FOLIAR FUNGAL ENDOPHYTES ASSOCIATED WITH NATIVE HAWAIIAN PLANTS AND THE BIOGEOGRAPHY OF THEIR INTERATCTIONS ACROSS THE ARCHIPELAGO

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI'I AT MĀNOA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BOTANY

MAY 2018

BY

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Keywords: Fungi, Foliar Endophytic Fungi, Community Ecology, Elevation Gradient

Acknowledgements

I would first and foremost like to express my sincere gratitude to my advisor and mentor Dr. Anthony S. Amend whose scientific insight, support, and leadership made this research possible. I would also like to extend my thanks to my graduate committee members, Dr. Nicole Hynson, Dr. Clifford Morden, Dr. Andrew Taylor, and Dr. Travis Idol for their guidance throughout my degree. Special thanks goes to Dr. Nhu Nguyen for his friendship, leadership, and lab training during my undergrad which prepared me greatly for graduate research.

I would also like to thank current and past members of the University of Hawaiʻi fungal lab groups including Richard O'Rorke, Geoff Zhan, Jack Darcy, Cameron Egan, Laura Tipton, Kamala Earl, Leah Tooman, Andre Boraks, Kama Chock, Sean Swift, Jerry Koko, Erin Datlof, and honorary member Patricia Sendao.

Thank you to my father Jose Maria Cobian for being a positive role model and showing me what hard work feels like. My mom and her partner, Julie Clifton and Susan Kolar, for supporting me and encouraging me throughout my lifetime. My grandparents, Delfina Cobian-Cisneros and Jose Maria Cobian for showing me how to work and be a man.

Last, and certainly not least, thank you to my incredible and amazing wife Kanade Oishi. Thank you for your constant support. Thank you for your partnership, positivity, and optimism. Thank you for being an amazing mother to our two kids, Sierra Cobian and Eli Cobian. Words could never describe how much you mean to me.

Abstract

Foliar fungal endophytes are a globally ubiquitous group of fungi that form species rich communities within plant host leaves. While uncertainty exists about the exact mechanisms determining fungal endophyte community assembly, communities have been shown to be structured by their host species as well as local environmental conditions. However, our understanding of the interaction between host specialization and local environmental conditions is limited, especially at broader spatial scales within the same host species. The aim of this dissertation was to address this knowledge gap, through the examination of fungal endophyte communities at the regional and landscape scale within the Hawaiian archipelago. Specific objectives were to determine whether (1) host selection or island is a stronger determinate of community structure at the regional scale, (2) habitat filtering or host selection is a stronger determinate of community structure at the landscape scale by communities within the same hosts across an elevation gradient on the island of Hawaìi, and (3) whether fungal endophytes follow similar distribution patterns as their plant hosts at the landscape scale.

Examination of fungal endophyte communities across the Hawaiian Archipelago revealed that communities are structured by both island and host, but more strongly by island. At the landscape scale, fungal endophyte communities were significantly structured by host species, with little to no environmental effect. Similar to other microbial studies, fungal endophyte species did not display similar patterns as larger organismal groups, and were largely random in their distribution, indicating that fungal endophytes and their hosts' distributions are regulated by different factors. Collectively, these studies indicate that fungal endophyte community structure is scale dependent. At regional spatial scales, in this case the Hawaiian archipelago, geographic location is a stronger determinate of community structure than host species, signifying the importance of local ecological conditions, such as local environmental conditions, dispersal limitations, and evolutionary history. Conversely, at

landscape scales, such as the elevation gradient examined, host was observed to be a

stronger determinant of community structure than geographic location.

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Chapter 1   Introduction

1.1   Foliar fungal endophytes

Foliar fungal endophytes are arguably one of the most ubiquitous symbiotic

relationships between fungi and plants, occurring in every examined plant lineage (Arnold and

Engelbrecht, 2007a). By definition these fungi are not pathogenic (Arnold et al., 2003;

Rodriguez et al., 2009) and have been shown to play important roles in plant biochemistry

(*reviewed*: Arnold, 2007), water conductance (Arnold and Engelbrecht, 2007a), and heat and

drought tolerance (Kannadan and Rudgers, 2008). Endophyte global diversity is estimated to

comprise of greater than one million species, but less than 1% have been described. In dicot

plant hosts, foliar endophytes are "hyperdiverse" containing communities of hundreds of

species of fungi coexisting within the leaves of a single host (Arnold et al., 2000; Arnold and

Lutzoni, 2007). Unlike the endophytes associated with monocot plants, endophytes associated

with dicots are horizontally acquired and not inherited from the parent plant (Arnold, 2007).

Additionally, knowledge is lacking on how endophytes associated with dicots are distributed

across varying landscapes.

It is likely that the intimate associations between hosts and foliar endophytes and the

variation in environmental factors contribute to the distribution of foliar fungal endophytes.

Indeed, both host and environment have been shown to correlate with foliar endophyte

community composition, but the importance of each largely understudied at varying spatial

scales. Additionally, foliar endophyte community composition correlates with environmental

factors such as elevation and precipitation (Zimmerman and Vitousek, 2012), and temperature

(Coince et al., 2014). Since both environment and host affect community composition of foliar

fungal endophytes, their distributions across space are likely susceptible to both stochastic

and deterministic filters.

1.2   Hawai'i as a model system

Archipelagos provide an opportune setting in which to examine species distributions and can provide insight into ecological and evolutionary dynamics determining how species are distributed across space (Martiny et al., 2006). The extreme geographic isolation of the Hawaiian Archipelago has led to a highly endemic flora, where some species encompass remarkably wide niches and elevational distributions (Wagner, 1999), allowing for the examination of how host identity and distance simultaneously impact communities of symbionts. Additionally, the Hawaiian Archipelago contains dramatic elevation gradients that facilitate the study of elevation effects on species distributions while minimizing the effects of distance (Raich et al., 1997). For example, Mauna Loa is a shield volcano located on Hawai'i Island, which is the largest and youngest of the Hawaiian Islands. Mauna Loa ranges in elevation from sea level up to approximately 4200 m above sea level in a relatively short distance. Also, *Metrosideros polymorpha*, an endemic tree species, can be found near sea level all the way up to 2500 m along the eastern slope of Mauna Loa (Vitousek et al., 1988). Co-occurring with *M. polymorpha*, there are other native woody plants that span large portions of the slope as well.

1.3   Research outline

In this dissertation, I used the Hawaiian Archipelago, the unique native flora, and the foliar fungal endophytes associated with native Hawaiian flora to examine the distribution patterns of foliar fungal endophytes. In Chapter 2, I investigate whether host selection or island is a stronger determinate of community structure at the regional scale (across the Hawaiian Archipelago). I use the unique native flora of Hawai'i to examine the importance of hosts and islands in determining endophyte community structure, the effects of distance on endophyte community structure, and the taxonomic and functional classification of fungi that are indicative of specific islands and hosts. I hypothesize that: 1) islands will be stronger

determinates than hosts of foliar fungal endophyte community structure at regional scales due to the limited distribution of endophytes, 2) isolation by distance will affect community structure of foliar fungal endophytes across the Hawaiian Archipelago, and 3) both islands and hosts will have indicator OTUs associated with them, meaning fungal species with restricted distributions within an island or host.

In Chapter 3, I investigate whether habitat filtering or host selection is a stronger determinate of community structure at the landscape scale when communities within the same hosts across an elevation gradient on the island of Hawaiʻi. I used the unique characteristics of the Hawaiian flora and the dramatic environmental gradients of Mauna Loa to isolate environment from both plant community and distance effects in order to test how environment impacts endophyte richness, community diversity, community similarity, host preference, and geographic distance. I hypothesize that 1) elevational gradients will affect fungal foliar endophytes community composition and richness and 2) host will play an important role within and among sites in shaping differences of fungal communities because different hosts provide different physiological environments for their symbiotic partners.

In Chapter 4, I investigate whether fungal endophytes follow similar distribution patterns as their plant hosts. I use the unique characteristics of the native Hawaiian flora and the dramatic elevation gradient of Mauna Loa to test Rapoport's Rule of elevational range distributions, spatial autocorrelation, and abundance-occupancy trends of individual foliar fungal endophytes along an elevation gradient. I hypothesize 1) that samples at higher elevations will contain species whose distributions, on average, span greater elevational ranges, 2) that fungal endophytes will be spatially autocorrelated, and 3) fungal endophytes will follow the abundance-occupancy trend. I will also examine fungi that demonstrate greater or less occupancy than local abundance would predict to glean insight into taxonomic and functional correlates with distribution patterns.

Chapter 2   Distribution of foliar fungal endophyte communities is driven by location at regional scales

2.1   Background

Scientists have postulated, historically, that microbes have unlimited dispersal capabilities, and that species distributions are determined solely by local environmental conditions (Baas-Becking hypothesis). However, multiple studies point to dispersal limitation in microbes. For example, the distribution of ectomycorrhizal fungi on tree-islands is limited by their ability to disperse across uninhabitable grasslands, reducing immigration rates to distant islands (Peay et al., 2012, 2010). Additionally, microbes associated with the built-environment have limited distributions among sampling location at small scales (< 500 m) despite the fact that communities were structured by outdoor environment ((Adams et al., 2013). Foliar fungal endophytes are an important plant symbiont, but our understanding of their distribution patterns is limited.

It is likely that the intimate associations between hosts and foliar endophytes and the variation in environmental factors contribute to the distribution of foliar fungal endophytes across the Hawaiian Archipelago. Indeed, both host and environment have been shown to correlate with foliar endophyte community composition, but the importance of each at broad regional scales in largely understudied. Additionally, foliar endophyte community composition correlates with environmental factors such as elevation and precipitation (Zimmerman and Vitousek, 2012), and temperature (Coince et al., 2014). At small spatial scales within Hawaii Islands there is no evidence of dispersal limitation, and host identity is the strongest driver of community composition (Cobian, Chapter 2). I sought to test the dispersal limitation of foliar endophytes at larger spatial scales and across ocean channels which presumably inhibit dispersal.

Archipelagos provide an opportune setting in which to examine species distributions and can provide insight into ecological and evolutionary dynamics determining how species are distributed across space (Martiny et al., 2006). The Hawaiian Archipelago is especially opportune as the native flora is host to a large number of endemic species, and many species can be found across all islands (Wagner, 1999), allowing for the examination of how host identity and distance simultaneously impact communities of symbionts. In this study, I use the unique native flora of Hawaiʻi to examine the importance of hosts and islands in determining endophyte community structure, the effects of distance on endophyte community structure, and the taxonomic and functional classification of fungi that are indicative of specific islands and hosts. I hypothesize that: 1) island will be a stronger determinate than host of foliar fungal endophyte community structure at the regional scale (across the archipelago) due to the limited distribution of endophytes, 2) Dispersal limitation will affect community structure of foliar fungal endophytes across the Hawaiian Archipelago, and 3) both islands and hosts will have indicator OTUs associated with them, meaning fungal species with restricted distributions within an island or host.

2.2   Methods

2.2.1 Sites/Fieldwork

To investigate the effects of both island and host on foliar fungal endophytes, I sampled 10 sites on five different islands, where three hosts co-occur across the Hawaiian Archipelago Figure 2.1; Table 2.1). Sites were chosen based on the co-occurrence of target host plants: *Metorsideros polymorpha, Leptecophylla tameiameiae* and plants in the genus *Cheirodendron* (within which species are either phylogenetically unresolved or difficult to identify in the field). Only seemingly healthy, mature, naturally recruited individuals were selected. Leaves were collected such that when combined, they covered a surface area roughly equivalent to two

adult-sized hands. The location of each plant was recorded with a GPS and plants were positively identified in the field and/or vouchered for subsequent identification (vouchers deposited at Joseph F. Rock Herbarium at the University of Hawai'i, Mānoa; HAW). Leaves were refrigerated until subsequent processing (within 72 hours of collection).

2.2.2 Molecular analysis

2.2.2.1 Surface sterilization

To eliminate fungi that may have been present on leaf surfaces, I surface sterilized all leaves prior to DNA extraction. To do this, I first collected forty leaf 'disks' per individual host by hole punching leaves using a surface sterilized standard paper single hole punch (approximately 0.5 cm diameter). I then placed leaf disks into loose-leaf tea bags and stapled them shut. Next, I surface sterilized the disk packets by rinsing the loose-leaf tea bags with 1% NaClO for 2 mins, then 70% EtOH for 2 mins, followed by two rinses with sterile water for 2 mins each (*adapted from*: (Zimmerman and Vitousek, 2012).

2.2.2.2 DNA Isolation

For DNA extraction, ten leaf disks were placed in MP Biomedical Lysing Matrix A tubes (MP Biomedical, Santa Ana, CA, USA) containing DNA isolation solutions from the MoBio PowerPlant® Pro DNA Isolation kit (Solution PD1, Solution PD2, Phenolic Separation Solution, and RNase A Solution; MO Bio, Carlsbad, CA, USA). Leaf disks were homogenized using a Mini-Beadbeater 24 (BioSpecs Inc. OK) at 3,000 oscillations per min for 2 mins. Genomic DNA was isolated using a modified MoBio PowerPlant® Pro DNA kit using the manufacturers protocol (centrifuged beaten tubes at 13,000 xg for 2 mins).

2.2.2.3 Amplification and Illumina Library Prep

The ITS1 region of the rDNA was amplified using fungal specific primers ITS1f and ITS2, combined with Illumina adaptors and Golay barcodes (Smith and Peay, 2014). Polymerase chain reactions (PCR) were carried out using the KAPA3G Plant PCR kit (KAPA Biosystems, Wilmington, MA, USA). All PCR products were purified and normalized using the just-a-plate 96 PCR Purification and Normalization Kit (Charm Biotech, San Diego, California, USA). Normalized PCR products were then pooled and concentrated using a streptavidin magnetic bead solution. Pooled and concentrated products were then sequenced at the Hawaiʻi Institute of Marine Biology's Genetics Core Facility (HIMB GCF, Kaneohe, HI, USA) using the 2 x 300 paired-end sequencing protocol on an Illumina MiSeq sequencing platform (Illumina Inc., Dan Diego, CA, USA).

2.2.3 Bioinformatics

Analysis of sequencing reads was conducted using the open-source software 'quantitative insights into microbial ecology' (QIIME; (Caporaso et al., 2010). Sequences were first demultiplexed to be identified to their respective samples using the 'split libraries' step. Even though paired-end sequencing was conducted, I observed low quality sequencing reads on our reverse sequences, forcing us to use only the forward reads for downstream analysis (*see* Nguyen et al., 2015). Reads were quality filtered using the VSEARCH algorithm (Rognes et al., 2016) implemented in QIIME to discard reads with an average quality score below 25. Next the ITSx program (Bengtsson-Palme et al., 2013) was used to extract the ITS1 region from quality-filtered reads.

Quality filtered reads were then assembled into operational taxonomic units (OTUs) using the UNOISE3 algorithm (Edgar, 2016). Sequences were first de-replicated at 100% similarity using the VSEARCH algorithm (Rognes et al., 2016), then zOTU (zero-radius

Operational Taxonomic Units) centroid sequences were picked and chimeric sequences were removed. Then, all sequences were mapped onto zOTU seeds to create a zOTU table using VSEARCH. Unlike *de novo* OTUs clustered at arbitrary identity cutoffs like 0.97 or 0.95, zOTUs are exact sequence variants (ESVs), which are better able to detect novel diversity while simultaneously filtering out artificial diversity caused by sequencing and PCR error (Callahan et al., 2017). Taxonomy was assigned to each zOTU using the UNITE v7 database (Kõljalg et al., 2013) and QIIME's assign_taxonomy.py script (Caporaso et al., 2010) with the BLAST method ; Altschul et al., 1990).

2.2.4 Statistical Analyses

2.2.4.1 Host and island specialization and selectivity

To determine the importance of island and plant-hosts in structuring foliar fungal endophyte communities I aggregated OTU tables into island networks. zOTUs were first aggregated by plant host and island. Next, to determine network specialization *H2'* (Blüthgen et al., 2006) was calculated using the *H2fun* function in the *bipartite* package in R (Dormann et al., 2008). To determine host selectivity of fungal endophyte communities, the *d'* (d-prime) index (Blüthgen et al., 2006) was calculated using the *dfun* function in the *bipartite* package in R. Both indices range from 0 to 1. Where a value of 0 indicates complete generalization and a value of 1 indicates complete specialization. Both indices take into account the interaction frequencies and are standardized to account for heterogeneity in the interaction strength and taxon richness. Observed *H2'* and *d'* values were compared to a null distribution of both indices with 1000 permutations.

2.2.4.2 Community composition

Effect of island vs host on community composition: To visualize how island and hosts structure foliar endophyte communities across the archipelago, the *vegdist* function was used from the *vegan* package (Oksanen et al., 2017) to calculate Bray Curtis dissimilarity distance matrix from our log transformed zOTU abundances and plotted NMDS plots. Bray Curtis dissimilarities range from 0.0, indicating identical communities, to 1.0, indicating completely different communities. To determine the extent to which plant-host and islands predict community composition, a PERMANOVA was carried out using the *adonis* function in vegan.

Effect of distance on community composition: A Mantel test was used to investigate the effect of distance on community composition across the archipelago. The *vegdist* function from the *vegan* package was used to calculate a spatial distance matrix and a Bray Curtis dissimilarity distance matrix. The dissimilarity values were compared between pairwise samples across the archipelago using the *mantel* function in *vegan* with 1,000 permutations.

2.2.4.3 Species indicator analysis

To determine whether foliar fungal endophyte taxa are significantly associated with specific islands or hosts species indicator analysis was conducted. Indicator analyses were carried out on zOTUs using the function *multipatt* from the R package *indicspecies* (Cáceres and Legendre, 2009) with 1000 permutations. The function assesses the strength and statistical significance of the relationship between species abundances and groups of sites (islands and species-island). Two different components are returned from the statistical test: 1) specificity value of the zOTU as indicator of a site group, indicated as 'A' and 2) sensitivity of the zOTU as indicator of the target site group, indicated as 'B'. Component A indicates the probability that the zOTU belongs to the target site; therefore, a value of 1.0 indicates that the zOTU only occurs at the target site group. Component B indicates the probability of finding a

particular zOTU at the site group; therefore, a value of 1.0 indicates that the zOTU is found at all site groups. For example, if zOTU1 was indicated for *M. polymorpha* with an A-value of 1.00 and a B-value of 0.82, this would indicate that zOTU1 only occurs with *M. polymorpha* but does not occur in all *M. polymorpha* samples. Also, if zOTU3 was indicated for *L. tameiameiae* with an A-value of 0.82 and a B-value of 1.00, this would indicate that zOTU3 is largely, but not completely, restricted to *L. tameiameiae*, and it occurs in all sampled *L. tameiameiae*. To explore the functional and taxonomic correlates of indicator fungal zOTUs, I used taxonomic assignments from our BLAST results and compared them to taxa identified on the functional database *FunGuild* program v1.1 (Nguyen et al., 2016). *FunGuild* uses strings in the assigned taxonomy to compare against a database of known ecological guilds in order to assign a functional guild to OTUs in OTU tables.

2.3   Results

2.3.1 Host and island specialization and selectivity

Both islands and hosts displayed high specialization (Table 2.2). Island networks were highly specialized, and this specialization was statistically significant (Table 2.2; $H2'_{all\text{-}islands} > 0.80$; $p_{all} < 0.001$) with network specialization increasing with island age. Island showed a high statistically significant specialization (Table 2.2; $d'_{all\text{-}islands} > 0.78$; $p_{all} < 0.001$), and island specialization decreased with island age with the exception of Hawai'i Island. Similarly, all plant hosts examined showed high host specialization (Table 2.2, $p < 0.001$ for all hosts d' values). On all islands, *M. polymorpha* had the lowest specialization values. Specialization ranged from 0.70 to 0.87, less than 0.90, but still highly significant (p<0.001). All d' values for both *Cheirodendron spp.* and *L. tameiameiae* were high ($d' > 0.90$).

2.3.2 Effect of distance on fungal community composition

Mantel test of pairwise comparisons of Bray-Curtis community dissimilarities explained less than 1% of variance and was not significant (Figure 2.2; r^2 = 0.003; p > 0.05). Additionally, Bray-Curtis values were high ($Bray\text{-}Curtis_{MEAN}$ = 0.97) across all pairwise distance differences. Mantel tests for both islands and sites were similar to those of the entire dataset (results not shown, p > 0.05).

PERMANOVA results indicate that plant host was a significant contributor to endophyte community composition and accounted for 10% of variance (Figure 2.3a; $stress$ = 0.09; r^2 = 0.10; p = 0.01). Additionally, islands were also significant in structuring endophyte communities and accounted for 17% of variance (Figure 2.3b; $stress$ = 0.09; r^2 = 0.17; p = 0.01). Endophyte communities from neighbor islands were more similar to each other than non-neigbor islands, except Kaua'i (Figure 2.3b).

2.3.3 Indicator OTUs

Indicator species analyses concluded that there were 15 indicator OTUs on three of the five sampled islands and one of the three host genera (Table 2.3). Kaua'i had one indicator OTU that only occurred on this island but only in 2/3 of the collections. Moloka'i had three indicator OTUs two of which only occurred on that island, and all three OTUs occurred in 2/3 of the island samples. Maui had nine indicator OTUs with six only occurring on this island and three found in all island samples. *Metrosideros polymorpha* was the only host to have indicator OTUs. Of the two indicator OTUs, neither were observed to only occur within *M. polymorpha* nor were they found in all the *M. polymorpha* samples. Of the 15 observed indicator OTUs, *FunGuild* was unable to assign a fungal guild to 1/3 of the OTUs. Of the other 2/3 that were assigned a guild, six were pathogens and five were saprotrophs (Table 2.3).

2.4   Discussion

In this study, plant hosts and islands had highly specialized communities associated with them. Additionally, both plant host and island were significant indicators of endophyte community composition across the Hawaiian Archipelago, but islands explained more variation. Despite the fact the endophytes were structured by both plant host and island, distance did not correlate with community dissimilarity across the archipelago. Indicator OTUs were observed in three of the five islands and one host.

This study indicates that foliar fungal endophyte community composition is structured differently at different scales. At the landscape scale (within-island), foliar fungal endophyte community composition is influence by plant host to a greater extent than environment (Cobian, Chapter 3). In this study I broadened the scale from landscape to the archipelago region and saw that location is a more important determinate of foliar endophyte community composition. Thus, the factors affecting community composition of foliar fungal endophytes work at disparate scales. It is likely that dispersal limitation has a greater influence at regional scales as opposed to landscape scales.

Our PERMANOVA results support the hypotheses that both host and location are important components in structuring foliar fungal endophyte community composition. Other studies suggest that foliar endophyte communities are structured via environmental factors such as elevation (Zimmerman and Vitousek, 2012), precipitation (Giauque and Hawkes, 2013; Zimmerman and Vitousek, 2012), and temperature (Coince et al., 2014; Zimmerman and Vitousek, 2012). While I did not take environmental parameters into account in this study, environmental parameters are typically confounded by distance. It is possible that environmental conditions are not the main parameters responsible for differences among communities.

Despite the historical dogma that "everything is everywhere" and the environment selects, fungal studies have determined that ectomycorrhizal fungi (Peay et al., 2012, 2010), the indoor microbiome (Adams et al., 2013), and airborne and soil fungi (Kivlin et al., 2014) are dispersal limited. However, our Mantel test results did not show the typical pattern of isolation by distance pattern (Figure 2.3). While the Mantel correlation trend is zero, community dissimilarly is very high across pairwise comparisons of distance. In fact, mean Bray-Curtis value was almost one (*Bray-Curtis$_{MEAN}$* = 0.97) across the entire study. While there are a number of processes that affect the distribution of organisms (Martiny et al., 2006), our results suggest that foliar fungal endophytes are extremely dispersal limited. It is expected that communities in close proximity will have more similar compositions while those that are further apart are expected to have more dissimilar compositions. This is the expected trend if dispersal limitation is not a major factor in the distribution of foliar fungal endophytes. However, this was not the observed trend in this study, and therefore, it is likely that these endophyte communities are dispersal limited.

Since foliar endophytes are potentially very dispersal limited, identifying indicator fungi could help in identifying which symbionts to focus attention at larger scales to determine which fungi may not be as dispersal limited. For example, *M. polymorpha* had one indicator species, and it could potentially be used as an environmental indicator to investigate how changes in environment affects its relationship with *M. polymorpha*. Indicator fungal OTU results showed that there were indicator fungi for Kaua'i, Moloka'i, Maui, and *M. polymorpha* suggesting that these fungi are potentially important symbionts for the host and/or locations and can be used as environmental indicators. Additionally, since approximately 88% of the Hawaiian flora is thought to be endemic to Hawai'i (Wagner, 1999), it is likely that some endophytes associated with endemic plants are also endemic. While further research would be needed to test this

hypothesis, indicator OTU analysis can help in determining which fungi may be endemic to a region or host.

2.5   Conclusion

I observed that 1) the drivers of foliar fungal endophyte community structure work at disparate scales, 2) foliar fungal endophytes are dispersal limited, and 3) indicator OTUs may be the first indications of endemic fungi. Thus, spatially explicit approaches might help to better understand the factors that influence foliar fungal endophyte distributions. Despite the challenges of working with foliar fungal endophytes because of their diverse taxonomic makeup and their cryptic lifestyle, foliar endophytes offer great potential for advancing our understanding of the factors that spatially structure communities and the ecological functioning of aboveground ecological communities.

3.6   Tables and Figures

Table 2.1: Site locations

	Longitude	Latitude	Site
Kauaʻi	-159.652	22.112	Ka
Oʻahu West	-158.145	21.513	Oa_W
Oʻahu Center	-157.996	21.625	Oa_C
Oʻahu East	-157.774	21.337	Oa_E
Molokaʻi	-156.921	21.112	Mo
Maui	-156.235	20.774	Ma
Hawaiʻi North	-155.737	20.09	Ha_N
Hawaiʻi Center1	-155.335	19.673	Ha_C1
Hawaiʻi Center2	-155.376	19.658	Ha_C2
Hawaiʻi South	-155.242	19.414	Ha_S

Table 2.1: Locations of our sampling sites across the Hawaiian Archipelago.

Table 2.2: Network, Island, and Host Specialization

	H2'	d'			
		Island	Cheirodendron	Leptecophylla	Metrosideros
Kauai	0.999	0.781	0.947	0.972	0.685
Oahu	0.955	0.843	0.922	0.912	0.829
Molokai	0.99	0.883	0.978	0.978	0.823
Maui	0.831	0.947	0.907	0.921	0.872
Hawaii	0.886	0.804	0.935	0.906	0.669

Table 2.2: Island network specialization (H2') for each island ($p_{all} < 0.001$). Island specialization (d') for each island ($p_{all} < 0.001$). Host specialization (d') for each host on each island ($p_{all} < 0.001$).

Table 2.3: Indicator OTUs and Guild Assignment

	A	B	p-value		Taxonomy	Ecological Guild
Kaui						
Zotu158	1.000	0.667	0.022	*	*Colletotrichum karsti*	Endophyte-Plant Pathogen
Molokai						
Zotu451	1.000	0.667	0.019	*	*Pseudoteratosphaeria ohnowa*	Unassigned
Zotu843	1.000	0.667	0.019	*	*Teratosphaeriaceae*	Unassigned
Zotu706	0.750	0.667	0.043	*	*Myriangium sp.*	Animal Pathogen
Maui						
Zotu437	1.000	1.000	0.001	**	*Wallemia sp.*	Undefined Saprotroph
Zotu418	0.999	1.000	0.001	**	*Aspergillus penicillioides*	Undefined Saprotroph
Zotu294	0.983	1.000	0.001	**	*Cutaneotrichosporon cutaneum*	Unassigned
Zotu40	1.000	0.667	0.029	*	*Ascomycota sp.*	Unassigned
Zotu1390	1.000	0.667	0.029	*	*Aspergillus sp.*	Undefined Saprotroph
Zotu2620	1.000	0.667	0.019	*	*Devriesia sp.*	Plant Pathogen
Zotu3474	1.000	0.667	0.029	*	*Aspergillus caesiellus*	Undefined Saprotroph
Zotu3848	1.000	0.667	0.029	*	*Fusarium keratoplasticum*	Plant Pathogen-Soil Saprotroph-Wood Saprotroph
Zotu442	0.997	0.667	0.005	**	unidentified Fungi	Unassigned
M. polymorpha						
Zotu2	0.997	0.700	0.003	**	*Pseudocercospora macadamiae*	Plant Pathogen
Zotu4	0.986	0.600	0.001	**	*Pseudocercospora macadamiae*	Plant Pathogen
Significance: < 0.001 ***, < 0.01 **, < 0.05 *						

Table 2.3: Predictive specificity value for OTU as an indicator to group (column "A"). Sensitivity of OTU as an indicator of the target group (column "B"). Ecological guild based on *FunGuild* results.

Figure 2.1: Sampling Locations
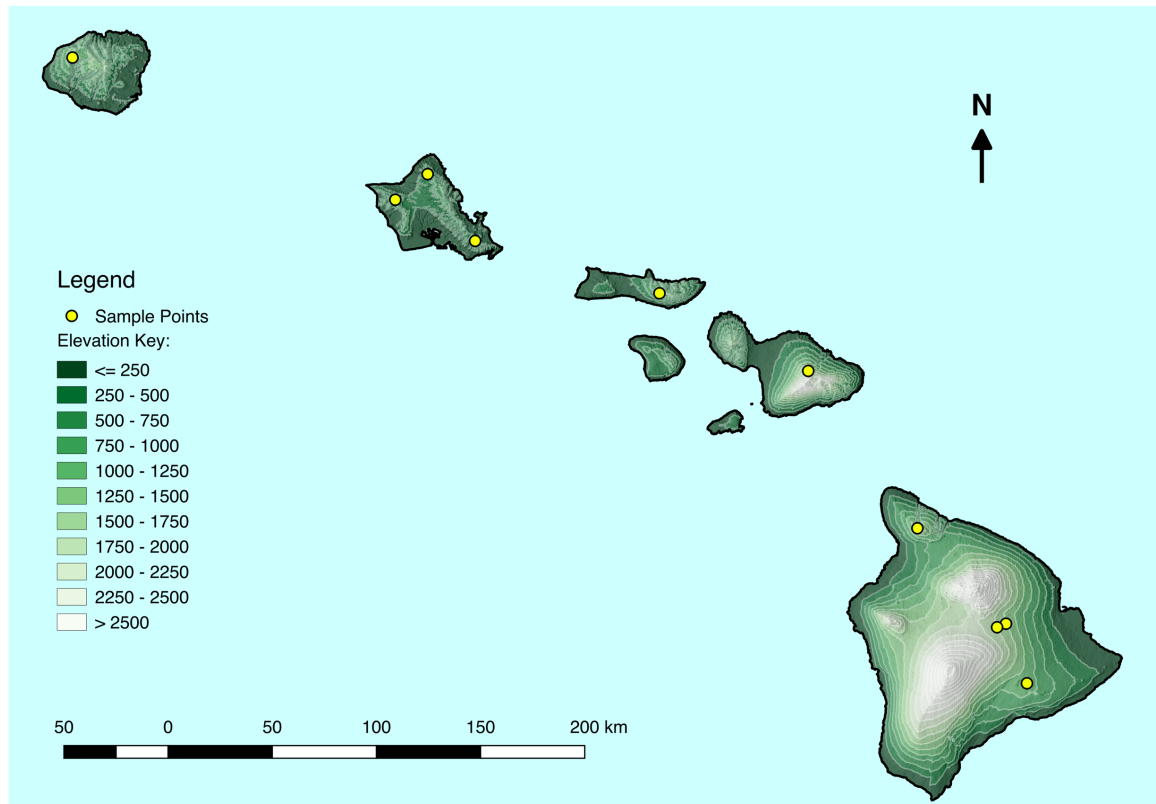
Figure 2.1: Ten sampling locations across the Hawaiian Archipelago are represented by dots. At each location, one *M. polymorpha*, one *L. tameiameiae*, and one *Cheirodendron spp.* were collected.

Figure 2.2: Mantel Distance Effect
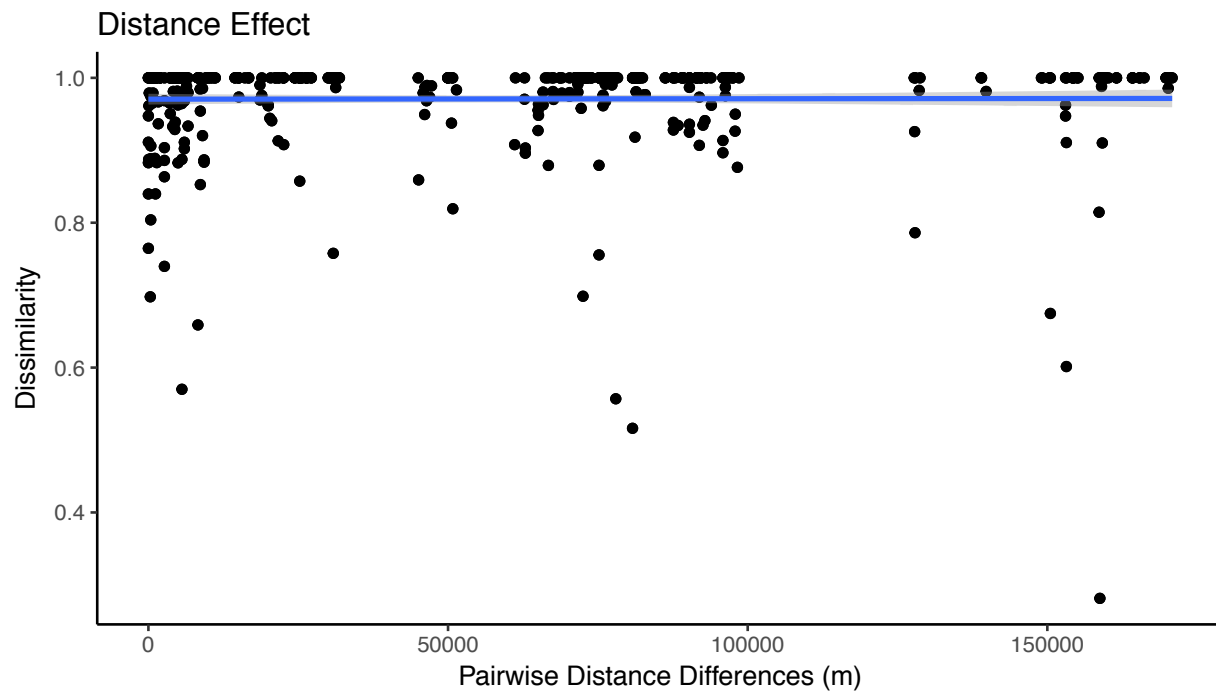
Figure 2.2: Mantel results for distance effect on foliar fungal endophyte community similarity. Distance between sampling points and community similarity were not correlated or significant ($r^2 = 0.003$; $p > 0.05$).

Figure 2.3: Community Composition

A.
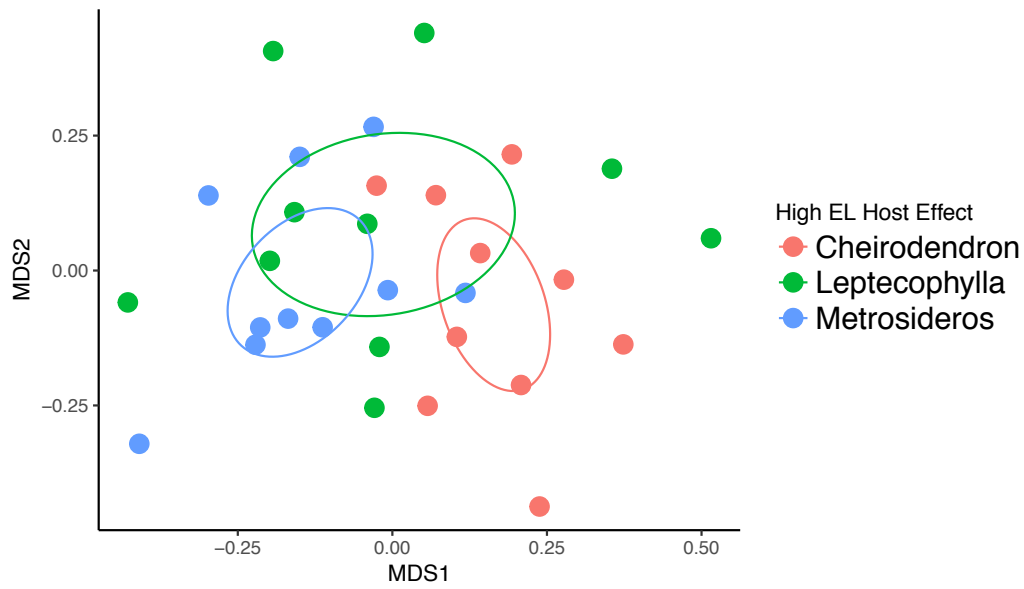
B.
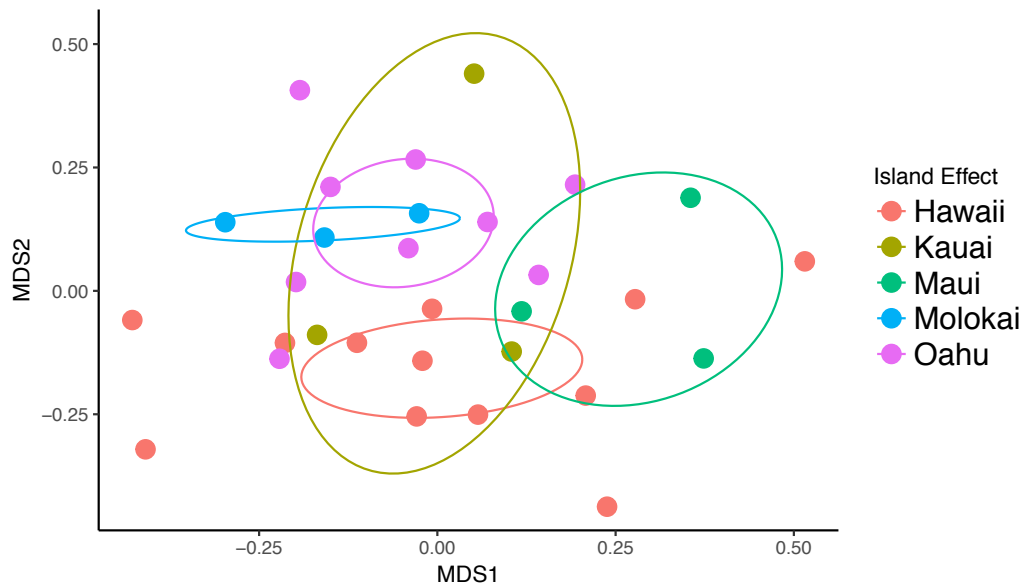
Figure 2.3: NMDS plots showing relationship between hosts (A) and islands (B) (*stress* = 0.09). A) Represent host structure of endophyte communities across archipelago. Ellipses represent standard error of the mean (95%) for each host (r^2 = 0.10; p = 0.01). B) Represents island structure of endophyte communities. Ellipses represent standard error of the mean (95%) for each island (r^2 = 0.17; p = 0.01).

Chapter 3   Host specialization has a strong influence on foliar endophytic fungi community composition along a steep elevation gradient

3.1   Background

As early as Carolus Linnaeus, researchers have been fascinated by the effects of mountain slopes on plant and animal communities (Bryant et al., 2008). Elevation gradients are thought to be analogous to latitudinal gradients where species richness increases towards the equator; similarly, richness decreases with increasing elevation (Stevens, 1992). Richness of plants and animals has been shown to decrease with increasing elevation (Pellissier et al., 2012). Studies looking at trends in microbial richness along elevation gradients have found varying results. Bryant et al. (2008) found a decrease in soil bacteria richness along an altitudinal gradient. On Mt. Fuji, Singh et al. (2012) observed mid-elevation richness peaks in soil bacteria communities as did Miyamoto et al. (2014) in ectomycorrhizal fungi with non-significant changes in richness between the lowest and highest elevations. Similarly, Fierer et al. (2011) investigated bacteria communities associated with soil and leaf surfaces, and they did not find a significant change in microbial richness along their elevation gradient.

The strength of specialization between host-microbe partners can be characterized by the frequency of the interaction between the two individuals along a continuum from complete generalization to full specialization (Blüthgen et al., 2006). Specialization can result in a restricted association of one or both partners. Some hosts only associate with specific symbiont species or clades such as mycoheterotrophic plants from the subfamily Monotropoideae where each plant species targets a different but specific ectomycorrhizal fungal host (*reviewed*: Bruns et al., 2002), and the ectomycorrhizal fungal genus *Rhizopogon* is specific to the Pinaceae family (Grubisha et al., 2002). Other symbiotic associations are more general in nature, such as most ectomycorrhizal fungi (Bruns et al., 2002) and arbuscular

mycorrhizal fungi (Klironomos, 2000). It has been shown that elevation gradients can influence biotic interaction (Schemske et al., 2009), and it is likely that environmental influences can also have an effect on host specialization.

The symbiotic foliar endophytic relationship between fungi and plants is potentially important and arguably one of the most ubiquitous plant-fungal symbiosis in nature, and researchers have yet to find a plant lineage lacking these cryptic microbial associates (Arnold and Engelbrecht, 2007a). By definition these fungi are not pathogenic (Arnold et al., 2003; Rodriguez et al., 2009) and have been shown to play important roles in plant biochemistry (*reviewed*: Arnold, 2007), water conductance (Arnold and Engelbrecht, 2007a), and heat and drought tolerance (Kannadan and Rudgers, 2008). Endophytes associated with eudicot plants are thought to be horizontally transmitted (Arnold and Herre, 2003). Foliar endophytes have also been shown to vary in their specialization with plant hosts. For example, (U'Ren et al., 2012) found host specialization at the plant host familial level across North America, while Vincent et al. (2016) found host specialization at the species level in New Guinea in lowland tropical forests. Additionally, Saunders and Kohn (2009) found that even host genotype was important in structuring endophyte communities.

Mauna Loa is a shield volcano that is located on Hawai'i Island, the largest and youngest of the Hawaiian Islands. It is an ideal location to examine host specialization and how it is influenced by environmental conditions because it offers dramatic environmental gradients at relatively short distances. Mauna Loa ranges in elevation from sea level up to approximately 4200 m above sea level in a relatively short distance on the eastern side of the island allowing us to study environmental effects of foliar endophytic communities while minimizing the effects of distance (Raich et al., 1997). Additionally, the very young geological age and isolation of the Hawaiian Archipelago allows for a small but endemic flora, some of which encompass remarkably wide niches and elevational distributions (Wagner, 1999). For example,

Metrosideros polymorpha, an ecologically important and endemic tree species, can be found near sea level all the way up to 2500 m along the eastern slope of Mauna Loa (Vitousek et al., 1988). There are a few other native woody plants that span large portions of the slope that co-occur with *M. polymorpha*. It is because I am able to separate environmental and host differences that makes Mauna Loa an ideal place to study the community dynamics of foliar endophytic fungi.

In this study, I used the unique characteristics of the Hawaiian flora and the dramatic environmental gradients of Mauna Loa to isolate environment from both plant community and distance effects in order to test how environment impacts endophyte richness, community diversity, community similarity, host preference, and geographic distance. Because environmental conditions along Mauna Loa become more stressful with increasing elevation (e.g. decreased precipitation and increased solar radiation), I expected fewer fungal species to be able to persist under these extreme conditions. Based on the results of previous microbial studies, I hypothesize that elevational gradients will affect fungal foliar endophytes community composition and richness, and that host will play an important role within and among sites in shaping differences of fungal communities because different hosts provide different physiological environments for their symbiotic partners.

3.2   Methods

3.2.1 Sites/Fieldwork

Sampling was conducted along the slopes of Mauna Loa Volcano on Hawaiʻi Island (19.4721° N, 155.5922° W). This shield volcano ranges from sea level up to 4200 m above sea level (masl), encompassing an environmental gradient over which temperature, rainfall and solar irradiance differ over a relatively short spatial distance (Figure 3.1). To examine the influence of environment on foliar endophyte host specialization I sampled three native

Hawaiian plants that co-occur along the gradient from 1100-2000 masl: *Leptecophylla tameiameiae* (pūkiawe) an indigenous species found throughout various Polynesian islands in the Pacific, *Metrosideros polymorpha* (ʻōhiʻa) an important endemic foundation species found on all the major Hawaiian Islands, and *Vaccinium reticulatum* (ʻōhelo) an endemic species and early colonizer after lava flows. *Metrosideros polymorpha* was the dominate tree species along the entire gradient. At 1100 masl, the understory was dominated by *Dicranopteris linearis* an endemic fern then it drastically started to thin out around 1500 masl. From about 1400-1600 masl, the forest is less fragmented, but above 1600 masl, it starts to thin and become fragmented due to old lava flows. I sampled hosts along the eastern slope of Mauna Kea from 1100 masl up to 2000 masl (Figure 3.2). I sampled every 100 m in elevation for a total of 10 sites and collected 4 samples for each host species at each site. To isolate environmental factors from those of distance, I selected an additional four sites at 1700 masl perpendicular to the elevation gradient and established a 2000 m transect, where I sampled every 500 m (168 sampled individuals, 56 per host species). To control for differences in biomass among target hosts, I collected 5 leaves from of Metrosideros and Vaccinium and 40 leaves from Leptecophylla, as Leptecophylla leaves are much smaller. In the field leaf samples were stored on ice until they could be and transferred to -20 °C freezer in the lab. A voucher specimen of each host species per site was collected (voucher specimens were deposited at the Joseph F. Rock Herbarium at the University of Hawaiʻi, Mānoa).

3.2.2 Molecular analysis

3.2.2.1 Surface sterilization

Prior to DNA extraction I surface sterilized leaves to exclude fungi present on leaf surfaces. First, I collected forty leaf 'disks' per individual host by punching leaves using a surface sterilized standard paper single hole punch (approximately 0.5 cm diameter), and then

placed leaf disks into loose-leaf tea bags that were subsequently stapled shut. I then surface sterilized the disk packets by rinsing the loose-leaf tea bags with 1% NaClO for 2 mins, then 70% EtOH for 2 mins, followed by two rinses with sterile water for 2 mins each.

3.2.2.2 DNA Isolation

For DNA extraction, ten leaf disks were placed in MP Biomedical Lysing Matrix A tubes (MP Biomedical, Santa Ana, CA, USA) containing DNA isolation solutions from the MoBio PowerPlant Pro DNA Isolation kit (Solution PD1, Solution PD2, Phenolic Separation Solution, and RNase A Solution; MO Bio, Carlsbad, CA, USA). Leaf disks were homogenized using a Mini-Beadbeater 24 (BioSpecs Inc. OK) at 3,000 oscillations per min for 2 mins. Genomic DNA was isolated using a modified MoBio PowerPlant Pro DNA 96well Isolation kit protocol (centrifuged beaten tubes at 13,000 xg for 2 mins).

3.2.2.3 Amplification and Illumina Library Prep

I amplified the ITS1 region of the rDNA using fungal specific primers ITS1f and ITS2, along with Illumina adaptors and Golay barcodes (Smith and Peay, 2014), using a dual indexing approach. Polymerase chain reactions (PCR) were carried out using the KAPA3G Plant PCR kit (KAPA Biosystems, Wilmington, MA, USA). All PCR products were purified and normalized using just-a-plate 96 PCR Purification and Normalization Kit (Charm Biotech, San Diego, California, USA). Normalized PCR products were pooled and concentrated using a streptavidin magnetic bead solution. Pooled PCR products were sequenced by GENEWIZ (GENEWIZ, South Plainfield, NJ, USA) using the 2 x 300 paired-end (PE) sequencing protocol on an Illumina MiSeq sequencing platform (Illumina Inc., Dan Diego, CA, USA).

3.2.3 Bioinformatics

Bioinformatic analyses were conducted using QIIME v1.9 (Caporaso et al., 2010) and Mothur v1.39 (Schloss et al., 2009). Initial quality filtering was conducted on 23,531,047 raw demultiplexed sequences, where sequences <75 bp and with an average Phred score <25 were removed, resulting in 18,370,578 sequences remaining for downstream analyses. Because the overall quality of the reverse reads was poor, I used only forward reads for subsequent processing and final analysis (see Nguyen et al., 2015 for a similar analysis). I employed a chain-clustering protocol to group sequences into operational taxonomic units (OTU) because this method tends to recover a more accurate OTU number than simply employing a single picking method (see Nguyen et al., 2015). Sequences were first grouped into *de novo* OTUs using a sequence similarity of ≥96% using USEARCH (Edgar, 2010) in QIIME. Sequences were also checked for chimeras using a reference based approach with the UNITE v7 database (Kõljalg et al., 2013) during clustering. A second round of *de novo* clustering was done using UCLUST (Edgar, 2010), again using 96% sequence similarity. OTUs were identified taxonomically by using the BLAST v2.6 algorithm (; Altschul et al., 1990) to identify representative sequences against the UNITE v7 database. Sequences with an aligned length divided by the total length of the query sequence <0.85 were removed from the OTU table. Additionally, OTUs found in the PCR negative controls were removed from each OTU by subtracting the number of sequences in the negatives from the sequence abundance from sample OTUs. This process removed almost 500,000 sequences from approximately 11.2 million sequences.

All downstream analyses were done in R (v 3.3.2; R Core Team, 2016). Data biom file with taxonomy and metadata was imported to R using the *biomformat* package (McMurdie and Paulson, 2016) and the functions *biom_data*, *observation_metadata*, and *sample_metadata* from package *biomformat* to extract the OTU table, taxonomy, and metadata, respectively. To

reduce the likelihood of tag switching, I removed OTUs from samples in which their abundance was < 0.1% of the max number of reads found in another sample. I then removed all OTUs with <10 reads in the dataset, and OTUs in a sample that do not have a minimum of 3 reads in that sample. This reduced the number of OTUs from 3486 to 2058 after removing suspicious and low abundant OTUs and OTUs that were not classified as fungi at the kingdom level. Lastly, I rarified the OTU table down to 2300 reads per samples to account for uneven sequencing depth across the dataset.

3.2.4 Statistical Analyses

3.2.4.1 Effect of elevation on local OTU richness and evenness

The effect of elevation on fungal richness and diversity in leaves was determined by using the *specnumber* function in the *vegan* package (Oksanen et al., 2017) for richness and by calculating Shannon's diversity using the *diversity* function. Evenness was also calculated by dividing Shannon's index value for each sample by log richness for each sample.

3.2.4.2 Effect of elevational difference and host on fungal community composition

To test the effect of elevation and distance on community composition, Bray Curtis dissimilarities index was used to test the effects of elevation and distance on community composition by using the *rankindex* function in *vegan* on log transformed OTU abundances. The dissimilarity values were compared between pairwise samples along the gradient and along the consent elevation transect using the *mantel* function in *vegan* with 10 000 permutations. Additionally, I used the *betapart* package (Baselga and Orme, 2012) to determine the proportion of fungal community differences attributable to either nestedness or turnover. To determine the extent to which host predicts community composition at each site along the elevation gradient I performed a PERMANOVA using the *adonis* function in vegan.

3.2.4.3 Host specialization and selectivity

To determine the degree of specialization within the plant-fungal network I aggregated each host species per site then calculated H2' (H-2-prime) index using the *H2fun* function in the *bipartite* package in R (Dormann et al., 2008). Additionally, using the same aggregated network I calculated the d' (d-prime) index (Blüthgen et al., 2006) to determine the extent of host selectivity using the *dfun* function in the *bipartite* package in R. Both functions range from 0.0, indicating complete generalization, to 1.0, indicating complete specialization. Both indices take into account the interaction frequencies and are standardized to account for heterogeneity in the interaction strength, and taxon richness. H2' and d' were plotted as a function of elevation and fitted with a loess curve. I then used a Pearson's product-moment correlation to test the fit of host selectivity from distance to mid-range along the elevation gradient.

3.3   Results

3.3.1 Effect of elevation on local fungal diversity (alpha-diversity)

There was no relationship between fungal richness and elevation (Figure 3.3a). Similarly, for *M. polymorpha* and *V. reticulatum*, there was not a significant decrease in Shannon's diversity with increasing elevation (Figure 3.3b). However, *L. tameiameiae* fungal diversity did decrease with increasing elevation (Figure 3.3b, $r=-0.325$, $p=0.041$).

3.3.2 Effect of elevational and host on fungal community composition (beta-diversity)

Our NMDS and ADONIS results show that fungal communities are significantly partitioned by host, except at 1700 and 2000 m, but more so at lower and mid elevation sites (Figure 3.4a and Table 3.1). Host identity explained the greatest amount of variance (R^2) at mid elevations (1300-1600) and ADONIS values attenuated towards the lowest and highest

elevations (Table 3.1). Additionally, host was a significant indicator of fungal community composition across the elevation gradient (Figure 3.5, ADONIS: $R2 = 0.104$, $P <0.001$).

Mantel tests for both *M. polymorpha* and *V. reticulatum* along the elevation gradient revealed that geographic distance between samples did not affect foliar endophytic fungal community composition (Figure 3.6a). However, for *L. tameiameiae* I observed a statistically significant but weak interaction between community composition and geographic distance between samples, where community similarity increased with decreasing distance ($r=0.105$, $p=0.02$).

Along the isocline transect where elevation was held constant, only a single species, *V. reticulatum*, showed a relationship between geographic distance and community dissimilarity ($r=0.188$, $p=0.02$; Figure 3.6b). Additionally, beta partitioning indicated that nearly all the observed dissimilarity along the gradient was driven by community turnover as opposed to community nestedness (Table 3.2).

3.3.3 Effects of elevation on host specialization

Our network analysis supported NMDS findings of host selectivity along the elevation gradient. Network specialization (*H2'*) was high (>0.6) for all sites except the highest site at 2000 masl (Table 3.1, Figure 3.4a, *H2'*=0.475). Despite the lower network specialization at 2000 masl, it was still significantly higher than the null for this site and all sites ($p <0.001$ for all sites). Individual host specialization (*d'*) was high and significant compared to the null for all hosts at all sites (Table 3.1, Figure 3.4b, $p <0.001$ for all hosts), showing that high network selectivity at sites was not driven by any one host (Table 1). Both *H2'* and *d'* for all hosts had the highest selectivity values around mid-elevation and selectivity decreased from the highest values towards the ends of the gradient (Figure 3.4). Pearson's correlation revealed that host specialization decreased as a function of distance to mid-range for both *L. tameiameiae* and *M.*

polymorpha (*r*=-0.676 *p*<0.05, *r*=-0.708 *p*<0.05, respectively, Figure 3.7) but not for *V. reticulatum* (*r*=-0.373 *p*>0.05, Figure 3.7).

3.4   Discussion

In this study, host was a significant indicator of endophyte community composition within sites, among sites, and across the entire gradient. Additionally, host specialization and alpha diversity had an inverse relationship with more diversity and less specialization at the ends of the gradient and lower diversity and more specialization towards the middle elevations. However, elevation was not a good predictor of community richness, alpha diversity, or community composition.

I determined that host was a significant component in structuring foliar fungal endophyte communities despite the fact that hosts of the same species in close proximity had large differences in communities that were almost entirely driven by turnover rather than community nestedness. Other studies also observe evidence of host specialization in the foliar endophyte symbiosis. U'Ren et al. (2012) found foliar fungal endophyte communities were structured by hosts within sites. Endophytic fungi grow faster on media containing host leaf extract than non-host leaf extract (Arnold and Herre, 2003; Lau et al., 2013), suggesting host leaf chemistry influences community composition. In fact, Saunders and Kohn (2009) found evidence of community partitioning at the host genotype level for maize-associated endophytes when the environment was tightly controlled. It's likely that hosts provide different biotic environments that select for different fungal partners.

Many endophyte studies investigating fungal community composition across varying environments find that differences in community structure are correlated with environmental differences. For example, Giauque and Hawkes (2013) found that environment played a significant role in structuring endophyte communities of grasses in central Texas along a

precipitation gradient. Also, U'Ren et al. (2012) looked at endophyte communities across North America associated with varying plant and lichen hosts and found that environment played key roles in community structure. Similarly, Zimmerman and Vitousek (2012) investigated the community composition of endophytes associated with a single host species across an environmental gradient in Hawaiʻi and found that precipitation and elevation were important drivers of fungal community composition.

Significant findings in these studies raises the question of how distance affects endophyte community composition. To address this question, I kept host species constant along a steep elevation gradient. Despite the fact that the gradient was positively correlated with solar radiation and negatively correlated with temperature, precipitation, humidity, cloud cover, and canopy and vegetation cover, I did not find evidence that endophyte communities are significantly structured by their abiotic environment. A likely explanation for these this is that findings in previous studies where environment affects community composition were confounded by distance between samples.

Species richness for both plants and animals has been shown to decrease with increasing elevation (Pellissier et al., 2012). However, this trend does not seem to hold true for microbial communities. Microbial richness did not significantly change from the lowest to the highest elevations on Mt. Fuji for either ectomycorrhizal fungi (Miyamoto et al., 2014) or soil bacteria (Singh et al., 2012). Studying the bacterial communities of soil and leafs along an elevation gradient, Fierer et al. (2011) did not find a significant change in microbial richness along their gradient. Studying foliar fungal endophytes associated with a single host species along the slopes of Mauna Loa, Zimmerman and Vitousek (2012) found no significant differences in richness among sites. Similarly, I did not observe any significant changes in foliar fungal endophyte richness along the elevation gradient.

I hypothesized that environment would affect host specialization, but I did not observe a linear trend in host specialization along the gradient. Instead, specialization peaked around mid-elevation and attenuated towards the extremes of the gradient distribution. Additionally, I observed that host specialization and alpha diversity had inverse relationships along the elevation gradient where alpha diversity was highest toward the edges of the gradient and lowest in the middle while host specialization was highest in the middle and lowest at the edges.

Since plant host populations at the edges of their niche distributions are likely to have lower survival rates than those near the middle of the distributions (Angert, 2009), it is likely that the hosts in this study are healthier toward the mid-elevation since it is the middle of two of the three hosts range distributions along the gradient. Additionally, fungal symbionts, such as arbuscular mycorrhizal fungi, tend to span the mutualist-antagonist continuum (Klironomos, 2003), and it is probable that foliar fungal endophytes also span this continuum. Therefore, it is feasible that plant hosts impose a top-down effect on foliar endophyte colonization in order to associate with the most beneficial symbionts. As a result, they would be more likely to associate with the best symbionts at mid-elevation which is why I observed high host specialization and lower diversity at mid-elevation. Conversely, at the edges of their distribution they would need to allocate resources elsewhere, so I observed lower host specialization and higher diversity.

Alternatively, it is possible that there were more generalist fungi at higher and lower elevations because of unmeasured environmental conditions along the gradient. *Metrosideros polymorpha* was the dominate canopy species along the entire gradient, but the understory and the degree of forest fragmentation varied. At lower elevations, the understory was dominated by the native fern *Dicranopteris linearis*, which is weedy in disturbed areas. The mid-elevations understories were not dominated by any single species but contained a mixture

of native shrubs, and the forest started to become fragmented due to past lava flows. At high elevations, the understory looked similar to mid-elevation, but with less plant diversity. The forest was very fragmented with little to no vegetation between vegetative patches. These differences in the surrounding communities could lead to differences in the availability and types of foliar fungal endophytes.

3.5   Conclusion

Foliar endophytic fungi are important plant associated microbes; however, I know little about the factors that affect community composition. In this study, I showed that the abiotic environment plays little to no role in fungal richness or community composition at the landscape scale within islands but plays a critical role in how fungi are distributed among hosts within sites, and host is an important determinant in community composition. Additionally, diversity and specialization along elevation were inversely related. This suggest that host may be important in determining which fungi a host will associate with. It is possible that optimum environments are important for specialization where at the ends of a hosts niche they are less selective as to which symbionts to associate with and more selective at the sweet spot of their niche.

3.6   Tables and Figures

Table 3.1

Site Elevation	d'			H2	adonis R^2
	Leptecophylla	Metrosideros	Vaccinium		
1100	0.638	0.548	0.782	0.665	0.296 **
1200	0.757	0.652	0.604	0.67	0.255 **
1300	0.968	0.836	0.748	0.85	0.325 **
1400	0.855	0.967	0.772	0.866	0.477 **
1500	0.862	0.981	0.851	0.868	0.451 ***
1600	0.966	0.715	0.807	0.829	0.410 **
1700	0.648	0.643	0.518	0.602	0.165
1800	0.712	0.814	0.386	0.636	0.339 *
1900	0.748	0.676	0.774	0.732	0.285 *
2000	0.555	0.545	0.395	0.497	0.173

Table 3.1: Host specialization (d'), network specialization (H2'), permutational multivariate analysis of variance (ADONIS, Site~Host) for each site. All specialization values were significant (p <0.001) against a null. ADONIS values were calculated using 10,000 permutations (significance: . >0.05, * <0.05, ** <0.01, *** <0.001).

Table 3.2

	Leptecophylla	Metrosideros	Vaccinium	
Turnover	0.959	0.942	0.947	S O R E N S E N
Nestedness	0.01	0.013	0.013	
Beta Diversity	0.969	0.955	0.96	
Turnover	0.979	0.97	0.973	J A C C A R D
Nestedness	0.005	0.007	0.007	
Beta Diversity	0.984	0.977	0.98	

Table 3.2: Beta diversity partitioning: Table indicates contributions of community turnover and community nestedness to observed beta diversity. Both Sorensen and Jaccard indicies gave the same results.

Figure 3.1
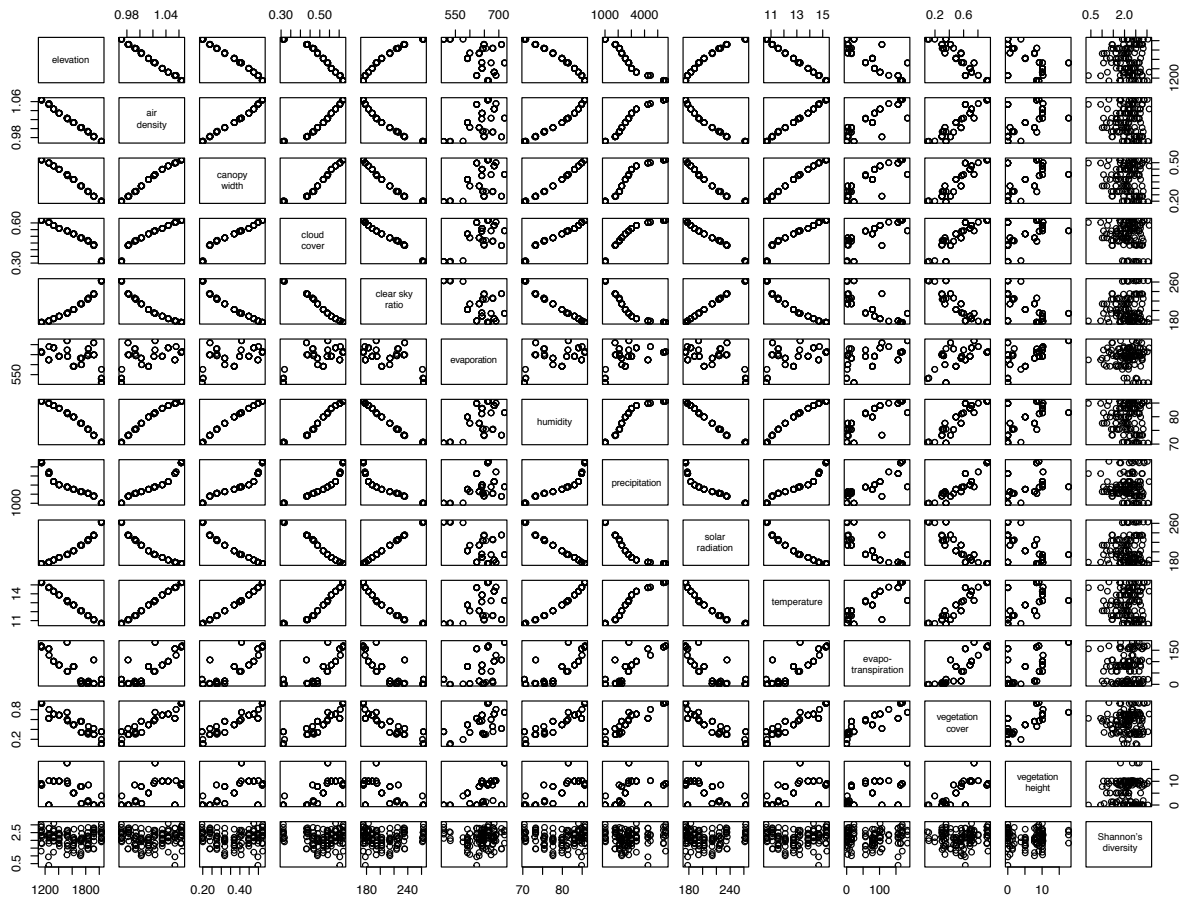
Figure 3.1: Matrix scatterplot shows the relationships of various environmental parameters of the elevation gradient. There were clear negative relationships between elevation and air density, canopy width, cloud cover, humidity, precipitation, temperature, evapotranspiration, and canopy cover. There were clear positive relationships between elevation and clear sky ratio and solar radiation.

Figure 3.2
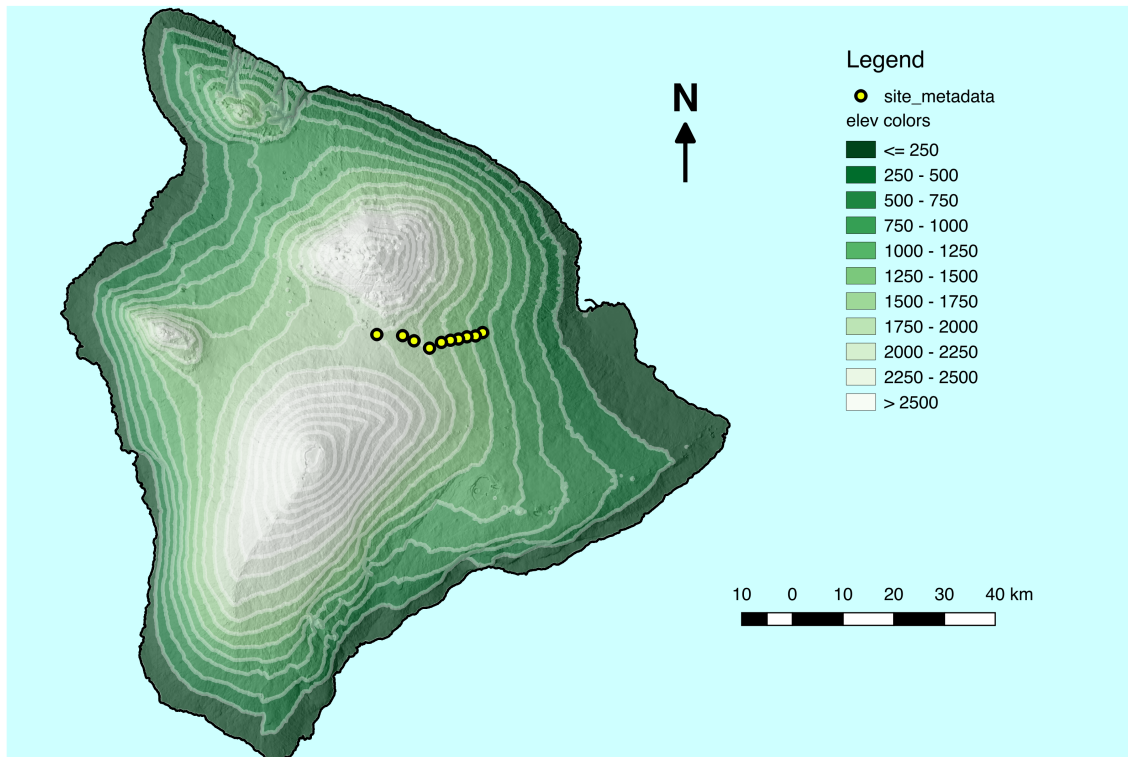
Figure 3.2: Each sampling location indicates were 12 leaf samples were collected (three plant host and four replicas per host). Sampling location were 100 m in elevation apart spanning approximately 20 km from the lowest elevation to the highest.

Figure 3.3

Fungal Richness

Alpha Diversity
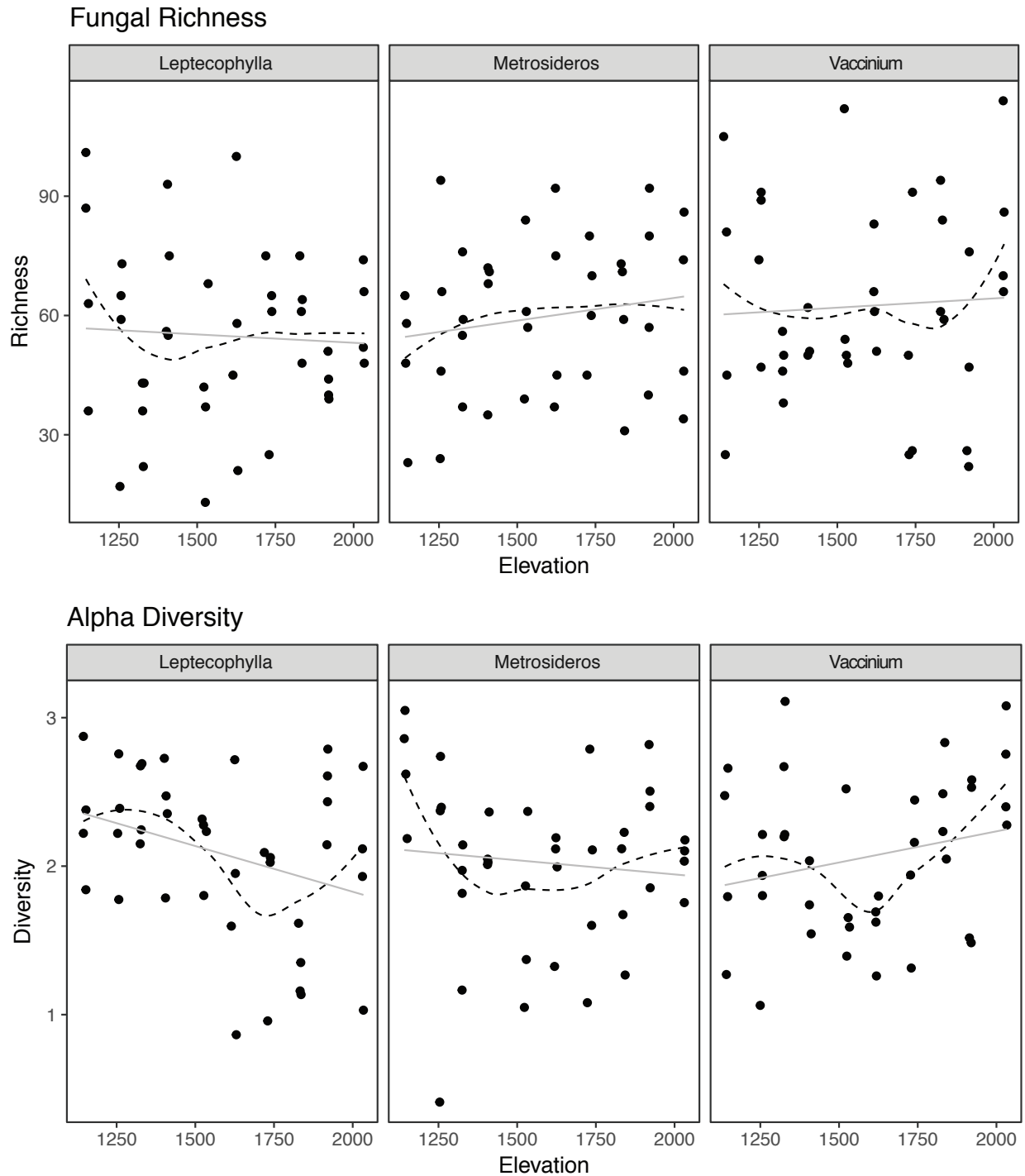
Figure 3.3: Richness: Fungal richness was not affected by elevation (*L. tameiameiae*: R^2=0.003, p>0.05; *M. polymorpha*: R^2=0.029, p>0.05; *V. reticulatum*: R^2= 0.055, p>0.05). B) Shannon's Diversity: There were no significant trends in the effects of elevation on Shannon's Diversity on *M. polymorpha* and *V. reticulatum* (R^2=0.01, p>0.05; R^2=0.052, p>0.05; respectively). However, the decrease in alpha diversity for *L. tameiameiae* was significant (R^2=0.105, p= 0.041).

Figure 3.4
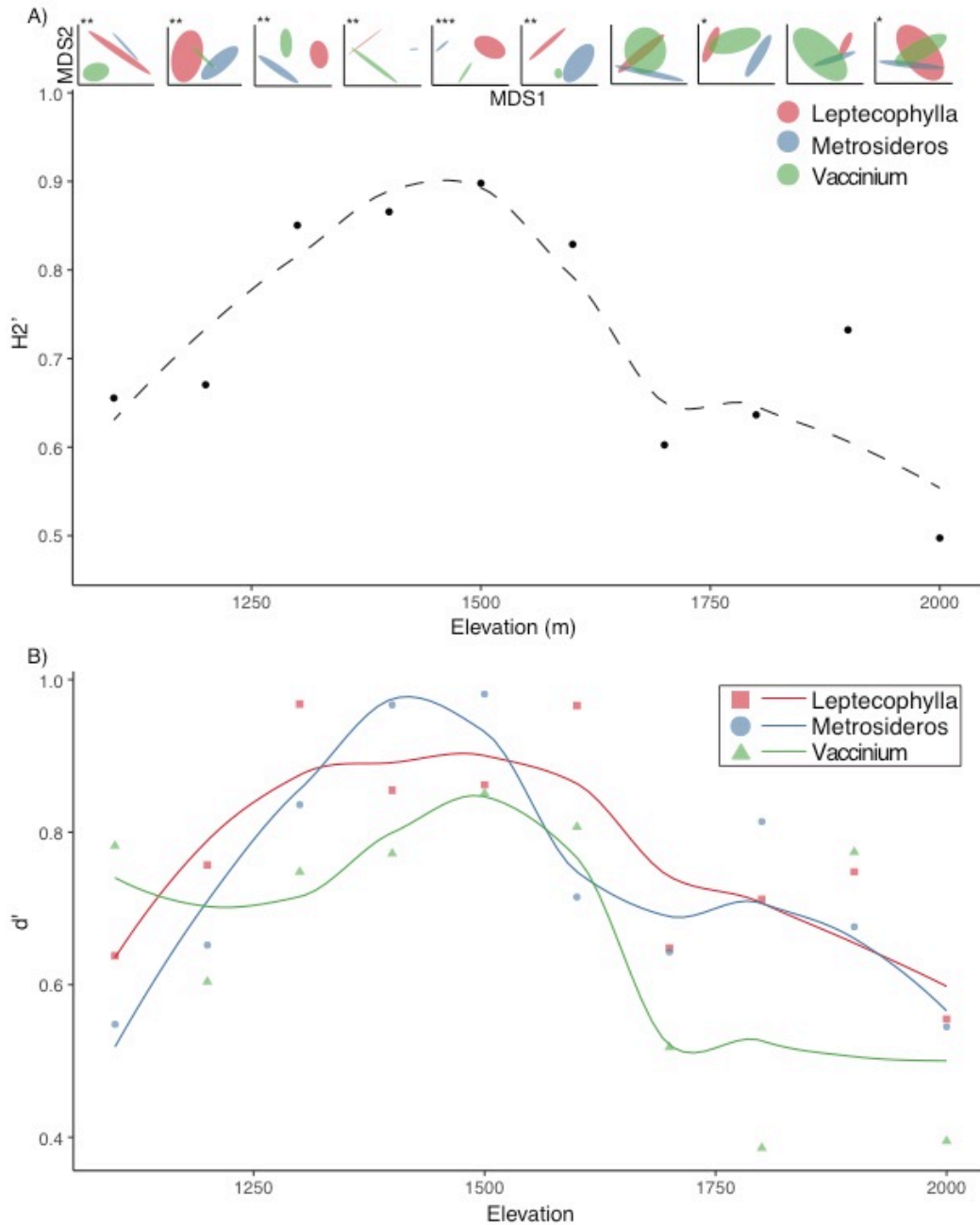
Figure 3.4: A) Top panel shows NMDS plots for each elevation site and illustrates how hosts structure endophyte communities. Shaded ellipses represent standard error of the mean (95%) for each host. Asterisk and dots above NMDS spots show significance from permutational multivariate analysis of variance (significance: * <0.05, ** <0.01, *** <0.001). Bottom panel shows network specialization plotted as a function of elevation. Each site was significant against a null (p<0.001 for all sites). B) Shows host specialization for each host plotted as a function of elevation. Each site was significant for each host against a null (p<0.001).

Figure 3.5
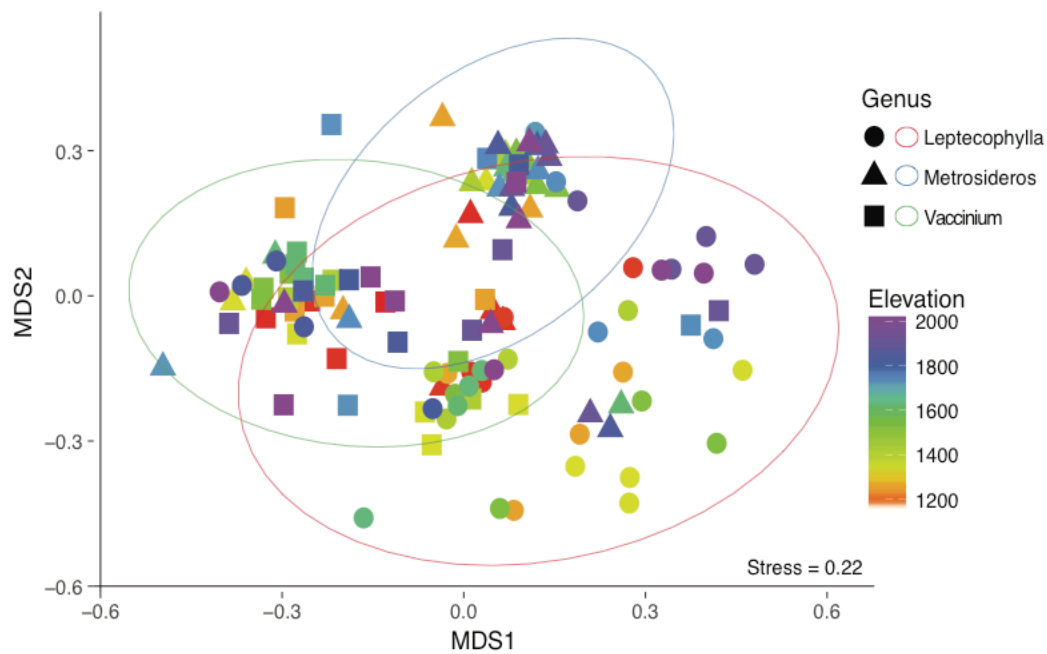
Figure 3.5: Host specialization as a function of the absolute value of the elevation difference from mid-elevation along the gradient (1500 masl). *L. tameiameiae* and *M. polymorpha* both showed a significant negative relationship between host selectivity and elevation difference (r=-0.676, p<0.05; r=-0.708, p<0.05; respectively). However, the relationship was not significant for *V. reticulatum* (r=-0.373, p>0.05).

Figure 3.6
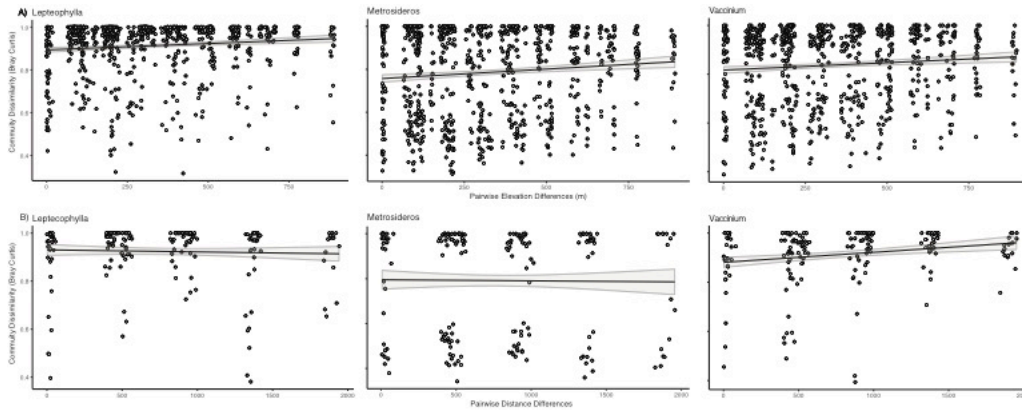
Figure 3.6: Distance decay plots A) shows community dissimilarity as a function of pair-wise elevation differences between two samples (points). There was no trend for either *M. polymorpha* or *V. reticulatum* after 10,000 permutations (r=0.10, p>0.05; r=0.09, p>0.05; respectively). There was a slightly significant trend for *L. tameiameiae* (r=0.105, p<0.05). B) shows community dissimilarity as a function of pair-wise distance difference between two samples. There was no trend for *L. tameiameiae* or *M. polymorpha* after 10,000 permutations (r= -0.035, p>0.05; r=0.011, p>0.05; respectively). There was a slightly significant trend for *V. reticulatum* (r=0.188, p>0.05).

Figure 3.7 Host Specialization
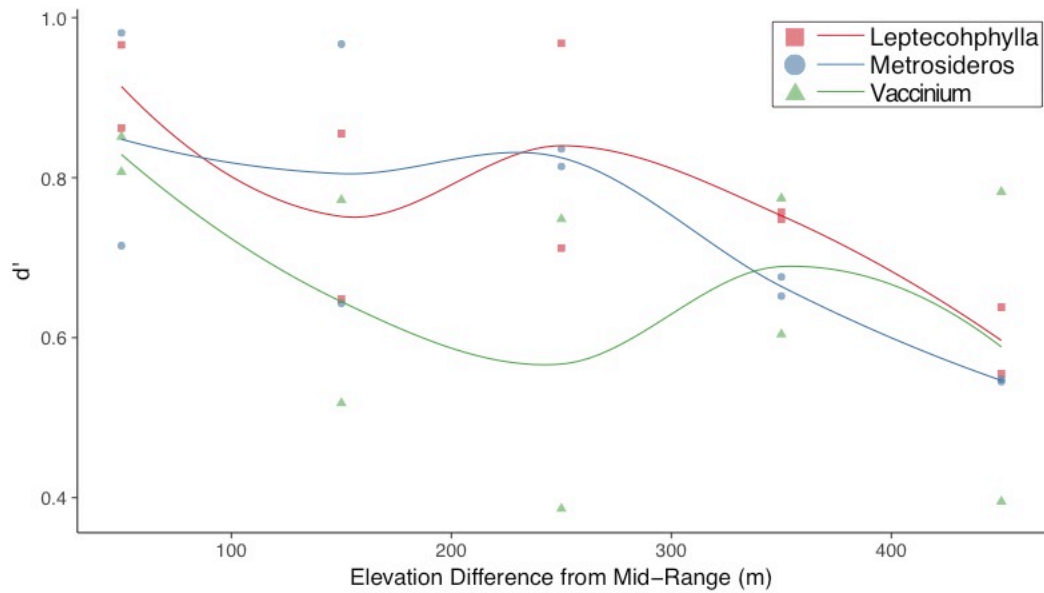
Figure 3.7: Host specialization as a function of the absolute value of the elevation difference from mid-elevation along the gradient (1500 masl). *L. tameiameiae* and *M. polymorpha* both showed a significant negative relationship between host selectivity and elevation difference (r=-0.676, p<0.05; r=-0.708, p<0.05; respectively). However, the relationship was not significant for *V. reticulatum* (r=-0.373, p>0.05).

Chapter 4   Foliar fungal endophytes follow macroecological distribution patterns across a landscape, but only on a case-by-case basis

4.1   Background

Foliar fungal endophytes are arguably one of the most ubiquitous symbiotic relationships between fungi and plants, occurring in every examined plant lineage (Arnold and Engelbrecht, 2007a). Most studies on foliar fungal endophyte community ecology are plant-centric, meaning that host plant distributions are used to understand fungal community structure. The emphasis of foliar fungal endophyte research has been on community composition as an index for distribution patterns, but the distribution of individual fungal endophytes has been largely overlooked.

At the global scale, microorganisms tend to follow Rapoport's rule, which states that species found at higher latitudes tend to have wider latitudinal ranges than species found closer to the equator, which have more confined latitudinal distributions (Amend et al., 2013; Sul et al., 2013). Rapoport's rule is often attributed to climactic stability at lower latitudes, leading to greater range distributions and lower species richness towards the poles. Elevation gradients have been hypothesized to follow similar species distribution patterns as latitudinal gradients, and as such, Rapoport's rule has been extended to hypothesize that species elevation ranges will increase with increasing elevation (Stevens, 1992). Indeed, studies have found that macroorganisms such as butterflies (Fleishman et al., 1998), ants (Sanders, 2002), and a variety of tree species in the Himalayas (Bhattarai and Vetaas, 2006) follow the elevational version of Rapoport's rule. However, it is unclear whether microbes exhibit similar distribution patterns as macroorganisms along elevation gradients (Bryant et al., 2008; Fierer et al., 2011).

In most host-endophyte systems, testing this hypothesis would be extremely difficult because a host plant species distribution is closely tied to elevation. Said another way, because the same host species cannot be found across a wide enough elevational gradient, the effect of host species identity on endophyte community structure cannot be disentangled from the effect of elevation. For example, several studies have found that plant host identity is an important component in determining the community composition of foliar endophytes (U'Ren et al., 2012; Vincent et al., 2016). However, these systems cannot be used to test for Rapoport's rule for two reasons: 1) they use different hosts species across their study site, and 2) their host species distributions are limited to narrow elevation ranges.

The extreme geographic isolation of the Hawaiian Archipelago has led to a highly endemic flora, where some species encompass remarkably wide niches and elevational distributions (Wagner, 1999). Additionally, the Hawaiian Archipelago contains dramatic elevation gradients that facilitate the study of elevation effects on species distributions while minimizing the effects of distance (Raich et al., 1997). For example, Mauna Loa is a shield volcano located on Hawai'i Island, which is the largest and youngest of the Hawaiian Islands. Mauna Loa ranges in elevation from sea level up to approximately 4200 m above sea level in a relatively short distance. Also, *Metrosideros polymorpha*, an endemic tree species, can be found near sea level all the way up to 2500 m along the eastern slope of Mauna Loa (Vitousek et al., 1988). Co-occurring with *M. polymorpha*, there are other native woody plants that span large portions of the slope as well. Because we are able to separate elevation and host differences, Mauna Loa is an ideal location to study the distribution of foliar fungal endophytes. Thus, this system is ideal for testing whether Rapoport's rule holds true for foliar fungal endophytes.

I hypothesize that foliar fungal endophyte community averages, along the elevational transect, will follow Rapoport's rule and will be significantly spatially autocorrelated, meaning

they will have clustered distributions along the gradient. I would expect OTUs to be spatially autocorrelated if there are strong environmental determinants or strong dispersal limitations among foliar fungal endophytes, but I would expect weak spatial autocorrelation if neither of those are important drivers of community composition. Patterns of elevational autocorrelation have been found for ectomycorrhizal fungi (Gorzelak et al., 2012) and for microbial eukaryote communities in aggregate (Darcy et al., 2017). Elevation has been shown to be a significant driver of fungal endophyte community structure in Hawaiʻi (Zimmerman and Vitousek, 2012). However, distribution patterns of foliar fungal endophytes have not been examined.

In this study, I use the unique characteristics of the native Hawaiian flora and the dramatic elevation gradient of Mauna Loa to test Rapoport's rule of elevational range distributions, spatial autocorrelation, and abundance-occupancy trends of individual foliar fungal endophytes along an elevation gradient. Based on Rapoport's rule, I hypothesize that samples at higher elevations will contain species whose distributions, on average, span greater elevational ranges. Additionally, I hypothesize that fungal endophytes will be spatially autocorrelated. Finally, I examine fungi that demonstrate greater or less occupancy than local abundance would predict to glean insight into taxonomic and functional correlates with distribution patterns.

4.2   Methods

4.2.1 Sites/Fieldwork

Sampling was conducted along the slopes of Mauna Loa volcano on Hawaiʻi Island. The elevation ranges from sea level to approximately 4200 m above sea level (masl) encompassing an environmental gradient over which temperature, rainfall and solar irradiance differ over a relatively short spatial distance. Since fungal endophyte community composition has been shown to be host specialized and I wanted to examine the influence of environment on fungal

distribution patterns, I sampled three native Hawaiian plants that co-occur along the gradient from 1100-2000 masl: *Leptecophylla tameiameiae* (pūkiawe) an indigenous species found throughout various Polynesian islands in the Pacific, *Metrosideros polymorpha* ('ōhi'a) an endemic foundation species found on all the major Hawaiian Islands, and *Vaccinium reticulatum* ('ōhelo) an endemic species and early colonizer after lava flows. *Metrosideros polymorpha* was the dominant tree species along the entire gradient. I sampled hosts along the eastern slope of Mauna Loa from 1100 masl up to 2000 masl. I sampled every 100 m in elevation for a total of 10 sites and collected 4 samples for each host species at each site (120 sampled individuals, 40 per host species). To control for differences in biomass among target hosts, 5 leaves from of *Metrosideros* and *Vaccinium* and 40 leaves from *Leptecophylla*, as *Leptecophylla* leaves are much smaller. In the field, leaf samples were stored on ice until they could be and transferred to -20 °C freezer in the lab. A voucher specimen of each host species per site was collected and deposited at the Joseph F. Rock Herbarium at the University of Hawai'i, Mānoa.

4.2.2 Molecular analysis

4.2.2.1 Surface sterilization

Leaves were surface sterilized to exclude fungi present on leaf surfaces prior to DNA extraction. First, I collected forty leaf 'disks' per individual host by punching leaves using a surface sterilized standard paper single hole punch (approximately 0.5 cm diameter), and then placed leaf disks into loose-leaf tea bags that were subsequently stapled shut. I then surface sterilized the disk packets by rinsing the loose-leaf tea bags with 1% NaClO for 2 mins, then 70% EtOH for 2 mins, followed by two rinses with sterile water for 2 mins each (Zimmerman and Vitousek, 2012).

4.2.2.2 DNA isolation

For DNA extraction, ten leaf disks were placed in MP Biomedical Lysing Matrix A tubes (MP Biomedical, Santa Ana, CA, USA) containing DNA isolation solutions from the MoBio PowerPlant Pro DNA Isolation kit (Solution PD1, Solution PD2, Phenolic Separation Solution, and RNase A Solution; MO Bio, Carlsbad, CA, USA). Leaf disks were homogenized using a Mini-Beadbeater 24 (BioSpecs Inc. OK) at 3,000 oscillations per min for 2 mins. Genomic DNA was isolated using a modified MoBio PowerPlant Pro DNA 96well Isolation kit protocol (centrifuged beaten tubes at 13,000 xg for 2 mins).

4.2.2.3 Amplification and Illumina library prep.

The ITS1 region of the rDNA was isolated and amplified by using fungal specific primers ITS1f and ITS2, along with Illumina adaptors and Golay barcodes (Smith and Peay, 2014), using a dual indexing approach. Polymerase chain reactions (PCR) were carried out using the KAPA3G Plant PCR kit (KAPA Biosystems, Wilmington, MA, USA). All PCR products were purified and normalized using just-a-plate 96 PCR Purification and Normalization Kit (Charm Biotech, San Diego, California, USA). Normalized PCR products were pooled and concentrated using a streptavidin magnetic bead solution. Pooled PCR products were sequenced by GENEWIZ (GENEWIZ, South Plainfield, NJ, USA) using the 2 x 300 paired-end (PE) sequencing protocol on an Illumina MiSeq sequencing platform (Illumina Inc., Dan Diego, CA, USA).

4.2.3 Bioinformatics

Bioinformatic analyses were conducted using QIIME v1.9 (Caporaso et al., 2010) and Mothur v1.39 (Schloss et al., 2009). Initial quality filtering was conducted on 23,531,047 raw demultiplexed sequences, where sequences <75 bp and with a Phred score <25 were removed, resulting in 18,370,578 sequences remaining for downstream analyses. Because the

overall quality of the reverse reads was poor, I only used forward reads for subsequent

processing and final analysis (see Nguyen et al., 2015 for a similar analysis). I employed a

chain-clustering protocol to group sequences into operational taxonomic units (OTU) because

this method tends to recover a more accurate OTU number than simply employing a single

picking method (see Nguyen et al., 2015). Sequences were first grouped into *de novo* OTUs

using a sequence similarity of ≥96% using USEARCH (Edgar, 2010) in QIIME. Sequences were

also checked for chimeras using a reference based approach with the UNITE v7 database

(Kõljalg et al., 2013) during clustering. A second round of *de novo* clustering was done using

UCLUST (Edgar, 2010), again using 96% sequence similarity. OTUs were assigned taxonomy

by using the BLAST v2.6 algorithm (Altschul et al., 1990) to identify representative sequences

against the UNITE v7 database. Sequences with an aligned length divided by the total length of

the query sequence <0.85 were removed from the OTU table. Additionally, OTUs found in the

PCR negative controls were removed from each OTU by subtracting the number of sequences

in the negatives from the sequence abundance from sample OTUs. This process removed

almost 500,000 sequences from approximately 11.2 million sequences.

All downstream analyses were done in R (v 3.3.2; R Core Team, 2016). Data biom file

with taxonomy and metadata was imported to R using the *biomformat* package (McMurdie and

Paulson, 2016) and the functions *biom_data*, *observation_metadata*, and *sample_metadata*

from package *biomformat* to extract the OTU table, taxonomy, and metadata, respectively. To

reduce the potential impacts of tag switching, I removed OTUs from samples in which their

abundance was < 0.1% of the max number of reads found in another sample. I then removed

all OTUs with <10 reads in the dataset. This reduced the number of OTUs from 3486 to 2018

after removing suspicious and low abundant OTUs and OTUs that were not classified as fungi

at the kingdom level. Lastly, The OTU table was rarified down to 2300 reads per sample to

account for uneven sequencing depth across the dataset. Since I was interested in how fungal

OTUs were distributed across space, I aggregated all OTUs by site, disregarding host identification.

4.2.4 Statistical Analysis

4.2.4.1 Effects of elevation on OTU range

To determine the effect of elevation on the range of OTUs, I first calculated the elevation range of each OTU by subtracting an OTU's minimum elevation from its maximum elevation and adding one to prevent elevation ranges of zero. Next, I took the average range for all OTUs occurring at each site along the elevation gradient weighted by the relative abundance of each OTU at each site. Finally, I use a linear regression model to test whether average elevational range per site increased as a function of site elevation in accordance with Rapoport's rule.

4.2.4.2 Effects of elevation on OTU spatial correlation

In order to test whether OTU distributions along the elevation gradient were spatially autocorrelated I calculated Moran's I for each OTU. Moran's I is a test for spatial autocorrelation which ranges from a value of 1 (perfectly clustered) to -1 (perfectly dispersed). Observed values were compared to a distribution of null values calculated by 1000 random reshuffles of the elevation distance matrix. Moran's I was calculated by using the *Moran.I* function in the *ape* package (Paradis et al., 2004).

4.2.4.3 Abundance-occupancy

To investigate fungal OTUs that demonstrate greater or less occupancy than local abundance would predict, I first determined if foliar endophytes follow the abundance-occupancy trend where OTUs that are more abundant will also occur at more sites. I defined

occupancy as the total number of sites in which an OTU was found. I then separately

calculated total relative abundance for each OTU by averaging the relative abundance at each

site that the OTU occurred. To determine the locally abundant outliers, I calculated the

residuals from the average abundance-occupancy linear model. I then chose the top 2.5%

quantile of OTUs with the greatest residuals and with an occupancy of less than five. There

were 18 OTUs that fit this definition of significantly high abundance, low occupancy OTUs.

Additionally, I chose the bottom 2.5% quantile of OTUs with the lowest residuals and with an

occupancy of greater than six. There were 39 OTUs that fit this definition of low abundance,

high occupancy OTUs. I defined these OTUs by functional guild using the FunGuild program

v1.1 (Nguyen et al., 2016).

4.3   Results

4.3.1 Rapoport's Rule and spatial autocorrelation

Foliar fungal endophyte distribution did not follow Rapoport's rule along the elevation

gradient, rather I found that based on Moran's I analysis foliar endophytes were, on average,

randomly distributed across the gradient (Figure 4.1 & 4.2). However, there were 33 OTUs that

were statistically more dispersed than expected, and 80 OTUs there were statistically more

clustered than expected (Table 4.1). Taxonomic assignments for the majority of these OTUs

resulted in low *BLAST* confidence (<97%). OTUs that were significantly more dispersed were

more likely to be plant pathogens (64%) while those that were significantly more clustered were

more likely to be unassigned a fungal guild (51%).

4.3.2 Abundance-occupancy and significant OTUs

There was a positive correlation between the average read abundance per site of a

given OTU and the number of sites at which that OTU occurs (Figure 4.3a; r^2 = 0.382; $p <$

0.001). Therefore, foliar endophytes that were at high local abundances also had a larger range distribution, following the abundance-occupancy trend. Similarly, more abundant OTUs were significatly more likely to occur in more hosts compared to those that were less abundant (Figure 4.3b; r^2 = 0.293; p < 0.001).

Each site had on average four OTUs that were significantly high abundant with low occupancy (locally abundant). Of the 13 locally abundant OTUs that occurred at more than one site, only six were in adjacent sites or with one absent site in between adjacent sites. Also, these locally abundant OTUs were less likely to be assigned a fungal guild (Table 4.1; 65% unassigned). For OTUs that were less abundant than expected and occurred at six or more adjacent sites (widespread and rare), only OTUs with an occupancy of nine and 10 had significantly low abundances. These widespread and rare OTUs were more likely to be plant pathogens (Table 4.1; 87% plant pathogens).

4.4   Discussion

To my knowledge, this was the first study to take a fungal-centric approach to investigating the distribution of foliar fungal endophytes. I used the leaf-endophyte system along a steep elevation gradient to test Rapoport's rule, the extent of spatial autocorrelation, and the abundance-occupancy trend of foliar fungal endophytes.

At global scales, plants (Stevens, 1989), animals (Hausdorf, 2006), and microbes (Amend et al., 2013) have been shown to follow Rapoport's rule where latitudinal ranges of taxa increase as a function of latitude. Additionally, macroorganisms tend to follow Rapoport's rule along elevation gradients (Fleishman et al., 1998; Sanders, 2002; Bhattarai and Vetaas, 2006).

Unlike plants and animals, elevation was not a significant indicator of fungal OTU distributions in this study (Figure 4.1). Similarly, bacterial distributions (Bryant et al., 2008) and

diversity patterns (Fierer et al., 2011) were shown to differ from those of macroorganisms along elevation gradients. The steep elevation gradient of Mauna Loa facilitated the investigation of the distribution of fungal endophytes at variable elevations with minimal distance between sampling locations. Like other microbial systems, foliar fungal endophytes do not mirror distribution patterns of macroorganisms along elevation gradients. It is likely that fungal endophytes and other microbes are regulated differently than plants and animals. Therefore, it is necessary to study their distribution patterns separately from their hosts.

Foliar fungal endophytes, on average, do not exhibit elevational autocorrelation (Figure 4.2). Despite this, I did observe outlier OTUs with distributions that were either more dispersed or more clustered than expected from the null model. Interestingly, OTUs that were more dispersed than expected and widespread and rare were more likely to be plant pathogens (Table 4.1). Also, OTUs that were more clustered or locally abundance were more likely to be unassigned a fungal guild (Table 4.1). Endophytes seem to straddle the continuum of plant-antagonist and plant-mutualist (Saikkonen et al., 1998). It is possible that OTUs that lean more towards the antagonistic side of the continuum are more likely to have wider distributions than those that are more mutualistic. Or perhaps, this is a simple case of an ascertainment bias. Foliar endophytes are defined as symptomless, and the only way they are detected is through molecular analysis or culturing, which happens less frequently than fungal plant pathogens. Therefore, foliar endophytes are less likely to be included in these databases. The study of plant pathology is economically important, and as a result, are much more easily identified. Therefore, plant pathogens are more likely to be in reference databases than those characterized as foliar endophytes.

4.5   Conclusion

Most foliar fungal endophyte studies take a plant-centric approach. In this fungal-centric approach, I investigated the distribution of endophytic fungi along an elevation gradient. My results support the hypothesis that microbial distributions along elevation gradients differ from those of plants and animals. It is likely that the distribution of foliar fungal endophytes is governed differently than their host plants. Despite the fact that, on average, endophytes were not spatially autocorrelated, there were a few fungal endophytes that fit spatial autocorrelation patters of macroorganisms. This could explain why foliar fungal endophytes as a group are functionally diverse; therefore, it is essential to study endophytes and their distributions individually, not just as communities.

4.6   Tables and Figures

Table 4.1: Outlier OTUs

	Guilds					
	Endophyte	**Saprotroph**	**Plant Pathogen**	**Fungal Parasite**	**Lichenized**	**Unassigned**
High Abundance Low Occupancy	0%	12%	18%	5%	0%	65%
Low Abundance High Occupancy	0%	0%	87%	0%	0%	13%
Dispersed Distribution	3%	3%	64%	0%	3%	27%
Clustered Distribution	3%	6%	38%	1%	1%	51%

Table 4.1: Outlier OTUs from both occupancy-abundance analysis (top two rows) and Moran's I spatial autocorrelation analysis (bottom two rows). There were 13 OTUs that had significantly higher abundance and lower occupancy, and 39 OTUs had significantly lower abundance and higher occupancy. There were 33 OTUs that were significantly over dispersed, and 80 OTUs that were significantly clustered.

Figure 4.1: Rapoport's rule
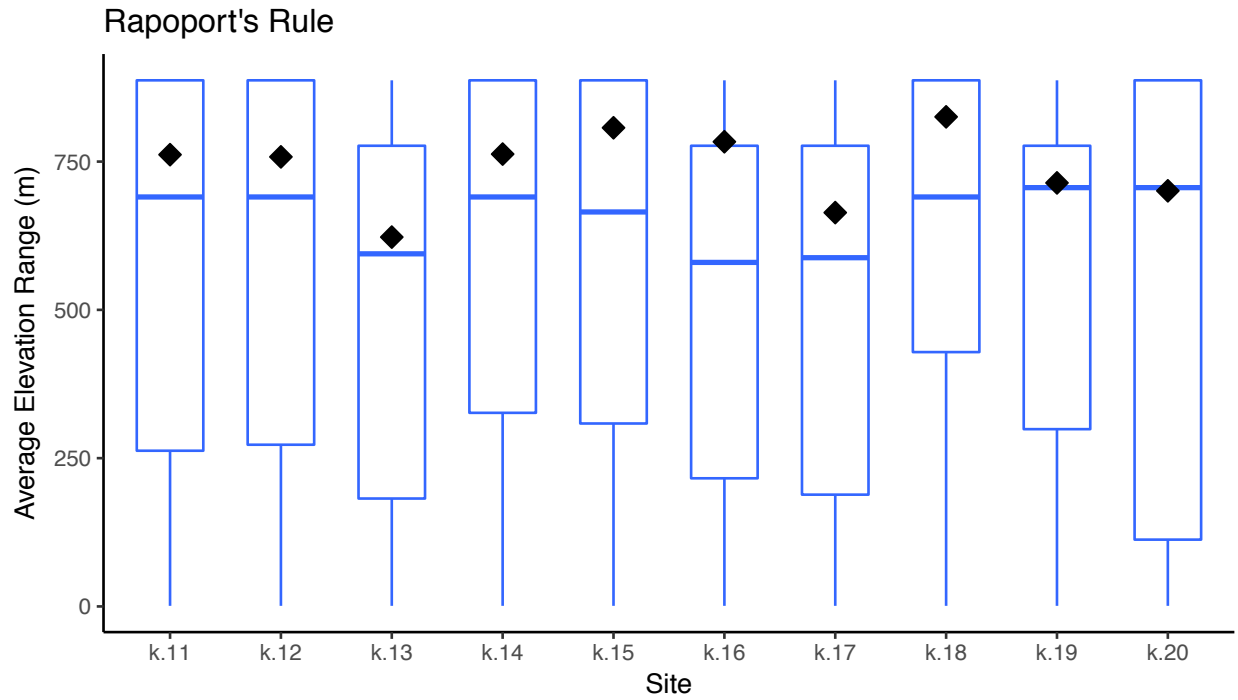
Figure 4.1: Average elevation range (blue lines) along the elevation gradient ($r^2 = 0$; $p > 0.1$). Average weighted mean along the elevation gradient ($r^2 = 0$; $p > 0.1$).

Figure 4.2: Spatial Autocorrelation

Null Distribution of Moran's I Values
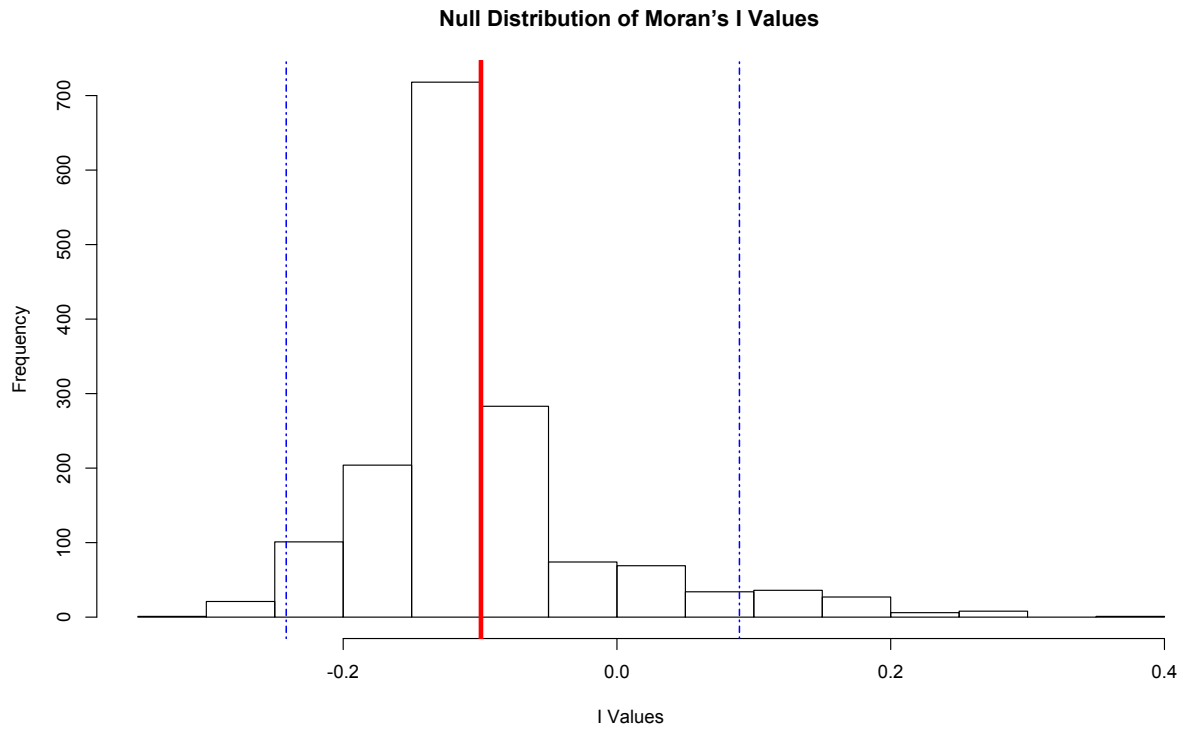
Figure 4.2: Null distribution of Moran's I. Histogram of null distribution of Moran's I from 100 permutations. Dashed vertical blue lines represent the lower and upper bounds of the 95% confidence intervals. The solid vertical red line represents the average observed Moran's I value (-0.099) for the entire gradient. The minimum null I-value was -0.339, the maximum was 0.384, and the medium was - 0.119.

Figure 4.3: Occupancy-Abundance

A) Site Occupancy-Abundance
Occupancy Abundance

B) Host Occupancy-Abundance
Occupancy Abundance
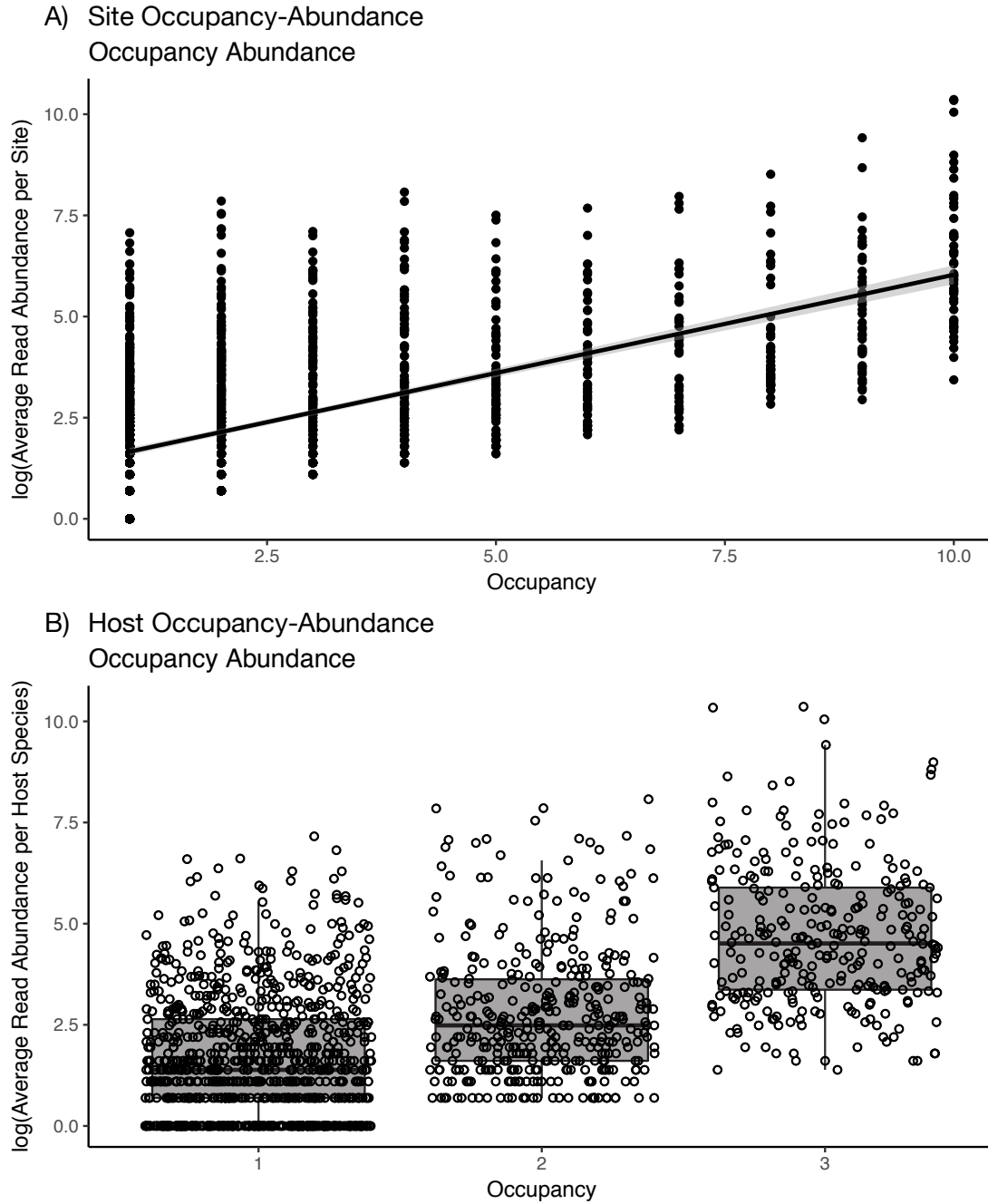
Figure 4.3: Occupancy-abundance a) the average read abundance per site for a given OTU and number of sites at which it occurs. Line represents linear regression with confidence 95% confidence interval (r^2 = 0.382; p < 0.001), and b) whisker-box plot showing the average read abundance per host for a given OTU and the number of hosts with which it associates (r^2 = 0.293; p < 0.001). Points on both plots represent individual OTUs.

Chapter 5 Synthesis

5.1 Foliar Fungal Endophytes

Fungal diversity is estimated between 2.2-5 million species globally (Blackwell, 2011; Hawksworth and Lücking, 2017). However, only about 120,000 have been described, and the vast majority of undescribed fungi are thought to live a cryptic lifestyle inside plants or animals (Hawksworth and Lücking, 2017). Foliar fungal endophytes are a polyphyletic group (Rodriguez et al., 2009) of cryptic fungi that live inside healthy looking leaves. These fungi occur in every major lineage of plants examined and are assumed to be non-pathogenic.

Tropical plants may represent a particular "hotspot" of fungal diversity (Arnold and Lutzoni, 2007; Zimmerman and Vitousek, 2012), making them a potentially a significant source of undescribed biodiversity (Porras-Alfaro and Bayman, 2011). Foliar fungal endophytes have been found in every major lineage of plants examined, and in studies of tropical forests, 100% of mature leaves contain endophytes (Arnold and Engelbrecht, 2007). Thousands of fungal species from at least seven fungal orders can coexist within the leaves of a single tropical plant species (Arnold et al., 2000; Zimmerman and Vitousek, 2012).

Foliar fungal endophytes are thought to be horizontally transmitted in dicot plants (Bayman et al., 1998). Therefore, these endophytes are susceptible to both environmental filters and host filters. Temperature (Coince et al., 2014), elevation and precipitation (Zimmerman and Vitousek, 2012) have shown to be significant factor in shaping endophyte communities. Additionally, foliar fungal endophytes have been found to be significantly host specific (Unterseher et al., 2012; U'Ren et al., 2012). However, studies of foliar endophytic community structure and biogeography have been relatively small in scale, opting to study endophytes within narrowly defined systems. In this dissertation, I used the unique characteristics of the Hawaiian Archipelago and the native flora to address the effects of

geographic scale on the distribution of foliar fungal endophytes at the regional scale (among islands), landscape scale (within an island), and among foliar fungal endophytes in aggregate.

5.2   Regional Scale

The Hawaiian Archipelago is especially opportune as the native flora is host to a large number of endemic species, and many species can be found across all islands (Wagner, 1999), allowing for the examination of how host identity and distance simultaneously impact communities of symbionts. In this study, I used the unique native flora of Hawaiʻi to examine the importance of hosts and islands in determining endophyte community structure, the effects of distance on endophyte community structure, and the taxonomic and functional classification of fungi that are indicative of specific islands and hosts. I hypothesized that: 1) island would be a stronger determinate than host of foliar fungal endophyte community structure at the regional scale (across the archipelago) due to the limited distribution of endophytes, 2) Dispersal limitation would affect community structure of foliar fungal endophytes across the Hawaiian Archipelago, and 3) both islands and hosts would have indicator OTUs associated with them, meaning fungal species with restricted distributions within an island or host.

In this study, plant hosts and islands had highly specialized communities associated with them. Additionally, both plant host and island were significant indicators of endophyte community composition across the Hawaiian Archipelago, but islands explained more variation. Despite the fact the endophytes were structured by both plant host and island, distance did not correlate with community dissimilarity across the archipelago. I also observed indicator OTUs for three of the five islands and one host. Therefore, spatially explicit approaches might help to better understand the factors that influence foliar fungal endophyte distributions. Despite the challenges of working with foliar fungal endophytes because of their diverse taxonomic makeup and their cryptic lifestyle, foliar endophytes offer great potential for

advancing our understanding of the factors that spatially structure communities and the ecological functioning of aboveground ecological communities.

5.3   Landscape Scale

In this study, I used the unique characteristics of the Hawaiian flora and the dramatic environmental gradients of Mauna Loa to isolate environment from both plant community and distance effects in order to test how environment impacts endophyte richness, community diversity, community similarity, host preference, and geographic distance. Because environmental conditions along Mauna Loa become more stressful with increasing elevation (e.g. decreased precipitation and increased solar radiation), I expected fewer fungal species to be able to persist under these extreme conditions. Based on the results of previous microbial studies, I hypothesize that elevational gradients will affect fungal foliar endophytes community composition and richness, and that host will play an important role within and among sites in shaping differences of fungal communities because different hosts provide different physiological environments for their symbiotic partners.

Host was a significant indicator of endophyte community composition within sites, among sites, and across the entire gradient. Additionally, host specialization and alpha diversity had an inverse relationship with more diversity and less specialization at the ends of the gradient and lower diversity and more specialization towards the middle elevations. However, elevation was not a good predictor of community richness, alpha diversity, or community composition. These findings suggest that host may be important in determining which fungi a host will associate with. It is possible that optimum environments are important for specialization where at the ends of a hosts niche they are less selective as to which symbionts to associate with and more selective at the sweet spot of their niche.

5.4   Individual Scale

In this study, I used the unique characteristics of the native Hawaiian flora and the dramatic elevation gradient of Mauna Loa to test Rapoport's Rule of elevational range distributions, spatial autocorrelation, and abundance-occupancy trends of individual foliar fungal endophytes along an elevation gradient. Based on Rapoport's Rule, I hypothesized that samples at higher elevations would contain species whose distributions, on average, span greater elevational ranges. Additionally, I hypothesized that fungal endophytes would be spatially autocorrelated. Finally, I examined fungi that demonstrate greater or less occupancy than local abundance would predict to glean insight into taxonomic and functional correlates with distribution patterns.

Most foliar fungal endophyte studies take a plant-centric approach. In this fungal-centric approach, I investigated the distribution of endophytic fungi along an elevation gradient. My results support the hypothesis that microbial distributions along elevation gradients differ from those of plants and animals. It is likely that the distribution of foliar fungal endophytes is governed differently than their host plants. Despite the fact that, on average, endophytes were not spatially autocorrelated, there were a few fungal endophytes that fit spatial autocorrelation patters of macroorganisms. This could explain why foliar fungal endophytes as a group are functionally diverse; therefore, it is essential to study endophytes and their distributions individually, not just as communities.

5.5   Synthesis

Results from both Chapter 2 and Chapter 3 expand the knowledge of the foliar fungal endophyte system by providing evidence that these fungal communities are structured

differently depending on scale. At the landscape scale (within-island; Chapter 3), foliar fungal

endophyte community composition is influence by plant host to a greater extent than

environment. In Chapter 2 the scale was broadened from landscape to the archipelago regional

scale (among islands), and I observed that location was a more important determinate of foliar

endophyte community composition. Thus, the factors affecting community composition of

foliar fungal endophytes work at disparate scales. It is likely that dispersal limitation has a

greater influence at regional scales as opposed to landscape scales. From an ecological

perspective, dispersal method for fungal endophytes will have a significant effect on local,

regional, and landscape scale diversity patterns. It is likely that these fungi disperse via multiple

mechanisms; additionally, the extent to which these mechanisms vary across environmental

conditions and ecological space remains to be explored.

Foliar fungal endophytes, on average, do not exhibit elevational autocorrelation,

meaning their distributions along the gradient are not clustered. Despite this, I did observe

outlier OTUs with distributions that were either more dispersed or more clustered than

expected from the null model. Interestingly, OTUs that were more dispersed than expected and

widespread and rare were more likely to be classified as plant pathogens. Also, OTUs that

were more clustered or locally abundance were more likely to be unclassified. Endophytes

seem to straddle the continuum of plant-antagonist and plant-mutualist (Saikkonen et al.,

1998). It is possible that OTUs that lean more towards the antagonistic side of the continuum

are more likely to have wider distributions than those that are more mutualistic. Or perhaps,

this is a simple case of an ascertainment bias. Foliar endophytes are defined as symptomless,

and the only way they are detected is through molecular analysis or culturing, which happens

less frequently than fungal plant pathogens. Therefore, foliar endophytes are less likely to be

included in these databases. The study of plant pathology is economically important, and as a

result, are much more easily identified. Therefore, plant pathogens are more likely to be in

reference databases than those characterized as foliar endophytes.

References

Adams, R.I., Miletto, M., Taylor, J.W., Bruns, T.D., 2013. Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. ISME J. 7, 1262.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Amend, A.S., Oliver, T.A., Amaral-Zettler, L.A., Boetius, A., Fuhrman, J.A., Horner-Devine, M.C., Huse, S.M., Welch, D.B.M., Martiny, A.C., Ramette, A., Zinger, L., Sogin, M.L., Martiny, J.B.H., 2013. Macroecological patterns of marine bacteria on a global scale. J. Biogeogr. 40, 800–811. https://doi.org/10.1111/jbi.12034

Angert, A.L., 2009. The niche, limits to species' distributions, and spatiotemporal variation in demography across the elevation ranges of two monkeyflowers. Proc. Natl. Acad. Sci. 106, 19693–19698.

Arnold, A.E., 2007. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fungal Biol. Rev. 21, 51–66. https://doi.org/10.1016/j.fbr.2007.05.003

Arnold, A.E., Engelbrecht, B.M.J., 2007a. Fungal endophytes nearly double minimum leaf conductance in seedlings of a neotropical tree species. J. Trop. Ecol. 23, 369–372. https://doi.org/10.1017/S0266467407004038

Arnold, A.E., Engelbrecht, B.M.J., 2007b. Fungal endophytes nearly double minimum leaf conductance in seedlings of a neotropical tree species. J. Trop. Ecol. 23, 369–372. https://doi.org/10.1017/S0266467407004038

Arnold, A.E., Herre, E.A., 2003. Canopy cover and leaf age affect colonization by tropical fungal endophytes: Ecological pattern and process in *Theobroma cacao* (Malvaceae). Mycologia 95, 388–398. https://doi.org/10.1080/15572536.2004.11833083

Arnold, A.E., Lutzoni, F., 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? Ecology 88, 541–549. https://doi.org/10.1890/05-1459

Arnold, A.E., Maynard, Z., Gilbert, G.S., Coley, P.D., Kursar, T.A., 2000. Are tropical fungal endophytes hyperdiverse? Ecol. Lett. 3, 267–274. https://doi.org/10.1046/j.1461-0248.2000.00159.x

Arnold, A.E., Mejía, L.C., Kyllo, D., Rojas, E.I., Maynard, Z., Robbins, N., Herre, E.A., 2003. Fungal endophytes limit pathogen damage in a tropical tree. Proc. Natl. Acad. Sci. 100, 15649–15654.

Baselga, A., Orme, C.D.L., 2012. betapart : an R package for the study of beta diversity: *Betapart package*. Methods Ecol. Evol. 3, 808–812. https://doi.org/10.1111/j.2041-210X.2012.00224.x

Bayman, P., Angulo-Sandoval, P., Báez-ortiz, Z., Lodge, D.J., 1998. Distribution and dispersal of Xylaria endophytes in two tree species in Puerto Rico. Mycol. Res. 102, 944–948.

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, I., de Sousa, F., Amend, A.S., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y.J.K., Sanli, K., Eriksson, K.M., Vik, U., Veldre, V., Nilsson, R.H., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol. Evol. n/a-n/a. https://doi.org/10.1111/2041-210X.12073

Bhattarai, K.R., Vetaas, O.R., 2006. Can Rapoport's rule explain tree species richness along the Himalayan elevation gradient, Nepal? Divers. Htmlent Glyphamp Asciiamp Distrib. 12, 373–378. https://doi.org/10.1111/j.1366-9516.2006.00244.x

Blackwell, M., 2011. The Fungi: 1, 2, 3 ... 5.1 million species? Am. J. Bot. 98, 426–438. https://doi.org/10.3732/ajb.1000298

Blüthgen, Nico, Menzel, F., Blüthgen, Nils, 2006. Measuring specialization in species interaction networks. BMC Ecol. 6, 9.

Bruns, T.D., Bidartondo, M.I., Taylor, D.L., 2002. Host specificity in ectomycorrhizal communities: what do the exceptions tell us? Integr. Comp. Biol. 42, 352–359.

Bryant, J.A., Lamanna, C., Morlon, H., Kerkhoff, A.J., Enquist, B.J., Green, J.L., 2008. Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. Proc. Natl. Acad. Sci. 105, 11505–11511.

Cáceres, M.D., Legendre, P., 2009. Associations between species and groups of sites: indices and statistical inference. Ecology 90, 3566–3574.

Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11, 2639.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336. https://doi.org/10.1038/nmeth.f.303

Coince, A., Cordier, T., Lengellé, J., Defossez, E., Vacher, C., Robin, C., Buée, M., Marçais, B., 2014. Leaf and Root-Associated Fungal Assemblages Do Not Follow Similar Elevational Diversity Patterns. PLoS ONE 9, e100668. https://doi.org/10.1371/journal.pone.0100668

Darcy, J.L., King, A.J., Gendron, E.M.S., Schmidt, S.K., 2017. Spatial autocorrelation of microbial communities atop a debris-covered glacier is evidence of a supraglacial chronosequence. FEMS Microbiol. Ecol. 93. https://doi.org/10.1093/femsec/fix095

Dormann, C.F., Gruber, B., Fründ, J., 2008. Introducing the bipartite package: analysing ecological networks. interaction 1, 0–2413793.

Edgar, R.C., 2016. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. BioRxiv 081257.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Fierer, N., McCain, C.M., Meir, P., Zimmermann, M., Rapp, J.M., Silman, M.R., Knight, R., 2011. Microbes do not follow the elevational diversity patterns of plants and animals. Ecology 92, 797–804.

Fleishman, E., Austin, G.T., Weiss, A.D., 1998. An empirical test of Rapoport's rule: elevational gradients in montane butterfly communities. Ecology 79, 2482–2493.

Giauque, H., Hawkes, C.V., 2013. Climate affects symbiotic fungal endophyte diversity and performance. Am. J. Bot. 100, 1435–1444. https://doi.org/10.3732/ajb.1200568

Gorzelak, M.A., Hambleton, S., Massicotte, H.B., 2012. Community structure of ericoid mycorrhizas and root-associated fungi of Vaccinium membranaceum across an elevation gradient in the Canadian Rocky Mountains. Fungal Ecol. 5, 36–45. https://doi.org/10.1016/j.funeco.2011.08.008

Grubisha, L.C., Trappe, J.M., Molina, R., Spatafora, J.W., 2002. Biology of the ectomycorrhizal genus *Rhizopogon* . VI. Re-examination of infrageneric relationships inferred from phylogenetic analyses of ITS sequences. Mycologia 94, 607–619. https://doi.org/10.1080/15572536.2003.11833189

Hausdorf, B., 2006. Latitudinal and altitudinal diversity patterns and Rapoport effects in north-west European land snails and their causes. Biol. J. Linn. Soc. 87, 309–323.

Hawksworth, D.L., Lücking, R., 2017. Fungal Diversity Revisited: 2.2 to 3.8 Million Species. Microbiol. Spectr. 5. https://doi.org/10.1128/microbiolspec.FUNK-0052-2016

Kannadan, S., Rudgers, J.A., 2008. Endophyte symbiosis benefits a rare grass under low water availability. Funct. Ecol. 22, 706–713. https://doi.org/10.1111/j.1365-2435.2008.01395.x

Kivlin, S.N., Winston, G.C., Goulden, M.L., Treseder, K.K., 2014. Environmental filtering affects soil fungal community composition more than dispersal limitation at regional scales. Fungal Ecol. 12, 14–25. https://doi.org/10.1016/j.funeco.2014.04.004

Klironomos, J.N., 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84, 2292–2301.

Klironomos, J.N., 2000. Host-specificity and functional diversity among arbuscular mycorrhizal fungi. Microb. Biosyst. New Front. 1, 845–851.

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.-H., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277. https://doi.org/10.1111/mec.12481

Lau, M.K., Arnold, A.E., Johnson, N.C., 2013. Factors influencing communities of foliar fungal endophytes in riparian woody plants. Fungal Ecol. 6, 365–378. https://doi.org/10.1016/j.funeco.2013.06.003

Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Øvreås, L., Reysenbach, A.-L., Smith, V.H., Staley, J.T., 2006. Microbial biogeography:

putting microorganisms on the map. Nat. Rev. Microbiol. 4, 102–112.

https://doi.org/10.1038/nrmicro1341

Miyamoto, Y., Nakano, T., Hattori, M., Nara, K., 2014. The mid-domain effect in

ectomycorrhizal fungi: range overlap along an elevation gradient on Mount Fuji, Japan.

ISME J. 8, 1739–1746. https://doi.org/10.1038/ismej.2014.34

Nguyen, N.H., Smith, D., Peay, K., Kennedy, P., 2015. Parsing ecological signal from noise in

next generation amplicon sequencing. New Phytol. 205, 1389–1393.

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S.,

Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community

datasets by ecological guild. Fungal Ecol. 20, 241–248.

https://doi.org/10.1016/j.funeco.2015.06.006

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R.,

O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H.,

2017. vegan: Community Ecology Package.

Paradis, E., Claude, J., Strimmer, K., 2004. APE: Analyses of Phylogenetics and Evolution in R

language. Bioinformatics 20, 289–290. https://doi.org/10.1093/bioinformatics/btg412

Peay, K.G., Garbelotto, M., Bruns, T.D., 2010. Evidence of dispersal limitation in soil

microorganisms: isolation reduces species richness on mycorrhizal tree islands.

Ecology 91, 3631–3640.

Peay, K.G., Schubert, M.G., Nguyen, N.H., Bruns, T.D., 2012. Measuring ectomycorrhizal

fungal dispersal: macroecological patterns driven by microscopic propagules:

MEASURING MYCORRHIZAL FUNGAL DISPERSAL. Mol. Ecol. 21, 4122–4136.

https://doi.org/10.1111/j.1365-294X.2012.05666.x

Pellissier, L., Fiedler, K., Ndribe, C., Dubuis, A., Pradervand, J.-N., Guisan, A., Rasmann, S., 2012. Shifts in species richness, herbivore specialization, and plant resistance along elevation gradients. Ecol. Evol. 2, 1818–1825. https://doi.org/10.1002/ece3.296

Porras-Alfaro, A., Bayman, P., 2011. Hidden Fungi, Emergent Properties: Endophytes and Microbiomes. Annu. Rev. Phytopathol. 49, 291–315. https://doi.org/10.1146/annurev-phyto-080508-081831

Raich, J.W., Russell, A.E., Vitousek, P.M., 1997. Primary productivity and ecosystem development along an elevational gradient on Mauna Loa, Hawai 'i. Ecology 78, 707–721.

Rodriguez, R.J., White Jr, J.F., Arnold, A.E., Redman, R.S., 2009. Fungal endophytes: diversity and functional roles: *Tansley review*. New Phytol. 182, 314–330. https://doi.org/10.1111/j.1469-8137.2009.02773.x

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584

Saikkonen, K., Faeth, S.H., Helander, M., Sullivan, T.J., 1998. Fungal endophytes: a continuum of interactions with host plants. Annu. Rev. Ecol. Syst. 29, 319–343.

Sanders, N.J., 2002. Elevational gradients in ant species richness: area, geometry, and Rapoport's rule. Ecography 25, 25–32.

Saunders, M., Kohn, L.M., 2009. Evidence for alteration of fungal endophyte community assembly by host defense compounds. New Phytol. 182, 229–238. https://doi.org/10.1111/j.1469-8137.2008.02746.x

Schemske, D.W., Mittelbach, G.G., Cornell, H.V., Sobel, J.M., Roy, K., 2009. Is There a Latitudinal Gradient in the Importance of Biotic Interactions? Annu. Rev. Ecol. Evol. Syst. 40, 245–269. https://doi.org/10.1146/annurev.ecolsys.39.110707.173430

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09

Singh, D., Takahashi, K., Kim, M., Chun, J., Adams, J.M., 2012. A Hump-Backed Trend in Bacterial Diversity with Elevation on Mount Fuji, Japan. Microb. Ecol. 63, 429–437. https://doi.org/10.1007/s00248-011-9900-1

Smith, D.P., Peay, K.G., 2014. Sequence Depth, Not PCR Replication, Improves Ecological Inference from Next Generation DNA Sequencing. PLoS ONE 9, e90234. https://doi.org/10.1371/journal.pone.0090234

Stevens, G.C., 1992. The elevational gradient in altitudinal range: an extension of Rapoport's latitudinal rule to altitude. Am. Nat. 140, 893–911.

Stevens, G.C., 1989. The latitudinal gradient in geographical range: how so many species coexist in the tropics. Am. Nat. 133, 240–256.

Sul, W.J., Oliver, T.A., Ducklow, H.W., Amaral-Zettler, L.A., Sogin, M.L., 2013. Marine bacteria exhibit a bipolar distribution. Proc. Natl. Acad. Sci. 110, 2342–2347.

Team, R.C., 2000. R language definition. Vienna Austria R Found. Stat. Comput.

Unterseher, M., Petzold, A., Schnittler, M., 2012. Xerotolerant foliar endophytic fungi of Populus euphratica from the Tarim River basin, Central China are conspecific to endophytic ITS phylotypes of Populus tremula from temperate Europe. Fungal Divers. 54, 133–142. https://doi.org/10.1007/s13225-012-0167-8

U'Ren, J.M., Lutzoni, F., Miadlikowska, J., Laetsch, A.D., Arnold, A.E., 2012. Host and
geographic structure of endophytic and endolichenic fungi at a continental scale. Am. J.
Bot. 99, 898–914. https://doi.org/10.3732/ajb.1100459

Vincent, J.B., Weiblen, G.D., May, G., 2016. Host associations and beta diversity of fungal
endophyte communities in New Guinea rainforest trees. Mol. Ecol. 25, 825–841.
https://doi.org/10.1111/mec.13510

Vitousek, P.M., Matson, P.A., Turner, D.R., 1988. Elevational and age gradients in Hawaiian
montane rainforest: foliar and soil nutrients. Oecologia 77, 565–570.

Wagner, W.L., 1999. Manual of the flowering plants of Hawai'i, Rev. ed.. ed, Bernice P. Bishop
Museum special publication; 97. University of Hawai'i Press: Bishop Museum Press,
Honolulu, HI.

Zimmerman, N.B., Vitousek, P.M., 2012. Fungal endophyte communities reflect environmental
structuring across a Hawaiian landscape. Proc. Natl. Acad. Sci. 109, 13022–13027.
https://doi.org/10.1073/pnas.1209872109