BALDCYPRESS (*TAXODIUM DISTICHUM*) ASSOCIATED MICROBES IN A DYNAMIC COASTAL LANDSCAPE

AN ABSTRACT
SUBMITTED ON THE SIXTH DAY OF MAY 2019
TO THE DEPARTMENT OF
ECOLOGY AND EVOLUTIONARY BIOLOGY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
OF THE SCHOOL OF SCIENCE AND ENGINEERING
OF TULANE UNIVERSITY
FOR THE DEGREE
OF
DOCTOR OF PHILOSOPHY

BY

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ABSTRACT

The influence of environment on microbial community structure is of increasing interest, especially in coastal habitats where climate change is rapidly altering the landscape. In this dissertation, I characterize the microbial communities associated with a key wetland species, the baldcypress tree (Taxodium distichum), and examine the relationship between environment, geographic distance, and microbial community composition. In a culture-based study of T. distichum leaf and root endophytes, I found that both salinity and flooding contributed to bacterial and fungal endophytic community composition. Additionally, I report that diversity and endophyte isolation frequency were higher in roots than in leaves, with leaf bacteria being almost negligible. Using 16S Illumina profiling, I found that geographic distance correlated with rhizosphere but not root endosphere bacterial communities and that mean water level, mean salinity, and the volume of woody debris were correlated with both endosphere and rhizosphere bacterial communities of T. distichum. Finally, using salt challenge assays, I isolated five strains of extreme halotolerant endophytes and eleven strains of moderately halotolerant endophytes— a necessary first step towards using endophytes for restoration, or towards understanding the functions of some of these organisms in situ. This dissertation demonstrates a connection between environmental variables, plant symbionts, and a key restoration species and may help in predicting future outcomes of sea level rise for endophytes communities in baldcypress and other wetland plants.

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INTRODUCTION

A fundamental goal of community ecology is to understand the patterns in the diversity, abundance, and composition of species in communities and the processes underlying these patterns (Vellend 2010). Drivers of macro-organismal community structure have been the focus of research and debate for over a century (Clements 1916; Diamond 1975; Hubbell 2001; Tilman 2004) while explorations into microorganism communities have received comparatively less attention. A central question in microbial ecology continues to be: what factors influence microbial community composition? The Baas Becking hypothesis (1934) posits that "everything is everywhere, and the environment selects," meaning that microbial species are globally dispersed (by wind, water, and animal/insect vectors) but environmental filtering (e.g. site condition, soil properties, water regimes) precludes some species from surviving in particular habitats. Others have proposed that microbes are in fact dispersal limited and that distance between sites is a more important driver of community assembly than environmental parameters (Martiny et al. 2006; Goldmann et al. 2016). These phenomena remain underexplored, especially for microbes associated with plants.

A key group of plant associated microbes are the endophytes, namely fungi and bacteria, which live entirely within the tissues of their host plants without causing apparent symptoms of disease (Wilson 1995; Friesen et al. 2011). Endophytes have been found in all plants examined to date and in all plant parts including leaves, roots, flowers, stems and buds (Friesen et al. 2011). Most endophytes are thought to be horizontally-transmitted to their host plant via wind, rain, insect vectors, soil and water (Rodriguez et al. 2009). Plants

may also use root exudates to recruit endophytes from the rhizosphere—the layer of soil directly surrounding the root (Compant et al. 2010). A mounting body of literature suggests that endophytes can have a substantial influence on a plant's performance, contributing to nutrient uptake and increased growth as well as a plant's ability to respond to abiotic stressors such as temperature changes, flooding and salinity (Arnold et al. 2003; Lemons et al. 2005; Mandyam & Jumpponen 2008; Deivanai et al. 2014).

Salinity and flooding stress are of particular concern in coastal areas, where the environment is being altered by climate change (Mimura 2013). Here, salt water inundation— the movement of salt water into previously fresh areas— is being exacerbated by sea level rise and storm surge (White & Kaplan 2017). As this occurs, bottomland hardwood forests and swamps are being degraded and transformed to open marshes or ghost forests where only standing dead or stressed trees remain amongst an understory of oligohaline plant species (Shaffer et al. 2009). In these landscapes, baldcypress trees (*Taxodium distichum*) are often the last remnants of the previously freshwater inhabitants. These deciduous conifers are the dominant trees in many swamp ecosystems and are crucially important to the Gulf Coast region as the major buffers against storm damage (Krauss et al. 2009; Shaffer et al. 2009). Little is known about the symbionts of this key restoration species and the extent to which salinity and flood regime structure endophytic community assembly. Because most plant species have narrow salinity tolerances, baldcypress' ability to span fresh water to brackish water as well as to persist in a variety of flooding regimes (Allen et al. 1996), make them an excellent candidate for studying interactions between endophytes and environmental variables associated with climate change.

My doctoral research investigates the endophytic and rhizosphere communities

associated with *T. distichum* in the southeastern United States. My first chapter characterizes the culturable fungal and bacterial endophytes in the roots and leaves of baldcypress trees at four sites with varying levels of salinity and flooding regimes in southeastern Louisiana. In my second chapter, I use 16S Illumina sequencing to characterize the communities of baldcypress root endosphere and rhizosphere bacteria along established salinity and flooding gradients in the greater southeastern United States. And finally, in my third chapter, salt challenge assays are applied to screen *T. distichum* and *Spartina alterniflora* (smooth cordgrass) endophytes for salt tolerance—a necessary first step towards implementing endophytes for restoration, or towards understanding the functions of some of these organisms *in situ*.

Despite their known influence on plants' phenotypes, ecological interactions, and community dynamics, little is known about what structures communities of endophytes within a host plant (Christian et al. 2015; Partida-Martínez & Heil 2011; Saunders et al. 2010). However, the unification of microbial technological advances with ecological theory is allowing us to elucidate the patterns underlying these cryptic and critical bacterial and fungal symbionts. My doctoral research is the first to investigate how environmental variables associated with climate change (specifically increased coastal inundation brought about by sea level rise) are structuring *T. distichum* microbial communities and is the first to use 16S Illumina sequencing to characterize these communities. Understanding the community dynamics of these key microbes will inform the fields of plant biology, plant ecology, and community ecology; may have implications for restoration; and will also help us to predict how microbial communities in coastal swamp ecosystems are shifting as climate change continues to alter this dynamic landscape.

CHAPTER 1. WATER LEVEL AND SALINITY DRIVE COMMUNITY STRUCTURE OF CULTURABLE BALDCYPRESS (*TAXODIUM DISTICHUM*) ENDOPHYTES IN SOUTHERN LOUISIANA

ABSTRACT

Little is known about effects of salinity and flooding on plant symbionts, including baldcypress trees (*Taxodium distichum*), the dominant trees in many swamp ecosystems in the southeastern US. In this study, we characterize the culturable fungal and bacterial endophytes in the roots and leaves of baldcypress trees at four sites with varying levels of salinity and flooding regimes in southeastern Louisiana. Both salinity and flooding (water level) contributed to endophytic community composition of leaves and roots. We found that diversity and endophyte isolation frequency were higher in roots than in leaves, with leaf bacteria being almost negligible. Our study demonstrates a connection between environmental variables, plant symbionts, and a key restoration species. This work may help in predicting future outcomes of sea level rise for endophytes communities in baldcypress and other wetland plants.

INTRODUCTION

Land use change, sea level rise, and associated changes in salinity, hydrology, and flood dynamics are global concerns. However, the effects are especially acute in coastal communities and subsiding areas near river deltas, as in southern Louisiana, where swamps and marshes have been isolated from their sustaining rivers and are being degraded into submerged marsh and open water (Shaffer et al. 2009). Little is known

about effects of salinity and flooding on a key group of plant symbionts, the endophytes – microscopic fungi and bacteria that live asymptomatically within tissues of host plants (Porras-Alfaro & Bayman 2011). These microbial symbionts have been shown to increase host plants' resilience to biotic and abiotic stressors such as herbivory, disease, salinity, and temperature (Rodriguez et al. 1997; Redman et al. 2002; Rodriguez et al. 2009; Friesen et al. 2011; Redman et al. 2011). Endophytes have been found throughout the tissues of all plants examined to date (Rosenblueth & Martınez-Romero 2006) including baldcypress trees (*Taxodium distichum*) (Kandalepas et al. 2010).

Baldcypress are dominant trees of Cypress-Tupelo swamp ecosystems in the southeastern US. These deciduous conifers are crucially important to the Gulf Coast region as major buffers against storm damage and as focal restoration species (Shaffer et al. 2009). Because most plants have narrow salinity tolerances, baldcypress's ability to span freshwater to slightly brackish water (2 ppt salinity) and persist in variable flooding conditions (Allen et al. 1996) makes it an excellent candidate species for studying interactions between salinity levels, flooding, and plant endophytes.

In this study, we characterized the culturable fungal and bacterial endophytes in the roots and leaves of baldcypress trees at four sites with varying levels of salinity and flooding in southeastern Louisiana. Understanding the factors that structure microbial community composition has been the focus of much research over the past decades (Christian et al. 2015). Previous studies have demonstrated that endophyte community composition is influenced by plant organ (i.e. root, stem, leaf, etc.) and environmental variables (Compant et al. 2010; Zimmerman & Vitousek 2012; Higgins et al. 2014; de Souza et al. 2016). For wetland plants, however, we do not know how interactions among environmental variables influence symbiont communities, including those variables

associated with sea level rise. We predicted that baldcypress roots and leaves would harbor diverse (species rich) communities of culturable endophytes and that differences in the community diversity, isolation frequency, and composition would be driven by plant organ, salinity, and flooding. Our work on baldcypress endophyte communities explores the connection between the environment, plant symbionts, and a key restoration species, and may help in making predictions about the effects of sea level rise on wetland endophyte communities.

METHODS

Study Sites/Sampling

In October 2014, we harvested root and leaf tissue from 12 mature *T. distichum* trees (>20 m height) from four unique sites (n = 48) along a degradation gradient in southern Louisiana (Table 1.1). We sampled apparently healthy leaves and roots from three locations on each tree. All trees within a site were ~100 m apart from each other and inundated with water, having roots completely submerged at the time of collection. Degree of degradation was determined by flooding regime, salinity, and anthropogenic access/disturbance. Permanently flooded and impounded sites were designated as more degraded than periodically flooded sites; and brackish sites were more degraded than fresh sites. The four sites, listed from the healthiest to most degraded, are: Tickfaw (TF), a periodically flooded freshwater swamp within the Tickfaw River floodplain; Jean Lafitte (JL), a permanently flooded freshwater site in Jean Lafitte National Historical Park and Preserve; Honey Island Swamp (HI), a permanently flooded freshwater swamp site along the Pearl River; and Bonnet Carre Spillway (LP), a brackish site on Lake Ponchartrain that is permanently inundated (Table 1.1). Salinity data for each site was collected from the USGS Coastwide Reference Monitoring System (CRMS) website

(Steyer 2010) and is the average of the measurements taken monthly 1 year prior to our collection dates.

Table 1.1 Salinity, flooding regime and degradation of four sites in southern Louisiana.

Site	**Mean Salinity /Salinity Range(ppt)**	**Flooding Regime**	**Degree of Degradation**
Tickfaw (TF)	Fresh (0.1ppt)/ (0-0.2ppt)	Periodic	LOW: apparently healthy trees of varying ages, closed to heavy foot traffic
Honey Island (HI)	Fresh (0.2ppt)/(0.1-0.5ppt)	Permanent	LOW-MEDIUM: apparently healthy trees of varying ages, along a flowing river, visited by humans
Jean Lafitte (JL)	Fresh (0.6ppt) /(0.3-1.0ppt)	Permanent	MEDIUM: healthy adult trees with no young trees established, water impounded, few human visitors
Lake Pontchartrain (LP)	Brackish (1.3ppt) (0.8-1.7ppt)	Permanent	HIGH: Stressed trees with no young trees established, flowing water along the Bonnet Carre spillway in a high use area, near an elevated highway

Culturing

Samples were placed on ice and transported into refrigeration at Tulane University for processing following well- established protocols (Arnold et al. 2003) within 48 h of collection. For leaves, a sterile blade was used to remove leaf tips and the remaining tissues were cut into 2 mm long leaflet pieces and then surface sterilized via serial immersion in 95% ethanol (10 s), 10% Clorox (5.25% NaOCl-; 2 min), and 70% ethanol (2 min). For roots, apparently healthy, fine roots were selected, cut into 2 mm length sections and serially immersed in 70% ethanol (10 s), 50% Clorox (2 min), and sterile water (2 rinses). A surface sterilization control plate was used for each plant in this collection to be sure that surface sterilization was complete. For each individual tree, 32

surface sterilized sections of roots and 32 sections of leaves were randomly selected for plating on growth media. Of these 32, 16 pieces were plated on 2% malt agar (2% MEA: 20 g of Difco Malt Extract and 20 g of Difco Agar per L of deionized water) which is selective for fungi (Fröhlich & Hyde 1999). The remaining 16 pieces were plated on a non-salt containing nutrient agar (BD Difco Nutrient agar containing: beef extract 3 g/L, peptone 5 g/L, agar 15 g/L) which is selective for bacteria. The total sampled was n = 768 leaf and root pieces plated to screen for fungal abundance and n = 768 leaf and root pieces plated to screen for bacterial abundance. Plates were sealed, incubated at room temperature, and monitored daily for 5 weeks for emergent fungi and bacteria. Emergent fungal and bacterial colonies were counted and isolated into pure cultures. Isolates were photographed and preserved in 50% glycerin and water, respectively, in the Van Bael laboratory at Tulane University.

Sequencing

Up to 50% of the surviving symbionts at each site were randomly selected for Sanger sequencing. Total genomic DNA was extracted using a MoBio Ultraclean DNA Isolation Kit. For fungi, we used primers ITS1F and LR3 to amplify the nuclear ribosomal internal transcribed spacers (nrITS) and 600 bp of the large ribosomal subunit (partial LSU) as a used primers 27F and 1492R to amplify the 16S rDNA gene DNA. All PCR products were submitted to Beckman Coulter Genomics for Sanger sequencing. Sequence editing was carried out with Sequencher v5.0 (Gene Codes Corporation, Ann Arbor, MI). Sequences with 97% similarity were considered to be representative of the same operational taxonomic unit (OTU). Representative sequences of each OTU were compared to NCBI archives through BLAST searches to assign putative taxonomic identities using Geneious version r9 (http://www.geneious.com, Kearse et al. 2012).

Voucher cultures of all OTUs were archived in the Van Bael lab at Tulane University, under accession numbers 1182– 1750. Accession numbers for sequences deposited in the NCBI Genbank are: MK036892-MK036991, as well as KY765153, KY765188, KY765159, KY765161, KY765168 referenced in Washburn & Van Bael (2017).

Environmental Data

Environmental data for each site was collected from the USGS Coastwide Reference Monitoring System (CRMS) website (Steyer 2010). Using google maps, we selected the CRMS stations that were within 3 miles of the collection sites. For the sites Lake Ponchartrain, Tickfaw, Honey Island and John Lafitte we used data from CRMS stations 6299, 0046, 6088, and 0234, respectively. The following environmental variables were used and accounted for data collected over a 1- year period prior to our plant tissue sampling date: site, date of collection, average salinity, maximum salinity, average water level, minimum water level, maximum water level, average temperature, minimum temperature, maximum temperature, tidal amplitude, and average time flooded.

Statistical Methods

Endophyte isolation frequency (EIF) was measured as a proportion of root/leaf pieces which grew a symbiont into culture over the total number of pieces plated in growth media and compared via Mann-Whitney U tests in PAST(PAlaeontological STatistics) (Hammer et al. 2001). All alpha diversity metrics were created with Fisher's Alpha (FA). We used Bray-Curtis dissimilarity to compare community composition and to assess among community (beta) diversity. We conducted a db-RDA in the R package vegan (Oksanen et al. 2016) to determine which environmental variables best explained the endosymbiont communities (in Bray-Curtis dissimilarity matrix format). We used the *ordistep* function to select from the environmental data variables. The *anova* function

was used to determine the significance per variable that contributed to the model (Table 1.2).

Table 1.2 ANOVA results based on R capscale model selection of all environmental variables. Bold indicates significance.

Organ	Symbiont Type	Max Water Level (F, p)	Average Salinity (F, p)
Roots + Leaves	Bacteria + Fungi	F = 3.02, ***p* = 0.001**	F = 1.64, ***p* = 0.041**
Roots + Leaves	Bacteria	F = 0.79, *p* = 0.671	F = 0.93, *p* = 0.585
Roots + Leaves	Fungi	F = 4.59, ***p* = 0.002**	F = 1.77, *p* = 0.092
Roots + Leaves	Fungi (*Eutypa lata* excluded)	F = 2.08, ***p* = 0.007**	F = 1.66, ***p* = 0.044**
Roots	Bacteria + Fungi	F = 1.56, ***p* = 0.032**	F = 1.46, ***p* = 0.058**
Roots	Bacteria	F = 0.79, *p* = 0.390	F = 0.93, *p* = 0.522
Roots	Fungi	F = 2.16, ***p* = 0.011**	F = 1.68, *p* = 0.065
Leaves	Bacteria + Fungi	F = 2.43, *p* = 0.065	F = 2.63, ***p* = 0.052**

RESULTS

We cultured 364 bacterial and fungal endophyte isolates from leaves and roots of 48 *T. distichum* trees from southern Louisiana. We acquired DNA sequences from a subset of 151 isolates: 113 fungal and 38 bacterial sequences, representing 43 fungal and 17 bacterial OTUs (Supplementary Table S1.1 and S1.2). All species names are putative taxonomic identities based on 97% sequence similarity. The most common fungal species were *Eutypa lata*, which is a known pathogen in sugarcane (*Saccharum* sp.) and grapes (*Vitis vinifera*) (Erincik et al. 2001) and *Metarhizium brunneum*, a fungal strain which is used as a biocontrol. The most common bacterial species were *Bacillus ceres*, a plant growth promotor known to increase salt tolerance in safflower (*Carthamus tinctorius*) plants (Reyad et al. 2017) and *Bacillus aryabhattai*, a plant growth promoting rhizobacteria (Park et al. 2017).

Plant Organ

Endophytic diversity, as calculated by Fisher's Alpha, was seven times higher in roots

(FA = 27.70) than in leaves (FA = 3.51). We observed a greater EIF of culturable endophytes in the roots than in the leaves of baldcypress (Mann Whitney U = 2225.5, n = 98, $p < 0.001$) (Fig 1.1).

Site

Among sites, Jean Lafitte had the highest diversity of endophytes (Fisher's Alpha: HI =12.50, JL = 29.18, LP = 12.66, TF = 8.83). Tickfaw and Lake Ponchartrain significantly differed from one another in EIF (Mann Whitney, U = 2225, n = 98, $p = 0.035$) and fungi were isolated more frequently than bacteria across all sites (Mann Whitney, U = 3920, n = 98, $p = 0.047$) (Fig 1.1).

Environmental Data

Maximum water level and average salinity in the year prior to collection explained a significant amount of variation in the culturable endophyte communities ($F = 3.02$, $p < 0.001$); ($F = 1.64$, $p < 0.041$) (Fig 1.2). For root communities alone, the maximum water level explained a significant amount of variation within the culturable endophyte communities ($F = 1.56$, $p < 0.032$) and average salinity was found to improve the model during model selection ($F = 1.45$, $p < 0.058$). Within cultured fungal communities (excluding bacteria), maximum water level in the year prior to collection explained a significant amount of variation in community composition ($F = 4.59$, $p < 0.02$).

DISCUSSION

Diversity and Isolation Frequency

We found that diversity and isolation frequency of culturable endophytes in baldcypress were greater in roots than in leaves. Leaf bacterial diversity and EIF were quite low when compared to studies on other conifers and other deciduous temperate trees examining

both leaves and roots (Izumi et al. 2008; Wilson 2015). This is the first study done on the leaves of a tree that is both deciduous and a conifer (and having high level of phenolic terpenoid compounds present in leaves (Falk & Wolkenstein 2017)) and we speculate that these factors could have some influence on the dearth of endophytes found. However, this could also be attributed to the difficulties of using culture-based methods to survey bacterial communities.

The leaf endophyte community was dominated by the putative fungal pathogen, *Eutypa lata* (Erincik et al. 2001). The greater endophytes isolation frequency observed at Tickfaw can also be attributed to the presence of *E. lata*, which comprised 52 of the 112 isolates. Even though we collected fungi from apparently healthy leaves, the *E. lata* isolates may actually represent pathogens, as *E. lata* is a known generalist pathogen infecting sugar cane, grapes, and woody fruit trees such as *Prunus* sp. (Lecomte et al. 2000). Further investigation into the relationship between *E. lata* and baldcypress is needed.

Environmental Variables

Both salinity and flooding (water level) contributed to endophytic community composition of baldcypress endophytes. Microbes have variable tolerances to salt, and only certain microbes are able to persist when conditions become more saline (Chowdhury et al. 2011). Changes in salinity alter the community composition of bacteria in soil samples and increases in salinity of 5% or more significantly decreased the genetic diversity of bacteria in soil samples (Omar et al. 1994). Fungal diversity and community composition are also altered in the presence of NaCl (Ke et al. 2013). For arbuscular mycorrhizal fungi, the presence of NaCl delayed germination of spores and reduced overall hyphal growth, the potential mechanism being the diversion of energy from

metabolism to osmoregulation (Juniper & Abbott 2006). Because many plants draw upon the rhizosphere/soil microbiome to assemble their own microbiomes (Compant et al. 2010), changes in the soil microbiome due to salinity shifts could be responsible for the change in plant microbial communities, particularly for root endophytes, which recruit microbes from the rhizosphere and soil in closest proximity to the roots.

Salinity and flooding have known negative effects on baldcypress physiology individually, and these effects are increased in combination, demonstrating an interactive effect which may influence baldcypress populations (Allen et al. 1996; Krauss et al. 1998, 1999). Some research supports the idea that with increased depth and duration of flooding, baldcypress growth declines and mortality is increased (Souther & Shaffer 2000), potentially due to a decrease in oxygen and nutrient availability (Conner & Day Jr. 1992). These changes in plant physiology may influence the tree's recruitment of endophytes or have weakened the tree's defenses to pathogens, thus influencing endophyte community assembly.

Limitations

Though our study utilized agar media recipes consistent with many other endophyte studies (Arnold et al. 2003; Mighell & Van Bael 2016; Kandalepas et al. 2015) the richness, source (plant vs. animal) and composition of nutrients could bias the selection of microbial isolates. Overall, culture-based work provides a very limited view of the endophyte community, as it is estimated that only a fraction of microbes, especially bacterial, can be grown in culture (Izumi et al. 2008; Ulrich et al. 2008). Future work should focus on using an amplicon-based approach to better assess true diversity. This study also failed to account for plant genotypes, which can be highly variable among baldcypress (Allen et al. 1996). Gehring et al. (2017) demonstrated that an interaction

between plant genotypes and their mycorrhizal fungal symbiont community was important for drought tolerance in pine trees and that mycorrhizal community composition was strongly driven by plant genetics. Interactions between plant genotype, endophytic communities, and environmental stress should be examined *in situ* or tested experimentally using advanced molecular methods.

CONCLUSION

Our study demonstrates a connection between environmental variables, plant symbionts, and a key restoration species. Baldcypress' culturable endophyte community composition was associated with both salinity and flooding. As coastal ecosystems change due to sea level rise, subsidence, and human activities, we can expect variability in water levels and greater saline incursions into previously freshwater areas. It is possible that the observed clines in salinity and differing hydrological regimes at these sites can be used to predict future outcomes of sea level rise for baldcypress endophytes and endophyte communities in other wetland species.

FIGURES

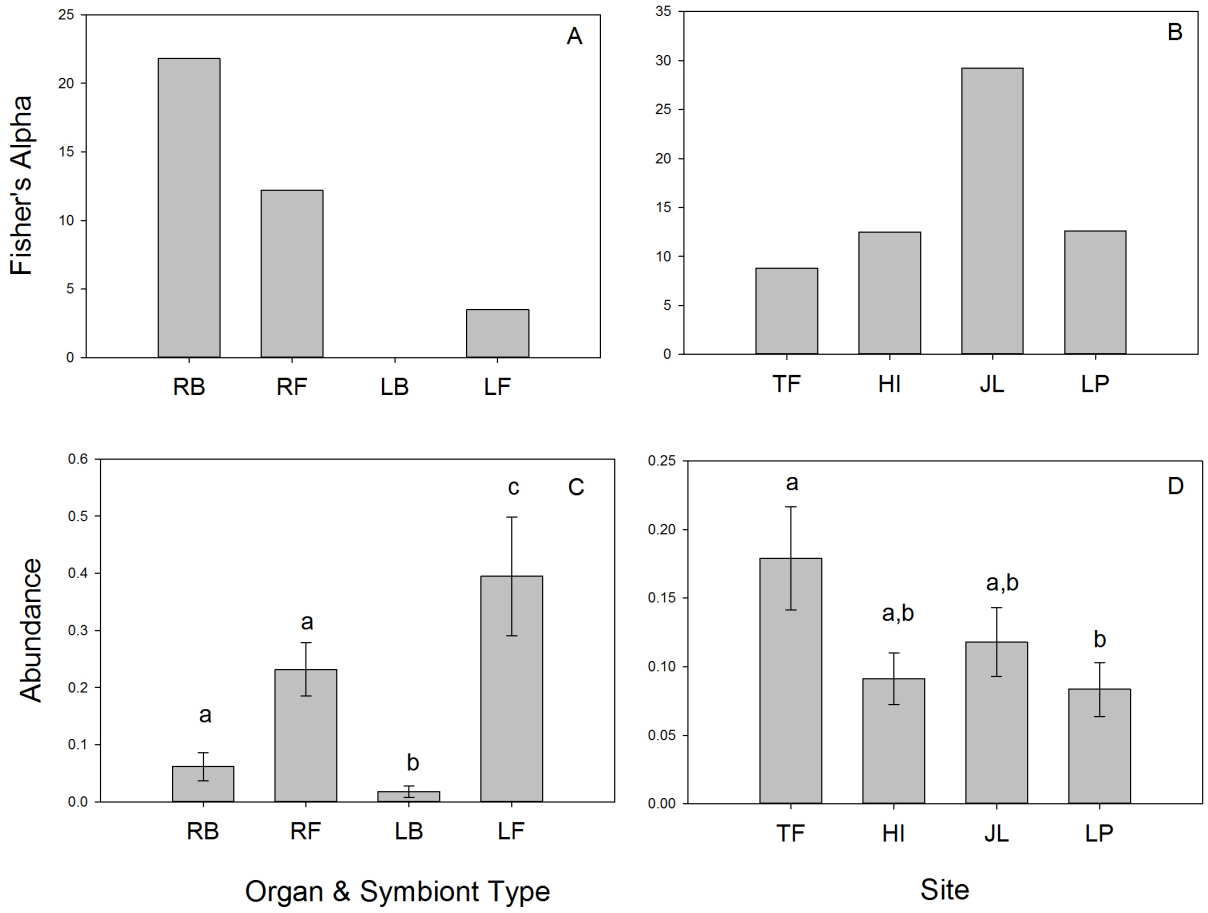

Figure 1.1 Plant organs and sites differed in endophyte isolation frequency (abundance) and diversity of endophytes (root bacteria = RB, root fungi = RF, leaf bacteria = LB, leaf fungi = LF; Tickfaw = TF, Honey Island = HI, Jean Lafitte = JL, Bonnet Carre Spillway at Lake Ponchartrain = LP). A) Diversity (measured by Fisher's Alpha) was highest in roots and for root bacteria. B) Among sites, diversity was highest at Jean Lafitte, the least disturbed area. C) Endophyte isolation frequency (abundance, mean ± standard error) was highest for leaf fungi and D) at Tickfaw, the least degraded site. Lower case letters show which groups are significantly different using a Bonferroni correction ($p<0.05$).

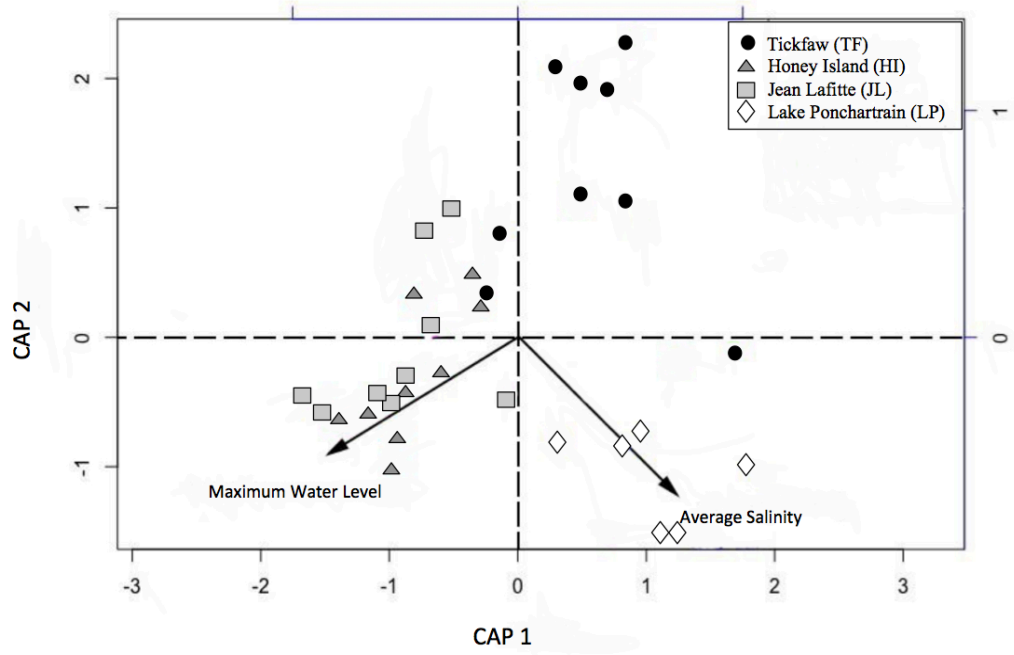

Figure 1.2 Sites differed in community composition of endophytes. Salinity and maximum water level described significant variation with community composition. Each data point represents the microbial community composition (of fungi and bacteria combined) of one tree and is based on Bray-Curtis similarity of communities at the OTU level. Vectors indicate the weight and direction of those environmental variables that were best predictors of endophyte community composition as suggested by the results of the db-RDA.

CHAPTER 2: SALINITY, WATER LEVEL, AND FOREST STRUCTURE CONTRIBUTE TO BALDCYPRESS TREE *(TAXODIUM DISTICHUM)* RHIZOSPHERE AND ENDOSPHERE COMMUNITY STRUCTURE

ABSTRACT

As climate change alters coastal ecosystems, it is of increasing interest to examine the influence of variables associated with saltwater intrusion on coastal communities. Using 16S Illumina profiling, we characterized the communities of baldcypress tree (*Taxodium distichum*) root endosphere and rhizosphere soil bacteria. Our study utilized established sites along salinity and flooding gradients in Georgia, Louisiana, and South Carolina. We hypothesized that environmental variables, namely salinity and water level, as well as distance between sites would be correlated with baldcypress-associated rhizosphere and root endosphere bacterial communities. Mean water level, mean salinity, and the volume of woody debris were associated with both endosphere and rhizosphere bacterial communities in baldcypress trees. The density of host trees was also associated with endosphere community composition. We found that geographic distance increased the difference between bacterial communities in soil rhizosphere more than it increased the difference in bacterial communities in the root endosphere, suggesting that the trees may have stabilized their root endosphere communities via recruitment of a more specific suite of taxa from the surrounding soil. Our study is the first to use 16S Illumina sequencing to characterize bacterial communities in baldcypress trees— a key restoration species in coastal swamp ecosystems under threat from climate change.

INTRODUCTION

Sea level rise, storm surge, and subsidence all contribute to saltwater intrusion into the freshwater forested wetlands (swamps) of the Gulf and Southeastern coastal regions of the United States. As saltwater moves inland, swamps are being converted to marsh, where only standing dead or stressed trees remain among an understory of saltwater-adapted plant species (Krauss et al. 2009; Shaffer et al. 2009). Many swamp ecosystems are dominated by the deciduous conifer, *Taxodium distichum* (hereafter baldcypress) which are able to persist in a range of conditions associated with a healthy, dynamic, coastal landscape. For instance, baldcypress can tolerate a relatively wide range of salinities — from freshwater to brackish (0-2 ppt salinity) and can also live in dry conditions or permanently, periodically, seasonally, or tidally flooded locations (Allen et al. 1996). The wide geographic range of baldcypress, as well as its ability to live across a spectrum of environmental conditions make it an ideal candidate species for examining plant-associated microbial community composition along a gradient that is shifting due to salt water inundation brought about by sea level rise.

Two prominent groups of plant-associated bacteria are endophytes (bacteria that live entirely within the tissues of their host plants in the endosphere) and rhizosphere bacteria (which reside in the layer of soil directly surrounding the roots) (Wilson 1995; Friesen et al. 2011; Philippot et al. 2013). Both endosphere and rhizosphere bacteria contribute to nutrient uptake, and increased growth, as well as a plant's abilities to respond to abiotic stressors such as temperature changes, flooding, and salinity (Rosenblueth & Martínez-Romero 2006; Jungwook et al. 2009; Philippot et al. 2013; Ryan et al. 2016; Yuan et al. 2016). Despite these known roles in plants' phenotypes

and, in turn, ecological interactions and community dynamics (Wilson 1995; Friesen et al. 2011; Christian et al. 2015), what drives community composition of these bacterial groups remains underexplored, especially for plants in wetland and coastal ecosystems.

Krauss et al. (2009) examined site condition, structure, and growth of baldcypress trees along five salinity gradients in the southeastern US (Figure 2.1). Long-term, background data from these established sites offer a unique opportunity to examine a suite of environmental variables as well as geographic distance on plant-associated microbes. Our research builds upon this work by characterizing the bacterial communities in the endospheres and rhizospheres of these same baldcypress trees.

In this study, we examine baldcypress root endosphere and rhizosphere bacterial community patterns with respect to environment and spatial distance across the southeastern US. We hypothesized that both geographic distance between host trees as well as environmental variables associated with salt water inundation would influence baldcypress-associated rhizosphere and root endosphere communities and predicted that the endosphere community would be less affected by geographic distance than the rhizosphere community because of the additional biotic filter imposed by the host tree. We characterized baldcypress root and rhizosphere bacterial communities and asked the following questions: 1) Is bacterial community composition correlated with environmental variables and/or geographic distance? 2)Which environmental factors explain a significant amount of the variation in bacterial community composition? Understanding how bacterial communities are structured in association with this key restoration species may help us to more accurately predict how microbial communities will shift due to salt water inundation brought about by sea level rise.

METHODS

Site descriptions

Baldcypress trees were sampled along four landscape gradients (hereafter referred to as transects) in the southeastern US (Figure 2.1). These transects, which range from freshwater to oligohaline (salinity ranged from 0.1 to ~3.4 ppt), were established by the USGS (US Geological Survey) and Clemson University in 2004 and have been continuously monitored for environmental data such as temperature, soil nutrients, and vegetation to provide long-term data on how *T. distichum* swamps are affected by climate change-induced salinization and flood regimes (Krauss et al. 2009). Each transect had one degraded site established alongside a marsh, and two sites located progressively upstream to represent moderately degraded and healthy stands for a total of three sites per transect (Figure 2.1). Each site was comprised of two 20 x 25 meter plots. South Carolina and Georgia transects (Waccamaw and Savannah transects hereafter) were strongly tidal and associated with rivers and tidal creeks. Louisiana transects (the Terrebonne and Barataria transects hereafter) were associated with more "complex systems of interconnected bayous and open water bodies" (Krauss et al. 2009) and had periodic wind tides.

Environmental Measurements

Within each plot, salinity and conductivity were measured from four salinity wells every four months in LA and monthly in SC and GA. Water level (the height of the water from the ground surface) was measured hourly. To characterize environmental conditions for each sample, we averaged measurements over the six to nine months preceding our collections. Baldcypress tree density (#/ha) was calculated based on the number of trees (individual >10 cm diameter at breast height [dbh]) per plot times 20. Woody debris volume (m^3/ha) (hereafter woody cover) was measured as the volume of downed wood 1

m above the surface (Krauss et al. 2018). We also considered whether a plot was historically tidally inundated (tidal history).

Sampling

In June of 2015, we collected root and rhizosphere samples from mature baldcypress trees along the South Carolina and Georgia transects. The two Louisiana transects were sampled in March of 2016. Each transect had three sites and we collected from five trees per site with the exception of one site, where we were only able to access three trees (n = 58 total trees). From each tree we collected a half liter Ziploc bag full of accessible roots from three points around the tree. Rhizosphere samples were obtained by using a gloved hand to pull soil from directly around a belowground or submerged root clump and placed into a sterile 50 mL falcon tube.

Processing

We sterilized the roots using the protocol described in Mugerwa et al. (2013) by submerging roots in 70% EtOH for 10 seconds, 50% Clorox (3.125% sodium hypochlorite) for 2 minutes and then rinsing twice with sterile DI water. We used sterile scissors to cut the roots into small ~2 mm long pieces. These pieces were transferred into empty microtubes and placed in -20^{o}C freezer for downstream processing.

Sequencing

We used MoBio PowerPlantPro DNA isolation kits and MoBio PowerSoil kits to isolate DNA from the root endopshere and rhizosphere soil, respectively. Bacterial ribosomal DNA was amplified with 799F and 1115R primers and tagged with an 8bp barcode indices as described in Hanshew et al. (2013) and Kembel et al. (2014). The PCR products were normalized with SequelPrep plates, pooled, and concentrated with

Agencourt AMPure beads. Amplicons were sent to Genewiz Inc. and sequenced on an Illumina MiSeq instrument using 300bp paired-end sequencing.

Bioinformatics

QIIME was used to demultiplex and quality-filter the sequences. Sequences were dereplicated and clustered into Operational Taxonomic Units (OTUs) at a 97% threshold, in an open reference manner using UCLUST and assigned taxonomy with the GreenGenes 13_5 reference sequences. Chimera detection and removal was conducted with the uparse algorithm in USEARCH (Edgar 2013). Mitochondrial 16S sequences were filtered out as well as samples with low sequencing success. Singleton OTUs (OTUs with sequence count = 1) were excluded from analyses. Prior to all analyses, we used *rrarefy* function in the R package vegan (Oksanen et al. 2013) to rarefy our data to n = 10000.

Statistical Methods

Collinearity of environmental variables was determined in R using the Pearson correlation coefficient. For pairs of variables with greater than 0.25 (or less than -0.25) Pearson's r value, one variable was removed from analyses. Variables used in our analyses were: temperature, tree density, woody debris volume, salinity, and water level. Environmental data were natural log-transformed, and all continuous variables were scaled to standard z-scores, with mean of 0 and standard deviation of 1 in order to compare estimates among variables. All analyses were conducted at the OTU level and separately for endosphere and rhizosphere OTUs.

Geographic Distance and Bacterial Community Structure

We examined the relationship between geographic/spatial distance and bacterial community composition relative to environmental variables by conducting a Multiple

Regression on distance Matrices (MRM) analysis of pairwise community similarities (Bray Curtis) as a function of geographic distance (Lichstein 2007). The rooted, weighted unifrac matrix used to describe bacterial composition was generated using the *beta_diversity.py* function in QIIME.

We aggregated OTUs from 3-5 trees at the site level, summed the OTU abundances, and measured pairwise site dissimilarity as an abundance-weighted, unifrac matrix. We used the *distGeo* function in the *geosphere* package (Hijmans et al. 2015) in R v.3.4 (R Development Core Team 2016) to calculate the pairwise geographic distances between plots. We also included the Euclidean differences in the environmental parameters to control for effects of environmental differences. Similarity matrices were regressed against the geographic distance matrix and the Euclidean distance matrices using *ecodist* (Goslee and Urban 2007).

Environmental Variables and Community Composition

We conducted a distance based redundancy analysis (Db-RDA) in the R package *vegan* (Oksanen et al. 2013) on both on both a Bray-Curtis dissimilarity matrix of 16S rhizosphere and root endophyte community data. We used the *ordistep* function to perform forward model selection, determining if any of the following variables explained the bacterial communities: mean temperature, percent woody cover, tree density, salinity, and water level. Models were then reduced by removing non-significant terms sequentially.

Diversity/Richness/Abundance

We calculated Shannon diversity in the R v.3.4 (R Development Core Team 2016) package vegan (Oksanen et al. 2013) using the *rare.d* function and OTU richness (i.e. number of OTUs present within a community) using the *rich.vegan* function. We ran

Mann Whitney U tests in PAST (PAlaeontological STatistics) (Hammer et al. 2001) to compare diversity among sites, transects and combinations of endosphere and rhizosphere communities in sites and transects. We ran a Kruskal-Wallace test to compare diversity between endosphere vs. rhizosphere communities. We used the multivariate statistical framework MaAsLin (Morgan et al. 2012) to find associations between relative abundance of specific microbial taxa and environmental data. All MaAsLin analyses were performed at the Class level, which allowed for best resolution of emergent patterns.

RESULTS

Geographic Distance and Bacterial Community Structure

Rhizosphere bacterial communities became more dissimilar with increasing geographic distance ($R^2 = 0.15$, $F = 3.65$, $p = 0.01$) (Figure 2.2). The root endosphere bacterial communities showed a trend towards dissimilarity with increasing geographic distance but was only marginally significant ($R^2 = 0.18$, $F = 4.47$, $p = 0.056$). Bacterial community dissimilarity matrices were regressed against the geographic distance matrix and the Euclidean distance matrices for the environmental variables to determine that the observed effect of distance was not correlated with differences among plots with respect environmental variables.

Environmental Variables and Community Composition

In rhizosphere bacterial communities, mean water level, mean salinity, and woody cover were selected as significant explanatory variables which explained a significant amount of variation in the model (77.4%) using the Bray-Curtis dissimilarity matrix (Figure 2.3). For root endosphere communities, mean water level, mean salinity, woody cover, and

tree density were variables which explained a significant amount of variation (75.4%) in the model using the Bray-Curtis dissimilarity matrix (Figure 2.4).

Table 2.1: Distance-Based Redundancy Analysis (Db-RDA) results and comparison between environmental variables correlated with bacterial community composition of endosphere and rhizosphere.

Endosphere	Rhizosphere
Mean Water Level ($F = 3.1$, $p < 0.001$)	Mean Water Level ($F = 4.4$, $p < 0.001$)
Mean Salinity ($F = 2.8$, $p < 0.001$)	Mean Salinity ($F = 4.7$, $p < 0.001$)
Woody Cover ($F = 2.2$, $p < 0.014$)	Woody Cover ($F = 2.2$, $p < 0.004$)
Tree Density ($F = 3.2$, $p < 0.011$)	

Diversity/Richness/Abundance

Our samples from the rhizosphere (n = 83) and endosphere (n = 45), contained 5,223,258 sequences, which clustered into 14,377 OTUs of bacteria and archaea. Endosphere communities were not a complete subset of the rhizosphere soil bacterial communities, though 40.7% of the overall OTUs were shared between roots and soil. Rhizosphere bacterial communities were significantly more diverse than endosphere communities (Mann-Whitney U = 857, n1 = 47, n2 = 83, $p < 0.0001$, two-tailed). There were no differences in diversity or richness of microbial communities among sites in the endosphere (Kruskal-Wallis H = 7.07, $p < 0.79$;) or rhizosphere (Kruskal-Wallis H = 3.64, $p < 0.97$). Likewise, no significant differences in diversity or richness were detected among transects in the endosphere (Kruskal-Wallis H = 0.66, $p < 0.88$) or rhizosphere (Kruskal-Wallis H = 1.24, $p < 0.74$).

The multivariate statistical framework MaAsLin detected significant differences in the relative abundance of several classes of microbial communities in the roots and rhizosphere including, most notably, higher abundances of Alphaproteobacteria (q = 1.72e-37), and Betaproteobacteria (q = 9.64e-11) in the roots compared to the rhizosphere. Additionally, water level was positively correlated with the abundance of Methanobacteria (q = 0.017) and mean salinity was positively correlated with the abundance of bacterial class TK17 (q = 0.019). (A q-value is a p value that has been adjusted for false discovery rates and significant values are less than or equal to 0.05).

DISCUSSION

Our study examines the relationship of geographic distance and environmental filtering to the community composition of baldcypress root-associated endosphere and rhizosphere bacteria along ecological gradients. We highlight three key findings. First, that community composition is more similar in sites that are closer together in the rhizosphere soil but not in the endosphere bacterial communities. Next, that rhizosphere community composition was correlated with mean salinity, mean water level, and woody cover and lastly, that root endosphere community composition was explained by the same three variables as the rhizosphere and additionally by tree density.

Distance correlated with rhizosphere but not endosphere bacterial communities

We found that rhizosphere but not root endosphere bacterial communities were correlated with distance when we used multiple linear regression analyses (MRM) analysis on bacterial community distance matrices regressed against geographic distance. The rhizosphere bacterial communities were more dissimilar the further away they were in physical space. This phenomenon, known as distance decay, has been observed in many

studies on soil bacteria (Goldman et al. 2016; Peay et al. 2010; Martiny et al. 2011). The root endophyte communities showed a similar trend toward distance decay, but the effect of distance was marginally significant. The roots of many plants select for/attract specific microbial communities from the rhizosphere by using root exudates, which contain nutrients for the microbes including carbohydrates, organic acids, and amino acids (Compant et al. 2010; Zarraonaindia et al. 2015). It is possible that this recruitment mechanism is occurring for baldcypress and that may have had a stabilizing effect on root endophyte communities, thus decreasing the influence of distance that was observed in the surrounding rhizosphere bacterial communities. Baldcypress trees are genetically diverse (Krauss et al. 1999), so these results might also be an indication that the root interior is a highly selective environment driven by highly conserved genes and pathways or may indicate a stable symbiosis of some microbial taxa with baldcypress trees.

Salinity correlated with endosphere and rhizosphere communities

We detected a correlation between the mean salinity and the bacterial community composition in both the endosphere and the rhizosphere. It has been well established that increased salinity alters microbial community composition and reduces microbial biomass and activity in the soil (Andronov et al. 2012; Setia et al. 2012; Yan et al. 2015; Thompson et al. 2017). Soluble salts in the soil increase osmotic potential, in-turn drawing water out from the cells of microbes and impeding some microbes' ability to acquire water from their surroundings (Oren 1999). Because plants recruit microbes from the rhizosphere and soil that is in closest proximity to the roots, changes in the rhizosphere microbial community due to salinity shifts could be responsible for the observed changes in endosphere microbial communities.

Water level correlated with endosphere and rhizosphere communities

We found that baldcypress endosphere and rhizosphere bacterial communities correlated with mean water level. Bacterial communities could be responding to different environments or to changes in the host plant brought about by environmental change. Though baldcypress can withstand periodic and permanent flooding, prolonged high-water levels can cause stress, and flooding and salt in combination have a known negative interactive effect on baldcypress (Allen et al. 1996). In other systems, stress due to flooding and changes in water regime have been demonstrated to alter the composition and amounts of plant root exudates (Henry et al. 2007; Wang et al. 2017). Stress-induced changes in the plant may influence the root microbiome of the plant by altering its capacity to select for a specific suite of microbes and stress can also increase plants' vulnerability to invasion by pathogens (De Conink et al. 2017).

Furthermore, it is known that baldcypress roots adapt morphologically to flooding via the production of water roots and adventitious roots, increasing intercellular air spaces in the roots, and developing shallower root systems (Megonigal & Day 1992). Though we did not systematically record root architecture or appearance for our root samples, we observed that roots collected from flooded/permanently inundated sites were often succulent in texture and/or arose from the trunk closer to the water's surface, as is more characteristic of the "water roots" and adventitious roots described for mature flooded baldcypress (Harms et al. 1980; Megonigal & Day 1992). Rhizosphere soil methods were standardized for each sample, however, it is possible that morphological differences in the roots may have influenced the root microbiome by altering the interior landscape inhabited by microbes.

It is also possible that the flooding conditions selected for a suite of anaerobic microbes which could survive underwater. Our MaAsLin analyses found that the abundance of one

class in particular, the Methanobacteria, which are most commonly anaerobic (Chen et al. 2008), was positively correlated with water level. Further explorations are needed into the life histories of specific taxa.

Woody cover correlated with endosphere and rhizosphere communities

Woody cover, the volume of wood in the layer up to one meter above the surface, correlated with bacterial community composition in the endosphere and the rhizosphere. Bacteria produce extracellular enzymes that degrade lignin/organic matter, and therefore thrive where there is plenty of substrate to decompose (de Gonzalo et al. 2016). Decaying wood provides a substrate for increased bacterial diversity and abundance and thus serves as a local source of microbial dispersal (Stokland et al. 2012). It follows that an increase in the amount of woody cover on a site would serve as fertile fodder for flourishing microbial communities which, in turn, could find their way into the rhizosphere and endosphere.

Tree density correlated with endosphere communities

The density of baldcypress trees at each site contributed to the bacterial community composition in the roots but not in the rhizosphere. Distance-decay, the decline in community similarity with increasing distance, has been demonstrated in bacterial communities over distances as short as a few centimeters apart (Martiny et al. 2011). Peay et al. (2010) used the framework of island biogeography to examine dispersal limitation of mycorrhizal fungi between individual trees referred to as “tree islands” and found that with increasing isolation of trees, species richness decreased significantly. Given the microscopic nature of endophytes and considering that each baldcypress tree would be a vector for dispersal of baldcypress endophytes, perhaps we are seeing a

similar “tree island” effect in our study system, where a greater number of trees in close proximity to one another are influencing community dynamics.

Limitations/Future Directions

While we did collect GPS coordinates at each 20x25 meter site areas, we were unable to retrieve our GPS coordinate measurements taken at the individual tree level which would have allowed for a finer resolution examination of the potential effects of distance decay and dispersal limitation. Additionally, samples were only taken at one time point in each location and were taken several months apart for samples in Louisiana versus the east coast (SC and GA). It has been demonstrated that microbial communities shift over time, so both concurrent sampling and longitudinal studies are needed to determine how communities are changing.

CONCLUSION

Other studies have noted that salinity and flooding are stressful to baldcypress. Our work, which is the first to use 16S Illumina sequencing to characterize endosphere and rhizosphere bacteria associate with baldcypress, suggests that stress associated with sea level rise leads to altered microbial communities and that geographic distance has a greater influence in structuring baldcypress associated bacteria in the rhizosphere than in the endosphere. Understanding the ways in which bacterial communities are structured along salinity and flooding gradients in this key restoration species could inform restoration and conservation planning and help us to predict how bacterial communities may shift in dynamic coastal ecosystems.

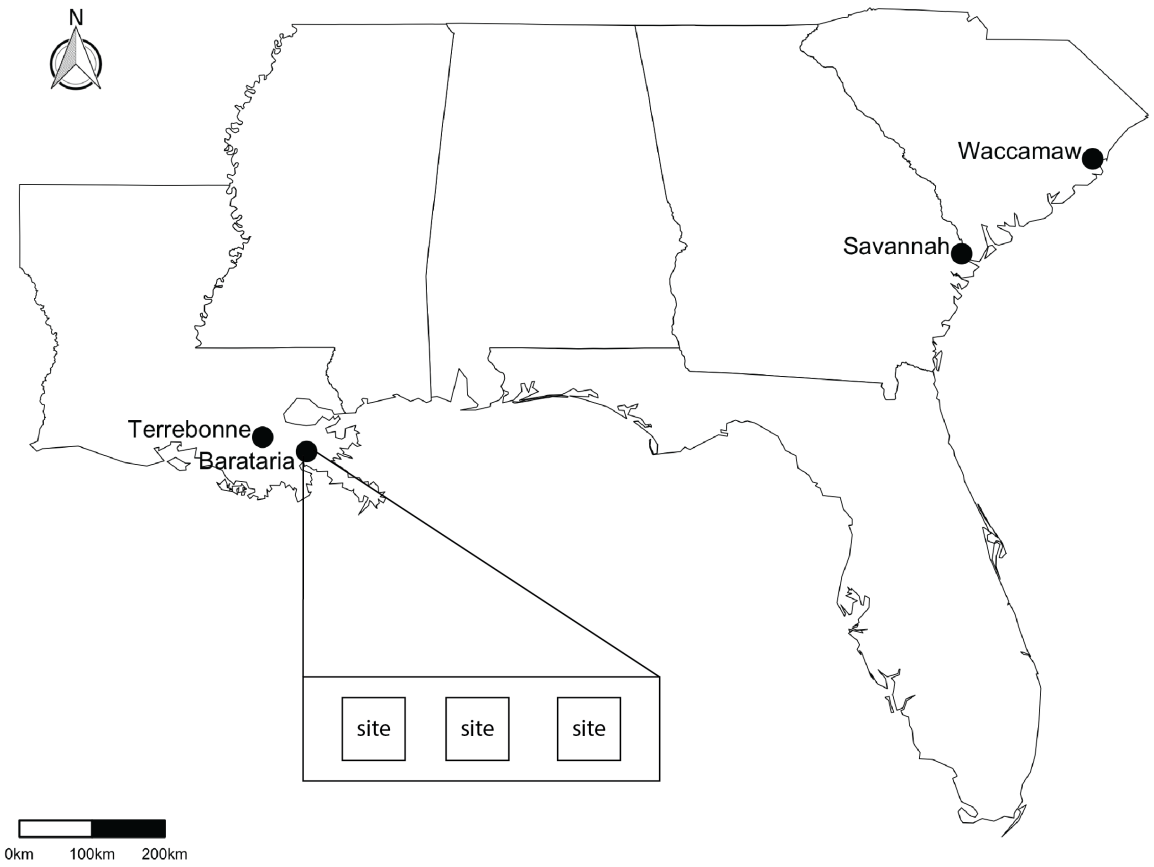

Figure 2.1 Map showing the location of the four transects established by Krauss et al. 2009. Each transect contains three sites comprised of two 20 x 25 meter areas.

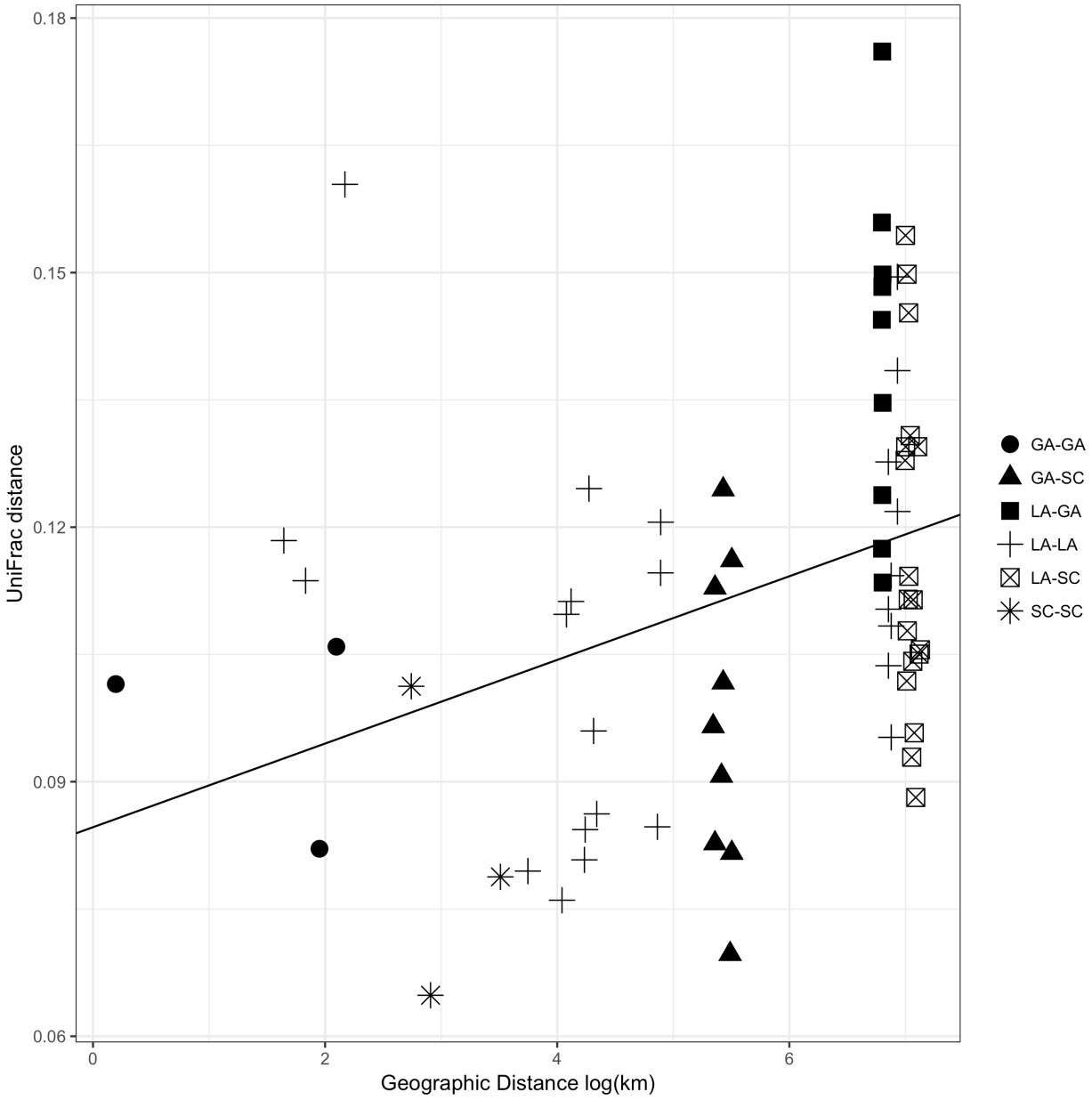

Figure 2.2 Rhizosphere bacterial communities were more dissimilar with increasing geographic distance (R^2=0.13, F = 9.6, p<0.009). Points represent pairwise comparisons between sites and are described at the state level (Louisiana, Georgia, South Carolina). The further apart the two points are the greater the unifrac distance between the bacterial communities. For example, the longest geographic distance will always be LA-SC. Those points show that the unifrac distance (between centroids of their clouds of microbial communities) is variable and is greater than SC-SC comparisons.

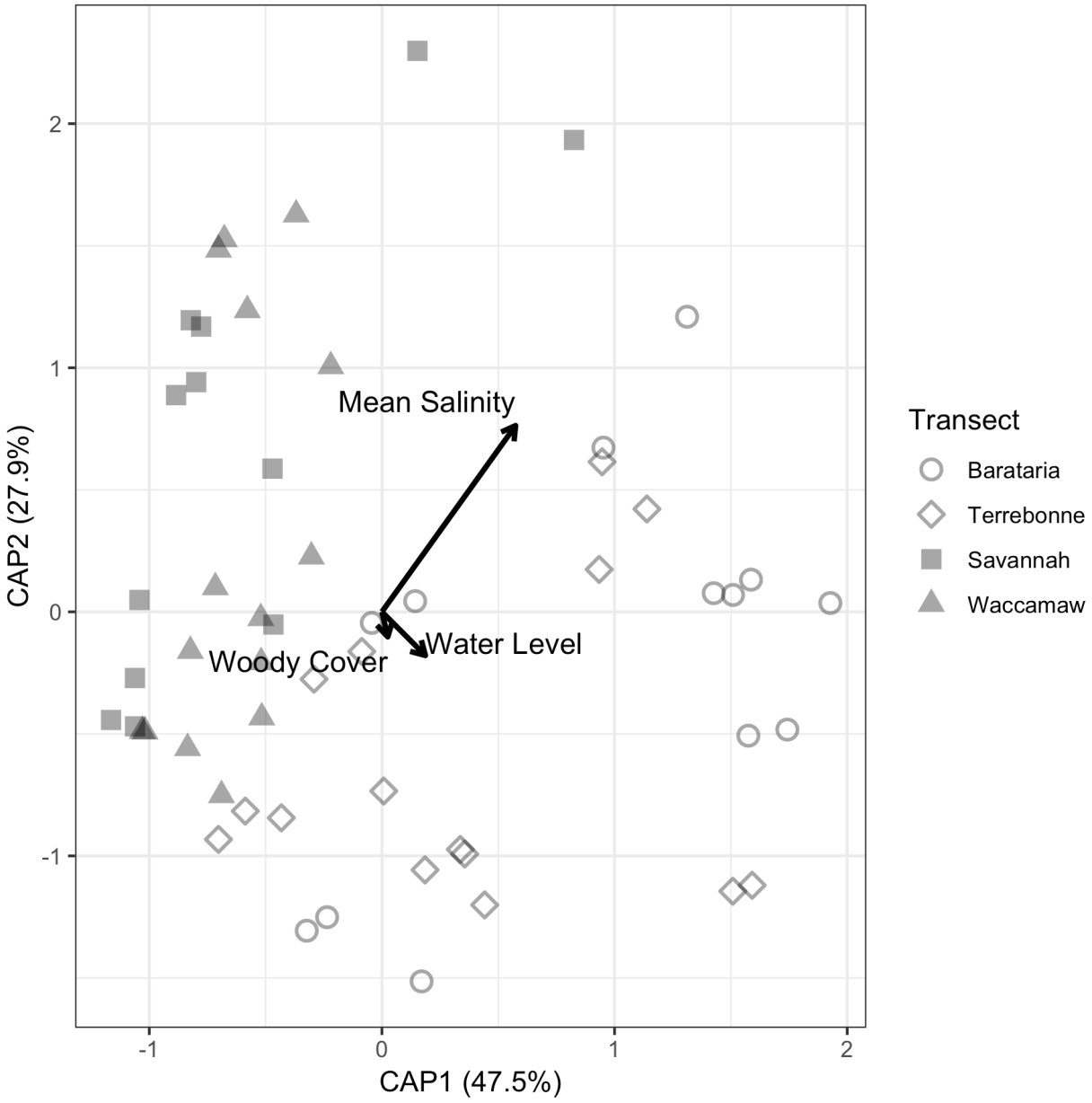

Figure 2.3 In rhizosphere bacterial communities, mean water level, mean salinity, and woody cover were selected as significant explanatory variables which explained a significant amount of variation in the model (77.4%) using the Bray-Curtis dissimilarity matrix in a Db-RDA analysis.

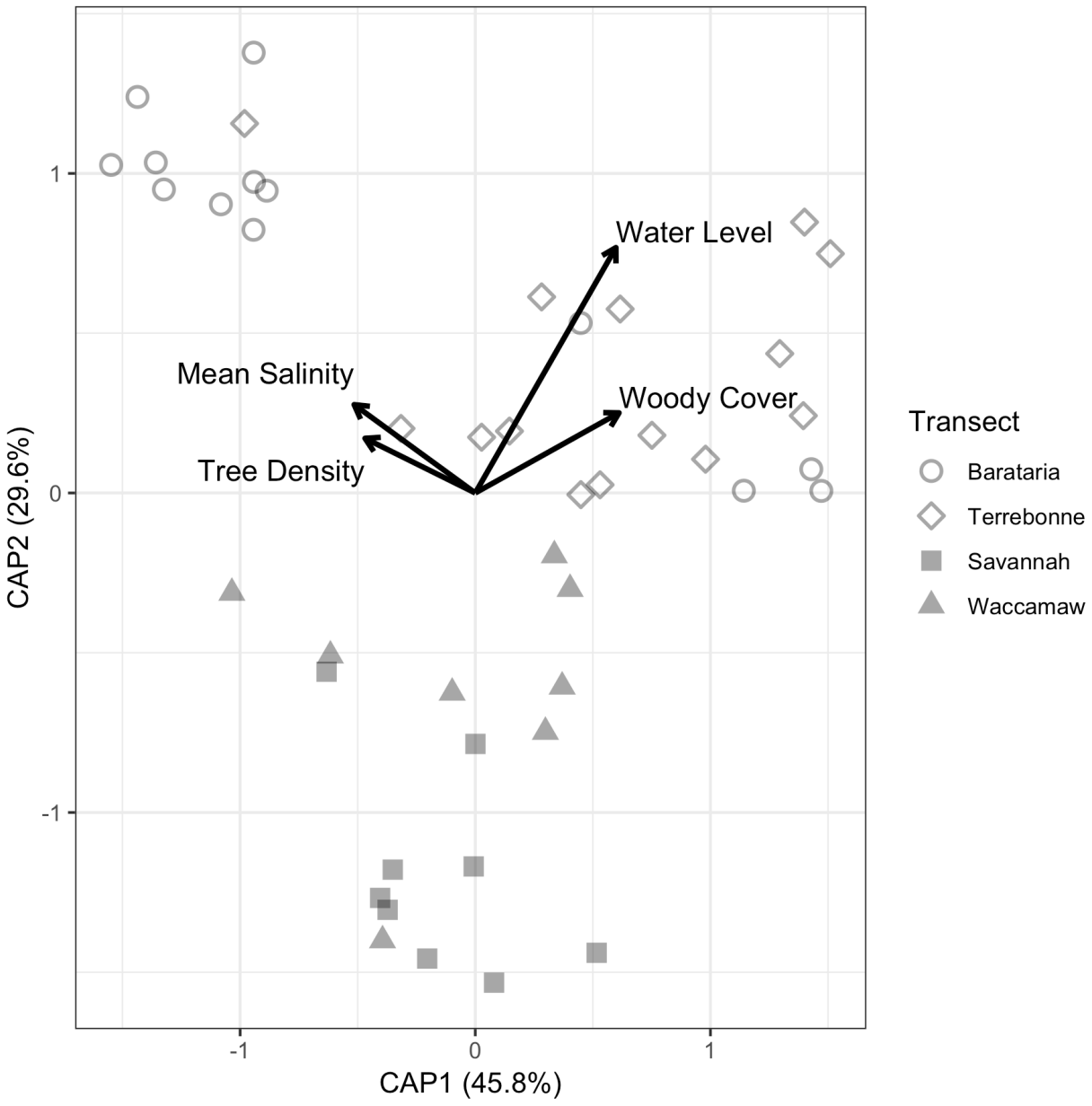

Figure 2.4 In root endosphere communities, mean water level, mean salinity, woody cover, and tree density were variables which explained a significant amount of variation (75.4%) in the community composition model using the Bray-Curtis matrix.

CHAPTER 3: HALOTOLERANT ENDOPHYTES ISOLATED FROM *TAXODIUM DISTICHUM* AND *SPARTINA ALTERNIFLORA*

ABSTRACT

Establishment of plant species in coastal areas, both naturally and through human restoration efforts, is complicated by climate changed induced salt water inundation and salt stress. Restoration efforts could be improved if salt tolerant endophytes were identified and used to mitigate salt stress in key restoration species, such as the baldcypress tree (*Taxodium distichum).* In this study, endophyte cultures were collected from *T. distichum* trees in fresh and brackish swamps and from *Spartina alterniflora* (smooth cordgrass) in saline marshes. A first step towards using endophytes for restoration, or towards understanding the functions of some of these organisms *in situ*, was to conduct salt challenge assays with our cultures to identify candidates for use in *in vivo* inoculation trials. We hypothesized that salt tolerant endophytes would be present in both *S. alterniflora* and *T. distichum*, and that those collected from plants in saline environments would be better adapted to grow in saline conditions. Using salt challenge assays, we identified five strains of extreme halotolerant endophytes and eleven strains of moderately halotolerant endophytes. We did not observe different growth patterns in endophyte cultures obtained from fresh versus brackish soils. Experiments which use halotolerant microbes to inoculate plants at risk for salt water damage are needed in both greenhouse and natural settings.

INTRODUCTION

Salt stress in plants represents a major disturbance to both agricultural and coastal ecosystems. An estimated 20% or greater of all irrigated agricultural lands worldwide are affected by salt and up to 50% of arable lands could be affected by 2020 (Pitman & Läuchli 2002; Jamil et al. 2011). Salt is also problematic where saltwater intrusion, the movement of saline water into freshwater sites, is converting freshwater forested wetlands into open marshes (Shaffer et al. 2009). For plants that are not adapted to handle salt stress, excess salt negatively influences the internal environment of the plant in several possible ways. Na+ and Cl- themselves inhibit many metabolic processes including protein synthesis and photosynthesis (Ouzounidou et al. 2014). Salt stress has also been linked to an increase of reactive oxygen species (ROS), the cell signaling molecules that regulate stress response mechanisms. With increased ROS levels, stomata close, transpiration is reduced, and net photosynthesis is reduced (Ouzounidou et al. 2014). Additionally, salt stress can cause a plant to release the stress-induced hormone ethylene which itself can cause considerable damage to the plant (Stearns & Glick 2003).

Endophytes— fungi and bacteria that live the majority of their lives within plant tissues without causing apparent damage—play major roles in mitigating the harmful effects to host plants of both biotic and abiotic stressors, including salinity stress (Compant et al. 2012; Philippot et al. 2013; Christian et al. 2015; Yuan et al. 2016). Several studies have found that plant growth promoting bacterial endophytes containing ACC deaminase facilitate plant growth by mitigating the damaging effects of ethylene (Saravanakumar & Samiyappan 2007, Siddikee et al. 2010, Ali et al. 2014). Redman et al. (2011) found that salt tolerance correlated with decreased ROS activity in rice plants inoculated with class 2 fungal endophytes. Khan et al. (2016) used inoculations of the

bacterial endophyte *Bacillus pumilus* to ameliorate salt stress in rice plants. For these types of studies, agricultural species have received the bulk of the attention and the roles of endophytes in mitigating salt stress in natural populations, especially in a restoration context, is underexplored.

In conditions of chronic inundation and salt stress, successful establishment of plant species, both naturally and through human restoration efforts, is determined by complex processes (Shaffer et al. 2009). Coastal restoration efforts could be improved if salt tolerant endophytes were identified and used to mitigate salt stress in key restoration species, such as the iconic baldcypress tree (*Taxodium distichum).* Previous work has resulted in hundreds of endophytic cultures from *T. distichum* trees as well as from the marsh grass *Spartina alterniflora* (Kandalepas et al. 2018; Kimbrough et al. 2018). Adult *T. distichum* can persist in conditions of 2 ppt salinity—the level at which swamp begins to convert to marsh (Lundberg et al. 2011; Krauss et al. 2012) — and *S. alterniflora* is adapted to highly saline environments, up to full-strength seawater (~35 ppt) (Tang et al. 2014).

A necessary first step towards using endophytes for restoration, or towards understanding the functions of some of these organisms *in situ*, was to conduct salt challenge assays and to identify salt tolerant endophyte candidates for use in *in vivo* inoculation trials. We grew endophytes cultures on agar plates containing 3 levels of salinity: fresh (0 ppt salinity), seawater-level salinity (35 ppt), and hyper-saline (200 ppt). We hypothesized that salt tolerant endophytes would be present in both *S. alterniflora* and *T. distichum*, and that those collected from plants in saline environments would be better adapted to grow in saline conditions, presenting evidence for local adaptation. Specifically, we predicted that endophyte growth rates would be greater in salt amended

media if their host plant was collected in a saline environment, while the growth rates would be greater in non-saline media if the host plant was collected in a freshwater environment.

METHODS

Taxodium distichum collections

We harvested root and leaf tissue of mature *T. distichum* trees (>20 m height) from 12 mature trees at four unique sites (n=48) with varying levels of mean salinity in southern Louisiana in October 2014 and in the summer of 2015. Apparently healthy leaves and roots were collected haphazardly from three locations on each individual tree.

Spartina alterniflora collections

S. alterniflora was collected in November and December of 2013 from two sites in southeast Louisiana: a marsh in Bay Jimmy in Northern Barataria Bay and a marsh behind Fourchon Beach, which lies in front of adjoining Caminada Bay. Both are salt marshes of predominantly organic soil with some clay and sand content.

Salinity data

Salinity data for *S. alterniflora* and the 2014 *T. distichum* collections were obtained from the USGS Coastwide Reference Monitoring System (CRMS) website (Steyer 2010) and are the mean of the measurements taken monthly one year prior to our collection. All sites were within 3 miles of a CRMS data collection site. Salinity data for the 2015 *T. distichum* data were collected from plots established by the United States Geological Survey (Krauss et al. 2009). Within each plot, salinity and conductivity were measured from four salinity wells every four months and the salinity value used in this study is the average of the 2 measurements prior to our sample collection date.

Culturing

Samples were placed on ice and transported into refrigeration at Tulane University for processing following protocols described in (Kandalepas et al. 2015) within 48 hours of collection. Pure cultures of bacterial and fungal isolates were photographed and preserved in 50% glycerin or water vouchers, respectively, in the Van Bael laboratory at Tulane University. In the spring of 2018, fungal and bacterial cultures were re-grown into pure culture from glycerin and water vouchers. Successfully growing voucher specimens were isolated a final time into pure culture and incubated at room temperature until the salt screening/growth rate measurement experiment.

Sanger Sequencing

Total genomic DNA was extracted using a MoBio Ultraclean DNA Isolation Kit. For fungi, we used primers ITS1F and LR3 to amplify the nuclear ribosomal internal transcribed spacers (nrITS) and 600bp of the large ribosomal subunit (partial LSU) as a single fragment (nrITS-partial LSU) following Higgins et al. (2011). For bacteria, we used primers 27F and 1492R to amplify the 16S rDNA gene DNA. All PCR products were submitted to Beckman Coulter Genomics for Sanger sequencing. Sequence editing was carried out with Sequencher v5.0 (Gene Codes Corporation, Ann Arbor, MI). Sequences with 97% similarity were considered to be representative of the same operational taxonomic unit (OTU). Representative sequences of each
OTU were compared to NCBI archives through BLAST searches to assign putative taxonomic identities using Geneious version r9 (http://www.geneious.com, Kearse et al. 2012).

Salt Screening/growth rate measurement experiment

Salt amended plates with 0 ppt, 35 ppt, and 200 ppt were created by combining Instant Ocean (Spectrum Brands Inc., Blacksburg, VA), DI water, either BD Difco Nutrient agar (for bacterial plates) or Difco Malt Extract and agar (for fungal plates). In sterile conditions, cultures were plated onto 2 replicates of each salinity level (n=6). Cultures were incubated in identical light and temperature conditions and the area of culture growth was outlined every other day for 6 days on the outside of the plate with black marker. Plates were photographed, and Image J (Schneider et al. 2012) was used to quantitatively calculate the area of growth for each measurement.

Statistical Methods

Growth rate areas were square-root transformed and growth rate was estimated as the slope of a linear regression for the changes in area through time. We averaged the data for the 2 replicates for each individual isolate in each of the salt-media levels (0 ppt, 35 ppt, 200 ppt) after calculating the slope (Estrada et al. 2013).

Estimated growth rates were compared using a Type III ANOVA, with 3 treatments (0 ppt, 35 ppt, 200 ppt), 2 levels (fresh or salt source) and growth rate was the response variable. Plants sourced in salinity levels less that 2 ppt were binned as "fresh sourced" and isolates over 3 ppt as "salt sourced." A Tukey test was used to perform multiple pairwise comparisons between significant groups. A residual versus fit plot was used to check the homogeneity of variances. Normality was verified by a Shapiro-Wilk test on the ANOVA residuals. These analyses were repeated excluding the 200 ppt, high saline treatment.

RESULTS

We screened 49 endophytes for salt tolerance in salt-amended media. In regard to host plant, 34 isolates were obtained from *T. distichum* and 15 from *S. alterniflora*. In regard

to source habitat, 31 isolates came from plants in fresh water sources (<1.5 ppt salinity) and 18 came from salt water sourced plants (>3 ppt salinity).

We did not observe differences in growth rates between endophytes sourced in fresh water vs. salt water locations and there was no interaction effect between source (salt vs. fresh) and treatment level (ppt) (Table 3.1, Figure 3.1). These results were consistent when the 200 ppt treatment was excluded from analyses.

Table 3.1: Type III ANOVA Results

Response: Growth Rate	Sum sq	Df	F Value	p
Source Salinity	0.14	1	1.34	0.24
Salt Assay Level (ppt)	10.99	2	52.7	<2e-16
Source x Assay	0.08	2	0.39	0.67

However, endophyte growth rate did significantly differ among salinity treatments (ppt) overall indicating that the treatment was effective. Of our screened cultures, all 49 isolates grew in 0 ppt. Sixteen cultures grew in 35 ppt salinity (Table 3.2), exhibiting moderate tolerance and of those 16 which grew in 35 ppt, we identified five which were able to grow in 200 ppt salinity and are therefore classified as extreme halophiles (Olivier et al. 1994) (Table 3.2).

Table 3.2. Halotolerant endophytes isolated from *T. distichum* and *S. alterniflora*

Isolate #	**Halotolerance**	**Host Plant**	**Organ**	**Type**	**Source Salinity (ppt)**
325	Extreme	*S. alterniflora*	Leaf	Fungi	11.00
462	Extreme	*S. alterniflora*	Leaf	Fungi	11.00
812	Extreme	*S. alterniflora*	Root	Bacteria	11.00
1423	Extreme	*T. distichum*	Leaf	Fungi	1.31
1633	Extreme	*T. distichum*	Leaf	Fungi	0.20
811	Moderate	*S. alterniflora*	Root	Bacteria	11.00
931	Moderate	*S. alterniflora*	Leaf	Bacteria	11.00
1206	Moderate	*T. distichum*	Root	Bacteria	0.10
1305	Moderate	*T. distichum*	Root	Bacteria	0.20

1472	Moderate	*T. distichum*	Root	Fungi	1.31
1635	Moderate	*T. distichum*	Root	Fungi	0.61
1710	Moderate	*T. distichum*	Root	Fungi	1.31
2004	Moderate	*T. distichum*	Root	Fungi	3.20
2028	Moderate	*T. distichum*	Root	Fungi	1.50
2036	Moderate	*T. distichum*	Root	Fungi	1.50
2039	Moderate	*T. distichum*	Root	Fungi	0.05

DISCUSSION

The primary goal of this study was to screen our available *T. distichum* and *S. alterniflora* endophyte cultures for salt tolerance. Nearly a quarter (16 out of 49) of our screened endophyte cultures were able to grow in 35 ppt salt amended media—the approximate salinity level of sea water (Cullum et al. 2016). This threshold represents the upper limit that the majority of organisms would encounter in the environment and thus the extent to which many would need to be halotolerant to survive. Of those screened cultures which were able to grow at 35ppt, five grew in 200ppt salinity. Organisms which can grow at a level of 4M salinity (~200ppt) or above are classified as "extreme halotolerant" Larsen (1986). Extreme halotolerant species are typically found in locations where the salinity is five times or greater than that of the ocean (i.e. the Dead Sea, Great Salt Lake) (Larsen 1986). Finding that roughly 10% of our isolates were able to persist in this extreme salt environment for six days speaks to the idea that the innate plasticity in endophyte genomes remains a great underexplored frontier in biology and that studies which assay microbes in various extreme conditions are of interest to understand the limits of these organisms.

Halotolerant endophytes

The five extreme halotolerant endophytes found in our assays were sourced primarily from leaves as opposed to roots. The eleven moderately salt tolerant endophytes (which grew in 35ppt salinity but not 200 ppt) were all sourced from the roots. The majority of the halotolerant species were fungi. It is unclear whether these patterns are due to stochastic processes or due to some sort of habitat filtering at the plant level. Endophytes can be vertically transmitted, as is often the case for grass, or horizontally transmitted, being dispersed by wind, water, insects, and animal vectors (Rodriguez et al. 2009). Once on the leaf surface, microbes can enter the leaves directly via stomatal openings or, in the case of fungi, by using a penetration peg to create an opening (Xi-Hu et al. 2014). Endophytes in the roots, however, are often recruited from the soil (Compant et al. 2010, Zarraonaindia et al.2015). One might expect to see more salt tolerant species sourced from roots as saltier soil would act as a habitat-imposed filter—a barrier inhibiting survival of all but the innately salt tolerant microbial species. However, the same could be said for the leaves of *S. alterniflora*, as the plant processes salt by secreting it out of the plant and onto the leaves to be washed away, creating a salt barrier (Yuan et al. 2016). Two extremely salt tolerant species of fungi were also sourced from *T. distichum* leaves, one from a salt sourced tree and one from a fresh sourced tree. We suggest that no plant level filtering is selecting for halotolerant microbes in the case of *T. distichum*, which processes salt by altering sap flow to cope with long-term-imposed osmotic gradients (Krauss & Duberstein 2010) and, as far as we know, involves the leaves only via lowering transpiration rates in saltier locations (Lauer 2013).

Lack of evidence for local adaptation

Overall, endophytes collected from plants in saline environments did not exhibit higher growth rates in salt amended plates, presenting a lack of evidence for local adaptation.

Although evidence for local adaptation in macro-organisms is widespread, very few studies have investigated local adaptation of microorganisms. Local adaptation has been observed for both pathogenic and mutualistic microbes, though pathogens have received the bulk of the attention as they often exhibit clear signs of fitness (i.e., fitness reduction and disease symptoms in the host) and are of economic importance (Dethlefsen et al. 2007, Walter & Ley 2011). In line with our findings, Chaia et al. (2006) found no evidence of local adaptation for differing genotypes of the bacterial root symbiont *Frankia* and likewise no evidence was detected for fungal symbionts of pine trees (Hoeksema & Thompson 2007). However, as Kraemer & Boynton (2017) pointed out in a review of the existing evidence for microbial local adaptation, a lack of evidence doesn't mean it is not occurring. Local adaptation is extremely difficult to study in microbes as we do not have the technology to track individual microbes in the field, and laboratory studies fail to mimic natural conditions. It is also important to consider that dispersal limitation, genetic drift and bottlenecks could all be playing a role in observed community patterns. Overall, more work combining lab and field studies are needed to determine whether mutualistic host associations exert selective pressures comparable to those of pathogens (Kraemer & Boynton 2017).

Bacillus pumilus: an anecdotal example

One species of bacteria in our study, *Bacillus pumilus,* was found in both *S. alterniflora* and *T. distichum* leaves. In our assays, the *S. alterniflora* sourced (salt-sourced) *B. pumilus* colony grew more rapidly at all levels of the salinity treatments than the *T. distichum*-sourced (fresh sourced) *B. pumilus* colony. This suggests that while there is no overall trend in our sampled communities, some of endophytes may indeed exhibit local adaptation either to the environment, host plant, or both. Khan et al. (2016) found that, in

rice plants, *B. pumilus* showed a positive potential for limiting the Na^+ resulting in the decreased plant antioxidation activity which in turn enhanced the rice plant's tolerance to salt. Future studies in our lab may test these two strains of *B. pumilus* by inoculating both *S. alterniflora* and *T. distichum* and examining plant performance of inoculated versus non-inoculated plants under salt stress.

Limitations

A larger sample size with more samples from salt-sourced *T. distichum* would have benefitted this study. We were limited to a relatively low sample size as it is difficult to re-grow bacterial and fungal strains from vouchered specimens. We recommend that future studies conduct more intensive surveys of culturable endophytes straight from collection onto salt amended media plates. Additionally, while Instant Ocean is a good proxy for sea water, which was relevant to the aim of this study, it is possible that other elements in the mixture may have increased or inhibited the growth of microbes. It is also known that certain abiotic factors such as salinity, water availability, and climate (Friesen et al. 2011) and even small differences in the host plant genome can significantly alter the outcome of interactions between the plant and the symbiont (Rodriguez et al. 2008). Future research may investigate the efficacy of using salt tolerant microbes to increase *T. distichum* salt tolerance by inoculating different genotypes of *T. distichum* trees with both individual and suites of salt tolerant microbes and measuring plant performance both in a sterile growth chamber and in the field.

CONCLUSION

Using salt challenge assays, we identified five strains of extreme halotolerant and eleven strains of moderately halotolerant endophytes. Bacterial and fungal endophyte cultures obtained from *S. alterniflora* grass sourced from salt marshes and from *T. distichum* trees

growing in both fresh and brackish soils did not exhibit different growth patterns in salt amended media. This suggests a lack of evidence for microbial local adaptation, though more studies explicitly testing local adaptation of microbes in both the field and the lab are needed. Experiments which use halotolerant microbes to inoculate plants at risk for salt water damage are needed in both greenhouse and natural settings. Coastal restoration efforts could be improved if these salt tolerant endophytes were employed to mitigate salt stress in key restoration species, such as the iconic baldcypress tree.

FIGURES

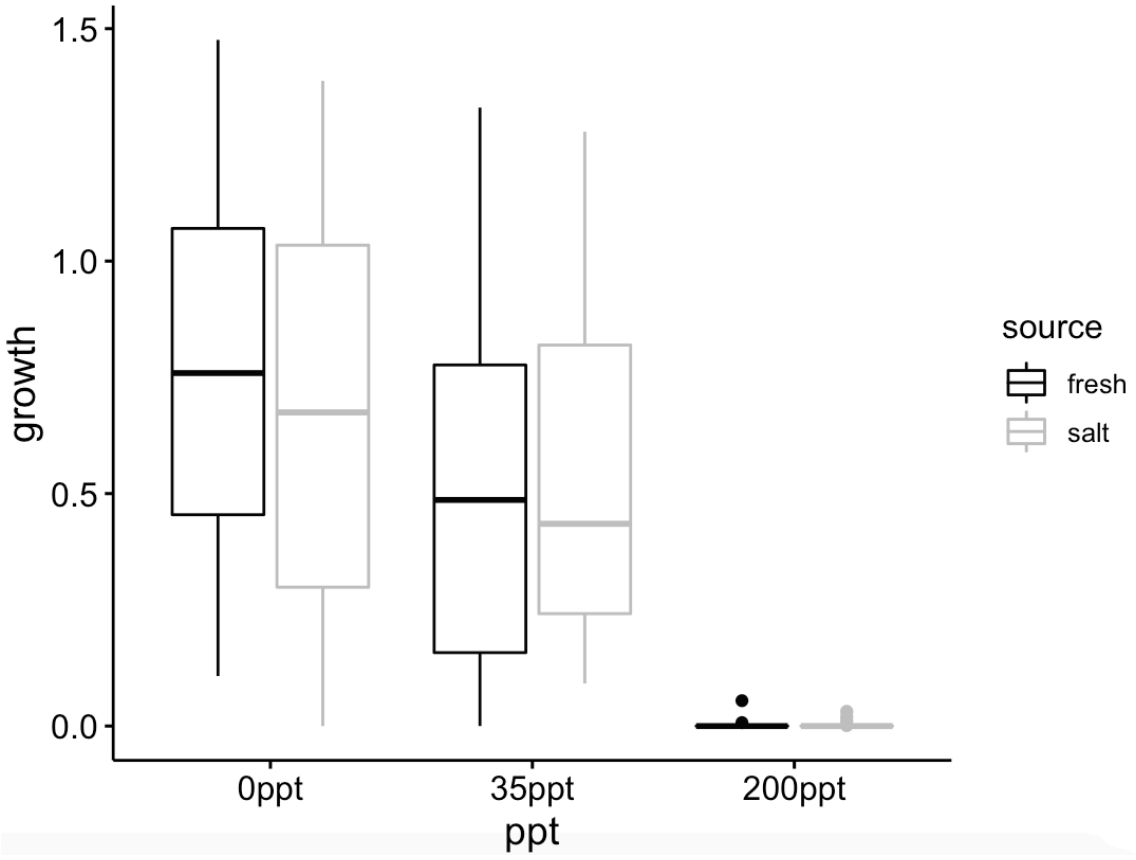

Figure 3.1 Growth rate (growth) did not significantly differ between endophytes sourced from plants in salt and fresh water among salt challenge assay levels (ppt).

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and Assessment For more information, please contact: Figure 4. Example of information provided in the basic viewer of the Web site for the Coastwide Reference Monitoring System.

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SUPPLEMENTARY

Table S1.1. Information on the nearest match in the NCBI Genbank database to our bacterial and fungal isolates from leaves and roots of baldcypress trees 4 sites in southeastern Louisiana, USA by plant organ and symbiont type.

		No. endophyte isolates/plant part					Geneious batch search information	
OTU	Putative taxonomic ID	Leaf Fungi	Root Bacteria	Root Fungi	Total	Isolate no	Accession of nearest match	Sequence Length
1	*Bacillus cereus*	1	6		7	1187	NZ_NUUR01000028	1401
2	*Bacillus altitudinis*		4		4	1232	NZ_PEKR01000032	1402
3	*Fictibacillus* sp.		4		4	1228	NZ_LMVC01000002	1382
4	*Bacillus aryabhattai*		4		4	1221	NZ_CP024035	1408
6	*Bacillus* sp.	2	1		3	1651	NZ_PDIY01000001	1413
7	*Diplodia corticola*	3			3	1420	NW_017971615	936
8	*Trichoderma virens*	21		6	27	1635	NW_014013678	1142
9	*Phialocephala scopiformis*	6		3	9	1702	NW_017263637	1031
10	*Penicillium digitatum*	2		13	15	1566	NW_014574576	1135
13	*Metarhizium brunneum*			2	2	1627	NW_014574716	1108
22	*Lysinibacillus xylanilyticus*		3		3	1308	NZ_MDDN01000041	1415
25	*Phanerochaete carnosa*			3	3	1511	NW_006767655	997
27	*Eutypa lata*	1		1	2	1597	NW_006915952	1134
29	*Trichoderma virens*	1		1	2	1465	NW_014013678	1154
30	*Olea europaea*			2	2	1579	NW_019266316	938

	31	*Eutypa lata*			2	2	1571	NW_006915952	1025
	39	*Eutypa lata*	3			3	1486	NW_006915952	945
	40	*Pseudovalsaria ferruginea*	1		2	3	1589	NG_042719	602
	41	*Fusarium verticillioides*	1		1	2	1556	NW_017387867	1085
	43	*Bacillus* sp.		3	1	4	1287	NZ_LMRU01000001	1416
	47	*Thielavia terrestris*			3	3	1472	NC_016459	602
	49	*Scedosporium apiospermum*			2	2	1474	NW_015971807	1150
	50	*Pseudomonas mosselii*		2		2	1253	NZ_LSLE01000030	1387
	52	*Bacillus subtilis*		2		2	1332	NZ_MOXE01000023	1412
	53	*Eutypa lata*	2			2	1499	NW_006915952	1163
Unassigned		*Aeromonas hydrophila*		1		1	1200	NZ_NBWY01000014	1404
Unassigned		*Alcaligenes faecalis*		1		1	1236	NZ_CP021641	1395
Unassigned		*Bacillus cereus*		1		1	1282	NZ_NUUR01000028	1405
Unassigned		*Bacillus cereus*		1		1	1667	NZ_NUJK01000025	1414
Unassigned		*Bacillus indicus*		1		1	1309	NZ_JGVU02000005	1400
Unassigned		*Bacillus novalis*		1		1	1235	NZ_KV440951	1416
Unassigned		*Bacillus* sp.		1		1	1279	NZ_PDIZ01000003	1405
Unassigned		*Bacillus* sp.		1		1	1320	NZ_AP013294	1417
Unassigned		*Chaetomium globosum*	1			1	1454	NT_166001	947
Unassigned		*Chaetomium globosum*			1	1	1481	NT_166001	917

Unassigned	*Chaetomium globosum*			1	1	1495	NT_166001	1121
Unassigned	*Endoxyla macrostoma*			1	1	1557	NG_042716	605
Unassigned	*Eutypa lata*	1			1	1427	NW_006915952	1151
Unassigned	*Eutypa lata*			1	1	1485	NW_006915952	933
Unassigned	*Eutypa lata*			1	1	1550	NW_006915952	1163
Unassigned	*Eutypa lata*	1			1	1564	NW_006915952	1142
Unassigned	*Eutypa lata*	1			1	1578	NW_006915952	1146
Unassigned	*Eutypa lata*	1			1	1582	NW_006915952	1141
Unassigned	*Eutypa lata*	1			1	1645	NW_006915952	946
Unassigned	*Eutypa lata*	1			1	1673	NW_006915952	944
Unassigned	*Eutypa lata*	1			1	1697	NW_006915952	1023
Unassigned	*Eutypa lata*	1			1	1705	NW_006915952	1025
Unassigned	*Eutypa lata*	1			1	1728	NW_006915952	1145
Unassigned	*Eutypa lata*	1			1	1737	NW_006915952	1136
Unassigned	*Fusarium graminearum*			1	1	1583	NW_001837910	1111
Unassigned	*Fusarium verticillioides*			1	1	1527	NW_017387867	1112
Unassigned	*Leptosphaeria maculans*			1	1	1520	NW_003533867	610
Unassigned	*Nostoc* sp.		1		1	1321	NZ_MTAX01000014	1415
Unassigned	*Paraburkholderia tropica*		1		1	1302	NZ_MSDZ01000062	1400
Unassigned	*Phaeoacremonium minimum*			1	1	1477	NW_006920967	955

Unassigned	*Phialocephala scopiformis*			1	1	1521	NW_017263637	1048
Unassigned	*Phialocephala scopiformis*			1	1	1524	NW_017263637	1044
Unassigned	*Rhizobium* sp.		1		1	1248	NZ_LMGD01000021	1319
Unassigned	*Thielavia terrestris*	1			1	1494	NC_016459	953
Unassigned	*Verticillium alfalfae*	1			1	1488	NW_003315035	978
Unassigned	*Xylaria hypoxylon*	1			1	1513	NG_027599	440
TOTAL		58	40	53	151			

Table S1.2. Information on the nearest match in the NCBI Genbank database to our bacterial and fungal isolates from leaves and roots of baldcypress trees 4 sites in southeastern Louisiana, USA by site

		Collection Site					Geneious batch search information	
OTU	**Putative taxonomic ID**	**Honey Island**	**Jean Lafitte**	**Lake Ponchartrain**	**Tickfaw**	**Total**	**Our isolate no**	**Accession of nearest match**
1	*Bacillus cereus*		5		3	8	1187	NZ_NUUR01000028
2	*Bacillus altitudinis*	3	1			4	1232	NZ_PEKR01000032
3	*Fictibacillus* sp.		3	1		4	1228	NZ_LMVC01000002
4	*Bacillus aryabhattai*	1	2		1	4	1221	NZ_CP024035
6	*Bacillus* sp.		1		2	3	1651	NZ_PDIY01000001
7	*Diplodia corticola*		1	1	1	3	1420	NW_017971615
8	*Trichoderma virens*	5	2	1	19	27	1635	NW_014013678
9	*Phialocephala scopiformis*				9	9	1702	NW_017263637
10	*Penicillium digitatum*	5	5	2	3	15	1566	NW_014574576
13	*Metarhizium brunneum*	1	1			2	1627	NW_014574716
22	*Lysinibacillus xylanilyticus*	1	1		1	3	1308	NZ_MDDN01000041
25	*Phanerochaete carnosa*	1	2			3	1511	NW_006767655
27	*Eutypa lata*	1			1	2	1597	NW_006915952
29	*Trichoderma virens*	1			1	2	1465	NW_014013678
30	*Olea europaea*				2	2	1579	NW_019266316

	31	*Eutypa lata*			2		2	1571	NW_006915952
	39	*Eutypa lata*			1	2	3	1486	NW_006915952
	40	*Pseudovalsaria ferruginea*		1	1	1	3	1589	NG_042719
	41	*Fusarium verticillioides*	1			1	2	1556	NW_017387867
	43	*Bacillus* sp.		3	1		4	1287	NZ_LMRU01000001
	47	*Thielavia terrestris*			3		3	1472	NC_016459
	49	*Scedosporium apiospermum*	2				2	1474	NW_015971807
	50	*Pseudomonas mosselii*		2			2	1253	NZ_LSLE01000030
	52	*Bacillus subtilis*				2	2	1332	NZ_MOXE01000023
	53	*Eutypa lata*	1		1		2	1499	NW_006915952
Unassigned		*Aeromonas hydrophila*	1				1	1200	NZ_NBWY01000014
Unassigned		*Alcaligenes faecalis*	1				1	1236	NZ_CP021641
Unassigned		*Bacillus cereus*		1			1	1282	NZ_NUUR01000028
Unassigned		*Bacillus indicus*	1				1	1667	NZ_NUJK01000025
Unassigned		*Bacillus novalis*				1	1	1309	NZ_JGVU02000005
Unassigned		*Bacillus* sp.			1		1	1235	NZ_KV440951
Unassigned		*Bacillus* sp.	1				1	1279	NZ_PDIZ01000003
Unassigned		*Chaetomium globosum*				1	1	1320	NZ_AP013294
Unassigned		*Chaetomium globosum*				1	1	1454	NT_166001

Unassigned	*Chaetomium globosum*				1	1	1481	NT_166001
Unassigned	*Endoxyla macrostoma*			1		1	1495	NT_166001
Unassigned	*Eutypa lata*			1		1	1557	NG_042716
Unassigned	*Eutypa lata*				1	1	1427	NW_006915952
Unassigned	*Eutypa lata*				1	1	1485	NW_006915952
Unassigned	*Eutypa lata*				1	1	1550	NW_006915952
Unassigned	*Eutypa lata*				1	1	1564	NW_006915952
Unassigned	*Eutypa lata*				1	1	1578	NW_006915952
Unassigned	*Eutypa lata*				1	1	1582	NW_006915952
Unassigned	*Eutypa lata*				1	1	1645	NW_006915952
Unassigned	*Eutypa lata*				1	1	1673	NW_006915952
Unassigned	*Eutypa lata*	1				1	1697	NW_006915952
Unassigned	*Eutypa lata*				1	1	1705	NW_006915952
Unassigned	*Eutypa lata*				1	1	1728	NW_006915952
Unassigned	*Fusarium graminearum*		1			1	1737	NW_006915952
Unassigned	*Fusarium verticillioides*				1	1	1583	NW_001837910
Unassigned	*Leptosphaeria maculans*	1				1	1527	NW_017387867
Unassigned	*Nostoc* sp.			1		1	1520	NW_003533867
Unassigned	*Paraburkholderia tropica*		1			1	1321	NZ_MTAX01000014
Unassigned	*Phaeoacremonium minimum*				1	1	1302	NZ_MSDZ01000062

Unassigned	*Phialocephala scopiformis*				1	1	1477	NW_006920967
Unassigned	*Phialocephala scopiformis*				1	1	1521	NW_017263637
Unassigned	*Rhizobium* sp.			1		1	1524	NW_017263637
Unassigned	*Thielavia terrestris*				1	1	1248	NZ_LMGD01000021
Unassigned	*Verticillium alfalfae*				1	1	1494	NC_016459
Unassigned	*Xylaria hypoxylon*	1				1	1488	NW_003315035
TOTAL		**30**	**33**	**19**	**69**	**151**		

BIOGRAPHY

Liz hails from the briar-patches of Alabama. At 18, she moved to Northern California to study Botany in the redwood trees at Humboldt State University. During the summer of 2008, while living on the La Selva Biological station in Costa Rica researching mycorrhizal fungi, grew interested in plant symbionts leading into her doctoral work with endophytes.

After graduating, Liz worked as a research assistant in the Peruvian Amazon, conducted rare plant surveys on Pacific Northwest timberlands, served as the first Travel Intern for Appropedia.org, worked as a tutor in Italy, taught science to kids in San Diego, and became a yoga teacher. Liz began interning for mongabay.com, a popular science and conservation news site, in July of 2012 and quickly discovered a passion for science communication.

Liz began graduate school at Tulane University in 2014. While in graduate school, Liz continued to work in the field of science journalism and as a grant writer. Liz's interests include: playing music, volunteering with youth, and working towards equity with a focus on LGBTQIA activism and anti-racism work.