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RESEARCH ARTICLE

Divergent biotic and abiotic filtering of root endosphere and rhizosphere soil fungal communities along ecological gradients

Candice Y. Lumibao[1], Elizabeth R. Kimbrough[1], Richard H. Day[3], William H. Conner[2], Ken W. Krauss[3] and Sunshine A. Van Bael[1,*,†]

[1]Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, 70118, USA, [2]Baruch Institute of Coastal Ecology and Forest Science, Clemson University, P.O. Box 596, Georgetown, SC, 29442, USA and [3]U.S. Geological Survey, Wetland and Aquatic Research Center, 700 Cajundome Blvd., Lafayette, LA, 70506, USA

*Corresponding author: 6823 St. Charles Avenue, Suite 400 Boggs, Department of Ecology & Evolutionary Biology, New Orleans, LA 70118, USA. Tel: 504-862-8291; E-mail: svanbael@tulane.edu

One sentence summary: Fungal communities associated with baldcypress trees differ along a salinity gradient.

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†Sunshine A. Van Bael, http://orcid.org/0000-0001-7317-3533

ABSTRACT

Plant roots assemble in two distinct microbial compartments: the rhizosphere (microbes in soil surrounding roots) and the endosphere (microbes within roots). Our knowledge of fungal community assembly in these compartments is limited, especially in wetlands. We tested the hypothesis that biotic factors would have direct effects on rhizosphere and endosphere assembly, while abiotic factors would have direct and indirect effects. Using a field study, we examined the influences of salinity, water level and biotic factors on baldcypress (*Taxodium distichum*) fungal communities. We found that endosphere fungi, unlike rhizosphere fungi, were correlated with host density and canopy cover, suggesting that hosts can impose selective filters on fungi colonizing their roots. Meanwhile, local abiotic conditions strongly influenced both rhizosphere and endosphere diversity in opposite patterns, e.g. highest endosphere diversity (hump-shaped) and lowest rhizosphere diversity (U-shaped) at intermediate salinity levels. These results indicate that the assembly and structure of the root endosphere and rhizosphere within a host can be shaped by different processes. Our results also highlight the importance of assessing how environmental changes affect plant and plant-associated fungal communities in wetland ecosystems where saltwater intrusion and sea level rise are major threats to both plant and fungal communities.

Keywords: baldcypress; endosphere and rhizosphere; fungal diversity; plant microbiomes; salinity and water level gradients; wetlands

INTRODUCTION

Identifying the ecological factors that shape the assembly of microbial communities across space and time is an on-going challenge (Arnold 2007; Borer *et al.* 2013). In plant-associated fungal communities, local abiotic and biotic environments as well as the regional species pool able to colonize a host can influence the diversity and composition of these communities. The relative importance of biotic (e.g. host density) and abiotic components of the environment on these communities, however, depends on the habitat type, the spatial scale (Bahram *et al.* 2012; Moll *et al.* 2016), and the host plant compartments that microbes

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are associated with, for instance, within roots or soil (de Souza *et al.* 2016). As microbes are key players in many processes such as organic matter recycling (Johnson *et al.* 2013), characterizing ecological factors underlying plant-associated microbiome distribution and assembly is paramount to understanding general ecosystem processes.

Ecological gradients have been used to study how natural variations in environmental conditions (i.e. those external to the host) shape fungal communities (Pellissier *et al.* 2014; Tian *et al.* 2017). In the sediments and water of wetland ecosystems, for example, salinity can be the major determinant of fungal community structure (Mohamed and Martiny 2011; Hammami *et al.* 2016), with greatest fungal richness occurring at intermediate salinity levels. Similarly, water regime histories can affect the function and composition of soil fungal communities (e.g. Frossard *et al.* 2015). Fungal communities often exhibit varying diversity patterns along environmental gradients due to different magnitudes of abiotic filtering; for example, hump-shaped or linear patterns along gradients of elevation (Bahram *et al.* 2012; Tian *et al.* 2017), salinity (Mohamed and Martiny 2011; Hammami *et al.* 2016) and water regime (Ma *et al.* 2018).

Host-associated fungal communities can also be directly affected by biotic factors via the host plant. Along ecological gradients, different stages of vegetation develop as environmental conditions change, thus modifying host plant structure such as host abundance and community structure (Krauss *et al.* 2009), which, in turn, alter their associated belowground microbiomes. For instance, density-dependent colonization of plants by fungi is common for pathogenic fungal infections (Burdon and Chilvers 1982; Gilbert, Hubbell and Foster 1994). Alternatively, increases in host density can increase the abundance of specialist microbes, thereby reducing microbial diversity as demonstrated in plant pathogens (i.e. the 'dilution' effects) (Mitchell, Tilman and Groth 2002; Keesing, Holt and Ostfeld 2006). Changes in abundance of the host relative to non-hosts (i.e. host cover) can also lead to reduced fungal richness and composition (Gilbert, Ferrer and Carranza 2002; de Araujo *et al.* 2017). While both abiotic and biotic processes contribute to the assembly of fungal communities (e.g. Hollister *et al.* 2010; Goldmann *et al.* 2016), the relative importance of each is not clear.

Spatial variation among fungal communities can arise, however, despite similarities in their environment. Similarities among communities are expected to decline with increasing geographic distance (i.e. distance–decay), potentially due to dispersal limitation exerting a stronger influence than environmental filtering (Kivlin *et al.* 2014). However, distance–decay patterns can vary depending on spatial scale. Some studies show that they can be most pronounced at small scales (Bahram *et al.* 2012) while others showed patterns most noticeable only between continents and not within continents, largely due to stronger effects of environmental filtering via soil properties (e.g. salinity and nutrient availability) than dispersal limitation (Kivlin, Hawkes and Treseder 2011).

In different compartments of a host plant, like the root endosphere and rhizosphere soil, fungal communities can be structured uniquely, reflecting their idiosyncratic interactions with biotic and abiotic factors. For instance, soil fungal communities tend to be structured by stochastic processes (Powell *et al.* 2015; Goldmann *et al.* 2016) and environmental filtering (Kivlin *et al.* 2014). In contrast, fungi in the root endosphere (i.e. fungal endophytes) can be strongly shaped by both abiotic and biotic factors via host filters (Alzarhani *et al.* 2019). Endophytes are recruited from the surrounding soil fungal communities and are therefore dependent on the local environment. Furthermore, acquisition of endophytic microbes in the root endosphere can be determined by host plant defense strategies, root structure and root exudation (Bulgarelli *et al.* 2012; Edwards *et al.* 2015). Thus, we expect that these communities will be influenced by biotic and abiotic factors of their environment in different ways and magnitudes.

Here, we examined the factors along salinity and hydrological regime gradients that contribute to shaping the endosphere and rhizosphere fungal communities associated with baldcypress (*Taxodium distichum*), a tree species dominant in wetlands along the southeastern coast of the USA. In coastal wetland ecosystems, salinity gradients and hydrological regimes occur over large spatial scales, shaping forest structure and densities of baldcypress (Krauss *et al.* 2009). By extension, associated root endosphere and rhizosphere fungal communities of baldcypress can be directly affected by biotic and abiotic factors along ecological gradients. We sought to: (i) address whether root endosphere and rhizosphere soil fungal communities exhibit parallel shifts in diversity and composition along ecological gradients and across space; and (ii) assess the relative influences of biotic (e.g. host tree density, canopy cover) and abiotic (e.g. salinity, water level or regime) factors on fungal communities along ecological gradients.

METHODS

Site and sample collection

Our study sites were located along landscape transects of coastal baldcypress-dominated swamp sites that were established in 2004 in GA, SC and LA, previously described in Krauss *et al.* 2009. Four landscape transects—one each from GA and SC, and two from LA—were selected (Table S1, see online supplementary material). Each transect includes two to four 1000 m^2 plots (paired 20 × 25 m plots, hereafter referred to as single 'plot'). The plots are located along salinity gradients that range from freshwater to mid-saline to oligohaline, and hydrological gradients ranging from non-tidal/tidal to strongly tidal.

Long-term measurements of salinity, conductivity, water level and water temperature have been recorded at each plot since 2004 (see online supplementary material). Tidal history—whether the plot was historically tidally inundated or not—was also recorded for each plot. To account for potential monthly variabilities, we took the average measurements of these variables within the 6–9 month-period prior to our sample collections (Table S1, see online supplementary material).

Baldcypress is a long-lived, deciduous tree that can grow up to 10–40 m tall. It has a wide distributional range within tidal-freshwater, forested wetlands as it can tolerate a wide range of soil and salinity conditions including dry to wet soil (Hook 1984; Krauss, Chambers and Creech 2007). We collected rhizosphere soil and roots from five baldcypress trees, ∼5 m apart, at each plot. Plots in SC and GA were sampled in June 2015, while samples from LA were collected in March 2016 ($n = 120$). Roots and soil were immediately stored at 4°C in the field, processed in the lab (see below) and transferred to −20°C prior to DNA extraction.

For biotic factors, we used host density, percent canopy cover and woody debris volume. Baldcypress tree density within a plot was measured as the number of individual trees >10 cm in diameter at breast height (dbh)/ha (Krauss *et al.* 2009). Percent canopy cover (a proxy for host cover relative to non-host species) was measured from each plot (see online supplementary material), along with woody debris volume (m^3/ha)—the volume of downed wood up to 1 m above the surface. Canopy cover and

woody debris volume measurements were taken in 2014. Baldcypress densities in GA and SC were measured in December 2014, while for LA plots, densities were recorded in 2012. We note that the scope of our study does not cover host genetic identity or genotype.

Fungal community profiling

Root samples were cut into 2–3 mm^2 pieces and then surface-sterilized by sequential immersion in 70% ethanol for 10 s, 3.125% sodium hypochlorite for 2 min, and rinsing with sterile distilled water twice. Roots were ground in liquid nitrogen using a mortar and pestle, and 30 mg of materials was used for extraction of total genomic DNA with a MoBio PowerPlant DNA isolation Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Soil surrounding the roots was collected and frozen immediately, and DNA extraction was carried out using the MoBio PowerSoil DNA isolation Kit.

Fungal community composition was characterized by an amplicon sequencing approach targeting the fungal 'barcode'18S rDNA Internal Transcribed Spacer (ITS1) region. Genomic DNA was first standardized to 20 ng before amplification. Libraries were generated by a two-step amplicon PCR approach using the standard ITS1 region primers ITS1F and ITS2 (adapted from Nguyen *et al.* 2015) modified with the Illumina TruSeq adapter, followed by the indexing PCR (see online supplementary material). Indexed libraries were purified using the Qiaquick PCR Purification Kit (Qiagen, Hilden, Germany), quantified using the Quant-iT® dsDNA HS Assay kit in Qubit Flourometer (ThermoFisher Scientific, Waltham, MA, USA) and pooled at equal amounts (20 ng) prior to sequencing. Purified libraries were run on the paired-end Illumina MiSeq platform at the Duke IGSP Genome Sequencing and Analysis Core Facility (Durham, NC, USA). Negative controls were also included.

MiSeq sequences were filtered for quality, and adaptors/distal priming sites were removed, keeping a minimum sequence length of 50 bp cutadapt v1.7.1 (Martin 2011). Further filtering of the sequences by removing homopolymers of up to 9 bases at both ends of sequences, short sequences (<125 bp were removed) and those containing ambiguous bases were done in mothur v.1.34.4 (Schloss *et al.* 2009). Cleaned-up sequences were then dereplicated and clustered into operational taxonomic units (OTUs) at a 97% threshold, with chimera detection and removal using the uparse algorithm in USEARCH (Edgar 2013). We excluded singleton OTUs (OTUs with sequence count = 1) as they can be artifacts of PCR and sequencing (Nguyen *et al.* 2015). Taxa assignment of OTUs was done by BLAST alignment against the reference database UNITE v.7.2 (Nilsson *et al.* 2018). A single representative sequence was first picked using the pick_rep_set.py command in QIIME2 (Caporaso *et al.* 2010), then representative sequences were assigned taxonomy in BLAST++ v 2.2 (Camacho *et al.* 2009) with a threshold of 80% hit length alignment and 80% identity. OTUs falling below the threshold were removed and were recorded as 'unidentified'. All succeeding analyses were done at the OTU level (i.e. all OTUs that can be identified as Fungi), except for the analyses describing taxonomic composition (see below). The sequencing data were deposited in the National Center for Biotechnology Information, Sequence Read Archive (SRA) database with the accession number PRJNA638111.

Statistical analyses

All analyses were conducted separately for root endosphere and rhizosphere soil samples using the R statistical computing platform v.3.5.1 (R Development Core Team 2016). Prior to analyses, we rarefied our community matrix data to $n = 2500$ sequences.

Within-community fungal diversity (equivalent to alpha-diversity) was expressed as Shannon diversity (Shannon 1948) and OTU richness (number of OTUs present within a community) and calculated in vegan (Oksanen *et al.* 2013). Data were natural log-transformed to fit model assumptions, and all continuous variables were scaled to standard z-scores where necessary, with mean of 0 and standard deviation of 1 in order to compare estimates among variables. We examined general patterns of fungal diversity along ecological gradients by first conducting linear regression analyses with Shannon diversity and richness as response variables and host density, salinity and water level as predictors. Given that salinity and fungal diversity might not have a linear relationship (i.e. fungal richness might reach the highest level at intermediate salinity levels (hump-shaped pattern)), we also conducted a separate quadratic regression model analysis on both Shannon diversity and OTU richness with salinity as the explanatory variable for endosphere and rhizosphere separately.

In order to assess shifts in fungal community composition in endosphere and rhizosphere (beta-diversity) at the OTU level relative to the biotic and abiotic factors, we examined community variation measured as abundance-weighted Bray–Curtis dissimilarity by conducting a permutational multivariate analysis of variance (PERMANOVA) at the plot level using the adonis function in vegan. We analyzed the main effects of plot nested within transect, host density, percent canopy cover, woody debris, tidal history, salinity and water level. Terms were added sequentially, with the resulting adjusted R^2 values for each factor plotted to visualize the effect size accounted for by these factors. Non-metric multidimensional scaling (NMDS) was also conducted to visualize patterns of among-community dissimilarities in vegan. We further analyzed the compositional (dis)similarities at the plot level by hierarchical clustering of the distributions of OTUs using the hclust function (Supporting Information), then visualized clustering as a dendrogram using dendextend (Galili 2015).

We also assessed the compositional differences between individual plants in the abundances of taxa representing different taxonomic levels, which allowed for evaluating whether compositional shifts based on the OTUs described above were mirrored by changes at specific taxonomic levels (e.g. phylum). OTUs were collapsed into specific taxonomic levels, and sequence counts were summed to that level. All unclassified OTUs were included in the analyses and were lumped together. We rarefied samples at $n = 600$ sequences and performed separate PERMANOVA analyses at each level of taxonomic group on the endosphere and rhizosphere community compositions for each individual plant, with abundance-weighted Bray–Curtis index calculated at each taxonomic level, using the same PERMANOVA model described above. We further assessed compositional changes in abundances of different fungal guilds by assigning guilds on OTUs that are only identifiable to genus level using the FUNGuild software (Nguyen *et al.* 2016), keeping only assignments with 'probable' and 'highly probable' confidence as recommended in Nguyen *et al.* (2016). We conducted PERMANOVA analyses (model as described above) to examine shifts in guild compositions due to environmental factors, and used the multivariate statistical framework MaAsLin2 (multivariate association with linear models) (Mallick *et al.* in review, 2020) to investigate whether a specific guild is significantly associated with the environmental factors. For MaAsLin, significant associations were assessed using the FDR-corrected values, the q-values.

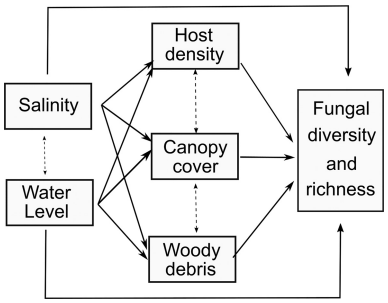

Figure 1. Metamodel or causal diagrams used as the basis for evaluating hypothesized direct and indirect effects of salinity and water level on endosphere and rhizosphere fungal diversity. The metamodel structure includes the indirect effects via multiple biotic pathways (host density, percent canopy cover and woody debris volume) of salinity and water level on fungal (Shannon) diversity and richness. Dashed lines represent correlated terms in the model.

As variation among fungal communities might be due to spatial distance, we examined distance–decay relationships among fungal communities. We evaluated how communities vary along spatial distances relative to other environmental differences by conducting a multiple regression on distance matrices (MRM) analysis of pairwise community similarities as a function of geographic and environmental distances. As we lacked the individual tree coordinates, we aggregated trees at the plot level and summed the OTU abundances at that level. Pairwise plot similarity was measured as abundance-weighted Bray–Curtis index. The pairwise geographic distances between plots were calculated with the distGeo function in geosphere (Hijmans, Williams and Vennes 2015). To control for effects of environmental differences, we also included the Euclidean differences in the environmental parameters (salinity and water level). Similarity matrices were regressed against the geographic distance matrix and the Euclidean distance matrices for differences in both salinity and water level using ecodist (Goslee and Urban 2007).

Path analysis (structural equation modelling)

As salinity and water level can directly and indirectly affect fungal diversity via biotic factors, we investigated the causal pathways upon which abiotic factors can influence fungal diversity (alpha-diversity) by constructing a meta-model and conducting a multilevel path analysis (Shipley 2009) using the package piecewiseSEM (Lefcheck 2016). Our meta-model consisted of combined direct and indirect effects via host density, percent canopy cover and woody debris of both salinity and water level on fungal diversity (Figure 1). First, we constructed separate models for host density, percent canopy cover and woody debris with salinity and water level as predictor variables. We then constructed two models that predicted fungal diversity—separate for richness and Shannon diversity—using salinity and water level (direct) and biotic factors (indirect) as predictor variables. The five models were then combined and tested with the d-separation test for global goodness-of-fit (Shipley 2009), and we derived an AIC score from Fisher's C statistic (CIC) and extracted the path coefficients from the models that fit. In order to meet assumption of residual normality, we log-transformed host density, percent canopy cover and woody debris volume. In addition, given that salinity and fungal diversity might not have a linear relationship, we explored both linear and non-linear (quadratic regression model) relationships of salinity with fungal diversity in determining the best fit model. We utilized a step-wise fitting procedure in which we first fit the model as specified in the metamodel, then added correlated error terms (between pairs of factors: salinity, water level, host density and canopy cover, canopy cover and wood debris) to improve model fit, based on estimates of path strength. We report standardized path coefficients and R^2 values for each downstream variable.

RESULTS

Root endosphere and rhizosphere soil fungal communities

We obtained 5.8 million sequences from 130 root and rhizosphere soil samples after quality filtering. Of these, we delineated 1105 fungal OTUs for roots and 7916 fungal OTUs in rhizosphere soil (Fig. 2A). Fungal community composition of the endosphere and rhizosphere exhibited clear separation based on the NMDS ordination analysis using the abundance-weighted Bray–Curtis dissimilarity index (Fig. 2B). In addition, endosphere communities were not a subset of the rhizosphere soil fungal communities (Figure 2B), although ~60% of the root endophyte OTUs were shared between endosphere and rhizosphere communities (Fig. 2A).

Taxa identification based on BLAST showed that most of the OTUs were classified as Ascomycota (41.7%), Basidiomycota (7.6%) or Glomeromycota (6.2%), with Zygomycota, Rozellomycota, Chytridiomycota and Blastocladiomycota each comprising <5%. About 40% of the sequences could not be assigned below phylum level. At the class level, Leotiomycetes, Sordariomycetes, Wallemiomycetes and Glomeromycetes were the most dominant classes present among endosphere communities, although the abundances of each class varied across different plots (Fig. S1, see online supplementary material). Most recovered taxa in the endosphere were assigned to undefined saprotrophs (45%) and arbuscular mycorrhizal (25%) guilds. In contrast, Archaeorhizomycetes and Sordariomycetes were dominant among rhizosphere soil communities. Based on guild assignments, soil and undefined saprotrophs (26% and 15%, respectively) followed by plant pathogens (13%) were the most abundant guilds recovered among rhizosphere communities.

Root endosphere and rhizosphere soil diversity along ecological gradients

Abiotic factors showed stronger filtering effects than biotic factors on root endosphere diversity. Salinity and water level had a strong influence on endosphere fungal OTU richness. Endophyte richness exhibited a hump-shaped pattern based on quadratic regression analysis ($R^2 = 0.226$, $P = 0.003$), with greatest richness at an intermediate salinity level (Fig. 3A), whereas it increased with increasing water level (Fig. 3B). On the other hand, endosphere richness significantly increased with increasing canopy cover ($R^2 = 0.11$, $P = 0.024$, Fig. 3C).

Rhizosphere diversity was less influenced by biotic and abiotic factors compared to root endosphere communities. Only water level showed a significant effect on rhizosphere OTU richness, with fungal richness decreasing as water level increased ($R^2 = 0.11$, $P = 0.015$), in contrast to the endosphere (Fig. 3B). Rhizosphere diversity remained the same along the salinity gradient. Although not statistically significant, based on quadratic regression analysis, rhizosphere fungal richness exhibited a U-shaped pattern, with lowest richness at an intermediate salinity level, in contrast to the root endosphere (Fig. 3A). Similarly, rhizosphere diversity and richness were not significantly correlated with host density.

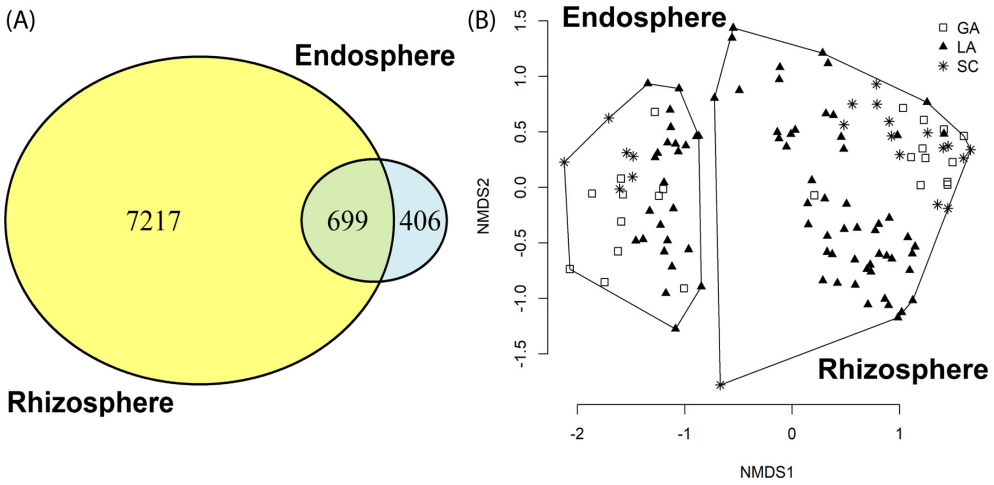

Figure 2. (**A**) Venn Diagram of OTU counts shared between and distinct within root endosphere and rhizosphere fungal communities; (**B**) NMDS ordination showing compositional differences between root endosphere and rhizosphere soil fungal communities across the three sites based on the Bray–Curtis dissimilarity index.

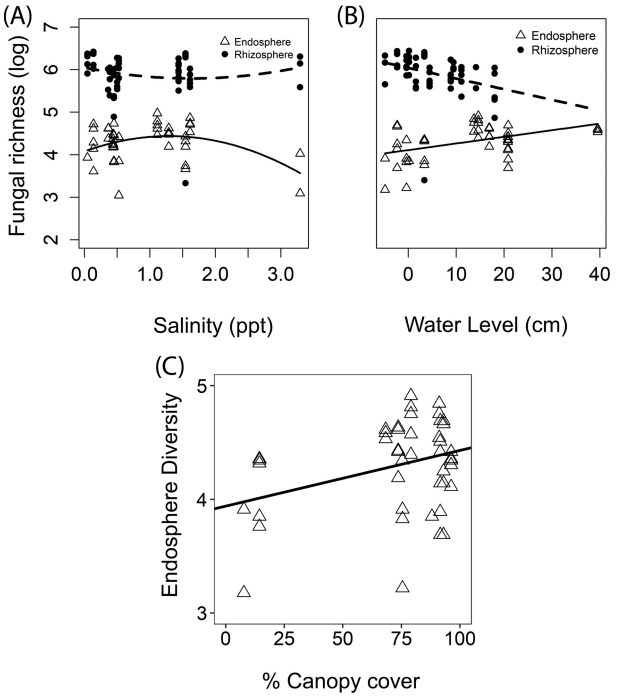

Figure 3. Rhizosphere soil and root endosphere fungal OTU richness exhibited contrasting patterns along (**A**) salinity and (**B**) water level gradients. (**C**) Root endosphere diversity increased with increasing percent canopy cover. Lines are regression lines (dashed for rhizosphere and solid line for root endosphere).

Path analysis: indirect and direct effects of salinity and water level on fungal diversity

In our structural equation modelling (SEM) analysis, the overall best model fit for endosphere data showed direct and indirect effects of salinity on fungal (alpha) diversity (Fisher's C = 3.616, $P = 0.164$, Fig. 4A). As predicted, the direct effects of salinity on endosphere diversity appear to be non-linear in nature (i.e. quadratic regression was significant in the SEM model). The indirect effects of salinity appeared to be mediated through changes in the percent canopy cover, where we found a significant positive correlation between canopy cover and fungal richness (Fig. 4A, Table S2, see online supplementary material). Thus, the total effect of salinity, combining both direct and indirect causal pathways, was a quadratic response where endosphere diversity was highest in the intermediate salinity range and low in the low and high ends of the salinity gradient (Figs 4A and 3A). In contrast, the metamodel for rhizosphere did not identify any causal mechanisms for the effects of water level on rhizosphere fungal diversity although we observed direct linear effects of salinity on fungal richness and diversity (Fisher's C = 2.925 $P = 0.232$, Fig. 4B, Table S3, see online supplementary material). Both the endosphere and rhizosphere metamodels also identified direct, negative effects of salinity on host density and percent canopy cover, while water level had positive direct effects on woody debris volume (Fig. 4).

Shifts in fungal community composition (beta diversity) along ecological gradients

Endosphere communities exhibited compositional shifts along environmental gradients based on PERMANOVA analyses (at the OTU level). Significant compositional shifts were observed along both salinity ($R^2 = 0.043$, $P < 0.001$) and water level ($R^2 = 0.040$, $P < 0.001$, Fig. 4A) gradients. Canopy cover and woody debris, though not host density, also exhibited a strong influence on endosphere communities, although the greatest source of variation was due to geographic distance (i.e. transect ($R^2 = 0.120$, $P < 0.001$) and plot ($R^2 = 0.031$, $P < 0.05$)). These shifts in endosphere composition were also reflected in changes at different taxonomic levels (e.g. phylum, class; Fig. S2, see online supplementary material). While we did not find significant associations between specific guild (e.g. saprotroph) and environmental factors based on MaAsLin analysis, there were significant changes in the composition of guilds due to woody debris ($R^2 = 0.07$, $P < 0.001$; Table S4, see online supplementary material).

Rhizosphere communities, however, showed significant turnover (OTU-level) along both water level ($R^2 = 0.034$, $P < 0.001$) and salinity ($R^2 = 0.032$, $P < 0.001$) gradients (Fig. 5B), as well as tidal history (Fig. 5B). Biotic factors also demonstrated strong effects on the structure of rhizosphere communities as canopy cover, host density and woody debris volume were significantly correlated with community shifts (Fig. 5B). We also observed community structuring due to distance, as transect and plot showed strong influence on among-community variations ($R^2 = 0.157$, $P < 0.001$).

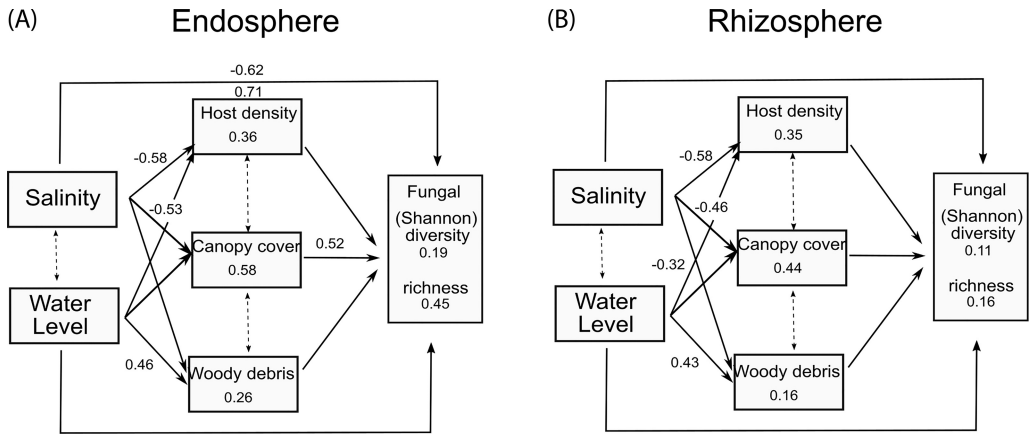

Figure 4. Path analysis testing the direct and indirect effects of salinity and water level on (**A**) endosphere and (**B**) rhizosphere fungal diversity and richness associated with baldcypress. Numbers inside the boxes represent R^2 values, while numbers associated with arrows are regression (path) coefficients that illustrate the magnitude and direction of the relationships between the variables. Only significant relationships have coefficients beside the arrows. Dashed lines represent terms that were designated as correlated when constructing the model. The regression coefficients for the effects of salinity on endosphere diversity in (A) were comprised of a positive and a negative value representing the non-linear (quadratic) nature of the relationship.

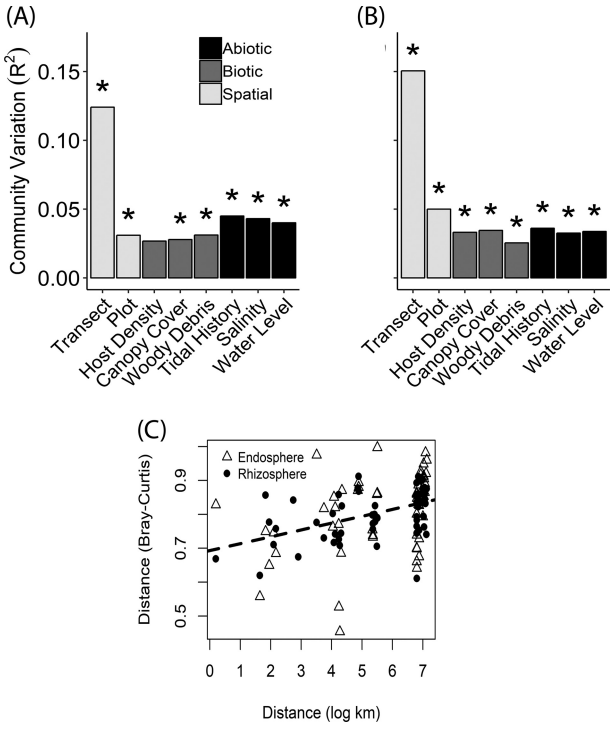

Figure 5. Sources of variation among fungal communities in (**A**) root endosphere and (**B**) rhizosphere soil based on PERMANOVA analyses. Only factors that generated estimates in the analysis were visualized (*$P < 0.05$). (**C**) Regional distance–decay relationships for root endosphere (open triangle) and rhizosphere soil (filled circle, dashed line) fungal communities aggregated at the plot level. Line is regression line.

Similar to the endosphere, PERMANOVA analyses of changes across different taxonomic levels mirrored shifts at the OTU level, although rhizosphere taxa appeared to be more correlated with biotic than abiotic factors. Both canopy cover and woody debris demonstrated significant influence on taxonomic shifts in soil communities from class to species level, while shifts in rhizosphere taxa from order to species levels were correlated with host density (Fig. S3, see online supplementary material). These changes were mirrored as shifts in abundant families along water regimes. For example, in the plots with low water level (<1 cm above ground surface), Mortierellaceae was the most abundant family in soil communities while Lasiosphaeriaceae and Archaeorhizomycetaceae were dominant across plots with greater water levels (>8 cm). Salinity showed significant correlation with shifts in rhizosphere taxonomic composition only at the species level.

We only observed significant association between undefined dung/wood/soil saprotrophs and baldcypress density (coefficient = −0.064, q = 0.009) based on MaAsLin analysis. However, salinity, canopy cover and woody debris volume all significantly correlated with shifts in guild compositions among rhizosphere fungi (Table S5, see online supplementary material).

Regional spatial structure: distance–decay relationships

Root endosphere fungi exhibited no significant spatial structure based on the MRM analyses (Fig. 5C). In contrast, rhizosphere communities showed spatial structure, demonstrating significant distance–decay relationships as plots closer to each other harbored more similar fungi than those plots farther away (i.e. decreasing similarities with increasing geographic distance, Fig. 5C). In addition, rhizosphere communities tended to harbor similar fungi in plots with little difference in water level regardless of geographic distance (Table S6, see online supplementary material).

DISCUSSION

Our study highlights the relative importance of biotic and abiotic factors along ecological gradients in shaping biodiversity of fungal communities of baldcypress occurring in coastal areas. We highlight three key findings. First, abiotic factors exhibited greater influence than biotic factors in shaping overall fungal diversity and composition. Compositional turnover among root endophytes occurred along salinity and water level gradients, accompanied by peak endosphere diversity at intermediate salinity levels. Salinity had both direct and indirect effects mediated through host plant community structure (canopy cover) on endosphere fungal diversity based on SEM analysis. In contrast, rhizosphere fungal richness was negatively correlated with water level, despite a high degree of compositional

turnover. Second, biotic factors influenced endosphere and rhizosphere fungal diversity and composition, though this was more evident among endosphere communities. Third, rhizosphere but not endosphere fungal communities were highly variable across geographic distance, exhibiting distance–decay patterns. Overall, these results show divergent filtering between root endosphere and rhizosphere soil communities along ecological gradients, suggesting that their assembly and structure were shaped by different processes.

Local abiotic conditions can regulate the assembly of fungal communities in varying magnitudes along ecological gradients. The endosphere and rhizosphere communities of baldcypress in wetlands were sensitive to water level changes. Other studies have found similar results for bacteria and fungi in wetland (Chambers *et al.* 2016; Ma *et al.* 2018) and temperate soils (Brockett, Prescott and Grayston 2012), attributing these community changes to the effects of soil moisture. Interestingly, we found endosphere diversity increased while, in contrast to other studies (e.g. Santos-Medellín *et al.* 2017), rhizosphere fungal diversity declined with increasing water level, suggesting potential selective enrichment of certain taxa from the rhizosphere to the endosphere. High water levels can induce stress, even for flood-tolerant baldcypress (Krauss *et al.* 2009), modifying the amount and composition of plant root exudates (Henry *et al.* 2007; Song *et al.* 2012), thus leading to selective promotion of specific taxa in the rhizosphere by the host plant. At the same time, under these high water level conditions, baldcypress might lose the ability to filter the endophytes that colonize their roots, thereby resulting in increased diversity, as observed in other studies (e.g. Kwaśna, Szewczyk and Behnke-Borowczyk 2016).

Shifts in abundant families were evident for soil and root communities. For instance, among endosphere communities, Lulworthiaceae, whose members are predominantly marine decomposers (Kohlmeyer, Spatafora and Volkmann-Kohlmeyer 2000), was abundant in high water level plots (>8 cm). However, it declined in abundance among low water level plots (<1 cm). The opposite pattern was found in Diatrypaceae (comprised mostly of pathogens). Again, this might be due to baldcypress losing the ability to filter colonizing endophytes such as pathogens into their roots under these high water level conditions. Among rhizosphere communities, Mortierellaceae was the most abundant in low water level plots. While recent work suggests that some endophytic members of this family can promote plant growth (Wani *et al.* 2017), the Mortierellaceae are primarily saprotrophs occurring in soil and decaying organic matter (Petkovits *et al.* 2011). Since we found Mortierellaceae in rhizosphere, and saptrotrophs were the most abundant guild observed in the rhizosphere, decomposition is a more likely role in our study. Under saturated soil conditions (high water level), the rates of aerobic microbial activity and breakdown of organic matter are suppressed (Hobbie *et al.* 2017), which might explain why saprotrophs are more abundant in soil with low water level in the rhizosphere soils.

The effects of water level on fungal communities can be confounded by the correlation with salinity. It is worth noting though, that in our study, plots with high water levels did not necessarily correlate with lower salinity. While salinity is generally considered a major factor structuring plant communities in wetland ecosystems (Brock, Nielsen and Crosslee 2005; Krauss *et al.* 2009), and by extension, the plant-associated fungal communities (Mohamed and Martiny 2011; Maciá-Vicente *et al.* 2012), here, the effect of salinity was more evident among endosphere than rhizosphere communities. Endosphere richness peaked at intermediate salinity levels but declined under high salinity, likely due to potential increases in the abundance of salt-tolerant fungal taxa. This pattern might reflect nutrient stress experienced by the host plant under high salinity (Zhai *et al.* 2018). This hump-shaped pattern of species diversity with salinity has been observed in fungi (Coince *et al.* 2014), bacteria (Claire Horner-Devine *et al.* 2003) and plants (Bryant *et al.* 2008). Our results are relevant given the magnitude of on-going environmental changes—including saltwater intrusion and increasing water levels—that can potentially affect host plant communities and their associated microbiomes.

Biotic factors related to the host plant can also regulate the diversity and composition of plant-associated fungal communities. Here, we found strong support for plant community structure (canopy cover) mediating the effects of salinity on fungal endosphere diversity. Surprisingly, canopy cover rather than host density exhibited a strong positive correlation with diversity of endosphere communities: plots with higher percent canopy cover tended to harbor more diverse root endophytes. In our study sites, canopy cover partly reflects plant (tree) community structure i.e. plant diversity. Although baldcypress is the dominant species at all sites, other tree species such as *Nyssa aquatica*, *Nyssa sylvatica* and/or *Nyssa biflora* also contributed to canopy cover. Other studies have also found that forest cover and plant diversity cover influenced colonization of foliar endophytes in the tropics (Arnold and Herre 2003; Arnold and Lutzoni 2007; Saucedo-Garcia *et al.* 2014).

Association between diversity of plants and endosphere diversity could arise from multiple pathways, including environmental filtering such as that imposed by salinity, host specialization (Joshee *et al.* 2009) or dispersal limitation (Oono, Rasmussen and Lefèvre 2017). Increased complexity or diversity in vegetation can create opportunities for organisms subsisting on or associated with them such as endosphere fungal species, and thus promote fungal diversity (Saikkonen 2007). Alternatively, greater canopy cover might also provide a better growth environment as the soil surface would not be subjected to higher light levels and thus, would have a more moderate temperature throughout the summer months (Allen *et al.* 2016). This can, in turn, enhance or promote fungal diversity. While we did not find strong support for correlation between host density and fungal diversity, it is worth noting that there was a strong correlation between host density and percent canopy cover, given that baldcypress dominates all plots.

In contrast to the endosphere, rhizosphere (alpha) diversity did not correlate with canopy cover or host density—although there was significant fungal community turnover along a range of canopy cover and host density values—suggesting that biotic effects found in the endosphere may not be as strong as in rhizosphere soil fungal communities. Some studies have demonstrated that biotic factors (i.e. host filters) tend to have stronger coupling with root endophytes than with soil fungal communities (e.g. Goldmann *et al.* 2016).

The high variability in rhizosphere fungal communities that we found across geographic distance suggests that, to a certain extent, dispersal limitation of fungi in soils can constrain the assembly of these communities along ecological gradients. Across similar local environments (e.g. plots with the same water level) but tens of kilometers apart, dispersal limitation can alter the species pool available to colonize a given host plant. Thus, similarities among communities in the rhizosphere declined with increasing geographic distance, similar to other studies (Kivlin *et al.* 2014; David, Seabloom and May 2016). On the other hand, the lack of spatial structure among endosphere communities further corroborates our findings that the

influence of biotic or host filters were more pronounced on root endophytes than on rhizosphere communities. Alternatively, it is possible that the spatial structure we found reflects other unmeasured environmental variables rather than dispersal limitation, which is more pronounced on rhizosphere communities, and less so with endosphere communities where they are more tightly influenced by biotic factors.

While the biotic and abiotic factors measured in this study were not comprehensive (e.g. host genotype was not measured but could be important as well), our study gives insights into the relative magnitude of their influence in shaping fungal diversity. Within individual hosts, fungal community assembly can be a product of complex interactions between these biotic and abiotic factors, which can lead to varying magnitudes of their effects for the root endosphere versus rhizosphere. While it is not clear how host-associated microbiomes confer benefits on host plant fitness, a basic understanding of factors shaping the diversity and composition of these communities is important for assessing how environmental changes affect plant and plant-associated fungal communities. For coastal wetland ecosystems, saltwater intrusion and sea level rise are major threats to both plant and fungal communities that can impact plant community and microbial functions (Chambers *et al.* 2016; Pettit, Bayliss and Bartolo 2016). In turn, these impacts can scale up to ecosystem level via changes in microbial activities and key processes such as organic matter decomposition and nutrient cycling (Jackson and Vallaire 2009; Morrissey *et al.* 2014). Quantifying the direct and indirect impacts of these forces can give insights into mitigating threats imposed by these environmental changes.

DATA ACCESSIBILITY

Illumina sequences are available in the NCBI SRA with accession number PRJNA638111. Other data information and R scripts used in the analyses are publicly available under github.com/VanBaelLab.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online

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AUTHOR CONTRIBUTIONS

S.A.V.B., W.H.C. and K.W.K. conceived and designed the experiment, E.R.K. collected root and soil samples, R.H.D. collected environmental data, C.Y.L. and E.R.K. conducted molecular work; C.Y.L. led the statistical analyses with assistance from all authors. C.Y.L. wrote the first draft of the manuscript and all authors contributed significantly to succeeding drafts.

Conflict of Interest. None declared.

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