

Good neighbors aplenty: fungal endophytes rarely exhibit competitive exclusion patterns across a span of woody habitats

MARISSA R. LEE,^{1,7} JEFF R. POWELL,² BRAD OBERLE,³ WILLIAM K. CORNWELL,⁴ MITCHELL LYONS,⁵ JESSICA L. RIGG,^{2,6} AND AMY E. ZANNE¹

¹*Department of Biological Sciences, The George Washington University, Washington, D.C. 20052 USA*
²*Hawkesbury Institute for the Environment, Western Sydney University, Penrith, New South Wales 2751 Australia*

³*Division of Natural Sciences, New College of Florida, Sarasota, Florida 34243 USA*

⁴*School of Biological, Earth & Environmental Sciences, Ecology and Evolution Research Centre, UNSW Australia, Sydney, New South Wales 2052 Australia*

⁵*School of Biological, Earth & Environmental Sciences, Centre for Ecosystem Science, UNSW Australia, Sydney, New South Wales 2052 Australia*

⁶*NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Woodbridge Road, Meanangle, New South Wales 2568 Australia*

Citation: Lee, M. R., J. R. Powell, B. Oberle, W. K. Cornwell, M. Lyons, J. L. Rigg, and A. E. Zanne. 2019. Good neighbors aplenty: fungal endophytes rarely exhibit competitive exclusion patterns across a span of woody habitats. *Ecology* 100(9):e02790. 10.1002/ecy.2790

Abstract. Environmental forces and biotic interactions, both positive and negative, structure ecological communities, but their relative roles remain obscure despite strong theory. For instance, ecologically similar species, based on the principle of limiting similarity, are expected to be most competitive and show negative interactions. Specious communities that assemble along broad environmental gradients afford the most power to test theory, but the communities often are difficult to quantify. Microbes, specifically fungal endophytes of wood, are especially suited for testing community assembly theory because they are relatively easy to sample across a comprehensive range of environmental space with clear axes of variation. Moreover, endophytes mediate key forest carbon cycle processes, and although saprophytic fungi from dead wood typically compete, endophytic fungi in living wood may enhance success through cooperative symbioses. To classify interactions within endophyte communities, we analyzed fungal DNA barcode variation across 22 woody plant species growing in woodlands near Richmond, New South Wales, Australia. We estimated the response of endophytes to the measured wood environment (i.e., 11 anatomical and chemical wood traits) and each other using latent-variable models and identified recurrent communities across wood environments using model-based classification. We used this information to evaluate whether (1) co-occurrence patterns are consistent with strong competitive exclusion, and (2) a priori classifications by trophic mode and phylum distinguish taxa that are more likely to have positive vs. negative associations under the principle of limiting similarity. Fungal endophytes were diverse (mean = 140 taxa/sample), with differences in community composition structured by wood traits. Variation in wood water content and carbon concentration were associated with especially large community shifts. Surprisingly, after accounting for wood traits, fungal species were still more than three times more likely to have positive than negative co-occurrence patterns. That is, patterns consistent with strong competitive exclusion were rare, and positive interactions among fungal endophytes were more common than expected. Confirming the frequency of positive vs. negative interactions among fungal taxa requires experimental tests, and our findings establish clear paths for further study. Evidence to date intriguingly suggests that, across a wide range of wood traits, cooperation may outweigh combat for these fungi.

Key words: coexistence; competition; facilitation; fungal endophytes; latent variable models; plant traits; species interactions; woody plants.

INTRODUCTION

Community assembly and stability arise from species' responses to each other and their environment (Weiher

et al. 2011, Ovaskainen et al. 2017). Ecologists have historically focused on how communities are organized by negative species interactions such as competition, in which one species increases its abundance at the expense of another (Connell and Slatyer 1977, Tilman 1982, Connell 1983). However, the importance of positive interactions (e.g., facilitation in which both species' abundances increase in each other's presence) in shaping communities has received mounting empirical and

Manuscript received 27 May 2018; revised 14 April 2019; accepted 6 May 2019. Corresponding Editor: Samantha Chapman.

⁷E-mail: marissaruthlee@gmail.com

theoretical evidence from across organisms and ecosystems (McIntire and Fajardo 2013, Zelezniak et al. 2015, Li et al. 2018). Specifically, the relative balance of positive and negative interactions in structuring diverse ecological communities remains an open question.

Much of our knowledge of how positive and negative species interactions influence assembly outcomes in complex communities is based on relatively simple assessments of pairwise interactions. However, these species usually represent only a subset of those in the community, chosen based on criteria that may limit the applicability of the results for predicting processes that emerge from species interactions such as community assembly. In particular, diverse communities, including tropical forests, coral reefs, and those of host-associated microbes are simply too specious, with interactions too complex to build an ecological understanding solely from experiments on species pairs. As such, there is an important role for observational data combined with models. These observational data can be used to identify signatures of ecological processes in highly complex communities that guide further inquiry with fine-scale manipulations. Inferring ecological processes from community patterns has a “checkered” history (Diamond 1975, Gotelli and McCabe 2002, Connor et al. 2013) because of an inability to disentangle environmental drivers of species abundance from biotic interactions. However, modern approaches that account for measured environmental gradients in co-occurrence pattern analyses offer an important view into a complex set of interactions. This has proven useful in tropical forests (Leach et al. 2018) and coral reefs (Brown and Hamilton 2018), and is now providing new insights into microbial communities (Björk et al. 2018, Maynard et al. 2018).

Fungal endophytes are fungi that reside inside healthy plant tissue with potentially far-reaching ecological impacts on terrestrial carbon storage based on their abilities to mediate plant health (Sieber 2007) and modify determinants of decay prior to plant death (Griffith and Boddy 1990, Parfitt et al. 2010, Song et al. 2016). Wood fungal endophytes, like many complex communities, are specious and occupy a broad range of environmental habitats, making them useful for studying the relative balance of positive and negative interactions in structuring diverse ecological communities. More specifically, these endophytes have access to many wood species in diverse forested systems. Among fungal endophyte taxa, host specificity and environmental tolerances can vary widely (Arnold 2007, U'Ren et al. 2012), and many have special adaptations to survive within their plant host (Friesen et al. 2008, Hamilton et al. 2012). Interactions among endophytes are rarely examined (Combès et al. 2012, Rodriguez Estrada et al. 2012), and no specific information exists regarding *in situ* interactions in woody tissue.

In order for species to interact, they must first be able to survive in a common environment, i.e., tolerate abiotic conditions and extract resources. For wood-dwelling fungal endophytes, wood traits—both the physical and

chemical characteristic of the wood—make up the environment that they experience; yet the specific physical and chemical traits of woody hosts to which fungal endophyte taxa are sensitive are not well known. As a barrier to the outer environment, bark thickness may filter endophyte colonization. Once beyond the physical barrier of the outer bark, endophytes must tolerate stressful abiotic conditions, including high water content that may limit gas exchange (Chapela and Boddy 1988), as well as biotic challenges including host plant defenses (Boller and Felix 2009). Nutrition is another challenge, because wood is typically low in macro- and micronutrient concentrations (Cornwell et al. 2009), and many compounds (e.g., cellulose and lignin) require specialized enzymes to digest (Parrent et al. 2009, Eastwood et al. 2011). Moreover, the density of the wood substrate may limit the ability of fungi to access resources. Although relatively little is known regarding interactions among endophytes, we can use fungi that occupy a similar habitat to wood endophytes, that is, saprotrophic fungi in decaying wood, to establish expectations about the direction and magnitude of species interactions. These taxa exhibit a diverse range of species interactions that include mycoparasitism (Boddy 2000), facilitation (Heilmann-Clausen and Boddy 2005, Tiunov and Scheu 2005), and, frequently, negative interactions. Direct combat and territoriality often result in the competitive exclusion of a subdominant or late-arriving taxon (Boddy 2000).

Classifications of fungi based on evolutionary history and resource acquisition (e.g., trophic mode: pathogen, symbiont, latent saprotroph) may aid in predicting success of fungal taxa in different wood environments and in the presence of other taxa. Some wood traits that are relevant to endophyte communities may be difficult to measure directly, for example, wood secondary metabolites, but their effects may correlate with plant phylogeny. Closely related wood species within clades may share commonly inherited interactions with their fungal residents (Petrini 1996, Ahlholm et al. 2002). Likewise, the success of fungal taxa in these wood environments may depend on fungal evolutionary history (Eichlerová et al. 2015). The principle of limiting similarity (Abrams 1983, 1996) predicts that taxa with similar traits are more likely to engage in negative interactions and lead to competitive exclusion. As such, taxa with similar evolutionary history or that belong to the same guild are less likely to coexist. Insight into the balance of positive and negative interactions among wood endophytes will bring us closer to understanding how communities are assembled in this system.

One very recent statistical advance for inferring the direction and magnitude of species interactions from observational data first partitions taxa responses to measured environmental variables and then uses latent variables to describe taxa responses to unexplained environmental gradients that include biotic interactions (Fig. 1, Hui et al. 2015, Warton et al. 2015). Similar

approaches have been used to characterize fungal communities previously (Ovaskainen et al. 2010, 2017, Maynard et al. 2018); however, to our knowledge, this is the first time that this technique has been used to infer processes that underlie community structure, such as competitive exclusion and coexistence in a microbial system. Microbial systems such as these are ideal for exploring community structuring with these models, as the variance explained by species interactions is constrained by the effects of environmental filtering. Because we are able to characterize so many of the key environmental gradients experienced by fungal endophytes (i.e., a multitude of physical and chemical traits of wood, a relatively closed environment), species' residual correlations represent a best approximation of the strength and direction of biotic interactions among taxa. We have two key expectations: Based on the principle of limiting similarity (Abrams 1983, 1996), we expect taxa responses to a

common, measured wood environment (hereafter “shared environment”) to be uncorrelated or negatively correlated (i.e., Fig. 1, Ex. 1, Habitat differences). We also expect residual taxa correlations to be primarily negative, having a distribution that supports a “competitive exclusion pattern” rather than a “coexistence pattern” (Fig. 1, Ex. 2 and 3) because fungi isolated from decaying wood generally exhibit combative behavior. Our pattern labels of “competitive exclusion” and “coexistence” rest on the assumptions that (1) spatial segregation, after accounting for environmental variables, is indicative of negative species interactions (e.g., Fuhrman et al. 2015, Rajala et al. 2019) and (2) negative interactions lead to competitive exclusion and positive interactions lead to species' coexistence over the long term (Gause 1934).

In this study, we combine environmental microbial sequencing and emerging statistical analyses to

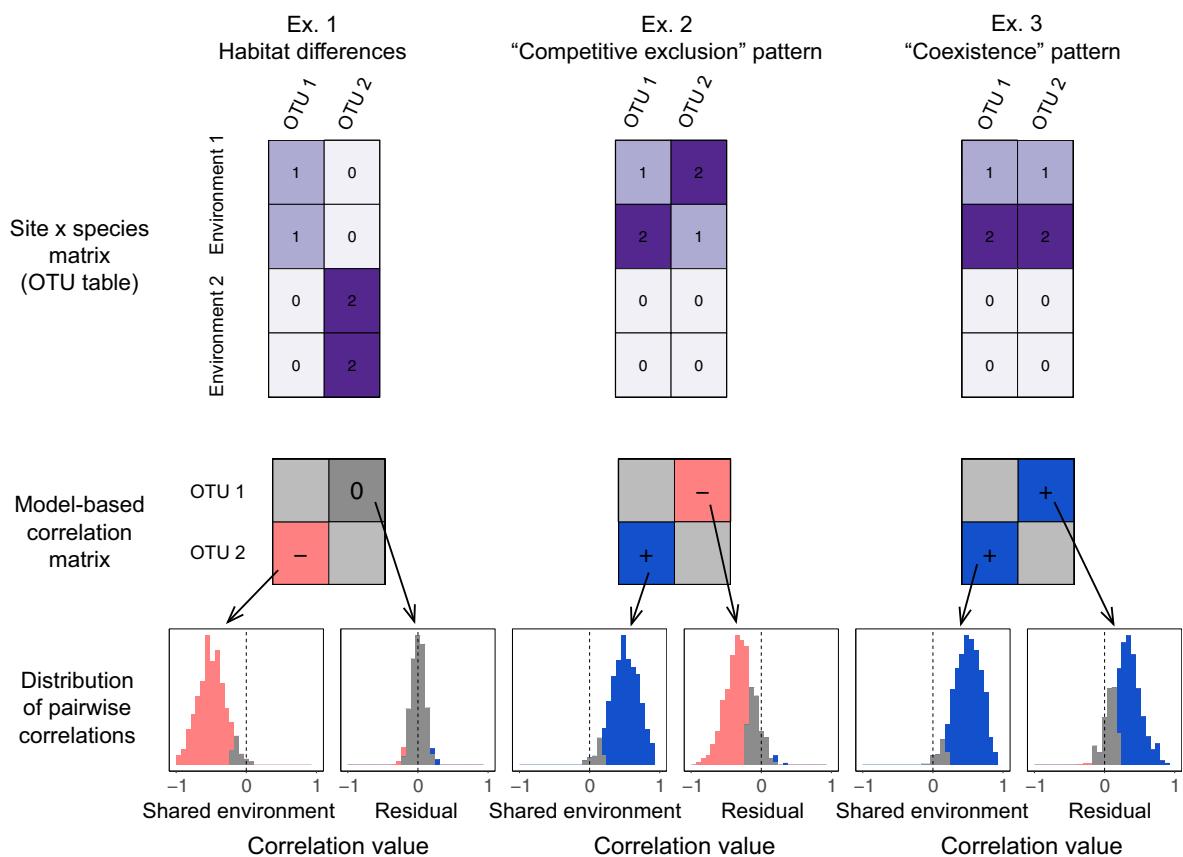


FIG. 1. Emerging analyses can be used to infer the signature of measured environmental variables and unmeasured drivers such as species interactions on community assemblages (Hui et al. 2015, Warton et al. 2015). Taxa-specific responses can be inferred from an operational taxonomic unit (OTU) table annotated with environmental metadata (i.e., Environments 1 and 2) and used to create model-based correlation matrices that summarize correlation in OTU responses based on “shared environment” and “residual” responses (displayed in lower and upper triangle of matrix, respectively). The distribution of pairwise “shared environment” correlations between all OTUs in the study system provides insight into the role of environment filtering in reducing (e.g., Ex. 1) or promoting (e.g., Ex. 2 and 3) species co-occurrence. Because OTU interactions are unmeasured, the distributions of pairwise “residual” correlations can indicate the strength and direction of OTU interactions in the study system, especially if key environmental variables have been measured and included as predictors. The predominance of negative vs. positive interactions leads to either a “competitive exclusion” (Ex. 2) or “coexistence” (Ex. 3) pattern, respectively.

understand the community assembly of wood-dwelling fungal endophytes across a wide range of wood traits from tree species found in woodlands near Richmond, New South Wales (NSW), Australia. We ask: (1) how do fungal taxa partition environmental niche space, and do residual taxa associations suggest predominantly a “competitive exclusion”—or “coexistence”—type pattern? We also (2) evaluate the ability of a priori classifications, such as trophic mode and phylum, to explain variation in fungal responses to their environment and each other with the expectation that shared trophic modes and clades will lead to higher instances of competitive exclusion. We (3) infer how communities of fungi, or consortia, are distributed using model-based classification. Last, we (4) evaluate the extent to which dissimilarities between fungal communities can be explained by wood functional and/or phylogenetic distance with the expectation that more similar communities are found in more similar wood.

METHODS

Wood sampling

Stems of 22 tree species were collected in 2013 from Agnes Banks Nature Reserve, Castlereagh Nature Reserve, and the Cumberland Plain SuperSite of the Terrestrial Ecosystem Research Network, all located in the Hawkesbury region of Sydney, NSW, Australia. Live stems from at least three individuals of each species were collected and divided into two stem-diameter classes: 1–2 cm (small size class; all species except *Eucalyptus seleirophylla*) and 5–9 cm (large size class; 12 species). Species that were not represented in the large size class do not naturally produce stems of that diameter size (Appendix S1: Fig. S1).

Sawdust was generated from three replicate stems each for both small and large wood samples and stored at -20°C until processing for DNA extraction and chemical analysis (see below). Shavings from each of the three stems were kept separate in subsequent analyses ($n = 3$). However, it was necessary to perform chemical analysis on a composite sample of all three small stems for each species because of limited sample volumes ($n = 1$). To assess wood density and bark thickness, three small 10-cm-length stem segments for each tree species were used (see below). To assess wood water content, at least four additional small and large stem segments for each species were used (see below).

Wood trait data collection

Elemental concentrations were measured on 150 mg of dried, milled wood (Appendix S2: Wood trait data collection). Carbon (C) and nitrogen (N) concentrations were measured using a TruMac CN Macro analyzer (Leco, St. Joseph, Michigan, USA). Concentrations of other elements (P, K, Ca, Mn, Fe, Zn) were measured

using an Epsilon 3^X X-ray fluorescence (XRF) spectrometer (PANalytical, Sydney, Australia). Other wood traits, including bark thickness, water content, and density, were measured within 2 weeks of stem collections, using methods described in Pérez-Harguindeguy et al. (2013) and Osazuwa-Peters and Zanne (2011; Appendix S2: Wood trait data collection).

DNA extraction, sequencing, and data processing

DNA was extracted from 60 mg of wood shavings using a modified CTAB DNA extraction protocol (Doyle and Doyle 1987, Soltis et al. 1991; Appendix S2: Modified CTAB DNA extraction protocol). DNA samples were submitted to the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, NSW, Australia). Amplicons to identify fungal taxa were generated using fITS7 (5'-GTGARTCATCGAATCTTG-3'; Ihrmark et al. 2012) and ITS4 (5'-TCCTCCGCTTATT GATATGC-3'; White et al. 1990), purified using the Agencourt AMPure XP system (Beckman Coulter, Lane Cove, NSW, Australia), and genomic libraries were prepared with the use of the Nextera XT Index Kit (Illumina, San Diego, California, USA). Paired-end (2×251 bases) sequencing was performed on the Illumina MiSeq platform.

To process the DNA sequencing data, we used the approach described by Bissett et al. (2016) with a few modifications (Appendix S2: OTU picking and mapping). Operational taxonomic unit (OTU) richness per sampling effort was evaluated by plotting rarefaction curves for each sample (using the R package *vegan*; version 2.4-1 Oksanen et al. 2015). Putative taxonomic identities for fungal OTUs were generated using the “classify.seqs” command in *mothur* on representative sequences for each OTU, using a reference database of fungal ITS sequences and taxonomic annotations obtained from UNITE (version 7.0; Abarenkov et al. 2010). Trophic modes of OTUs that were assigned to taxa were then inferred using FUNGuild (version 1.0; Nguyen et al. 2016). Trophic mode assignments should be considered with caution, because they are primarily based on taxa observed outside of the wood endophyte system. Nevertheless, the assignments formalize what we do know about taxa (and their close relatives) identified in our samples and allow us to test whether this information can explain observed co-occurrences.

Redundancy analysis and regressions for multivariate data

To determine which wood traits were important for characterizing fungal community composition, we initially conducted a distance-based redundancy analysis in the R package *vegan* on the fungal taxa abundance matrix using Bray-Curtis distances. We used stem size class and 11 wood species traits (density, water percent, bark thickness, C, N, P, Ca, Fe, K, Mn, Zn) to build models and perform model selection in a stepwise search

with permutation tests in the constrained ordination (“ordistep” in the R package *vegan*). Traits that were identified in the best model were investigated in more detail with the following analyses.

To estimate the multivariate response of fungal taxa to individual wood traits, we used an approach that involves fitting a single negative binomial regression model to each response variable (OTUs) with a common set of predictors (Wang et al. 2012). In contrast to common multivariate analyses (e.g., the redundancy analysis previously described), this approach improves statistical power and accounts for the mean–variance relationship in abundance data (Wang et al. 2012, Warton et al. 2012). To determine which traits most affect fungal taxa, we contrasted a base model that included stem size as a predictor variable against models with stem size in addition to 1 of the 11 candidate wood traits. Model quality was calculated by summing Akaike information criteria (AIC) across generalized linear models corresponding to each species in the community as in Lyons et al. (2016) such that the lower the “sum-of-AIC” value, the better overall model. Candidate models with lower “sum-of-AIC” relative to the base model were used to identify key traits. This analysis was implemented using the R package *mvabund* (version 3.11.9; Wang et al. 2012).

Correlated response model

To examine drivers of fungal co-occurrence, we used a type of generalized latent-variable model that we refer to as a correlated response model (Hui 2016). The correlated response model fits a generalized linear model to each OTU, but additionally deals with the multivariate nature of the data by including latent-variable predictors to deal with residual correlation between OTUs (Hui 2016; Appendix S2: Correlated response model). We parameterized the model to include size class and 11 wood traits as predictors, two latent variables (to mimic a traditional 2D ordination), and sample identity as a random effect. We used a negative binomial error structure to deal with the overdispersed OTU counts. The model was fit with the R package *boral* (Hui 2016), which uses Bayesian Markov chain Monte Carlo sampling for estimation (see Appendix S2: Correlated response model for details on prior specification and parameterization). The same model was run 12 times with different seed values to ensure repeatability of results. We evaluated parameter estimate convergence using Holm-adjusted Gekewe diagnostics (Hui 2016; Appendix S1: Fig. S2).

The key parameter estimates that result from this model are OTU-specific coefficients associated with each environmental predictor variable as well as the latent variables. The environmental variable coefficients allow quantification of shared environmental responses among OTUs, and the latent-variable coefficients allow quantification of shared residual correlations (Fig. 1, Model-based correlation matrix; Hui 2016) and used histograms to display the distribution of correlations values due to the large matrix

size (Fig. 1, Distribution of pairwise correlations). Correlations are considered significantly positive or negative if the 95% credible interval does not include zero. The distribution of shared environment correlations indicates the role of environment filtering (via wood trait predictors) in reducing (Fig. 1, Ex. 1) or promoting (Fig. 1, Ex. 2 and Ex. 3) species co-occurrence. The distribution of residual correlations, on the other hand, hint at the frequency and strength of negative and positive OTU interactions that collectively form either a “competitive exclusion” or “coexistence” pattern, respectively (Fig. 1).

To evaluate whether OTUs within or between a priori group classifications (trophic mode and phyla) are more frequently correlated in how they respond to their environment, we determined the frequency of significant positive and negative shared environment and residual correlations within and between groups. Wood-associated saprotrophs in the phylum Basidiomycota tend to have more extensive wood-degrading abilities than Ascomycota (Eichlerová et al. 2015). Differences in saprotrophic abilities between these phyla do not directly translate to functional differences in an endophyte context, but since many of these taxa are latently present we hypothesized that phyla identity may inform co-occurrence patterns. Only OTUs that were (1) classified by FUNGuild to a trophic mode as “Probable” or “Highly Probable”, and (2) classified as either “saprotroph” or “pathotroph” were used; for example we excluded OTUs with a “saprotroph–pathotroph” assignment (45 of 7,260 pairs). To evaluate phylum groupings, only OTUs that were assigned as either Ascomycota or Basidiomycota (1,953 of 7,260 pairs) were used.

Model-based classification

We used model-based classification to infer how fungal communities partition niche space. This approach simultaneously models multivariate abundance data and environmental covariates to classify samples into groups, where each group has a distinct profile of fungal taxa in a distinct region of environmental space (Foster et al. 2017, Lyons et al. 2017; Appendix S2: Model-based classification). The classification is probabilistic, where group membership is treated as a latent variable within a mixture model. The classified groups can be thought of as representing observable fungal communities. The models are fit with the R package *RCPmod* (Foster et al. 2013). Size class and all 11 wood traits were included as covariates. We subjectively chose five groups to diagnose further using a combination of BIC and the relative occurrence of poor fits (Appendix S1: Fig. S3, see Appendix S2: Model-based classification for details).

Dissimilarity and distance analyses

To determine whether wood samples with high functional dissimilarity foster highly dissimilar fungal communities, we calculated pairwise wood functional

distances, wood phylogenetic distances, and fungal community distances among samples of the same size class (i.e., we did not include comparisons of samples between size classes; Appendix S2: Distances). The capacity of wood functional distance and wood phylogenetic distance to explain variation in fungal community distance were determined using a linear model that included wood functional distance, wood size class, and their interaction as fixed effects. The squared value of wood functional or phylogenetic distance was added to the model to accommodate nonlinear relationships. Wood phylogenetic distance and functional distance were regressed to evaluate their relationship.

Code and data needed to reproduce statistical analyses are available on Zenodo (see Data Availability statement).

RESULTS

A total of 3,021 OTUs were observed. Sampling effort was sufficient for near-complete coverage (Appendix S1: Fig. S4) with $24,753 \pm 753$ (mean \pm SE) reads and 140 ± 5 OTUs per sample on average. Almost 90% of OTUs were observed in fewer than 10 samples. Many OTUs (66%) did not match a genus-level taxonomic assignment in UNITE (Fig. 2a, b). Of those OTUs that did match, 30% also matched to a “Probable” or “Highly Probable” trophic mode assignment in the FUNGuild database.

Redundancy analysis and regressions for multivariate data

All 11 wood traits were included in the model that best explained variance in fungal community composition (Appendix S1: Table S1, Fig. S5). We found that C concentration and water percent improve models of fungal taxa abundance the most, followed by bark thickness, density, and Ca (Table 1).

Correlated response model

Only 6% of all OTUs were not responsive to one or more wood traits that were included as predictors in the correlated response model (Table 2). There was no clear relationship between a taxon’s assignment to a trophic mode or phylum and its wood trait responses (Appendix S1: Fig. S6).

Most fungal taxa pairs (52%) were not significantly correlated in their shared environment or residual responses. The average shared environment correlation was positive (median = 0.199, mean = 0.186, SE = 0.003) and ranged from -0.733 to 0.931. The average residual correlation was also weakly positive (median = 0.007, mean = 0.027, SE = 0.002) and ranged from -0.538 to 0.852. Of the 36% of significant correlations relating how taxa responded to their measured environment, positive correlations had a higher absolute mean correlation value and were about five times more frequent than negative ones

(Fig. 2a). Of the 23% of significant correlations relating taxa residual responses, positive correlations had a higher absolute mean correlation value and were about 50% more frequent than negative ones (Fig. 2b). More than 97% of significantly correlated OTU pairs had a consistent correlation sign across the 12 independent model runs. Results from the correlated response model that was built with a reduced set of predictor variables (size, C concentration, water percent, and bark thickness) supports the trend toward positive shared environmental and residual correlation values (Appendix S1: Fig. S7).

For the fraction of taxa pairs where both taxa could be classified to the genus level (Appendix S1: Table S2), almost all environmental (Fig. 2c) and residual (Fig. 2d) correlations were positive. The most strongly and positively correlated OTU pairs exhibited shared preferences for small stems with low densities and water concentrations (*Psathyrella candolleana* and *Coprinus* sp., $r = 0.84$) and stems with thin bark and high N concentrations (*Harknessia proteae* and *Mollisia* sp., $r = 0.78$; Appendix S1: Table S2). Notably, two OTUs belonging to genera *Fellomyces* and *Coprinus* were the only pair with a significant negative shared environmental correlation ($r = -0.64$) that could both be identified to the genus level (Fig. 2c; Appendix S1: Table S2). Both OTUs classify to the phylum Basidiomycota, with the *Coprinus* OTU classifying as a saprotroph. The *Fellomyces* OTU occurred more often in high N and large-stem host environments whereas the *Coprinus* OTU more commonly occupied small stems with low wood density (Appendix S1: Fig. S6).

Regarding residual correlations among taxonomically identified OTUs, OTUs in the Ascomycetes *Cladophialophora* sp. and *Rhytidhysteron* sp. were most strongly and positively correlated ($r = 0.61$) and were frequently co-located in a diverse set of woody hosts including *Allocasuarina littoralis*, *Ricinocarpos pinifolius*, and *Leucopogon ericoides*. Some of the strongest negative residual correlations were detected among *Neodevriesia* sp. and the following taxa: *Rhytidhysteron* sp. ($r = -0.52$), *Cladophialophora* sp. ($r = -0.45$), and *Teichospora trabicola* ($r = -0.36$; Appendix S1: Table S2). The distribution of the *Neodevriesia* sp. is unique because it is frequently, but not exclusively, found in the woody host *Acacia parramattensis*.

As predicted from the principle of limiting similarity, fungal taxa within the same trophic mode (e.g., both saprotrophs) were more frequently negatively correlated in how they respond to a shared environment than taxa with different trophic modes (Fig. 3a). None of the OTU pairs with sufficient trophic information ($n = 45$) were significantly positively correlated in how they responded to a shared environment (Fig. 3a), nor were any significantly residually correlated (Fig. 3b). We did not find similar support for limiting similarity when we examined taxonomic groups. Within and between phyla, the frequency of significantly negative taxa correlations to a shared environment and residual factors were

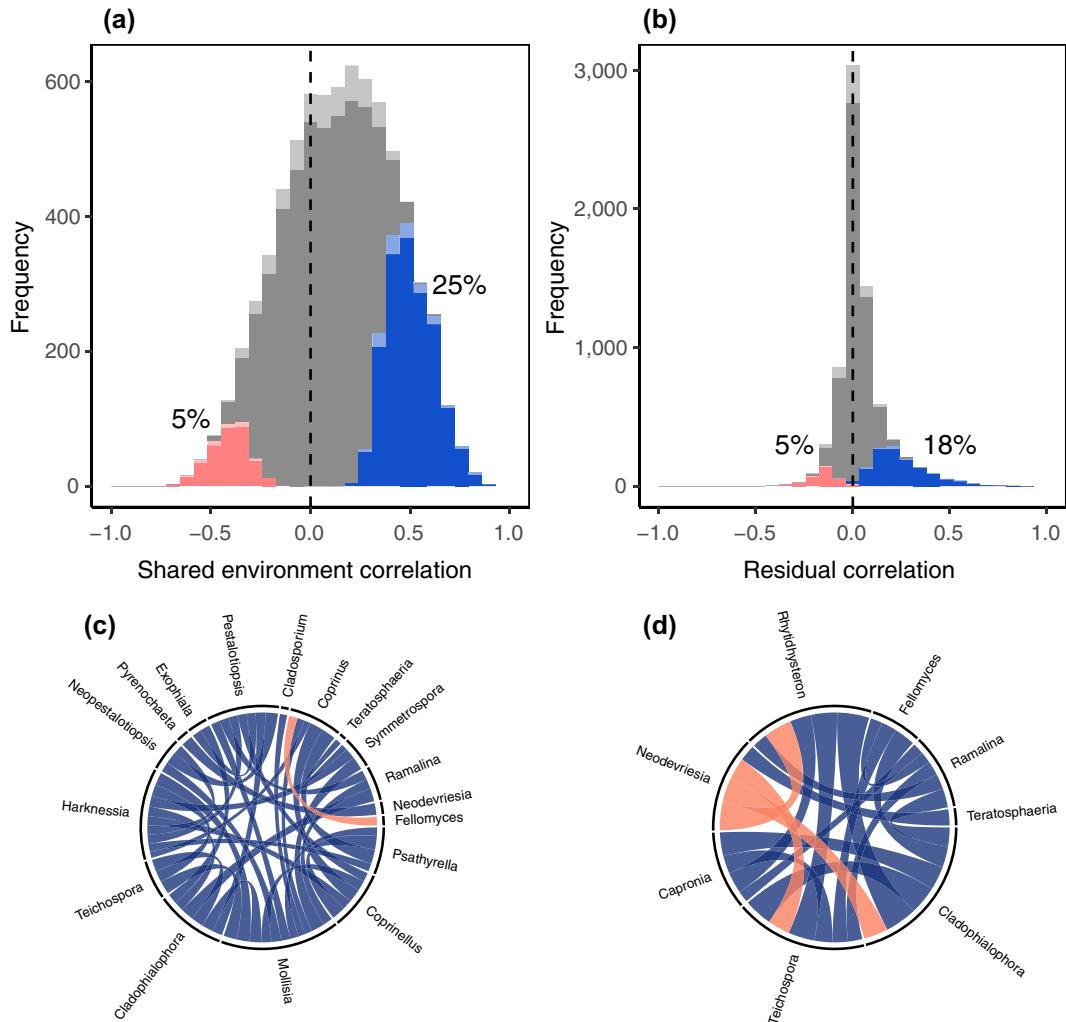


FIG. 2. The distribution of (a) shared environmental correlations and (b) residual correlations among fungal endophyte operational taxonomic unit (OTU) pairs indicates a bias toward positive values in this system. For OTU pairs with known genus-level taxonomic information (light shades in panels a and b), circular correlation diagrams show the relative strength (ribbon width) of significant (c) shared environmental and (d) residual correlations between OTUs by genus. Color indicates correlations that are negative (red), positive (blue), and not significantly different from zero (gray).

approximately equivalent (Fig. 3c, d). We found a slightly higher frequency of positive taxa correlations between than within fungal phyla due to shared environmental factors (Fig. 3c), but not residual factors (Fig. 3d).

Model-based classification

Modeled communities (Groups 1–5) varied in OTU membership and relative abundance (Appendix S1: Fig. S8). Certain OTUs occurred at relatively high abundances in more than one group, for example, *Didymosphaeria variabile* in Groups 1 and 3. Among modeled communities, no differences in the relative abundance of particular phyla or trophic modes were apparent (Appendix S1: Fig. S8).

The partial environmental effects for each modeled fungal community (Groups 1–5) demonstrated that communities occupied distinct ranges of environmental space (Fig. 4; Appendix S1: Fig. S9). Communities varied in their representation in small vs. large stem size classes. Groups 1 and 5 tended to occupy small stems (Fig. 4a–d), whereas Groups 2 and 3 tended to occupy large stems (Fig. 4e–h). Additionally, individual groups varied in how extensively they occupied niche space (broad vs. narrow). For example, Group 1-like communities were commonly found in small stems with a wide range of water, density, and C conditions (Fig. 4). The most abundant taxon in this group was an Ascomycete in the genus *Cladosporium* with an unclassified trophic mode (Appendix S1: Fig. S8). Other highly abundant taxa in this group included *Mollisia* sp. and *Teichospora*

TABLE 1. Ranking of key wood environmental variables that explain fungal endophyte operational taxonomic unit (OTU) relative abundances. Results are based on model comparisons using multivariate generalized linear mixed models (GLMMs) and Akaike information criteria (AIC). ΔAIC is equal to difference between the base and candidate model AIC values (base – candidate), where the base model includes the size class of the wood sample and the candidate model also includes a candidate wood environmental variable.

Model	AIC	ΔAIC
C	70,244	85
Water percent	70,247	83
Bark thickness	70,274	56
Density	70,278	52
Ca	70,302	28
Base	70,330	0
P	70,350	-20
N	70,370	-41
K	70,398	-69
Zn	70,424	-94
Fe	70,870	-540
Mn	72,483	-2,154

TABLE 2. Percent of fungal endophyte operational taxonomic units (OTUs; $n = 121$) that respond significantly to wood environmental variables based on the correlated response model. A response is considered significant if the 95% credible interval of posterior coefficient estimates does not overlap zero. Less than 10% of OTUs demonstrated no response to any wood trait. Results from a representative model run are shown.

Wood trait	All OTUs (%)	
	-	+
Size (small)	22	30
Density	3	2
Water percent	21	9
Bark thickness	31	3
C	21	2
N	7	12
P	24	4
Ca	23	18
Fe	15	10
K	10	25
Mn	31	9
Zn	5	19

trabicola (Appendix S1: Fig. S8), which are classified as pathotroph–symbiotrophs and saprotrophs, respectively. In contrast, Group 5-like communities tended to occur in small stems with particularly high water content and particularly low carbon content. This group was dominated by an OTU identified as an Ascomycete in the genus *Rhytidhysteron* (Appendix S1: Fig. S8).

The five fungal groups we modeled also had clear relationships with wood species (e.g., Group 4 frequently observed in *Eucalyptus* species). Group membership across wood species was overall not strongly organized

by wood phylogenetic relatedness; however, Groups 1 and 5 tended to be associated with woody hosts in Proteaceae and Myrtaceae families, respectively (Fig. 5).

Dissimilarity and distance analyses

Fungal community distance was positively correlated with wood trait distance (Fig. 6a) and wood phylogenetic distance (Fig. 6b). Samples of the same species and size class were highly variable with on average more than 50% dissimilarity between fungal communities (Fig. 6). Beyond the same species and size class, fungal community distance saturated quickly with increasing wood phylogenetic distance and wood trait distance (Fig. 6). Nonlinear relationships were supported as the additional second-degree polynomial terms improved model fit for both the wood functional and phylogenetic distance models (Appendix S1: Table S3). Small stem samples tended to have smaller fungal community distances than large stem samples at intermediate values of wood functional trait and phylogenetic distances (Fig. 6). Surprisingly, wood trait distances and wood phylogenetic distances were not correlated (Fig. 6c).

DISCUSSION

This study applies emerging community analysis approaches to infer processes that underlie community structure such as competitive exclusion and coexistence in a microbial system. Application of these approaches to microbial communities can lead to new views that refine our expectations about the role of species interactions in shaping taxa co-occurrence. The structure of fungal communities in living Australian woodland trees exhibited four general patterns. First, fungal taxa were more commonly positively than negatively correlated, exhibiting patterns consistent with “coexistence” as opposed to “competitive exclusion.” Second, fungal lifestyle information such as membership to a trophic mode may improve our understanding of species associations since negative associations appear to be more common among fungal taxa from the same trophic mode than between modes. Further, we have gained a greater understanding of wood as a fungal endophyte habitat, finding that—third—water content and carbon concentrations are key gradients and—fourth—evolutionary history for host trees structures fungal communities independent from measured chemical and physical traits.

Many positively associated taxa

Species pairs that share environmental tolerances are more likely to co-occur. Many more taxon pairs responded in a coordinated (25%) rather than in an opposing fashion (5%; Fig. 2a) to the same environmental conditions. This result suggests that coexisting taxa in this system have highly similar habitat and resource needs. Life in living wood tissue is not easy, with high

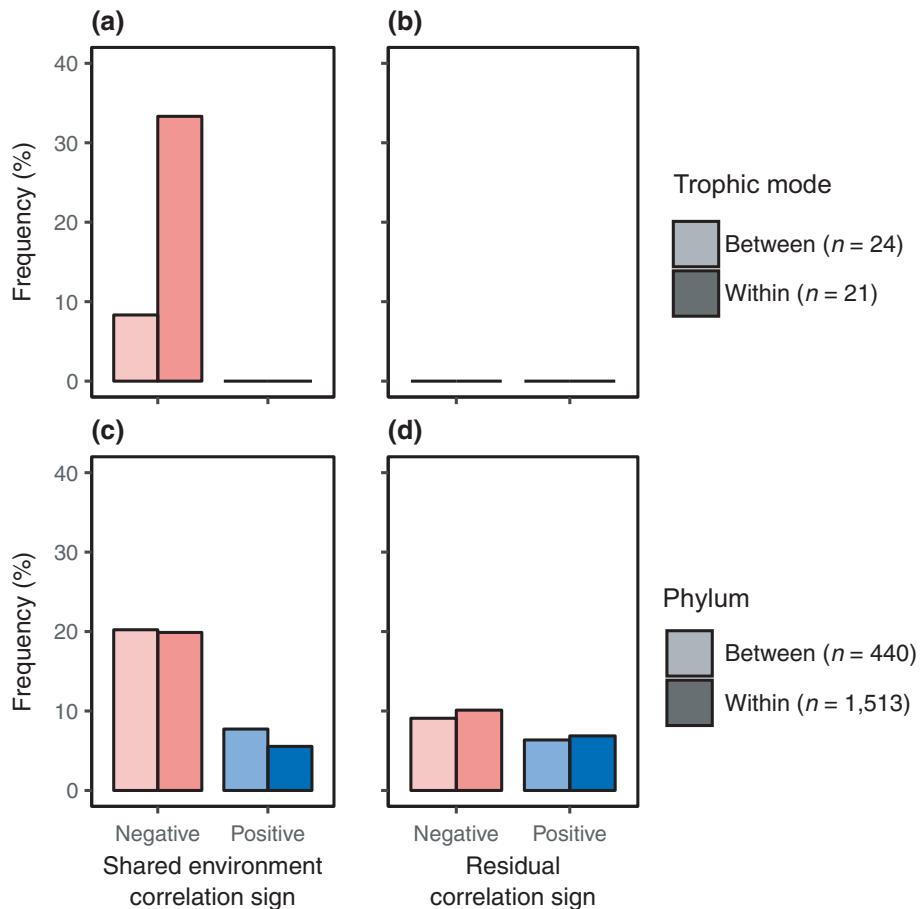


FIG. 3. Frequency of significant (a), (c) shared environmental and (b), (d) residual correlations among fungal endophyte operational taxonomic units (OTUs) within and between (a), (b) trophic mode and (c), (d) phylum. Total numbers of OTU pairs per category are shown in the legend.

osmotic and aeration stress (Boddy 2001) and low nutrient availability (Cornwell et al. 2009). However, all samples were diverse (Appendix S1: Fig. S1) with fungal communities occupying distinct regions of wood trait space (Fig. 4, Appendix S1: Fig. S9), which suggests that fungi exist in consortia. Each set of similarly diverse species occupied a consistent and unique slice of environmental space. For example, one consortium occupied large stems with intermediate water content and relatively low C (Group 4, Fig. 4); others occupied small stems (Groups 3 and 5, Fig. 4). These results suggest that communities are filtered based on their preferences/tolerance of particular environmental conditions (Keddy 1992). Further characterization of fungal communities along environmental gradients may improve our understanding of fungal natural history and ecological functioning.

Although habitat filtering explains some grouping in fungal communities, positive species interactions can also explain co-occurrence among fungal taxa. Almost one-fifth of OTU pairs had positive residual correlations, with positive correlations being more than three times more frequent than negative ones (Fig. 2b). The

relatively low frequency of negative correlations is surprising given the documented examples of combative-ness among fungi isolated from decaying wood (Boddy 2000, Heilmann-Clausen and Boddy 2005). Isolated fungi may represent a subset biased toward combative-ness based on their ability to compete during culturing (Zambell and White 2017). Furthermore, the simplified environment of lab-based studies may promote negative interactions that lead to competitive exclusion (Toljander et al. 2006). For these reasons, it is also important to investigate species responses in complex natural settings such as with environmental sequencing and statistical inference.

Living vs. deadwood may also present different environments for fungal communities to interact. In living wood, the host plant maintains the environmental conditions that fungi experience, potentially making those conditions more stable than conditions experienced in decaying wood where fungi must defend and exploit more territory for resources (Boddy 2000, 2001). A recent wood decay study, however, detected a large proportion of positive associations among fungal taxa after

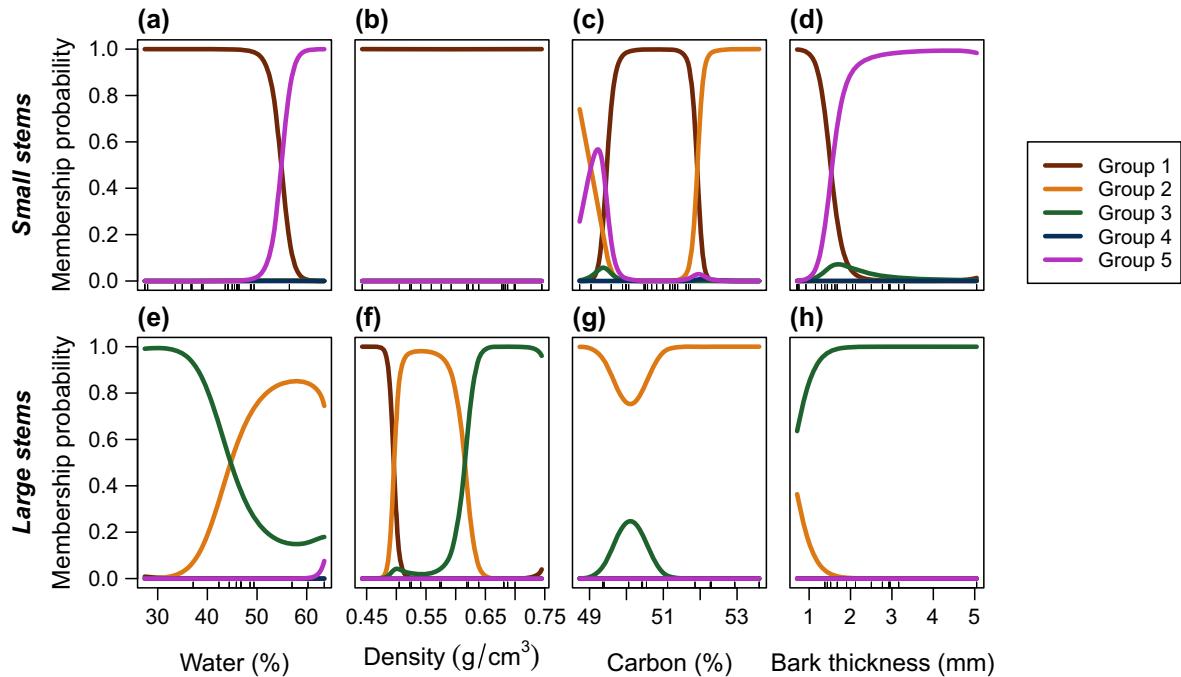


FIG. 4. Model-based fungal endophyte operational taxonomic unit (OTU) groups occupy different regions of wood environmental niche space. Curves show the response of the groups to wood environmental variables including (a)–(d) small and (e)–(h) large stem size; (a), (e) water (%); (b), (f) density (g/cm^3); (c), (g) carbon (%); and (d), (h) bark thickness (mm). Groups were created based on model estimates of individual OTU responses to 12 wood environmental variables (partial effects plots for remaining variables are in Appendix S1: Fig. S9). Ticks along the x-axis show the distribution of environmental data in the samples.

2 yr of decay in *Acer rubrum* (Maynard et al. 2018). Moreover, this study found that more positive associations were linked with less mass loss and higher fungal activity (Maynard et al. 2018). Similar analyses are needed at time points before and during wood decay to ascertain how ecological conditions determine whether fungal communities are structured more by negative than positive taxa associations.

The preponderance of positive residual correlations in our system suggests that fungi engage in facilitation, specialized predation/parasitism, and/or are responding in a coordinated fashion to unmeasured environmental characteristics. Facilitation can occur through cooperative or cross-feeding interactions such as beneficial metabolic exchanges (Zeleznak et al. 2015). In addition, fungi can secrete secondary metabolites that can have stimulatory (or inhibitory) effects on other fungi (Heilmann-Clausen and Boddy 2005). Ecological theory suggests that endophytes may more frequently engage in mutualistic interactions that support species coexistence than their decay-associated counterparts, because the live-wood habitat is arguably more stressful because of aeration stress and host-plant defenses (e.g., the stress gradient hypothesis; Bertness and Callaway 1994). Positive associations may also reflect negative interactions such as specialized predation and parasitism. In addition, co-occurring taxa may be engaged in intransitive competitive relationships among dominant individuals that do not readily lead to

competitive exclusion (Boddy 2000, Laird and Schamp 2006, Maynard et al. 2018). Our findings call for targeted tests to identify exactly why key species in this system co-occur in similar environments (Fig. 2d).

Taxa in this residual interaction pool may also be responding to unmeasured characteristics of the environment in this study. For instance, some studies have found that pH, which we did not measure, is important for explaining variation in fungal community composition in decaying wood (Baldrian et al. 2016, Heilmann-Clausen et al. 2016, Purahong et al. 2016). Additionally, wood secondary chemistry is difficult to measure, but is an important way in which plants regulate pathogens. Studies that extend the range of wood traits measured and how they shape the fungal communities can improve our understanding of wood as a fungal habitat.

Negative associations between trophically similar fungi

Taxa responses to the environment depended, in part, on how similar they were ecologically. Members with the same trophic mode have shared resource requirements and greater potential for competitive interactions if they occupy the same habitat (Blondel 2003). We found that taxa from within the same trophic mode had more significant negative environmental correlations than taxa with classifications that differed (Fig. 3a). Even though many of these taxa had not previously been observed in

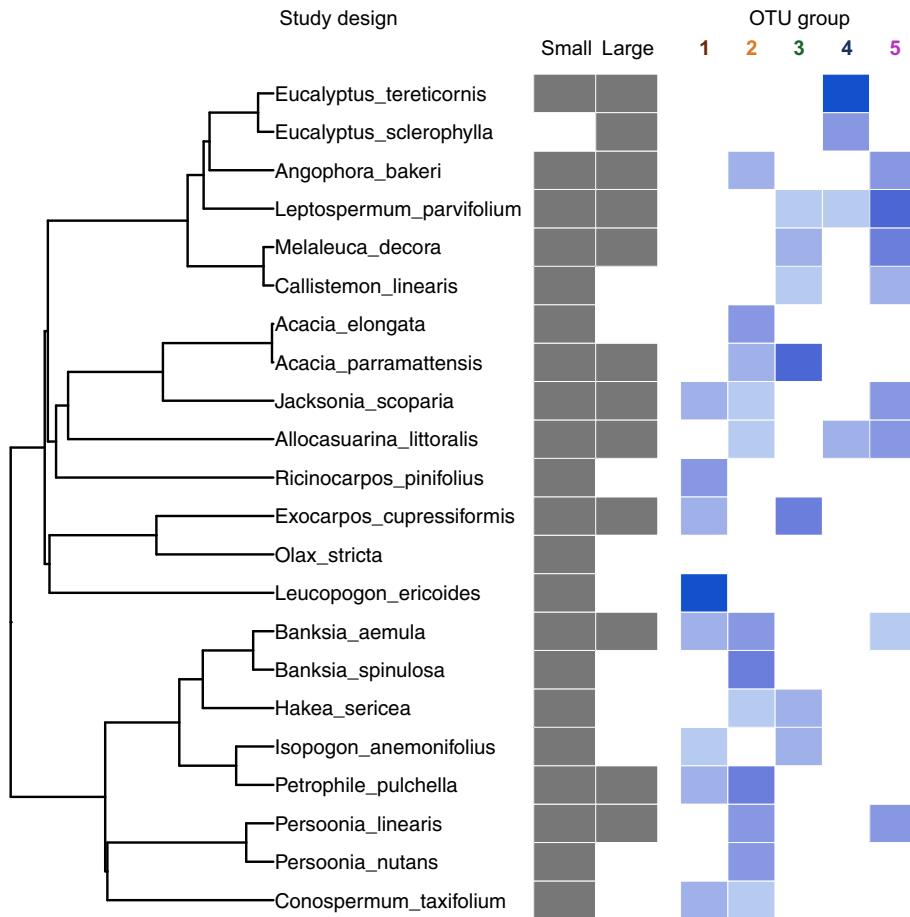


FIG. 5. Modeled operational taxonomic unit (OTU) groups tend to occur in particular wood species and are not strongly organized by wood species' phylogenetic relatedness. Gray boxes indicate which wood species and size classes were sampled. Blue-hued heat map represents the number of samples for which the fungal endophyte community was probabilistically classified to a given OTU group by wood species identity; darker shades signify more samples. OTU group numbers are colored as in Fig. 4.

living wood (i.e., were not classified as endophytes), similarities in trophic modes from other settings predicted the nature of interactions as expected. Although trophic mode predicted the direction of significant shared environmental correlations, it did not explain individual taxon responses to the environment (e.g., Appendix S1: Fig. S6). The weak relationship between trophic mode and environment could reflect context-dependent nutritional behaviors and reduced statistical power for the subset of taxa with trophic annotations (45 of 7,260 OTU pairs). More precise classifications possible with growing databases will allow for more powerful tests for trait-based predictors of fungal taxa associations (Aguilar-Trigueros et al. 2014, 2015).

Although functionally similar fungi had significantly more negative associations, fungi from the same phylum did not exhibit similar responses (Fig. 3c, d). The weak association between phylum and environment may reflect relatively more variation in fungal lifestyles within vs. between these ancient and diverse lineages. More detailed phylogenetic analyses could resolve which traits

might underlie different associations between major fungal lineages.

Wood traits and phylogeny independently influence endophytes

Of the traits considered, wood water percent and C concentration were the most influential predictors of variation in fungal taxa abundance in living wood. Not surprisingly, C (Rajala et al. 2011, Hoppe et al. 2015) and moisture (Chapela and Boddy 1988, Boddy et al. 1989, Rajala et al. 2011, 2012, Hoppe et al. 2015, Kubartová et al. 2015) are also critical controls on fungal communities in decaying wood. In addition, C percentage, which is linked with wood density, reflects the plant's ecological strategy (Wright et al. 2004, Chave et al. 2009) in ways that may influence fungal endophyte communities. Fast-growing tree species tend to have low-density wood with relatively few defense compounds compared to slow-growing, highly defended, and carbon-rich tree species (Chave et al. 2009). In response,

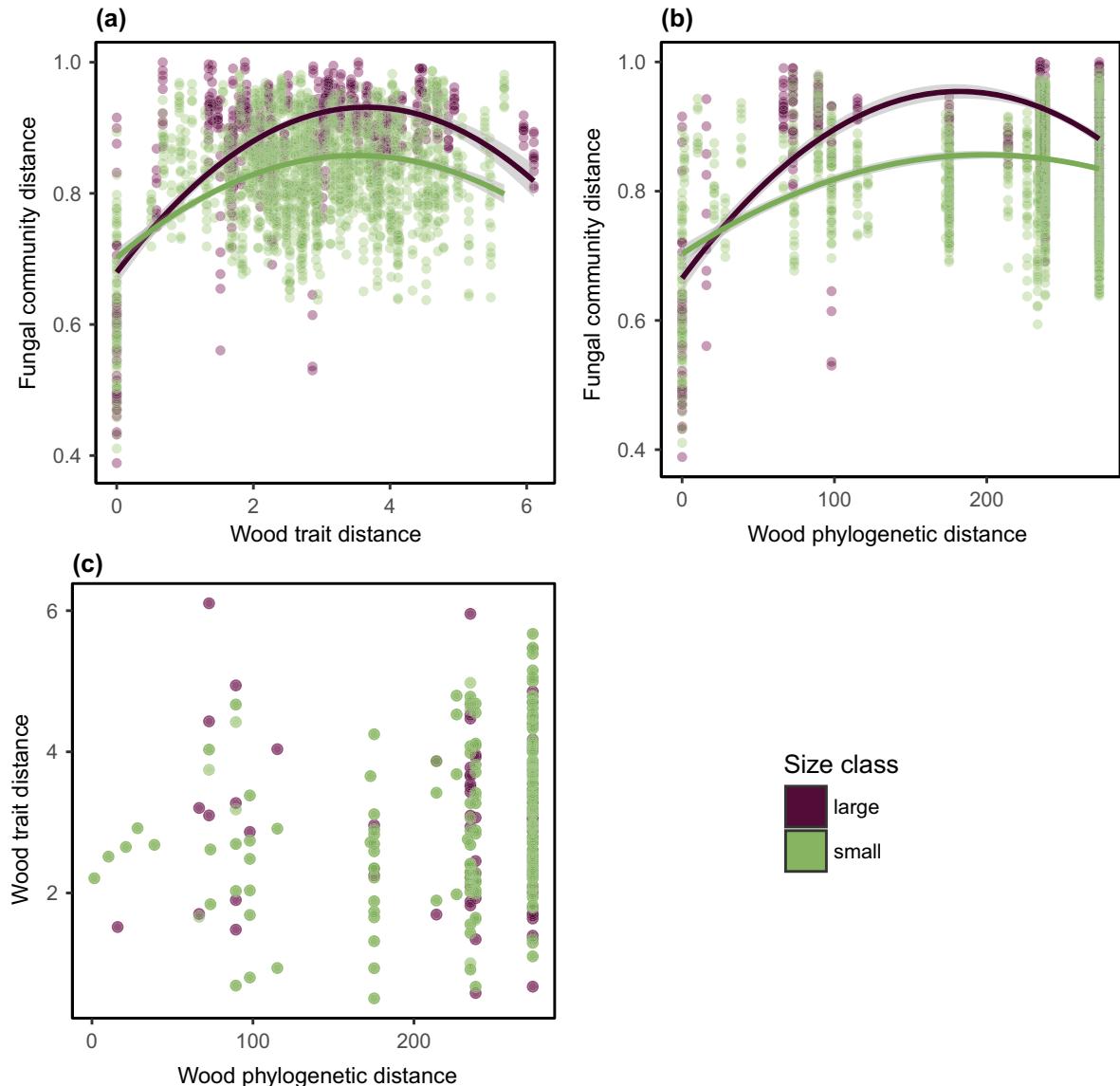


FIG. 6. Fungal community distance saturates with wood (a) functional trait and (b) phylogenetic distance; (c) wood functional trait and phylogenetic distances are unrelated. Data from large and small stem samples are shown in purple and green, respectively. Trend lines are second-degree polynomial fits with 95% confidence intervals.

some fungi may thrive in poorly defended and short-lived tree species, whereas others are especially suited to live in well-defended and long-lived woody tissue.

We detected 2–31% of taxa had significant associations with a given trait. Slightly more fungal taxa were associated with small stems, low wood density, thin bark, and low P concentrations (Table 2), suggesting that these traits might indicate environments that support greater abundances of these fungi. Tree species with thin bark and low wood density may be easier for fungi to colonize and explore. More diverse fungal communities in wood with low P concentrations suggests that this element is less strongly limiting to fungal taxa than has been previously thought. Another explanation is that

high P levels have indirectly led to fungal diversity declines by increasing productivity and P limitation for all but a few dominant taxa (Tilman 1987, Wang et al. 2016). Alternatively, these patterns may reflect an underlying unmeasured gradient, e.g., a preponderance of harmful secondary metabolites that contain P. Teasing apart these hypotheses will require further work using approaches that allow estimation of absolute fungal taxa abundances in more controlled experiments.

Phylogenetic distances could help explain dissimilarities in fungal communities because wood traits should be similar in closely related taxa. Surprisingly, measured traits lacked phylogenetic structure. Phylogenetic relatedness can, however, capture variation in traits that are

difficult to measure (Webb et al. 2002), such as secondary metabolite composition. Entire classes of secondary compounds (i.e., formylated phloroglucinol compounds) are virtually exclusive to the *Eucalyptus* genus (Eyles et al. 2003). Other more common secondary metabolites such as phenolics and terpenes occur in clade-specific combinations (Chave et al. 2009). Relatedness can act as a proxy for those traits, and statistically, relatedness may be even more effective for cases, such as this one, when the traits involved are likely to be highly dimensional (Kraft et al. 2007).

The cryptic nature, high diversity, and habitat complexity of many microbial systems, such as the wood fungal endophyte system, can make experimental manipulations infeasible. As such, statistical methods combined with environmental sequencing represent the best approach to develop evidence-based inferences regarding the ecological processes that structure fungal communities *in situ*. Because DNA sequencing does not distinguish active from dormant taxa (Lennon and Jones 2011, Blagodatskaya and Kuzyakov 2013) and the presence of dormant saprotrophs in wood tissue is well known (Boddy 2001, Parfitt et al. 2010), future studies will benefit from characterizing the active community at multiple time points.

Here, we have shown how co-occurrence may be shaped both by habitat and by species interactions in a cryptic, diverse, and understudied microbial system. The approaches used to study the endophyte system may serve as an example for other more difficult-to-measure communities, as well as illuminating paths for targeted experimentation and hypothesis testing of key ecological processes that are relevant to diverse ecological communities more generally. Specifically, these insights into endophyte community assembly may be of functional consequence: fungal communities in living wood can control tree health (Sieber 2007) and ecologically “set the stage” for the patterns and rate of wood decay (Griffith and Boddy 1990, Parfitt et al. 2010, Song et al. 2016)—a critical component of the global carbon cycle.

ACKNOWLEDGMENTS

An Australian Research Council Discovery Grant (DP160103765), Western Sydney University International Research Initiatives Scheme Visiting Fellowship, and The George Washington University, all contributed to support this work. We thank Brendan Choat and Peter Reich for contributing to the initial project design. We would also like to thank those who helped improve the manuscript: Steve Allison and Andrew Letten.

LITERATURE CITED

- Abarenkov, K., et al. 2010. The UNITE database for molecular identification of fungi—recent updates and future perspectives. *New Phytologist* 186:281–285.
- Abrams, P. 1983. The theory of limiting similarity. *Annual Review of Ecology and Systematics* 14:359–376.
- Abrams, P. 1996. Limits to the similarity of competitors under hierarchical lottery competition. *American Naturalist* 148:211–219.
- Aguilar-Trigueros, C. A. et al. 2015. Branching out: towards a trait-based understanding of fungal ecology. *Fungal Biology Reviews* 29:34–41.
- Aguilar-Trigueros, C. A., J. R. Powell, I. C. Anderson, J. Antonovics, and M. C. Rillig. 2014. Ecological understanding of root-infecting fungi using trait-based approaches. *Trends in Plant Science* 19:432–438.
- Ahlholm, J. U., M. Helander, J. Henriksson, M. Metzler, and K. Saikkonen. 2002. Environmental conditions and host genotype direct genetic diversity of *Venturia ditricha*, a fungal endophyte of birch trees. *Evolution* 56:1566–1573.
- Arnold, A. E. 2007. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biology Reviews* 21:51–66.
- Baldrian, P., P. Zrůstová, V. Tláskal, A. Davidová, V. Merhautová, and T. Vrška. 2016. Fungi associated with decomposing deadwood in a natural beech-dominated forest. *Fungal Ecology* 23:109–122.
- Bertness, M. D., and R. Callaway. 1994. Positive interactions in communities. *Trends in Ecology and Evolution* 9:191–193.
- Bissett, A. et al. 2016. Introducing BASE: the Biomes of Australian Soil Environments soil microbial diversity database. *GigaScience* 5:21.
- Björk, J. R., F. K. C. Hui, R. B. O’Hara, and J. M. Montoya. 2018. Uncovering the drivers of host-associated microbiota with joint species distribution modelling. *Molecular Ecology* 27:2714–2724.
- Blagodatskaya, E., and Y. Kuzyakov. 2013. Active microorganisms in soil: critical review of estimation criteria and approaches. *Soil Biology and Biochemistry* 67:192–211.
- Blondel, J. 2003. Guilds or functional groups: Does it matter? *Oikos* 100:223–231.
- Boddy, L. 2000. Interspecific combative interactions between wood-decaying basidiomycetes. *FEMS Microbiology Ecology* 31:185–194.
- Boddy, L. 2001. Fungal community ecology and wood decomposition processes in angiosperms: from standing tree to complete decay of coarse woody debris. *Ecological Bulletins* 49:43–56.
- Boddy, L., E. M. Owens, and I. H. Chapela. 1989. Small scale variation in decay rate within logs one year after felling: effect of fungal community structure and moisture content. *FEMS Microbiology Letters* 62:173–183.
- Boller, T., and G. Felix. 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual Review of Plant Biology* 60:379–406.
- Brown, C. J., and R. J. Hamilton. 2018. Estimating the footprint of pollution on coral reefs with models of species turnover. *Conservation Biology* 32:949–958.
- Chapela, I. H., and L. Boddy. 1988. Fungal colonization of attached beech branches. *New Phytologist* 110:47–57.
- Chave, J., D. Coomes, S. Jansen, S. L. Lewis, N. G. Swenson, and A. E. Zanne. 2009. Towards a worldwide wood economics spectrum. *Ecology Letters* 12:351–366.
- Combès, A., I. Ndoye, C. Bance, J. Bruzaud, C. Djediat, J. Dupont, B. Nay, and S. Prado. 2012. Chemical communication between the endophytic fungus *paraconiothyrium variabile* and the phytopathogen *Fusarium oxysporum*. *PLoS ONE* 7:e47313-11.
- Connell, J. H. 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. *American Naturalist* 122:661–696.
- Connell, J. H., and R. O. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. *American Naturalist* 111:1119–1144.

- Connor, E. F., M. D. Collins, and D. Simberloff. 2013. The checkered history of checkerboard distributions. *Ecology* 94:2403–2414.
- Cornwell, W. K., J. H. C. Cornelissen, S. D. Allison, J. Bauhus, P. Eggleton, C. M. Preston, F. Scarff, J. T. Weedon, C. Wirth, and A. E. Zanne. 2009. Plant traits and wood fates across the globe: Rotted, burned, or consumed? *Global Change Biology* 15:2431–2449.
- Diamond, J. M. 1975. Assembly of species communities. Pages 342–444 in M. L. Cody and J. M. Diamond, editors. *Ecology and evolution of communities*. Belknap Press, Cambridge.
- Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* 19:11–15.
- Eastwood, D. C. et al. 2011. The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. *Science* 333:762–765.
- Eichlerová, I., L. Homolka, L. Žifcákova, L. Lisá, P. Dobíšová, and P. Baldrian. 2015. Enzymatic systems involved in decomposition reflects the ecology and taxonomy of saprotrophic fungi. *Fungal Ecology* 13:10–22.
- Eyles, A., N. W. Davies, and C. Mohammed. 2003. Novel detection of formylated phloroglucinol compounds (FPCs) in the wound wood of *Eucalyptus globulus* and *E. nitens*. *Journal of Chemical Ecology* 29:881–898.
- Foster, S. D., G. H. Givens, G. J. Dornan, P. K. Dunstan, and R. Darnell. 2013. Modelling biological regions from multi-species and environmental data. *Environmetrics* 24:489–499.
- Foster, S. D., N. A. Hill, and M. Lyons. 2017. Ecological grouping of survey sites when sampling artefacts are present. *Journal of the Royal Statistical Society: Series C (Applied Statistics)* 44:139.
- Friesen, T. L., J. D. Faris, P. S. Solomon, and R. P. Oliver. 2008. Host-specific toxins: effectors of necrotrophic pathogenicity. *Cellular Microbiology* 10:1421–1428.
- Fuhrman, J. A., J. A. Cram, and D. M. Needham. 2015. Marine microbial community dynamics and their ecological interpretation. *Nature* 13:133–146.
- Gause, G. F. 1934. Experimental analysis of Vito Volterra's mathematical theory of the struggle for existence. *Science* 79:16–17.
- Gotelli, N. J., and D. J. McCabe. 2002. Species co-occurrence: a meta-analysis of J. M. Diamond's assembly rules model. *Ecology* 83:2091–2096.
- Griffith, G. S., and L. Boddy. 1990. Fungal decomposition of attached angiosperm twigs I. Decay community development in ash, beech and oak. *New Phytologist* 116:407–415.
- Hamilton, C. E., P. E. Gundel, M. Helander, and K. Saikkonen. 2012. Endophytic mediation of reactive oxygen species and antioxidant activity in plants: a review. *Fungal Diversity* 54:1–10.
- Heilmann-Clausen, J., and L. Boddy. 2005. Inhibition and stimulation effects in communities of wood decay fungi: exudates from colonized wood influence growth by other species. *Microbial Ecology* 49:399–406.
- Heilmann-Clausen, J., P. K. Maruyama, H. H. Bruun, D. Dimitrov, T. Laessøe, T. G. Frøslev, and B. Dalsgaard. 2016. Citizen science data reveal ecological, historical and evolutionary factors shaping interactions between woody hosts and wood-inhabiting fungi. *New Phytologist* 212:1072–1082.
- Hoppe, B., W. Purahong, T. Wubet, T. Kahl, J. Bauhus, T. Arnsdorf, M. Hofrichter, F. Buscot, and D. Krüger. 2015. Linking molecular deadwood-inhabiting fungal diversity and community dynamics to ecosystem functions and processes in central European forests. *Fungal Diversity* 77:367–379.
- Hui, F., S. Taskinen, S. Pledger, S. D. Foster, and D. I. Warton. 2015. Model-based approaches to unconstrained ordination. *Methods in Ecology and Evolution* 6:399–411.
- Hui, F. K. C. 2016. Boral–Bayesian ordination and regression analysis of multivariate abundance data in r. *Methods in Ecology and Evolution* 7:744–750.
- Ihrmark, K., et al. 2012. New primers to amplify the fungal ITS2 region—evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology* 82:666–677.
- Keddy, P. A. 1992. Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science* 3:157–164.
- Kraft, N. J. B., W. K. Cornwell, C. O. Webb, and D. D. Ackerly. 2007. Trait Evolution, Community Assembly, and the Phylogenetic Structure of Ecological Communities. *American Naturalist* 170(2):271–283. <http://doi.org/10.1086/519400>
- Kubartová, A., E. Ottosson, and J. Stenlid. 2015. Linking fungal communities to wood density loss after 12 years of log decay. *FEMS Microbiology Ecology* 91.
- Laird, R. A., and B. S. Schamp. 2006. Competitive intransitivity promotes species coexistence. *American Naturalist* 168:182–193.
- Leach, E. C., C. J. Burwell, D. N. Jones, and R. L. Kitching. 2018. Modelling the responses of Australian subtropical rainforest birds to changes in environmental conditions along elevational gradients. *Austral Ecology* 43:490–501.
- Lennon, J. T., and S. E. Jones. 2011. Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature* 9:119–130.
- Li, X., Z. Zhong, D. Sanders, C. Smit, D. Wang, P. Nummi, Y. Zhu, L. Wang, H. Zhu, and N. Hassan. 2018. Reciprocal facilitation between large herbivores and ants in a semi-arid grassland. *Proceedings of the Royal Society B* 285:20181665–9.
- Lyons, M. B., S. D. Foster, and D. A. Keith. 2017. Simultaneous vegetation classification and mapping at large spatial scales. *Journal of Biogeography* 44:2891–2902.
- Lyons, M. B., D. A. Keith, D. I. Warton, M. Somerville, and R. T. Kingsford. 2016. Model-based assessment of ecological community classifications. *Journal of Vegetation Science* 27:704–715.
- Maynard, D. S., K. R. Covey, T. W. Crowther, N. W. Sokol, E. W. Morrison, S. D. Frey, L. T. A. van Diepen, and M. A. Bradford. 2018. Species associations overwhelm abiotic conditions to dictate the structure and function of wood-decay fungal communities. *Ecology* 99:801–811.
- McIntire, E. J. B., and A. Fajardo. 2013. Facilitation as a ubiquitous driver of biodiversity. *New Phytologist* 201:403–416.
- Nguyen, N. H., Z. Song, S. T. Bates, S. Branco, L. Tedersoo, J. Menke, J. S. Schilling, and P. G. Kennedy. 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* 20:241–248.
- Oksanen, J., F. G. Blanchet, R. Kindt, and P. Legendre. 2015. “vegan”: community ecology package Version 2.5.5. <http://CRAN.R-project.org/package=vegan>
- Osazuwa-Peters, O. L., and A. Zanne. 2011. Wood density protocol. <http://prometheuswiki.org/tiki-index.php?page=Wood+density+protocol&highlight=wood%20density>.
- Ovaskainen, O., J. Hottola, and J. Siitonen. 2010. Modeling species co-occurrence by multivariate logistic regression generates new hypotheses on fungal interactions. *Ecology* 91:2514–2521.
- Ovaskainen, O., G. Tikhonov, A. Norberg, F. Guillaume Blanchet, L. Duan, D. Dunson, T. Roslin, and N. Abrego. 2017. How to make more out of community data? A conceptual framework and its implementation as models and software. *Ecology Letters* 126:269–316.
- Parfitt, D., J. Hunt, D. Dockrell, H. J. Rogers, and L. Boddy. 2010. Do all trees carry the seeds of their own destruction? PCR reveals numerous wood decay fungi latently present in sapwood of a wide range of angiosperm trees. *Fungal Ecology* 3:338–346.
- Parrent, J., T. Y. James, R. Vasaitis, and A. F. Taylor. 2009. Friend or foe? Evolutionary history of glycoside hydrolase

- family 32 genes encoding for sucrolytic activity in fungi and its implications for plant–fungal symbioses. *BMC Evolutionary Biology* 9:148.
- Pérez-Harguindeguy, N. et al. 2013. New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* 61:167–168.
- Petrini, O. 1996. Ecological and physiological aspect of host specificity in endophytic fungi. Pages 87–100 in S. C. Redlin and L. M. Carris, editors. *Endophytic fungi in grasses and woody plants*. APS Press, St. Paul, Minnesota, USA.
- Purahong, W., T. Arnstadt, T. Kahl, J. Bauhus, H. Kellner, M. Hofrichter, D. Krüger, F. Buscot, and B. Hoppe. 2016. Are correlations between deadwood fungal community structure, wood physico-chemical properties and lignin-modifying enzymes stable across different geographical regions? *Fungal Ecology* 22:98–105.
- Rajala, T., S. C. Olhede, and D. J. Murrell. 2019. When do we have the power to detect biological interactions in spatial point patterns? *Journal of Ecology* 107:711–721.
- Rajala, T., M. Peltoniemi, J. Hantula, R. Mäkipää, and T. Pennanen. 2011. RNA reveals a succession of active fungi during the decay of Norway spruce logs. *Fungal Ecology* 4: 437–448.
- Rajala, T., M. Peltoniemi, T. Pennanen, and R. Mäkipää. 2012. Fungal community dynamics in relation to substrate quality of decaying Norway spruce (*Picea abies* [L.] Karst.) logs in boreal forests. *FEMS Microbiology Ecology* 81:494–505.
- Rodriguez Estrada, A. E., W. Jonkers, H. Corby Kistler, and G. May. 2012. Interactions between *Fusarium verticillioides*, *Ustilago maydis*, and *Zea mays*: an endophyte, a pathogen, and their shared plant host. *Fungal Genetics and Biology* 49: 578–587.
- Sieber, T. N. 2007. Endophytic fungi in forest trees: Are they mutualists? *Fungal Biology Reviews* 21:75–89.
- Soltis, D. E., P. S. Soltis, T. G. Collier, and M. L. Edgerton. 1991. Chloroplast DNA variation within and among genera of the Heuchera group (Saxifragaceae): evidence for chloroplast transfer and paraphyly. *American Journal of Botany* 78:1091–1112.
- Song, Z., P. G. Kennedy, F. J. Liew, and J. S. Schilling. 2016. Fungal endophytes as priority colonizers initiating wood decomposition. *Functional Ecology* 31:407–418.
- Tilman, D. 1982. Resource competition and community structure. Princeton University Press, Princeton, New Jersey, USA.
- Tilman, D. 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. *Ecological Monographs* 57:189–214.
- Tiunov, A. V., and S. Scheu. 2005. Facilitative interactions rather than resource partitioning drive diversity-functioning relationships in laboratory fungal communities. *Ecology Letters* 8:618–625.
- Toljander, Y. K., B. D. Lindahl, L. Holmer, and N. O. S. Höglberg. 2006. Environmental fluctuations facilitate species co-existence and increase decomposition in communities of wood decay fungi. *Oecologia* 148:625–631.
- U'Ren, J. M., F. Lutzoni, J. Miadlikowska, A. D. Laetsch, and A. E. Arnold. 2012. Host and geographic structure of endophytic and endolichenic fungi at a continental scale. *American Journal of Botany* 99:898–914.
- Wang, J., F. Pan, J. Soininen, J. Heino, and J. Shen. 2016. Nutrient enrichment modifies temperature–biodiversity relationships in large-scale field experiments. *Nature Communications* 7:1–9.
- Wang, Y., U. Naumann, S. T. Wright, and D. I. Warton. 2012. mvabund—an R package for model-based analysis of multivariate abundance data. *Methods in Ecology and Evolution* 3:471–474.
- Warton, D. I., S. T. Wright, and Y. Wang. 2012. Distance-based multivariate analyses confound location and dispersion effects. *Methods in Ecology and Evolution* 3:89–101.
- Warton, D. I., F. G. Blanchet, R. B. O'Hara, O. Ovaskainen, S. Taskinen, S. C. Walker, and F. K. C. Hui. 2015. So many variables: joint modeling in community ecology. *Trends in Ecology and Evolution* 30:766–779.
- Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. *Annual Review of Ecology and Systematics* 33:475–505.
- Weiher, E., D. Freund, T. Bunton, A. Stefanski, T. Lee, and S. Bentivenga. 2011. Advances, challenges and a developing synthesis of ecological community assembly theory. *Philosophical Transactions of the Royal Society B* 366:2403–2413.
- White, T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 in M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, editors. *PCR protocols*. Academic Press, San Diego, California, USA.
- Wright, I. J. et al. 2004. The worldwide leaf economics spectrum. *Nature* 428:821–827.
- Zambell, C., and J. White. 2017. Community assembly of phyllosphere endophytes: a closer look at fungal life-cycle dynamics, competition and phytochemistry in the shaping of the fungal community in J. Dighton, and J. White, editors. *The fungal community*. Fourth edition. CRC Press, Boca Raton, Florida, USA.
- Zelezniak, A., S. Andrejev, O. Ponomarova, D. R. Mende, P. Bork, and K. R. Patil. 2015. Metabolic dependencies drive species co-occurrence in diverse microbial communities. *Proceedings of the National Academy of Sciences* 112:6449–6454.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.2790/supplinfo>

DATA AVAILABILITY

Data are available on Zenodo: <https://doi.org/10.5281/zenodo.3234129>