | Between-year variation

We found that fungal community composition significantly varied between study years (Figure 3a); however, the magnitude of this effect varied among study sites. In particular, when community composition was analysed at the site level, it varied with year at all 5 eastern sites, but only 1 of the 5 western sites (Appendix S11, Figure 7). Shannon diversity and estimated richness (Chao1) also varied from between study years (both were lower in 2014), and this variation was not region-dependent (i.e. year × region interaction was not significant) (Appendix S10, Figure 5).

4 | DISCUSSION

4.1 | Spatial/environmental structure

P. trichocarpa occupies a broad geographic area in Western North America, encompassing a wide range of environmental conditions. Within the distribution of *P. trichocarpa*, the Cascade Range has, since the Pliocene, marked a particularly sharp transition in the abiotic and biotic environment, moving from a wet and mild climate to the west into a dry and more continental climate to the east, as well as acting as a potential physical barrier to dispersal of plants and animals (Brunsfeld et al., 2001; Daubenmire, 1975). For example, the coastal and inland varieties of Douglas fir are consistent with divergence since the Pliocene (Gugger, Sugita, & Cavender-Bares, 2010). We found that foliar fungal community composition was highly variable across the range of *P. trichocarpa*. Specifically, communities varied among sites and between regions (east vs. west of the Cascades), and fungal alpha diversity was higher in the wetter, milder sites west of the Cascades.

While the observational nature of this study precluded us from explicitly separating the effects of space and climate on foliar fungi, variation partitioning allowed us to address their relative effects. We found that fungal community composition was primarily explained by the correlated components of space and climate. However, we also found that additional variation in fungal community composition could be explained by variation in climate that was independent from spatial distance among sites. This finding makes sense given

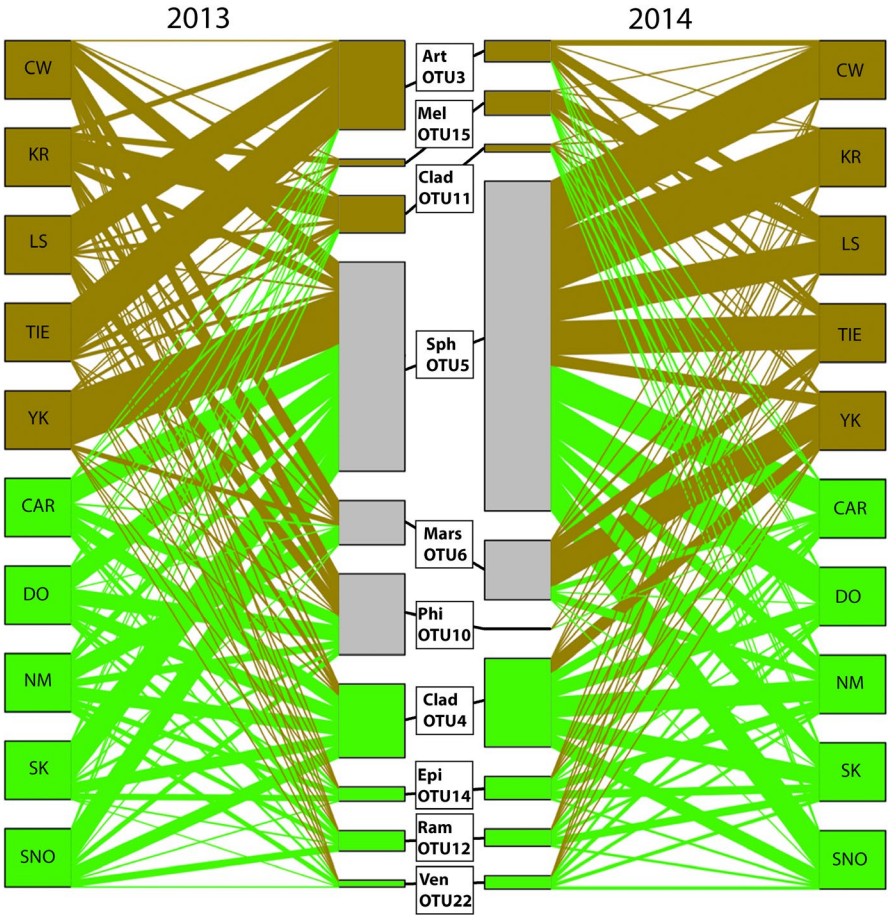

FIGURE 4 Associations between the 10 most abundant *Populus trichocarpa* foliar fungal OTUs (% of total OTU abundance) and sites over two years. Line width indicates the relative abundance of a fungal OTU (central nodes) in amplicon sequencing data for each site (outer nodes). The colour of site nodes indicates whether they are located east (brown) or west (green) of the Cascades. Coloured OTU nodes indicate taxa that were significant indicators of the study regions, east (brown) or west (green) of Cascades. Art = *Articulospora*, Mel = *Melampsora*, Clad = *Cladosporium*, Sph = *Sphaerulina*, Mars = *Marssonina*, Phi = *Phialocephala*, Epi = *Epicoccum*, Ram = *Ramularia*, Ven = *Venturia*

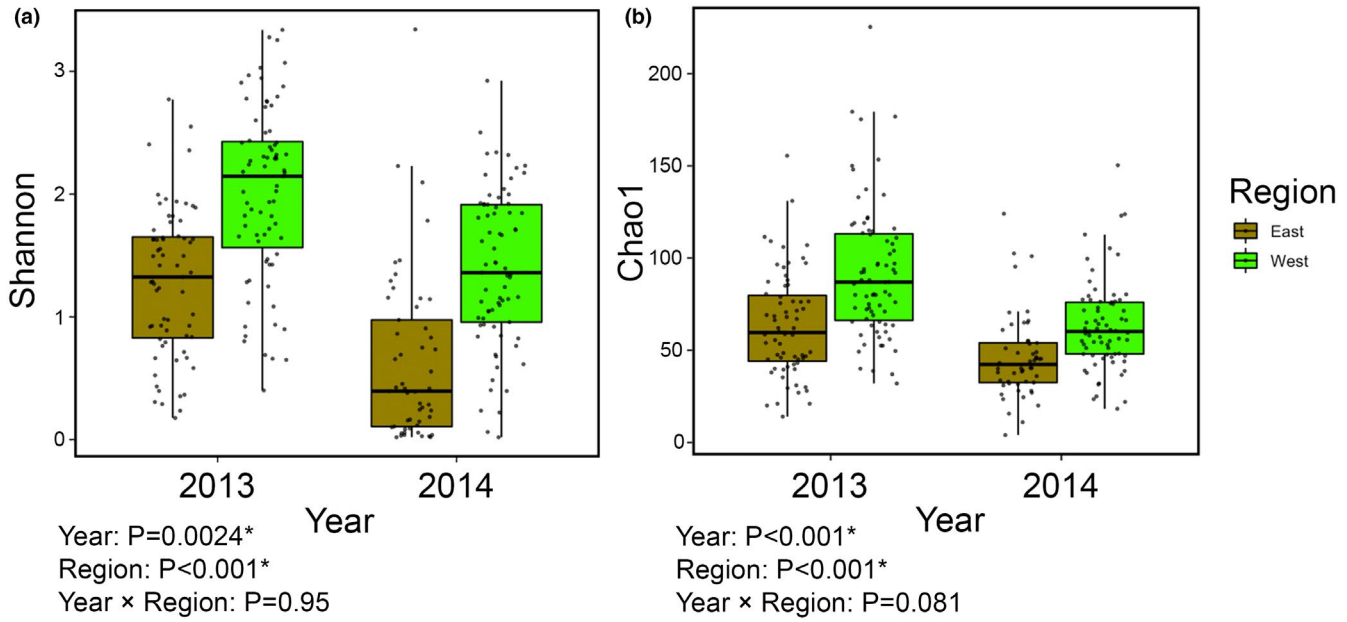

FIGURE 5 Box plots of Shannon diversity (a) and Chao1 richness (b) of foliar fungi associated with *Populus trichocarpa* in 2013 and 2014. Points represent richness and diversity estimates of individual samples. Box colour indicates whether samples came from east (brown) or west (green) of the Cascades. *significant difference in the response at alpha = 0.05

the study system, in which the Cascade Range marks a sharp transition in climate over a short geographic distance. This finding is also in agreement with previous studies of foliar endophyte communities that have examined turnover in foliar endophyte community composition both at a continental scale (U'Ren et al., 2012) and at a smaller landscape scale over an elevational gradient (Zimmerman &

Vitousek, 2012). Thus, it appears that spatial patterns of foliar fungal communities may be different than soil fungal communities, which display greater dispersal limitation (Talbot et al., 2014), and environmental filtering by the soil matrix (David, Seabloom, & May, 2015). The Cascade Range may also act as a physical barrier to spore dispersal, which may have led to some of the differences in community composition we observed in this study. However, because there is evidence of pollen (not seed) exchange across the Cascades (Gugger et al., 2010), and pollen is wind-borne like spores of fungi, we suspect that the Cascades do not entirely prohibit fungal dispersal.

The biotic environment, or host plant population, may have also influenced spatial variation in foliar fungal communities (Bálint et al., 2013). Previous studies have found evidence for *P. trichocarpa* population genetic structure at the scale of our study, but along a north–south gradient (Evans et al., 2014), as well as differences in *P. trichocarpa* leaf traits (e.g. defence, epidermal structure; Dunlap & Stettler, 1996, 2001) between eastern and western tree populations. Because leaf traits can influence colonization by foliar fungi (Kembel & Mueller, 2014), phenotypic variation among tree populations could have contributed to observed variation in foliar fungi in our study. Future, manipulative studies (e.g. reciprocal common garden design) are needed to disentangle the effects of host genetic variation, geography and climatic gradients on foliar fungi.

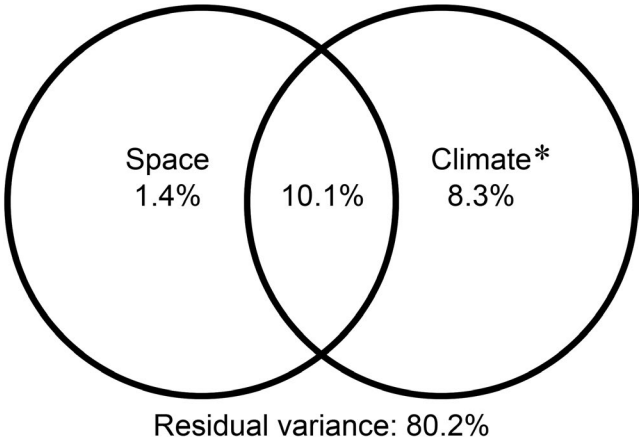

FIGURE 6 Results of analyses partitioning the variation in *Populus trichocarpa* foliar fungal community composition explained by spatial versus climatic factors. *unique fraction significant at alpha = 0.05

4.2 | Between-year variation

Sampling the same *P. trichocarpa* populations in two consecutive years allowed us to investigate variation in fungal community composition between study years. Our observational study did not directly address the specific factor(s) driving site-level community shifts between years. However, we found greater variation in foliar fungal community composition between study years in dry, continental (eastern) versus wet, maritime (western) sites, suggesting that environmental context may influence temporal variability. Major differences among eastern and western sites that could have led to the pattern we observed include moisture availability, landscape connectivity and foliar fungal diversity. Overall, a more stochastic and therefore less deterministic community assembly process is predicted for each of these characteristics: moisture limitation and habitat isolation (Fukami, 2005, 2015), and limited diversity (Levine & D'antonio, 1999), all of which characterize eastern sites. However, given that sampling was conducted in only two years, we cannot draw general conclusions regarding the drivers of year-to-year variation. Additional sampling (i.e. multi-year) would be necessary to reveal whether differences in the magnitude of variation in community composition that we observed between these two years are representative and whether the variation has a deterministic cause (e.g. climate). Additionally, manipulative experiments would be necessary to identify and tease apart the factors playing a prominent role in year-to-year variation of the foliar fungal community.

Differences in fungal community composition, the number of OTUs, sequencing depth and Shannon and Chao1 between years may also have been influenced by differences in sampling and sequencing methodology. For example, different trees in the study

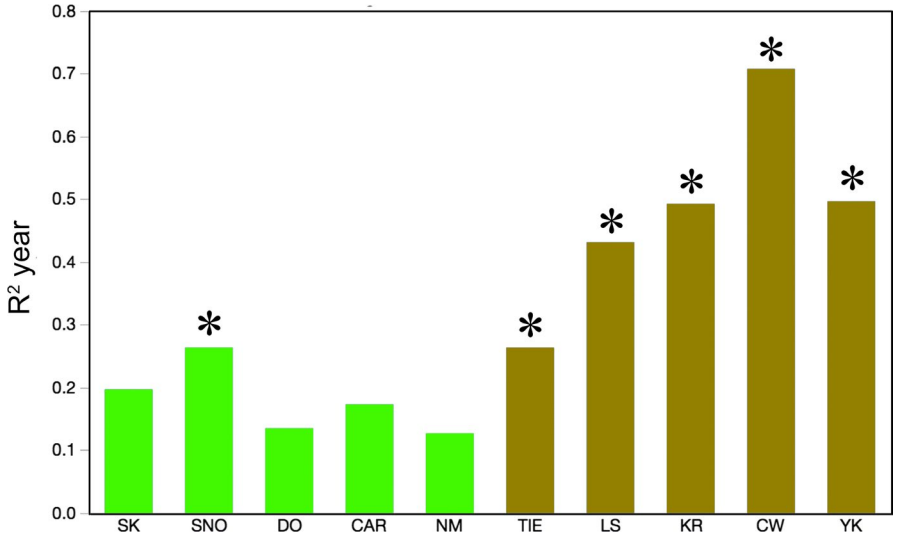

FIGURE 7 Bar graph showing the proportion of variation in *Populus trichocarpa* foliar fungal community composition (PERMANOVA R^2) explained by year at each study site. Bar colour indicates whether study site is east (brown) or west (green) of the Cascades. *significant at alpha = 0.05

populations were sampled in the different years, and we made minor modifications to DNA extraction protocols, PCR library preparation and sequencing in the second study year. In particular, we noticed a difference in the number of observed OTUs (786 OTUs in 2013 and 849 OTUs in 2014) and alpha diversity between years, which could be attributed to differences in molecular methods between years. However, these methodological differences would have affected all samples within each year, limiting potential effects on variation in the magnitude of year-to-year variation among sites. Additionally, despite methodological changes, we identified many of the same dominant taxa in each year and found consistent patterns of variation in community composition and alpha diversity.

4.3 | Conclusions

While substantial progress has been made in understanding how foliar fungi are distributed across space (Arnold & Lutzoni, 2007; Bálint et al., 2013; Christian et al., 2016; U'Ren et al., 2012; Zimmerman & Vitousek, 2012), few studies have attempted to tease apart spatial from environmental factors influencing these communities or tested the degree to which communities vary from year to year in the leaves of deciduous plants. Answers to these questions are important given the critical functional roles played by foliar fungi in both wild and crop plant systems. For example, common foliar endophytes of *P. trichocarpa* are capable of modifying the expression of foliar disease (Busby, Peay, et al., 2016). Our results reveal that the community composition of *P. trichocarpa* foliar fungi is spatially variable throughout the plant's native range. In particular, we found distinct communities of foliar fungi when comparing sites on opposite sides of the Cascade Range, which marks a sharp climatic transition. This division is consistent with our finding that spatial patterns of fungal community composition among sites were also largely correlated with variation in climate. However, climate also explained additional variation in community composition that was not associated with spatial distance. Moreover, the degree of site-level variation in community composition between study years depended on environmental context. Sites in the drier, more continental region were associated with higher variation in fungal composition between study years in comparison with sites in the wetter, more maritime region.

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AUTHORS' CONTRIBUTIONS

PEB and GN conceived the idea for the study. PEB collected and processed the samples with assistance from KP. EB and DL analysed the data with assistance from PB. EB wrote the paper with contributions from all authors.

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BIOSKETCH

Edward Barge is a PhD student in the Botany and Plant Pathology Department at Oregon State University. He is working with mentor Posy Busby to characterize the taxonomic structure and ecological function of microbial communities that live in and on plant tissues (https://bpp.oregonstate.edu/users/posy-busby).

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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