Received: 27 July 2017 | Revised: 17 October 2017 | Accepted: 25 October 2017

DOI: 10.1111/mec.14414

ORIGINAL ARTICLE

WILEY MOLECULAR ECOLOGY

Environmental filtering by pH and soil nutrients drives community assembly in fungi at fine spatial scales

Sydney I. Glassman[1,2,3] | Ian J. Wang[1] | Thomas D. Bruns[1,3]

[1]Department of Environmental Science Policy and Management, University of California, Berkeley, CA, USA

[2]Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, USA

[3]Department of Plant & Microbial Biology, University of California, Berkeley, CA, USA

Correspondence
Sydney I. Glassman, Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, USA.
Email: sglassma@uci.edu

Funding information
National Science Foundation Graduate Research Fellowship Program; National Science Foundation Doctoral Dissertation Improvement Grant, Grant/Award Number: 1403215; NSF Dimensions of Biodiversity Grant, Grant/Award Number: 1046115

Abstract

Whether niche processes, like environmental filtering, or neutral processes, like dispersal limitation, are the primary forces driving community assembly is a central question in ecology. Here, we use a natural experimental system of isolated tree "islands" to test whether environment or geography primarily structures fungal community composition at fine spatial scales. This system consists of isolated pairs of two distantly related, congeneric pine trees established at varying distances from each other and the forest edge, allowing us to disentangle the effects of geographic distance vs. host and edaphic environment on associated fungal communities. We identified fungal community composition with Illumina sequencing of ITS amplicons, measured all relevant environmental parameters for each tree—including tree age, size and soil chemistry—and calculated geographic distances from each tree to all others and to the nearest forest edge. We applied generalized dissimilarity modelling to test whether total and ectomycorrhizal fungal (EMF) communities were primarily structured by geographic or environmental filtering. Our results provide strong evidence that as in many other organisms, niche and neutral processes both contribute significantly to turnover in community composition in fungi, but environmental filtering plays the dominant role in structuring both free-living and symbiotic fungal communities at fine spatial scales. In our study system, we found pH and organic matter primarily drive environmental filtering in total soil fungal communities and that pH and cation exchange capacity—and, surprisingly, not host species—were the largest factors affecting EMF community composition. These findings support an emerging paradigm that pH may play a central role in the assembly of all soil-mediated systems.

KEYWORDS
beta-diversity, ectomycorrhizal (ECM) fungi (EMF), generalized dissimilarity modelling (GDM), Illumina MiSeq, pH, Yosemite National Park

1 | INTRODUCTION

A long-standing goal of ecology is to identify the processes structuring ecological communities. Ecologists endeavour to determine the biotic and abiotic factors that cause changes in beta-diversity, or species turnover, because higher beta-diversity produces greater regional species richness (Kraft et al., 2011). Still debated is whether niche processes, like environmental filtering, vs. neutral processes, like dispersal limitation, are more important in driving community composition (Gravel, Canham, Beaudet, & Messier, 2006). Only recently have ecologists begun explicitly testing for the prominence of environmental determinism or dispersal limitation (Kristiansen et al., 2012; Landesman, Nelson, & Fitzpatrick, 2014; Tuomisto, Ruokolainen, & Yli-Halla, 2003) and typically only at broad spatial

scales and in macro-organisms (Tuomisto et al., 2003). Hence, studies that simultaneously quantify the relative effects of environmental filtering and dispersal limitation, especially at fine spatial scales, are integral to understanding how local processes drive patterns of biodiversity.

Microbial communities are excellent systems in which to examine the drivers of community assembly because of their high diversity, central role in many ecological processes and tractability for replication. Microbes are essential in driving many ecosystem processes like decomposition and carbon cycling (van der Heijden, Bardgett, & van Straalen, 2008; Martiny et al., 2016). In addition, microbes play critical roles in symbiosis—for instance, mycorrhizal fungi helped drive the initial terrestrial colonization by plants (Humphreys et al., 2010).

Free-living and symbiotic microorganisms could be affected by both dispersal limitation and environmental filtering, including filtering by host or habitat. Yet, whether they are more strongly driven by niche vs. neutral processes might depend on their ecology. Examples of microbial dispersal limitation have been found in free-living archaea (Whitaker, Grogan, & Taylor, 2003) and bacteria (Martiny et al., 2006), and it is also clear that dispersal limitation affects community composition of both symbiotic (Glassman et al., 2015; Talbot et al., 2014) and nonsymbiotic fungi (Adams, Miletto, Taylor, & Bruns, 2013; Amend, Seifert, Samson, & Bruns, 2010). Microbial symbionts such as mycorrhizal fungi can be affected by host specificity (Hausmann & Hawkes, 2010; Tedersoo, Mett, Ishida, & Bahram, 2013), soil habitat (Dumbrell, Nelson, Helgason, Dytham, & Fitter, 2010; Peay et al., 2015) and large-scale differences in biogeography (Glassman et al., 2015; Talbot et al., 2014). Additionally, evidence for environmental filtering by soil nutrients and pH exists for both fungi (Dumbrell et al., 2010; Erlandson, Savage, Cavender-Bares, & Peay, 2016) and bacteria (Griffiths et al., 2011; Jones et al., 2009). Yet, while it has been understood for over a decade that bacteria are most strongly driven by pH (Fierer & Jackson, 2006), the processes that predominate in structuring symbiotic and free-living fungal communities remain elusive (Rousk, Brookes, & Baath, 2010).

Quantifying the relative influence of niche vs. neutral processes on microbial community composition is a major challenge. Environment and geography are often difficult to disentangle and can inadvertently be conflated at all levels of biological organization (Ferrier, Manion, Elith, & Richardson, 2007; Fitzpatrick & Keller, 2015; Wang, Glor, & Losos, 2013). Moreover, attempting to model nonlinear effects with linear statistical models can lead to weak inferences. However, studies identifying drivers of beta-diversity can overcome this issue by explicitly accounting for both environmental dissimilarity and geographic distance between assemblages (Warren, Cardillo, Rosauer, & Bolnick, 2014). Generalized dissimilarity modelling (GDM; Ferrier et al., 2007), a form of nonlinear matrix regression, is an excellent method for distinguishing between environment and geography because it accounts for nonstationary rates of turnover along environmental gradients (Warren et al., 2014). GDM is thus a powerful new approach to analyse nonlinear effects and to quantify the relative importance of the many factors affecting community composition (Fitzpatrick & Keller, 2015; Fitzpatrick et al., 2013). By combining recent advances in molecular sequencing technologies with GDM, we can now study drivers of turnover in natural, complex microbial communities. For example, the first and only application of GDM to microbes to date helped explain a remarkable 77% of the variation in soil bacterial community composition, with soil properties, particularly pH, explaining the largest proportion of variation (Landesman et al., 2014).

Here, we apply GDM and amplicon metabarcoding to a natural experimental system in a subalpine basin in Yosemite National Park, containing isolated pine tree "islands," to determine whether free-living vs. symbiotic fungal community diversity results primarily from environmental filtering or dispersal limitation. Ectomycorrhizal fungi (EMF), tree root symbionts (Tedersoo et al., 2013) that play essential roles in forest functioning (Read, 1991), are obligately symbiotic with the pine trees but cannot associate with the surrounding herbaceous plants in the basin (Peay, Garbelotto, & Bruns, 2010). The two pine species *Pinus albicaulis* and *P. contorta subsp. murrayana* are the only EMF hosts in the basin in a matrix of nonectomycorrhizal sedges and grasses (Cole, Van Wagtendonk, McClaran, Moore, & McDougald, 2004) and the individual tree "islands" of these two *Pinus* species are interspersed (Figure 1).

This system is ideal for determining the strongest drivers of community composition at the fine scale for both symbiotic and free-living fungi as the islands are discrete patches in which it is possible to fully characterize local soil conditions. We focused explicitly on examining effects of dispersal limitation and edaphic filtering at the fine scale (<1 km) because it is well established that fungal communities are structured by biogeography at the continental scale (Talbot et al., 2014) and evidence exists for fine-scale (<1 km) dispersal limitation in fungi (Adams et al., 2013). Twenty individuals of both *P. albicaulis* and *P. contorta* were selected to represent a range of sizes, ages and distances from each other (pairwise geographic distance) and from the forest edge (forest distance; Figure 1) so that the effect of host could be separated from other potential environmental drivers. We expected that EMF would primarily disperse to the tree islands from the surrounding forest edges while free-living fungi could exist continuously in the soil matrix.

We analysed variation in fungal community composition, as determined by Illumina MiSeq sequencing of Internal Transcribed Spacer (ITS) amplicons (Glassman, Levine, DiRocco, Battles, & Bruns, 2016; Smith & Peay, 2014), and determined whether its strongest predictors were soil properties, pairwise geographic distance, forest distance or tree species, age or size using GDM. We compared total fungal and EMF communities to determine how processes differ in the host-associated subset of microbial symbiont communities vs. the total fungal community. Previous knowledge of fungal community composition (Adams et al., 2013; Talbot et al., 2014) led us to predict that total fungal communities would be most strongly structured by dispersal limitation, followed by edaphic environmental filtering. In contrast, we expected EMF to be structured primarily by host filtering, followed by dispersal limitation. We did not expect edaphic environmental filtering to have a large effect on EMF community turnover at this scale due to expected environmental

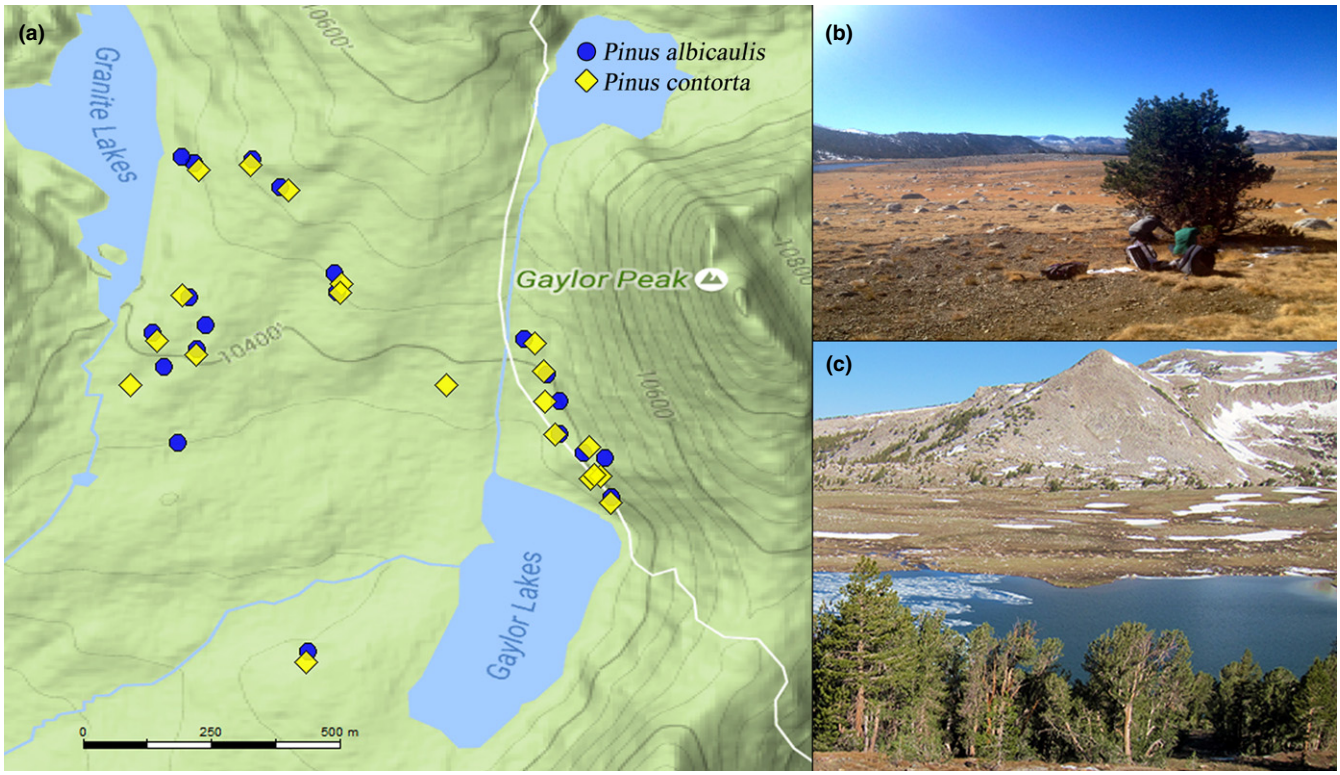

FIGURE 1 (a) Map of isolated trees "islands" of *Pinus albicaulis* (*n* = 20) and *Pinus contorta* (*n* = 20) in Gaylor Lake Basin located in Yosemite National Park, California. (b) Example of an isolated tree "island" in a matrix on nonectomycorrhizal sedges and grasses. (c) View from surrounding forest edge looking down into Gaylor Lake Basin (view from Gaylor Peak in figure a). Isolated tree "islands" are located in the basin but not visible from the picture

homogeneity within the basin. We also hypothesized that both total and EM fungal communities might differ by season based on previous studies showing high rates of fungal turnover before and after snowmelt in alpine plant communities (Schmidt et al., 2007).

2 | MATERIALS AND METHODS

2.1 | Study site and species

We used isolated single pine tree "islands" in Gaylor Lake Basin (37°54′47.4″N; 119°16′07.8″W), located near Tioga Pass, in Yosemite National Park, California, USA. Gaylor Lake Basin was selected due to the presence of two distantly related *Pinus* species that were isolated and intermixed in the subalpine basin (Figure 1). The "islands" consist of isolated individual trees of either *Pinus contorta* or *Pinus albicaulis*, with each pine species occurring approximately in paired distances from each other and the forest edge (Figure 1). Elevation averaged 3,176 m and ranged from 3,147 to 3,200 m. The basin is seasonally wet, with a mean annual precipitation of 64.18 cm that occurs mostly as snow (Klikoff, 1965); late summer is often very dry.

The two pine species *Pinus albicaulis* and *P. contorta subsp. murrayana* are the only EMF hosts in the basin in a matrix of nonectomycorrhizal sedges and grasses, primarily *Carex exserta* (Cole et al., 2004). The trees are members of subgenera *Strobus* and *Pinus*, respectively, which diverged approximately 80–70 mya (Wang, Tank, & Sang, 2000), and each subgenus is known to have specialized EMF associates, particularly in the genera *Suillus* and *Rhizopogon*, which are largely specific to the Pinaceae (Bruns, Bidartondo, & Taylor, 2002; Kretzer, Li, Szaro, & Bruns, 1996; Molina & Trappe, 1982). For example, *Suillus brevipes* is considered to be specific to *Pinus contorta* (Nguyen, Vellinga, Bruns, & Kennedy, 2016). However, species of *Cortinarius* and other Boletales including *S. subalpinus*, *S. sibericus*, *R. evadens* and *Chroogomphus* have been ascribed as *P. albicaulis* associates (Mohatt, Cripps, & Lavin, 2008).

2.2 | Tree selection and sampling

Twenty individuals of both *P. albicaulis* and *P. contorta* (total of 40 single trees) were selected to represent a range of sizes, ages and distances from each other (pairwise geographic distance) and from the forest edge (forest distance) so that the effect of host could be separated from other potential environmental drivers. We expected that EMF would primarily disperse to the tree islands from the surrounding forest edges while nonsymbiotic fungi could exist continuously in the soil matrix. We sampled the fungal community of each tree island in October 2012, before the first snow, and in June 2013, approximately three weeks after snowmelt because we expected high turnover of species with snowmelt (Schmidt et al., 2007). Soils were collected and DNA was extracted with MoBio PowerSoil DNA Isolation Kit (Carlsbad, CA, USA) as described in

Glassman, Lubetkin, Chung, and Bruns (2017). Tree size was estimated as total tree photosynthetic volume, and ages were estimated using a combination of node counts and tree ring analysis as described in Glassman et al. (2017).

2.3 | Soil chemistry

In June 2013, we collected a soil sample from a randomly selected subset of five trees for soil chemistry analysis. In October 2014, after receiving additional funds, a soil sample was collected for each of the 40 trees, to conduct nutrient analysis. A pooled sample per tree was collected by combining ~250 ml of soil collected from approximately the same locations as previous samples. Two days after collections, each of the 40 soil samples was homogenized and sieved in a 2-mm sieve, dried in a fume hood and sent to A&L Western Laboratories, Inc. (Modesto, CA, USA) for analysis (soil test suite S1B, http://www.al-labs-west.com/fee-schedule.php?section=Soil%20Analysis) including ppm of sulphate, ppm of ions of potassium, magnesium, calcium and sodium, soil pH, cation exchange capacity (CEC), hydrogen concentration, organic matter (lbs/A), phosphorous (weak Bray and sodium bicarbonate-P; ppm) and C:N ratio. Sulphate and all nutrient cations were extracted using neutral 1M ammonium acetate: potassium (ppm), magnesium (ppm), calcium (ppm) and sodium (ppm). Soil pH, CEC and hydrogen concentration (in meq 100 per g) were measured on soil solutions. Although we acknowledge a time gap between the collection of the soil nutrient analysis for the 40 trees and the sampling of the DNA for fungal community analysis, the per cent organic matter and soil pH were over 80% correlated for the subset of soils collected in both June 2013 and October 2014.

2.4 | Distance calculations

The location of each tree was determined with a handheld GPS unit and used to calculate distances. As not every tree in the basin was sampled, every stem of each tree species located within a 10-m-radius circle of a focal tree was counted and the sum was used as an isolation index for the tree. Pairwise geographic distance, a matrix of pairwise Euclidean distances (lat lon) between focal trees, was calculated in R (R Core Team 2017). We calculated forest distance in GIS and R (Wang, 2012) to be the distance to the nearest forest edge averaged to all possible forest edges, and we accounted for topographic resistance, or the effect of topographic barriers to dispersal such as elevation changes or lakes, as previously described (Glassman et al., 2017). We visually estimated crown cover, and drew forest polygons by hand using Google Earth imagery in ArcGIS where forest cover exceeded >20% (Fig. S1). This definition is consistent with the Kyoto Protocol definition of 10%–30% minimum crown cover (Lund, 2002).

2.5 | Amplification, Illumina sequencing and bioinformatics

Soil extracts were PCR-amplified using Illumina sequencing primers designed by Smith and Peay (2014) using the ITS1F forward (Gardes & Bruns, 1993) and ITS2 reverse (White, Bruns, Lee, & Taylor, 1990) primer pair to target the ITS1 spacer. The ITS1 spacer is a part of the internal transcribed spacer region (ITS) that serves as the universal DNA barcode for fungi (Schoch et al., 2012). PCR mixtures for amplification contained 0.130 μl of HotStar Taq Plus (5 units/μl) DNA polymerase (Qiagen, Valencia, CA, USA), 2.5 μl of 10 × PCR buffer supplied by manufacturer, 0.50 μl 10 × each dNTPs (200 μM), 0.50 μl each of 10 μM forward and reverse primer and 1 μl of DNA template (diluted to 1:20 to overcome inhibitors), and water up to 25 μl. PCR conditions were as follows: denaturation at 95°C for 5 min; 29–34 amplification cycles of 30 s at 94°C, 30 s at 51°C, 1 min at 72°C; followed by a 10-min final extension at 72°C. Triplicate amplifications for each barcoded sample were pooled, cleaned with AMPure magnetic beads (Beckman Coulter Inc., Brea, CA, USA), quantified fluorescently with the Qubit dsDNA HS kit (Life Technologies Inc., Gaithersburg, MD, USA) and pooled at equimolar concentration. Negative controls of empty MoBio Power soil tubes were also extracted and amplified, and gels were examined for PCR products. Libraries were quality-checked for concentration and amplicon size using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) at the Functional Genomics Laboratory, University of California, Berkeley, CA, USA. Sequencing was performed with Illumina MiSeq PE 2 × 250 at the DNA Technologies Core, UC Davis Genome Center Davis, CA, USA in August 2013.

Illumina data were processed using a combination of the UPARSE (Edgar, 2013) and QIIME (Caporaso et al., 2010) based on the methods of Smith and Peay (2014) and using the same pipelines as previously described (Glassman et al., 2016, 2017). The Illumina MiSeq run of 120 samples yielded 20.6 M reads; 80 of those samples were used for this study. Sequences were then subjected to extensive quality control. The first processing step was to remove distal priming/adapter sites from the ends of reads using cutadapt (Martin, 2011). The remaining untrimmed low-quality regions were trimmed from the ends of reads using Trimmomatic (Bolger, Lohse, & Usadel, 2014). The forward and reverse reads were then paired using the fastq_mergepairs command in usearch/v7 (Edgar, 2013). The sequences that failed to merge the first time were trimmed and cleaned again by employing the homerTools command from the HOMER (Hypergeometric Optimization of Motif EnRichment) module (Heinz et al., 2010). We then used the fastq_mergepairs command in usearch to try to pair the sequences that failed to merge the first time. The trimmed, paired, sequences were then quality-filtered using the fastq_filter command in UPARSE and employing a maximum expected number of errors of 0.15. After quality control, there were 8,256,583 sequences for downstream analyses. Trimmed, paired, high-quality sequences were grouped into operational taxonomic units (OTUs) based on 97% similarity in UPARSE (Edgar, 2013) and taxonomy assignments were made in QIIME based on the UNITE database (Koljalg et al., 2013). Only OTUs that were identified to the Kingdom Fungi were maintained, and after removing all OTUs with no BLAST hit, we were left with 2,788,753 sequences. EMF were separated from the OTU table bioinformatically based on genera determined to be ectomycorrhizal (Tedersoo, May, & Smith,

2010) as detailed in Appendix S1: Methods S1–S3. Sequences were submitted to the National Center for Biotechnology Information Sequence Read Archive under Accession no. SRP079403.

2.6 | Statistical analysis

The primary goal of this study was to disentangle the effects of the different potential environmental drivers of fungal species composition in this system. Specifically, we tested the relative magnitude and contribution of various aspects of geographic distance, soil nutrient environment, tree host and seasonality on fungal community composition. We quantified the pairwise differences between sampled fungal communities by calculating a matrix of Bray–Curtis and Jaccard dissimilarities in species composition using the "VEGAN package" in R (Oksanen et al., 2012). All analyses were conducted on the total fungal OTU table rarefied to 39,721 sequences (n = 40), the lowest common number of sequences per sample, and on the EMF OTU table, rarefied to the bottom 10% quantile of sequencing depth, with four samples removed due to low sequencing yields, and the rest subsampled to an even 647 sequences/sample (n = 36).

To quantify the environmental and geographic drivers of fungal species composition, we employed generalized dissimilarity modelling (GDM), in R using the "GDM" package (Manion, Lisk, Ferrier, Nieto-Lugilde, & Fitzpatrick, 2015). In GDM, predictor variables are first transformed using a series of I-spline basis functions, which are constructed from piece-wise polynomial functions that possess a high degree of smoothness at the places where the polynomial pieces connect (knots), and models are fit using maximum-likelihood estimation (Ferrier et al., 2007). Variables are standardized, so they can be directly compared with one another, and GDM is highly robust to multicollinearity among predictors. In a GDM model, the coefficient for each variable describes the proportion of compositional turnover explained by that variable and is determined by the maximum height of its I-spline (Ferrier et al., 2007; Fitzpatrick et al., 2013). The slope of the I-spline indicates the rate of compositional turnover and how this rate varies at any point along the gradient concerned, while holding all other variables constant (Landesman et al., 2014).

We first constructed variance–covariance matrices to remove highly correlated variables from analysis in the GDM model. For the soil cations, we retained only Na and CEC, due to high correlations among Mg, Ca, K and CEC (Fig. S2). Our primary concern was to disentangle the effect of soil chemistry vs. pairwise geographic distance. We expected soil chemistry to affect fungi and EMF similarly, while forest distance would only affect EMF strongly because the forest is the main source of EMF propagules (Glassman et al., 2017). Soil fungi can exist continuously in the soil matrix and thus should be less affected by forest distance. Pairwise geographic distance was not correlated with OM (Fig. S3a) and was only slightly correlated with pH (r = .23, p < .01; Fig. S3b), and pH and OM were not correlated with each other (Fig. S3c). Of secondary concern was the effect of forest distance as it is mainly expected to affect EMF as a source of propagules. OM was not correlated with forest distance, OM and pH were slightly correlated (r = −.3, p = .06), and pH was correlated with forest distance (r = .52, p < .01; Fig. S4), which possibly drives the changes in pH across the basin (Fig. S5). This pH gradient could be driven by distance from forest edge due to a reduction in acidic pine needle litter with distance from forest edge, or it could be due to the parent material or an underground seep.

We started with the full model that included species, size and age of tree host, pairwise geographic distance, forest distance, isolation index, pH, organic matter (OM), phosphorous (P), sulphur (S), C:N ratio, Na and cation exchange capacity (CEC). To prune this down to a set of predictor variables for each GDM, we used backward elimination (Ferrier et al., 2007), beginning with the full model, removing the variable with the lowest coefficient at each step and calculating the change in the deviance information criterion (DIC). DIC is based on the variance explained by each model in a hierarchical set, penalized by its effective number of parameters. For each GDM analysis, the results included (i) a unique fitted I-spline for each predictor variable describing its relationship with community turnover and (ii) deviance explained by the model (the metric used by GDM to assess model fit).

To disentangle potential correlations among pH and pairwise geographic distance, we ran a GDM fitted with all variables except pH and with all variables except pairwise geographic distance, following a similar approach to Dumbrell et al. (2010). We also ran a similar analysis fitting a multivariate distance-based linear model (DistLM), to compare results with an analogous linear nonparametric model, in Primer 6+ (Anderson, Gorley, & Clarke, 2008), and employed Multiple Matrix Regression Randomization (MMRR; Wang, 2013) on each tree host separately. MMRR performs multiple regression on distance matrices; because pairwise distances are nonindependent, MMRR permutes the rows and columns of the response matrix while holding all others constant to generate a null distribution with which to perform significance testing (Wang, 2013). We employed traditional multivariate linear models in addition to the nonlinear GDM to show that the tests yield similar results although the nonlinear models improve model fit and explain more variance. To complement the multivariate analyses, we used Mantel tests to compare each fungal community dissimilarity matrix with the pairwise geographic distance matrix, used Adonis tests to contrast the fungal communities associated with the two host species and visualized effect of tree host identity on fungal community composition using NMDS. These analyses were conducted with the "VEGAN" package in R (Oksanen et al., 2012).

3 | RESULTS

3.1 | Tree information

Average tree age was 65 ± 33 years (mean ± SD), with no significant difference between *Pinus contorta* (71 ± 31) and *P. albicaulis* (59 ± 34; t test, t = −1.1761, p = .25). Average size, estimated as canopy volume, was 12 ± 14 m^3, with no significant difference between *Pinus contorta* (11 ± 13 m^3) and *P. albicaulis* (14 ± 15; t test, t = 0.63106, p = .53). Forest distance ranged from 308 to

837 m, with no significant differences between *Pinus contorta* (630.5 m) and *P. albicaulis* (628.7 m; *t* test, $t = -0.037919$, $p = .97$). Pairwise geographic distance ranged from 8 to 1073 m. For isolation index, nearly half the sampled trees (18 of 40) had no neighbours within 10 m, with the vast majority (29 of 40) having fewer than five neighbouring stems (of either *Pinus* species) within 10 m. Location, age, size, forest distance and degree of isolation for all 40 trees are summarized in Table S1, and soil chemistry in Table S2.

3.2 | Fungal community richness

After extensive quality control, our Illumina MiSeq run yielded 2.8 M sequences for 80 samples. Because seasonality explained neither total fungal community dissimilarity (Adonis $R^2 = .0036$, $p = .934$; Fig. S6a) nor EMF community dissimilarity (Adonis $R^2 = .0038$, $p = .983$; Fig. S6b), we combined the OTUs from the June and October sampling dates to produce a single sample per tree. We found a total of 3,265 fungal OTUs after rarefying to a depth of 39,721 sequences per sample.

We selected EMF OTUs (238,895 sequences; 96 OTUs), and rarefied to a common sequencing depth of 647 sequences/sample. This yielded 76 EMF OTUs (51 OTUs under *P. albicaulis* and 54 OTUs under *P. contorta)*, with an average of 9 ± 3.7 EMF OTUs per tree. Five of the ten most frequent EMF OTUs belonged to the genus *Rhizopogon*, with the rest belonging to the genera *Cadophora*, *Tricholoma* and *Suillus* (Fig. S7).

3.3 | Effect of dispersal limitation on fungal community composition

Dissimilarity of the total fungal communities increased with geographic distance (Bray–Curtis; Mantel $r = .33$, $p < .01$; Jaccard; Mantel $r = .42$, $p < .01$; Fig. S8a). In contrast, dissimilarity of EMF communities was not correlated with distance (Bray–Curtis; Mantel $r = .07$, $p = .08$; Jaccard; Mantel $r = .0008$, $p = .5$; Fig. S8b). Analysing the two host trees separately yielded similar results for total fungal communities (PA: Bray–Curtis; Mantel $r = .37$, $p < .01$; PC: Bray–Curtis; Mantel $r = .28$, $p < .01$), whereas the effects on the EMF communities differed by host tree (PA: Bray–Curtis; Mantel $r = .096$, $p = .1$; PC: Bray–Curtis; Mantel $r = .25$, $p < .05$; Table S3).

3.4 | Effect of tree host identity on fungal community composition

Tree host identity (*P. contorta* vs *P. albicaulis*) significantly affected EMF community composition (Bray–Curtis; ADONIS $R^2 = .15$, $p < .01$; Fig. S9; Jaccard; ADONIS $R^2 = .13$, $p < .01$) but did not significantly affect total fungal community composition (Bray–Curtis; ADONIS $R^2 = .02$, $p = .46$; Jaccard; ADONIS $R^2 = .02$, $p = 0.58$). The EM fungi identified by the "envfit" function in the VEGAN package to most strongly differentiate between host trees were *Suillus brevipes*, *Rhizopogon salebrosus and Helvella aff. lacunosa* (Fig. S9), with *Suillus brevipes* largely favouring *P. contorta* and *Rhizopogon salebrosus* and *Helvella aff. lacunosa* slightly favouring *P. albicaulis*. *Tricholoma myomyces* was equally common on *P. contorta* and *P. albicaulis* but drove the taxonomic differences in six of the trees (trees PA23, PA27, PA29, PA30, PC15, PC20; Figs S7 and S9b). Those trees are generally in the same geographically clustered, and the mean pH for those size trees is 4.7 ± 0.26 (SD).

3.5 | Disentangling environmental drivers of total fungal community composition

GDM helped disentangle the relative contributions of geographic and environmental drivers of total fungal community composition. Our model was a strong fit to the data and explained a large fraction of the variance in fungal community composition (deviance explained = 0.70; Table 1; Figure 2a). After backward elimination model selection, we retained OM, pH, pairwise geographic distance, P, tree size and cation exchange capacity (CEC) as significant predictors of total fungal community composition (Table 1). The optimal fit includes three I-splines for each predictor, as can be seen by the nonlinear shapes of the curves (Figure 2c). Neither forest distance nor isolation index was retained as significant factors in the model, which was not surprising given that forest distance and isolation index are only expected to affect EMF composition as the forest is only expected to be the main propagule source for EMF. Organic matter explained the largest proportion of fungal community turnover (coefficient = 0.38), closely followed by pH (coefficient = 0.34). Per cent OM ranged from 2.4 to 33.6 among the trees, and pH ranged from 3.8 to 5.1. We saw substantial changes in community compositional turnover at pH differences of approximately 0.6. Pairwise geographic distance, phosphorous, tree size and CEC all explained smaller (coefficients ranging from 0.07 to 0.11) but significant proportions of fungal community compositional turnover (Table 1; Figure 2c).

To deal with potential confounding effects of correlations among pH and pairwise geographic distance (Fig. S3b), we also fitted GDMs with all factors included except pH and with all factors included except pairwise geographic distance. Removing geography as a predictor negligibly affected the results of the model, but removing pH greatly reduced the model's explanatory power (Table S4). To complement our findings from GDM, we ran a linear multivariate regression DistLM (Anderson et al., 2008), which found pH to be the largest driver of fungal community composition (Table S5), but the proportion of explained variance attributable to pH (10.27) is likely an underestimation due to the linear fitting of the model. We were unable to include pairwise geographic distance as a predictor because DistLM does not allow for explanatory variables to be matrices, but we found that pH, OM, P, tree age and CEC all contributed strongly to fungal community composition, with smaller contributions from forest distance, C:N ratio and tree species (Table S5). We also ran multiple matrix regression randomization (MMRR; Wang, 2013), another linear model, on each host tree species, and we found that soil chemistry was the greatest predictor of total fungal community dissimilarity (Table S6). While both linear models

TABLE 1 Results of generalized dissimilarity modelling (GDM) on fungal community composition. Community composition was measured as Bray–Curtis community dissimilarity for either the total fungal or ectomycorrhizal fungal (EMF) community composition. Only predictors that were retained as significant in the backwards elimination model selection are included

All fungi—Best model Predictor	Deviance explained = 0.70 Coefficients
Organic matter	0.38
pH	0.34
Pairwise geographic distance	0.11
Phosphorous	0.11
Tree volume	0.11
Cation exchange capacity	0.07
EMF—Best model Predictor	**Deviance explained = 0.26 Coefficients**
pH	0.61
Cation exchange capacity	0.46
Tree age	0.33
Phosphorous	0.32
Tree species	0.17

found soil chemistry to be the strongest predictor of fungal community compositional turnover, the explanatory power of the MMRR (R^2 = .32 for PA and R^2 = .22 for PC) and DistLM (R^2 = .21) was less than the GDM (vs. deviance explained of 0.70), likely because GDM can account for nonconstant rates of turnover whereas DistLM and MMRR cannot.

3.6 | Disentangling environmental drivers of ectomycorrhizal fungal community composition

In contrast to the total fungal community composition, in which nearly three quarters of the deviance was explained, the models explained approximately one quarter of the deviance in EMF community dissimilarity (deviance explained = 0.26; Table 1; Figure 2b). This is likely due to the reduced variance among trees in their EMF vs. total fungal communities and because many fewer sequences were retained. It is likely that the reduced number of EMF sequences, and thus reduced deviance explained, is an artefact of the fact that we assessed EMF by sequencing the soil rather than sequencing EMF root tips. Nevertheless, despite EMF reads being only a small fraction of the total soil community, they are likely of disproportionate importance to the pine tree hosts than other soil fungal species. The predictors retained in the model explaining EMF compositional turnover included pH, CEC, tree age, P and tree species (Table 1; Figure 2d). By far the largest proportion of EMF compositional turnover was explained by pH (coefficient = 0.61), followed by CEC (0.46), tree age (0.33) and phosphorous (0.32). The optimal fit includes three splines for each predictor, as can be seen by the nonlinear shapes of the curves (Figure 2d). Again, we found that removing pairwise geographic distance negligibly affected the model, but removing pH decreased the explanatory power of the model to 0.16 (Table S4). DistLM explained a similar degree of the variance (R^2 = .24), and retained tree species, pH and Na as the strongest contributors to EMF composition (Table S5). Tree species (9.48) explained a slightly larger proportion of the variance than pH (7.72) and Na (5.98), potentially because pH and Na were underfitted by the linear model due to their nonlinear rates of turnover (nonlinearity of pH demonstrated in Figure 2c,d). Results of the MMRR for EMF for each tree analysed separately were not significant, although soil chemistry had the highest coefficients.

4 | DISCUSSION

To our knowledge, this was the first study to apply GDMs to the study of fungal community composition (Table 1), and it yielded unexpected insight into fine-scale structuring of fungal communities. With traditional multivariate linear models, we accounted for less than half of the variance explained by the GDM for the total fungal community, although similar patterns were detected (Tables S5 and S6). With traditional Mantel and ADONIS tests, we would have concluded that dispersal limitation primarily structured fungal communities (Fig. S8) and host primarily structured EMF communities (Fig. S9). However, by simultaneously accounting for both environment and geography, and allowing for nonconstant rates of turnover, our results explained a large proportion of the deviance for total fungal communities (0.70) and showed that pH, organic matter and soil minerals are the strongest drivers of fungal communities at fine spatial scales.

4.1 | Effects of environmental filtering on fungal community composition

Despite the complexity of the soil microbiome, this study lends clear support to the hypothesis that environmental filtering is the strongest driver of soil microbial communities, even at this fine spatial scale. Previous studies have shown that environmental filtering is significant in shaping both fungal and bacterial communities over broad spatial scales (Prevost-Boure et al., 2014). For fungal communities in particular, strong effects of abiotic filtering have been shown at regional (Kivlin, Winston, Goulden, & Treseder, 2014) and global scales (Tedersoo et al., 2014). Nevertheless, little is known about the effects of environmental filtering at fine spatial scales, and we had predicted a priori that soil environmental heterogeneity would have limited effects on fungi at the fine spatial scale of our study (<1 km). The difference between our prediction and our results may be due to the high heterogeneity of soil in Gaylor Lake Basin.

4.1.1 | Organic matter

The gradient in per cent organic matter (OM) was surprisingly quite large (2.4%-33.6%) across the forty trees in this study and was the largest predictor of total fungal community composition. This range in OM is comparable to the variation in OM in forests across the

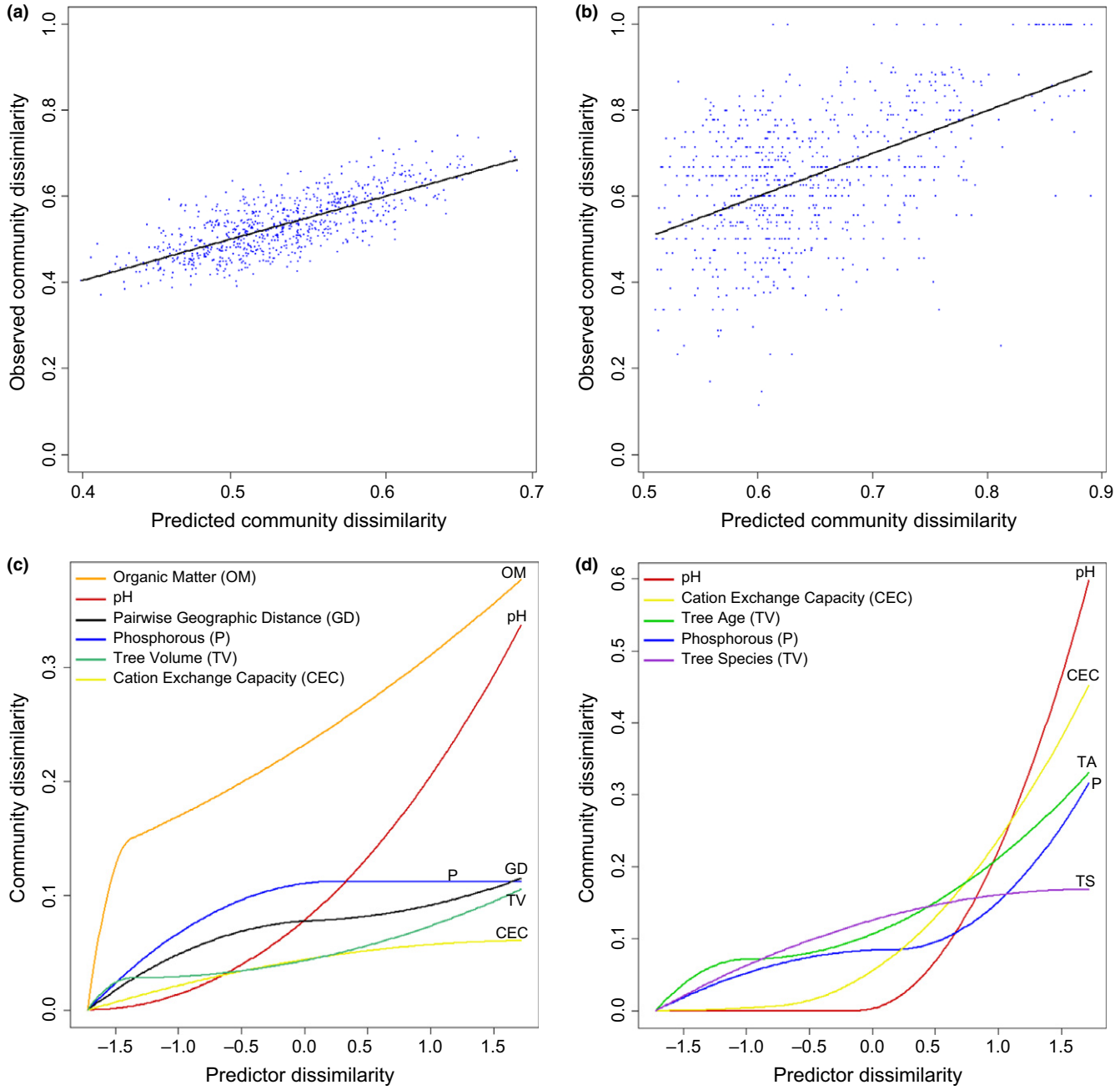

FIGURE 2 Relationships between observed compositional dissimilarity and predicted community dissimilarity between site pairs for (a) the total fungal community and (b) EMF community, based on generalized dissimilarity modelling (GDM) analysis. For GDM-fitted I-splines (partial regression fits) for variables significantly associated with (c) total fungal beta-diversity and (d) ectomycorrhizal fungal (EMF) beta-diversity, the maximum height reached by each curve indicates the total amount of compositional turnover associated with that variable (i.e., its relative contribution to explaining beta-diversity). The shape of each I-spline indicates how the rate of compositional turnover varies with increasing differences in a given predictor variable between sites. Predictor dissimilarity indicates the rates of turnover among trees for each of the predictors indicated in the legend. Only predictors that were retained as significant in the backwards elimination model selection are shown; GD indicates pairwise geographic distance

entire Japanese archipelago (scale >3,000 km); 36 of 38 forest plots from a meta-analysis ranged from 1.7 to 33.9 Mg/ha in OM layer amount (Urakawa et al., 2016). However, the OM range in our study is not as large as in another study at a similar scale, in which variation in per cent OM among seven willow (*Salix*) species ranged from (0.9% to 76.92%) among the sampled trees (Erlandson et al., 2016). In that study, the strong correlation between EMF compositional turnover with OM was attributed to differences in utilization of OM by EMF species, which vary in their ability to produce proteolytic enzymes that are used to scavenge soil OM for nutrients such as nitrogen (Courty, Franc, & Garbaye, 2010; Courty, Pritsch, Schloter, Hartmann, & Garbaye, 2005). Thus, it is likely that variation in per

cent OM can be quite high even at fine spatial scales, and is a significant driver of fungal community compositional turnover due to variation in enzymatic capabilities among fungal taxa.

Regardless of fungal guild, adaptation to soil nutrient environment is a niche process that can affect many species due to the variance in their ability to break down recalcitrant forms of organic matter (Courty et al., 2005; Talbot et al., 2013). Although we do not know the range of OM substrates available at this site, it is possible that the strong effect of OM on fungal turnover could be due to intraspecific differences among fungal species in their ability to compete with bacteria across a range of OM abundance and substrates (Rousk, Brookes, & Baath, 2010). Ecological theory predicts that high spatial and environmental heterogeneity will lead to high diversity and promote species coexistence in plants (Amarasekare, 2003). It is also clear that environmental heterogeneity correlates with high diversity (3,265 fungal OTUs in this study) and high compositional turnover among fungal communities in this system.

4.1.2 | Seasonality

Contrary to our expectations, seasonality did not have a significant effect on either total or EMF community turnover (Fig. S6). Although high fungal turnover has previously been reported before and after snow in alpine environments (Schmidt et al., 2007), we now know that temporal and seasonal changes are less likely to be found when samples are more deeply sequenced (Caporaso, Paszkiewicz, Field, Knight, & Gilbert, 2012). Indeed, we expect that the lack of changes in fungal communities before and after snowmelt may simply be due to relic DNA (Carini et al., 2017), which may be especially well preserved in this subalpine environment where soil is under snow many months of the year. As the summer is quite dry in Gaylor Lake Basin (Klikoff, 1965), it may also be that fungal DNA turnover is also slow over the summer where lack of moisture limits the production of fungal fruiting bodies which require water to be produced.

4.1.3 | pH

Perhaps most broadly unifying across many levels of biological organization was the result that pH was the second strongest driver of total fungal communities and the single largest driver of EMF turnover. At much broader spatial scales and wider pH ranges, previous work identified pH as the most important predictor of soil bacterial communities (Fierer, 2017; Fierer & Jackson, 2006; Landesman et al., 2014) and of fungi at a global scale (Prober et al., 2015; Tedersoo et al., 2014). The strong correlation between pH and EMF community in this study (coefficient = 0.61; Table 1) is impressive because the pH differences in our study area (3.8–5.1) are not as large as in other studies that attributed strong correlations in microbial turnover to very large ranges in pH values (from pH 4 to pH >8) (Dumbrell et al., 2010; Fierer & Jackson, 2006; Griffiths et al., 2011; Lauber, Hamady, Knight, & Fierer, 2009; Rousk, Baath, et al., 2010).

Thus, our study now contributes to a growing literature showing that pH can strongly shape ectomycorrhizal (Ge, Brenneman, Bonito, & Smith, 2017; Hung & Trappe, 1983; Kjoller & Clemmensen, 2009) and total soil fungal communities (Tedersoo et al., 2014). In particular, pH is thought to have a strong effect on a group of fungi in the ascomycete order Pezizales (Ge et al., 2017; Kluber et al., 2012). However, as our study was largely dominated by fungi in the phylum Basidiomycota with a marked lack of Ascomycota in the Pezizales (Fig. S7), we show that pH has a strong effect on a broader array of fungal phylogenetic groups than previously thought.

There is a rich literature examining the effects of pH on plant (Craine, 2009) and aquatic systems (Tilman, Kilham, & Kilham, 1982). In addition, for at least a decade, it has been part of the dogma of microbial ecology that pH is the strongest driver of bacterial communities (Fierer, 2017; Fierer & Jackson, 2006) while its impacts on fungi are thought to be weaker (Barberan et al., 2015; Rousk, Baath, et al., 2010). Yet, it now appears that pH is a unifying factor strongly shaping the community structure of plants, bacteria and fungi, regardless of spatial scale. We propose that this is due to the fact that pH is an excellent integrator of soil nutrient availability. Both anion and cation exchange capacity, the ability of negatively and positively charged materials in soils to hold charged ions, are directly affected by pH (Sylvia, Hartel, Fuhrman, & Zuberer, 2005). Phosphorous availability is particularly affected by pH and becomes less biologically available with decreases in soil pH (Kluber et al., 2012; Thomas & Hargrove, 1984). In our study, pH ranged from 3.8 to 5.1 (Table S2); this corresponds to a 20 × difference in hydrogen ion concentration and would affect the availability of all soil nutrients. The dominant effect of pH is extremely powerful because if plants, bacteria and fungi all respond to the same primary environmental cues, then that greatly simplifies and increases our power to model and understand their distributions and predict community responses to environmental change.

4.1.4 | Host

We had expected EMF assemblages to be primarily structured by tree host species and for edaphic environmental filtering to be weak at this fine spatial scale. Although EMF communities did exhibit some significant differentiation among tree hosts (Fig. S9), they were primarily structured by pH, soil minerals and tree age, with host identity as the weakest significant predictor of community composition (Table 1). Thus, our expectation that host-dependent EMF communities would differ between different tree species was correct, but the effect was modest (GDM coefficient = 0.17) compared to pH (0.61) and CEC (0.46; Table 1). High tree host specificity of EMF taxa is exhibited in western Amazonia (Tedersoo, Sadam, Zambrano, Valencia, & Bahram, 2010), among wooded savannas in Africa (Tedersoo et al., 2011), and in Tasmania (Tedersoo et al., 2008). In general, it appears that the degree of host specificity is correlated with phylogenetic distances between hosts for EMF symbionts (Tedersoo et al., 2013). Thus, it is likely that plant host effects would have been stronger if more phylogenetically distantly related hosts were tested (Tedersoo et al., 2016).

However, despite the fact that our hosts were congeneric, they are distantly related within the genus (Wang et al., 2000), and certain EMF genera did show distinct host preferences (Fig. S9). For instance, *Suillus brevipes* showed a strong preference for *P. contorta*, which is supported by the literature (Nguyen et al., 2016), while the occurrence of *Rhizopogon salebrosus* decreased under *P. contorta* (Fig. S9), which was unexpected. Both the linear (Table S5) and non-linear models (Table 1) found a significant effect of both tree host and pH despite the hosts belonging to the same genus. Pinaceae is an ancient plant family (approximately 140 mya) with many phylogenetically distant genera (Wang et al., 2000), and the split between the *Strobus* and *Pinus* subgenera of *Pinus* is estimated to be approximately 80 mya (Wang et al., 2000). Indeed, ectomycorrhizal associates of the two pine subgenera have been known to exhibit host specificity for decades (Molina & Trappe, 1982), but the importance of host relative to other possible factors structuring EMF assemblages was unknown. We thus show that although host specificity can be an important factor structuring symbiotic fungal communities (Fig. S9), it is simply not as large of a factor as local soil environmental parameters in this system where the host gradient is binary and based on distantly related congeneric species. In other cases of less phylogenetically distinct hosts, such as among seven willow (*Salix)* species (Erlandson et al., 2016), or within the hard pines (subgenus *Pinus*)(Glassman et al., 2015), the effect of host relative to the effects of soil chemistry and biogeography is undetectable.

4.2 | Effects of geographic distance on fungal community composition

We expected dispersal limitation (i.e., geographic distance) to be the strongest predictor of total fungal communities and to be a significant predictor of EMF assemblages, but in fact it was only the third largest predictor of total fungal community turnover after organic matter and pH, and did not contribute significantly to EMF community composition in this system (Table 1). Studies of fungal composition across North America (Talbot et al., 2014) and bacterial composition across France (Ranjard et al., 2013) both found significant distance decay in microbial communities across broad spatial scales. Distance decay has also been exhibited among fungal communities at fine spatial scales very similar to those in our study (Adams et al., 2013; Peay et al., 2010), and decreasing richness of EMF taxa with distance from forest edge was found at this study site (Glassman et al., 2017). Thus, the smaller effect of both pairwise geographic distance and forest distance on soil fungal and EMF beta-diversity in this system was unexpected.

It is possible that the autecology of EMF dominating the trees in this system indicates that more dispersal limitation is occurring in this system than we can detect with statistical tests. We note that the EMF genus dominating the isolated tree "islands" in this system is *Rhizopogon*, an EMF that produces hypogeous fruiting bodies which are an adaptation to drought (Bruns, Fogel, White, & Palmer, 1989). *Rhizopogon* are dispersed by small mammals (Frank et al., 2009), which are prevalent at high elevations in Yosemite National Park (Moritz et al., 2008). While Gaylor Lake Basin is not a desert, it is a seasonally dry habitat, with late summers typically being very dry, and most precipitation is received as snow rather than rain due to its high elevation (Klikoff, 1965). Thus, it is possible that EMF that disperse primarily by wind by making epigeous mushrooms are outcompeted by hypogeous fungi dispersed primarily by mycophagy because epigeous mushrooms require several days of rain in order to fruit. Members of the *Rhizopogon* genus are also known for their long-lived spores (Bruns et al., 2009), which allow them to maintain a large, viable spore bank. The fact that fungal communities did not differ by season (Fig. S6), which was contrary to our initial expectations, indicates a strong legacy effect and suggests that tree island communities might have been colonized from a persistent spore bank (Glassman et al., 2015) that is frequently replenished by small mammal activity. Moreover, *Rhizopogon* is known to form long-distance exploration types of mycelium (Agerer, 2001), which could potentially enable it to move more rapidly to the isolated trees in the basin than other EMF taxa. Thus, the lack of a signal of distance decay among EMF in this system could be due to the harshness of the high elevation setting, in which lack of precipitation as rain prevents the abundant growth of epigeous mushrooms that disperse spores via wind.

5 | CONCLUSIONS

Similar to macro-organisms (Gravel et al., 2006), both niche and neutral processes significantly contribute to community compositional turnover in fungi. Yet, our results provide strong evidence that environmental filtering, instead of dispersal limitation, plays a dominant role in structuring both free-living and symbiotic fungal beta-diversity at fine spatial scales. Moreover, by quantifying the effects of individual environmental variables, we found that organic matter and pH primarily drive environmental filtering in total soil fungal communities and that, surprisingly, host specificity was not the largest factor affecting EMF beta-diversity. Our findings support an emerging paradigm that pH may be a master switch in all soil-mediated systems, including fungi (Ge et al., 2017; Kjoller & Clemmensen, 2009), in addition to bacteria (Fierer, 2017; Fierer & Jackson, 2006; Landesman et al., 2014) and plants (Craine, 2009). Important future directions include determining which groups specifically respond to changes in pH and organic matter, and better understanding the mechanisms by which pH and organic matter affect fungal communities.

ACKNOWLEDGEMENTS

Thanks to JA Chung, L Boddy, A Carver, L Forero, A DiRocco and NH Nguyen for help with fieldwork. Thanks to JA Chung for assistance in setting up the Illumina MiSeq library, and to KG Peay and D Smith for providing primers, molecular protocols and bioinformatics assistance. Thanks to JJ Battles, EL Simms, L. Tedersoo and seven anonymous reviewers for providing helpful discussions and comments on the manuscript. Thanks to Yosemite National Park for

allowing us to sample, to K. Lubetkin and the Battles laboratory at UC Berkeley for use of cross-validation equipment and to the University of Arizona Laboratory of Tree Ring Research for curating the tree cores. SIG was supported by the National Science Foundation Graduate Research Fellowship Program. This project was funded by the National Science Foundation Doctoral Dissertation Improvement Grant to SIG and TDB (Award: 1403215) and an NSF Dimensions of Biodiversity Grant to TDB (Award 1046115).

AUTHOR CONTRIBUTION

S.I.G. and T.D.B. designed the study, S.I.G. collected the data, S.I.G. performed the molecular work and bioinformatics, and S.I.G. and I.J.W. analysed the data and made the figures. S.I.G. wrote the first draft of the manuscript and all authors contributed substantially to revisions.

DATA ACCESSIBILITY

Sequences were submitted to the National Center for Biotechnology Information Sequence Read Archive under Accession no. SRP079403. All ecological data are included in the supplements and are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.q0rv9.

ORCID

Sydney I. Glassman http://orcid.org/0000-0001-9115-3026

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Glassman SI, Wang IJ, Bruns TD. Environmental filtering by pH and soil nutrients drives community assembly in fungi at fine spatial scales. *Mol Ecol*. 2017;00:1–14. https://doi.org/10.1111/mec.14414