

Changes in the phyllosphere and rhizosphere of a cloud forest tree fern along an elevation gradient

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Abstract

The diversity of plant-associated microbial communities is shaped by both host factors and the environment. Natural environmental gradients, specifically elevational ones, can serve as study systems to understand community and ecosystem responses to environmental changes, however the relationship between elevation and microbial diversity is not completely understood, especially in non-model systems such as wild plants. In this paper we explored the role of environmental factors in shaping the diversity and structure of the rhizosphere and phyllosphere of the cloud forest tree fern *Cyathea fulva*. Samples of phyllosphere, rhizosphere and soil were collected from 15 individual tree ferns across five forest plots along an elevation gradient ranging from 1978 to 2210 meters above sea level. Physicochemical soil data were collected, along with environmental data of all plots. Using 16S rRNA and ITS1 amplicon sequencing, we tested for differences in diversity and composition of bacterial and fungal communities and their potential abiotic drivers. We found that bacterial alpha diversity decreased with elevation in the phyllosphere and rhizosphere, but for fungi this pattern was only found in the rhizosphere. We also observed significant changes in community structure and composition with elevation in both the fungal and bacterial phyllosphere and rhizosphere. Our results suggest a close relationship between elevation and the overall microbial structure associated with tree ferns. We envision this information will help to further understand the dynamics between microbiota and wild plants, contributing to the conservation of necessary interactions for plants and ecosystems wellbeing.

INTRODUCTION

The microbiome of plants, including the rhizosphere, phyllosphere, and endosphere, performs important functions for plant growth and health [1], including nutrient uptake, growth promotion, pathogen resistance, and abiotic stress mitigation [2]. The diversity and structure of plant microbiomes can be influenced by both host-associated (*e.g.*, taxonomic identity, genotype [3] or age) and environmental (*e.g.*, climate, soil type or cultivation practices [4]) factors.

Research on plant-associated microbiota has focused mostly on soil microorganisms and those associated with domesticated plants [5]. Studies on the microbial diversity of wild plants as diverse as cacti [6], magnolia trees [7], carnivorous plants [8], and agaves [9], have shown that environmental factors (*e.g.*, temperature, humidity, precipitation and soil characteristics) play an important role in structuring microbial communities associated with different plant compartments (*e.g.*, phyllosphere, rhizosphere, and endosphere). In addition, these microbial communities provide plants with tolerance to stress from environmental changes [10]. However, the degree to which spatial and temporal environmental change affects microbial communities is poorly understood. Environmentally-driven changes in microbial communities have been documented particularly for the soil component [11]. These changes have been attributed to variation in factors such as temperature, humidity, plant-species richness, and soil characteristics (*e.g.*, pH, available carbon) [12, 13]. Studies on the structure of plant-associated microbiota along elevation suggest that microbial diversity varies with elevation and that community dissimilarity increases with elevational distance among host individuals [14–17]. However,

great variation in the patterns of microbial diversity along elevation gradients has been documented, with evidence of diversity increasing, decreasing or following stochastic or more complex patterns [18]. The observed patterns of microbial diversity can vary even within the same elevation gradient and environmental conditions, where different microbial guilds (*e.g.*, fungi, bacteria, archaea) follow distinct and often contrasting patterns [19].

Therefore, questions remain on the degree to which different factors impact the diversity of plant-associated microbiota, especially in understudied non-model wild plants of tropical mountains. Tropical montane ecosystems are known for their steep environmental gradients, where several climatic, topographic, and edaphic factors drastically vary over short spatial distances [20]. This spatial heterogeneity is often associated with species turnover in plants and animals, but little is known about how these gradients impinge on microbial communities. In an attempt to understand the factors driving plant-associated microbial diversity in tropical mountains, we analyze the rhizosphere and phyllosphere of a species of tree fern along an elevation gradient. In particular, tree ferns (Cyatheaceae) exhibit relevant characteristics for studying plant-associated microbiota. Tree ferns (Cyatheaceae) represent an important structural component of Neotropical montane forests and play a significant role in regeneration and modulation of light conditions, nutrient availability, and soil water content of forest understories [21]. Tree ferns are an ideal system to explore the structure of plant-associated microbiota. These ferns show sharp elevational turnover of species and strong intra-population genetic structure [22, 23], which often reflect on microclimatic differences and morphological variation within and between species. To date there are no studies exploring the microbiota associated with fern species in general or tree ferns in particular. Research on interactions between ferns and microorganisms have had mostly a macroevolutionary emphasis and focused on specific microbial taxa (*e.g.*, symbiosis; [24, 25]), which neglects the ecological scale of fern-associated microbiota and their environmental drivers. To address this knowledge gap, the aim of the present project is twofold: (1) characterize the diversity and structure of the rhizosphere and phyllosphere in a tree fern species along an elevation gradient; and (2) assess the association between environmental factors and variation in these microbial communities.

METHODS

Study site and sampling

We carried out the sampling in the municipality of Santiago Comaltepec, Oaxaca, specifically in the cloud forest locality of Cerro del Relámpago [23]. This cloud forest is considered a highly conserved dense broadleaf forest with complex stratification and a high degree of epiphytic growth; multiple species of tree ferns (Cyatheaceae) inhabit the forest with a marked elevational turnover [23]. The locality has a mean temperature of 13–15.2°C, annual precipitation > 2000mm, high levels of relative humidity [23, 26], and acidic soils (Acrisol) rich in organic matter and low nutrient retention capacity [26, 27]. Both climatic and edaphic conditions are highly heterogeneous [23, 28].

We conducted the sampling during the rainy season (July 2021) along an elevation gradient ranging from 1978 to 2210 meters above sea level (masl). We established plots of 20 x 4 meters at five different elevations (1978, 2007, 2018, 2178, and 2210 masl), in which we measured and mapped all individual tree ferns. We placed four sensors in each plot: three measuring temperature and light intensity (HOBO MX2202), and one measuring temperature and relative humidity (HOBO MX2301A). We programmed sensors to measure every two hours for thirty days.

We obtained samples for the phyllosphere and rhizosphere of the tree fern *Cyathea fulva* from three individuals per plot (15 individuals in total), keeping a minimum distance of two meters between individuals. In sum, we obtained 15 phyllosphere, 15 rhizosphere, and 5 soil samples. For the phyllosphere, we swabbed the adaxial surface of a mature and healthy pinnule of each individual after an initial rinse with distilled water and preserved swabs in RNAlater (Sigma-Aldrich). For the rhizosphere, we sampled root-adjacent soil at three points for each individual, which were later combined into a single composite sample per individual. We also obtained single soil samples within each plot at points that maximized the distance from any tree fern, but with a minimum distance of two meters. For each rhizosphere and soil sample, we added a small portion into 15mL centrifuge tubes containing RNAlater (Sigma-Aldrich). The remaining portions of samples were stored in plastic bags and then processed and analyzed for the following physicochemical variables: pH, electrical conductivity (EC), organic matter content, N, C_{total}, P_{total}, P, K, Ca, Mg, Na, cation-exchange capacity (CEC), NO₃, NH₄ and Fe. Soil analyses were conducted by the Soil Fertility and Environmental Chemistry Laboratory of the Colegio de Postgraduados, Institución de Enseñanza e Investigación en Ciencias Agrícolas.

Sample processing and DNA sequencing

We extracted DNA from rhizosphere and soil samples using the Qiagen DNeasy PowerSoil kit (Qiagen, Valencia, USA) according to manufacturer instructions but with the following modifications: 0.5g of initial sample and 4°C-incubation periods were increased to 20 minutes. We extracted DNA from phyllosphere samples using the Qiagen Blood and Tissue kit (Qiagen, Valencia, USA) following manufacturer instructions, adding an initial lysozyme incubation step at 37° for one hour. We measured the concentration and quality of DNA using NanoDrop (NanoDrop technologies, Wilmington, USA).

Each of the 35 DNA samples was sequenced twice, once for the 16S rRNA gene and once for the fungal ITS1 region, obtaining a total of 70 sequence samples. Amplicon libraries of the 16S gene spanning the V4 region (primers 505F/806R) were constructed following the Earth Microbiome Project standard protocol (www.earthmicrobiome.org). In brief, samples were PCR amplified in triplicate plus one negative control per sample, which were verified with 1% agarose gels. Triplicate PCR products were pooled per sample and quantified using a Qubit 4.0 fluorometer (Invitrogen, Thermo Fisher Scientific, Waltham, USA); all samples were pooled in a single amplicon library at a concentration of 240 ng/mL. The final pool was cleaned using the QIAquick PCR clean up kit (Qiagen, Valencia, USA) and sequenced in a single run of MiSeq (2x250) (Illumina, Inc., San Diego, CA, USA) at Macrogen Inc. (Seul, Korea). The fungal ITS1

library preparation and sequencing were performed at the University of Minnesota Genomics Center using a single run of MiSeq (2x300) (Illumina, Inc., San Diego, CA, USA)

Bioinformatics processing

We did not obtain sequencing data for three out of the 70 DNA samples due to poor sequencing quality (Table S1). More specifically, we removed one fungal rhizosphere sample (2007 plot) and two bacterial phyllosphere samples (2018 plot). Sequences for the remaining samples were demultiplexed by the service providers with the exception of the 16S phyllosphere samples, which were demultiplexed using the BBMap *demuxbyname* pipeline [29]. We processed sequence reads using the QIIME 2.0 pipeline (Bolyen et al., 2019), removing low-quality reads, chimeras, and chloroplast and mitochondrial DNA using the *q2-dada2* plugin, and grouped reads into Amplicon Sequence Variants (ASVs). We ran the pipeline for all bacterial 16S and fungal ITS1 separately, combining sequences for the phyllosphere and rhizosphere. We performed taxonomic assignment of the sequence reads with the *q2-feature-classifier* plugin using the GREENGENES2 [30] database for bacterial 16S and UNITE [31] database for fungal ITS1.

Microbial diversity and statistical analysis

We estimated ASV richness per sample and performed rarefaction to the minimum number of ASVs found across samples using the *phyloseq* [32] package in R [33] for each dataset (16S and ITS). We calculated alpha diversity (Shannon Index) using the *microbiome* [34] package in R [33], and used QIIME 2.0 *q2-diversity* plugin to estimate beta diversity (Weighted and Unweighted UniFrac). We conducted all statistical analysis and visualizations in R [33].

To determine if there were significant differences in alpha diversity across elevations and by sample type (*i.e.*, phyllosphere, rhizosphere, soil) we performed two-way Analysis of Variance (ANOVA) using Shannon Index as the response variable and sample type and elevation as predictors. We visualized beta diversity through an ordination of the entire community implementing a Principal Coordinates Analysis (PCoA) based on Weighted and Unweighted UniFrac distances using the *phyloseq* [32] package in R [33]. We assessed the contribution of elevation and sample type to beta diversity with a Permutational Analysis of Variance (PERMANOVA) using 9999 permutations as implemented in the *vegan* [35] package in R [33]. We searched for shared bacterial and fungal genera among all samples across elevation plots and sample types (phyllosphere and rhizosphere) using the *microeco* [36] package in R [33]. We considered these shared genera as part of the core microbial community of tree ferns, only when these were present in all phyllosphere and rhizosphere samples, but absent from soil samples.

We evaluated differences in community composition among elevation plots using the Analysis of Similarity test (ANOSIM) in the *vegan* [39] package in R [33]. We further explored microbial community differences with the Linear Discriminant Analysis Effect Size (LEfSe) method [37] implemented in the *microeco* package in R [36]; this method identifies taxa with differential abundances among samples and

applies discriminant analyses to test for taxa that can statistically discriminate among sample classes (*i.e.*, elevation plots).

To explore the relationship between elevation and the measured environmental variables, we performed an Analysis of Variance (ANOVA) using environmental variables as the response and plot identity as the predictor; we further estimated the correlation between elevation and each variable using Spearman correlations. We employed Redundancy Analysis (RDA) using the *microViz* [38] package in R [33] to assess the relationship between microbial relative abundances across elevation and environmental variables. Briefly, the RDA allows to examine how much variation in the response variable (in this case abundances) can be explained by a given set of explanatory variables; we performed RDA on fungal and bacterial phyllosphere and rhizosphere separately (four analyses in total).

RESULTS

In total, we identified 26,819 bacterial ASVs (2,341,774 reads in total) and 17,464 fungal ASVs (2,275,982 reads in total) (Supplementary data 1 and 2) and performed rarefaction to the minimum number of ASVs found across samples: 10,847 bacterial and 26,919 fungal ASVs, respectively. The relative abundance of bacterial and fungal taxa differed between the phyllosphere and the rhizosphere. Soil was included in the figure to serve as a negative control, presenting more similarities to the rhizosphere than to phyllosphere. (Fig. 1). The most abundant bacterial phylum considering all samples was Proteobacteria (29–64%), followed by Acidobacteriota (4.7–39.6%) and Actinobacteriota (3.3–15.6%); other frequent phyla were Verrucomicrobiota, Bacteroidota, Firmicutes, and Planctomycetota. The most abundant classes were Alphaproteobacteria and Acidobacteriae (Fig. 1a). The most abundant fungal phyla were Ascomycota (36.1–95.3%) and Basidiomycota (4.6–54.9%), whereas Mortierellomycota (1.7–29.7%) was almost exclusively found in the rhizosphere and soil. The fungal phyllosphere showed high abundances of Eurotiomycetes, Dothideomycetes, and Leotiomyces, whereas the rhizosphere and soil were dominated by Agaricomycetes, Archaeorhizomycetes, and Mortierellomycetes (Fig. 1b).

Diversity and structure of tree fern microbial communities

Bacterial and fungal alpha diversity varied between sample types (Fig. 2). In bacteria the rhizosphere was more diverse than the phyllosphere, whereas in fungi it was the opposite (Supplementary data 1 and 2). We found that alpha diversity significantly varied with elevation for the bacterial communities ($F_{(4)} = 5.588$, $p = 0.00419$; Fig. 2a-b), but not for the fungal communities ($F_{(4)} = 1.165$, $p = 0.357375$, Fig. 2c-d).

We found significant differences in both bacterial and fungal beta diversity among elevation plots and sample types (Table 1). A Principal Coordinates Analysis (PCoA) using Unweighted UniFrac dissimilarities showed a pattern of microbial turnover with elevation, where samples from within plots were more similar than samples from different plots (Fig. 2). For the phyllosphere, the first two axes explained 23.9% and 17.1% of the total variation of bacterial communities (Fig. 2a), and 14.2% and 9.1% of the variation of fungal communities (Fig. 2c). For the rhizosphere, we found the first two axes

explained 14.1% and 8.6% of the total variation for bacterial communities (Fig. 2b) and 16.4% and 9.7% for fungal communities (Fig. 2d). Trends of increasing turnover with elevational distance were also evident when using Weighted UniFrac (Fig. S1).

We employed an ANOSIM to test for differences in microbial composition among samples and found strong dissimilarities ($R > 0.4$) among plots in the phyllosphere and rhizosphere for both bacterial and fungal communities (bacterial phyllosphere: $R = 0.5126$, $p = 0.0048$; bacterial rhizosphere: $R = 0.4859$, $p = 0.0025$; fungal phyllosphere: $R = 0.5822$, $p < 0.0001$; fungal rhizosphere: $R = 0.7535$, $p < 0.0001$). To further explore bacterial and fungal taxa driving differences across elevation plots, we used a LEfSe to identify taxa that were significantly differentially abundant across elevations. We found differential abundances of 124 bacterial taxa and 165 fungal taxa. (Fig. 4).

Table 1
Differences in bacterial and fungal communities of the tree fern *Cyathea fulva* across elevation and sample type (phyllosphere and rhizosphere). Estimates derived from permutational multivariate analysis of variance (PERMANOVA).

	Df	R ²	Mean Sq	F Value	Pr(> F)
Bacterial community					
Sample type	2	2.9652	0.27543	7.9518	0.001 ***
Elevation	4	1.6954	0.15748	0.15748	0.008 **
Fungal community					
Sample type	2	1.4722	0.1154	2.1421	0.001***
Elevation	4	1.6807	0.13175	1.2228	0.031*

We found several bacterial and fungal genera that were uniquely present in plant-associated samples from particular plots (Fig. 5). We identified bacterial genera shared among samples of the phyllosphere (44 genera) and rhizosphere (266 genera). In turn, we also found fungal genera shared among samples of the phyllosphere (129 genera) and the rhizosphere (114 genera). From shared bacterial genera, the most abundant included *Lichenihabitans*, *Sphingomonas*, *Terriglobus*, and *Methylobacterium* in the phyllosphere, and *Acidoferrum* and other candidate Acidobacteria in the rhizosphere. From the shared fungal genera, the most abundant were *Trichomerium*, *Brycekendrickomyces* and *Zymochalara* in the phyllosphere, and *Archaeorhizomyces*, *Mortierella*, and *Podila* in the rhizosphere (Supplementary data 3). Overall, we found 20 bacterial genera and 119 fungal genera that were present across all of the tree fern-associated samples, irrespective of plot or sample type, but were not found in the soil samples (Fig. S2).

Environmental drivers of the microbial communities of tree ferns

To test for differences in the mean value of environmental variables across elevation plots, we performed Analysis of Variance (ANOVA) and found that 11 out of 18 variables had significant differences among plots (Fig. S3). Subsequently, we tested for the correlation between elevation and each variable and found that several of these (*i.e.*, temperature, relative humidity, pH, electrical conductivity, P_{total} , NH_3 , and Fe) correlated with elevation ($r > 0.35$, $p < 0.05$). We performed a Redundancy Analysis (RDA) using all environmental variables and those soil variables correlating with elevation. The first two axes of the RDA explained 23.5% and 11.6% of the total variation in the bacterial phyllosphere and 24.8% and 9.1% of the total variation in the bacterial rhizosphere. For the fungal community, the first two axes of the RDA explained 15.1% and 11.6% of the total variation in the phyllosphere and 24.1% and 12.4% of the total variation in the rhizosphere (Fig. 6).

The proportion of constrained inertia for the bacterial communities was 64.2% for the phyllosphere and 60% for the rhizosphere, whereas for the fungal communities constrained inertia amounted to 59.7% and 64.9% for the phyllosphere and rhizosphere, respectively. The RDA showed that the structure of microbial communities at lower elevations was positively associated with temperature, relative humidity, pH, and P_{total} , whereas communities at higher elevations were positively associated with electrical conductivity and light intensity (Fig. 6). The abundance of several bacterial and fungal classes closely associated with environmental variables; such is the case of the phyllospheric Polyangiia and Orbiliomycetes, which increased with pH, relative humidity, and reduced luminosity. For the rhizosphere, the abundance of bacterial taxa such as Acidimicrobiia and Actinomycetia, and fungal taxa such as Archaeorhizomycetes, were closely associated with higher electrical conductivity, increased luminosity, and lower levels of relative humidity.

DISCUSSION

The diversity and structure of microbial communities along elevational gradients is not well understood [39]. In this study we analyzed bacterial and fungal communities of the phyllosphere and rhizosphere of the tree fern *Cyathea fulva* along an elevation gradient. We found that microbial diversity and composition varied consistently with elevation. Our results add to the mounting body of evidence that geographically small gradients can lead to changes in the composition of microbial communities [40]. Furthermore, our results provide novel information about the drivers of microbial diversity in non-model plant species in tropical mountains.

The decreasing pattern of bacterial alpha diversity with elevation, especially for the phyllosphere, is consistent with previous data from other ecosystems showing the same pattern of decreasing bacterial diversity in the phyllosphere with increasing elevation [16, 41]. On the other hand, fungal alpha diversity did not vary with elevation. This agrees with previous reports for both the rhizosphere [42–44] and phyllosphere [45] of wild plants, but differs from the observation that soil fungal diversity within cloud forests declines with elevation [46]. However, existing evidence points to turnover being more important for variation in fungal diversity across elevation than richness or abundance [47]. In Mexican cloud forests, high turnover rates have already been reported for culturable soil fungi [48], mirroring diversity

patterns in the soil and phyllosphere reported for elevation gradients that include montane and montane cloud forests [49, 50]. Indeed, we found that both bacterial and fungal communities consistently differentiate with elevation. Our data showed higher dissimilarity among elevation plots for fungi than for bacteria, but in general microbial communities from within the same plot showed more similar compositions than among plots. Differences among taxonomic guilds can be explained by fungi being more sensitive to temperature changes and having less dispersion than bacteria, thus accounting for stronger patterns of elevational differentiation [50]. Nevertheless, a significant percentage of the fungal ASVs were taxonomically unclassified due to lack of information in the UNITE database, implying that the fungal communities are largely under-described.

Studies concerning the phyllosphere have shown that temperature is of great importance to the structuring of microbial communities and can affect different properties of these communities, such as composition and abundance [45, 51, 52]. Phyllosphere microbial communities have shown increased abundances of Actinobacteria due to leaf temperature [53], which agree with our report of enrichment of these taxa in the samples from the lowermost plot with higher temperatures. However, studies of the phyllosphere of cloud-forest plant species are not available to serve as a comparison. Furthermore, the observation that the highly abundant Acidobacteriota appears enriched at the highest elevations is consistent with the more acidic nature of the soil in these plots ($\text{pH} < 3.75$), which is closely associated with the presence of Acidobacteriota in other montane ecosystems [54]. Other less abundant taxa were identified as markers for some of the plots according to LEfSe analysis: Gemmatimonadota enriched in lower elevations consistent with the phylum's preferences for higher pH and plant richness [55].

Fungal communities in both phyllosphere and rhizosphere were dominated by Ascomycota and Basidiomycota, which is consistent with previous studies of local soil fungi that detected the same taxa within the same forest plots [28]. More specifically, we observed highly abundant taxa such as Eurotiomycetes, Dothideomycetes, and Leotiomyces in the phyllosphere, and Agaricomycetes, Archaeorhizomycetes, and Mortierellomycetes in the rhizosphere. Interestingly, some of the aforementioned fungal taxa had differential abundances across elevation plots which are likely related to changes in temperature, one of the main factors accounting for variation in the abundance of fungal taxa in multiple ecosystems [56, 57]. Changes in other elevation-related variables such as pH, C:N ratio, moisture content, and phosphorus availability, have also been reported as influencing the fungal communities within cloud forests [46, 58, 59]. Although soil Ascomycota have been reported to increase in abundance with elevation in other ecosystems [60], our results align with studies in cloud forest showing higher abundances at mid-elevations (1000–2000 masl) [61].

Despite the elevation turnover of tree-fern-associated microbial communities, we found shared bacterial and fungal taxa across elevations and sample types, suggesting that host related factors are likely playing a role in microbial composition [4]. We also identified microbial genera only present at particular elevations, but, given the small number of samples in our study, a more detailed exploration is not possible. Our results are consistent with prior research showing that environmental heterogeneity

influences which microorganisms compose the shared microbial community, even in the phyllosphere that is usually thought to recruit microorganisms stochastically from the soil pool [15].

In montane gradients, the observed changes in climate, edaphic factors, and soil nutrients are the principal drivers of microbial composition [39], this also being true for tropical cloud forests [46, 62]. Indeed, our RDA showed that between 53–59% of the inertia was constrained by environmental variables, meaning that a significant proportion of variation in microbial composition can be explained by the environment. These results are consistent with previous reports on soil fungi [28] and culturable microbes [27], and point to the environmentally-driven deterministic processes that impact microbial community assembly in the microbial communities associated with tree ferns. Our study in tree ferns evinces the likely impact of small-scale environmental variation to the filtering of the microbial communities associated with cloud forest plants, possibly acting through changes to leaf surface temperatures, availability of nutrients, humidity, and light incidence [41, 63]. The large remaining unexplained variation could be the result of stochastic assembling processes or unconsidered variables such as biological interactions, vegetation changes or host intrinsic properties. For instance, seasonality has been reported as an important factor in microbial community structure [64–66], where there is a great dynamism of microbial communities to temporal environmental changes.

This study represents a first step towards understanding the microbial communities associated with tree ferns, yet the reduced sampling precludes more nuanced analyses of their drivers. Including additional tree fern species across a wider elevation gradient would be necessary to untangle host-related effects from environmental factors that remain largely unexplored in plants in wild settings. Overall, we found that the microbial communities associated with tree ferns varied with elevation gradient and that a significant proportion of this variation can be explained by environmental heterogeneity. Our findings suggest that elevation and associated variables influence the composition and diversity of the tree fern microbial communities, but with varying responses depending on sample type and taxonomic guild. Given their importance for plant health, understanding plant-associated microbial communities and the factors influencing them across space and time is highly relevant for under-studied and threatened plant species of tropical mountains.

Declarations

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

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Author Contribution

S.R.-B., E.A.R. and M.A. planned, designed, and funded research; S.R.-B. and MA selected sampling sites and collected samples; E.A.R., M.A., and M.V.-M. processed the samples and prepared sequence libraries; M.V.-M. processed the sequencing data, analyzed and visualized the data, and wrote the manuscript; all authors read, revised, and approved the final manuscript.

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Data Availability

Sequence data are publicly available in NCBI under the Bioproject PRJNA1180585. The QIIME2 code, R codes, samples metadata and supporting data are available at <https://github.com/marianavelezmunera/FernsMicrobiome>.

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Figures

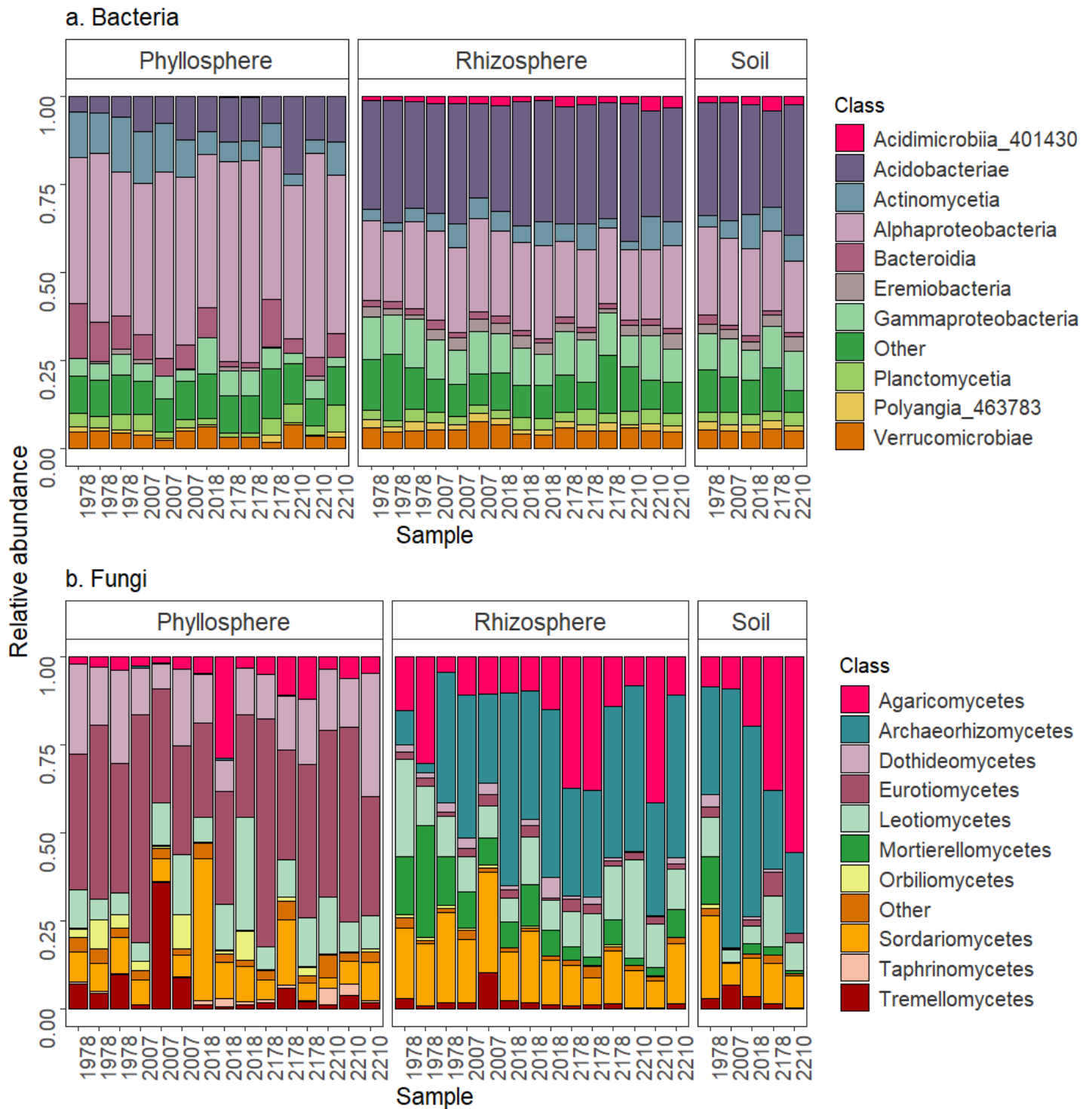


Figure 1

Relative abundances of bacterial (a) and fungal (b) classes in the microbial communities of the tree fern *Cyathea fulva* (Cyatheaceae) across elevation. Bar plots depict the microbial taxonomic composition (relative abundances) at class level for the phyllosphere (bacteria N=13; fungi N=15), rhizosphere (bacteria N=15; fungi N=14), and soil samples (bacteria N=5; fungi N=5). Sample names represent the elevation of the corresponding plot (1978, 2007, 2018, 2178, and 2210 masl). The ten most abundant classes are shown separately, whereas all other classes are combined into a single category ('Other').

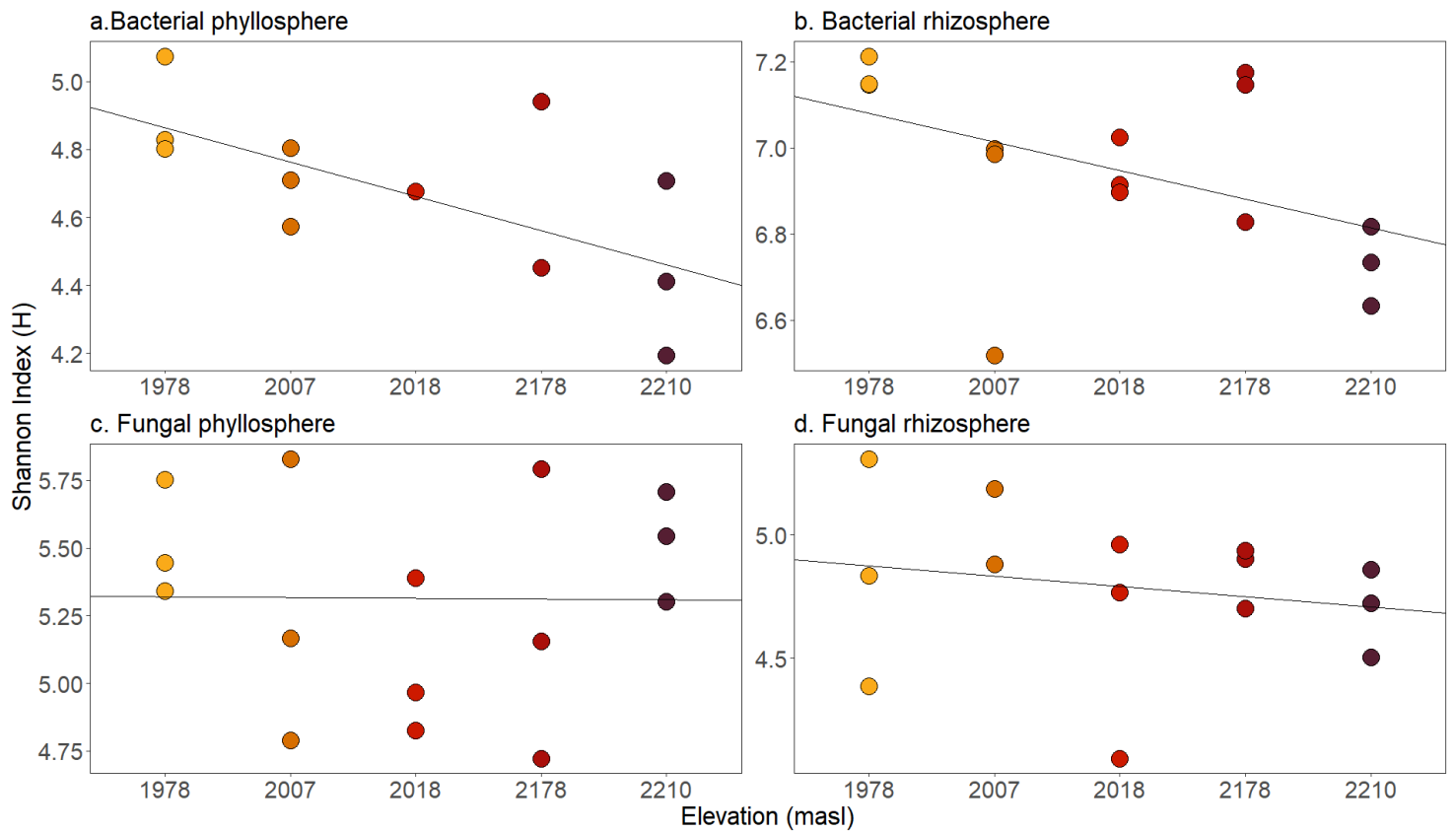


Figure 2

Microbial alpha diversity for bacterial and fungal communities of the tree fern *Cyathea fulva* across elevations. Shannon's diversity index (H) for individual samples of bacterial phyllosphere (N=13) (a) and rhizosphere (N=15) (b), and fungal phyllosphere (N=15) (c) and rhizosphere (N=14) (d). Solid line depicts the overall trend of varying alpha diversity as a function of elevation treated as a categorical variable (plots).

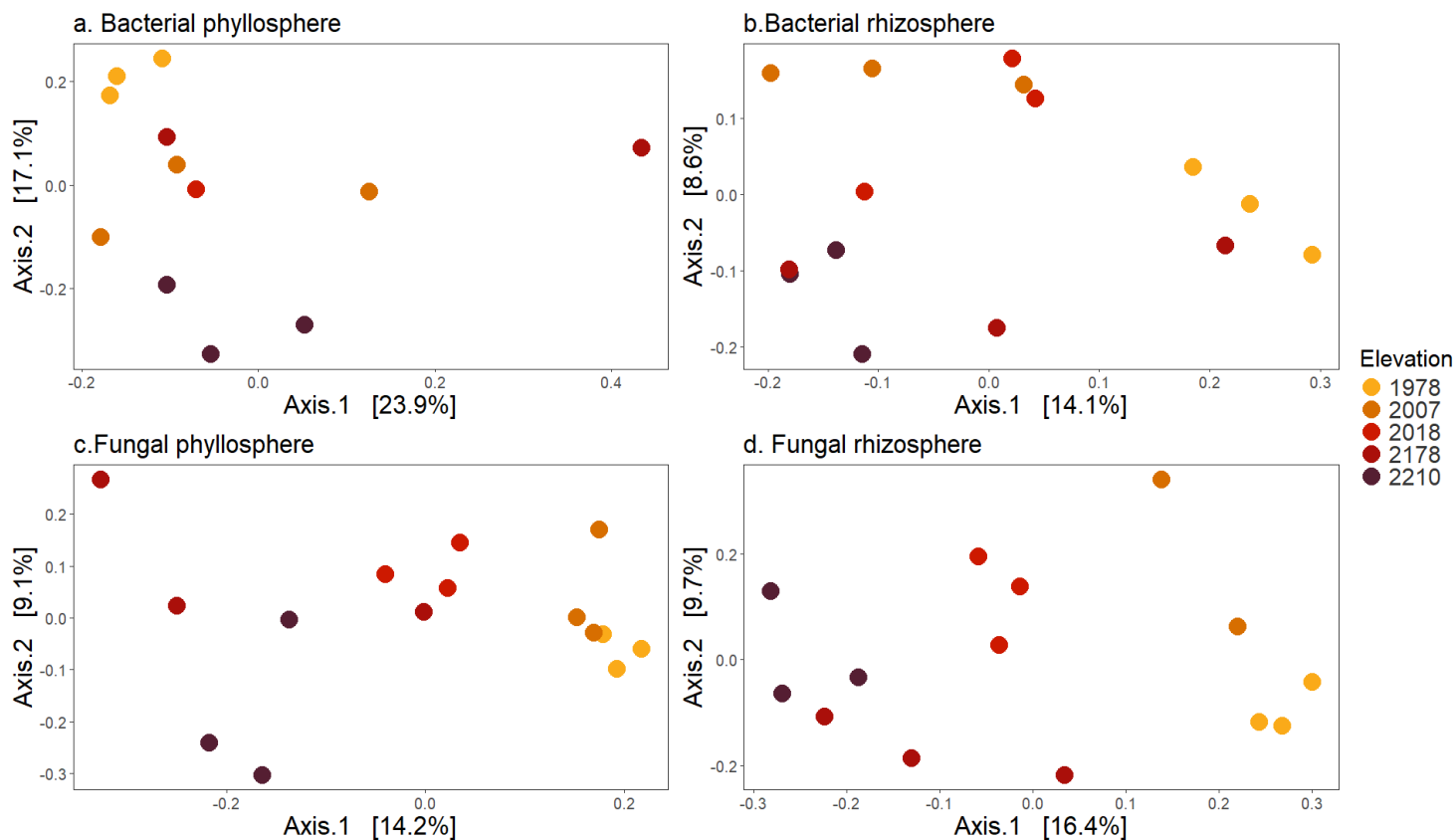


Figure 3

Principal Coordinate Analysis (PCoA) of bacterial and fungal communities of the tree fern *Cyathea fulva* across elevation. Ordination of pairwise community dissimilarities for bacteria (a, b) and fungi (c, d) based on Unweighted UniFrac distances for the phyllosphere (bacteria N=13; fungi N=15) (a, c) and the rhizosphere (bacteria N=15; fungi N=14) (b, d). Percentage variation explained by each PCoA is indicated on the axes. Colors indicate different elevations (masl).

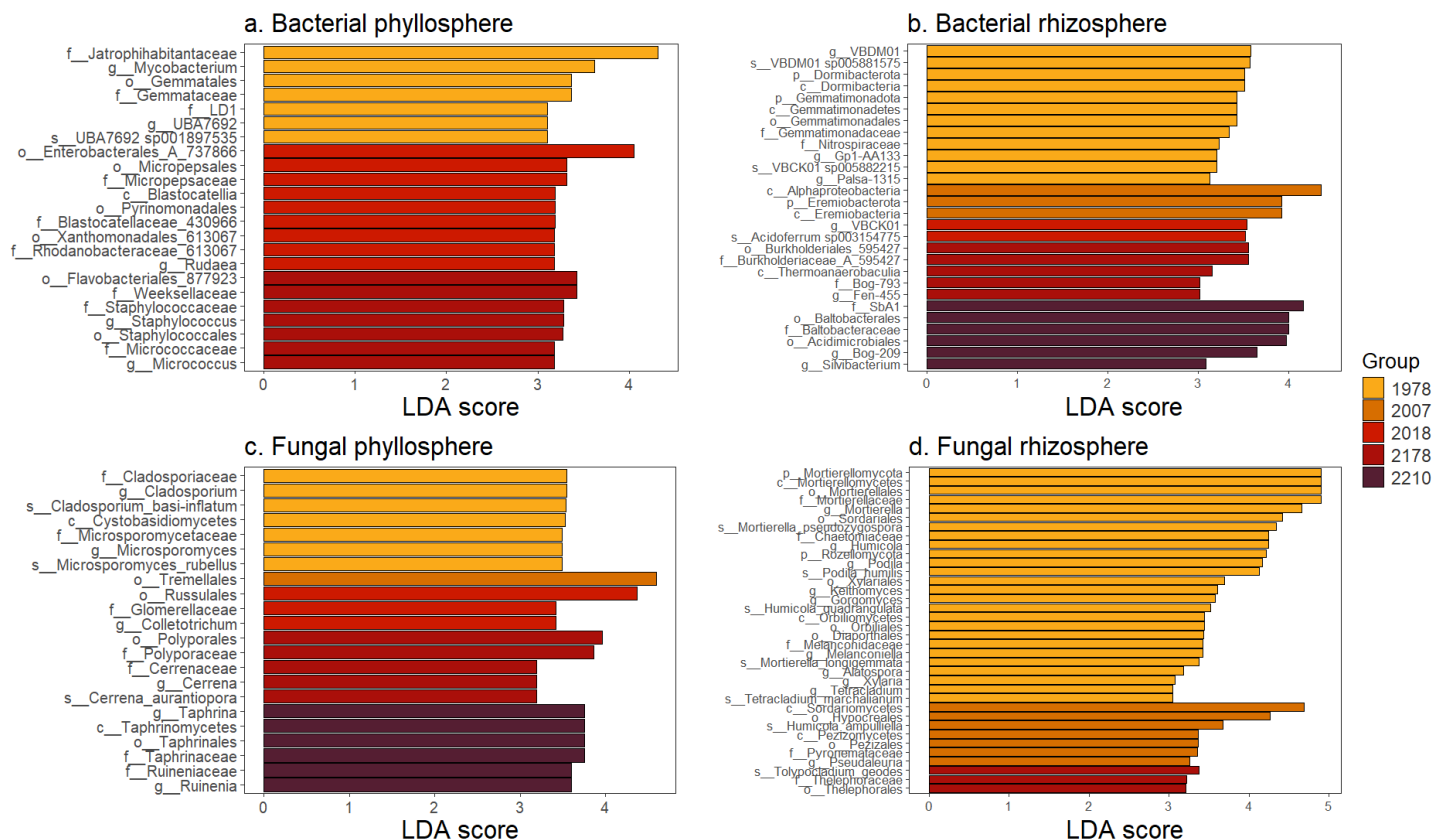


Figure 4

Differentially abundant taxa of the bacterial and fungal communities of the tree fern *Cyathea fulva* across elevation. Linear discriminant analysis (LDA) scores for differentially abundant bacterial (phyllosphere N=13; rhizosphere N=15) (a and b) and fungal (phyllosphere N=15; rhizosphere N=14) (c and d) taxa as determined by LefSE. Only taxa with an LDA score > 3 were considered as differentially abundant and plotted.

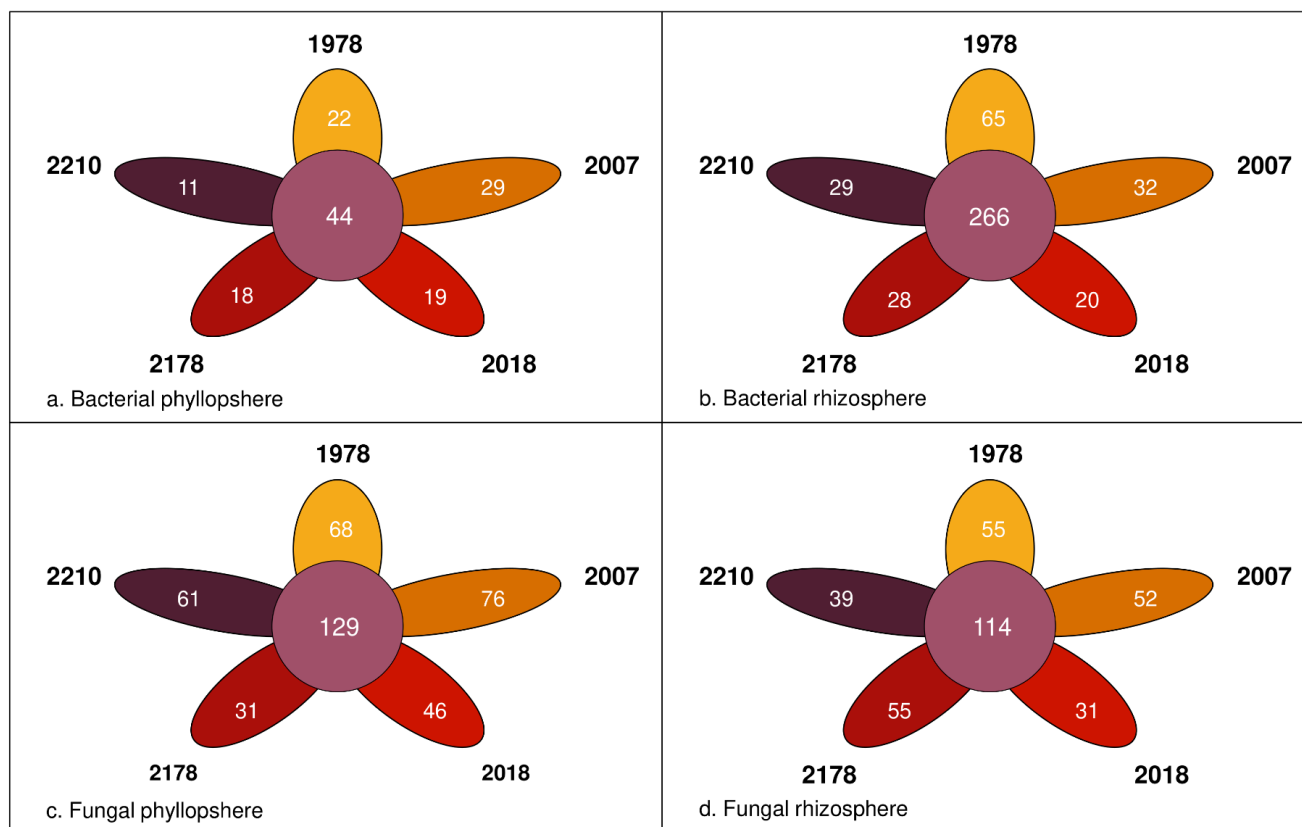


Figure 5

Diagrams showing the numbers of shared and unique bacterial and fungal genera in the phyllosphere and rhizosphere of the tree fern *Cyathea fulva* across elevation. The petals depict the numbers of unique microbial genera in each elevation plot (bold numbers). The central circle depicts the number of shared microbial genera across all elevation plots. (a) Bacterial phyllosphere (N=13), (b) bacterial rhizosphere (N=15), (c) fungal phyllosphere (N=15) and (d) fungal rhizosphere (N=14).

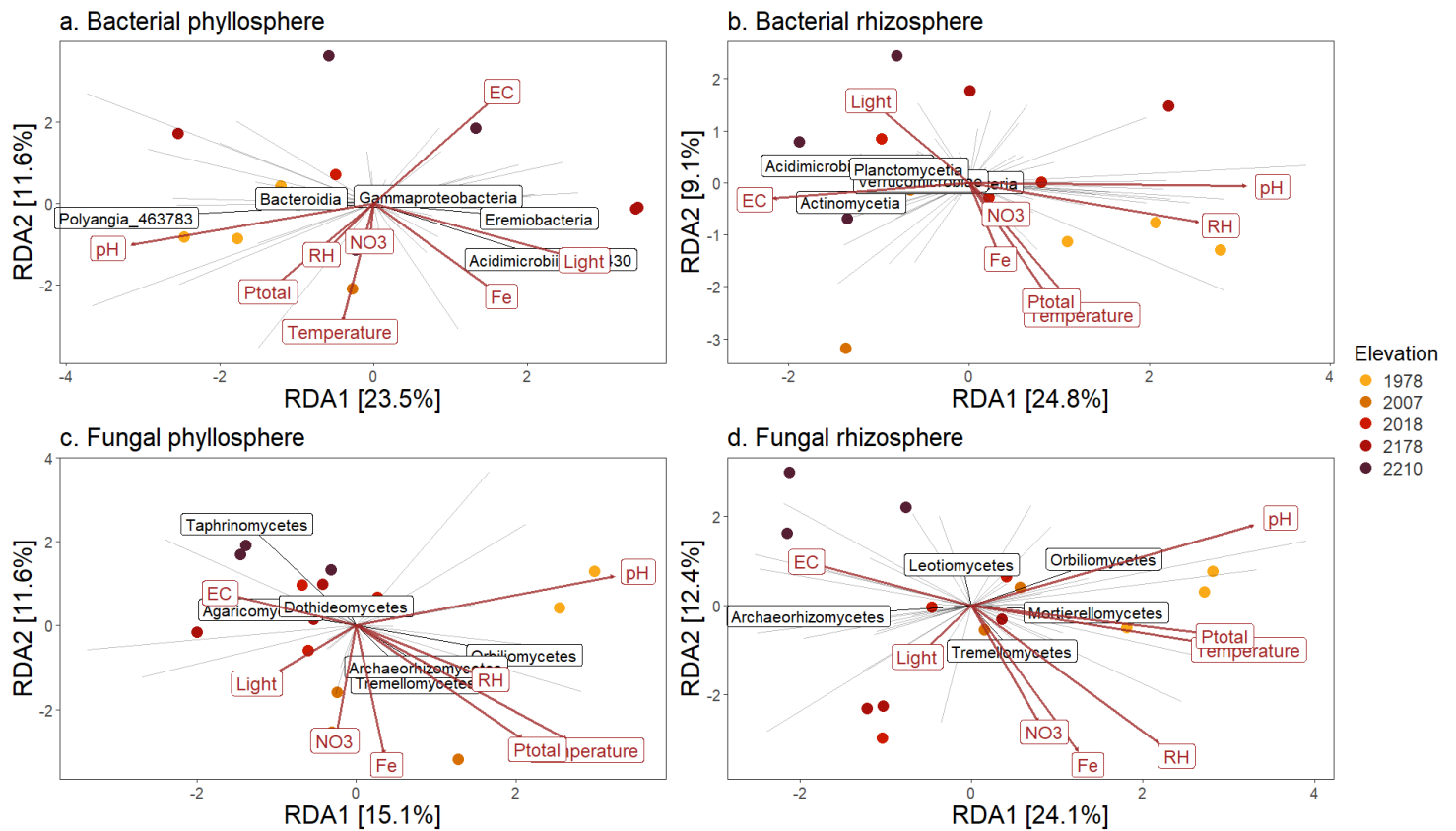


Figure 6

Redundancy Analysis (RDA) of bacterial and fungal communities of the tree fern *Cyathea fulva* across elevation. Ordinations for the phyllosphere (bacteria N=13; fungi N=15) (a, c) and rhizosphere (bacteria N=15; fungi N=14) (b, d) showing the relationships among the top most abundant microbial bacterial (a, b) and fungal (c, d) classes (black arrows), and measured environmental variables (red arrows). Only environmental variables and soil variables correlated to elevation were included in the RDA (temperature, relative humidity, pH, EC, total phosphorus, NO₃ and Fe). Samples are colored by their corresponding elevation plot.

Supplementary Files

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