ECOLOGY AND DIVERSITY OF THE LICHEN SYMBIOSIS: FOLLOWING ESTABLISHED PATTERNS, OR AN EXCEPTION TO THE RULE?

By

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ABSTRACT

ECOLOGY AND DIVERSITY OF THE LICHEN SYMBIOSIS: FOLLOWING ESTABLISHED PATTERNS, OR AN EXCEPTION TO THE RULE?

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Few phenomena of biodiversity have perplexed researchers as much as the latitudinal diversity gradient. Though many taxa have been shown to follow this pattern of high species richness in the tropics and lower species richness towards the poles, no consensus has yet been reached regarding the drivers of this global pattern. Symbiosis, a long term and physically intimate interaction between two organisms, is a prevalent biotic interaction across the tree of life, yet few studies of the latitudinal diversity gradient have looked at symbiotic organisms. One example of symbiosis is the lichen symbiosis, an association between a filamentous fungal partner and a photosynthetic partner of green algae or cyanobacteria. Little is known about the latitudinal diversity gradient of lichens, yet their terrestrial ubiquity and symbiotic nature could provide insight into the drivers of this global pattern.

To assess whether lichenized fungi follow a latitudinal diversity gradient, I compiled a dataset from three repositories for digitized herbarium specimen data: the Consortium for North American Lichen Herbaria, the Global Biodiversity Information Facility, and the Institutos Nacionais de Ciencia e Tecnologia. The fully compiled and quality-controlled dataset contained over 900,000 datapoints representing over 8,000 species. The raw species richness data revealed a peak in richness north of the equator outside of the tropics, however, this pattern mirrored the number of collections per latitudinal band. To correct for sampling effort biases in the digitized herbarium data, I rarefied species richness. I further corrected the rarefied species richness for land area, given the wide range of land area per latitudinal band in the Americas. This rarefied and land-area-corrected species richness data supports a latitudinal diversity gradient of lichenized fungi in the Americas. In a comparison to lichen checklist data at the country or state level, I revealed that tropical regions are underrepresented in the digitized herbarium data.

To test the influence of sampling effort bias on the patterns revealed by the digitized herbarium data, I designed a field sampling approach directly targeted at the question of whether epiphytic lichens follow a latitudinal diversity gradient. This approach can help remove bias present in digitized herbarium data because they result from the compilation of many studies, each of which had its own taxonomic, regional, or ecological focus. To do so, I sampled from nine lowland forest sites across a 70-degree span of latitude in the Americas. At each site, I randomly chose ten plots, and sampled from ten trees within each plot for a total of nine hundred trees sampled. At each tree, I randomly chose a cardinal direction and placed a 20x40cm grid on the tree, collecting all lichens that fell within that grid. Thus, each site had the same amount of area surveyed for epiphytic lichen diversity. Lichens were identified to species in the lab. Data from systematic field sampling corroborate the latitudinal diversity gradient of (epiphytic) lichens. In a mixed effects model including tree and climate data, I found that this pattern is largely explained by host tree (substrate) diversity.

With increasing land use change and impacts from climate change across the globe, it is increasingly important for us to set a baseline of patterns of diversity at large scales, as I did in my first two chapters, to then assess how these impacts are affecting the diversity of symbiotic organisms at different scales. For my third chapter, I applied my knowledge of lichen diversity to assess the impacts of three tropical forest restoration treatments on epiphytic lichens. The natural regeneration treatment had a small cohort of lichen species likely specialized to the high light and dry environment. The plantation and nucleation treatments had a mix of light and shade tolerant species and experienced higher competition from epiphytic bryophytes. The overall highest diversity of epiphytic lichens was found in the nucleation treatment, supporting this as the combination of the most cost-effective strategy that restores the greatest amount of tropical biodiversity.

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CHAPTER ONE:
Digitized herbarium data support a latitudinal diversity gradient in New World lichens

INTRODUCTION

The latitudinal diversity gradient (LDG) is a pattern of increasing biological diversity from the poles to the equator. This phenomenon has been recognized since the time of Humboldt (1807), Darwin (1859) and Wallace (1878), and has been demonstrated across a number of taxa, both marine and terrestrial (Hillebrand 2004). Over the 200 years that the LDG pattern has been researched, numerous hypotheses have been proposed to explain it (Pianka 1966, Fine 2015). These fall broadly into five categories: spatial or null mechanisms (e.g., Colwell & Lees 2000, but see Currie & Kerr 2008), mechanisms involving climate and metabolic energy balance (e.g., Hawkins et al. 2003), evolutionary or historical mechanisms (e.g., Mittelbach et al. 2007), time-integrated species-area effects (e.g., Fine & Ree 2006), and biotic mechanisms such as the biotic interactions hypothesis (e.g., Schemske et al. 2009, Smith et al. 2012). These mechanisms are not mutually exclusive, and to date no consensus has been reached regarding the driving mechanism of the LDG (Brown 2014, Schemske & Mittelbach 2017). More investigation of both taxonomic and ecological groups at broader geographic scales is needed to determine whether this lack of consensus would be resolved by investigating more organisms in more detail, or whether it is the case that each taxonomic group that follows the LDG might have its own particular underlying mechanisms.

Studies establishing the latitudinal diversity gradient often involve a synthesis of data from a variety of sources, including species range maps, state or regional floras, checklists, museum or herbarium collections, observation data, georeferenced molecular data, direct field sampling, and reports from the literature (Qian & Ricklefs 2007, Archibald et al. 2010, Rolland et al. 2014, Visser et al. 2014). Some studies, to enhance the robustness of their underlying data, combine some of the data sources listed above. For example, Smith et al. (2016) used a combination of GBIF (Global Biodiversity Information Facility) and GenBank data to test the accuracy of taxonomic identification while investigating global patterns of *Usnea longissima*. Recently, digitization efforts have made the tremendous resource of herbarium specimen data available for the investigation of broad-scale patterns of species richness such as the LDG (Holt et al. 2015, McLean et al. 2016, James et al. 2018).

The majority of studies on both the patterns and mechanisms of the LDG had a particular emphasis on plants and animals and a lack of data on fungi and other microorganisms. Taxa for which the LDG has been demonstrated include woody angiosperms (Barthlott et al. 2007, Kerkhoff et al. 2014), birds (Hawkins et al. 2007), swallowtails, (Condamine et al. 2012), amphibians (Pyron & Wiens 2013) and marine bivalves (Jablonski et al. 2013), among others (Fischer 1960). Some possible exceptions include conifers (Fragnière et al. 2015) and mosses (Mateo et al. 2016). Within the fungi, foliar fungal endophytes (Arnold & Lutzoni 2007) and coprophilous (dung-loving) fungi (Richardson 2001) follow the LDG, while ectomycorrhizal fungi (Tedersoo & Nara 2010) and indoor fungal communities (Amend et al. 2010) are cited as exceptions. More work is needed on whether different fungal clades or functional groups follow the LDG and why.

One particularly interesting system in which to investigate the latitudinal diversity gradient are lichenized fungi, a highly diverse and polyphyletic group defined by the nutritional habit in the form of symbioses with algae or cyanobacteria (Lücking et al. 2017, Lücking & Nelsen 2018). Lichens are a symbiotic association between a fungus and one or more photosynthetic partners, either green algae or cyanobacteria. Lichenized fungi are obligate symbionts, lending themselves to tests of biotic interactions hypotheses of the LDG. Lichens are ubiquitous, found in every terrestrial habitat on earth -- from tidelines to mountaintops, from deserts to rainforests, from under the ice in Antarctica to ground cover in savannas (Nash 2008). Their global distribution and symbiosis make them great candidates for LDG studies.

Though lichenized fungi have been studied for centuries (Linnaeus 1767, Acharius 1799, Tuckerman 1882), the relationship between diversity and latitude has not yet been investigated in detail in lichens at a global scale. Prior studies of lichen diversity suggest that patterns of lichen diversity may differ depending on the geographic extent of the study, the spatial and the taxonomic scales investigated. For example, a U-shaped pattern of diversity along the western coast of the United States was found using lichen herbarium collections data (Holt et al. 2015), while a reverse latitudinal pattern of diversity was found across North America using propagule sequence data from dust collected from door frames (Tripp et al. 2016). In a study across the Atlantic Forest of Brazil, LDG patterns varied by lineage: Trypetheliaceae and Graphidaceae followed more of a classic LDG, whereas species richness values peaked outside of the tropics for Lobariaceae and Parmeliaceae (Menezes et al. 2018).

There are several reasons to hypothesize that lichenized fungi will not display LDG patterns. The first is their symbiotic nature. Within the lichen symbiosis, the photosynthetic partner provides carbohydrates to the fungal partner, which is usually dominant in biomass, leading to the characteristic slow growth in lichens, though this varies by species (Reiter et al. 2008). This symbiotic balance also leads lichens to perhaps prefer environmental conditions conducive to photosynthesis (Will-Wolf et al. 2004) - medium temperatures, ample light, and sufficient humidity - conditions which are not actually met in the hotter wetter climates of the tropics. Lichens also fill a variety of niches - growing on bark, leaves, soil, rocks, sand, and on many manmade materials as well (Ellis 2012). Lichens are poikilohydric, capable of enduring extremely stressful conditions in the desiccated stage (Nash 2008), enabling them to be the main colonizers of extreme habitats such as deserts and the rocky nunataks of Antarctica (Seymour et al. 2005, Raggio et al. 2016). Thus, in this study, we asked whether lichenized fungi follow a classic latitudinal gradient despite the ancient nature of the lichen symbiosis, its terrestrial ubiquity, and the photosynthesis requirements combined with the ability of many lichens to tolerate extreme environmental conditions.

In this study, we consolidated and analyzed large-scale herbarium specimen data across North and South America to investigate latitudinal patterns of New World lichenized fungi. Specifically, we asked: (1) Do lichenized fungi follow a latitudinal diversity gradient? (2) What are the likely environmental drivers of lichenized fungal species richness across a latitudinal gradient? and (3) Do data from digitized herbarium collections show the same species richness patterns as other data sources such as regional checklists?

METHODS

Data

To assess latitudinal patterns of lichen diversity using collections data, we gathered data from the following repositories: the Consortium of North American Lichen Herbaria (CNALH), the Global Biodiversity Information Facility (GBIF), and the Institutos Nacionais de Ciência e Tecnologia in Brazil (INCT). The focus of these data repositories is on North American, global, and South American records, respectively. Since CNALH contains only lichen collection data, we simply used a bounding box search for the Americas; from 168W to 26W and from 72S to 83N. We used the same bounding box for GBIF and INCT; however, since these repositories contain more than just lichen data, we filtered by taxonomic group, using classes, orders, families, and genera that contain only lichenized fungi. The list comprised Lecanoromycetes, Arthoniomycetes, Lichinomycetes, Monoblastiales, Pyrenulales, Strigulales, Trypetheliales, Verrucariales, Lyrommataceae, *Acantholichen*, *Cora*, *Corella*, *Dictyonema*, *Lichenomphalia* and *Multiclavula*. Within each repository we filtered the search by specimen data only, excluding data based solely on observations with no accompanying specimen.

The compilation from all three repositories resulted in a dataset containing 991,098 records. All data filtering and analyses were conducted in R (version 3.3.2, 2016-10-31). We removed records with incomplete or missing georeference data. We filtered out redundant records (the same records from multiple data sources, e.g. present in both GBIF and CNALH) using the 'duplicated' function in R and verified that this removal did not impact the number of species, only the number of records. The resulting filtered dataset contained 712,798 records.

Do lichenized fungi follow a latitudinal diversity gradient?

To assess latitudinal patterns, we used the 'floor' function in R to divide the map into one-degree latitudinal bands. We converted our filtered dataset to a species-by-latitude matrix, with latitudinal bands as rows and species as columns. We created species ranges by filling in species presence values between the maximum and minimum latitude for which a species was recorded. This approach assumes that a lack of records for a given species collected in an area that falls within the min and max distribution limits represent missing data rather than true absence. In addition, this approach allows only for interpolation rather than extrapolation of species distributions, meaning that missing data at the extremes of the distribution will not be captured. We used this interpolated species-by-latitude matrix for our analyses.

We then binned the one-degree bands of our species-by-latitude matrix into 3-degree bands; three degrees being considered an intermediate amount of coverage compared to other LDG studies (Hawkins et al. 2003, Jablonski et al. 2013, Kerkhoff et al. 2014). By binning latitude into 3-degree bands, we mitigated the zero-bias that may have resulted from latitudinal bands for which no lichen records exist.

We first assessed the relationship between species richness and latitude using a Pearson's correlation using the 'cor.test' function in R. We also assessed the relationship between the number of species and the number of records using a Pearson's correlation. Because there was a strong correlation between number of records and species richness (Table S1), we rarified our data to estimate the number of species per latitudinal band given equal sampling effort across bands. We ran a rarefaction analysis using the 'vegan' package in R with the default parameters in the function 'rarefy.' We used these rarefied species richness values for the remainder of our analyses unless otherwise noted. We used a Pearson's correlation to assess the relationship between the rarefied species richness values and latitude. We also assessed the relationship between the rarefied number of species and the log land area. Rarefied species richness was significantly positively correlated with land area; because we were interested in comparing standardized species richness across latitudes, we calculated species richness per unit area for each 3-degree latitudinal band, and used these values in the remainder of our analyses.

What are the likely environmental drivers of lichenized fungal richness across a latitudinal gradient?

We assessed the potential impact of the climatic variables of mean annual temperature, annual precipitation, annual temperature range, and precipitation of the driest quarter on rarefied species richness per unit area. We included the annual temperature range and precipitation of the driest quarter to investigate the impact of extremes in either temperature or precipitation on lichenized fungal richness. All bioclim data (https://www.worldclim.org/) were downloaded at the 2.5 minutes resolution using the 'raster' package in R. We placed rectangular polygons over the map at three-degree latitudinal intervals, extracted the bioclim data from within those polygons, and calculated the means for each of the climate variables. We first assessed relationships between each of our five climatic factors individually with rarefied species richness using Pearson's correlation, with a Bonferroni adjustment. Due to the nonlinear nature of the relationships in the data, we then assessed the impacts of the climatic variables using generalized additive models with a negative binomial distribution. Poisson is the typical distribution for count data such as species richness; however, our data were overdispersed using this method.

Do data from digitized herbarium collections show the same species richness patterns as other data sources such as regional checklists?

We compared country checklist data compiled by Feuerer (2013) and total and georeferenced data from CNALH. We used just CNALH data to compare full lichen lists at the country level, rather than searching the other databases by orders, families, and genera. For this comparison, we downloaded data from the Consortium of North American Lichen Herbaria by country for all North, Central and South American countries. CNALH data were assigned to latitude by using state centroids for U.S. states and country centroids elsewhere. We used a Pearson's correlation to assess the relationship between the CNALH non-georeferenced and georeferenced datasets. We then calculated species richness per unit area for the CNALH and checklist data, and ran a Pearson's correlation to determine the similarity in patterns of species richness from the two data sources. Finally, we used a generalized additive model with a negative binomial distribution to assess the impact of latitude on species richness per unit area from the checklist data.

RESULTS

Do lichenized fungi follow a latitudinal diversity gradient?

New World lichen species richness shows a pronounced and significant (0.0024) latitudinal diversity gradient, with rarefied richness declining with increasing latitude in both hemispheres (Figure 1; observed species richness plotted in Figure S1). The peak in values is within the tropics, north of the equator.

What are the likely environmental drivers of lichenized fungal richness across a latitudinal gradient?

Using model comparison of GAMs of our climate predictor variables, the best model, based on R-squared values, was the model that included the full set of bioclim variables (mean annual temperature, annual precipitation, annual temperature range, and precipitation in the driest quarter) as well as latitude (Table 1). This model explained up to 73% of the variation in the rarefied species richness of lichenized fungi across a latitudinal gradient. The generalized additive model that included the means of temperature and precipitation in addition to latitude explained 69% of the variance in rarefied species richness of lichenized fungi. None of the smooth terms in the generalized additive models were significant.

Do data from digitized herbarium collections show the same species richness patterns as other data sources such as regional checklists?

Comparison of the checklist, total and georeferenced datasets revealed that species richness values were consistently lowered when non-georeferenced specimens were removed from the dataset. However, the non-georeferenced and georeferenced CNALH collections data were highly correlated (Figure 2A, Pearson's correlation coefficient = 0.945, t = 23.095, p<<0.0001). Species richness per unit area from the country checklist data had a much weaker but significant correlation with species richness per unit area from the georeferenced collections data (Figure 2B, Pearson's correlation coefficient = 0.4003, t = 3.495, p = 0.001), and actually had higher species richness than either non-georeferenced or georeferenced collections data for some countries (Argentina, Venezuela, Cuba, Panama, Guyana, Colombia, Uruguay and Nicaragua). Checklist data had higher species richness than just the georeferenced collections data for Costa Rica.

Latitude of the centroid was not a significant predictor of species richness per unit area from the country checklist data in the generalized additive model (Chi-squared=1.647, p=0.554, model R-squared=0.0523). Thus, the checklist data alone do not support a latitudinal diversity gradient of lichenized fungi (Figure 3).

DISCUSSION

This is the first study to assess a pattern of lichenized fungal richness across both hemispheres in the Americas. We compiled a large data set encompassing all lichenized fungi across two continents, as well as associated bioclimatic data. We found strong support for a latitudinal diversity gradient: rarified richness of lichenized fungi per area was highest near the equator (Figure 1). Including bioclimatic data revealed both temperature and precipitation as having strong predictive power of lichenized fungal diversity. These climatic correlations are similar to those found for other taxonomic groups that follow the latitudinal diversity gradient (Fischer 1960).

Lichenized fungal rarefied species richness was significantly correlated with both mean annual temperature and land area (Table S1). Mean annual temperature itself is correlated with latitude and is a main component of the climate and energy hypotheses of the LDG (Belmaker & Jetz 2015), and we would expect a greater area to support a greater number of species (Connor & McCoy 1979). Notably, the model that explains the greatest amount of variation in the rarefied species richness per unit area of lichenized fungi is the one that includes all of the climate variables we considered, in addition to latitude. Studies of lichenized fungal richness along other environmental gradients such as altitudinal gradients, frequently reveal a hump-shaped pattern of richness from low to high altitude, however the pattern often changes above the treeline (Grytnes et al. 2006, Baniya et al. 2010). This reveals a complex of interacting drivers including substrate and microclimatic factors (Nascimbene & Marini 2015). Extreme environmental conditions, such as steep seasonal swings in temperature or extreme periods of dry, may not explain lichenized fungal richness on their own, but may nevertheless play a role in driving lichenized fungal richness along a latitudinal gradient. Lichens are able to tolerate extreme conditions, including salinity (Delmail et al. 2013), freezing temperatures (Kappen et al.

1996), dryness (Kranner et al. 2008), and even the extremes of the vacuum of space (Brandt et al. 2015). The current consensus is that the LDG is driven by a number of interacting ecological and evolutionary factors (Jablonski et al. 2017), which should be incorporated into future investigations of the lichen LDG.

The raw species richness from the three data repositories was strongly and significantly correlated with number of records, with a high peak north of the equator, indicating a bias for collecting outside of the tropics. A potential temperate bias in lichen collection and digitization efforts is supported by the number of resources available for North American lichenized fungi (Brodo et al. 2001, Nash et al. 2007), while the number of resources available for lichenized fungi in the New World tropics remain fairly decentralized, with species lists, reports of new records, and keys spread across individual papers. A northern temperate collecting bias is prevalent in other groups of fungal symbionts, including arbuscular mycorrhizal fungi (Öpik et al. 2010) and Trichomycete gut fungi (Lichtwardt 2012), supporting the need for further sampling in the tropics as we continue to investigate global biogeographical patterns in these groups. As evidenced by the checklists having higher species richness than collections data in many tropical countries, there is also a need for more digitization of herbarium collections data. Furthermore, as indicated by the non-georeferenced data from CNALH having higher species richness values than the georeferenced data, there is a need for more georeferencing of specimen data. A previously cited exception to the LDG, ichneumonid wasps (Sime & Brower 1998), resulted from a lack of data in the tropics (Veijalainen et al. 2012), rather than an actual lack of species. We therefore stress the importance of further tropical sampling, and digitization of data from these tropical collections.

Herbarium collections are critical resources in studies of taxonomy (Bebber et al. 2010), phenology (Robbirt et al. 2011), and biogeography (Wollan et al. 2008). Herbarium collections are nevertheless underutilized; as of 2013, less than 2% of herbarium specimens worldwide had been used in these studies (Lavoie 2013). Studies of potential collection bias in herbarium collections reveal that these can be overcome with the incorporation of field data or expert consultation (Loiselle et al. 2008), emphasizing the usefulness of these resources even when biases exist. While inclusion of non-georeferenced collections data in our analyses increased the number of lichenized fungal species per latitudinal band, non-georeferenced and georeferenced data followed the same pattern. However, discrepancies found between the country checklist data and the georeferenced collections data warn that some collections data may be incomplete. Considering the countries for which checklist data had a higher species richness than either non-georeferenced or georeferenced collections data, the tropics and southern hemisphere are likely still underrepresented in digital repositories of specimen data.

In meta-analyses of the generality of the latitudinal diversity gradient across taxa, the LDG was shown to be a pervasive pattern, though weaker than expected in slope for some taxa (Hillebrand 2004, Kinlock et al. 2017). These meta-analyses included studies of latitudinal ranges from as small as 10 degrees to global spans. Previous work on the latitudinal diversity gradient in lichenized fungi outside of the tropics had revealed patterns incongruent with the classic pattern followed by many taxa that peak in richness in the tropics (Holt et al. 2015, Tripp et al. 2016). These more geographically restricted studies indicate that at smaller scales other drivers of lichen diversity may be important and should be explored more

Previous studies addressing a temperate-tropical LDG in lichens and including the tropics, were limited to one of the hemispheres and used either only checklist data (Lücking et al. 2011; north of the equator) or a limited set of taxa (Menezes et al. 2018; south of the equator). These studies of lichen diversity which incorporate or focus on the tropics show species richness increasing towards the equator (Lücking et al. 2011), though this depends on the taxonomic group (eg. Menezes et al. 2011: Graphidaceae versus Parmeliaceae). We emphasize here the importance of including both hemispheres, particularly including the tropics, when investigating a latitudinal diversity gradient.

With each new survey, lichenologists are finding undescribed species and high species richness of lichenized fungi in both tropical (Lücking et al. 2014a) and extratropical (Dillman et al. 2012, Fryday & Øvstedal 2012) environments. Tropical lichen floras are not as developed as temperate lichens in terms of taxonomic revisions and determination of cryptic species (Lücking et al. 2014b), which suggests that the collections data used in this study may not only be influenced by a collecting bias, but by a taxonomic bias as well. However, we did not directly test for taxonomic biases in the current study. Lichenized fungi, and fungi more generally, lag behind other taxonomic groups in terms of digitized data availability (eg. plants, Allen et al. 2019). We suggest the need for more collecting of lichens, taxonomic work on lichens, and digitization of lichen specimen data, especially in the tropics, such that we can continue to investigate these large-scale questions of diversity and distributions with greater and greater precision.

CONCLUSION

Lichens are an ideal system for investigating the pattern and process of the LDG due to their terrestrial ubiquity, their niche breadth, and the fact that lichens are themselves biotic interactions. This is the first study of the latitudinal diversity gradient of lichenized fungi to encompass data from both the Northern and Southern hemispheres. We consolidated data from both the northern and southern hemispheres, controlling for collection effort and area. Rarefied species richness from herbarium collections data reveal a classic latitudinal diversity gradient in lichenized fungi. This gradient is predicted by climatic factors of temperature and precipitation, possibly supporting climate and energy hypotheses of the LDG. Potential taxonomic and geographic biases in the collections data will be improved with increased collecting and research of lichenized fungi in the tropics.

APPENDIX

CHAPTER ONE TABLES AND FIGURES

Table 1.1 Comparison of Generalized Additive Models (GAMs) with a negative binomial distribution of the impact of latitude and climate data on rarefied species richness per unit area.

Model	Variables	R-squared(adj.)
Lat_Climate	Latitude, Mean Annual Temperature, Mean Annual Precipitation, Annual Temperature Range, Precipitation in the Driest Quarter	0.733
Lat_Means	Latitude, Mean Annual Temperature, Mean Annual Precipitation	0.695
Climate	Mean Annual Temperature, Mean Annual Precipitation, Annual Temperature Range, Precipitation in the Driest Quarter	0.667
Means	Mean Annual Temperature, Mean Annual Precipitation	0.198
Latitude	Latitude	0.119

Table 1.S1 Pearson's correlations between species richness or rarefied species richness (SR) of lichenized fungi and latitude, number of records, and environmental variables.

Variables	Correlation Coefficient	t-test	p-value	Bonferroni adjusted p-value
Species Richness X |Latitude|	-0.2659	-1.851	0.071	0.142
Species Richness X Number of Records	0.8083	9.211	<<0.00001***	0.00002***
Rarefied Species Richness X |Latitude|	-0.4935	-3.81	0.0004***	0.0024**
Rarefied Species Richness X Land Area (log)	0.7171	6.9028	<<0.00001***	0.00006***
Rarefied Species Richness X Mean Annual Temperature	0.4141	3.0516	0.0038**	0.0228*
Rarefied Species Richness X Annual Precipitation	0.2722	1.8978	0.064	0.384
Rarefied Species Richness X Annual Temperature Range	0.089	0.5994	0.552	1.00

Table 1.S1 (cont'd)

Rarefied Species Richness X Precipitation in Driest Quarter	-0.043	-0.2887	0.774	1.00

Figure 1.1 Rarefied species richness divided by log land area plotted against latitude. Extent of the tropics is marked by vertical dashed grey lines. One southern point (latitude = -60) was removed due to the small amount of land area and the fact that the log-area slope parameter changes between small and large values.

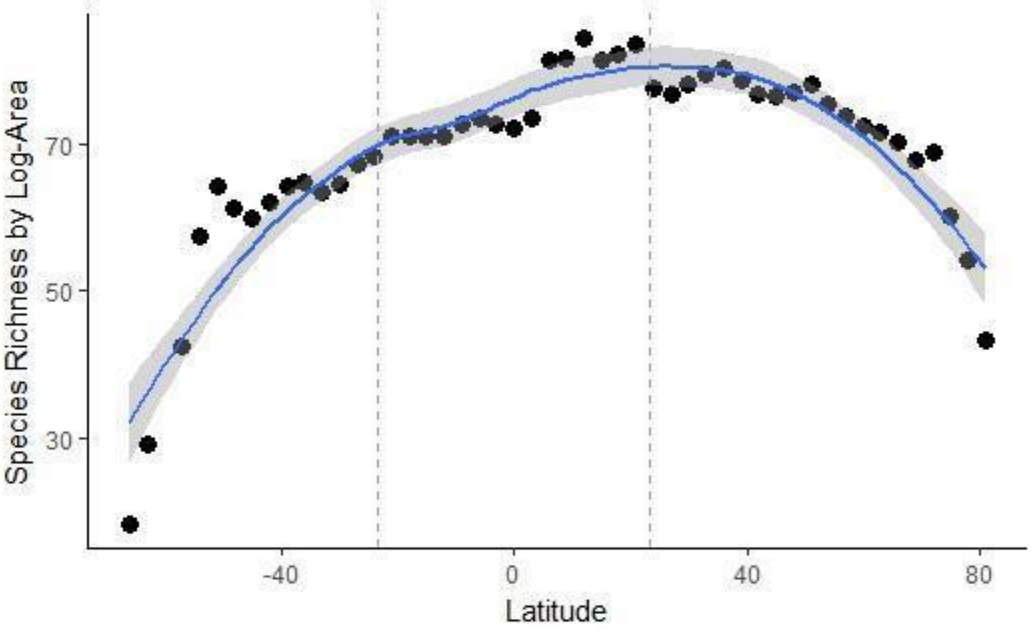


Figure 1.2 (A, above) Plot of lichenized fungal species richness per country using all data (non-georeferenced) versus just georeferenced data from the Consortium of North American Lichen Herbaria (CNALH) database. (B, below) Lichenized fungal species richness per unit area based on lichen checklist data by country versus georeferenced data from CNALH.

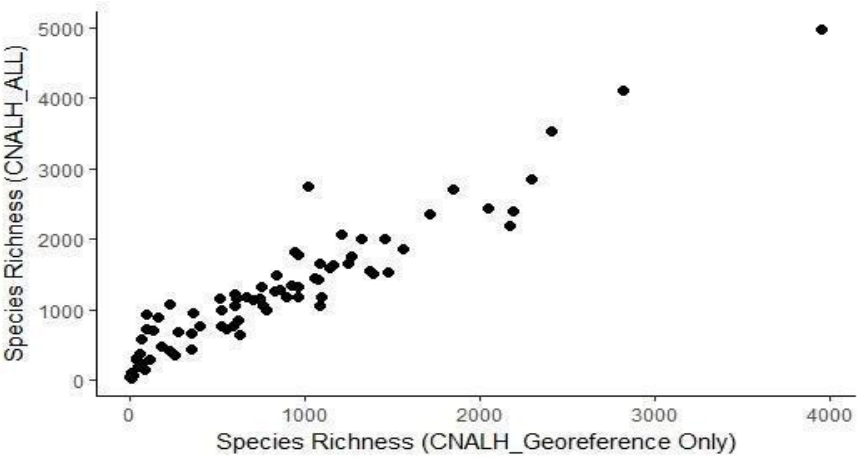


Figure 1.2 (cont'd)

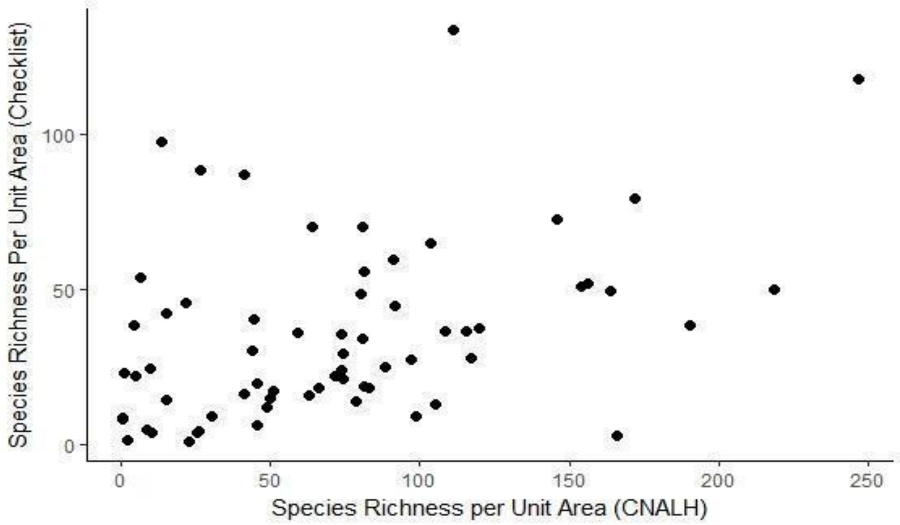


Figure 1.3 Species richness per unit area of lichenized fungi according to country (and US state) checklist data plotted against latitude, as determined by country and US state centroids.

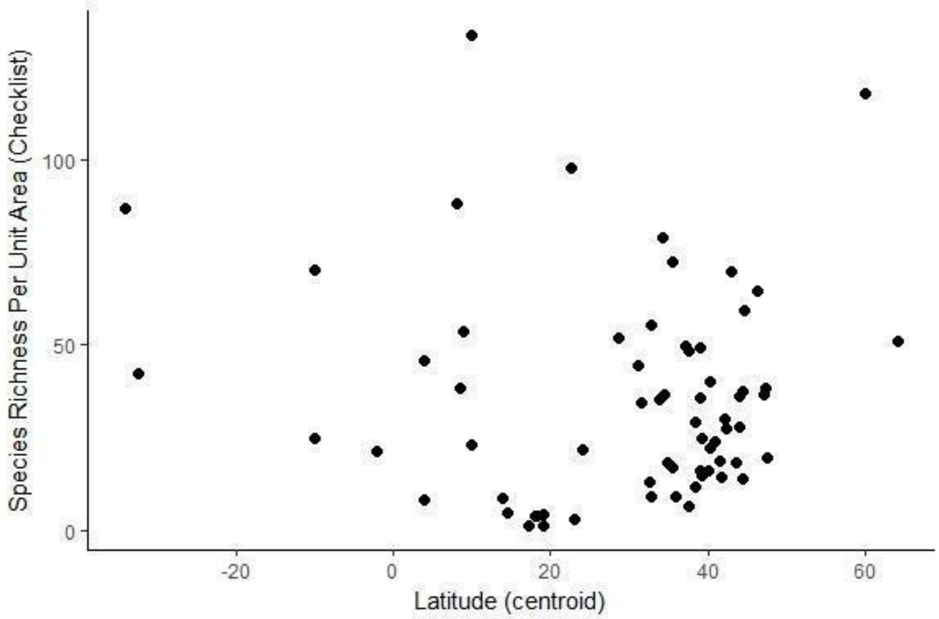


Figure 1.S1 The observed species richness in the herbarium specimen data plotted against latitude. Extent of the tropics is marked by vertical dashed grey lines.

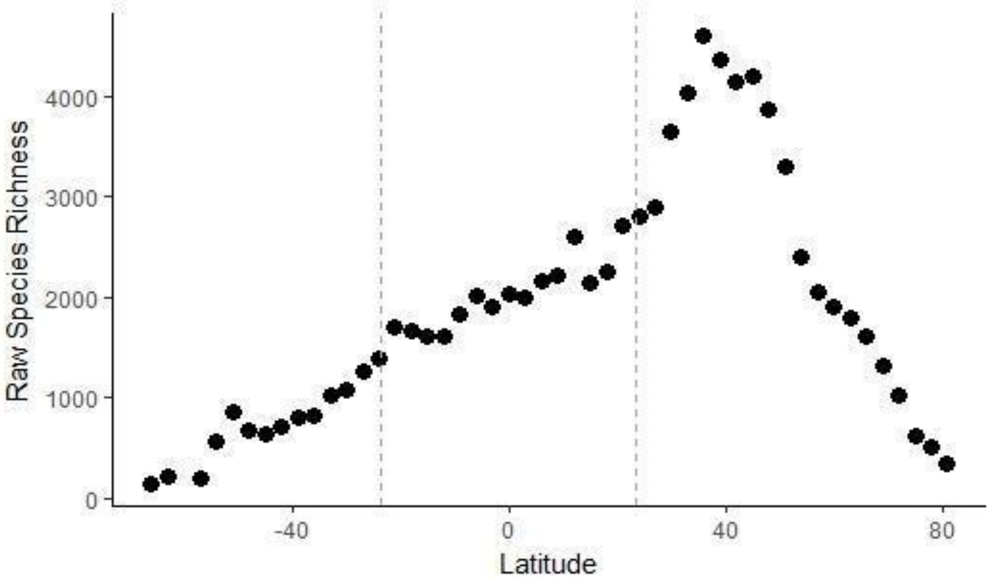
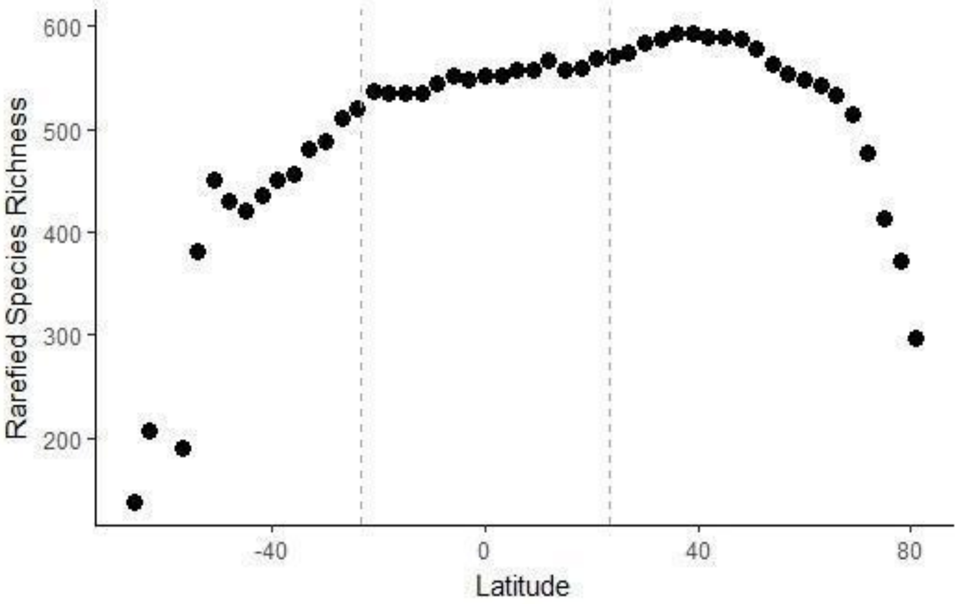


Figure 1.S2 Rarefied species richness in the herbarium specimen data plotted against latitude. Extent of the tropics is marked by vertical dashed grey lines.



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Pyrenulales (INCT) *Arizona State University Lichen Herbarium* (ASU-Lichen), *Herbário Irina Delanova Gemtchújnicov* (BOTU), *Herbário da Fundação Universidade Federal de Mato Grosso do Sul* (CGMS), *Coleção de Fungos INPA* (INPA-Fungos), *Herbário ISE* (ISE), *Herbário do Museu da Pontifícia Universidade Católica do Rio Grande do Sul* (MPUC), *Herbário Pe. Camille Torrand* (URM), *Wisconsin State Herbarium* (WIS) available at *species*Link network (http://inct.splink.org.br) in 2018, Feb 06.

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CHAPTER TWO:
Testing the latitudinal diversity gradient of New World epiphytic lichens using a systematic field sampling approach

INTRODUCTION

Symbiosis, the intimate association of two dissimilar organisms over space and time, is a biotic interaction that is critical across taxonomic groups, yet frequently gets a minimal treatment in studies of ecology, evolution, and biogeographical patterns, such as the latitudinal diversity gradient (Scharnagl 2019). The latitudinal diversity gradient (LDG) is a global pattern of increasing richness with decreasing latitude. The richness and diversity of many taxa peak within the tropics around the equator; this pattern has been demonstrated for plants (Kerkhoff et al. 2014) and animals (amphibians, Pyron & Wiens 2013; birds, Hawkins et al. 2007), and ranging from the terrestrial (Rolland et al. 2014) to the marine (Jablonski et al. 2013, Floeter et al. 2004). Of the few symbiotic associations that have been investigated, we find support for a LDG in both stony corals (Spano et al. 2016) and in arbuscular mycorrhizal fungi (Davison et al. 2015). In this study, we propose to expand upon our current understanding of the biogeography of symbioses through the investigation of the latitudinal diversity gradient of lichens.

Lichens are cryptic but ubiquitous terrestrial composite organisms (Nash 2008), which comprise symbiotic associations between a mycobiont (fungal partner), photobiont (photosynthetic partner; green algae and/or cyanobacteria), and as has been more recently discovered, a possible third partner as well (Cyphobasidiales yeast; Spribille et al. 2016). Beyond the primary symbionts, lichens contain additional algae, fungi, and bacteria; each lichen thallus is a microcosmic ecosystem (Eymann et al. 2017). Though lichens can be found from the poles to the equator, from tidelines to mountaintops, they vary in terms of their habitat and substrate preferences (Smith et al. 2009, Cornelissen et al. 2007). The lichen symbiosis is relatively ancient, and lichenized fungi are a polyphyletic group in which lichenization likely evolved multiple times independently (Gargas et al. 1995, Honegger 2018, Lücking & Nelsen 2018).

Despite the prevalence of the latitudinal diversity gradient across taxa, no consensus has been reached regarding what drives the latitudinal diversity gradient (Schemske & Mittelbach 2017, Palmer 1994). In general, the hypotheses for the LDG can be placed into three main categories: historical, ecological, and evolutionary (for a thorough review, see Mittelbach et al. 2007). Historical hypotheses relate to time and area; at different points in earth's history, more of the earth's surface experienced a tropical climate, enabling more species to diversify within a tropical environment (Fine & Ree 2006). One example of this is the tropics-as-museum hypothesis, in which the ranges of formerly widespread and diverse taxa were reduced into the contemporary latitudinal bands of the tropics as the earth's climate shifted. Another, related historical hypothesis is that of tropical niche conservatism, which emphasizes the importance of temperature, proposing that there may be a physiological hurdle to overcome if species are to move from the tropics to extra-tropical regions. Ecological hypotheses also consider climate, and include the species-energy hypothesis, which connects climate and energy with primary productivity. Higher primary productivity leads to a higher carrying capacity for more individuals, which can lead to higher species richness (Wright 1983). Another ecological hypothesis, proposed by Pianka (1966), proposes that tropical environments contain higher heterogeneity or complexity of physical/spatial factors, which in turn leads to a higher number of available habitats for species in the tropics. Evolutionary hypotheses discuss the differences in diversification rates between tropical and extratropical regions: for example, speciation rates may be equal in both regions but there are higher extinction rates in extra-tropical regions due to extreme climatic conditions (Mittelbach 2012). Though there is ample support for the latitudinal diversity gradient in different taxonomic groups, and its correlation with climate (temperature), no single proposed hypothesis has been able to match the species accumulation rate in the tropics (Antonelli & Sanmartin 2011): when species richness is plotted based upon models proposed in the LDG hypotheses, the predicted curve falls short of the curve of observed species richness increase with decreasing latitude (Currie, Mittelbach et al. 2004). One hypothesis that attempts to account for this discrepancy in species accumulation rates is the biotic interactions hypothesis.

Wallace (1878), Dobzhansky (1950) and Fischer (1960) each proposed the idea that in the tropics, organisms were freed from the abiotic constraints of the higher latitudes. Temperate regions experience extreme and unpredictable fluctuations in temperature and other climatic events not experienced in the tropics, tending to lead towards, as Dobzhansky postulated, "a few generalists." In the tropics, however, species are freed from this constraint, and the importance of biotic interactions increases. Schemske et al. (2009) expanded upon this idea; demonstrating that many species interactions, including parasitism, mutualisms, herbivory, predation and other indicators such as sexual selection, often showed a greater prevalence and importance in the tropics. However, many areas of species interactions require further research. The importance of species interactions would ideally be measured by looking at the proportion of fitness due to biotic interactions (Schemske 2009). Since this is often difficult, alternative measures discussed in Schemske et al. (2009) include looking at the frequency of interactions (eg. the richness of species involved in biotic interactions), the expression of interactions (eg. possessing traits involved in biotic interactions such as plants using chemical defenses against herbivory), and the strength of interactions (eg. the specificity or generality of species interactions).

Although the latitudinal diversity gradient has been demonstrated for most plant and animal groups, fungal patterns of diversity along a latitudinal gradient vary strongly by group. For example, while pathogenic fungi follow the LDG (Peay et al. 2016), indoor fungi follow an inverse pattern (Amend et al. 2010), and ectomycorrhizal fungi actually have a bimodal pattern (Tedersoo & Nara 2010). Previous studies that have investigated diversity of lichenized fungi (the mycobionts in the lichen symbiosis) across latitudes have also revealed a variety of patterns. In a study using herbarium collections data along a gradient in the Western continental United States, Holt et al. (2015) found a U-shaped pattern of diversity. Lichen richness was high in the southwestern USA, dipped down at mid-latitudes, and peaked again in the Pacific Northwest. In a study using a molecular dataset of lichen propagules collected in dust samples from door and window frames from across the USA, Tripp et al. (2016) found an inverse latitudinal gradient, with lichen species richness increasing with increasing latitude. In a study of patterns of lichen richness in the Brazilian Atlantic Forest, Menezes et al. (2018) found an overall latitudinal diversity gradient of lichens supported, though the patterns did vary when assessed by lineage (at the fungal family level). In a recent study of lichen richness based on herbarium specimen data across both hemispheres in the Americas, Scharnagl (Chapter One 2019) expanded the latitudinal range investigated for a lichen LDG, and demonstrated that lichens do indeed follow a latitudinal diversity gradient. However, multiple potential gaps in the herbarium collections data were identified, revealing the need for more data and other approaches in order to verify this lichen LDG.

The majority of LDG studies are based upon a compilation of data sources, including herbarium specimen data, observation databases, and reviews of the literature (Scharnagl Chapter One 2019). While these approaches enable the researcher to study a larger area or have a more comprehensive taxon sampling, these data are based upon multiple studies; multiple researchers and multiple approaches. In this study we set out to determine how a field approach, using a systematic sampling design and a single primary researcher, investigating the latitudinal diversity gradient of lichens compares to studies based upon the aforementioned larger datasets. Using data collected from nine sites along a 70-degree New World latitudinal gradient, we asked (1) what are the patterns of lichen richness, diversity, beta diversity, and functional diversity along a latitudinal gradient, and (2) what are potential drivers of lichen richness along a latitudinal gradient?

METHODS

Site Selection

Given the habitat and substrate versatility of lichens, we narrowed our sampling to focus on epiphytic lichens in (relatively) lowland forest habitats. Epiphytic lichens are those that grow on the bark of living trees. A total of nine sites were selected along a New World latitudinal gradient from approximately 12 degrees south to 61 degrees north (Table 1). Sites that included long term forest research plots (eg. RainFor, ForestGEO/CTFS, Bosques) were targeted. Where these were not available, sites were selected based upon level of protection (forests under long term protection, eg. State Preserve, Biological Station or National Forest) in addition to accessibility. Sites were also selected based upon maximal coverage along a latitudinal gradient. Based upon these selection criteria, sites were relatively evenly spaced latitudinally but varied a good deal longitudinally (Figure 1). Tree data and local climate data was obtained for the majority of sites. In three of the sites, trees were identified by the researcher or a field assistant.

Field Sampling

At each site we surveyed and sampled epiphytic lichens from 100 individual trees, for a total of 900 trees across nine sites (Figure 1). At most sites we sampled trees greater than 5cm diameter breast height (DBH); however, at some sites, such as the Scotty Creek site in the boreal, we had to amend this approach. Most of the long term forest sites were laid out in plots or grids; we used these to select which trees to sample from using a random number generator. At sites without plots or grids, we used a map of the site and trails with a grid overlay to randomly select trees for sampling. Once a tree was selected using the random number generator, we sampled from that tree and its nine closest neighbors for clusters of ten, before using the random number generator to sample from a different part of the forest plot. GPS coordinates were recorded for each cluster of ten trees.

Each tree species was either identified in the field, or its tag number was recorded to be matched with a tree database following field work. At each tree, we measured DBH and used a random number generator to choose whether to sample from the N, NE, E, SE, S, SW, W or NW side of the tree. Once a side was selected, we placed a 20 x 40cm open plastic grid over the trunk at 1.5 m above ground. The grid was photographed as a reference, then all lichens within the grid were collected. Lichens were collected using a field knife and stored in paper packets.

Lichen Identification

Lichen samples were first sorted into individual paper packets - one packet per lichen species. Packets were labeled with site information, then lichens were sorted by morphological characteristics. These include thallus type (crustose, foliose, fruticose, squamulose, leprose), reproductive structures (sexual: apothecia, perithecia, lirellae, asexual: isidia, soredia), and where easily identifiable, photobiont (green algae, cyanobacteria). From these categories, lichen samples were further sorted into similar morpho-groups. Lichens were then keyed out using dichotomous keys (Lendemer et al. 2013, Sipman 2005, Brodo et al. 2001). For some lichen identification, spot tests using iodine, bleach, or potassium hydroxide were necessary, and for some, spore characteristics were also obtained. Tropical lichen sample identification was provided by Robert Lücking at the Berlin Botanic Garden. Lichens that could not be identified to the species level were assigned to a morphospecies. Some lichens were recorded as indeterminable morphospecies either due to old or sterile thalli, and were excluded from further analyses. The final lichen dataset contained: collection numbers, lichen species identification and higher taxonomic classifications, site information including name and geographic coordinates, lichen morphological descriptions including growth form and reproductive mode, notes on any spore characteristics or spot tests, and tree data including tree species identification, tree tag numbers and DBH. Lichen voucher specimens are housed at the Michigan State University Herbarium.

Analyses

All statistics were conducted in R (R Core Team 2019). To assess three measures of diversity (richness, Simpson's diversity, and beta diversity), we used a species by site presence/absence matrix in the vegan package (Oksanen et al. 2019) using functions 'specnumber,' 'diversity,' and 'betadiver,' respectively. Richness was assessed at the site level. To determine dissimilarity and evenness within a site, beta diversity and Simpson's diversity were calculated at the plot level, then the mean value was calculated for each site. Each of the relationships between diversity and latitude were assessed using a generalized linear model with a negative binomial distribution. Analyses were conducted using the MASS package (Venables & Ripley 2002).

As lichen specimens were identified they were assigned to a variety of growth forms and reproductive modes. Growth form refers to the vegetative body of the lichen, the thallus (Brodo et al. 2001). The growth form categories assigned in this dataset included: crustose (flat, crust-like), foliose (three-dimensional, leaf-like), fruticose (long and three-dimensional, sometimes pendulous), leprose (loose association, almost fluffy-looking), squamulose (thallus composed of many small scales), and filamentous (thallus composed of thin filaments, often closely appressed to the substrate). To compare across latitudes, we lumped growth forms into two categories: two-dimensional and three-dimensional. Two-dimensional refers to the crustose growth form. Three-dimensional consisted of foliose, fruticose and squamulose. Leprose and filamentous growth forms were rare within the dataset, and were excluded from this analysis. We then calculated the ratio of two-dimensional to three-dimensional growth form categories for each site.

In this study, reproductive mode refers to the reproductive structures visibly apparent on the lichen thallus. These can include spore-producing structures such as apothecia, perithecia, and lirellae, or asexual propagules such as soredia and isidia. As with growth form, we lumped reproductive mode into two categories: sexual (spore-producing) and asexual (propagules composed of fungal hyphae and algal cells). Lichens that exhibited both sexual and asexual reproductive modes on the same thallus were counted in both categories; these represented less than 0.05% of the dataset. Sterile lichens, those for which no reproductive structures were identified on the thallus, were excluded from this analysis. We then calculated the ratio of these reproductive mode categories for each site. The relationship between each ratio (growth form, reproductive mode) and latitude was assessed using a generalized linear model with a negative binomial distribution.

Tree species richness, the number of host tree species from which lichens were collected, was calculated per site using the specnumber function in the vegan package in R. We calculated the species richness of lichens per individual tree, and assessed the impact of face on lichen species richness using a generalized linear mixed effects model with plot as a random variable using the lme4 package (Bates et al. 2015). We calculated the mean and standard deviation of DBH per site. We downloaded bioclim data at the 10 minute resolution (Fick & Hijmans 2017) using the raster package (Hijmans 2019) and extracted climate data for each of our nine sites. We used a generalized linear mixed model to assess the potential drivers of latitude, tree species richness, mean DBH, variance in DBH, temperature of the coldest quarter, and precipitation of the driest quarter on lichen species richness. We chose climate data from the coldest and driest quarters as representing potential constraints on photosynthesis. We also assessed the impact of different climate variables (mean annual temperature, annual precipitation, temperature seasonality, precipitation seasonality, temperature in the coldest quarter, temperature in the warmest quarter, precipitation in the driest quarter, and precipitation in the wettest quarter) on the ratio of two-dimensional to three-dimensional growth forms, using generalized linear models with a negative binomial distribution using the MASS package. A post-hoc analysis of deviance test was conducted for each of the variables in the models using the Anova function.

As a first look into the within-lichen patterns of diversity, we selected a subset of lichen individuals within the family Parmeliaceae from three of our northern sites. Total genomic DNA was extracted directly from each sample using the REDExtract-N-Amp kit (Sigma-Aldrich). After being soaked in acetone, our selected small fragments of lichen thalli were allowed to air dry. Twenty µL of extraction buffer was added to each sample, and the sample was then ground in the extraction buffer using a micropestle. Samples were then warmed to

95°C for twelve minutes, and brought back to room temperature. Twenty µL of dilution buffer were then added to each sample. Samples were centrifuged for 5 minutes, which brought all of the solids to the bottom of the tube. The elution was then used to create a 1:20 dilution using nuclease-free water. This 1:20 dilution was then used for PCR. We used specifically designed primers for algae within Trebouxiophyceae (Piercey-Normore & DePriest 2001) for the internal transcribed spacer (ITS) region. Each 20.8 µL of reaction mixture included 10 µLof REDExtract-N-Amp PCR Reaction Mix (Sigma-Aldrich), 8.5 µL PCR-grade water, 0.5 µL each of forward and reverse primers, and 1.3 µL of [1:20] extracted DNA solution. Microtubes were then placed in a BioRad T100 thermocycler and the following cycling reaction was run: 94°C for 5 min, 94°C for 30 sec, 45°C for 30 sec, 72°C for 2 min, 44 cycles at [94°C for 30 sec, 45°C for 30 sec, 72°C for 2 min], 72°C for 5 minutes, then kept at 4°C until retrieved. DNA bands were detected using ethidium bromide on a 0.7% agarose gel; all positive products yielded a single band. PCR products were normalized and submitted for sequencing on the Illumina MiSeq platform within the Michigan State University Genomics Core. Sequences were assembled, trimmed and BLASTed using Qiime2 and GENEious Prime. Patterns of specificity between lichenized fungi and their algal photobionts were analyzed using the bipartite package in R (Dormann et al. 2008).

RESULTS

Latitudinal Patterns in Lichen Diversity

As latitude increases, species richness of epiphytic lichens across our nine sampled sites decreases (Figure 2). Lichen species richness across nine sites from 12 degrees south to 61 degrees north in the Americas follows a latitudinal diversity gradient (for all measures of correlation between latitude and diversity, see Table S1). Simpson's diversity of lichens does not follow a latitudinal pattern; a diversity measure that includes both richness and evenness of lichens does not significantly change along a latitudinal gradient. Latitude is significantly correlated with beta diversity along two axes; as latitude increases, the number of species shared among plots within a site increases, whereas the number of species not shared among plots within a site decreases (Figure 3). The ratio of lichen species to genera decreases with increasing latitude (Figure S1).

The ratio between two-dimensional, crust-like growth forms and three-dimensional growth forms significantly changes along a latitudinal diversity gradient (Figure 4a, estimate= -0.0515, $p << 0.0001$). As latitude increases, crust-like growth forms are replaced by more three-dimensional growth forms including foliose, fruticose, and squamulose. The ratio between sexual and asexual reproductive modes did not significantly change along a latitudinal gradient (Figure 4b, estimate= -0.0043, p=0.69). The ratio of two-dimensional to three-dimensional growth forms was driven by climatic variables. The climate extremes model was the best fit, but only precipitation variables had a significant association with growth form ratios (Table 2).

Drivers of Lichen Diversity

In the full GLMM, only tree species diversity was a significant predictor (Chi-squared=3.76, p=0.033) of lichen species richness along a latitudinal gradient; mean and variance of DBH, climate variables, and latitude were not significant predictors of lichen richness. The face of the tree from which the lichens were collected had no significant impact upon lichen species richness at any latitude (AIC=3152, all p-values for the different faces >0.3, See Supp Fig2).

A Preliminary Look within the Lichen Symbiosis

Our sequencing approach yielded five algal amplicon sequence variants (ASVs) from across eight lichen species at the North Carolina site (Figure S4), three ASVs from across seven lichen species at the Harvard Forest site (Figure S5), and seven ASVs from across four lichen species at the Scotty Creek site (Figure S6). The mean specificity index of photobionts increases with increasing latitude across our three examined sites; however, latitude is not a significant predictor of photobiont specificity (F=0.614, p=0.557, Figure S7A). The mean specificity index of mycobionts does not follow a latitudinal pattern and latitude is also not a significant predictor of mycobiont specificity (F=0.123, p=0.73, Figure S7B). We found no overlap in photobiont ASVs among our three sites (Figure S8).

DISCUSSION

New World epiphytic lichenized fungi follow a latitudinal diversity gradient, with the highest species richness at and around the equator, and decreasing species richness with increasing latitude. The pattern revealed here by the systematic field sampling approach corroborates the latitudinal diversity gradient of lichens revealed from herbarium collections data (Scharnagl Chapter One 2019). Beta diversity of lichenized fungi also followed a latitudinal gradient, with lichens within plots becoming more similar to one another with increasing latitude. This latitudinal gradient in beta diversity has been observed in other groups as well, including vascular plants (Qian & Ricklefs 2007) and mammals (Qian et al. 2009). Interestingly, beta diversity between trees within a single plot did not follow a latitudinal pattern. Rather, as one moves from one tree to another within a plot, similarity remains low until we get to our two boreal forest sites (Figure S3). Schemske et al. (2009) proposed that the richness of organisms engaged in tight biotic interactions could be used as a proxy for the frequency of biotic interactions at different latitudes. The patterns of lichen diversity found in this study support the Biotic Interactions Hypothesis in terms of frequency of biotic interactions.

Lichen species richness generally increased with increasing host tree species richness, and tree species richness was the sole significant driver in the full model assessing drivers of lichen richness across our nine sites. Host specificity, even host genotype, has been implicated as an important driver in other epiphytic (Zytynska et al. 2011) and endophytic (Hoffman & Arnold 2008) groups, often interacting with other factors such as locality. In one group of lichenized fungi, Resl et al. (2018) found multiple transitions from specialist to generalist and back again in terms of substrate types (eg. soil, rock, bark, wood), though overall more transitions from generalist to specialist. In a study in the Brazilian Atlantic rainforest, the specificity of epiphytic lichen communities was somewhat influenced by bark characteristics and microclimate, but the largest factor seemed to be stochastic effects of lichen dispersal, suggesting less emphasis on substrate specificity within a substrate type, such as the bark of trees (Cáceres et al. 2007). Further investigation is needed into the relationship between lichen richness, tree richness, and lichen substrate specificity in order to determine the role of tree richness in driving lichen richness along a latitudinal gradient.

The ratio of two-dimensional to three-dimensional growth forms of lichens followed a latitudinal gradient across the nine sampled sites. Similar patterns in epiphytic lichens have been found across other gradients, such as elevational gradients; the percentage of crustose lichens decreases with increasing altitude, while the percentage of fruticose and vegetative lichens increases (Dietrich & Scheidegger 1997). The same pattern may also prevail at the scale of vertical gradients along a single tree (Scharnagl, *unpublished data*). These patterns are likely a result of environmental gradients in moisture availability or aridity (and other parameters (Matos et al. 2015)), as growth form has been associated with water uptake ability (Rundel 1978). Across multiple studies, both lichen richness and the diversity of lichen growth forms are driven primarily by light intensity and moisture availability (Harris 1971, Brodo 1961). The latitudinal gradient in growth form ration in our study supports climatic stability hypotheses of the LDG. The tropics have a relatively stable climate (Gates 1962) and have been less impacted by extreme events such as glaciation events compared to northern latitudes (Dynesius & Jansson 2000). The hypothesis of climatic stability states that the more stable climate in the tropics supports higher species richness through greater specialization and narrower niches. The converse of that being that the lower stability and higher variability of extratropical climates would support lower species richness (Pianka 1966). The ratio of lichen growth forms is best explained by the climate extremes model, supporting the idea that fewer species better adapted to higher climate variability may dominate outside of the tropics. The importance of species tolerance to climatic extremes at northern latitudes, and narrower tropical niches as an enforcing factor of isolation and diversification, is further discussed in Janzen's (1967) idea of mountain passes being higher in the tropics (Sheldon et al. 2018). Another symbiotic group, the stony corals, have a latitudinal gradient driven by a combination of history and climatic stability; more transitions from temperate to tropical have taken place than the reverse (Spano et al. 2016). Likewise, arbuscular mycorrhizal fungi follow the "sun worshipper hypothesis," in which the higher light availability and less extreme climate in the tropics leads to higher incidence and specificity of arbuscular mycorrhizal plants, thus driving arbuscular mycorrhizal diversity (Veresoglou et al. 2019).

The ratio between sexual and asexual reproductive modes did not vary along a latitudinal gradient, though these have been shown to be influenced by factors such as moving from forested to non-forested habitats or length of time following a disturbance such as fire (Nelson et al. 2015).

In our preliminary look into biotic interactions within the lichens themselves, we do not find evidence for a latitudinal pattern of specificity across three sites from either the mycobiont (fungal partner) or photobiont perspective. Specificity and selectivity of the mycobionts to the photobionts is driven largely by abiotic factors (Leavitt et al. 2015), though there is evidence for both photobiont-mediated (climate drivers, Peksa & Skaloud 2011) and mycobiont-mediated (lineage-driven, Yahr et al. 2004) guilds. Evidence from corals in the Indian Ocean suggest that partnerships within this symbiosis are also driven by abiotic factors (LaJeunesse et al. 2010). Partner switching may in fact be a mechanism by which these symbiotic organisms overcome physiological hurdles, eg. transitions from tropical to temperate environments. Mycobiont and photobiont specificity were not aligned at any of our sites. It is possible that this is an artefact of a small sample size or that mycobiont and photobiont specificity are driven by different factors in these northern latitudes, though other studies have found high levels of reciprocal specificity in lichens even at intercontinental scales (Otalora et al. 2010). We did find complete turnover of photobiont ASVs across our three sites, suggesting localized (locally adapted?) pools of available partners. We are interested to see whether these localized pools become narrower or broader in a tropical environment.

We find some support for a historical hypothesis of the LDG in lichens based upon a higher species:genus ratio in the tropics and a lower species:genus ratio with increasing latitude (Figure S1). Tropical lichens may have had more opportunities for speciation by isolation (Janzen 1967) before coming together again, leading to many closely related lichen species being found close together spatially in the tropics (Lücking & Matzer 2001).

As in other groups (Schemske & Mittelbach 2017), the LDG of lichens is likely driven by multiple interacting factors, including geological history, gradients in light, temperature and moisture availability, and substrate diversity. It is important to note here that these patterns and drivers are based on epiphytic lichen diversity.

Trees are a relatively new substrate for lichens evolutionarily speaking, and it is likely that lichens specialized to other substrates, such as saxicolous lichens (lichens growing on rocks) that tend to dominate in extreme environments such as arctic and alpine tundra (Wang et al. 2017), may follow a different pattern.

CONCLUSION

Systematic field sampling of one hundred individual trees across ten plots at each of nine sites spread across a 70-degree latitudinal gradient in the Americas supports a latitudinal gradient of species richness, beta diversity, and growth form ratio of lichenized fungi. Notably, Simpson's diversity and reproductive mode ratio do not follow a latitudinal gradient. The latitudinal gradient of lichen species richness is significantly driven by tree species richness, but not by tree size or face. This study provides further support that lichenized fungi do indeed follow a latitudinal diversity gradient and explores some of the underlying mechanisms of history, climate, and biotic interactions.

APPENDIX

CHAPTER TWO TABLES AND FIGURES

Table 2.1 The nine sites used in this study; the type of long term research or protection status of the forest; the latitude and longitude; the country in which the site was located; and the method or dataset by which tree species were identified.

Site	Forest Type	Latitude	Longitude	Country	Tree Species Identification
Cocha Cashu	Biological Station & Long term research plots	-11.8 S	-71 W	Peru	RAINFOR
Yasuni	Biological Station & Long term research plots	0.9 N	-76 W	Ecuador	ForestGEO
La Selva	Biological Station & Long term research plots	10 N	-84 W	Costa Rica	Bosques
Los Tuxtlas	Biological Station	18N	-95 W	Mexico	Not obtained
Fakahatchee	State Preserve	26 N	-81 W	USA	Identified by Klara Scharnagl
Nantahala	National Forest	35 N	-83 W	USA	Identified by Matthew Chansler
Harvard Forest	Biological Station & Long term research plots	42 N	-72W	USA	ForestGEO
BC Boreal	Public Forest	52 N	-119 W	Canada	Identified by Toby Spribille & Klara Scharnagl
Scotty Creek	Biological Station & Long term research plots	61 N	-121 W	Canada	ForestGEO

Table 2.2 Climate models for predicting lichen growth forms along a latitudinal gradient. Significance levels of each variable are reported from the generalized linear models and from the post-hoc analysis of deviance of the generalized linear models.

Model	**Variables (estimate & significance)**	**AIC**	**R-squared**
Climate Means	Mean Annual Temperature (0.0118 ***/***) Annual Precipitation (0.0001 ns/ns)	46.441	0.574
Climate Seasonality	Temperature Seasonality (-0.0021 ***/***) Precipitation Seasonality (0.0372 **/***)	37.399	0.862

Table 2.2 (cont'd)

Climate Extremes	**Temp Coldest Quarter (-0.0001 ns/**)** **Temp Warmest Quarter (0.0708 ns/ns)** **Precip Driest Quarter (0.0036 **/***)** **Precip Wettest Quarter (-0.0047 ***/***)**	**37.872**	**0.928**

Table 2.S1 Coefficients of correlation and significance tests between latitude and measures of epiphytic lichen diversity.

Correlation between Latitude and:	**Correlation Coefficient**	**t-value**	**p-value**
Species Richness	-0.802	-3.554	0.009
Simpson's Diversity	-0.416	-1.212	0.265
Similarity	0.678	2.44	0.044
Dissimilarity	-0.583	-1.901	0.01

Figure 2.1 Sampling schematic. Nine sites (red circles) were sampled along a latitudinal gradient. Ten plots (green circles) were sampled at each site. Ten trees were sampled in each plot; a randomly selected focal tree (black rectangle), and its nine nearest neighbors (brown rectangles).

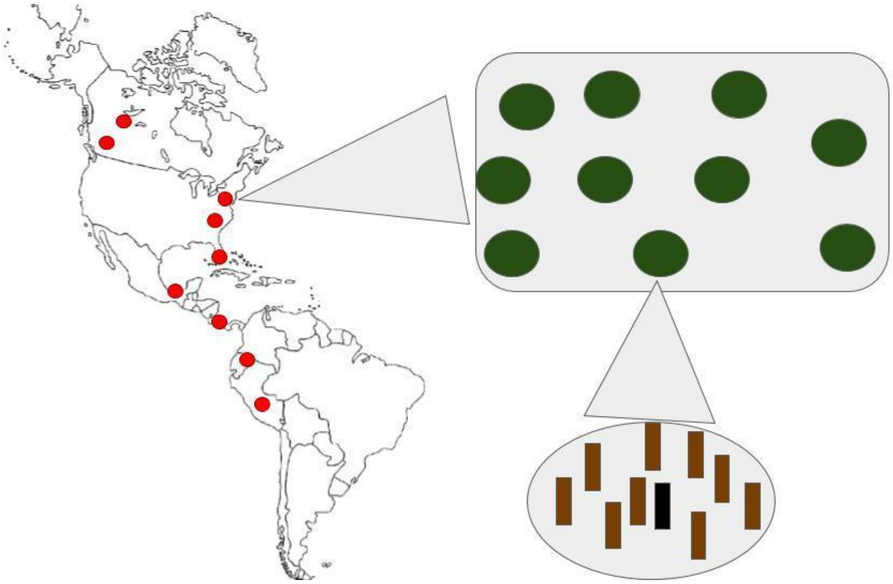


Figure 2.2 Epiphytic lichen species richness per site from nine forest sites along a latitudinal gradient. Line is fit from generalized linear model with 95% confidence intervals.

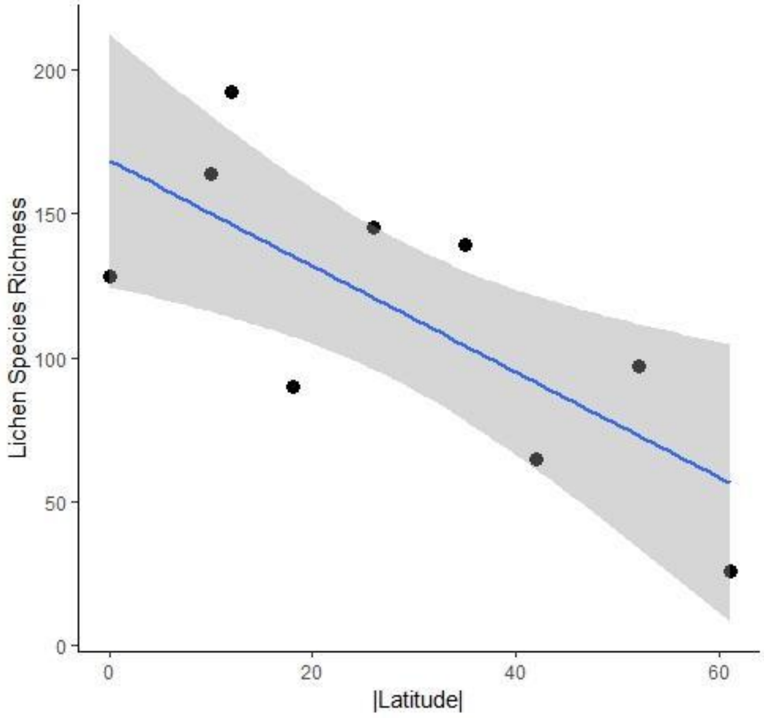


Figure 2.3 Lichen beta diversity (dissimilarity) plotted against latitude.

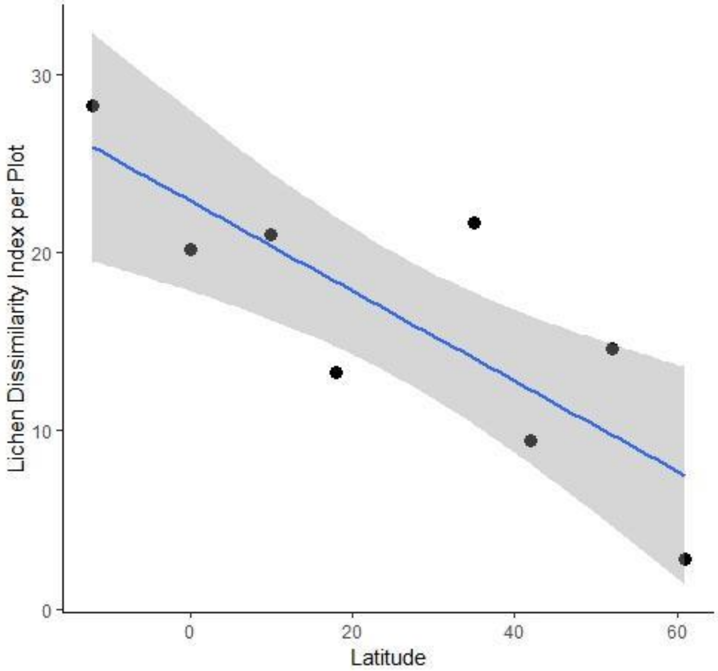


Figure 2.4 (a) Left, The proportion of two-dimensional or crust-like growth forms (light blue) to three-dimensional growth forms (dark blue) across latitudes. **(b)** Right, The proportion of asexual (light green) reproductive modes to sexual reproductive (green) modes across latitudes.

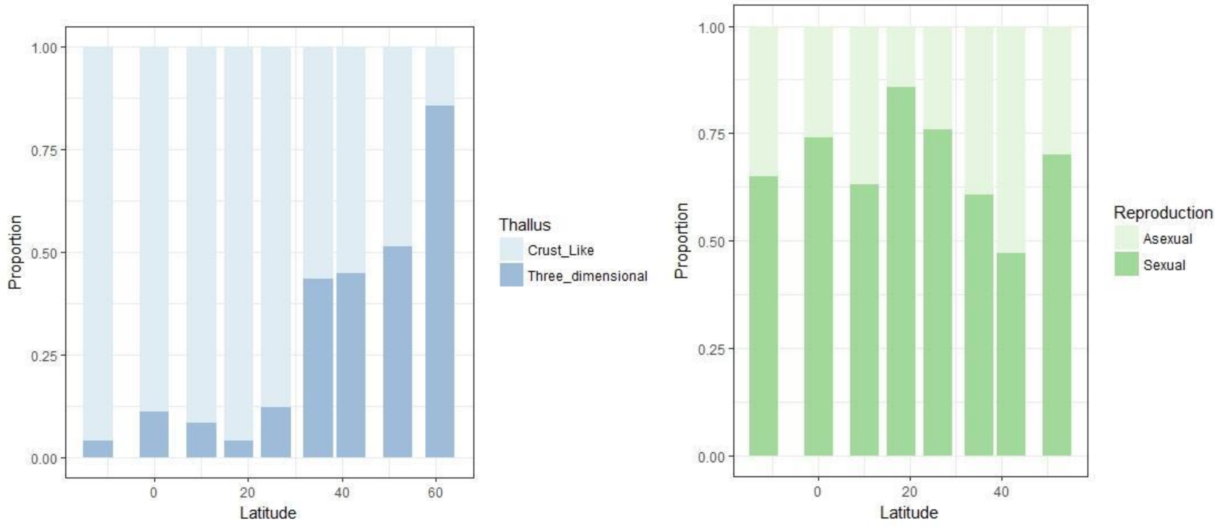


Figure 2.S1 Epiphytic lichen species to genus ratio plotted against latitude.

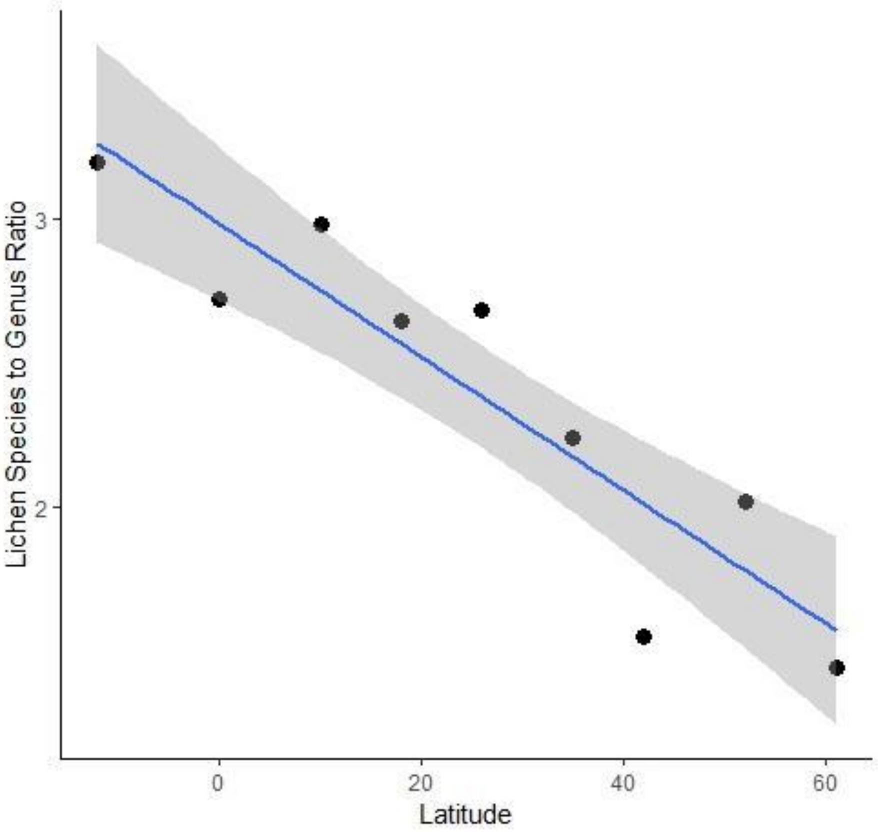


Figure 2.S2 The influence of face (cardinal direction) on lichen species richness per tree.

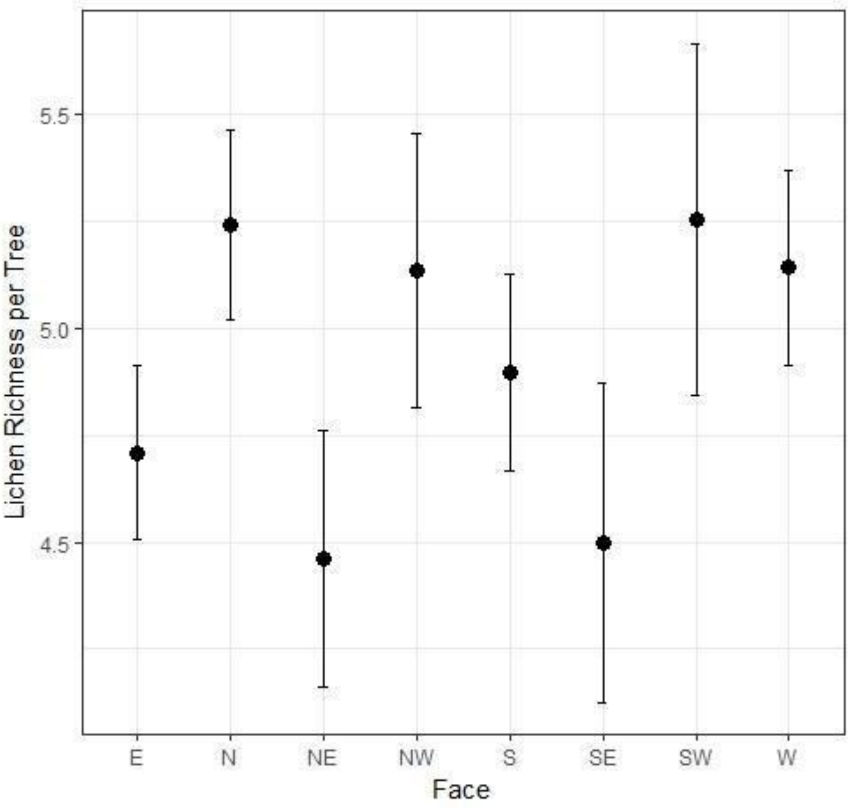


Figure 2.S3 Similarity of lichen species from one tree to another within a plot averaged across all plots within a site, plotted against latitude.

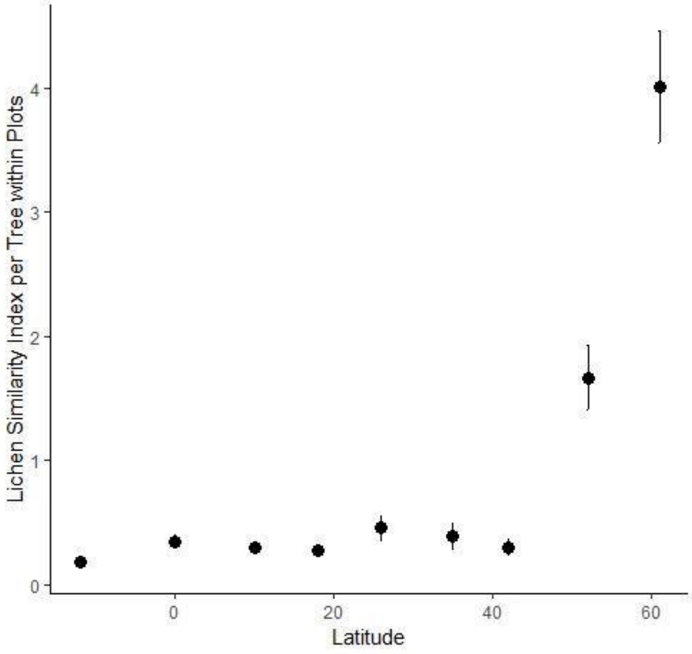


Figure 2.S4 Interaction network between mycobionts (8 species) and photobionts (5 ASVs) at the North Carolina site.

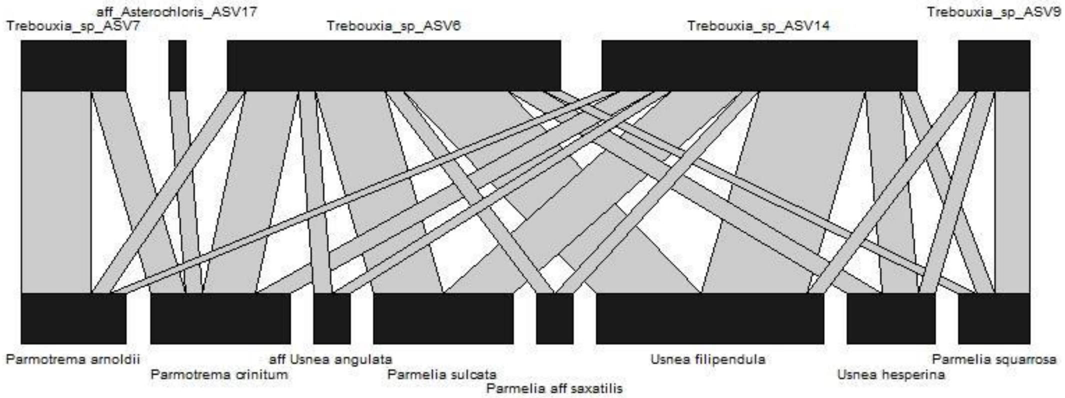


Figure 2.S5 Interaction network between mycobionts (7 species) and photobionts (3 ASVs) at the Harvard Forest site.

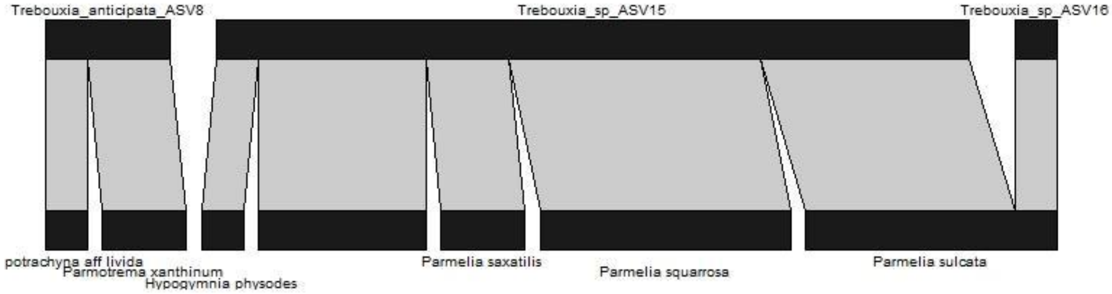


Figure 2.S6 Interaction network between mycobionts (4 species) and photobionts (7 ASVs) at the Scotty Creek site.

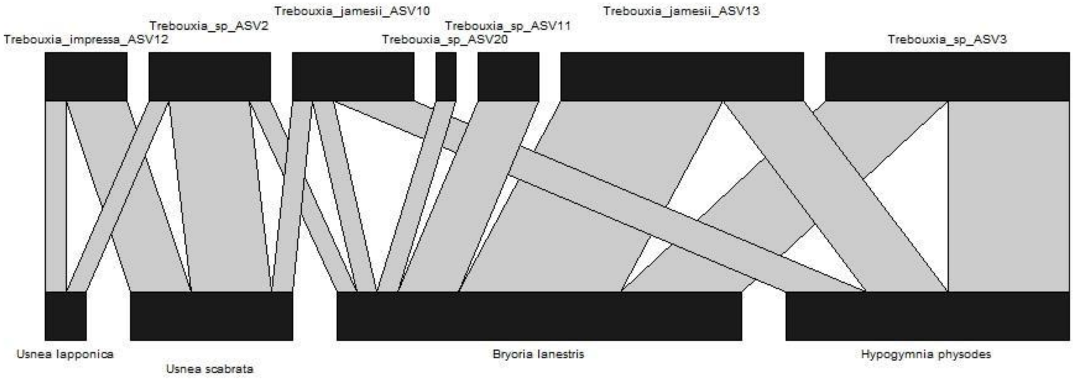


Figure 2.S7 (A, above) Specificity index of mycobionts across our three northern sites. (B, below) Specificity index of photobionts across our three northern sites. Lines represent standard error.

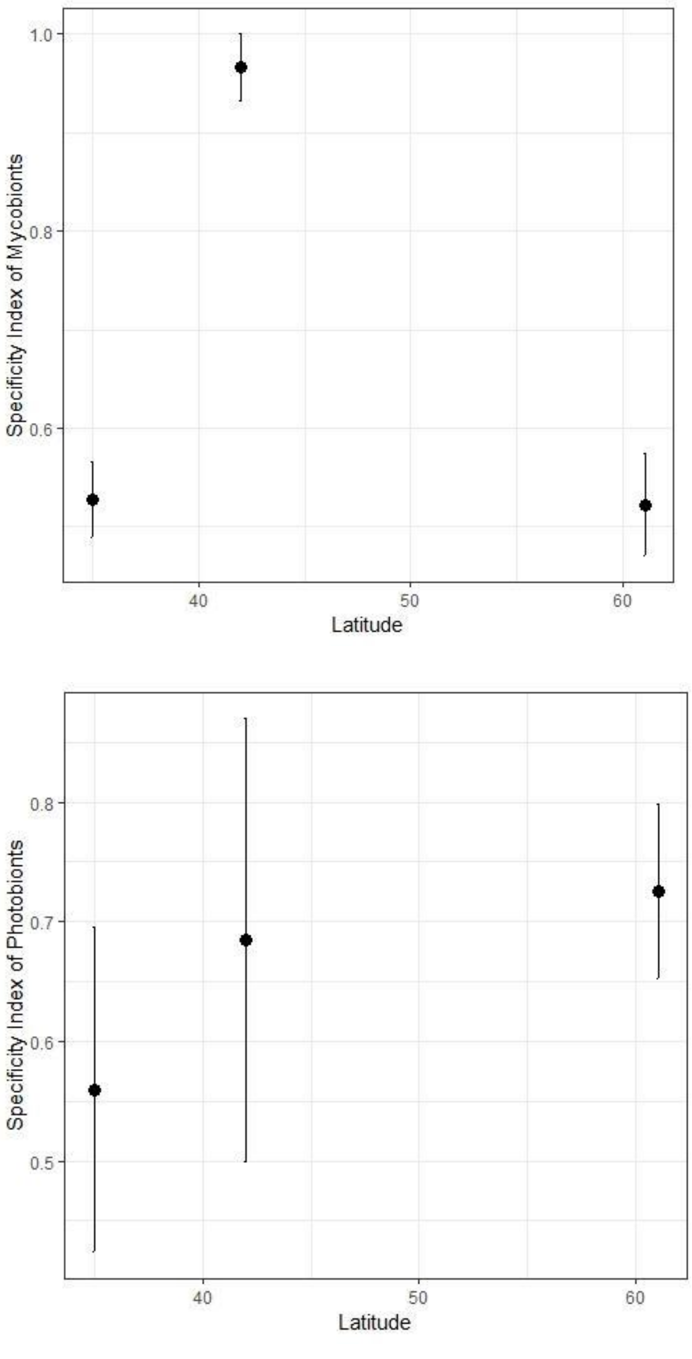
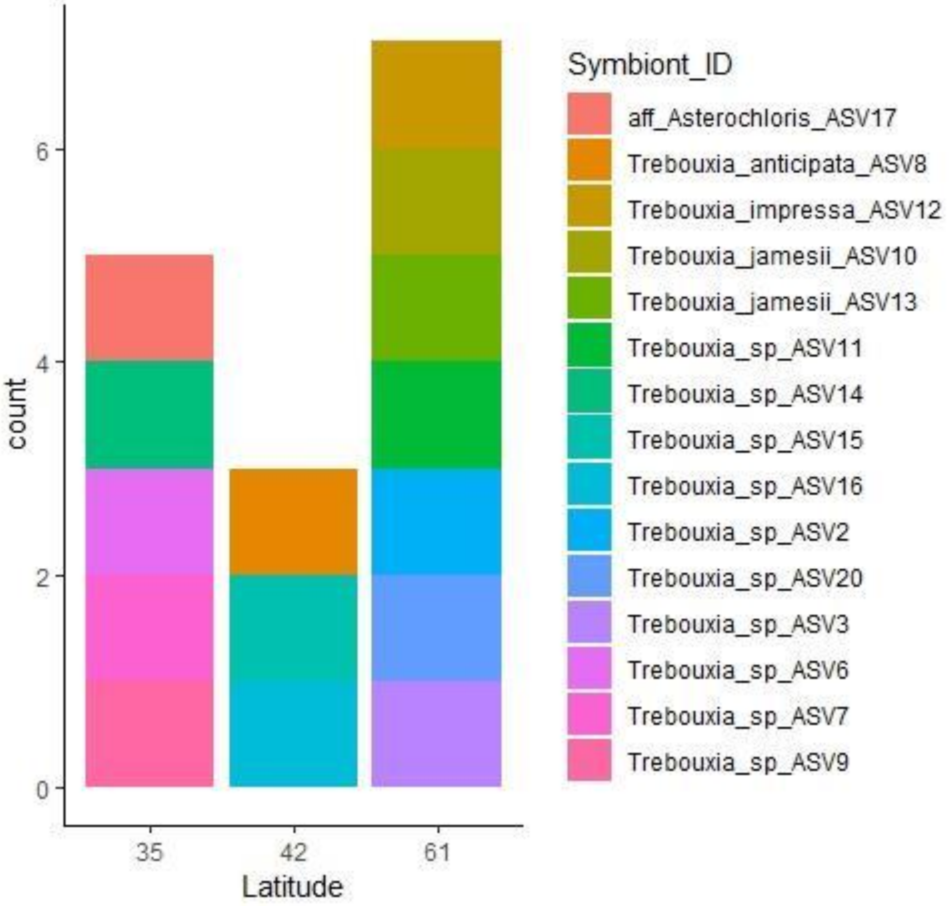


Figure 2.S8 Differences in community composition of photobiont ASVs. Symbiont ID is provided as a combination of the highest BLAST hit from NCBI and a unique amplicon sequence variant (ASV) number. There is no overlap of ASVs across the three sites.



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CHAPTER THREE:
Lichen diversity across tropical forest restoration strategies

INTRODUCTION

Tropical forests are global biodiversity hotspots and important sources of natural products (Myers et al. 2000), yet over half of the world's tropical forests have been converted to other land uses (Lewis et al. 2015). Tropical landscapes that were once dominated by primary tropical forests (Gibson et al. 2011) now exist as patchworks of secondary forests and other land uses (Zahawi et al. 2015), mainly agricultural (Henders et al. 2015). Conversion to other land uses is not only leading to irrevocable biodiversity loss, it is also leading to dramatic shifts in the global carbon budget (Norris 2016). It is therefore critical to work on restoring these tropical forest ecosystems, and to understand the many dynamics involved in different tropical forest restoration strategies.

The primary push in tropical reforestation has been for natural regeneration; however, this strategy is slow (Chazdon & Guariguata 2016), and may contain long term costs that lead to abandonment or disturbance of this forest restoration strategy (Zahawi et al. 2014). Tropical reforestation is challenging due to the initial costs involved in more active strategies such as tree plantations (Holl et al. 2011). In addition, most tropical reforestation is taking place on land that had been used for agriculture, which alters the seed bank (Reid et al. 2015), soil nutrient composition, and even the mycorrhizal communities (Holste et al. 2016), which may inhibit recruitment and growth of tropical trees.

Tropical forests are biodiversity hotspots for a multitude of organisms, and the symbiotic associations between fungi and a photosynthetic partner (green algae or cyanobacteria) known as lichens are no exception (Aptroot & Sipman 1997). Lichens are terrestrially ubiquitous organisms (Nash 2008), occurring from tidelines to mountaintops, from lush tropical forests to the arctic tundra, yet biodiversity of lichenized fungi seems to peak in the tropics (Scharnagl Chapter One & Chapter Two, Lücking & Matzer 2001). While our understanding of tropical lichen diversity is on the rise (Lücking et al. 2009) and we continue to discover and describe new tropical species (Lücking et al. 2014), our understanding of the ecology and the impact of deforestation on these composite organisms lags behind.

The photosynthetic requirements of lichens makes them sensitive to microclimatic gradients in light and moisture availability (Ellis et al. 2012, Fryday 2001). Lichens are poikilohydric, obtaining moisture and nutrients directly from the air, which makes them sensitive to subtle changes in these conditions. Lichens have long been used as indicators of air quality (Munzi et al. 2014), but more recently have been used as indicators of climate change (Aptroot 2009), biodiversity (Jovan 2008), and ecosystem disturbance (Leavitt & St. Clair 2015, Stoffer et al. 2006). A few studies have proposed the use of lichens as indicators of disturbance in tropical forest ecosystems (Benitez et al. 2012, Andersson & Gradstein 2005, Wolseley 1994). Rivas Plata et al. (2008) took the idea of lichens as indicators beyond total lichen richness, and rigorously assessed the use of corticolous (growing on the bark of trees) tropical lichen families as either indicators of disturbed/exposed forest habitat, or as indicators of more intact/continuous forest habitat. Of the three families (Thelotremataceae, Porinaceae, Letrouitiaceae) found to be indicators of ecological continuity of tropical rainforest habitats (preference for undisturbed primary and older secondary forest, shaded to somewhat exposed microhabitats, and mature trees), Rivas Plata et al. (2008) chose Thelotremataceae alone to be used in their IEC analyses, as it had a wider altitudinal range and larger number of genera and species compared to the other two families. Within their analyses, Rivas Plata et al. (2008) also identified lichenized fungal families that were indicators of more disturbed or pioneer habitats; these included Lecanoraceae, Pertusariaceae, Physciaceae, and Teloschistaceae.

In this study, we quantified lichen colonization in a long-term restoration study established in southern Costa Rica to test three forest restoration strategies: the active strategies of plantation-style planting and the nucleation or “island” style tree planting, and the passive strategy of natural regeneration. Within the framework of this study we addressed the following questions: (1) What is the impact of different restoration strategies on tropical lichen diversity and community composition?; and (2) Can lichens be used as indicators of ecological continuity within each of these restoration strategy treatments? We censused lichens in the three restoration strategies to address these questions.

METHODS

Study Sites

This study was conducted using twelve forest restoration sites located within a ~100 km^2 area in southern Costa Rica between the Las Cruces Biological Station (8° 47' N, 82° 57' W) and the town of Agua Buena (8° 44' N, 82° 55' W). This area is comprised of hilly terrain between 1060 to 1430 m asl, with annual precipitation of 3000-4000mm and mean annual temperature of 21℃. The native ecosystem is tropical premontane rainforest (Holdridge et al. 1971), but the majority of forest was cleared for coffee growing between 1960 and 1980 (Zahawi et al. 2015). The current landscape is highly fragmented into mixed-use agricultural fields, pastures, forest patches and riparian corridors.

The forest restoration sites were established in 2004-2006 on post-agricultural lands that had been farmed for 18 or more years (Holl et al. 2011). Each site contained three 0.25-ha (50x50 m) randomly assigned treatment plots. Each plot was assigned one of three restoration strategies: (1) natural regeneration, (2) applied nucleation or tree "islands," and (3) tree plantations. Natural regeneration plots were fenced to prevent livestock grazing but were otherwise left alone. Island plots contained six patches; two large islands (12x12 m, 25 seedlings planted), two medium islands (8x8 m, 13 seedlings planted), and two small islands (4x4 m, 5 seedlings planted). Plantation plots contained 313 seedlings planted throughout the 50x50 m area. Within the two active restoration strategies of island and plantation, seedlings were separated by 2.8 m from one another. Seedlings of the following tree species were planted in the island and plantation treatments: *Erythrina poeppigiana* (Fabaceae), *Inga edulis* (Fabaceae), *Terminalia amazonia* (Combretaceae), and *Vochysia guatemalensis* (Vochysiaceae). Plots within a site were separated by at least 5 m, and sites were separated by at least 0.7 km.

Field Surveys of Lichens

We conducted our lichen survey during the dry season in early 2019. We focused on two planted native species: *Terminalia amazonia* (J.F. Gmel.) Exell (Combretaceae), and *Vochysia guatemalensis* Donn. Sm. (Vochysiaceae), and three recruit species: *Cecropia obtusifolia* Bertol. (Urticaceae), *Miconia schlimii* Triana (Melastomataceae) and *Heliocarpus appendiculatus* Turcz. (Malvaceae). These were chosen as they were well distributed across treatments and sites, though, particularly in the natural regeneration treatment, not all species were found.

Within each treatment plot, a maximum of five individual trees per tree species were surveyed, and this method was replicated across the twelve sites. For each survey, the lower trunk of each tree, from the ground up to 1.8 m, was visually surveyed for epiphytic lichens. The diameter at breast height (DBH) was measured and recorded for each tree. The percent cover of lichens, bryophytes, and other [pooled cover of algae, non-lichenized fungi, vascular epiphytes, bare bark] were visually estimated and recorded for each tree. Each lichen in the field was assigned to a tentative morphospecies and a description was recorded; some were photographed for later identification.

Lichens were identified to genus or morphospecies based upon field notes descriptions and photographs using a combination of tropical lichen guides (fieldguides.fieldmuseum.org), tropical lichen keys (Sipman 2005), and a reference collection from a previous study in these plots [collected by K. Scharnagl, and identified with the help of Robert Lücking; voucher specimens from this study are housed in the Michigan State University Herbarium]. About half of all the morphospecies identified could be assigned to a family. Lichen community composition and indicator species were analyzed based upon these morphospecies identifications.

Analyses

All analyses were conducted in R (R Core Team 2019). Morphospecies richness was assessed using the specnumber function in the vegan package (Oksanen et al. 2019). Drivers of richness at both the tree and treatment level were analyzed using generalized linear mixed effects models using a poisson distribution, with site as a random variable, in the lme4 package (Bates et al. 2015). The significance of each of these effects was analyzed using the Anova function, which performs a Type II Wald Chi-squared test, in the car package (Fox & Weisberg 2011).

Lichen morphospecies were assigned to different functional groups based on photobiont (cyanobacteria or green algae), growth form (two-dimensional crust-like or three-dimensional macrolichens), and reproductive mode (asexual or sexual). Asexual lichens were those that produced fragments including soredia, isidia, and phyllidia. Sexual lichens were those that produced spore-bearing structures including apothecia, perithecia, and lirellae. Some functional diversity was recorded as indeterminable based upon what was observed in the field. For example, many lichens observed contained neither sexual or asexual reproductive structures and were therefore recorded as "N/A". We calculated the ratio of each functional group per treatment. For each functional group, the ratio was calculated as rare/dominant (eg. cyanobacteria/green algae, macrolichens/crusts, asexual/sexual). Effect of treatment was assessed using analysis of variance (ANOVA) and differences among treatments were assessed using a Tukey test (TukeyHSD in R).

Lichen community composition was assessed using a non-metric multidimensional scaling (NMDS) ordination. We further assessed drivers of lichen community similarity and dispersion using the adonis (permutational multivariate analysis of variance) function in the vegan package; permutational multivariate analysis of variance enables us to assess drivers using a community or dissimilarity matrix as the response variable.

We used the Index of Ecological Continuity (IEC) equation provided in Rivas Plata et al. (2008), as follows:

$\text{IEC} = 100 \times n/N_{max}$,

with n = the number of morphospecies detected in a given treatment, and N_{max} = the maximum number of morphospecies across all treatments/sites. IEC was determined for each treatment: plantation, island, and natural regeneration. To test the efficacy of Thelotremataceae alone as an indicator of ecological continuity as found in Rivas Plata et al. (2008), we first ran this equation for just morphospecies within Thelotremataceae. We then ran the equation for morphospecies within Thelotremataceae, Letrouitiaceae, and Porinaceae combined, to see how all three families performed as indicators within our forest restoration sites. Finally, we calculated the ratio of indicators of disturbed and exposed habitats (members of the Lecanoraceae, Pertusariaceae and Physciaceae families) to indicators of more continuous tropical forest habitats (members of the Thelotremataceae, Letrouitiaceae, and Porinaceae families) for each treatment.

RESULTS

In total, we surveyed 527 individual trees across 12 sites, observing 3093 lichen individuals that represent approximately 369 morphospecies (Table S1). Morphospecies richness per tree was highest in the natural regeneration treatment (Figure 1). Lichen percent cover per tree was also highest in the natural regeneration treatment, and followed an inverse pattern to bryophyte percent cover per tree (Figure 2). Total morphospecies richness at the 0.25 ha scale was lowest in the natural regeneration treatment, and highest in the island treatment (Figure 3). Both lichen morphospecies richness and percent lichen cover were highest on the naturally recruiting species *Heliocarpus appendiculatus* and *Cecropia obtusifolia* (Figure 4a-b).

At the individual tree level, treatment (Chi-squared=14.65, df=4, p=0.0007), DBH (Chi-squared=9.29, df=1, p=0.0023), and tree species (Chi-squared=548.11, df=4, p<0.0001) all had significant effects on lichen morphospecies richness. Elevation (Chi-squared=0.645, df=1, p=0.422) and surrounding forest cover (Chi-squared=0.325, df=1, p=0.57) were not significant predictors of lichen morphospecies richness at the individual tree level (Figure 5a). Elevation (Chi-squared=4.84, df=1, p=0.027), surrounding forest cover (Chi-squared=54.21, df=1, p<0.0001), and treatment (Chi-squared=4848.4, df=2, p<0.0001) were all significant predictors of lichen species richness at the 0.25 ha level (Figure 5b).

We found substantial overlap in lichen communities among sites. We found a significant influence of both treatment (adonis(treatment) F=2.13, R2=0.114, p=0.001, Figure S1) and elevation (adonis(tree species) F=2.80, R2=0.075, p=0.001, Figure S2), and no significant effect of surrounding forest cover (adonis(surrounding forest cover) F=1.14, R2=0.031, p=0.281, Figure S3) on lichen community composition.

There was a significant effect (F=32.92, df=2, p<0.001) of treatment on the ratio between lichens containing cyanobacteria and lichens containing green algae (more common). The tukey's test revealed a significant difference among all three treatments (Figure 6a); natural regeneration had significantly more lichens containing cyanobacteria than either islands or plantations, and islands had significantly more lichens containing cyanobacteria than plantations (p<0.05). Treatment also had a significant effect (F=12.96, df=2, p<0.0001) on the ratio between macro lichens and crust lichens. The natural regeneration treatment had significantly more macro lichens than both the island and plantation treatments, and the island and plantation treatments were not significantly different from one another (Figure 6b). There were no significant differences among treatments on the ratio between lichens with asexual modes of reproduction to lichens with sexual modes of reproduction.

The IEC score based on Thelotremataceae alone was the same for island and natural regeneration treatments (Table 1), and lowest in the plantation treatment. The IEC score based on the three families Thelotremataceae, Letrouitiaceae, and Porinaceae, was highest in the plantation, followed by the island, and lowest in the natural regeneration. For these two assessments, a higher IEC score corresponds to a higher score for ecological continuity. The ratio of families indicating disturbed or exposed environments (Lecanoraceae, Pertusariaceae, Physciaceae) to families indicating forest continuity (Thelotremataceae, Letrouitiaceae, Porinaceae) was highest in the plantation treatment, followed by the island treatment, and lowest in the natural regeneration treatment. In this assessment, a higher ratio is indicative of a more exposed or disturbed habitat, and a lower ratio (fewer morphospecies representing disturbed habitats relative to morphospecies representing continuous or undisturbed habitat) is indicative of more ecological continuity.

DISCUSSION

Our lichen survey across twelve sites in southern Costa Rica yielded approximately 369 lichen morphospecies. This likely represents a conservative estimate of lichen richness, as there are many cryptic lichen species in the tropics, which may have been lumped under a single morphospecies in our surveys (Cáceres et al. 2008, Emmerer & Hafellner 2004). Forest restoration treatment (nucleation, plantation, or natural regeneration) was a significant predictor of lichen morphospecies richness at both the tree and 0.25-ha levels, and of lichen community composition. Other studies have found forest stand structure, as replicated by our forest restoration strategies, to be the strongest influence on lichen diversity (Moning et al. 2009), even more than other predictor variables such as climate.

The highest overall morphospecies richness was found in the active restoration strategy of nucleation, followed by the active restoration strategy of plantations, and then by the passive restoration strategy of natural regeneration. Though there was overlap of lichen communities across sites and treatments, we found the greatest spread in our ordination of the island treatment communities, and treatment overall had a significant effect on lichen community composition. The island or nucleation strategy may have the most heterogeneous microclimatic landscape compared to the plantation or natural regeneration strategies, which could lead to more heterogeneous lichen communities and therefore overall higher morphospecies richness in the island treatments. A similar pattern was observed for other groups in these treatments; both arthropod (Cole et al. 2016) and vascular epiphyte (Reid et al. 2016) diversity were higher in the islands than in the other treatments.

At the level of the individual tree we found a different pattern, with the highest lichen morphospecies richness per tree in the natural regeneration, followed by the nucleation, then the plantation. Lichen cover per tree followed the same pattern, which was the inverse of the pattern of bryophyte cover per tree. Bryophytes are more shade tolerant whereas lichens may have the competitive advantage on substrates exposed to higher UV radiation and aridity (Ranius et al. 2008). Thus we find the highest lichen cover and lowest bryophyte cover on trees in the natural regeneration treatment, where many trees are surrounded by lower vegetation, their trunks exposed on all sides to high levels of light and wind. In both the island and plantation interiors, there is much higher shade, and we find lower lichen cover and higher bryophyte cover per tree. While we find the highest lichen morphospecies richness and cover per individual tree in the natural regeneration treatment, we also find the lowest overall species richness in this treatment, suggesting that there may be a species pool specialized to this more gap-like environment.

We found the highest morphospecies richness and lichen cover per tree on the naturally recruiting species *Heliocarpus appendiculatus* and *Cecropia obtusifolia.* Species such as *Cecropia* are known pioneers, and have a large leaf area ratio and high growth rate (Pompa & Bongers 1988). While we found both *H. appendiculatus* and *C. obtusifolia* across our treatments, they were often the only trees found in the natural regeneration plots, where lichen cover and richness were generally high, which may explain the overall high richness and cover reported for these species. Epiphytic lichen composition and richness is also known to be driven by bark characteristics, including pH and texture (Lamit et al. 2015, Cácares et al. 2007). It is therefore possible that the naturally recruiting species *H. appendiculatus* and *C. obtusifolia* have more favorable bark characteristics for lichen growth than the planted species. In addition, their strategy of fast growth often led to higher DBH measurements than those recorded for the other tree species, and we found DBH to be a significant predictor of lichen species richness at the individual tree level. Other research has shown that both the mean and variance of DBH predict lichen species richness at multiple scales, including across a latitudinal gradient (Scharnagl Chapter Two), supporting a species-area interaction for lichens (He & Legendre 1996).

We did not find a significant effect of surrounding forest cover, at either a short distance of 100-150m or a wider distance of 500-550m, on lichen morphospecies richness per tree. Likewise, a study on seedling recruits across the three treatments found a weak effect of surrounding forest cover, compared to the effects of treatment and elevation (Holl et al. 2017). We did find a significant effect of surrounding forest cover at the 100-150m distance on lichen morphospecies richness at the 0.25-ha scale. Interestingly, there was a negative slope in this relationship; increasing surrounding forest cover led to a slight decrease in lichen morphospecies richness. This is the opposite trend of that found for vascular epiphytes in these plots, where vascular epiphyte richness increased with increasing surrounding forest cover (Reid et al. 2016). In addition, surrounding forest cover did not predict lichen community composition. These patterns may be driven by dispersal modes of lichens and the ability of lichen propagules to travel on the wind; this enables many lichen species to disperse over long distances (Gjerde et al. 2015, Muñoz et al. 2004). Lichen community composition and succession are in fact more limited by establishment parameters than by dispersal (Ronnås et al. 2017, Werth et al. 2006). We suggest that more data is needed on the establishment criteria of tropical lichens before we can understand the role of surrounding forest cover on lichen recruitment into these plots.

Elevation was a significant predictor of both lichen morphospecies richness and community composition at the 0.25 ha level. Overall, as elevation increased, lichen morphospecies richness decreased, which is again opposite to that found for vascular epiphytes (Reid et al. 2016). It is possible that lichens and vascular epiphytes respond to different environmental drivers along an elevational gradient, or even that there is direct competition between these two epiphytic groups. Studies in temperate regions found increasing lichen richness with increasing elevation (Bässler et al. 2016, Bruun et al. 2006), and it is unclear whether our results are representative of total lichen richness along an elevation gradient, or whether there is an interaction between elevation and forest restoration impacting the observed pattern.

We found the highest ratio of cyanolichens to lichens containing green algal photobionts per plot in the natural regeneration treatment, followed by the island treatment, then the plantations. In a study of lichen functional traits as ecological indicators, Benitez et al. (2018) found cyanolichens to be an indicator of more continuous forest. However, they also found certain macrolichens such as fruticose and foliose species with narrow lobes to be more abundant in disturbed forest habitats. As such, it is difficult to use the ratios we found in our plots as indicators of forest disturbance or continuity. However, if we consider the microclimates experienced by lichens in the different treatments to fall along a gradient from highest light and variability of moisture and temperature in the natural regeneration treatments (Zahawi & Augspurger 2006) to lowest light and variability in the plantation (Holl et al. 2011), we find parallels to patterns of lichen diversity across larger ecological gradients. In a study on the latitudinal diversity gradient of lichens in the Americas, Scharnagl (Chapter Two, 2019) found a higher ratio of macro lichens to crusts with increasing latitude, which coincides with increasing variability in temperature and precipitation. We found no significant differences in the ratios of asexual to sexual reproductive modes along an environmental gradient.

New species of lichens are continually being discovered and described (Lumbsch et al. 2011), both in the tropics and in the temperate regions (Fryday & Hertel 2014, Fryday & Ovstedal 2012). There is therefore much work to be done to understand both the diversity and the ecology of tropical lichen communities. As revealed in this study, there are multiple interacting factors affecting the recruitment patterns of tropical lichen communities, and these tropical forest restoration plots provide an ideal setting in which to investigate these dynamics. We aim to continue to monitor these plots for lichen succession as fast growing recruits or microhabitat specialists are replaced or joined by other species.

Our preliminary indicator measurements support that the plantation is a more open/"disturbed" habitat type whereas islands and natural regeneration more closely mimic continuous forest, but we suggest that more data is needed to make use of the lichen indicators proposed by Rivas Plata et al. (2008). Our indicator analyses support the island treatment as an intermediate between potential microclimatic extremes of the natural regeneration and plantation treatments. Islands, with their multiple edges and interiors and more heterogeneous microclimates, are likely the most suitable environments for supporting the widest array of lichen niches and therefore the highest overall morphospecies richness. As we continue to monitor these tropical forest restoration strategies over time, we propose that lichens may become increasingly important as indicators of both ecological continuity and forest health (McCune 2000) within these plots.

APPENDIX

CHAPTER THREE TABLES AND FIGURES

Table 3.1 IEC scores for Thelotremataceae, for combined Thelotremataceae, Porinaceae and Letrouitiaceae, and the ratio of lichen species in families indicating exposed or disturbed habitats to lichen species in families indicating forest continuity. Higher IEC scores mean more ecological continuity, whereas a higher indicator ratio means less continuity and more disturbance.

Treatment	Thelotremataceae EIC	Combined EIC	Indicator Ratios
Island	80	68.75	0.36
Plantation	60	75	0.42
Natural Regeneration	80	25	0.25

Table 3.S1 The number of morphospecies and number of individual lichens observed per lichenized fungal family.

Family	Number of Species	Number of Samples
Arthoniaceae	19	379
Arthopyreniaceae	1	2
Caliciaceae	1	1
Coccocarpiaceae	1	3
Coenogoniaceae	20	163
Collemataceae	25	155
Crocyniaceae	1	2
Graphidaceae	65	290
Hygrophoraceae	2	7
Lecanoraceae	1	1
Letrouitiaceae	1	1
Lobariaceae	6	27
Malmideaceae	12	130
Monoblastiaceae	2	14
Opegraphaceae	2	2
Parmeliaceae	5	17
Peltigeraceae	1	5
Pertusariaceae	1	5
Physciaceae	4	51
Pilocarpaceae	3	15

Table 3.S1 (cont'd)

Porinaceae	10	102
Pyrenulaceae	8	113
Ramalinaceae	5	58
Roccellaceae	7	32
Stictidaceae	1	1
Thelotremataceae	5	27
Trypetheliaceae	2	3
Unassigned	158	1487

Figure 3.1 Lichen morphospecies richness per tree within each forest restoration treatment.

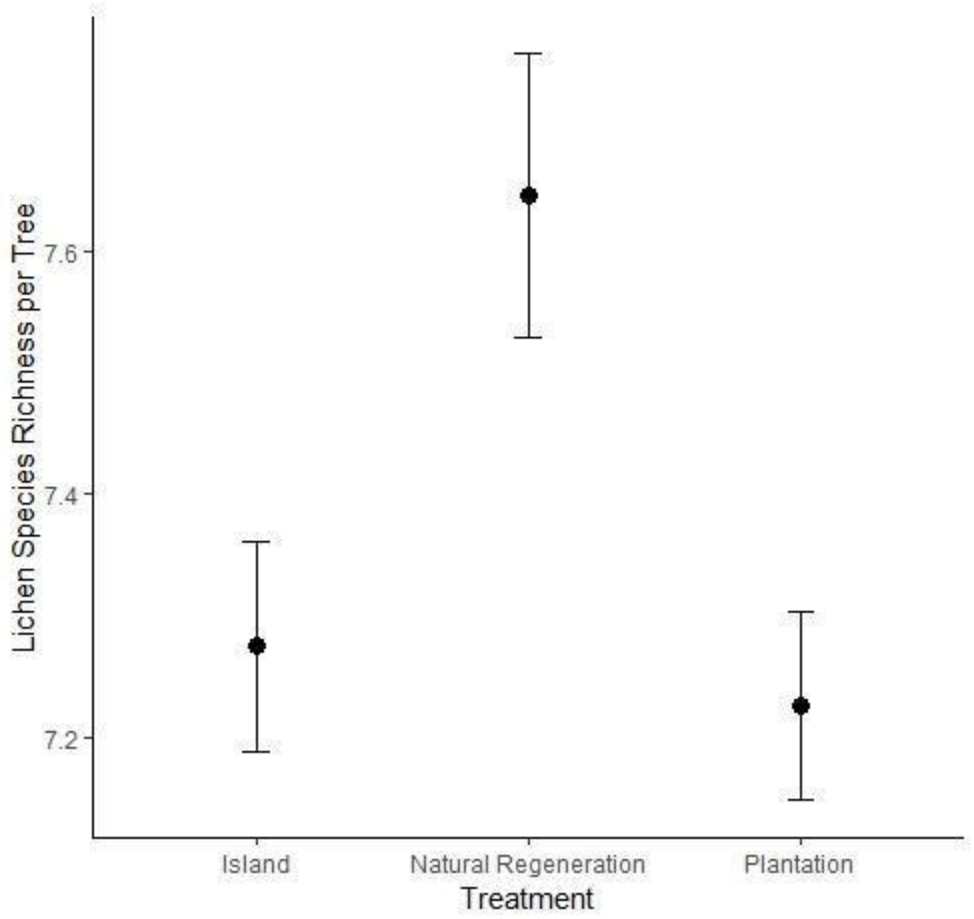


Figure 3.2 Percent cover per tree of lichens (triangles) and bryophytes (circles).

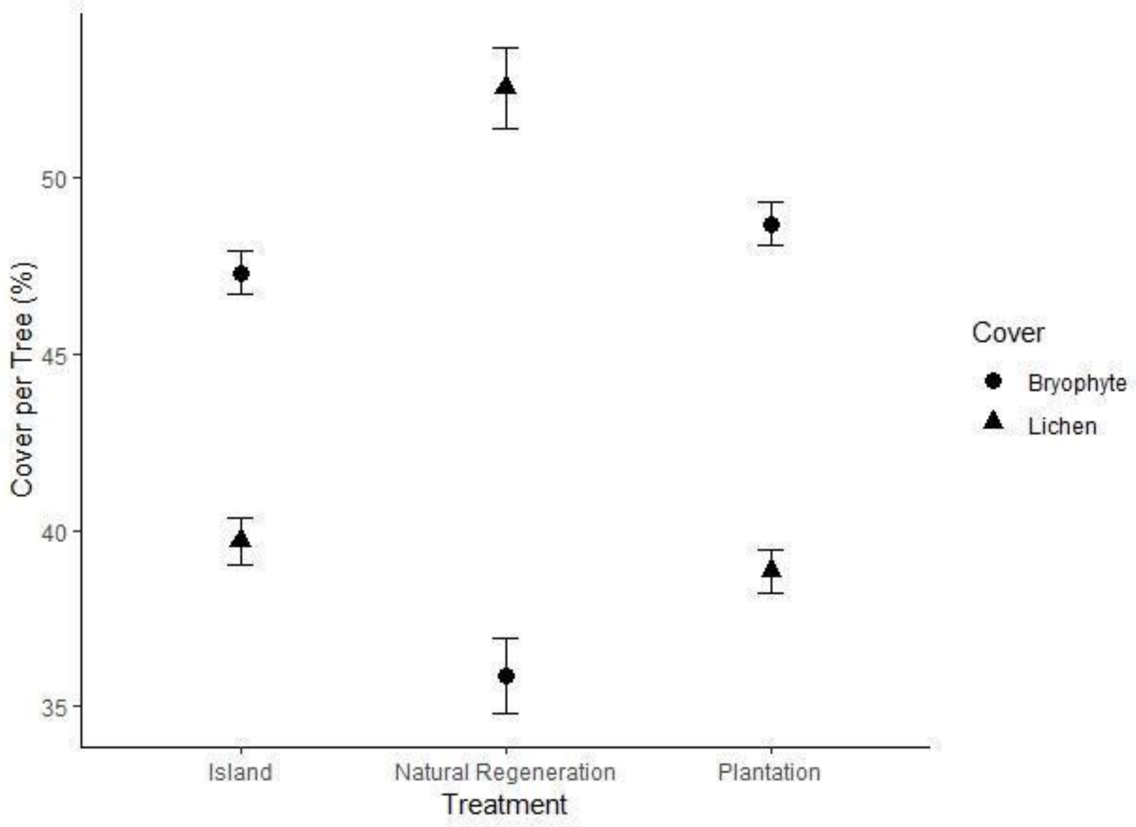


Figure 3.3 Total lichen morphospecies richness at the 0.25 ha scale.

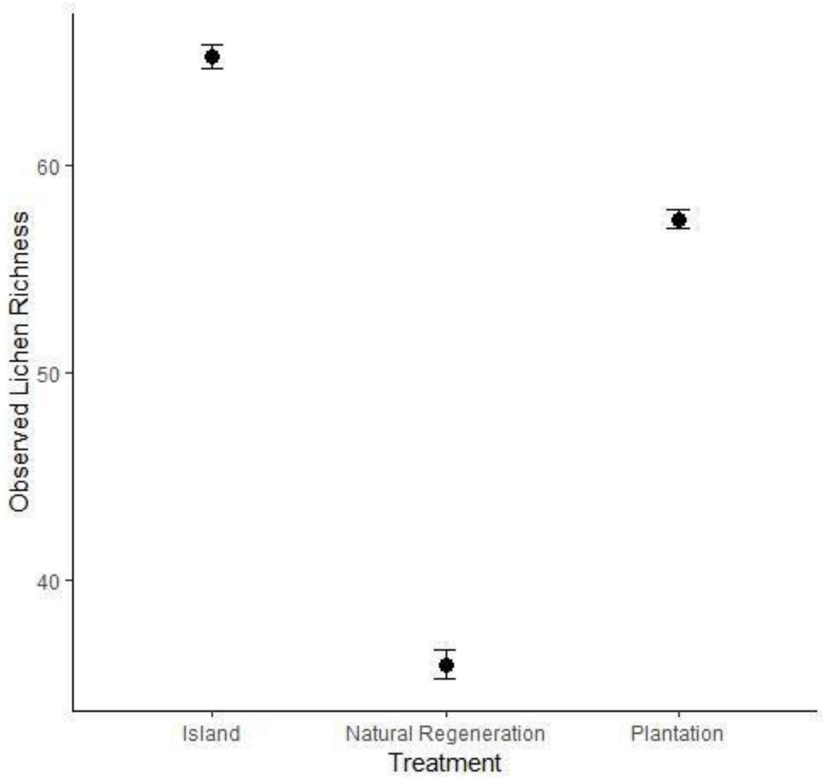


Figure 3.4 (a) Lichen morphospecies richness on each tree species across plots. (b) Lichen percent cover on each tree species across plots.

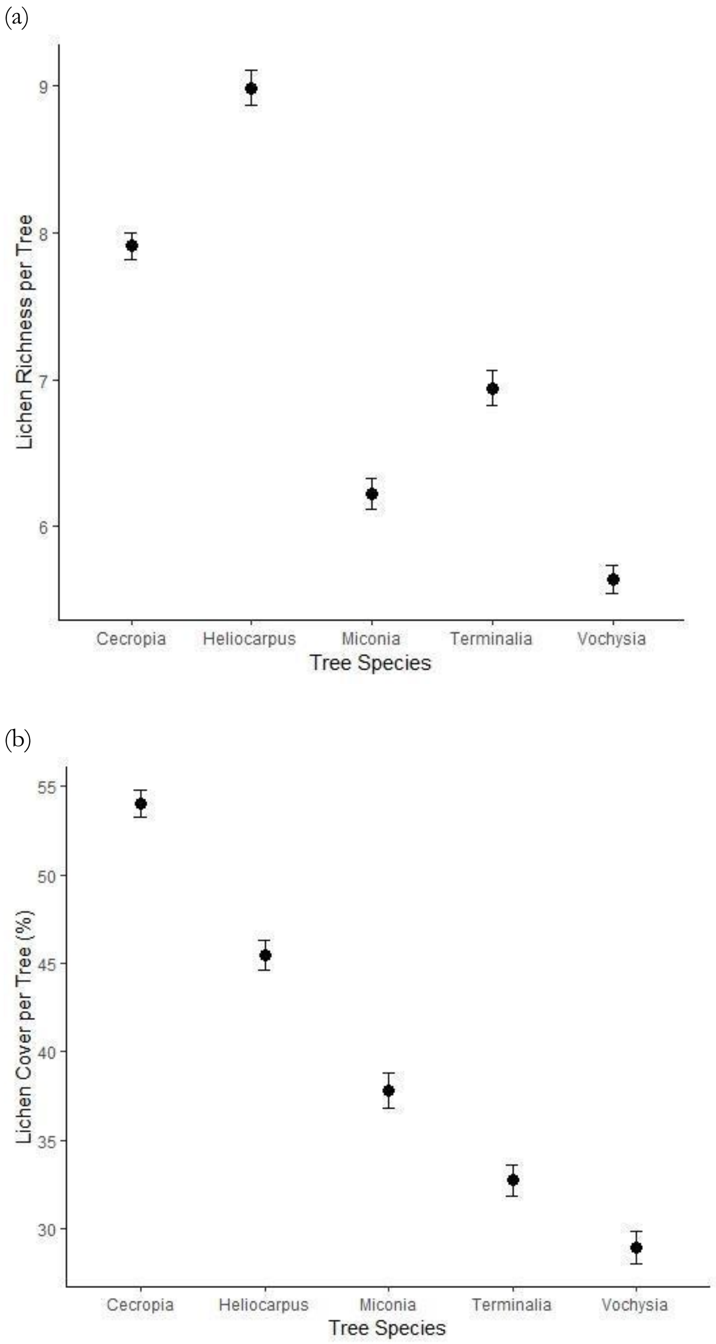
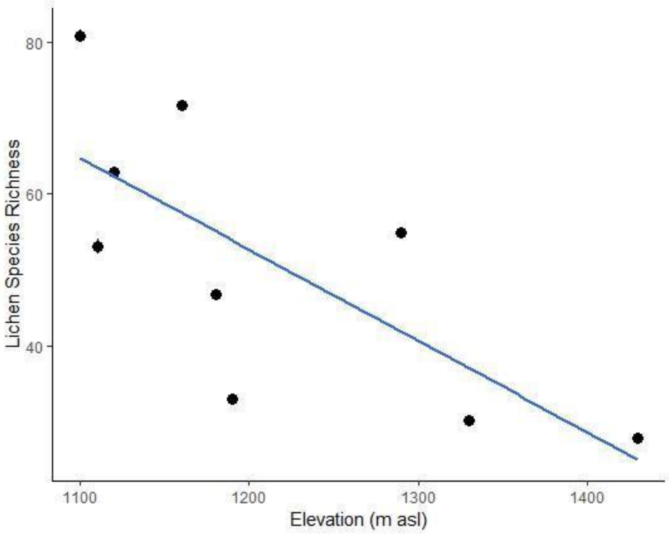


Figure 3.5 Effect of elevation (a) and surrounding forest cover at 100-150m (b) on lichen morphospecies richness at the 0.25 ha level. Line of fit based on GLMER models.

(a)



(b)

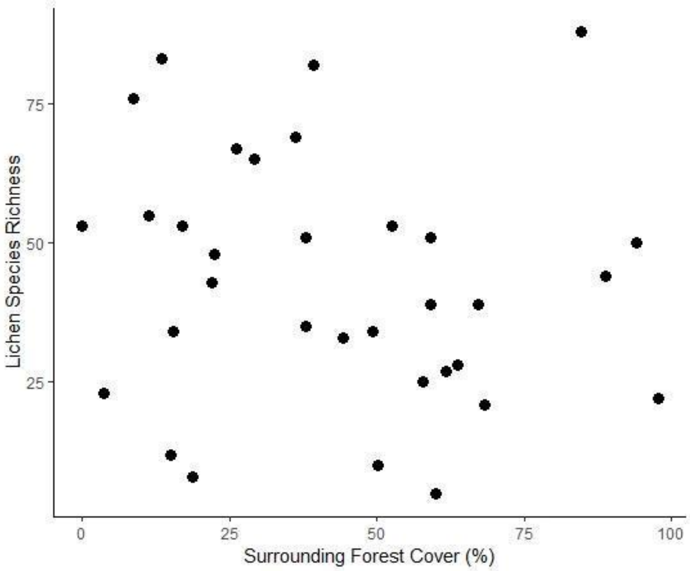


Figure 3.6 (a) Ratio of lichens containing cyanobacteria to lichens containing green algal photobionts across treatments. (b) Ratio of three-dimensional macro lichens to two-dimensional crust lichens across treatments.

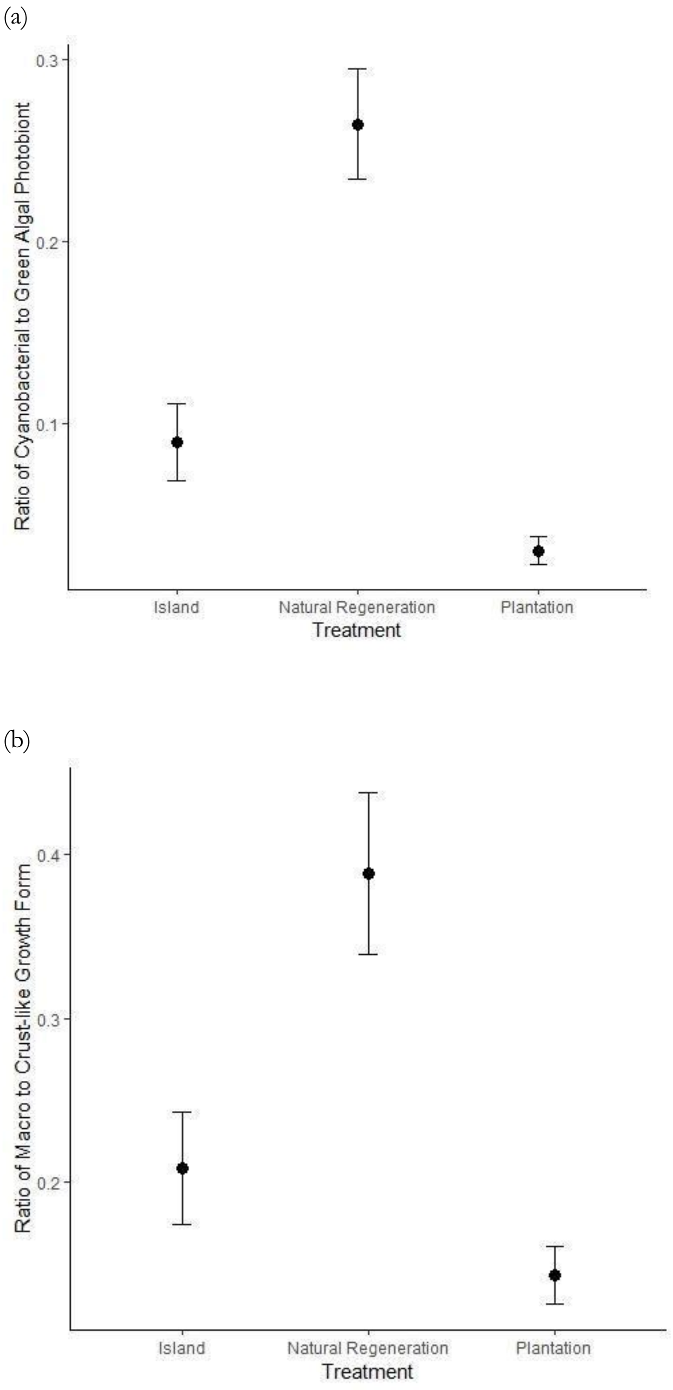


Figure 3.S1 Ordination plot of lichen communities, colors represent treatments.

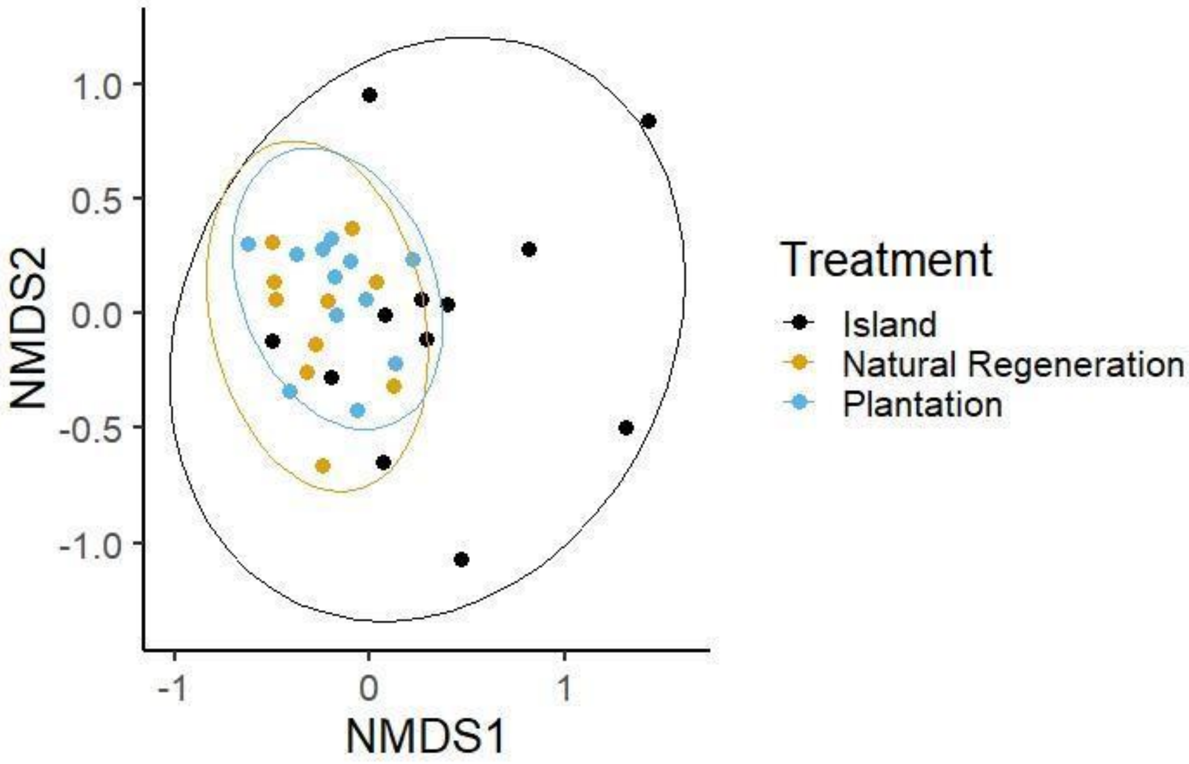


Figure 3.S2 Ordination plot of lichen communities, colors represent elevation.

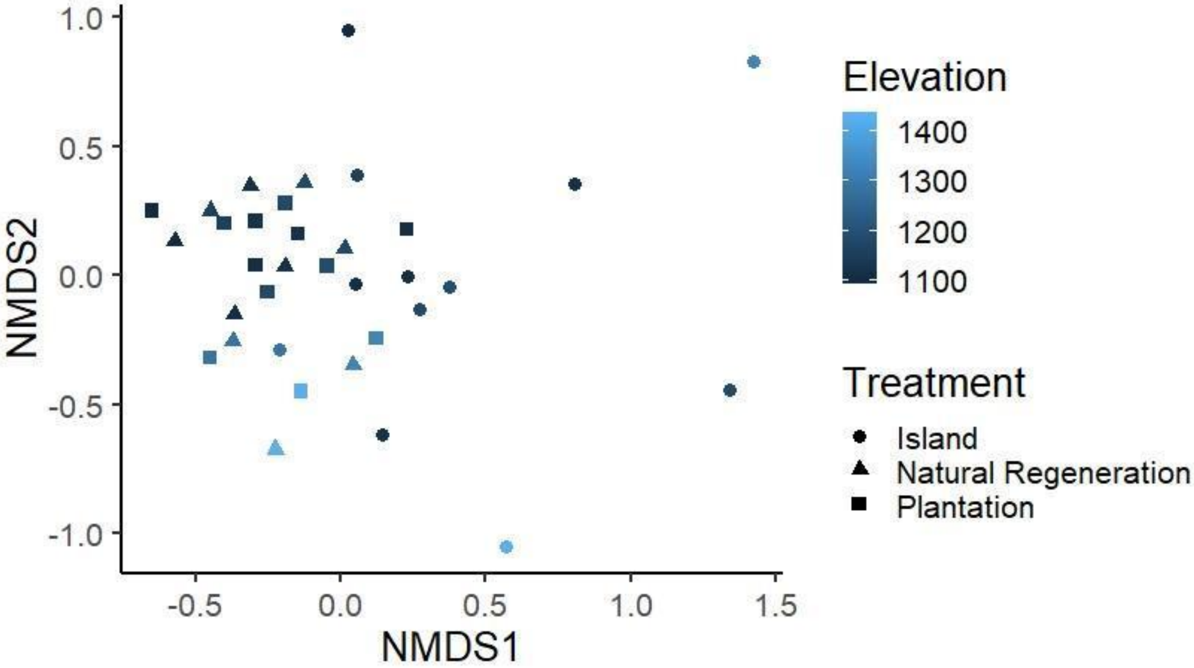
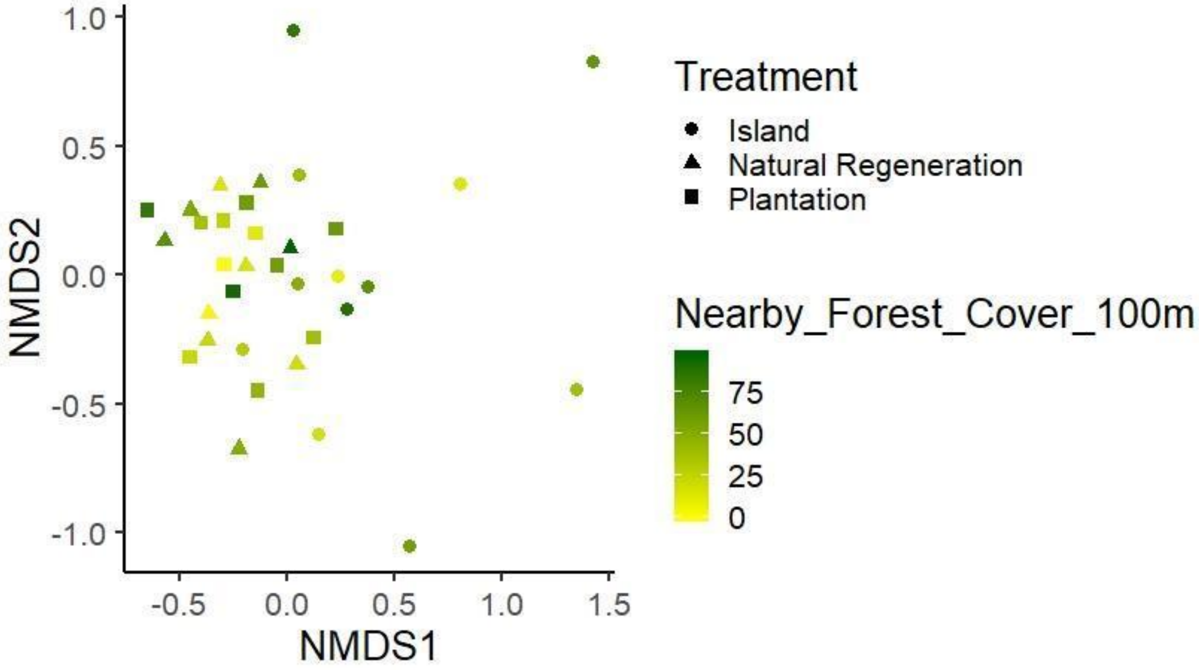


Figure 3.S3 Ordination plot of lichen communities, colors represent surrounding forest cover.



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