Characterization of Bacterial and Fungal Communities Reveals Novel Consortia in Tropical Oligotrophic Peatlands

Elise S. Morrison [1,2] · P. Thomas [3] · A. Ogram [1] · T. Kahveci [3] · B. L. Turner [4] · J. P. Chanton [5]

Abstract
Despite their importance for global biogeochemical cycles and carbon sequestration, the microbiome of tropical peatlands remains under-determined. Microbial interactions within peatlands can regulate greenhouse gas production, organic matter turnover, and nutrient cycling. Here we analyze bacterial and fungal communities along a steep P gradient in a tropical peat dome and investigate community level traits and network analyses to better understand the composition and potential interactions of microorganisms in these understudied systems and their relationship to peatland biogeochemistry. We found that both bacterial and fungal community compositions were significantly different along the P gradient, and that the low-P bog plain was characterized by distinct fungal and bacterial families. At low P, the dominant fungal families were cosmopolitan parasites and endophytes, including *Clavicipitaceae* (19%) in shallow soils (0–4 cm), *Hypocreaceae* (50%) in intermediate-depth soils (4–8 cm), and *Chaetothyriaceae* (45%) in deep soils (24–30 cm). In contrast, high- and intermediate-P sites were dominated by saprotrophic families at all depths. Bacterial communities were consistently dominated by the acidophilic *Koribacteraceae* family, with the exception of the low-P bog site, which was dominated by *Acetobacteraceae* (19%) and *Syntrophaceae* (11%). These two families, as well as *Rhodospirillaceae*, *Syntrophobacteraceae*, *Syntrophorhabdaceae*, *Spirochaetaceae*, and *Methylococcaceae* appeared within low-P bacterial networks, suggesting the presence of a syntrophic-methanogenic consortium in these soils. Further investigation into the active microbial communities at these sites, when paired with CH_4 and CO_2 gas exchange, and the quantification of metabolic intermediates will validate these potential interactions and provide insight into microbially driven biogeochemical cycling within these globally important tropical peatlands.

Keywords Metabarcoding · Microbial networks · Bacterial and fungal communities · Peat · Phosphorus

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Introduction

Soil microbial communities are key drivers of ecosystem functioning [1], but our understanding of microbial community structure, diversity, and interactions is incomplete. Few studies have investigated the microbial communities of tropical peatlands, and those that have been studied are based predominantly in Southeast Asia, with few focused on Central and South America [2]. However, understanding the structures and activities of microbial communities within tropical peatlands worldwide is critical for understanding global biogeochemical cycles, as tropical peatlands play a key role in the global carbon (C) cycle [3], and sequester approximately 40 to 90 Gt of C globally [4]. In addition to C, nutrient availability can be a key driver of peatland function and can subsequently control net primary productivity and shape vegetative and microbial communities [5, 6], thereby influencing biogeochemical processes, including C cycling and greenhouse gas

production [7, 8]. Phosphorus (P) is a critical element in soils [9], and previous studies have shown that P-availability can alter microbial communities [5], C quality, CO_2, and CH_4 emissions [8], enzyme activities [5, 6], and decomposition rates in tropical wetlands [10]. Understanding microbial community structure, including bacteria, archaea [2], and fungi [11] within tropical peatlands, can provide insights into their roles in peatland biogeochemistry and ecosystem function. In addition, peatland fungal communities are understudied, particularly in the tropics [11], although they can participate in a variety of interactions with wetland vegetation [12] and play important roles in C, nitrogen, and P cycling.

In recent years, novel tools, including trait-based approaches and network analyses, have been developed, providing insights into microbial ecological strategies and community structure. One such trait-based approach is the ribosomal RNA operon number (rrn) [13], which has been used as a measure of bacterial ecological strategies and succession [14]. Bacteria with a high rrn operon number generally have a greater capacity to respond to high-nutrient conditions than those with more streamlined genomes and lower rrn operon numbers [14]. Microbial networks are another useful tool for exploring interactions within microbial communities and elucidating community patterns that may otherwise not be apparent [15]. Within microbial networks, microorganisms are frequently treated as nodes, and edges denote relationships between taxa. Network attributes, such as the number of nodes and edges, network membership, and degree centrality (i.e., how connected a node is), as well as network membership can be useful for evaluating ecological relationships between taxa. The exploration of microbial networks may be particularly insightful for the exploration of methanogenic-syntrophic consortia in tropical peat soils, as the production of methane in peatlands is often driven by methanogens and their relationships with primary and secondary (syntrophic) fermenters [16].

Here, we incorporate trait-based approaches and network analyses with metabarcoding of the 16S rRNA gene and internal transcribed spacer (ITS2) regions [17] to investigate the bacterial and fungal community structure within a tropical, ombrotrophic peat dome. These two genetic markers were used to (1) characterize community composition, diversity, rrn operon number, and network attributes within tropical peat soils along a naturally occurring P gradient, and (2) explore differences in peatland community traits between high- and low-P sites. Our overarching hypothesis was that community composition, diversity, rrn operon numbers, and network attributes would differ between high- and low-P communities. Specifically, we hypothesized that (1) low-P sites would be characterized by oligotrophic taxa and low diversity, (2) the rrn operon copy number would decline along the transect, with lower values in low-P sites indicating greater oligotrophy, and (3) low-P sites would be characterized by

lower network membership and fewer interactions, possibly due to decreased microbial activity and P-limitation.

Methods

Study Site, Sample Collection, and Soil Chemical Properties

The San San Pond Sak wetland is located in the Bocas del Toro province, Panama, and is comprised of several different wetland types, including the Changuinola peat deposit [18]. This site is an unimpacted RAMSAR site of scientific interest [19] and has been well characterized with regard to vegetation communities [5], carbon quality [20], greenhouse gas fluxes [8], and enzyme activities. Additionally, an initial characterization of bacterial communities was conducted using denaturing gradient gel electrophoresis (DGGE) [5]. The deposit is an ombrotrophic peat dome that exhibits a well-characterized, naturally occurring P gradient that transitions from a high-P region (total P 1028 mg kg^{-1}) [21] at the exterior of the dome that gradually transitions to a P-limited (total P 442 mg kg^{-1}) region in the center [5, 8, 21], with subsequent changes in vegetation communities [22]. The freshwater region of the swamp consists of four concentric vegetation, or phasic, zones: (I) a *Raphia taedigera* palm swamp; (II) a mixed forest swamp; (III) *Campnosperma panamensis* forest swamp; and (IV) a *Myrica-cyrilla* bog plain [23, 24]. The water table is consistently at or above the peat surface [18], and peat (organic matter > 30%) with these sites extends to at least 180 cm [20] but can be as deep as 9 m [23].

Sample Collection

Samples were collected from nine sites on a 2.7-km transect along this gradient in June 2013 (Fig. 1), spanning the four aforementioned vegetation zones. Despite the change in nutrient status, the system exhibits little change in pH along the gradient [6, 8], which allows investigation of microbial community dynamics that would otherwise be masked by pH changes. At each site, three replicate soil cores were taken within 0.5 m of each other using a 10-cm thin-walled, polycarbonate core with stainless steel tip. Cores were sectioned into shallow (0–4 cm), intermediate (4–8 cm), and deep (24–30 cm) depths and frozen at − 80 °C upon return to the laboratory (< 4 h). Due to the extensive peat deposit at the site [22], there were no distinct horizons within the 30-cm cores. Each replicate was homogenized and environmental DNA was isolated using a PowerSoil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). Supporting data were compiled from previous studies and used to aid interpretation of metabarcoding results (Table S1).
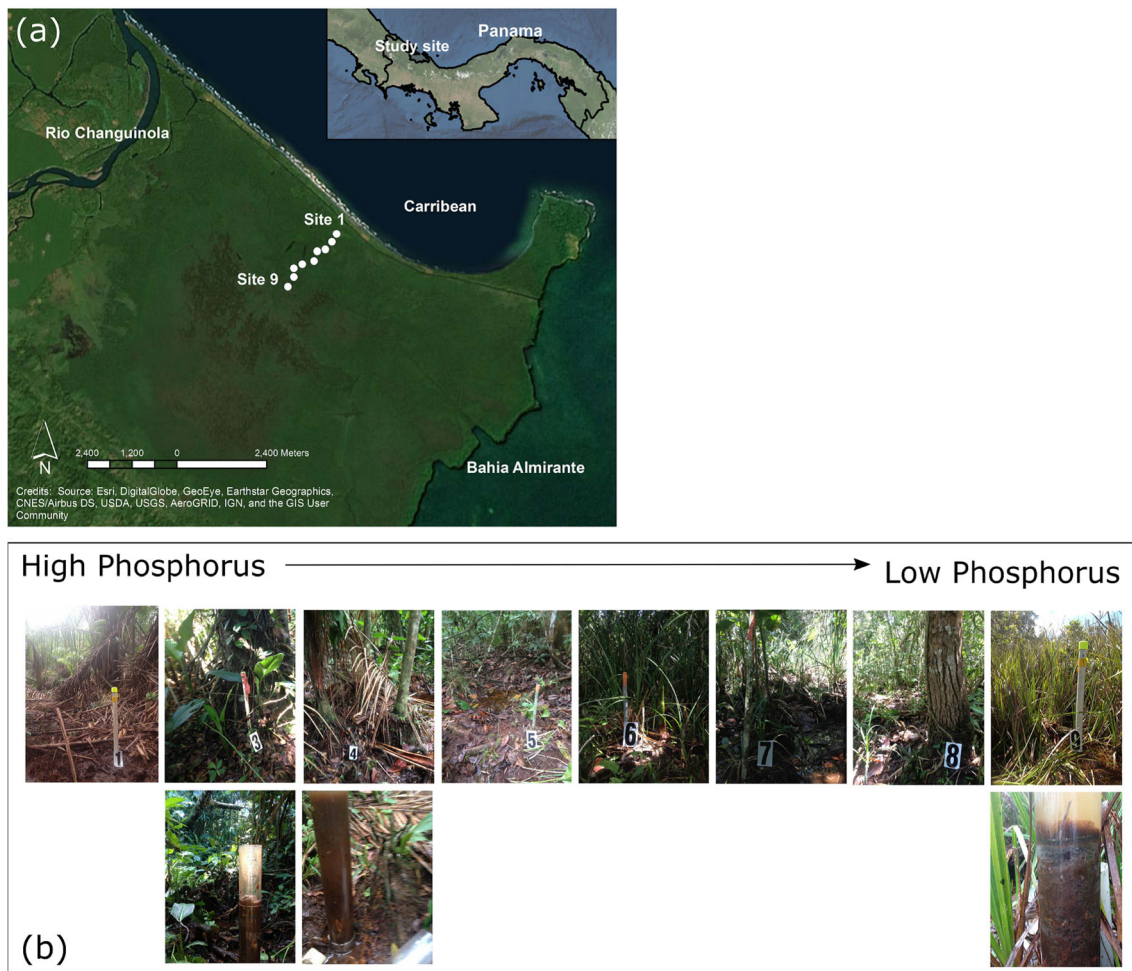
Fig. 1 (**a**) Map of the transect and (**b**) photographs of all sites (with the exception of site 2) with photographs of cores below each site, when available

Sequencing: ITS and 16S rRNA

Bacterial community composition was analyzed for all sites and depths along the transect ($n = 81$), while fungal community composition was analyzed for a subset of sites along the transect due to sequencing limitations (specifically, sites 2 and 8 were excluded and one sample was lost in transit, $n = 62$). To target the ITS2 region, the primers fITS7 (forward) and ITS4 (reverse) [17] were used, with the addition of an Illumina adapter sequence (Table S2). For ITS, amplicons were prepared using a modified version of Illumina's 16S rRNA gene sequencing protocol, with cycling conditions [25] of 95 °C for 30 s, 30 cycles of 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 2 min, followed by one cycle of 72 °C for 10 min. For 16S rRNA, library preparation and sequencing was conducted according to Illumina's 16S rRNA gene sequencing protocol (Illumina technical document, Part#15044223 Rev.B). Primers targeted the hypervariable V3 and V4 regions of the 16S rRNA gene. Primers were comprised of an Illumina adapter and Bakt_341f Bakt_805r primers from

[26] (Table S2). Cycling conditions for the amplicon PCR was as follows: 95 °C for 3 min, then 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by one cycle of 72 °C for 5 min. Barcoding was conducted for both ITS and 16S rRNA using Nextera indexing kits (Illumina Inc., San Diego, CA) with the following cycling conditions: one cycle of 95 °C for 3 min, 8 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by one cycle of 72 °C for 5 min.

Both ITS and 16S rRNA amplicons were quantified using QuantIT PicoGreen double-stranded DNA assay kit (Invitrogen, Carlsbad, CA, USA), and amplicons were pooled with equimolar concentrations prior to submission for sequencing. Separate libraries were created for ITS and 16S rRNA amplicons. The pooled libraries were sequenced at the University of Florida's Interdisciplinary Center for Biotechnology Research (ICBR) on an Illumina MiSeq, for 2×250 bp (ITS) and 2×300 bp (16S rRNA) paired-end reads per sample (Illumina, Inc., San Diego, CA). Sequences were demultiplexed by ICBR, and paired-end reads were retrieved from Illumina's BaseSpace platform.

Sequence Processing

ITS sequences were analyzed using the PIPITS pipeline version 1.3.6 [25] to conduct quality filtering and assign taxonomy to reads. Paired-end reads were joined with the function pipits_prep, which joins reads with PEAR v. 0.9, and quality filtered with the command fastq_quality_filter, using the FASTX-toolkit v. 0.0.14. The ITS2 subregion was extracted by using ITSx [27], with HMMER3 v. 3.1b2 to compare input sequences with ITS2 subregions. Sequences were then clustered into OTUs using VSEARCH 20151022 through the PIPITS pipeline based on 97% sequence identity. Representative sequences for each OTU cluster were assigned and chimera detection and removal was conducted using the UNITE UCHIME reference dataset retrained for PIPITS. Sequences were then taxonomically assigned with the RDP classifier v. 2.1 [28] with the UNITE fungal ITS reference dataset. Prior to any analyses, samples were rarefied to the lowest number of reads for all samples with a threshold of > 10,000 reads, using Quantitative Insights Into Microbial Ecology (QIIME)'s function single_rarefaction.py, and samples with less than 10,000 reads were discarded and not included in downstream analyses. The number of reads and alpha diversity measures are presented in Tables S2 and S3.

16S rRNA gene sequences were quality filtered and assigned to OTUs using QIIME v. 1.9.1 [29]. Paired-end reads were joined using the function join_paired_ends.py, and quality filtering was conducted with multiple_split_libraries.py, by discarding any sequences smaller than 200 nucleotides long and discarding reads that had an average equal score less than 25. OTUs were assigned with the function assign_taxonomy.py, using the UCLUST consensus taxonomy assigner, with a minimum percent query coverage of 90%. Prior to any analyses, datasets were rarefied to the lowest number of reads for all samples, with a minimum threshold of > 10,000 reads. All samples with too few reads were discarded. The resulting rarefied OTU table was used as input for subsequent analyses in R version 3.4.0. The number of reads and alpha diversity measures are presented in Tables S2 and S3.

Libraries for 16S rRNA genes and ITS were summarized with the command summarize-table in biom version 2.1.5. Rarefied OTU tables for 16S rRNA genes and ITS were imported into R version 3.4.0 for further analysis. Nontarget reads, such as those for chloroplasts, were removed from both datasets prior to downstream analyses in R. OTUs that were not assigned to family were discarded prior evaluating the relative abundance of each family. For both ITS and 16S rRNA genes, percent relative abundance was calculated for all families along the gradient, and families that comprised < 1% of the community were aggregated to ease interpretation. Significant differences between OTU abundance across the transect were determined for each OTU with a Kruskal-Wallis rank sum test in QIIME (group_significance.py), filtered for those OTUs that only had significant differences along the transect (Benjamini Hochburg corrected $p < 0.1$) and then identified and summed based on their family taxonomic rank, for each depth, to determine which families had the greatest number of OTUs that changed significantly along the gradient. Non-metric dimensional scaling (NMDS) was run on a Bray-Curtis dissimilarity matrix to determine differences in either fungal or bacterial structure between sites, depths, and vegetation zones, and an ADONIS test was run to test for significant differences with R^2 values as a by term calculation. The phyloseq function estimate_richness was used to calculate α-diversity measures for each sample, [30] using the function estimate_richness, and summarized with dplyr [31]. For both 16S rRNA genes and ITS, distance from centroid (a measure of β-diversity) was calculated by running a PERMDISP analysis on a Bray-Curtis dissimilarity matrix with vegan [32], and significant differences in multivariate dispersion were calculated with a Kruskal-Wallis test. Additional data processing, statistical analyses (i.e., Kruskal-Wallis tests), and figure plotting were run using R version 3.2.2 [33], with phyloseq, ggplot2 [34], and Hmisc [35].

SPIEC-EASI Networks

Bacterial networks were developed using SPIEC-EASI [15] in R version 3.4.0. Bacterial abundance data was subset by site, and networks created for both the high- and low-P site sites (sites 1 and 9 respectively, $n = 9$). To increase the number of samples included in the networks, samples from shallow, intermediate, and deep soils were included in each site's network. Site explained more variability in community composition when compared with depth (discussed below), suggesting that there may be minimal bias to networks run with pooled depths. However, to further minimize this potential bias, we only selected OTUs present in all samples to increase the conservatism of our networks. For future studies considering incorporating network analyses at this location, we would recommend additional sample replication to improve the robustness of future network analyses. Abundance data was not pre-processed prior to processing with SPIEC-EASI, as data normalization is run internally within SPIEC-EASI. Networks were created with the Meinshausen and Buhlmann (MB) network selection method in SPIEC-EASI, with an nlambda penalty value of 20. SPIEC-EASI networks were then converted to igraph objects for computing network attributes using the igraph package [36] in R version 3.4.0, and networks were visualized with phyloseq in R version 3.4.0. Network modularity was evaluated using the cluster walktrap method within igraph. Due to the low annotation of fungal families to family, fungal networks were not developed using the ITS dataset.

Bacterial rrn operon copy numbers

For bacterial communities, ribosomal RNA operon copy numbers (*rrn*) were estimated with methods from [14]. OTUs were picked with QIIME's closed reference OTU picking to ensure that the OTU table was compatible with downstream analyses. The *rrn* operon copy number for each OTU was determined from PiCRUSt's database [37], and OTU abundances were normalized to *rrn* operon numbers by dividing OTU abundance by *rrn* operon copy number, and abundances were recalculated. Then the product of the relative abundance and *rrn* operon copy number was taken for each OTU and used to calculate the mean operon copy numbers (i.e., "community trait value") for each sample. A Kruskal-Wallis test was run to determine significant differences between rrn operon number and sites.

Sequence Accession Numbers

Both ITS and 16S rRNA gene sequences were submitted to NCBI under BioProject number PRJNA376032, with accession numbers SRR311893 to SRR312034. Analysis scripts are available at: https://github.com/elisemorrison/MECO_Panama_peat_microbiome.

Results

Sequencing Depth and Diversity for Fungi and Bacteria

The mean number of reads for the 16S rRNA gene dataset ranged from 19,631 ($\pm$ 8690) (site 1, shallow soil) to 179,386 ($\pm$ 30,618) (site 5, intermediate soil), while the mean

number of reads for the ITS dataset ranged from 1040 ($\pm$ 1706) (site 6, deep soil) to 145,964 ($\pm$ 204,382) (site 9, intermediate soil). Rarefaction curves are presented in Figure S1. There was a total of 2934 taxa assigned to the fungal kingdom after rarefaction and filtering non-target reads. Only 28.9% of fungal taxa were assigned to family level (848 taxa). There were 5502 taxa assigned to the bacterial kingdom after rarefaction and filtering non-target reads, and 99% of bacterial taxa were annotated to the family level (5451 taxa). Additional summary data for fungal and bacterial libraries, including the number of singletons and doubletons, are available in Tables S2 and S3. There was no significant difference in α-diversity measures along the gradient for fungi at shallow or deep depths ($p > 0.05$). However, at intermediate depth (4–8 cm), two of the mixed forest sites (sites 4 and 5) exhibited significantly greater α-diversity when compared with one of the *Campnosperma* sites (site 6). In addition, one of the mixed forest sites (site 4) exhibited significantly greater α-diversity than the low-P bog (site 9) (Fig. 2; Table S3). For bacterial α-diversity, in shallow soils, one of the *Campnosperma* sites (site 7) exhibited a significantly greater α-diversity when compared with a high-P *Raphia* (site 1) and low-P bog site (site 9) sites. Within deep soils, α-diversity declined along the transect, and the high-P *Raphia* sites (sites 1 and 2) exhibited significantly greater α-diversity measures than the intermediate (mixed forest and *Campnosperma*) and low-P bog sites (Fig. 2; Table S3). For fungal communities, there were no significant differences in α-diversity along the gradient in shallow or deep soils; however, in intermediate soils, two of the mixed forest sites (sites 4 and 5) exhibited significantly greater α-diversities than one of the *Campnosperma* forest sites (site 6). Site 4 also exhibited significantly greater α-diversity than the bog site (site 9). Measures of α-diversity are summarized in Fig. 2 and Table S3.

Fig. 2 Differences in the number of observed OTUs for both fungi (**a**) and bacteria (**b**) along the gradient. Site 1 is the high-P site, while site 9 is the low-P site. When available, mean values ($n = 3$) are presented $\pm$ standard error. Points are colored by vegetation zone and significant differences are denoted with an asterisk. Additional diversity measures are presented in Table S3
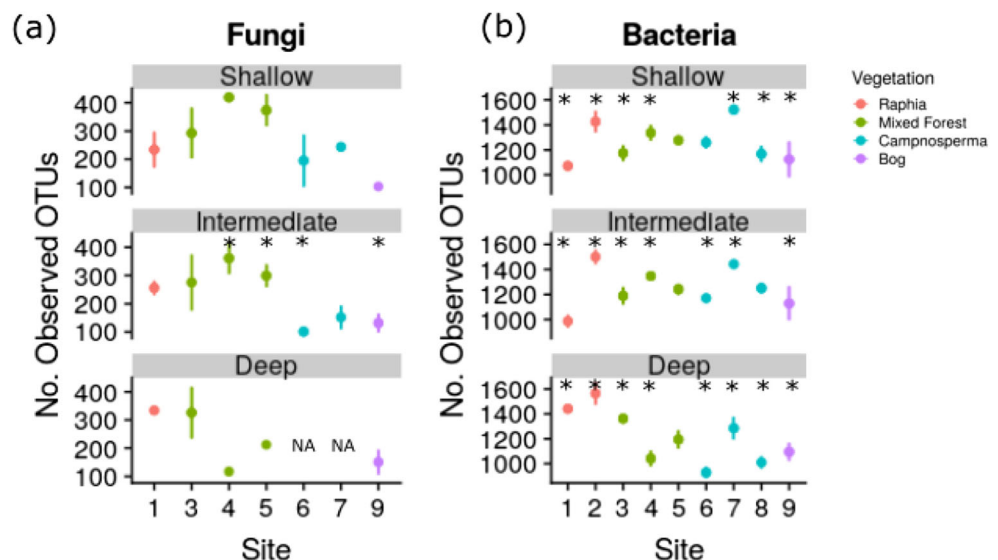
Fungal Community Structure and Composition

Fungal community composition was significantly different among sites (ADONIS, $p = 0.001$, $R^2 = 0.48$), vegetation zones (ADONIS, $p = 0.001$, $R^2 = 0.27$), but not among depths (ADONIS, $p = 0.04$, $R^2 = 0.04$; Fig. 3a). The most abundant fungal families exhibited considerable variability between sites and depths (Fig. 4a). In the high-P *Raphia* site (site 1), the fungal family *Mortierellaceae* (20%) was dominant in shallow and deep soils, while *Mycenaceae* (21%) was dominant in intermediate soils. In mixed forest (sites 3–5), *Herpotrichiella* (33–72%), *Nectriaceae* (15–26%), and *Trichocomaceae* (61%) were the most abundant fungal families. In *Campnosperma* forest sites (sites 6 and 7) the most abundant families were *Cordycipitaceae* (94%), *Annulatascaceae* (26%), *Teratosphaeriaceae* (14%), and *Sympoventuriaceae* (32%) in shallow and intermediate depths, although there were insufficient reads for deep soils, such that samples were discarded after rarefaction. For site 9 (bog), *Clavicipitaceae* (19%) was the most abundant taxon in shallow soils, *Hypocreaceae* (50%) was most abundant in intermediate soils, and *Chaetothyriaceae* (45%) was the most abundant family in deep soils. Relative abundance data are summarized in Table S4. Based on a Kruskal-Wallis test with a Benjamini Hochberg correction for multiple comparisons, there were no fungal families that significantly changed along the transect (corrected $p < 0.1$). However, this lack of significant difference is likely due to this dataset being statistically underpowered for this comparison. Thus, we suggest future studies increase replication and their experimental design [38] to allow for additional statistical comparisons of fungal communities in this system.

## Bacterial Community Structure and Composition

The composition of bacterial communities was significantly different among sites (ADONIS, $p = 0.001$, $R^2 = 0.49$), vegetation zones ($p = 0.001$, $R^2 = 0.35$), and depths ($p = 0.001$,

$R^2 = 0.10$; Fig. 3b). Position along the P gradient explained more variation in community composition than depth. For all sites and depths, with the exception of surface soils in the low-P bog (site 9), *Koribacteraceae* was the dominant bacterial family (16–54%). The most abundant family in site 9 surface soils was *Acetobacteraceae* (19%). However, the second-most abundant bacterial families shifted along the gradient, particularly in deep soils (Fig. 4b). *Solibacteraceae* was consistently the second-most abundant family within shallow and intermediate soils of sites 1–3 (*Raphia* and mixed forest) and 7 (*Campnosperma* forest), as well as in the intermediate soils of sites 6 and 8 (*Campnosperma* forest) (9–13%). The second-most abundant family in shallow and intermediate soils was *Acidobacteriaceae* in sites 4 and 5 (mixed forest) and site 8 (*Campnosperma* forest) (11–12%). For site 6 (*Campnosperma* forest), an undescribed *Verrucomicrobia* family (Ellin515) was the second-most abundant family in shallow soils. *Koribacteraceae* was the second-most abundant family in site 9 (bog) shallow soils (18%), while *Acetobacteraceae* was the most abundant in site 9 (bog) intermediate soils (18%). Along the gradient, deep soils had particularly interesting shifts in the second-most abundant bacterial family. *Solibacteraceae* was the second-most abundant family (12–9%) in deep soils of sites 1–4 (*Raphia* and most mixed forest sites) and sites 7–8 (*Campnosperma* forest sites). However, in deep soils of sites 5 (mixed forest) and 6 (*Campnosperma* forest), *Thermodesulfovibrionaceae* was the second-most abundant family (10%, 11%, respectively), and in site 9, *Syntrophaceae* (11%), was the second-most abundant family in deep soils. Bacterial abundance data are summarized in Table S5.

When tested for significant differences along the gradient, only shallow soils exhibited significant differences in bacterial OTU abundance (Kruskal-Wallis, Benjamini-Hochberg corrected p value < 0.1) between sites. However, as with the fungal communities, the lack of significant difference seen in intermediate and deep soils is likely due to insufficient statistical power of this dataset. In shallow soils, the top 10 bacterial
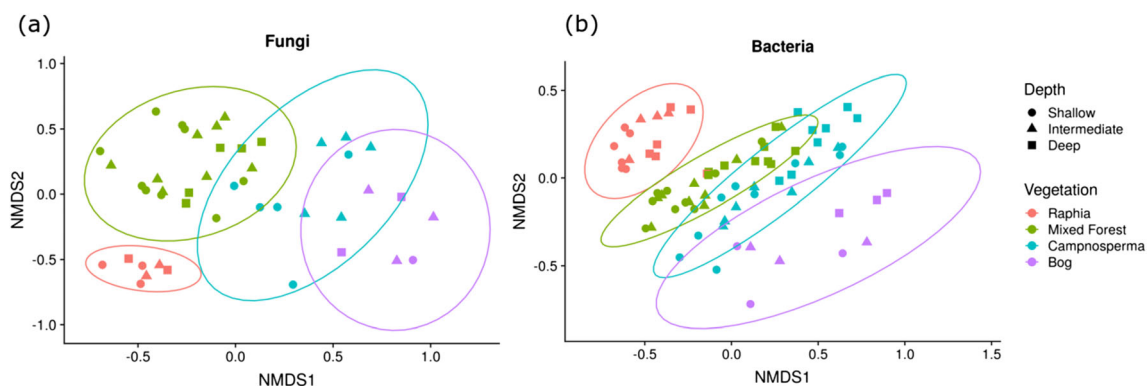
Fig. 3 Non-metric dimensional scaling (NMDS) based on Bray-Curtis dissimilarities for (**a**) fungal (stress = 0.18) and (**b**) bacterial (stress = 0.12) communities. Ellipses reflect 95% confidence regions based on vegetation zones and point shapes denote soil depth
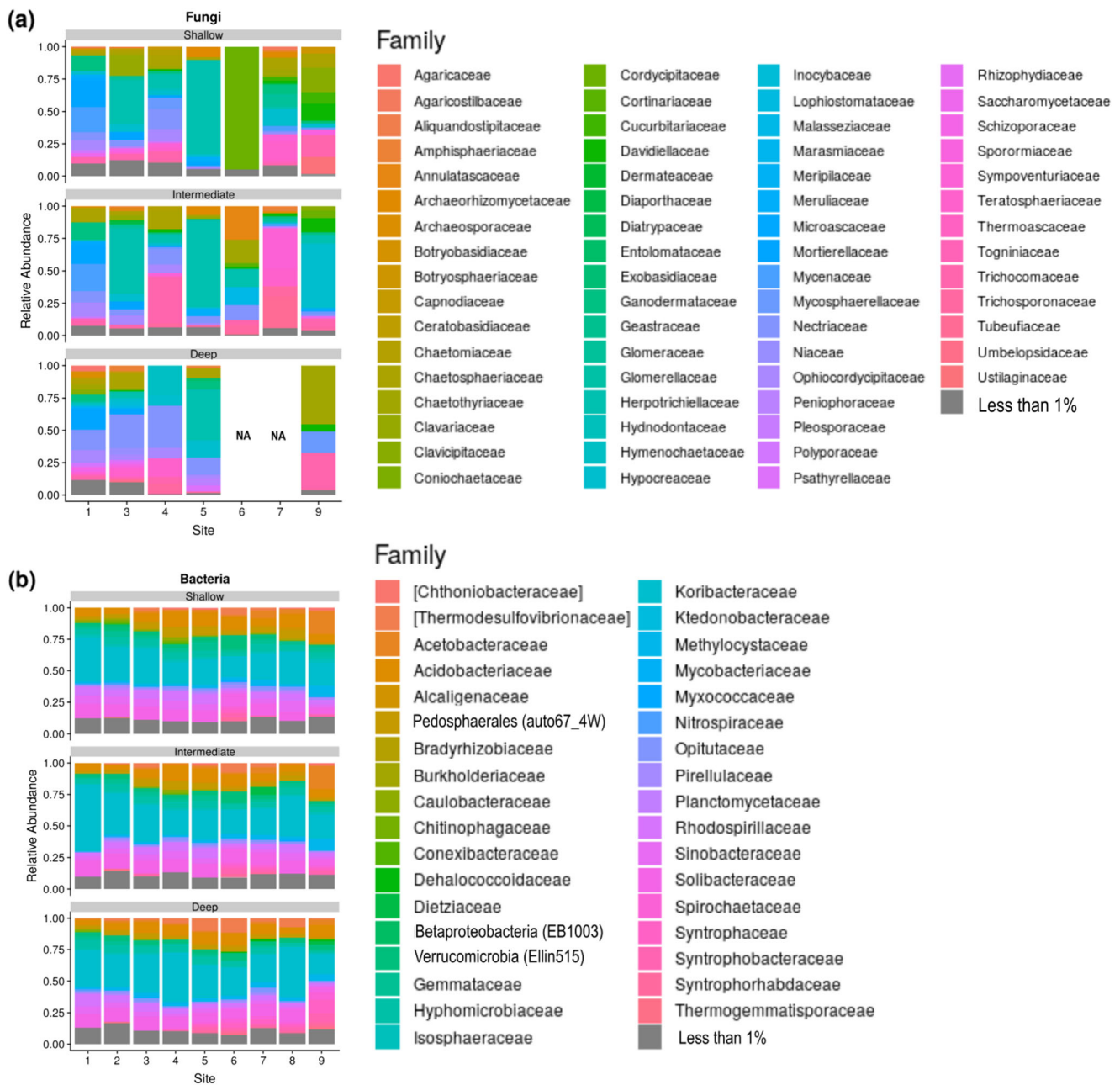
Fig. 4 Percent relative abundance of all (**a**) fungal and (**b**) bacterial families seen along the gradient, for each depth. Site 1 corresponds to the high-P site, while site 9 corresponds with the low-P site. Bars are colored by family. For fungal communities, deep soils for sites 6 and 7 did not have sufficient number of reads (i.e., < 10,000) for rarefaction.

OTUs not assigned to family were omitted. Taxa that were present in less than 1% abundance were grouped into the category "less than 1%." Undescribed families are indicated in bold, labeled with nearest known taxonomy and putative family names in parentheses

families with the most OTUs that significantly changed along the gradient were *Rhodopirillaceae* (8.5% of families that changed along the gradient), *Koribacteraceae* (7.3%), undescribed *Verrucomicrobia* (Ellin515) (5.7%), *Pirellulaceae* (4%), *Chitinophagaceae* (4.1%), *Acidobacteriaceae* (3.7%), *Solibacteraceae* (3.7%), *Syntrophobacteraceae* (3.7%), undescribed *Pedosphaerales* (auto67_4W) (3.3%), and *Sinobacteraceae* (3.3%). The top

20 bacterial families that significantly changed along the gradient are plotted in Fig. 5.

Network Attributes

The bacterial network at the high-P site (site 1) had 682 edges, of which 293 (42.9%) were negative. In contrast, the low-P network (site 9) had 704 edges, of which 273 (38.7%) were
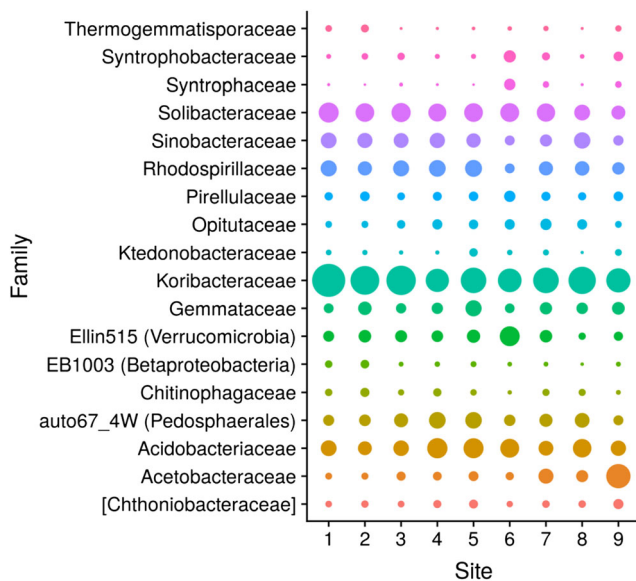
Fig. 5 Changes in relative abundance for top 20 bacterial families that had the greatest number of OTUs that changed significantly along the gradient (Kruskal-Wallis, Benjamini-Hochberg corrected $p < 0.1$). Only the surface soils of the bacterial datasets had families that changed significantly along the gradient, likely due to other soils being statistically underpowered

negative. The bacterial network at the high-P site (site 1) had 294 nodes (i.e., OTUs in the network), while the bacterial network at the low-P site (site 9) had 291 nodes (Fig. 6; Table S6). The average degree centrality for the high-P network was 13.7, while the average degree centrality was 14.2 for the low-P network. Although the average degree centrality differed between the high and low-P network, the degree distribution was similar for both (Figure S2). The network modularity for the high-P site was 0.306 while the modularity of the low-P site was 0.32. The most abundant families in the high-P bacterial network were taxa not assigned to family (43%), followed by *Rhodospirillaceae* (8%), *Koribacteraceae* (6%), and *Solibacteraceae* (5%). The most abundant families in the low-P bacterial network were also taxa not assigned to family (34%), as well as *Solibacteraceae* (6%), *Koribacteraceae* (5%), and undescribed *Pedosphaerales* (auto67_4W) (5%). Other taxa of particular note in the low-P network were *Rhodospirillaceae* (5%), *Acetobacteraceae* (4%), *Syntrophobacteraceae* (3%), *Syntrophorhabdaceae* (2%), *Spirochaetaceae* (2%), *Methylococcaceae* (1%), and *Syntrophaceae* (1%).

Community Level Attributes

The average *rrn* operon copy number ranged from 1.15 ($\pm$ 0.012) in site 8 (deep soils) to 1.49 ($\pm$ 0.031) in site 9 (shallow soils; Figure S3 and Table S7). Significant differences were seen in *rrn* operon copy numbers between sites for all depths

(shallow $p = 0.0197$; intermediate $p = 0.016$; deep $= 0.008$), namely, in shallow soils, site 1 (*Raphia*) was significantly lower than sites 4 (mixed forest), 7 (*Campnosperma*), and 9 (bog); and site 9 (bog) was significantly higher than sites 6 and 8 (*Campnosperma*). In intermediate soils, site 1 (*Raphia*) was significantly lower than sites 4 (mixed forest) and 7 (*Campnosperma*); and site 9 was significantly greater than sites 1, 2 (*Raphia*), 6, and 8 (*Campnosperma*). For deep soils, site 1 (*Raphia*) was significantly greater than sites 4, (mixed forest) 6, and 8 (*Campnosperma*); site 2 (*Raphia*) was significantly greater than sites 4 (mixed forest), 6, and 8 (*Campnosperma*); and site 3 (mixed forest) was significantly greater than site 8 (*Campnosperma*).

Average multivariate dispersion (a measure of β-diversity) for the fungal communities ranged from 0.327 ($\pm$ 0.08) in the intermediate soils of the mixed forest site (site 4) to 0.552 ($\pm$ 0.09) in the intermediate soils of the mixed forest site (site 3) and ranged from 0.108 ($\pm$ 0.03) in site 1 to 0.168 ($\pm$ 0.11) in site 9 shallow soils for the bacterial communities (Figure S4 and Table S8). Although the bacterial communities of shallow soils had a general increase in β-diversity along the gradient (Figure S4 and Table S8), a Kruskal-Wallis test indicated no significant difference in β-diversity between sites for either bacterial or fungal communities.

Discussion

Fungal Microbiome Composition

Although few significant differences were seen between sites and depths with respect to fungal α-diversity, intermediate sites within the mixed forests (sites 4 and 5) exhibited the greatest α-diversity (Fig. 2), particularly in shallow and intermediate depths, likely because site 5 has the greatest canopy diversity of all sites along the transect ($H' = 1.65$; note: canopy diversity was not quantified in site 4) [5]. There was a high proportion of fungi not assigned to family (71%), especially when compared with bacteria. This is likely due to database limitations, as fungal communities in tropical peatlands are understudied [11], highlighting the necessity for further characterization of fungal community composition and function within peatland soils. In addition, the dominant fungal families identified here were all cosmopolitan [39], further indicating that the dominant fungi identified were those that have been described throughout the globe, and further highlighting that tropical fungal diversity is understudied.

Within the taxa that were assigned to family, the dominant fungal families showed considerable variability along the P transect, particularly when compared with bacterial communities (Fig. 3). When tested with an ADONIS test, position along the transect explained more community variability than vegetation zones. In general, fungal ecological strategies shift
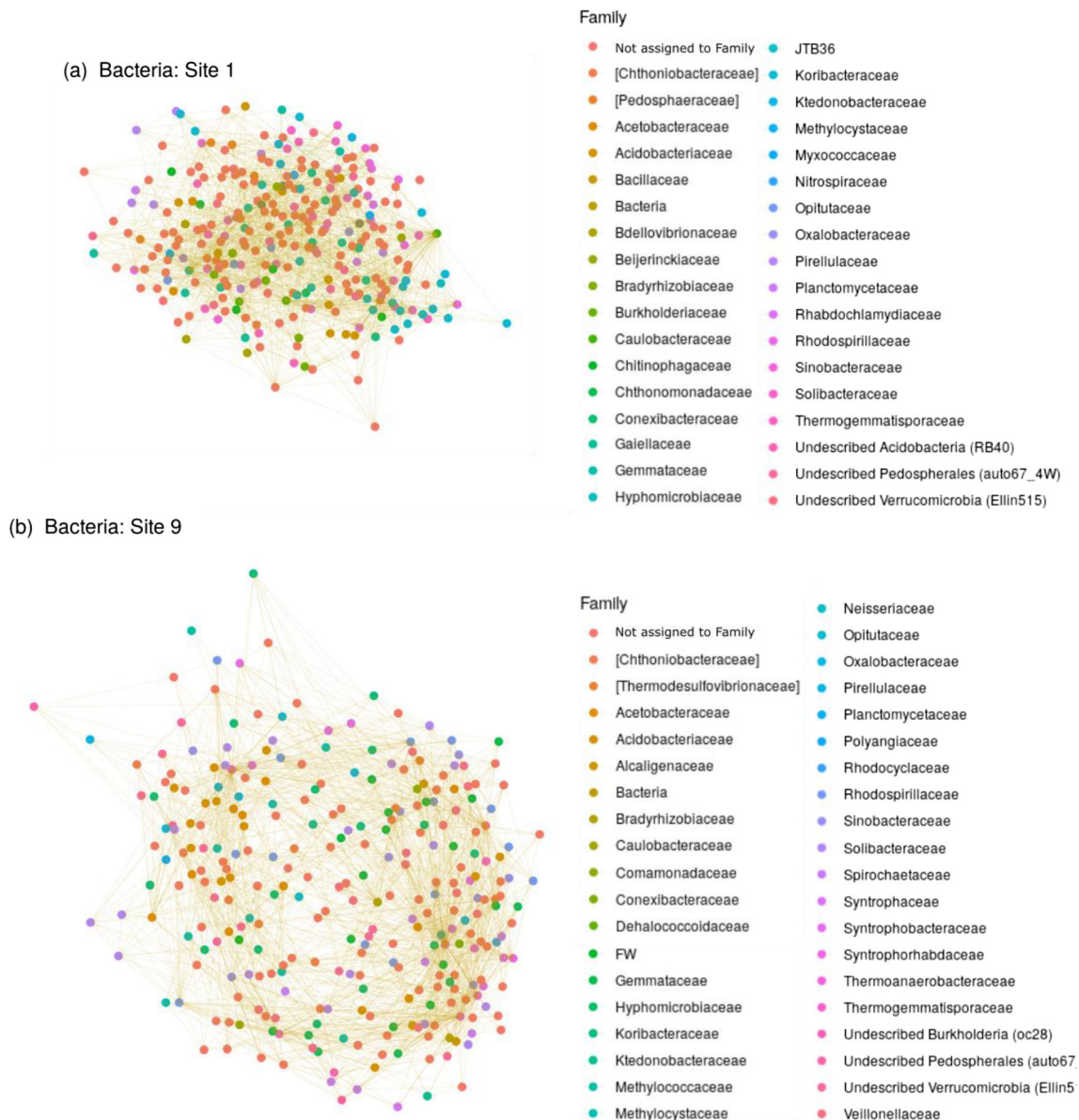
Fig. 6 Networks for bacterial communities at both high-P (**a**) and low-P (**b**). Nodes represent bacterial OTUs and are colored based on bacterial families. Edges represent putative relationships between OTUs and edge color is consistent for every edge (i.e., it does not represent any specific network attribute)

along the gradient: saprotrophic families were found in the high- to intermediate-P sites (sites 1–6; *Raphia* to *Campnosperma* forests), a mix of saprobic and parasitic families dominated intermediate-P sites (sites 6 and 7, *Campnosperma* forest). The low-P site (site 9, bog) was dominated by biotrophic, endophytic, and epiphytic families. Specifically, the cosmopolitan saprobes dominant in the high-P *Raphia* sites included *Mortierellaceae* and *Mycenaceae*. *Mortierellaceae* have been described in boral peat bogs and forests [40, 41], and members (specifically, the genus *Mortierella*) can harbor bacterial endosymbionts, often *Betaproteobacteria* [42]. Thus, future studies of bacterial-fungal associations within this system would be well

served to investigate relationships between these taxonomic groups. *Mycenaceae* is a cosmopolitan saprobe family that is an early colonizer of detritus, with a few species that may be mycorrhizal [39]. Cosmopolitan saprobes dominated the mixed forest sites (sites 3–5) including *Herpotrichillaceae*, *Nectriaceae*, and *Trichomaceae*. *Herpotrichillaceae* is common in extreme environments and has a diversity of ecological strategies [39]. Interestingly, a species within this family, *Heteroconium chaetospira*, can form mutualistic relationships with non-mycorrhizal plant species that exchange N for sucrose [43, 44], suggesting that additional study into plant-fungal interactions is warranted at these sites. *Nectriaceae* is commonly a saprobe but can also be parasitic, and members of

this family have been found associated with palm litter in Thai peat swamps [45] and can be associated with litter and other fungi [39]. *Trichocomaceae* are often aggressive colonizers [39] and include members of commonly known genera such as *Aspergillus*, and members of this family have been found in tropical peat soils cultivated for palm oil and in peat primary forests [46]. The most dominant families in the *Campnosperma* forest sites (sites 6, 7) have been reported to have a variety of ecological strategies including saprotrophy and parasitism, and include *Cordycipitaceae*, *Annulatascaceae*, *Teratosphaeriaceae*, and *Sympoventuriaceae*. Both *Teratosphaeriaceae* and *Cordycipitaceae* are commonly described as parasitic [47], but *Teratosphaeriaceae* can also include saprobes and extremotolerant species, including those that can tolerate low pH [48]. *Annulatascaceae* is a common tropical saprobe found associated with rotting wood in freshwaters [39], and *Sympoventuriaceae* can be saprobic or parasitic [49]. The low-P bog site (site 9) was dominated by families that include parasites, endophytes, or epiphytes, and one family that is a common saprobe. *Clavicipitaceae* is common in the tropics and can have parasitic, endophytic, or epiphytic relationships with insects, fungi, or grasses (*Gramineae*) [39]. Members of *Clavicipitaceae* can also be associated with sedges such as *Cyperus virens* [50] and may be associated with the dominant sedge, *Cladium* spp., within this low-P bog. *Hypocreaceae* is a common saprobe that has been found on decomposing vegetation and can also parasitize other fungi. Similarly, *Chaetothyriaceae* has a wide distribution, is commonly found in the tropics, and is characterized as epiphytic or biotrophic parasites on leaves [39].

Overall, the differences between fungal families along the gradient indicate that fungal ecological strategies shift as well, with saprobes dominant in the majority of sites and a gradual transition to more fungal parasites, endophytes, and epiphytes within low-P regions. Additional characterization of fungal community structure and function within tropical peatlands, as well as additional studies of the active fungi within this peatland, would provide further insights into the role of fungi in mediating organic matter turnover and their relationship with other biogeochemical cycles.

Bacterial Microbiome Composition

Bacterial community composition was significantly different between sites, vegetation zones, and depths, although differences between sites explained the greatest variation in community composition. Generally, bacterial α-diversity in shallow and intermediate soils was greater in high-P (*Raphia*) to intermediate sites (mixed forest and *Campnosperma*). Interestingly, α-diversity in deep soils declined significantly along the gradient, possibly due to P-limitation (Fig. 2). There were only significant differences in bacterial OTU abundance within surface soils. However, as with the fungal communities, the lack of significant differences in OTUs within intermediate and deep soils is likely due insufficient statistical power to capture significant differences within these depths, which is common for metabarcoding studies [38].

In shallow soils, the most notable families that changed significantly along the gradient were *Acetobacteraceae*, *Syntrophaceae*, and *Syntrophobacteraceae* (Fig. 5). *Acetobacteriaceae* was greatest in the low-P bog site, while *Syntrophaceae* and *Syntrophobacteraceae* increased in the shallow soils of the low-P site, but had the greatest abundance in the *Campnosperma* forest (site 6), likely due to differences in redox zonation at these sites. As discussed below, *Syntrophaceae* was found in greater abundance in deeper soils in the low-P bog site, but this increase in abundance was not statistically significant. However, the significant changes in these taxa in shallow soils, as well as changes in family abundance in deeper soils, have potential implications for methanogenesis pathways within this site.

Although the abundances of some families changed significantly along the gradient, the most abundant family, *Koribacteraceae*, was consistently dominant in all sites and depths, with notable exception of the intermediate and deep soils of the low-P bog site. *Koribacteraceae* is an acidophilic family that has been reported in peatlands from Finland [51] to Indonesia [52], but few studies have characterized this family. *Koribacteraceae* are members of the class *Acidobacteriia*, which is a dominant bacterial class in many acidic peatlands [5, 53], and a few members of this class have been isolated and characterized [51]. They are generally thought to be aerobic, but some members have been found to grow in anaerobic conditions [54]. Members of this class have one or two copies of gene encoding 16S rRNA and have relatively slow growth, suggesting that at least some members of this family may be oligotrophs [55]. Interestingly, *Acetobacteraceae* and *Syntrophaceae*, but not *Koribacteraceae*, were the dominant bacterial families in the intermediate and deep soils of the low-P bog. *Acetobacteraceae* has previously been described in peatlands along the Sanjiang Plain in China [56], and genera within this family have been categorized as the acetic acid bacteria (AAB). The AAB are aerobic bacteria that are found in acidic environments and are particularly well studied in food science. They partially oxidize carbohydrates and produce compounds including aldehydes, ketones, and organic acids [57]. In addition, these taxa can also act as plant-growth promoting bacteria, as they can form associations with vegetation and fix N_2 [58]. Interestingly, fungal communities also showed a greater abundance of families engaged in plant associations at the low-P bog site, suggesting that microbial-plant relationships may be heightened within the low-P regions of this gradient.

The high abundance of both *Acetobacteraceae* and *Syntrophaceae* in the low-P bog has implications for the

methanogenic food web at this site [59]. A previous study found that the majority of CH_4 produced in this system was predominantly produced through hydrogenotrophic methanogenesis [7]. However, this previous study only included the vegetation zones within the *Raphia* and mixed forest sites that were studied here, and the dominant pathway of methanogenesis in the low-P bog site is currently unknown. The community composition data presented here suggests that conditions within the low-P bog site may promote a dynamic syntrophic-methanogenic consortia (discussed further below) and that future studies should consider evaluating isotopic composition and production pathways of CH_4 at this site, as well as the active bacterial and methanogenic archaeal community.

Network Attributes and Membership

There were distinct differences between high- and low-P network attributes, suggesting potential differences in microbial interactions between high- and low-P sites in this system. Contrary to our hypothesis, the low-P bacterial network had more edges when compared with the high-P network, but they had almost the same number of nodes (291 and 294, respectively), suggesting that there may be more interactions between taxa within the low-P site. In addition, the low-P site had a lower proportion of negative edges when compared with the high-P site, suggesting that there may be more mutualistic interactions among taxa within the low-P site.

Network membership, as well as the shift in the most abundant taxa within the low-P bog described above, further indicated that microbial associations, particularly relating to methanogenesis, are likely important in the low-P bog. In addition to the most abundant taxa, *Acetobacteraceae* and *Syntrophaceae*, networks revealed that other taxa in the low-P site are likely important components of the microbial community. Bacterial fermenters and syntrophs are critical metabolic guilds involved in the production of substrates for methanogenesis [16, 60]. Bacterial families that are likely involved in methanogenic food webs and were found in our low-P bacterial networks include *Rhodospirillaceae*, *Acetobacteraceae*, *Syntrophobacteraceae*, *Syntrophorhabdaceae*, *Syntrophaceae*, and *Methylocystaceae*. *Rhodospirillaceae* can produce H_2 from formate and members of *Acetobacteraceae* have also been shown to assimilate formate [59], suggesting that these families may be consuming formate within this site. The turnover of the fermentation products butyrate, ethanol, and propionate is thermodynamically favorable for syntrophs if H_2 and formate concentrations are sufficiently low, which is often maintained by the methanogenic partner of the consortium [60]. However, *Acetobacteria*, and possibly *Rhodospirillaceae*, may play a role in keeping formate low in this system as well at this location, although further experimental evidence is

needed to confirm. In addition, acetogenesis has not been well characterized in *Acetobacteraceae*, but it is possible that they may have undescribed acetogenic capacity [59].

We found several syntrophic bacterial families, including *Syntrophobacteraceae*, *Syntrophorhabdaceae*, and *Syntrophaceae* in the low-P network, suggesting that they conditions are favorable for syntrophy and that they may be producing substrates for methanogens, which has been seen previously in the sub-tropical Everglades peatland [61]. Lastly, *Methylocystaceae* was also observed in the low-P network, which is an aerobic family that has been found in acidic peatlands and is capable of conducting N_2 fixation and methanotrophy [62, 63]. The presence of *Methylocystaceae* suggests that methanotrophs may also be an important component of the low-P bog community and supports previous work that found alterations in methanotroph community composition with changes in P-availability [64, 65]. Overall, the families seen within the low-P site, and particularly within the low-P network, indicate that there is likely a syntrophic-methanogenic consortium within this system and that further characterization of the metabolisms and activities of these families at this location will provide additional insights into the assembly of syntrophic-methanogenic consortia within tropical peatlands. Specifically, further investigation into the networks of active microbial communities at these sites could provide additional insights into the behavior of microbial communities within this unique peatland, particularly when incorporated with CH_4, CO_2 fluxes, and isotopic composition, as well as quantification of metabolic intermediates such as H_2, acetate, butyrate, ethanol, and propionate. Additionally, although the evaluation of *Archaea* was outside the scope of this study, additional characterization of *Archaea*, particularly methanogens, is warranted in this system.

rrn Operon Number

Community aggregate trait values are used to evaluate broad community responses to changes in succession [14] or ecological strategies [13, 66]. One such indicator is the mean number of *rrn* operons within a community, which has a strong relationship with microbial growth rates [67] and can provide insights into microbial ecological strategies and resource utilization [13]. Communities with high mean *rrn* operon copies are thought to be dominated by fast-growing microorganisms, while communities with low *rrn* operon copies may be dominated by slow-growing organisms with streamlined genomes that are less capable of monopolizing high resource availabilities [13, 68], although further validation in field conditions is needed. Across the gradient, the average numbers of *rrn* operons were consistently within the range of those from oligotrophic bacteria [13, 69], which agrees with the "oligotrophic" characterization of the dominant bacterial family, *Koribacteraceae*, in previous studies [55].

Community *rrn* operon copy numbers changed slightly along the gradient, depending on the soil depth. Interestingly, surface soils (0–4 and 4–8 cm) showed an increase in *rrn* number along the P gradient, while deep soils (24–30 cm) showed a decrease in *rrn* number along the P gradient (Figure S3), suggesting that further validation within field conditions is warranted. In surface soil, the intermediate sites along the transect had the highest α-diversity measures, for both bacterial and fungi. However, in deep soil (24–30 cm depth), α-diversity was highest in the high-P sites, for both bacteria and fungi. It is possible that vegetation diversity drives an increase in bacterial and fungal α-diversity within surface soils, as intermediate sites along the transect are characterized by the greatest canopy diversity as well [5]. Although not statistically significant, bacterial community multivariate dispersion, a measure of β-diversity, increased along the gradient in shallow soils (Figure S4), indicating an increase in community heterogeneity along the gradient and a subsequent increase in β-diversity [14, 68], suggesting that bacterial communities within low-P zones may be more heterogenous than those in high-P zones, which was also supported by the presence of heterogeneous consortia within low-P microbial networks.

Conclusions

Overall, we found that both bacterial and fungal communities changed in a systematic manner along the gradient and that members of low-P bog communities had distinct ecological strategies. For instance, fungal communities within the low-P bog were dominated by fungal families classified as parasites, endophytes, and epiphytes, while fungal communities in other sites were largely dominated by saprobes. Further investigation into fungal-plant interactions, particularly within the low-P bog site, would provide additional insights into fungal ecological strategies within oligotrophic tropical peatlands. Additionally, with community abundance data and bacterial networks, we found that, unlike the majority of sites that were dominated by the acidophilic *Koribacteraceae* family, the bacterial community of the low-P bog site contained families such as *Acetobacteraceae*, *Rhodospirillaceae*, *Syntrophobacteraceae*, *Syntrophorhabdaceae*, *Syntrophaceae*, and *Methylocystaceae*, which are likely members of syntrophic-methanogenic consortia at this site. Further characterization of the active microbial community, including *Archaea*, as well as CH_4 and CO_2 fluxes and isotopic composition, and metabolic intermediates, would provide further insights into CH_4 and CO_2 production within oligotrophic tropical peatlands and the microorganisms driving greenhouse gas fluxes. Additionally, we found that the low-P site exhibited higher β-diversity and greater network edges possibly indicating that this is a heterogeneous system with many potential microbial interactions. This work is an initial step in characterizing the diversity, composition, and potential interactions of bacterial and fungal communities within this ombrotrophic peat dome, yet additional investigation into the functional capacity of microbial communities within this site is needed to further understand the relationships between soil microorganisms and their role in driving tropical peatland biogeochemistry.

Compliance with Ethical Standards

Conflict of Interest   The authors declare that they have no conflicts of interest.

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