THESIS

BIOGEOGRAPHIC VARIATION AND RESPONSES TO DRYING IN LOWLAND TROPICAL FOREST SOIL MICROBIAL COMMUNITIES

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ABSTRACT

BIOGEOGRAPHIC VARIATION AND RESPONSES TO DRYING IN LOWLAND TROPICAL FOREST SOIL MICROBIAL COMMUNITIES

Soil contains more carbon (C) than terrestrial vegetation and the atmosphere combined, with some of the largest terrestrial C stocks in tropical rainforests. Soil microbes decompose organic matter, playing a vital role in the storage or loss of soil C. With climate change, drought conditions are predicted to increase in many tropical regions, including both chronic drying and extended drought, potentially influencing these processes. This project explored the effects of chronic and seasonal drying on soil microbial communities across four distinct tropical forests in a long-term drying experiment. In this thesis, I investigate the effects of a chronic drying manipulation on soil microbial community abundance and variation across different forests and seasons. I also compared my findings with previously published data from these forests after short-term drying. This project used soils from a long-term drying experiment established in 2018 across four seasonal lowland forests in Panama. Soils were collected from 0 – 10 cm depths during three seasonal periods in control and drying plots in 2024 and 2025 from a total of 32 plots (n = 4 per forest per treatment). The forests varied in baseline rainfall and soil fertility. For microbial community assessment, the 16S V4 rRNA region was amplified to identify bacteria, and the ITS1 rRNA region was used for fungi. I calculated alpha and beta diversity indices, and compared taxonomic community composition. I found significant biogeographic variation in microbial diversity and taxonomy, with significant differences across the forests and significant

effects of the drying treatment. For bacteria, some trends were consistent with prior findings after shorter-term drying, and I also identified new trends, such as a robustly significant increase in Actinomycetota and marginally significant increase of Pseudomondata within the drying plots across the forests, as well as more differences within the forests individually, suggesting the development of a drought microbiome. While fungal communities were resilient under shorter-term drying, I observed a longer-term shift toward a robust significant increase in relative abundance of drought-tolerant communities such as Agaricomycetes and Eurotiomycetes, and marginal increases of Dothideomycetes and Sordariomycetes. To balance these increases, I also observed marginally significant declines in the fungal classes Glomeromycota Incertae sedis and Rhizophydiomycetes. These results suggest that drying in tropical forests will have a significant long-term impact on soil microbial diversity, with potential downstream effects on soil C cycling and storage.

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Introduction

Tropical forests hold a massive amount of carbon (C) (Sundqvist et al., 2024), and within these forests, tropical soils account for some of the largest C stocks on Earth. (Crowther et al., 2019; Jackson et al., 2017). Climate change can threaten these carbon cycles (Mitchard, 2018; Sullivan et al., 2020), and tropical regions are predicted to experience more frequent and intense droughts (Chadwick et al., 2016; Easterling et al., 2000; Meehl et al., 2006). Within these tropical regions, drought can also affect nutrient cycling (O'Connell et al., 2019), as well as potentially cause declines in carbon storage as suggested in several large-scale reviews (Cusack et al., 2011; Gatti et al., 2014; Phillips et al., 2009). Soil moisture plays a critical role in influencing belowground biogeochemical cycles, as illustrated in a study on the effects of drought on belowground processes within Puerto Rican tropical forests (Bouskill et al., 2016). A decline in soil moisture results in a change in the water potential and physical soil matrix, thus causing osmotic stress on the organisms within (Schimel et al., 2007; Moyano et al., 2013). These environmental changes within the soil, caused by drought, can impact the community composition of the microbial communities (Bouskill et al., 2013; Chacon et al., 2023), as well as their functions (Bouskill et al., 2016; Callahan et al., 2016; Buscardo et al., 2021), which can impact the biogeochemical processes within (Manzoni and Katul, 2014).

Heterotrophic soil microbes derive energy primarily from organic carbon compounds. These microbes drive the carbon cycle by transforming organic carbon through decomposition to carbon dioxide (CO_2) and returning C to the atmosphere (Raza et al., 2023). Tropical soils act as a major carbon sink, and about 30% of their carbon emissions come from soil respiration (Jandl et al., 2007; Anderson-Teixeira et al., 2016). Thus, microbes provide a control point over ecosystem carbon cycling (Huang et al., 2020).

Microbial community resiliency is often shaped by historical climate (Evans and Wallenstein, 2014; Hawkes and Keitt, 2015), making historical precipitation and dry season lengths important to understanding how microbes may react to drought (Azarbad et al., 2020). The impact of drying on tropical soil microbial communities remains poorly understood, as does the importance of microbial diversity in driving carbon cycling. Understanding the relationship between soil microbial diversity and long-term drought is critical to understanding the future of carbon cycling in tropical forests.

Previous studies have shown that microbial community composition is distinct across lowland tropical forest sites that vary in rainfall and soil fertility (Chacon et al. 2023). Soil microbial communities have also been shown to have distinct responses to drying. For example, a drying experiment that excluded all throughfall in Puerto Rican rainforests led to a shift in microbial communities and parallel changes in extracellular enzymes that decompose organic matter (Bouskill et al, 2016), suggesting a potential change in carbon metabolism and biogeochemical cycling. The taxonomic responses of microbes during drought can vary greatly, with gram-positive bacteria typically being more drought-tolerant than gram-negative bacteria (Manzoni et al., 2012; Uhlírova et al., 2005). Gram-positive bacteria are typically thought to be more drought-resistant due to their thick peptidoglycan cell walls. These act as a barrier to osmotic stress and can allow cells to maintain functioning even during dry periods (Manzoni et al., 2012). But there have been some exceptions to this within grassland ecosystems, with gram-negative bacteria, *Acidobacteria, Verrucomicrobiota,* and *Alphaproteobacteria* showing tolerance towards drought, and the gram-positive *Actinobacteria* showing sensitivity to drought (de Vries et al., 2018). The community response of fungi can greatly vary as well, with grassland groups such as *Ascomycota* and *Glomeromycota* observed to be drought-tolerant, while

lower-level taxonomic groups, such as *Mortirelleceae* family, being more drought sensitive (de Vries et al., 2018). Within tropical forests, *Sordariomycetes* (*Ascomycota*) and *Agaricomycetes* (*Basiodiomycota*) have increased during a decline in precipitation caused by throughfall exclusion experiments (Buscardo et al., 2021). These results suggest that within tropical forests and other ecosystems, there may be consistent shifts in membership of bacterial and fungal groups within communities due to drought after shorter periods of drying.

In this project I used a precipitation throughfall manipulation in four seasonally dry Panamanian forests which vary in rainfall and soil nutrient levels. The project was established in 2018, and microbial community data were initially characterized after an initial throughfall exclusion period of 1-2 years. After this short period, there was already an overall shift in the bacterial communities in the three forests on infertile soils toward what the authors termed a "drought microbiome." There was almost no shift in a forest with fertile soils, and no shifts in fungal communities in any forest (Chacon et al., 2023). In the Panama experiment, there were increases in various gram-positive and gram-negative bacterial taxa during the first two years. Specifically, the relative abundance of the Phylum *Acidobacteria* had a significant increase with drying in the infertile experimental sites, and the Phylum *Proteobacteria* also increased in two of the forests. These data from the first two years of drying indicate that bacterial communities responded quickly to drying, while fungal communities were resilient. However, it is unclear if these initial responses are indicative of longer-term drying effects, or were only initial short-lived responses to drought.

This project was conducted within the same four Panamanian forests to follow up on the Chacon et al. 2023 study and assess longer-term effects of rainfall exclusion on microbial communities. Soils were collected during three seasonal timepoints after 6 years of prolonged

experimental drought during 2024-2025. I used DNA metabarcoding to calculate the alpha and beta diversity metrics within the control and treatment plots, and to assess taxonomic changes in the community. Alpha diversity metrics allowed for analysis of the number of different taxa within samples and beta diversity provides insights on differences in community compositions between samples within forests, both providing unique insights on the variation in these soil microbial communities. This experiment tested two hypotheses related to the structure of these microbial communities across forests and their resilience to long-term chronic drying within tropical forest ecosystems. The hypotheses were:

H1. Distinct microbial communities are present across forest biogeographic variations, with shifts corresponding to soil characteristics like fertility and pH, because soil properties select for locally adapted taxonomic groups.

H2. Prolonged chronic drying changes tropical forest soil microbial communities, including bacteria and fungi, toward stress-resistant microbes, because long periods of drying promote taxa with greater drought tolerance.

Methods

Site information

This study leveraged a long-term chronic drying experiment in the Isthmus of Panama at four different lowland tropical forests with varying rainfall and fertility: the Panama Rainforest Changes with Experimental Drying (PARCHED) manipulation, established in 2018 (Figure 1 and Table 1, see experimental details below).

The experiment is set at four forests along the Isthmus of Panama, which have strong variation in rainfall and soil properties. In general, the lowlands of Central Panama have a monsoonal climate with a ~3-month dry season, typically from mid-December to late-April, followed by a rainy season the remainder of the year, but the length and strength of the dry season vary spatially according to total mean annual precipitation (MAP). There is a rainfall gradient from South to North, with an increase in annual precipitation from 1750 mm MAP in the South to 34000 mm MAP in the Caribbean to the North. The PARCHED experimental forests span a range of 2350 mm MAP to 3400 mm MAP, representing the wetter half of the rainfall gradient. During the dry seasons, the monthly annual rainfall can fall as low as 60 mm (Leigh 1999; Leigh et al. 1996). The Isthmus of Panama also has strong variation in geological substrate (Stewart et al. 1980), which causes unique variations in soil fertility spanning from 0.50 to 8.44 mg of phosphorous in the soil (Table 1). Because of the extreme geological variation, soil fertility does not correlate with rainfall variations (Pyke et al., 2001; Turner and Engelbrecht, 2011), which is somewhat unusual among rainfall gradients.

The four PARCHED forests vary in rainfall and soil fertility, representing a large portion of the broader Isthmus variation. The Sherman Crane site in the north was the wettest with ~3421 mm MAP (Paton 2021a), located in the San Lorenzo National Park. There are two “sister”

sites on the Buena Vista Peninsula, P12 and P13, with rainfall of ~2590 mm MAP, but with strong differences in soil fertility due to different geological substrates, with P13 on soil much more fertile in phosphorus [P] and base cations than the other three sites (Table 1). The driest site is on the Gigante Peninsula with ~2335 mm MAP. Gigante, P12, and P13 are located in the Barro Colorado Island Nature Monument (BCNM) and near Barro Colorado Island (BCI), which has a STRI meteorological station used as a reference for the MAP of these forests (Paton 2021b). Sherman Crane rainfall data were also collected at the Smithsonian Tropical Research Institute (STRI) meteorological station (Paton 2021a).

Three of the PARCHED forests are located on strongly weathered soils and are infertile with respect to rock-derived nutrients (P12, GIG, SC). The most fertile forest, P13, has higher concentrations of base cations, P, and mineral nitrogen (N) compared to the other infertile sites (Table 1, Cusack et al., 2018, 2019), although nitrogen (N) levels are relatively high across all of the sites, typical of lowland tropical forests (Table 1).

Field throughfall exclusion experiment

Each of our study forests had four throughfall exclusion plots paired with four control plots. Plots were 10 m x 10 m, with 4 plots each of exclusion and control laid out in pairs. At each forest, there are four sets of pairs, with each pair having similar topography, spatial proximity, and tree cover. Exclusion structures are covered with clear plastic laminates covering part of the area, originally designed to exclude ~50% of the natural rainfall, and increased to exclude ~70% of throughfall in 2022 (Figure 3). The structures have a slope of 11.3˚ to angle the rainfall away from the plots, which reduces the amount of precipitation that reaches the soil in the experimental plots, causing chronic drying. The plots all have a 50 cm trench surrounding

them, which was lined with a heavy plastic and then filled with soil to prevent any diffusion of water from soil outside the plots, as well as to prevent roots from searching for water outside the designated plots, with further construction details in Dietterich et al. (2022). The laminates are cleaned and maintained monthly by a full-time technician to ensure that there is no light exclusion, as well as to repair damage from tree and limb falls.

Across the four forests for our sampling periods, the throughfall-exclusion plots had a statistically significant average 17% reduction in soil moisture compared to the control plots (SI Figure 1, $p < 0.05$), which is less than but approaching the drying that occurred during the dry versus wet season for the same time period (23% seasonal drying, SI Table 1).

Field sampling

I sampled the soils at each forest in December 2024, in March 2025, and in May 2025. The December time point correlates with the end of the rainy season, March represents the peak of the dry season, and the May sampling point correlates with the early wet season. For each sampling time point, soil microbial samples were collected from both the control and throughfall exclusion (i.e. drying) plots at all four forests within one week. The samples were taken at a depth of 0-10 cm, with a soil corer with a diameter of 2.2 cm. Three subsamples were taken at each plot, added to one bag, and the soil was then homogenized by hand. The samples were collected while wearing gloves, and the soil corer was rinsed with DI water and wiped with a towel between each plot to avoid cross-contamination. The samples were put on ice in a cooler during each day of field sampling, then stored in a -20˚C freezer after transport to the laboratory. Along with the microbial samples, additional soil samples at 0-10 cm and 10-20 cm were taken with a soil corer with a diameter of 3.81 cm from three locations within each plot, for a total of

six samples per plot (three 0-10 cm and three 10-20 cm) to calculate gravimetric soil moisture, and for chemical analysis. These soils were stored at room temperature in the field and then placed in a 4˚C fridge for storage. The soils collected for microbial community analysis were brought to the STRI Naos Molecular Laboratory for biological analysis. The rest of the soils were shipped to Colorado State University for physical and chemical analysis.

Temporal variation in precipitation and soil moisture during sampling period

To characterize temporal variation in rainfall and soil moisture during this study, I referred to weekly precipitation and soil moisture data from the BCI Lutz catchment, which is part of the long-term research monitoring program at the Smithsonian Tropical Research Institute. Monthly precipitation and soil moisture (gravimetric, 0-10 cm) from 2022-2025 show annual variation in rainfall from year to year (Figure 2). While none of the studied forests are located on BCI, three of the four (GIG, P12, and P13) are nearby, which makes this data a useful gauge of the overall seasonal trends. 2024 and 2025 were both wetter years than the 100-year average, with greater MAP (Figure 2a). Soil moisture was similarly higher than average for our sampling period, even throughout the dry season (Figure 2b). While our sampling points in December of 2024 and March of 2025 were not taken while the precipitation was higher than the average, all three of the timepoints sampled had greater soil moisture than the long-term average since 1972, and our sampling timepoints followed periods of higher than usual rainfall (STRI Physical Monitoring Program, 2025).

DNA extraction and amplicon sequencing

Microbial soil samples were thawed the day of use first on ice, then at room temperature; the rest of the samples remained in the -20˚C freezer. Total genomic DNA was extracted from 0.25 g of each soil sample using the DNeasy PowerLyzer PowerSoil Kit (QIAGEN) following the manufacturer's instructions until the final step, when the flow-through was removed from the collection tube, placed back on the membrane of the spin column, and centrifuged a final time to ensure maximum DNA was extracted. Each day of extractions, a negative control was added to check for any cross-contamination. A random selection of samples was checked daily on a Nanodrop spectrophotometer (ThermoFisher) to confirm that there was DNA present.

DNA metabarcoding, using a two-stage PCR amplification protocol, was used to construct sequencing libraries. PCR1 amplified the target locus and added partial Illumina adaptors, and PCR2 was performed to add sample barcodes and additional Illumina adaptors. The 16S V4 rRNA gene was amplified for the identification of bacteria and archaea, and the ITS1 rRNA region for the determination of fungi and archaea present. The 515F-806R primer pair (Caporaso et al. 2011) was used for the 16S sequences, and the ITS1F-ITS2R primer pair (White et al. 1990) was used for the ITS1. PCR reactions were performed in triplicate, with a water negative control run with each round. The PCR1 reactions were performed under a hood, with all equipment and supplies UV sterilized for 20 minutes prior to the start of each day. Reactions were done in 12.5 µL volumes with the following reagents: sH2O (5.9 µL), 2X Platinum Taq MasterMix (5 µL), bovine serum albumin (0.2 µL), 10 mM 515F primer (0.2 µL), 10 mM 806R primer (0.2 µL), and 1µL of DNA template. The ITS1 reactions were slightly modified by using only 4.9 µL of sH2O and 2 µL of the DNA template. The 16S gene amplification was done with the following settings on the thermocycler: 94 °C for 3 minutes,

then 25 cycles of 94 °C for 45 seconds, 50 °C for 1 minute, and 72 °C for 90 seconds, then a final extension cycle of 72 °C for ten minutes. The ITS1 amplifications were done with these thermocycler settings: 94 °C for 3 minutes, 30 cycles of 94 °C for 30 seconds, 51 °C for 30 seconds, and 72 °C for 30 seconds with a final addition at 72 °C for ten minutes. The first PCR1 replicate for each locus was fully checked via gel electrophoresis to confirm amplification of the selected genes (SI Figure 2), then a random selection of samples was gel checked from replicates two and three. PCR1 triplicates were pooled using 3 µL from each replicate for 16S and 8 µL of each replicate from ITS1. Bead washes of the ITS1 pools were done prior to performing PCR2 to remove the primer dimers that were visible on the agarose gel; this step was not done for the 16S samples. Every ITS1 sample was Nanodropped after the beadwash to confirm that DNA was still present.

PCR2 reactions were done at a sterile lab bench with 10 µL reactions using the following reactants: sH2O (2 µL), 2X Platinum Taq MasterMix (5 µL), 2.5 mM F primer (1 µL), 2.5 mM R primer (1 µL), and the pooled DNA product from PCR1 (1 µL). For ITS, 4.9 µL of sH2O was used, then 2 µL of the pooled ITS1 DNA from PCR1 was added. The sH2O and Taq MasterMix were made and mixed first, then aliquoted into each tube before the correct F and R primers and the DNA were added. Unique barcodes were used for each sample and recorded in a table to track which barcode belongs to each DNA sample. The PCR2 amplification was done with these thermocycler settings: 94 °C for 3 minutes, 94 °C for 30 seconds, 50 °C for 30 seconds, and 72 °C for 30 seconds for 6 cycles, with a final addition of 72 °C for ten minutes, with the ITS1 annealing stage performed at 51 °C. A random selection of PCR2 products for both ITS and 16S was checked with gel electrophoresis, run next to the corresponding PCR1 product, confirming larger bands for the PCR2 products, to show successful addition of the Illumina barcodes. The

PCR2 products were then pooled into single tubes to construct sequencing libraries, including pooling 3 μL of each 16S sample and 3 μL of each ITS1 sample, with an additional 3 μL added to each ITS1 sample that had faint lines on the gel. 16S had a total of 240 μL of pooled samples, and ITS1 had 270 μL; these were split into two tubes to be purified using KAPApure magnetic beads. Two washes were performed for both ITS1 and 16S to ensure maximum DNA retrieval, eliminate any primer dimers, and concentrate the libraries. The final ITS and 16S libraries were checked on the Nanodrop spectrophotometer (ThermoFisher) to confirm their DNA concentration. The samples were then diluted in elution buffer based on the Nanodrop concentrations to obtain a 5-6 ng/ μL concentration in 30 μL of diluted DNA for both 16S and ITS1.

Final quality control checks included checking the size of the libraries on a Bioanalyzer (Agilent) and quantifying concentrations on a Qubit spectrophotometer. Samples were sequenced in single runs per library (16S and ITS separate) on an Illumina MiSeq using 2x300 bp paired-end sequencing at the STRI Naos Molecular Laboratory in Naos, Panama.

Microbial community composition - data processing

I followed a series of protocols to clean and check the quality of the data. I also created three main data products for microbial diversity: 1) alpha diversity, measured as a Shannon Diversity Index (Shannon, 1948); 2) beta diversity, measured with Bray-Curtis, Unifrac, and Weighted Unifrac metrics (Bray and Curtis, 1957; Lozupone and Knight, 2005; Lozupone et al., 2007); and 3) taxonomic relative abundances (Magurran, 1988). Microbial and fungal community analysis was done in R version 4.5.2 using *DADA 2* (Callahan et al. 2016), phyloseq

(McMurdie et al. 2013), and associated R packages. Details for cleaning, quality assurance/control, and diversity products are described below.

DADA 2 analysis was done at Naos Molecular Laboratory to filter, de-noise, call amplicon sequencing variants (ASV), remove chimeric sequences, and assign taxonomy using the SILVA v.138 reference dataset for bacteria (Yilmaz et al. 2013) and the UNITE database for fungi (Abarenkov et al. 2023). The bacterial ASV table was filtered to retain only sequences with a size range of 250-256 bp; all fungal ASVs were retained. Once ASV sequence tables and associated taxonomy files were created, a sample metadata table was constructed and imported into *phyloseq* for diversity and community analysis.

After creating a phyloseq object, the ASV sequences were added to the phyloseq object and given short names such as ASV1, ASV2, ASV3, etc. A new column was added to the sample metadata that had the number of sequences per sample for further reference. The library size was checked and then graphically shown using the package ggplot2 (Whickham 2016) to confirm that the negative controls did not contain contaminants. Contamination was checked using *decontam* (Davis et al. 2018); nine contaminants were determined, but all were very rare in the dataset, so they were not removed. The presence of mitochondria or chloroplasts was checked for the 16S data, with there being no instances of either. Following this, ASVs with no remaining counts were removed, and samples were checked to ensure that they still contained sequences. All samples were retained, giving a total of 96 samples (4 sites x 2 treatments x 4 subplots x 3 seasons). Finally, the taxonomy was cleaned to add names for every sample. If a taxonomic level was unknown, such as the Family, the next most descriptive factor was filled in, such as the Order. This was done to ensure the best possible taxonomic resolution for each ASV. Once

completed, a phylogenetic tree was added to the phyloseq object for further analysis of the 16S data only.

For alpha diversity, rarefaction curves were created to evaluate the adequacy of sequence coverage for each locus. Data was then rarefied to 25,000 sequences per sample, which retained the greatest amount of taxa and samples for both the 16S and ITS1 datasets. Alpha diversity estimates were calculated using Shannon metrics, and a box plot was then created. Shapiro tests were used to check normality, which was confirmed. As the season was found not to be significant, this was repeated with the dataset averaged by plot (n = 32), and with subsets by each forest (n = 8 per forest over three seasons, total n = 32).

For beta-diversity, the full non-rarefied dataset was used, and a Hellinger transformation was performed to minimize the effect of outliers. Principal coordinates analysis (PCoA) was done using the Bray-Curtis metric for both bacteria and fungi, then the UniFrac and Weighted-UniFrac metrics for just bacteria. PCoA plots were then made for all of these metrics, showing the forests and treatments. The Bray-Curtis metric quantifies differences between distinct sites based on community composition. The Unifrac metric is similar to the Bray-Curtis, yet incorporates the phylogenetic tree when calculating distances between communities among distinct sites. The Weighted-Unifrac metric does the same as the Unifrac metric, but also adds in the relative abundance of microbes present. This allows for a better representation of community structure, while the Unifrac method is more sensitive to rare communities. This was repeated with subsets by season to observe the community composition over sampling months (n = 32). For microbial taxonomic relative abundances, relative abundance plots were then created for bacterial and fungal taxonomic groups, with the full dataset, comparing the most abundant taxa across the forests. Bacteria were analyzed at the phylum level, and fungi were analyzed at the

class level, because preliminary analyses determined that these were the taxonomic levels with the most differences among forests and treatments. Relative abundance histograms were made for both fungi and bacteria, showing the top 11 taxonomic groups, with the rest of the taxa grouped and labeled as "other," for phylum and class, respectively, across the four forests. More relative abundance plots were created for each site subset, comparing treatments for both bacteria and fungi. Finally, FUNGuildR (Nguyen et al., 2016) was used to identify potential functional traits of the common and significant fungal ASVs.

Statistical analyses

To test H1 and H2, we used a series of statistical analyses with each of the three types of microbial data as the response factors, and biogeography (forest and season), and drying treatment as predictors.

First, I used analysis of variance (ANOVA) with Shannon diversity scores as the response factor, and forest site, season, treatment, and all interactions as predictors. This was conducted for the full dataset, and then repeated for each forest, because the forest site was the strongest predictor of Shannon diversity. Seasonal timepoint was not significant in any test, so we then repeated this analysis using plot-averaged Shannon indices across the three timepoints, for a $n = 4$ per forest, per treatment, and a total $n = 32$ plot-scale Shannon indices (averaging the 96 samples taken across three timepoints). Post-hoc and Tukey Honesty Significance Differences (HSD) tests were then used to assess differences in means among groups, and to understand interacting effects. All ANOVA and Tukey HSD test significance levels were set to a $p < 0.05$, both for the overall model and significant factors and interactions.

I then tested the hypotheses with respect to beta diversity using the full dataset across the three timepoints (n = 96). I used a non-parametric permutational analysis of variance (ADONIS), and pairwise ADONIS were then conducted comparing forests and treatments on each of the seasonal subsets. This was done using every distance metric: Bray-Curtis for bacteria and fungi, then Unifrac and Weighted-Unifrac for bacteria. Because the main effect on beta diversity was forest (with no effect of season), we conducted further tests exploring treatment effects within each forest at each seasonal timepoint. To test if the community differences between the forests were from variance within each forest or differences in average values, betadispersion and permutation tests (1000 permutations) were completed.

We then tested our hypotheses with respect to taxonomic relative abundances. Differential abundance analysis was used to identify specific taxa with significant differences among forests, seasons, and treatments using linear discriminant analysis effect size (LefSe) (Segata et al. 2011), as implemented in Microbiome Analyst (Lu et al. 2023). This test was first used on the entire dataset to identify which taxa varied according to forest, season, or treatment. We then repeated follow-up analyses within each forest, because forest was the most significant overall effect, with no effect of season. Robust significant difference levels were set at $p < 0.05$ after FDR correction, and linear discriminant analysis (LDA) scores of >3. Using these levels for significant LDA scores is consistent with the alliteration, with some microbial studies using an LDA score >3 as their standard to identify significant community shifts (Wei-hua et al., 2023), and other studies using an LDA score >4 to identify significant community shifts (Dao et al., 2023).

Because of strong taxonomic differences among the four forests, I then repeated these analyses for each forest separately, at each seasonal time point, assessing seasonal and treatment

differences. Because season was never significant in these tests, I averaged by plot across the seasonal data, and repeated by forest to assess the treatment effects, with an n = 4 per forest, per treatment. For these follow-up analyses with small sample numbers, there were no robust significant differences, so I report trends that were marginally significant findings. To identify marginally significant results, I used a non-corrected p-value < 0.05 and an LDA score of >3.

Results

Sequencing summary

Across four forests and three seasons, a total of 96 soil samples were analyzed for bacterial (16S) and fungal (ITS1) community composition. There was an average of 88,528 sequences per sample for bacteria and 74,989 sequences per sample for fungi. Sequence numbers were relatively even across the four forests for bacteria and fungi (SI figures 3 and 4).

Biogeographic effects on community composition

Bacteria

Overall, forest had a significant effect on bacterial biodiversity, but seasonal time points did not. Shannon species richness of bacterial communities was significantly different across all the forests (ANOVA, $p < 0.05$). Since bacterial species richness was not significantly different by season, samples were averaged across seasons to give one Shannon value per plot ($n = 32$) for the alpha-diversity analyses. There was significantly higher alpha diversity at P13 (Figure 4a), and pairwise comparisons (Tukey, $p < 0.05$) showed that all the forests were significantly different from P13. This shows alpha-diversity is primarily driven by forest level differences, with fertility having a positive relationship with species richness.

Beta diversity metrics indicated that all the forests had significantly different bacterial communities for each of the three timepoints (Figure 5, Table 2, ADONIS $p < 0.05$). Pairwise ADONIS tests revealed that all sites had significantly different bacterial communities from one another ($p < 0.05$). Similar results were found using three metrics of beta-diversity: Bray-Curtis, Unifrac (SI figure 5), and Weighted Unifrac (SI figure 6). Betadispersion and permutation analysis confirmed that all differences were among forests, and not from variance within groups.

Season was also analyzed for biogeographic variation across time, but was not significant in any analyses (ADONIS, $p > 0.05$). Thus, similar to alpha diversity, beta diversity of bacteria varied primarily by forest, yet, with beta-diversity analysis there was a stronger signal, as all forests were significantly different from another.

Differential abundance analysis indicated significant shifts in some bacterial groups across the four forests. Most of the significant differences were at the phylum level, so these are presented here. For the top 11 bacterial phyla present, all had robust, significant differences across the four forests. Community differences among forests were driven by shifts in the relative abundance of the following phyla: Acidobacteriota, Actinomycetota, Bacillota, Bacteroidota, Candidatus Eremiobacterota, Chloroflexota, Entotheonellaeota, GAL15, Gemmatimonadota, Latescibacterota, NB1-j, Nitrospirota, MBNT15, Methylmirabilota, Myxococcota, Planctomycetota, Pseudomondota, RCP2-54, Thermodesulfobacteriota, and Verrucomicrobiota (LefSe LDA > 3, FDR-adjusted $p < 0.05$, Figure 6 and SI Table 2). These trends were sustained throughout lower taxonomic levels with many taxa within these phyla having robust, significant differences across the forests (Online SI, McLaughlin et al., 2026). Thus, there were broad-scale differences in bacterial communities across the rainfall and fertility gradients.

Fungi

Overall, forests had a significant effect on fungal biodiversity, similar to bacteria. The four forests had significantly different fungal species richness for Shannon diversity metrics (ANOVA, $p < 0.05$, Figure 4b). Season was not significant in the ANOVA, so the samples were averaged by plot across the three seasonal time points for the remainder of the alpha-diversity

analyses (n = 32 plots, averaged across season). Pairwise comparisons (Tukey, $p < 0.05$) showed that Sherman Crane tended to have marginally significantly higher alpha-diversity ($p < 0.1$) compared with the driest forest (Gigante) and the fertile forest (P13), with no significant differences from P12. Thus, fungi had broad-scale differences in alpha-diversity among the four forests, with the wettest forest (SC) having marginally greater fungal species richness than the other forests.

Beta-diversity metrics gave similar results for fungi as bacteria. The PERMANOVA on Bray-Curtis distances for fungi indicated significant differences among the four forests (Figure 7, Table 2, ADONIS $p < 0.05$) and pairwise ADONIS revealed that all forests had significantly different communities from one another for Bray-Curtis ($p < 0.05$). When samples were analyzed separately at each seasonal timepoint there were no differences in beta diversity of fungi across the timepoints (ADONIS, $p > 0.05$). Similar to alpha diversity of fungi, the beta diversity differences were primarily driven by forest.

Differential abundance analysis of soil fungi showed significant differences across the forests. Fungal community differences among forests were driven by shifts in the relative abundance of many taxa (Figure 8 and SI Table 2), with differences presented at the phylum and class levels. These included robust significant differences across all forests (n = 96) for phyla Ascomycota, Basidiomycota, Glomeromycota Kickxellomycota, and Rozellomycota. For the top 11 classes of fungi present, 9 had robustly significant differences across the four forests (LefSe LDA > 3, FDR-adjusted $p < 0.05$), specifically, Agaricomycetes (Basidiomycota), Archaeorhizomycetes (Ascomycota), Eurotiomycetes (Ascomycota), Glomeromycetes (Glomeromycota), Lecanoromycetes (Ascomycota), Rozelomycotonia class Incertae sedis

(Rozelomycota), Sordariomycetes (Ascomycota), Tremellomycetes (Basidiomycota), and the phylum Ascomycota.

Some less abundant classes also showed robust significant differences across all the forest, such as, Atractiellomycetes (Basidiomycota), Chytridiomycetes (Chytidiomycota), Chytridiomycota Incertae sedis (Chytidiomycota), Cystobasidiomycetes (Basidiomycota), Leotiomycetes (Ascomycota), Saccharomycetes (Ascomycota), Ramicandelaberomycetes (Kickxellomycota), and the phyla Glomeromycota and Chrytidiomycota. These trends were sustained throughout lower taxonomic levels with many taxa within these phyla and classes having robust, significant differences across the forests (Online SI, McLaughlin et al., 2026). In addition to the robust significant differences there were also marginally significant differences across all the forests (LDA > 3, non-corrected p-value < 0.05) in the phylum Chytidiomycota, and classes Dothideomycetes (Ascomycota), Mortierellomycetes (Mortierellomycota), and Rhizophydiomycetes (Chytidiomycota). Thus, there were broad-scale differences in fungal communities across the biogeographic gradients.

The FUNGuildR analysis revealed the functional traits of the fungal ASVs, and provided insights on the functions of the taxa that were significantly different among forests. The most abundant ASVs that were different among forests and treatments were most likely saprotrophs as well as some symbiotrophs, yet many of the significant classes had broad functional designations, or the possibility of many different functions (Online SI, McLaughlin et al., 2026).

Drying effects on microbial community composition

Bacteria

The drying treatments did not have a significant effect overall on bacterial alpha-diversity with Shannon metrics across forests, but there were some differences within forests. To better understand differences within forests, analyses were done for seasons and treatments within each forest (n = 24, 8 plots per forest x 3 sampling points). There was a significant drying treatment effect in the fertile forest (P13), where control plots had significantly higher alpha diversity for bacteria than the drying plots across time points (Figure 9a). There was a significant treatment*season interaction in the driest forest (Gigante), where the treatment effect was prominent in the in May timepoint. In both the fertile forest (P13) and in the driest forest (Gigante), a significant drying effect was characterized by higher species richness in control versus drying plots (Figure 9b). Thus, while there was not an overall drying effect on bacterial alpha-diversity, two of our forests had suppression of bacterial diversity with drying.

Similar to the drying effect on alpha-diversity, there was only a drying effect on beta diversity in some of the forests. The drying treatment did not have a significant overall effect on beta diversity of the bacteria using Bray-Curtis, Unifrac, and Weighted Unifrac metrics (Pairwise ADONIS $p > 0.05$), but there was an interaction between forest and treatment across all forests (Table 2). This interacting effect appeared to be due primarily to differences in the level of variance for control versus drying plot data, rather than a difference in the means.

Differential abundance analysis of soil taxonomy across all four forests revealed that one bacterial phylum in particular drove community composition differences between treatments. Across all the forests, the phylum Actinomycetota was robustly significantly enriched in soil from the drying plots (Figure 10a-d, SI Table 3, LefSe, LDA > 4, FDR adjusted $p < 0.05$). In

addition to this robust significant group, phylum Pseudomondata had a marginally significant increase within the drying plots across all the forests (LDA > 3, non-corrected p-value < 0.05). These trends persisted at other taxanomic levels, with many lower level groups within phyla Actinomycetota and Pseudomondata, which had robust, or marginally significant increases within the drying versus control plots. Thus, the drying treatment was found to have a significant effect on the bacterial community compositions across the forests.

Because of the strong biogeographic differences among the four forests, we also assessed drying effects within each forest separately. Assessing forests separately for each seasonal time point revealed that various taxonomic groups had a marginally significant response to the drying treatment (LDA score > 3, non-corrected p-value < 0.05). Here, differences followed similar trends as found across the entire data set, with some new diverging trends. Similar to what was found across all the forests, lower taxonomic members of the Actinomycetota phylum, such as the class Actinobacteria, order Micromonosporales, and family Micromonosporaceae had a marginal increase within the drying versus control plots for three forests (Gigante, P12, and P13). Phylum Pseudomonata also increased marginally in drying versus control plots for three forests (Gigante, P12, and P13). Thus, the broadest and most robust taxonomic shift with drying was an increase in the Actinomycetota phylum across forests and increases in lower level taxa within this phylum at some of the forests. This further shows that in addition to changes from drying across all the forests, there were similar trends within the forests when analyzed separately, showing that drying had a significant effect on the community composition at all levels of analysis.

In contrast to the increases in Actinomycetota with drying, other phyla and lower taxonomic groups declined in relative abundance with drying within specific forests. Phyla with

marginally significant declines in drying versus control plots included: Bacteroidota, Chloroflexota, Cyanobacteriota, and Plantomycetota. Some lower taxonomic levels within these phyla also had marginally significant declines with drying (Online SI, McLaughlin et al., 2026). Thus, in addition to the robust increase in Actinomycetota with drying, and number of groups had trends toward declines with drying, indicating that drying can cause both an increase and decline of different bacterial communities.

The above taxonomic results used all data across seasons. However, since season was not a significant predictor of taxonomic abundances, I also averaged by plot across season (n = 32 plots). These results were similar to the larger dataset, but more muted. Various taxa still had marginally significant differences between treatments for this averaged dataset (LDA score > 3, non-corrected p-value < 0.05). Specifically, the phylum Actinomycetota was enriched in drying versus control plots in the fertile forest (P13), while phylum Latescibacterota and MBNT-15 were depleted in drying versus control plots in this same forest. The phylum Myxococcota was depleted in drying versus control plots in the wettest forest (Sherman Crane), and phylum Bdellovibrionota was depleted in drying versus control plots at P12. Some lower taxonomic levels also declined with drying, such as class Polyangiia (Myxococcota), at Sherman Crane and Gigante. Other lower-level taxonomic groups had further marginally significant shifts within a single forest (Online SI, McLaughlin et al., 2026). Thus, the phylum Actinomycetota continued to be marginally and positively responsive to drying when all data were averaged by plot, with other phylum responsive in only one forest, and many lower-level shifts in response to drying.

Fungi

The drying treatment showed few impacts on fungal alpha-diversity. There was no significant overall effect of the drying treatment on alpha diversity overall (as measured with the Shannon metric), nor was there when analyzed within individual forests. Similarly, fungal beta-diversity Bray-Curtis metrics showed no overall effect of drying (Bray-Curtis, Pairwise ADONIS $p > 0.05$). However, there was a significant interaction between forest and treatment (Table 3). This interacting effect was due primarily to differences in the level of variance for control versus drying plot data, rather than a difference in the means.

In contrast to the broader diversity indices above, differential abundance analysis of soil taxonomic groups showed significant differences between drying and control plots for some fungal groups. Similar to results for biogeographic patterns, most drying effects on fungi occurred at the class level. Across all the forests, the class Agaricomycetes (Basidiomycota), had a robustly significant increase in abundance in drying versus control plots (Figure 11a-d, SI Table 4, LDA 4, FDR adjusted $p < 0.05$) as well as the class Eurotiomycetes (Ascomycota) (SI Table 4, LDA > 3, FDR adjusted $p < 0.05$). Also, the classes Dothideomycetes (Ascomycota), and Sodariomycetes (Ascomycota), had a marginally significant increase in abundance in drying versus control plots across the forests (Figure 11a-d, SI Table 4, LDA 3, non-corrected $p < 0.05$). In addition to robust and marginal increases in these fungal classes from the drying treatment, there were also some significant declines with drying. Classes Glomeromycota Incertae sedis (Glomeromycota) and Rhizophydiomycetes (Chytridiomycota) showed a robustly significant decline within the drying plots (LDA > 3, FDR adjusted $p < 0.05$). While there was less significant lower level taxonomic changes within these classes than observed for bacteria, there were still a couple taxa that had robust and marginally significant increases or declines across all

forests. Thus, several classes of fungi both increased and declined from drying across these four forests.

Because season was not a significant predictor of fungal taxonomic groups across the entire data set, we also analyzed forests for each seasonal timepoint, and we averaged data across the seasons for each plot. Assessing forests separately for each seasonal time point revealed more fungal taxonomic groups that had marginally significant responses to drying (LDA > 3, non-adjusted p-value < 0.05). Some lower taxonomic levels of Agaricomycetes (Basidiomycota), had marginally significant enrichment in the drying versus control plots within different forests such as the families Entolomataceae, Pterulaceae, and Geastraceae. Other fungal groups declined with drying, in the forests such as marginally significant declines for the classes Archaeorhizomycetes, Cladochytriomycetes, and Rhizophydiomycetes, as well as many lower taxonomic levels within these classes (Figure 11a-d). This shows that along with the significant changes across the forests as a whole, there are many marginally significant differences from drying across the forests individually.

When the fungal samples were averaged by plot across seasons (n = 32), results were similar, but more muted than results for the entire data set. Some taxa had marginally significant shifts with drying (LDA score > 3, non-corrected p-value < 0.05). Specifically, the family Geastraceae had enrichment in drying versus control plots for Gigante and P13. The family Entolmataceae was also enriched in drying versus control plots for Sherman Crane and P12. Other lower-level taxonomic groups had marginally significant shifts as well, but typically only within one of the forests (Online SI, McLaughlin et al., 2026). Thus, drying had the most robust positive effects on fungal classes Agaricomycetes and Sodariomycetes, with parallel enrichment

in the families Geastraceae and Entolmataceae, with these trends present across the forests as a whole, the forests, individually, and within the forests when averaged by season by plot.

Of the classes that were significantly different within the drying compared to the control plots, FUNGuildR provided insights on potential functions of fungi that increased and decreased in abundance. The groups that increased were most broadly, such as Agaricomycetes, were saprotrophs, but had some possible symbiotroph and pathotroph functions (Online SI, McLaughlin et al., 2026). Eurotiomycetes and Dothideomycetes had few confident functions, and had the possibility of being saprotrophs, symbiotrophs, and pathotrophs. Sordariomycetes were mostly identified as saprotrophs, but also had some ASVs as potential symbiotrophs or pathotrophs. For the groups that decreased, Glomeromycota Incertae sedis were found to be symbiotrophs and Rhizophydiomycetes had very little assigned functions, but some could be possible saprotrophs.

Discussion

Overview

Across the four forests, there were significant effects of both chronic drying and biogeography on microbial diversity overall. The drying experiment effects on microbial diversity in four tropical forests are particularly novel and showed robust and broad effects on bacterial metrics of diversity, as well as shifts in community composition and high taxonomic orders. The drying effects on bacteria included declines in alpha diversity within certain forests, shifts in bacterial community structure (in interaction with forest site), as well as broad scale increases in the phylum Actinomycetota. The stronger effects on bacteria during years 6 – 7 of the experiment follow some initial trends observed during year 1 (Chacon et al 2023). A new finding was that drying also shifted the taxonomic composition of the fungal community at higher taxonomic levels, even though overall alpha biodiversity did not respond to drying. Fungal community structures responded to drying in interaction with forest sites, similar to the pattern for bacteria. The emergence of a fungal response to chronic drying, in particular increases in the classes Agaricomycetes, Dothideomycetes, Eurotiomycetes and Sodariomycetes, as well as declines in classes Glomeromycota Incertae sedis and Rhizophydiomycetes suggests that stronger shifts in fungal diversity might occur in the longer term.

As seen across other landscapes (McGuire et al., 2012, Oliverio et al., 2020), I also found very strong biogeographic variation in both soil bacterial and fungal communities. The clear differences in soil microbial community structure and composition across large-scale shifts in MAP and soil nutrients highlight the importance of baseline conditions in governing soil biodiversity. Perhaps most interesting in this study is the coordinated shift in some bacterial and

fungal groups in responses to drying, despite the strong community-scale differences among the four forests.

Surprisingly, there were no differences among the three seasonal time points in this data, despite strong variation from the dry to the wet season for other soil properties at these sites (Dietterich et al., 2022) and across the Isthmus of Panama more broadly (Cusack et al., 2019). With the strong variations in soil moisture in the forests between sampling seasons (SI figure 1), and changes in average precipitation between the wet and dry season near the forests (Figure 2), I would have expected to see changes in diversity and communities between these large differences in soil moisture and rainfall. Yet, my sampling points were during years with increased precipitation and soil moisture than the historical average, so this may account for the lack of a seasonal difference.

While the fertile forest and fungi appeared to be resilient to drought at the 1-year timepoint (Chacon et al., 2023), taxa are now changing, indicating that the temporal scale of community change under chronic drought is longer than one year, with shifts ongoing. The functional consequences of these changes in microbial communities for carbon and nutrient cycling are likely. Below I discuss the observed changes in more detail, and suggest ways in which microbial shifts might impact ecosystem function.

Drying effects on microbial community composition

Alpha diversity

Drying caused a significant decrease in bacterial alpha diversity in some forests, although there was not an overall effect, which shows that drying can impact belowground species richness in tropical ecosystems. Within the fertile forest (P13) the drying plots had consistently

decreased diversity in comparison to the control plots. Within the driest site (Gigante) there was decreased alpha diversity in the drying plots only in May, when the rains began to come back. Previous work has shown that drought significantly reduces the diversity of soil microbial communities across tropical rainfall exclusion experiments in Puerto Rico(Bouskill et al., 2013). With forests drying out and then rewetting every year at our sites, it may take a longer to see overall diversity effects from the drying treatment. It is interesting that the strongest response in alpha-diversity to drying was in the most fertile forest, suggesting that it is less resistant to drought over this time period than the more infertile forests. In the fertile forest (P13), which had the highest bacterial alpha diversity among sites, bacterial species diversity remained consistently higher over the year in the control versus drying plots, and the drying plot diversity declined over the three sampling periods.

In general, drying has been found to cause stress to soil microbes. In other studies, it has been found that when soils dry, solutes can concentrate and cause stress on the bacteria in soil, as shown in arid ecosystems(Malik and Bouskill, 2022; Schimel, 2018). In response to this stress, microorganisms have been found to increase their demand for nitrogen and phosphorus (Buscardo et al., 2021), to alter their cell walls (Williams and Rice, 2007), and maintain turgor pressure to maintain cellular integrity during osmotic stress (Bremer and Kramer, 2019). However, these changes can be energy-expensive for the microbes (Oren, 1999), and likely are only used when the substrate availability can meet the energetic cost of this stress response (Manzoni et al., 2014). In the fertile site (P13) drought plots, there may be a larger impact of drought on soil nutrients than in the infertile sites after experiencing long-term chronic drying. A study in Puerto Rico showed that drought can disrupt nutrient cycling by decreasing inorganic P, increasing organic P, and cause a decline in carbon dioxide emission within the soils of tropical

ecosystems (O'Connell et al., 2018). The microbes in the control plots have more consistent access to water and may have higher nutrient availability from not needing to expend these resources as often as the drought plots. This may allow them to maintain a higher species diversity and respond to the absence and presence of rainfall, while the drought plots do not respond to this and remain constant over the seasons.

Drying did not change the alpha diversity of fungal communities within the forests. It is possible that the increased soil moisture above average during the sampling points allowed for increased resilience to changes in species richness. Within temperate grassland soils, it has been shown that fungi can have decreased alpha-diversity due to drought (Hannula and Veen, 2025). Yet, there is very little research on the effect of drought on fungal species richness in tropical ecosystems, so this can act as a baseline for fungal species richness across forests and during drying events.

Beta diversity

Across the seasons, treatment was not found to significantly affect the beta diversity of bacteria or fungi. However, there was a significant interaction between the forests and treatment for both bacteria and fungi. Specifically, the interaction between treatment and forests was shown to account for more of the community composition differences between the forests when looking at just the forests alone. So, although there was no treatment effect on its own, the drought does play a role in affecting the microbial community compositions across the forests.

Taxonomic shifts

Both bacteria and fungi had taxa that showed robust and marginal significant effects of the drying treatment. The bacterial phylum Actinomycetota was found to have robustly significant enrichment within the drying plots across the forests. In addition to this, several other groups were found to have a marginally significant increase or decline due to drying. Actinomycetota, previously referred to as Actinobacteria, are a gram-positive phylum of bacteria that are responsive to drying in soils. Within tropical ecosystems, Saltonstall et al., (2025), found that dry forests had decreased relative abundance of Actinomycetota than moist forests, similarly, de Vries et al. (2018) found this in grassland ecosystems during induced drought events. Suggesting that this phylum is drought adapted. Gram-positive bacteria have thick peptidoglycan cell walls, providing a barrier to osmotic stress and allowing them to maintain function when there is a decline in water (Manzoni et al., 2012), so they are likely drought-tolerant. Actinomycetes are also spore-forming bacteria (Bhatti et al., 2017), which further enhances their ability to survive during low moisture conditions. A review of Actinomycetes describes them as being known to enhance nutrient availability through decomposition of organic matter, metabolites, and plant growth regulators within soils across ecosystems (Bhatti et al., 2017). With these properties, they have an important functional role often active in the root microbiome, and may provide enhanced benefits to plants during drier periods.

In addition to these phylum-level changes, many other bacterial groups had marginally significant enrichment or decline with drying in some of the forests. Lower taxonomic levels within the Actinomycetota phylum were also found to have marginal enrichment in three forests (P13, P12, and GIG), further indicating that groups within this phylum may increase under drought conditions due to their ecological resilience. Another bacterial group that was marginally

enriched in dry plots was phylum Pseudomondata, previously known as Proteobacteria. This phylum was enriched in the drying plots of P13, P12, and GIG, yet they were all enriched at different sampling points. Many lower taxonomic levels were also trending towards an increase across P13, P12, and GIG as well. Pseudomondata is a diverse group, and many of its members can resist osmotic stress associated with drought through an increase in biofilm production (Romling and Galperin, 2015). The production of biofilms can protect the embedded cells from fluctuations in water within the surrounding soil (Flemming et al., 2016). In a review many Pseudomondata strains have also been associated with alleviating various climate-related stressors within plants, such as seasonal droughts across agricultural and other varieties of ecosystems (Zboralski and Filion, 2023). Shifts toward an increase in the phylum Pseudomondata bacteria can likely be attributed to their ability to withstand drought conditions and benefit the plant community.

To balance the increase in Actinomycetes and Pseuodomondata with drying, there were declines in the relative abundance of several other phylum. For example, I saw marginally significant declines in Bacteroidota, Chloroflexota, Cyanobacteriota, and Plantomycetota with drying. The phylum, Bacteroidota and lower taxonomic levels were shifting towards decline within P13 during May. Other studies have found that this group can decline under drought conditions within grassland ecosystems (Fan et al., 2023), yet in tropical forests, Bacteroidota has previously been found to increase within drying forests in comparison to wetter ones (Saltonstall et al., 2025), contrasting with these findings. Phylum Chloroflexota and lower taxonomic levels were found to be shifting in Gigante, most notably, but some lower taxa were trending towards decline at Sherman Crane and P12. This contrasts with previous findings in arid ecosystems that Chloroflexota are generally drought-tolerant species and often increase in

abundance in drying conditions (Maisnam et al., 2023). Finally, the last notable bacterial group shifting towards decline was the phylum Plantomycetota, which was trending as well as lower taxa, to a decline within Gigante and P13 across the sampling months. Previous studies in grasslands have noted that this phylum declined with induced drought (de Souza et al., 2024). Yet, their response to drought in the tropics remains poorly understood. Other groups such as phylum Cyanobacteria have no prior research on their response to drought in any soil environments, thus this research provides an important baseline of how these bacterial groups that are lacking in tropical research, such as, Cyanobacteria, Chloroflexota, and Plantomycetota, may decline from drought in tropical ecosystems. This shows how bacteria are beginning to change due to long-term drought, and provides insights into what the future microbial communities may be.

Regarding fungal taxonomic groups, the differential abundance analysis revealed that the fungal class Agaricomycetes and Eurotiomycetes were robustly significantly enriched in drying versus control plots across the forests, and that Sordariomycetes and Dothideomycetes were marginally significantly enriched in drying versus control plots across the forests. Within the tropics, specifically, the Amazon rainforest Agaricomycetes have been known to increase in abundance during decreased precipitation from rainfall exclusion experiments (Buscardo et al., 2021). Yet, in other ecosystem types, particularly grasslands, Agaricomycetes have been found to decline with drought conditions (Hannula and Veen, 2025; Bei et al., 2019). This is a broad group, and it is likely that these contrasting findings are due to different shifts at lower taxonomic levels. In the tropics, Agaricomycetes are known to be associated with the roots of tropical plants, such as seen in a study looking at fungal communities within tropical rainforest sites across Hainan Island, China (Ji et al., 2025). Specifically, some Agaricomycetes are

ectomycorrhizal fungi, and can help aid in bringing the plants nutrients after drought events (Liu et al., 2022). FUNGuildR analysis mainly identified Agaricomycetes as saprotrophs and symbiotrophs, correlating with these findings. Thus, with an increase in Agaricomycetes from the long-term drought across the forests, fungal competition may be changing to allow for plant resilience to osmotic stress. The other group that had robust significant increase within the drying plots, class Eurotiomycetes does not have much background research on drought tolerance within tropics or other ecosystem. They are a diverse class of fungi and have been shown to be negatively correlated with rainfall within China rainforests, and play a role in decomposition (Ji et al., 2025). FUNGuildR did not provide many insights on the functions of Eurotiomycetes, so it is likely a class that has many broad functions and can play different roles in the ecosystem. Overall, these robust significant increases across the forests may be to aid in nutrient uptake and decomposition within the soil during drought events.

Within the tropics of the Amazon rainforest, the fungal class Sordariomycetes have also been shown to increase in abundance during decreased precipitation from rainfall exclusion experiments (Buscardo et al., 2021). Yet, within some subtropical ecosystems, Sordariomycetes were found to decline in abundance with precipitation-altering experiments (He et al., 2017). FUNGuildR identified most Sordariomyetes as probable saprotrophs. Sapatrophic fungi play an important role in decomposing organic matter within soils, as well as redistributing key nutrients such as carbon, nitrogen, and phosphorus throughout the soils (Cairney, 2005). They have also been observed to increase within a grassland ecosystem experiment during drought conditions, due to an increase in plant litter when plants desiccate (Lozano et al., 2021). The increase in Sordariomycetes in this project is consistent with other trends within tropical drought experiments and could be due to the drought tolerance of this class and potentially the role that

this fungus plays in decomposition, specifically, with an increase in fungi that play a role in decomposition may allow for nutrients to still be distributed throughout the ecosystem, which can aid the plants during drought. The other fungal class that had marginal increase from drying was Dothideomycetes. In drylands Dothideomycetes has been found to increase during seasonal drought events (Ascota-Martinez et al., 2014), but there is little research on this classes response to drying in the tropics. They are a very broad group of fungi and are known to be plant pathogens, mycorrhizal, and saprotrophic (Haridas et al., 2020), so understanding how the increase in Dothideomycetes may impact tropical ecosystems is difficult and may require more insights into their functional traits.

To balance the increase in Agaricomycetes, Eurotiomycetes, Sodariomycetes, and Dothideomycetes with drying, there were declines in the relative abundance of several other classes For example, I saw robustly significant declines in classes Glomeromycota Incertae Sedis and Rhizophydiomycetes. and marginal declines of Archaeorhizomycetes and Chytridiomycota. The class Glomeromycota Incertae Sedis are arbuscular mycorrhizal fungi (AMF) within the phylum Glomeromycota that can not be classified into specific families or orders. Prior research within these forests has found that AMF colonization declined in the drying plots (Cordeiro et al., 2025), so a decline in the relative abundance of the class Glomeromycota is consistent with prior findings in these forests. AMF form symbiotic relationships with the roots within tropical forests and help the plants take up nutrients and water. A study within Panama rainforest has found that they can aid in decomposition in the soil and play a role in releasing substantial amounts of carbon dioxide into the atmosphere (Nottingham et al., 2010). It is well recognized that AMF play a key role in cycling nutrients through the ecosystem, benefiting the plant communities, and have a major role in the global carbon cycle. A decline in these fungi could

possibly be due to the increase in other saprotrophic fungi, but may also have major implications on the ecosystem as a whole, as AMF are important in aiding the roots of tropical plants. The other class with robust declines in the drying plots was Rhizophydiomycetes, there is not much prior research on this class of fungus response to drying in the tropics or other ecosystems. Rhizophydiomycetes are broadly categorized as saprotrophic fungi within tropical environments (Hurdeal et al., 2024) and were found to be possible saprotrophs by FUNGuildR, yet not much more information is known about this class. While the groups that had marginally significant declines varied by forest and season, one of the most notable ones were within Archaeorhizomycetes which shifted within P13 during May. There is little known about Archaeorhizomycetes, yet some research suggests they may be important saprotrophic decomposers in soil (Rosling et al. 2011). The only other group of note with a marginal decline is the phylum Chytridiomycota and lower taxonomic levels across all forests at varying time points. Within grassland ecosystems, orders within Chytridiomycota are more abundant in watered plots compared to drought plots (Hannula and Veen, 2025). Yet, there is very little research on the phylum as a whole, especially within drought conditions in tropical ecosystems. Overall, fungi did not have as many taxonomic groups with marginally significant enrichment or decline from drought within the forests as bacteria did, yet there were still many classes shown to have robust and marginal increases and declines in abundance now after 6 years of drying treatment, showing that sustained reductions in soil moisture can impact fungal community compositions in the tropics are altered from drought.

Biogeographic effects on microbial community composition

Across the four forests, the bacterial and fungal communities were found to all have significantly different taxonomic community composition, showing that biogeographic variations such as fertility, pH, and rainfall play a significant role in creating the microbial community differences across the landscape. The community compositions revealed more variation between the forests than species richness did, with both bacteria and fungi having unique communities across all the forests. Every forest was significantly different from one another across every sampling season. This is consistent with prior research in the Panamanian forests (Chacon et al, 2023; Saltonstall et al., 2025; McGuire et al., 2012; Oliverio et al., 2020). As our forests all have great variation in rainfall, soil nutrients, and pH, significantly different microbial communities can be expected. Furthermore, despite the major difference in communities across these forests , I found consistently different taxa in drying plots compared to the control plots, suggesting that soil microbes can respond to drying in similar ways across different abiotic and biotic environments.

Alpha diversity of the bacterial communities showed that there were significant differences between some of the forests. Specifically, bacterial communities were found to have significantly increased species richness within the most fertile forest and forest with the highest pH, P13, in comparison to the other three infertile, and more acidic soils forests. This implies that fertility and pH may be the main drivers of bacterial species richness within these tropical forests, a finding that is consistent with patterns identified in other studies. Among tropical forests across Southeast Asia, soil pH was found to be positively correlated with alpha diversity, and tropical forest bacterial communities were determined to evolve largely independently based on their local environment (Ito et al., 2017). Prior findings have also found that a higher pH and

increased fertility tend to relate to increased microbial diversity in tropical ecosystems (Costa et al., 2024, Saltonstall et al., 2025). Overall, this indicates that abiotic properties of soil, such as pH and soil fertility can significantly impact microbial alpha diversity across different forest types and biogeographic realms.

Fungal communities were found to have significantly increased species richness at the wettest site, Sherman Crane, which was significantly different from two of the drier sites. This implies that annual rainfall may be a main driver of fungal species richness within tropical forests. Previous findings within the Isthmus of Panama have shown that fungal community composition is more significantly affected by precipitation than other ecosystem factors, such as plant communities and soil nutrients, with taxonomic diversity increasing with higher precipitation (McGuire et al., 2012, Saltonstall et al., 2025). Overall, our results contribute some new insights to the drivers of tropical soil biodiversity, such as that both bacterial and fungal communities are significantly different across tropical forests and that alpha diversity of microbial communities is significantly correlated with differences in fertility, pH, and rainfall within the tropics.

Comparison to short-term drying findings

With long-term drying, I found some similar trends that were found in the short-term dataset (Chacon et al., 2023), but there are also some new divergent trends. While the fertile forest, P13, was previously found not to have any bacterial community composition shifts from short-term drying, it is now shifting similarly to the other three infertile forests. Fungi were found to be resilient to short-term drying, but after an additional 5 years of drying, fungi are now shifting toward more drought tolerant taxa, similarly to the bacterial communities. Overall, the

short-term drought findings revealed that bacteria community composition was beginning to shift within the three infertile forests, Sherman Crane, Gigante, and P12, but the fertile forest, P13, did not have any bacterial community differences between the control and drying plots. Fungal communities did not have any changes from the short-term drought across any of the forests (Chacon et al., 2023). Chacon et al. noted Pseudomondata (Proteobacteria) to be significantly enriched within the treatment plots of P12 and Gigante. While my findings did not show that Pseudomondata have a robustly significant increase, it was found to have marginally significant enrichment within these forests. The main group that had shifted after six years of drought, Actinomycetes, was found to be enriched after short-term drought within Sherman Crane and Gigante, but not in P12 or P13. Overall, there were more differences between short-term and long-term drying. There were more taxa that were deemed to be significantly different from the short-term drying than were found after long-term drying. However, there were some major differences now after six years of drying. The fertile forest, P13, was predicted to be resistant to short-term drought, as there were no community shifts, but it is now shown that P13 has a changing community in response to drought, just as the other forests do. This may indicate that while fertility may be a predictor of short-term drought tolerance, it is not a predictor of long-term drought tolerance. Another major difference between the short-term and long-term drought impacts is that after 6-years of drying, the fungal communities are shifting, while they were not after the short-term drought. This is a significant difference as fungal communities were thought to be more drought-tolerant (Chacon et al. 2023). However, now, after a long-term drought, the fungal community composition is significantly changing. Overall, some aspects of the community composition for bacteria were similar to those of prior findings, and some trends found after a short-term drought were not present after six years of drying. But community

composition still has changed from drying, which supports the Chacon et al. hypothesis that there is a shift toward a "drought microbiome." Now, after long-term drying, this shift is present across all forests, and not just the three infertile ones, showing that fertility cannot be a predictor of how bacterial communities will change after experiencing long-term chronic drying. Finally, for fungi, the communities were found to have a change in community composition after long-term chronic drying. While they were previously predicted to be resilient to short-term drought, it is now apparent that fungi also shift towards a "drought microbiome" after long-term chronic drying.

Community compositions were found to be significantly different across the forests, similarly to what Chacon et al. (2023) found for bacteria. However, community composition of fungi across forests were previously found to not be significantly different from one another (Chacon et al. 2023), but now the forests were found to have significantly different fungal communities.

Future research

This study has shown that long-term drying impacts microbial community composition. With microbial communities playing a critical role in tropical soil biogeochemical cycles, research is urgently needed to understand the consequences these community shifts will have on carbon and nutrient cycling in these tropical ecosystems. Microbial diversity plays a critical role in the overall functioning of the ecosystem within the soil, and previous research in tropical forests has identified that changes within both bacteria (Bouskill et al., 2016) and fungal diversity (Buscardo et al., 2021) can cause shifts within the biogeochemical processes within soils. Both fungal and bacterial communities were found to be changing in community

composition from long-term drought, yet the effects that this may have on the biogeochemical processes within the soil, and how this may affect the tropical ecosystems as a whole, remain unknown. While both fungi and bacteria are shifting toward an increase in microbes that may benefit plant resilience to drought, which could help the above-ground drought tolerance, it is unknown which and how below-ground processes may change from a shift in microbial community composition. Future research looking into the functional traits that are changing in the forests and how this plays a role in nutrient cycling is critical to gain a better understanding of how chronic drying will affect tropical ecosystems. These findings suggest that soil fungi and bacteria are changing from chronic drying to a "drought microbiome," and changes in community structure can cause shifts in the microbial pathways. So, understanding how these traits are changing and the effects this will have on the biogeochemical cycling within the ecosystem is critical. Within the tropics, changes in the soil microbial community are known to disrupt nutrient cycling within the soils (O'Connell et al., 2018) as well as decrease the carbon storage in tropical ecosystems. Specifically, within Panama, drought has been found to decrease soil carbon stocks (Cusack et al., 2011). This was also seen within rainforest plots in the Amazon (Gatti et al., 2014; Phillips et al., 2009). Further research looking into the relationship that this change in microbial community has to these changes in nutrient cycling can be key to understanding how tropical forests' carbon stocks may change with climate change.

Other projects within the PARCHED project are studying the way that carbon storage, sequestration, and fluxes are affected by drought within these tropical forests. This research can help aid in better understanding the shifts within these processes in these forests, and the implications this may have on the ecosystem, as well as the global carbon cycle.

Limitations

The sampling points were during years that had greater annual precipitation than the average; with this increase in precipitation, there is an increase in the soil moisture that tends to stick around throughout the year. Due to this project being completed when soil moisture was greater than the average, it is possible that the results do not fully depict the typical community patterns during a year with less precipitation. This also could explain why some taxonomic groups were found to be significantly enriched or depleted after short-term drought, yet no longer had these trends after six years of drying. Another limitation is that the short-term drought data in Chacon et al. were based on the rainfall exclusion structures, keeping out about ~50% of rainfall, while in 2022, these were increased to exclude ~70% of rain. This may explain the main changes that were found between the short-term and long-term drought, specifically that bacteria within P13 are now changing, and fungal communities are now changing, as these trends were not previously present after 1-2 years of exclusion.

Another limitation with this project comes biases and limitations associated with DNA metabarcoding. With large microbial datasets, absolute quantification can be a challenge as technical biases can make it difficult to obtain an accurate community structure. There is always the possibility of primer bias and amplification errors inflating or lowering the relative abundance of certain taxonomic groups. In addition, although the most current taxonomic reference databases were used; these are subject to change with new and ongoing research.

Conclusion

This project has shown that microbial communities do significantly shift toward different taxonomic communities under induced long-term drought conditions. I found a potential "drought microbiome" forming for both bacteria and fungi after six-years of drying, with some taxa likely having the ability to provide increased resistance toward osmotic stress and even potentially aiding the plant communities during drought stress. This pattern was seen across all four of my rainforest sites, despite their having significantly different soil microbial community compositions, with differences corresponding to biogeographic variations such as fertility, pH, and rainfall. This provides further emphasis on the importance of these findings and how significant it is that the effects of long-term drying are causing similar changes across all of these unique forests. As climate change is predicted to bring longer and stronger dry-periods to the tropics, studies examining the impacts of drought within tropical rainforests are becoming increasingly important. Longer term studies, such as this one, which capture differences in annual precipitation patterns, including extreme events, across a large biogeographic area are particularly important as shorter term studies may not adequately characterize the cumulative effects of stressors such as drying. With soil microbes playing key roles in plant growth, biogeochemical cycling and ecosystem processes, further research on what drives community shifts in these taxa is critical to understand how this changes in belowground community composition will affect tropical ecosystems and global carbon cycles during a changing climate.

Tables and Figures

Site	Soil Fertility	MAP (mm)	Soil order	Above-ground biomass (Mg/ha)	Resin-extractable P 0-10 cm (mg soil)	Soil pH 0-1 m	Soil organic C 0-1 m	Soil total N 0-1 m (kg/m^2)
Gigante	Infertile	2350	Oxisol	201.8	0.75	4.99	19	2.01
Plot 12	Infertile	2600	Ultisol	212.8	1.60	4.96	19	1.45
Plot 13	Fertile	2600	Alfisol	260.2	8.44	5.93	12	1.01
Sherman Crane	Infertile	3400	Oxisol	304.9	0.50	4.85	13	0.96

Table 1. Site characteristics of the four PARCHED tropical forests. MAP = Mean annual precipitation

ADONIS - December	**R^2**	**P-value**
Site	0.25691	**0.000999**
Treatment	0.03704	0.2597
Treatment*Site	0.36128	**0.000999**
ADONIS - March	**R^2**	**P-value**
Site	0.31852	**0.000999**
Treatment	0.0267	0.6424
Treatment*Site	0.40929	**0.000999**
ADONIS - May	**R^2**	**P-value**
Site	0.3277	**0.000999**
Treatment	0.02729	0.6553
Treatment*Site	0.42294	**0.000999**

Table 2: ADONIS results for bacteria, comparing forests (site), treatment, and their interaction for each sampling season.

ADONIS - December	**R^2**	**P-value**
Site	0.23571	**0.000999**
Treatment	0.02666	0.9021
Treatment*Site	0.33663	**0.000999**
ADONIS - March	**R^2**	**P-value**
Site	0.20932	**0.000999**
Treatment	0.02718	0.9381
Treatment*Site	0.3125	**0.000999**
ADONIS - May	**R^2**	**P-value**
Site	0.25139	**0.000999**
Treatment	0.0257	0.9201
Treatment*Site	0.34677	**0.000999**

Table 3: ADONIS results for fungi, comparing forests (site), treatment, and their interaction for each sampling season.

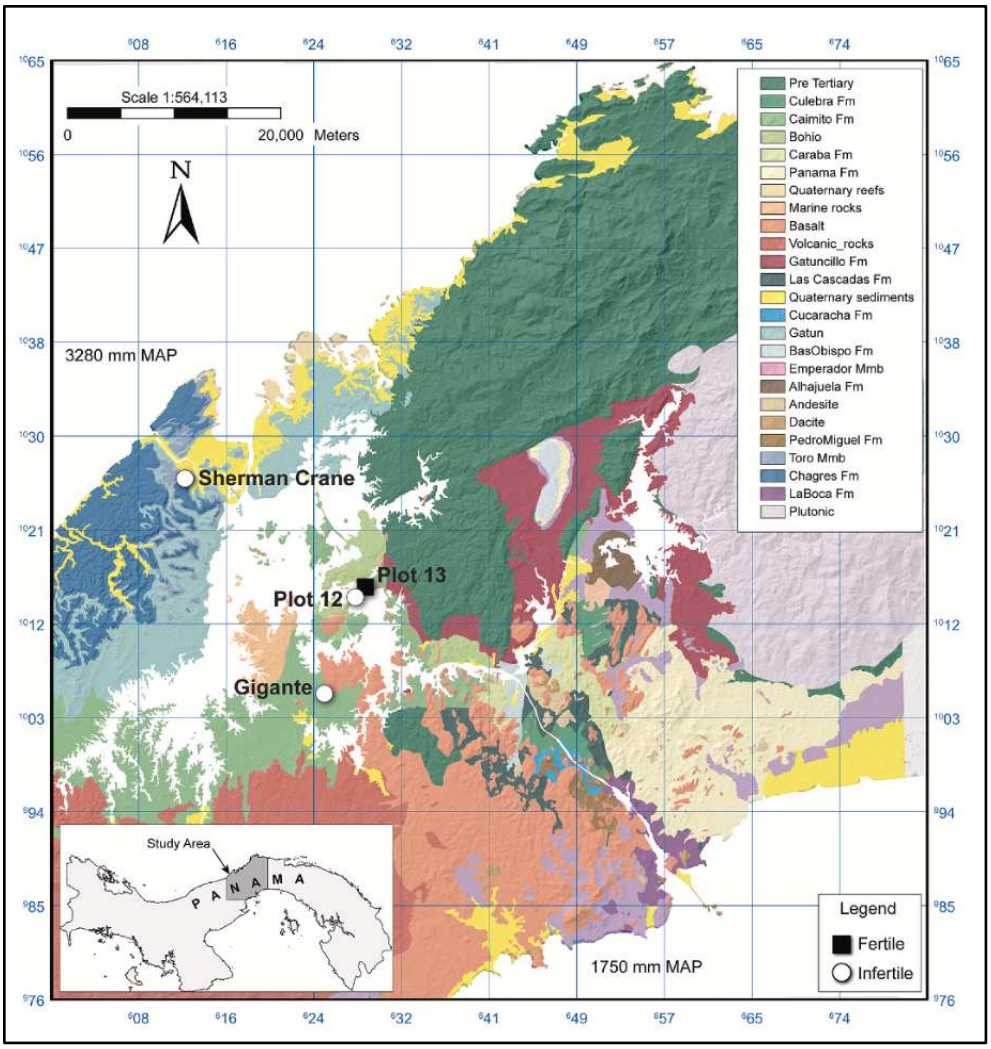

Figure 1. Locations of the four PARCHED experimental rainfall exclusion study forests in the Panama Canal Watershed

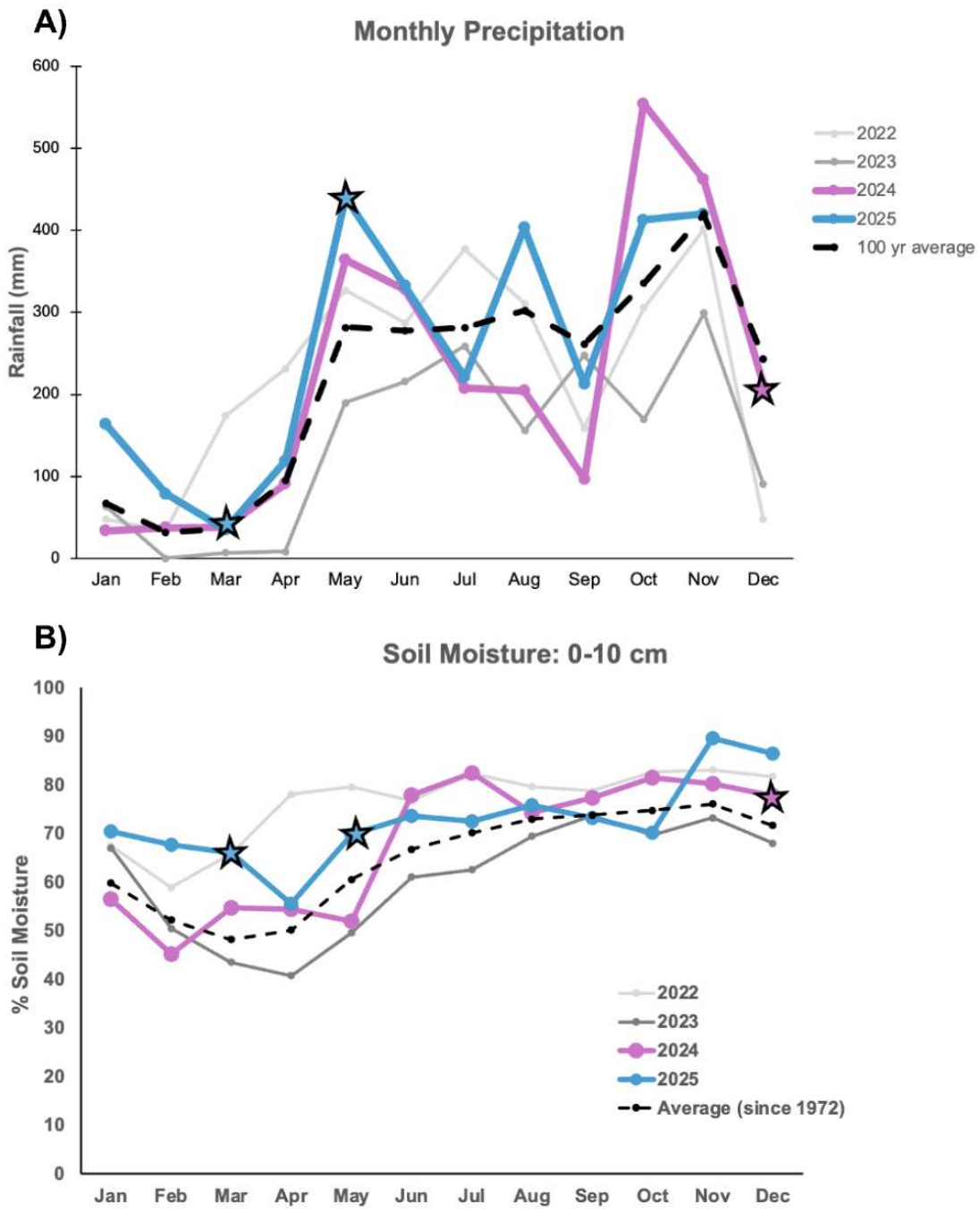

Figure 2: A) Monthly precipitation and B) soil moisture from the Barro Colorado Island Lutz catchment. Soil moisture was calculated using gravimetric analysis of 10 sample cores. Sampling months for this study are indicated with stars.

Figure 3: Partial throughfall exclusion structures are shown at the four PARCHED forest sites. a) Gigante; b) P12; c) P13; d) Sherman Crane

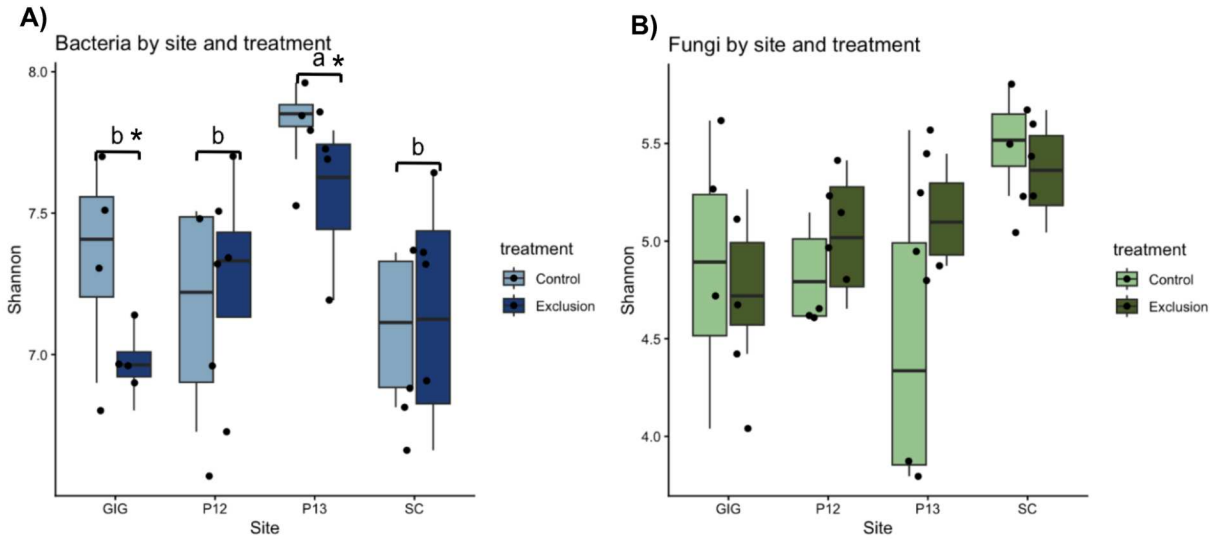

Figure 4: Shannon diversity of bacteria (A) and fungi (B) at each forest, comparing the control and drying treatments. Samples were combined within plots at the forest level (n = 8). In (A) bacterial diversity was significant by forests (p = 0.00562) and asterisks indicate forests with significance by treatment. In (B) fungal diversity was also significant by forests (p = 0.0476).

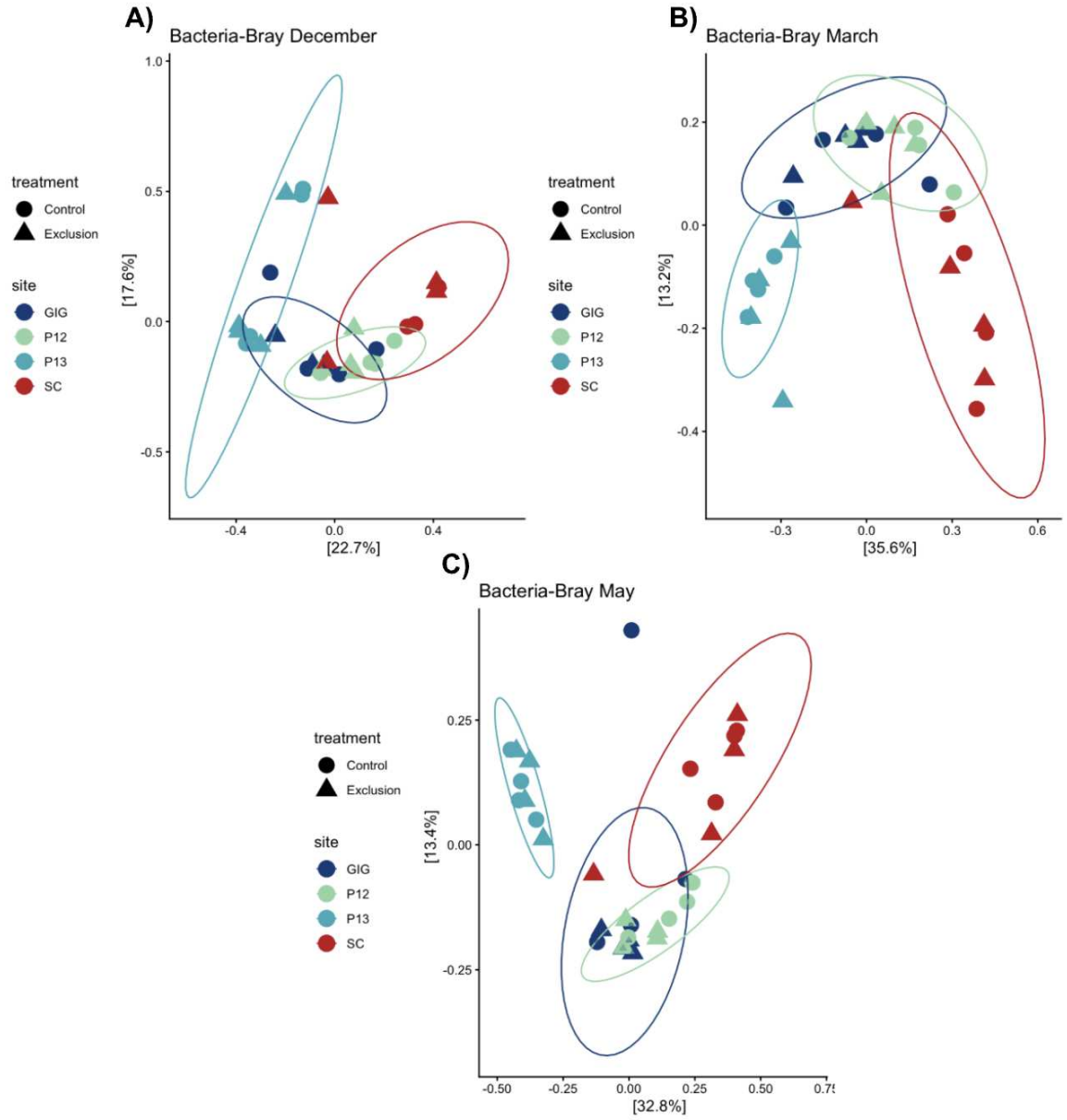

Figure 5: Principal coordinate analysis of bacteria using the Bray-Curtis metric. Sampling forest is shown by color, and treatment is indicated by shape, ellipses indicate grouping at a 95% confidence level (8 plots x 4 forests, n = 32 at each season).

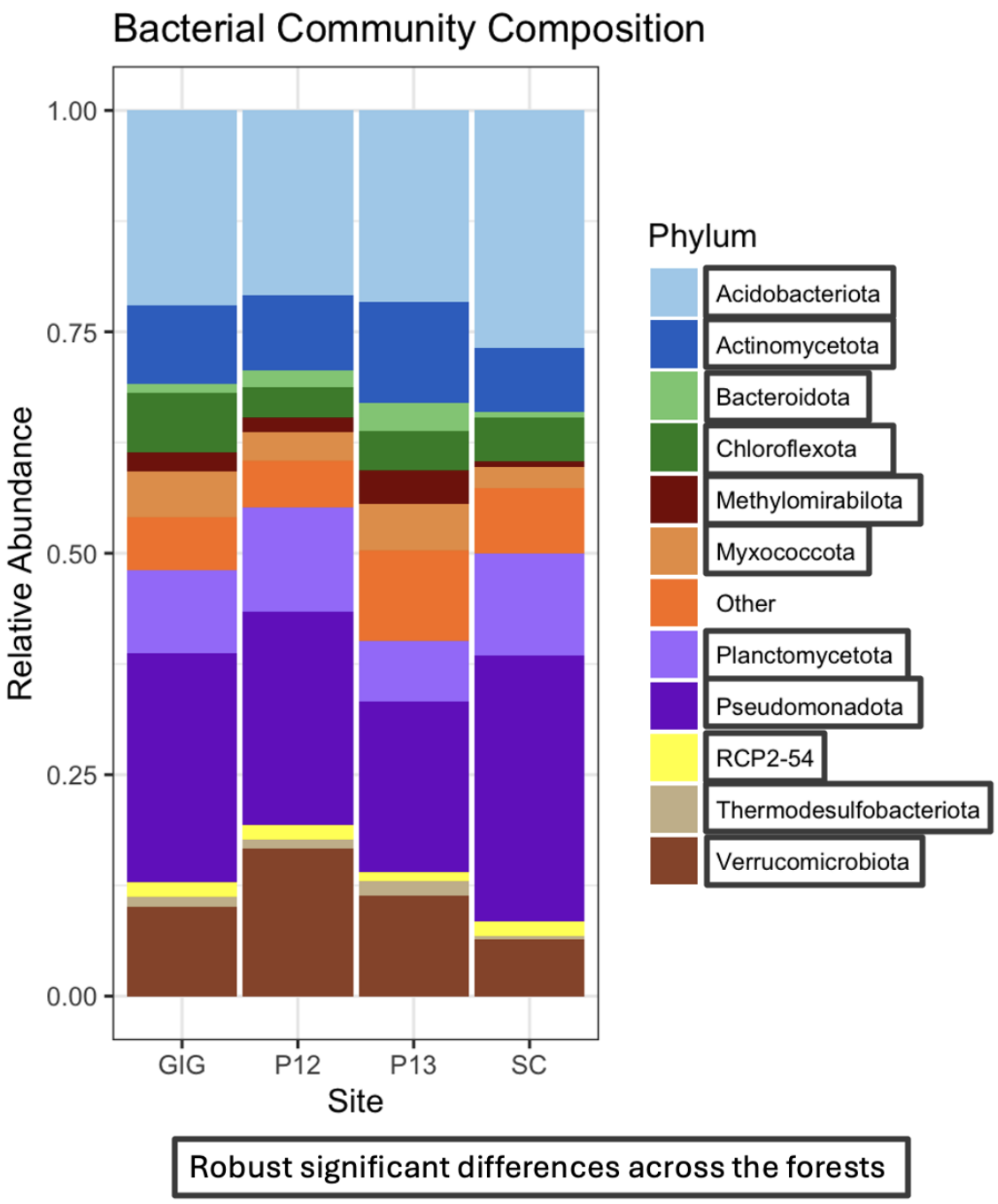

Figure 6: Relative abundances of dominant bacterial phyla across the four experimental forests. Phyla highlighted in black are the taxa that varied significantly across forests, with an LDA score greater than three, and a FDR-corrected p-value less than 0.05. The "Other" category includes all of the phyla that had a lower relative abundance than the top 11 shown (average relative abundance of all samples at each forest).

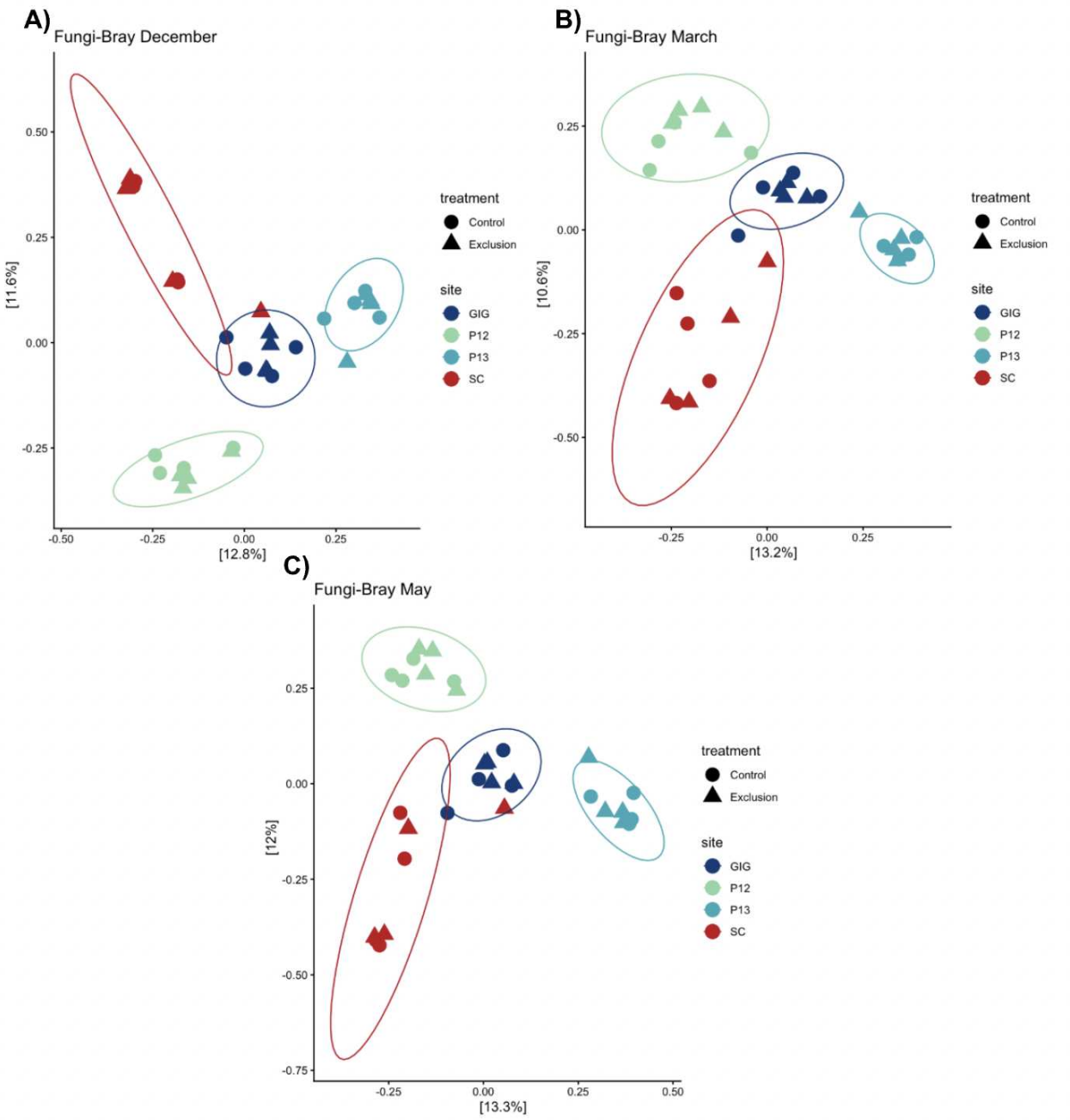

Figure 7: Principal coordinate analysis of fungi using the Bray-Curtis metric. Sampling forest is shown by color, and treatment is indicated by shape, ellipses indicate grouping at a 95% confidence level (8 plots x 4 forests, n = 32 at each season).

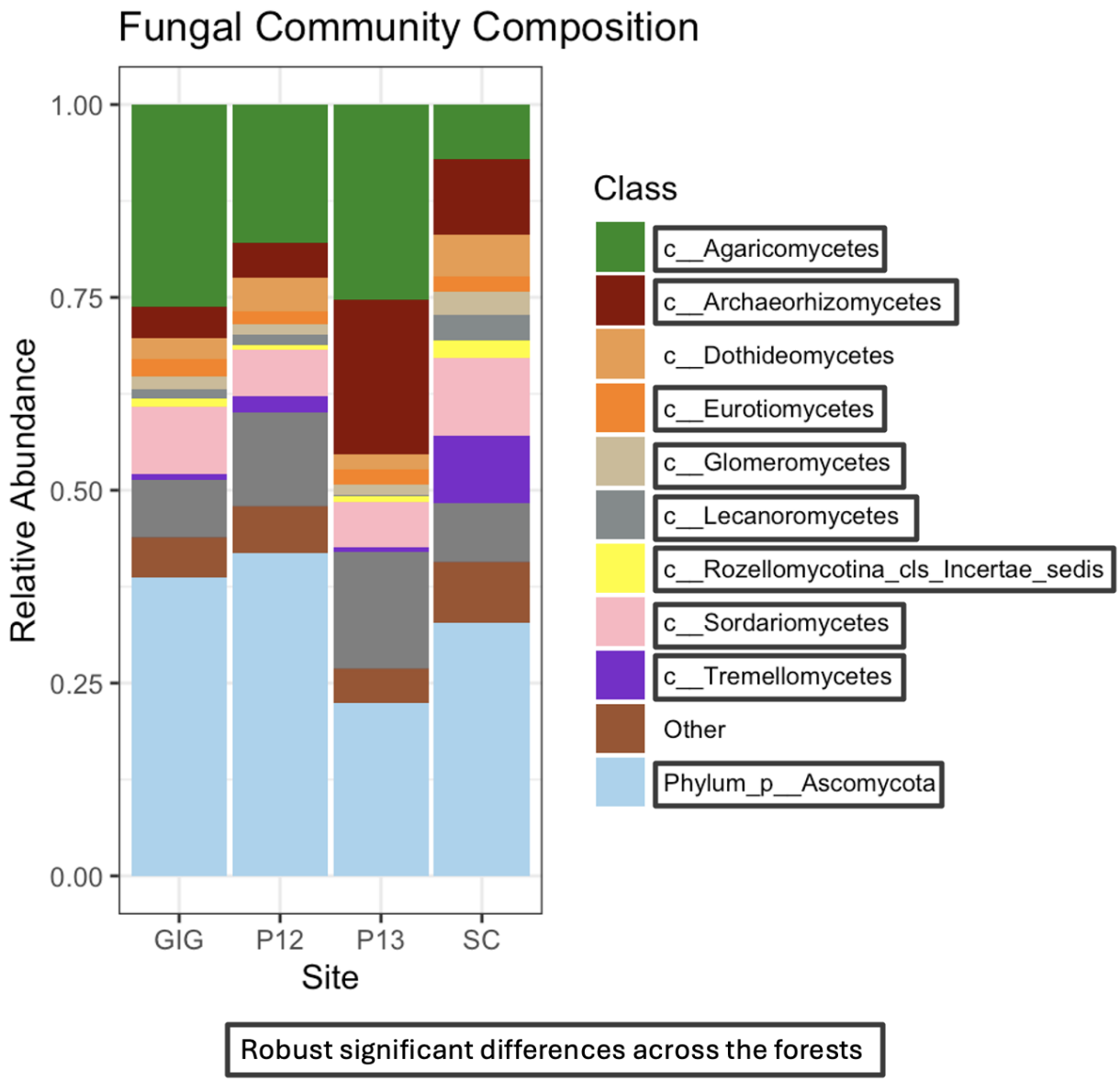

Figure 8: Relative abundances of the fungal classes across the four experimental forests. Classes highlighted in black are the taxa that varied significantly across forests, with an LDA score greater than three, and an FDR-corrected p-value less than 0.05. The "Other" category includes all of the fungal classes that had a lower relative abundance than the top 11 shown (average relative abundance of all samples at each forest).

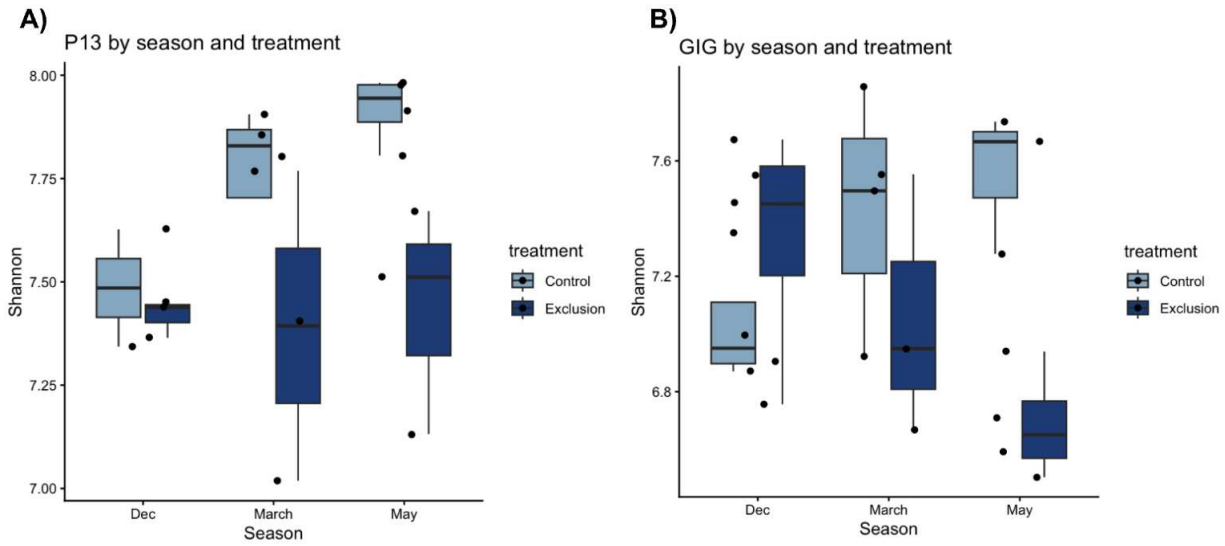

Figure 9: Shannon diversity of bacteria within A) P13 and B) GIG over the three sampling months, comparing control and drying plots. Treatment within A) P13 was significant ($p = 0.00941$). The treatment and season interaction within B) GIG was also significant ($p = 0.0198$), with the TukeyHSD showing control compared to drying during May being significant ($p = 0.0451271$) (8 plots at each seasonal timepoint, $n = 8$).

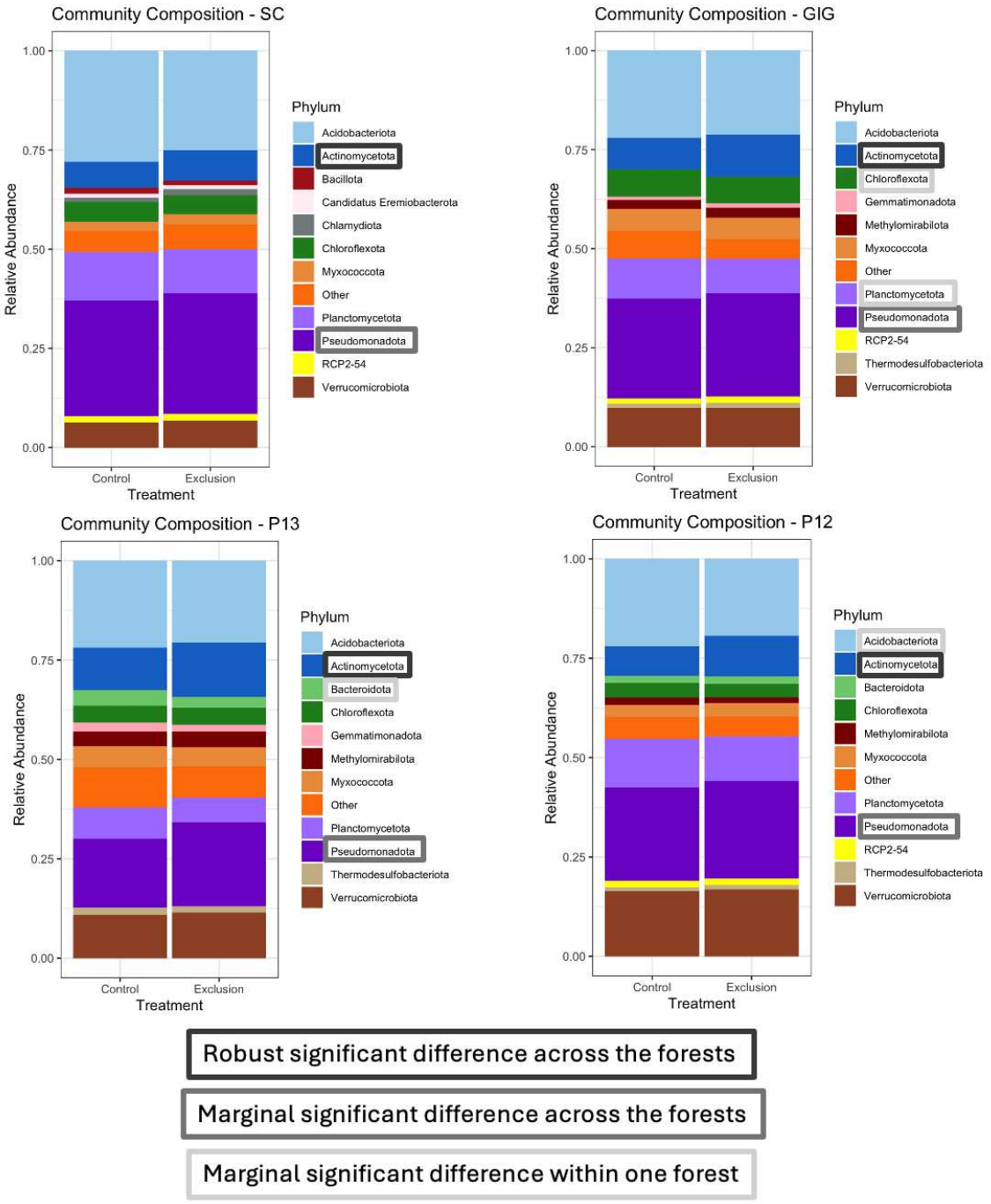

Figure 10: Relative abundances of bacterial phyla at each forest site. Phyla highlighted in black squares are significantly different between control and drying plots across all the forests, as determined by an LDA greater than 3, and an FDR-corrected p-value < 0.05. Phyla highlighted in light grey have marginally significant differences between treatments within the specified forest, as determined by a non-corrected p-value < 0.05, and an LDA score above 3. The "Other" category includes all of the phyla that had a lower relative abundance than the top 11 shown (average relative abundance values in the control vs drying plots at each forest across all seasons).

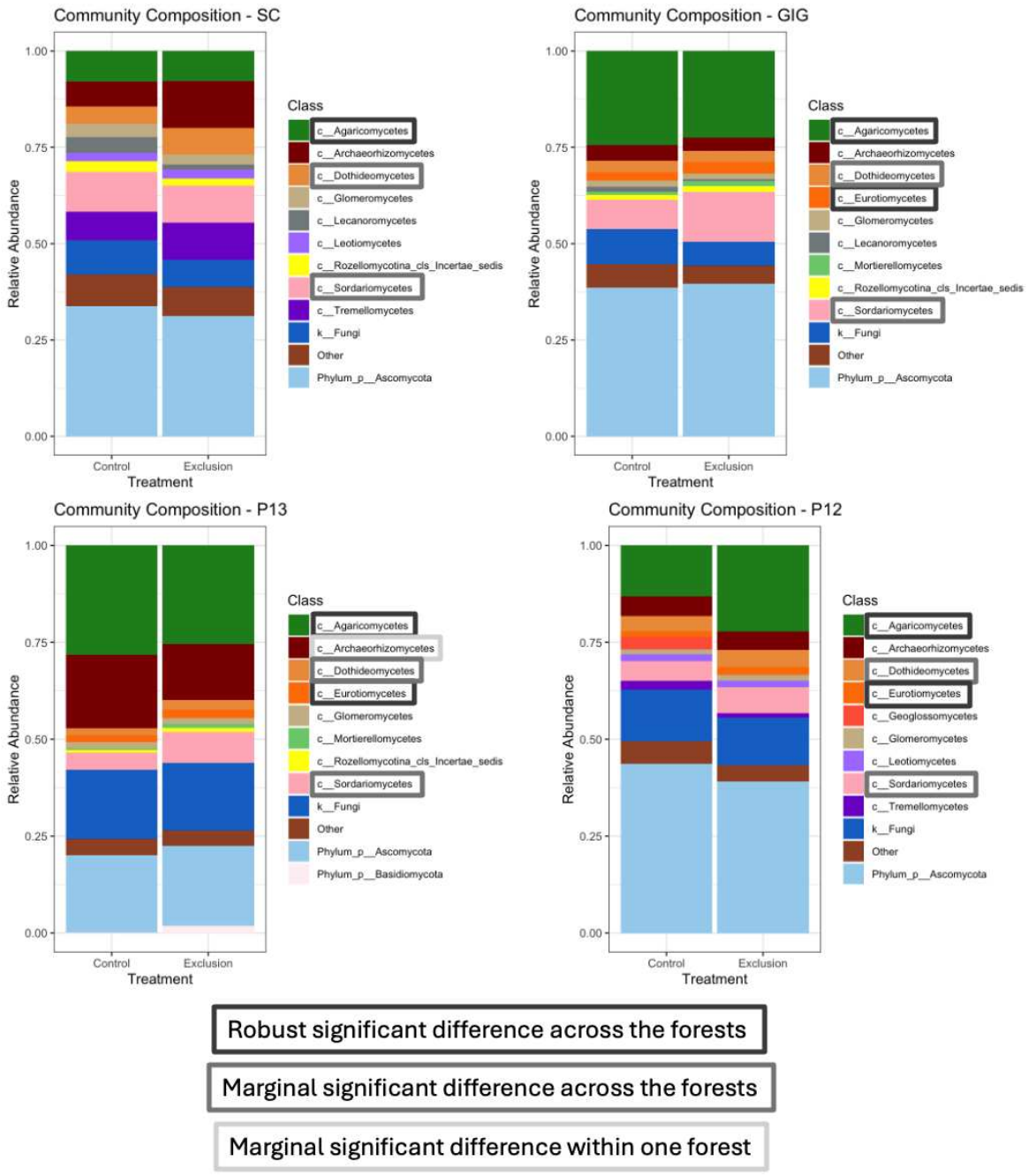

Figure 11: Relative abundances of fungal classes at each forest site. Classes highlighted in black squares are significantly different between control and drying plots across all the forests, as determined by an LDA greater than 3 and an FDR-corrected p-value < 0.05. Classes highlighted in grey have marginally significant differences between treatments across all the forests, as determined by a non-corrected p-value < 0.05, and an LDA score above 3. Classes highlighted in light grey have marginally significant differences between treatments within the specified forest. The "Other" category includes all of the classes that had a lower relative abundance than the top 11 shown (average relative abundance values in the control vs drying plots at each forest across all seasons).

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APPENDIX

Month	Year	Forest	Plot	Treatment	Depth cm	Mean(Bulk density (g-dw/cm3))	Mean(Soil Moisture g-water/g-dry soil)
Dec	2024	GIG	1C	C	0-10	0.75563411	0.54792639
Dec	2024	GIG	2E	Ex	0-10	0.79350673	0.48798927
Dec	2024	GIG	3C	C	0-10	0.52276058	0.49552501
Dec	2024	GIG	4E	Ex	0-10	0.73170245	0.46706367
Dec	2024	GIG	5C	C	0-10	0.66872133	0.54585237
Dec	2024	GIG	6E	Ex	0-10	0.7033754	0.47611444
Dec	2024	GIG	7C	C	0-10	0.76160448	0.43395935
Dec	2024	GIG	8E	Ex	0-10	0.82366315	0.4202469
Dec	2024	P12	1C	C	0-10	0.57519871	0.66752344
Dec	2024	P12	2E	Ex	0-10	0.78276936	0.47029459
Dec	2024	P12	3C	C	0-10	0.6355967	0.56181317
Dec	2024	P12	4E	Ex	0-10	0.78135785	0.40812693
Dec	2024	P12	5C	C	0-10	0.87977189	0.53272371
Dec	2024	P12	6E	Ex	0-10	0.75726701	0.45863915
Dec	2024	P12	7C	C	0-10	0.77619284	0.51766266
Dec	2024	P12	8E	Ex	0-10	0.89993471	0.46809539
Dec	2024	P13	1C	C	0-10	0.66231784	0.58525475
Dec	2024	P13	2E	Ex	0-10	0.59822734	0.54625681
Dec	2024	P13	3C	C	0-10	0.55263466	0.70980156
Dec	2024	P13	4E	Ex	0-10	0.4706116	0.62975778
Dec	2024	P13	5C	C	0-10	0.60731941	1.04840769
Dec	2024	P13	6E	Ex	0-10	0.6321616	0.56116191
Dec	2024	P13	7C	C	0-10	0.88314195	0.58374549
Dec	2024	P13	8E	Ex	0-10	0.6974938	0.5165801
Dec	2024	SC	1C	C	0-10	0.43016448	0.84230672
Dec	2024	SC	2E	Ex	0-10	0.51187484	0.77014139
Dec	2024	SC	3C	C	0-10	0.5344383	0.73082473
Dec	2024	SC	4E	Ex	0-10	0.57977071	0.61836599
Dec	2024	SC	5C	C	0-10	0.87102228	0.46095078
Dec	2024	SC	6E	Ex	0-10	0.77328428	0.66160671
Dec	2024	SC	7C	C	0-10	0.61534972	0.80649337
Dec	2024	SC	8E	Ex	0-10	0.58830718	0.79736922
March	2025	GIG	1C	C	0-10	0.76420033	0.42928047
March	2025	GIG	2E	Ex	0-10	0.72766039	0.3793623
March	2025	GIG	3C	C	0-10	0.7229203	0.41460548
March	2025	GIG	4E	Ex	0-10	0.82275374	0.39917629
March	2025	GIG	5C	C	0-10	0.75827228	0.38147583
March	2025	GIG	6E	Ex	0-10	0.8449705	0.39760116
March	2025	GIG	7C	C	0-10	0.86577096	0.33720932
March	2025	GIG	8E	Ex	0-10	0.96977361	0.33073149
March	2025	P12	1C	C	0-10	0.83936421	0.44610048
March	2025	P12	2E	Ex	0-10	0.7500693	0.33773634

March	2025	P12	3C	C	0-10	0.86837518	0.39666973
March	2025	P12	4E	Ex	0-10	0.69291603	0.40173357
March	2025	P12	5C	C	0-10	0.85249098	0.36342023
March	2025	P12	6E	Ex	0-10	0.7584465	0.44229658
March	2025	P12	7C	C	0-10	0.91064184	0.35818475
March	2025	P12	8E	Ex	0-10	0.87052054	0.38844308
March	2025	P13	1C	C	0-10	0.68004384	0.42538745
March	2025	P13	2E	Ex	0-10	0.82834129	0.40494857
March	2025	P13	3C	C	0-10	0.61089343	0.51227627
March	2025	P13	4E	Ex	0-10	0.71111058	0.39835028
March	2025	P13	5C	C	0-10	0.79334236	0.44907554
March	2025	P13	6E	Ex	0-10	0.67798196	0.43247953
March	2025	P13	7C	C	0-10	0.77265597	0.48184981
March	2025	P13	8E	Ex	0-10	0.63585222	0.4352102
March	2025	SC	1C	C	0-10	0.56988305	0.59849124
March	2025	SC	2E	Ex	0-10	0.66580003	0.58055167
March	2025	SC	3C	C	0-10	0.55987194	0.5994325
March	2025	SC	4E	Ex	0-10	0.55517908	0.52001493
March	2025	SC	5C	C	0-10	0.60271938	0.7409971
March	2025	SC	6E	Ex	0-10	0.54741507	0.62408631
March	2025	SC	7C	C	0-10	0.69667138	0.6803941
March	2025	SC	8E	Ex	0-10	0.8215909	0.41168399
May	2025	GIG	1C	C	0-10	0.66544918	0.53742721
May	2025	GIG	2E	Ex	0-10	0.56615822	0.4946158
May	2025	GIG	3C	C	0-10	0.56460794	0.54162709
May	2025	GIG	4E	Ex	0-10	0.6824234	0.49870678
May	2025	GIG	5C	C	0-10	0.4893639	0.55025425
May	2025	GIG	6E	Ex	0-10	0.55586266	0.5403175
May	2025	GIG	7C	C	0-10	0.71027109	0.46629468
May	2025	GIG	8E	Ex	0-10	0.73310826	0.4121682
May	2025	P12	1C	C	0-10	0.90037918	0.59364294
May	2025	P12	2E	Ex	0-10	0.54012964	0.52394206
May	2025	P12	3C	C	0-10	0.64772944	0.5347024
May	2025	P12	4E	Ex	0-10	0.67112773	0.42875342
May	2025	P12	5C	C	0-10	0.76568947	0.52954106
May	2025	P12	6E	Ex	0-10	0.73529963	0.44857335
May	2025	P12	7C	C	0-10	0.77194723	0.48617188
May	2025	P12	8E	Ex	0-10	0.77898704	0.49826123
May	2025	P13	1C	C	0-10	0.5790185	0.63548854
May	2025	P13	2E	Ex	0-10	0.59072453	0.62738479
May	2025	P13	3C	C	0-10	0.58296016	0.70197252
May	2025	P13	4E	Ex	0-10	0.50220673	0.61011296
May	2025	P13	5C	C	0-10	0.64910335	0.68285387
May	2025	P13	6E	Ex	0-10	0.60057285	0.57140833
May	2025	P13	7C	C	0-10	0.62553021	0.67124686
May	2025	P13	8E	Ex	0-10	0.65065052	0.56388659
May	2025	SC	1C	C	0-10	0.55553798	0.71804256
May	2025	SC	2E	Ex	0-10	0.57212857	0.66304902
May	2025	SC	3C	C	0-10	0.56298308	0.78062564

May	2025	SC	4E	Ex	0-10	0.56340821	0.58077732
May	2025	SC	5C	C	0-10	0.60479751	0.78290059
May	2025	SC	6E	Ex	0-10	0.59250861	0.8053757
May	2025	SC	7C	C	0-10	0.70262663	0.72182206
May	2025	SC	8E	Ex	0-10	0.98572038	0.4011817

SI Table 1: Table of soil moisture values from bulk density across all sampling timepoints.

Phylum	Pvalue	FDR	LDAs core	RA SC	RA GIG	RA P12	RA P13	Significance
Verrucomicrobiota	2.50E-11	2.63E-10	4.76	6.55%	9.80%	16.60%	11.20%	LDA 4, p < 0.05
Pseudomonadota	7.55E-07	9.94E-07	4.71	29.81%	25.63%	24.11%	19.29%	LDA 4, p < 0.05
Acidobacteriota	5.71E-05	6.80E-05	4.56	26.49%	21.65%	20.67%	21.25%	LDA 4, p < 0.05
Methylomirabilota	3.47E-11	2.63E-10	4.4	0.58%	2.34%	1.64%	3.82%	LDA 4, p < 0.05
Planctomycetota	5.99E-09	1.25E-08	4.39	11.75%	9.48%	11.62%	7.02%	LDA 4, p < 0.05
Actinomycetota	0.00042782	0.00046502	4.24	7.18%	9.29%	8.87%	12.18%	LDA 4, p < 0.05
Chloroflexota	7.21E-11	3.01E-10	4.21	4.91%	6.76%	3.51%	4.35%	LDA 4, p < 0.05
Myxococcota	5.84E-10	1.82E-09	4.17	2.65%	5.52%	3.31%	5.12%	LDA 4, p < 0.05
Bacteroidota	4.94E-09	1.13E-08	3.99	0.70%	0.90%	1.83%	3.25%	LDA 3, p < 0.05
Thermodesulfobacteriota	9.16E-09	1.43E-08	3.79	0.41%	1.16%	1.03%	1.66%	LDA 3, p < 0.05
Gemmatimonadota	5.26E-11	2.63E-10	3.73	0.62%	1.00%	0.42%	2.02%	LDA 3, p < 0.05
Nitrospirota	6.58E-09	1.27E-08	3.71	0.22%	0.44%	0.24%	0.92%	LDA 3, p < 0.05
Entotheonellaeota	3.34E-08	4.90E-08	3.6	0.18%	0.43%	0.51%	1.04%	LDA 3, p < 0.05
Bacillota	0.042621	0.042621	3.6	1.34%	0.66%	0.93%	0.88%	LDA 3, p < 0.05
RCP2_54	6.33E-05	7.19E-05	3.58	1.62%	1.45%	1.63%	0.89%	LDA 3, p < 0.05
MBNT15	4.83E-09	1.13E-08	3.46	0.12%	0.31%	0.26%	0.59%	LDA 3, p < 0.05

Candidatus_Eremiobacterota	4.93E-11	2.63E-10	3.41	0.95%	0.26%	0.25%	0.01%	LDA 3, p < 0.05
GAL15	2.55E-10	9.12E-10	3.2	0.30%	0.22%	0.12%	0.03%	LDA 3, p < 0.05
Latescibacterota	4.99E-09	1.13E-08	3.13	0.19%	0.45%	0.33%	1.27%	LDA 3, p < 0.05
Bacteria	9.57E-07	1.20E-06	3.1	0.41%	0.37%	0.30%	0.50%	LDA 3, p < 0.05
NB1_j	7.43E-09	1.33E-08	3.04	0.15%	0.16%	0.16%	0.71%	LDA 3, p < 0.05
Bdellovibrionota	4.57E-11	2.63E-10	2.76	0.49%	0.21%	0.41%	0.28%	NS
Cyanobacteriota	2.58E-07	3.59E-07	2.72	0.35%	0.15%	0.27%	0.12%	NS
Chlamydiota	8.04E-09	1.34E-08	2.69	1.23%	0.67%	0.45%	0.41%	NS
Armatimonadota	0.0044659	0.004652	2.21	0.18%	0.27%	0.19%	0.31%	NS

SI Table 2: Table of all the bacterial phyla present across the forests with the differential abundance (LefSe) values shown as well as relative abundance at each site. The LefSe values provided by MicrobiomeAnalyst include the p-value, FDR value, and LDA score.

Class	Pvalues	FDR	LDA score	RA SC	RA GIG	RA P12	RA P13	Significance
Phylum_p__Ascomycota	3.47E-11	4.17E-10	5.42	32.48%	39.14%	41.43%	20.32%	LDA 4, p < 0 0.05
c__Archaeorhizomycetes	2.93E-07	7.82E-07	5.22	9.32%	3.73%	4.96%	16.67%	LDA 4, p < 0 0.05
c__Tremellomycetes	4.67E-09	1.60E-08	5.2	8.46%	0.86%	1.71%	0.64%	LDA 4, p < 0 0.05
c__Sordariomycetes	0.00017254	0.00023288	4.76	10.10%	10.25%	5.94%	6.20%	LDA 4, p < 0 0.05
c__Agaricomycetes	0.0019006	0.0024008	4.29	7.93%	23.48%	17.65%	26.87%	LDA 4, p < 0 0.05
c__Rozellomycotina_cls_Incertae_sedis	4.03E-07	9.67E-07	4.28	2.25%	1.30%	0.80%	0.84%	LDA 4, p < 0 0.05
c__Dothideomycetes	0.064383	0.065373	4.21	5.67%	2.98%	4.15%	2.14%	NS
c__Eurotiomycetes	0.00011875	0.00017812	4.2	2.00%	2.56%	1.77%	2.03%	LDA 4, p < 0 0.05
c__Glomeromycetes	0.034621	0.039567	4.07	3.01%	1.54%	1.37%	1.52%	LDA 4, p < 0 0.05
c__Leotiomycetes	6.50E-12	1.56E-10	4	2.26%	0.33%	1.71%	0.06%	LDA 4, p < 0 0.05
c__Cystobasidiomycetes	1.27E-08	3.80E-08	3.95	0.03%	0.19%	0.03%	0.17%	LDA 3, p < 0.05
Phylum_p__Glomeromycota	8.17E-07	1.64E-06	3.95	0.49%	0.33%	0.49%	0.40%	LDA 3, p < 0.05
c__Ramicandelaberomycetes	3.38E-09	1.35E-08	3.82	0.09%	0.30%	0.15%	0.35%	LDA 3, p < 0.05
c__Mortierellomycetes	0.065373	0.065373	3.77	1.47%	1.10%	0.51%	0.73%	NS
c__Chytridiomycota_cls_Incertae_sedis	2.58E-10	1.55E-09	3.65	0.03%	0.03%	0.01%	0.14%	LDA 3, p < 0.05
c__Atractiellomycetes	2.61E-06	4.48E-06	3.52	0.03%	0.17%	0.03%	0.08%	LDA 3, p < 0.05

c__Chytridiomycetes	9.87E-05	0.00015792	3.46	0.07%	0.05%	0.02%	0.06%	LDA 3, p < 0.05
c__Rhizophydiomycetes	0.056012	0.061104	3.44	1.12%	0.29%	0.33%	0.37%	NS
Phylum_p__Chytridiomycota	1.85E-09	8.90E-09	3.28	0.31%	0.21%	0.21%	0.25%	LDA 3, p < 0.05
c__Saccharomycetes	2.48E-06	4.48E-06	3.13	0.25%	0.24%	0.08%	0.09%	LDA 3, p < 0.05
c__Lecanoromycetes	6.96E-07	1.52E-06	3.1	2.70%	0.87%	1.27%	0.22%	LDA 3, p < 0.05
c__Orbiliomycetes	7.59E-11	6.07E-10	2.9	0.16%	0.30%	0.23%	0.26%	NS
c__Endogonomycetes	0.00017466	0.00023288	2.78	0.18%	0.26%	0.19%	0.04%	NS
c__Glomeromycota_cls_Incertae_sedis	0.015234	0.018281	2.08	0.52%	0.24%	0.23%	0.05%	NS

SI Table 2: Table of all the fungal classes present across the forests with the differential abundance (LefSe) values shown as well as relative abundance at each site. The LefSe values provided by MicrobiomeAnalyst include the p-value, FDR value, and LDA score.

Phylum	Pvalues	FDR	LDAscore	RA Control	RA Exclusion	Signifigance
Acidobacteriota	0.052159	0.22354	4.08	23.47%	21.55%	NS
Planctomycetota	0.10225	0.30674	3.71	10.59%	9.34%	NS
Bacteroidota	0.116	0.31637	3.41	1.88%	1.46%	NS
Entotheonellaeota	0.39941	0.57058	2.81	0.59%	0.49%	NS
Cyanobacteriota	4.38E-05	0.0013148	2.68	0.29%	0.15%	NS
Bdellovibrionota	0.00058015	0.0058894	2.68	0.43%	0.27%	NS
Candidatus_Eremiobacterota	0.26426	0.51642	2.67	0.42%	0.31%	NS
MBNT15	0.76662	0.90377	2.64	0.36%	0.28%	NS
NB1_j	0.39736	0.57058	2.58	0.33%	0.26%	NS
Chloroflexota	0.90377	0.90377	2.32	4.95%	4.80%	NS
Latescibacterota	0.86591	0.90377	2.31	0.63%	0.49%	NS
Bacteria	0.22944	0.51642	2.26	0.43%	0.36%	NS
Myxococcota	0.8203	0.90377	2.14	4.19%	4.11%	NS
Thermodesulfobacteriota	0.89507	0.90377	1.86	1.05%	1.10%	NS
Elusimicrobiota	0.02301	0.17258	1.83	0.13%	0.10%	NS
GAL15	0.53818	0.70198	1.83	0.17%	0.16%	NS

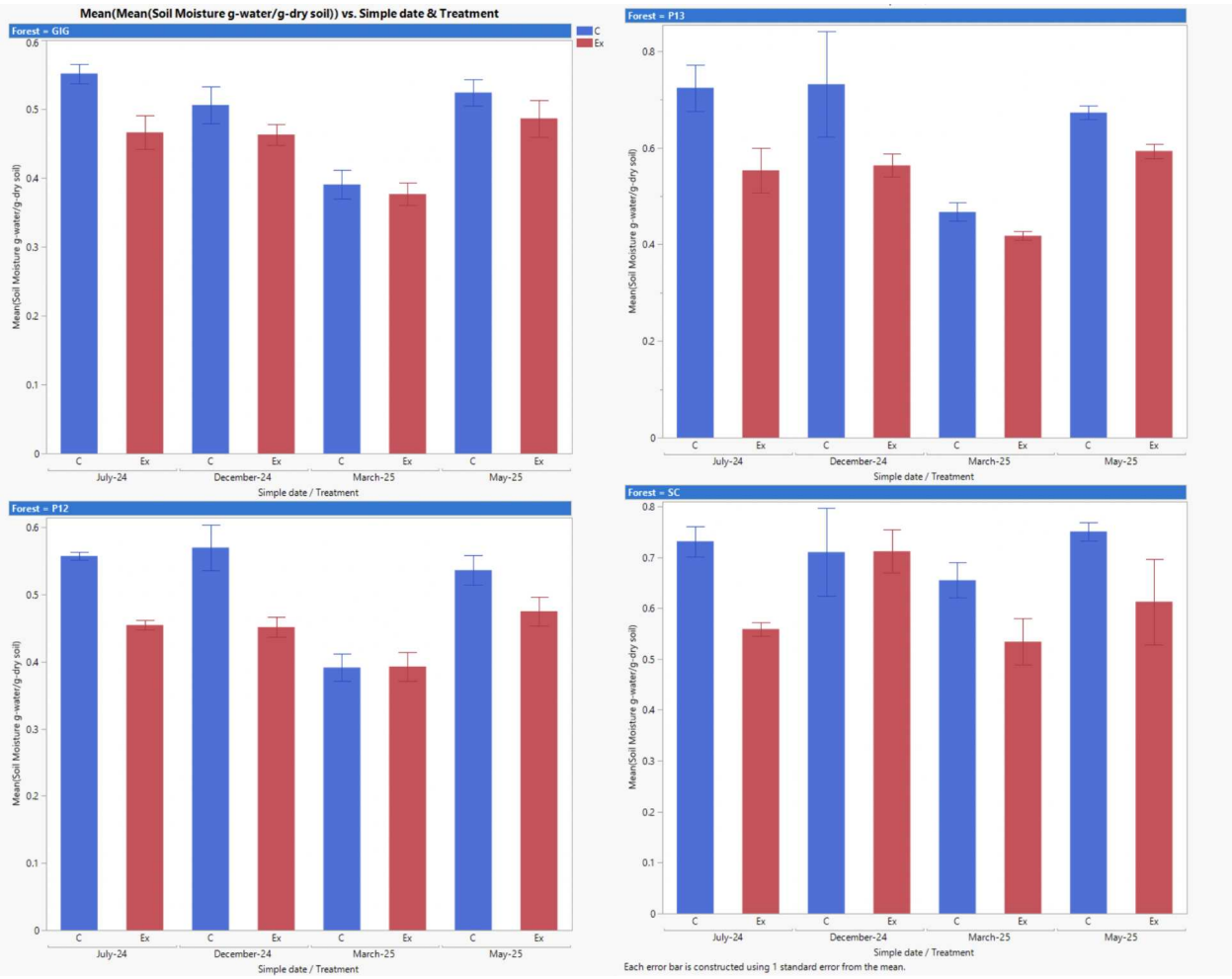
Chlamydiota	0.079873	0.27358	1.76	0.76%	0.62%	NS
Armatimonadota	0.27161	0.51642	1.76	0.26%	0.22%	NS
Candidatus_Kryptonia	0.082075	0.27358	1.46	0.08%	0.02%	NS
Dependentiae	0.42388	0.57802	1.38	0.22%	0.21%	NS
Candidatus_Kapabacteria	0.035895	0.18715	1.12	0.03%	0.02%	NS
WS4	0.27542	0.51642	0.581	0.01%	0.01%	NS
Gemmatimonadota	0.32977	0.56799	-1.15	0.97%	1.05%	NS
Bacillota	0.37725	0.57058	-2.17	0.95%	0.96%	NS
RCP2_54	0.8489	0.90377	-2.53	1.37%	1.44%	NS
Nitrospirota	0.19212	0.48031	-2.79	0.42%	0.49%	NS
Methylomirabilota	0.3408	0.56799	-3.06	2.02%	2.19%	NS
Verrucomicrobiota	0.826	0.90377	-3.33	10.83%	11.25%	NS
Pseudomonadota	0.03743	0.18715	-3.94	23.84%	25.58%	NS
Actinomycetota	0.00058894	0.0058894	-4.02	8.15%	10.60%	LDA 4, $p < 0.05$

SI Table 3: Table of all the bacterial phyla present across the forests with the differential abundance (LefSe) values shown as well as relative abundance within the control and esxlusion samples. The LefSe values provided by MicrobiomeAnalyst include the p-value, FDR value, and LDA score.

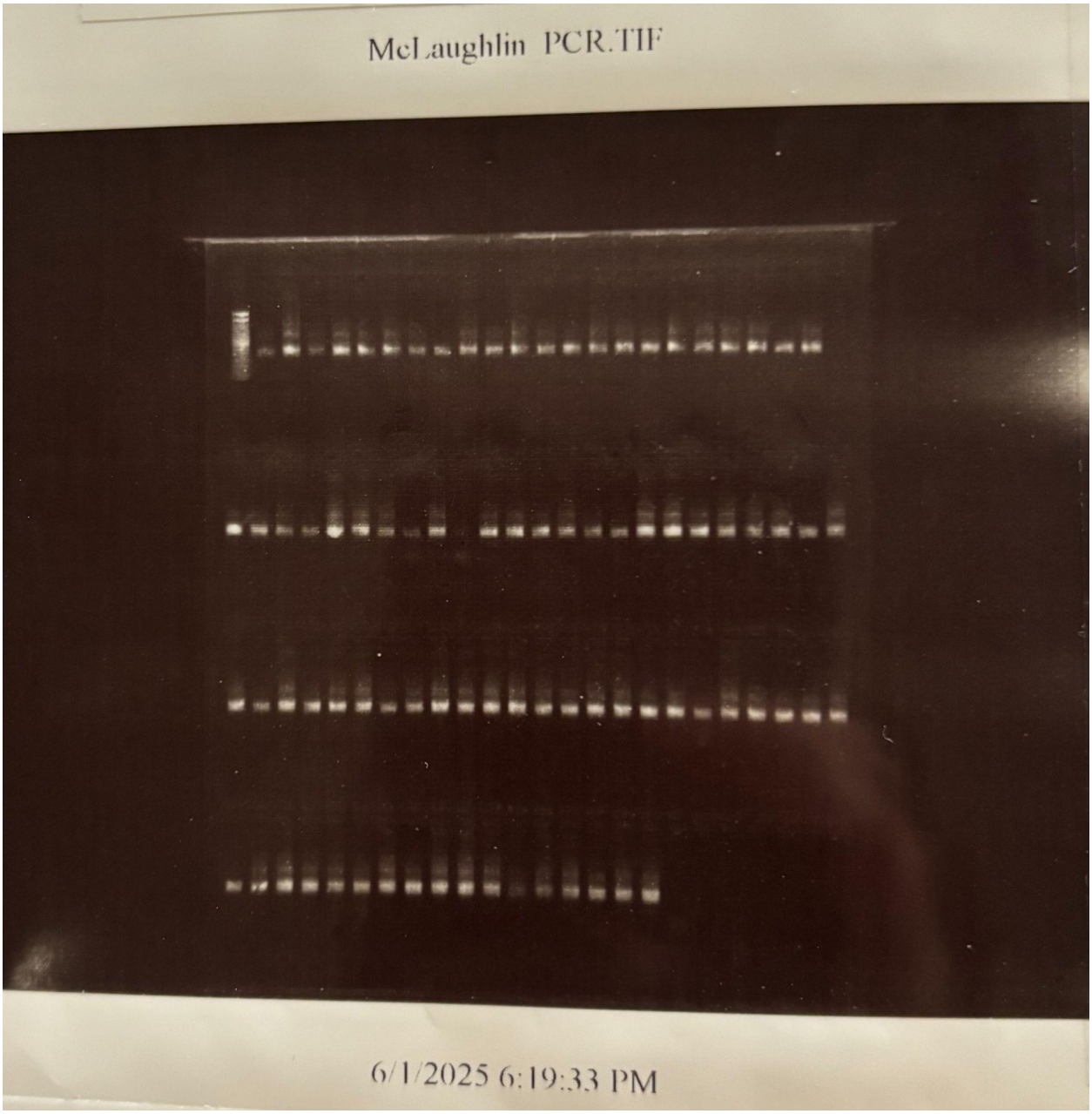
Class	Pvalues	FDR	LDAscore	RA Control	RA Exclusion	Signifigance
Phylum_p__Ascomycota	0.16385	0.49464	4.53	34.00%	32.68%	NS
c__Archaeorhizomycetes	0.30495	0.57179	4.24	8.65%	8.69%	NS
c__Lecanoromycetes	0.91237	0.97754	3.4	1.71%	0.81%	NS
c__Rozellomycotina_cls_Incertae_sedis	0.51528	0.8136	3.3	1.49%	1.10%	NS
c__Glomeromycota_cls_Incertae_sedis	0.0021832	0.016374	3.27	0.33%	0.19%	LDA 3, $p < 0.05$
c__Rhizophydiomycetes	0.00042024	0.012607	3.22	0.70%	0.35%	LDA 3, $p < 0.05$
c__Glomeromycetes	0.54793	0.82189	3.08	1.98%	1.74%	NS
c__Cladochytriomycetes	0.16488	0.49464	3.05	0.09%	0.02%	NS
c__Mortierellomycetes	0.3004	0.57179	3.02	0.83%	1.08%	NS
c__Endogonomycetes	0.42657	0.75277	3.01	0.23%	0.10%	NS
c__Ramicandelaberomycetes	0.90027	0.97754	2.85	0.21%	0.23%	NS
Phylum_p__Glomeromycota	0.99123	0.99123	2.52	0.45%	0.40%	NS
c__Chytridiomycetes	0.027173	0.15607	2.45	0.06%	0.05%	NS
c__Leotiomycetes	0.82753	0.97754	2.03	1.07%	1.11%	NS
c__Orbiliomycetes	0.96865	0.99123	1.64	0.24%	0.24%	NS
c__Chytridiomycota_cls_Incertae_sedis	0.65597	0.9371	1.17	0.06%	0.04%	NS

c__Saccharomycetes	0.51049	0.8136	1	0.15%	0.18%	NS
c__Rozellomycota_cls_Incertae_sedis	0.72524	0.94596	0.647	0.05%	0.10%	NS
c__Pucciniomycetes	0.22335	0.55837	-1.63	0.05%	0.06%	NS
Phylum_p__Chytridiomycota	0.89905	0.97754	-1.76	0.30%	0.18%	NS
c__Microbotryomycetes	0.26926	0.57179	-2.06	0.05%	0.05%	NS
c__Umbelopsidomycetes	0.88195	0.97754	-2.15	0.02%	0.02%	NS
c__Atractiellomycetes	0.13854	0.49464	-2.59	0.06%	0.09%	NS
c__Cystobasidiomycetes	0.22269	0.55837	-3.31	0.06%	0.15%	NS
c__Eurotiomycetes	0.0017544	0.016374	-3.39	1.81%	2.37%	LDA 3, $p < 0.05$
k__Fungi	0.27815	0.57179	-3.9	12.31%	10.78%	NS
c__Tremellomycetes	0.71408	0.94596	-3.91	2.89%	2.95%	NS
c__Dothideomycetes	0.031215	0.15607	-3.98	3.30%	4.16%	NS
c__Agaricomycetes	0.00097573	0.014636	-4.06	18.44%	19.52%	LDA 4, $p < 0.05$
c__Sordariomycetes	0.044668	0.19143	-4.37	6.89%	9.33%	LDA 4, $p <0.05$

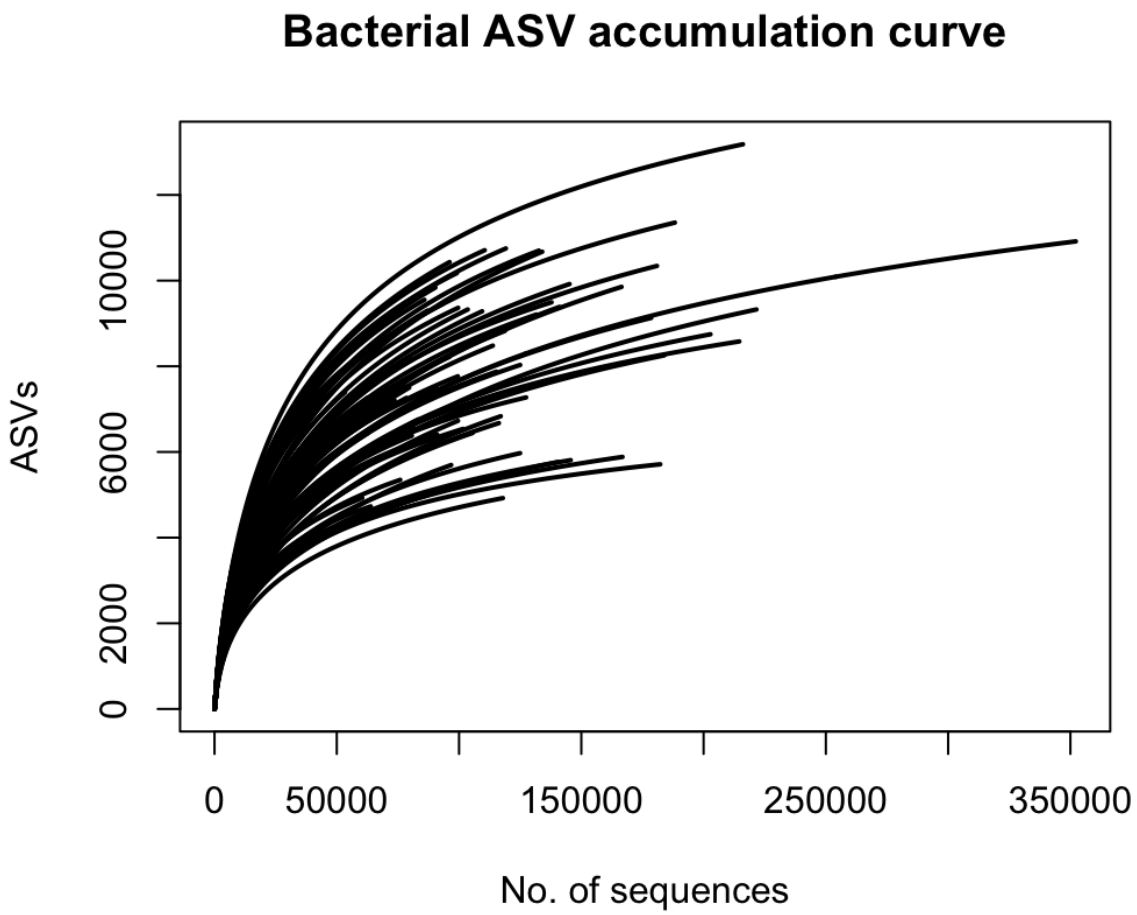
SI Table 4: Table of all the fungal classes present across the forests with the differential abundance (LefSe) values shown as well as relative abundance within the control and exclusion samples. The LefSe values provided by MicrobiomeAnalyst include the p-value, FDR value, and LDA score.



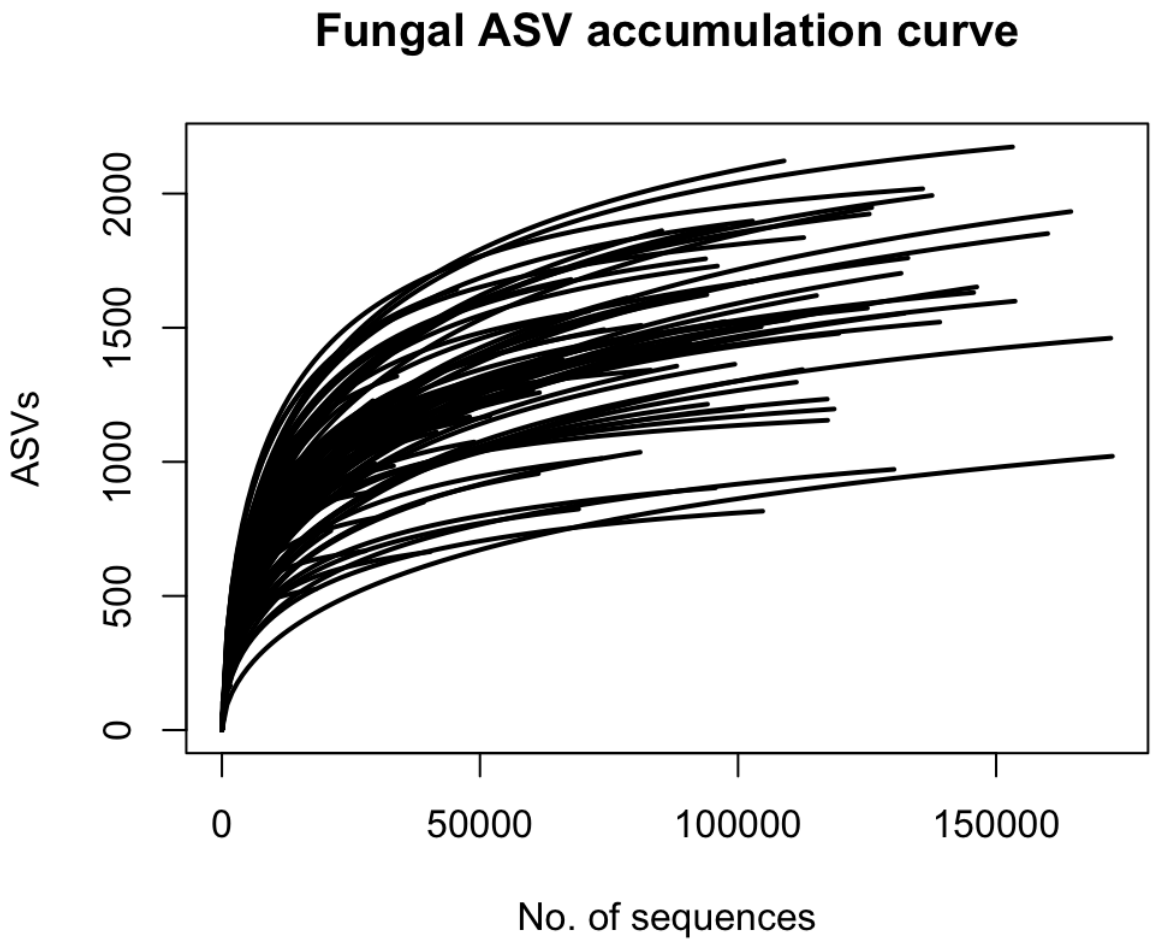
SI Figure 1: Soil moisture from the four forests within this project, GIG, P13, P12, and SC. Control plots are shown in blue, and exclusion plots are shown in red. The error bars are constructed with one standard error from the mean.



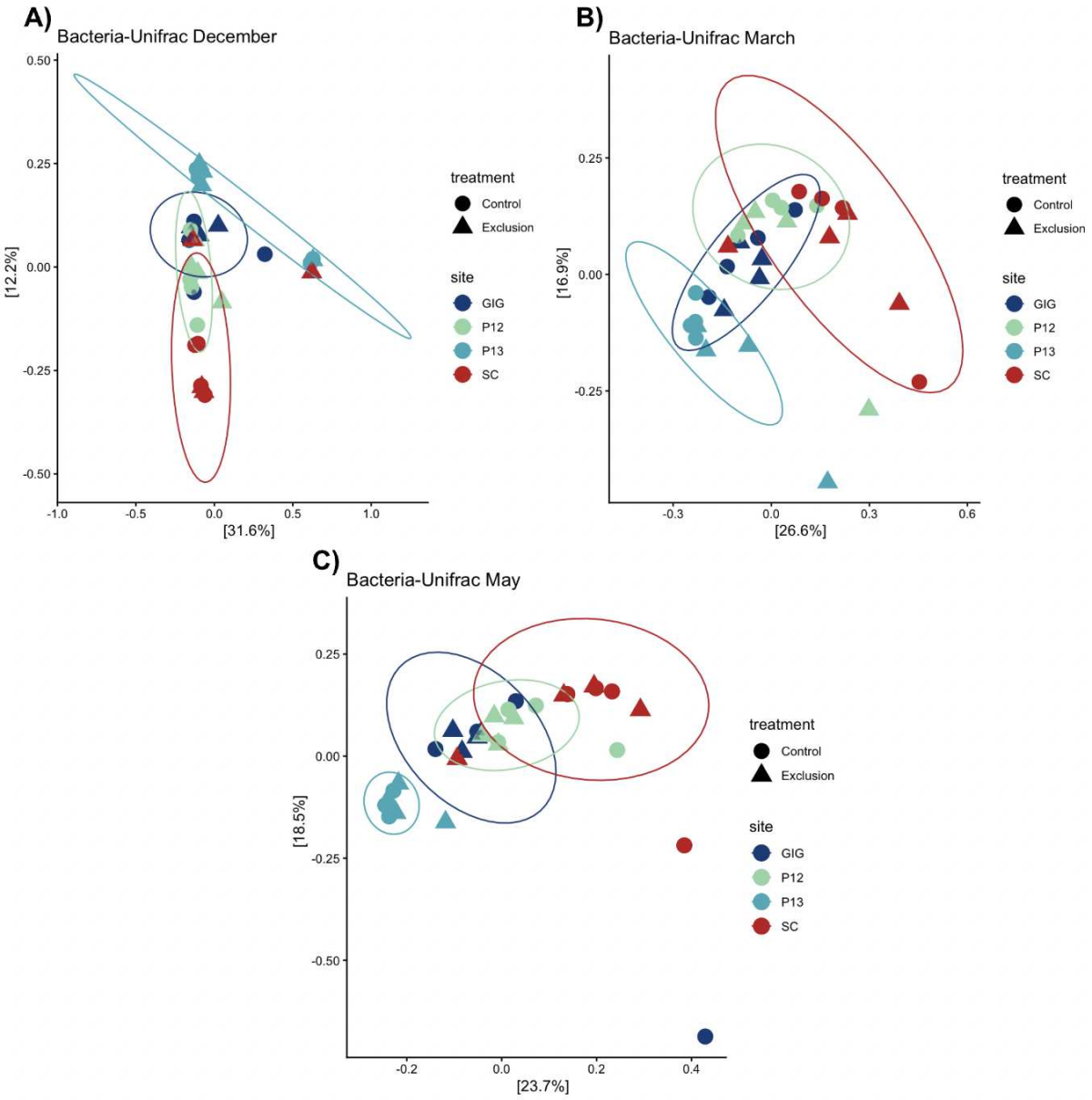
SI Figure 2: Image of results from a gel electrophoresis of the full bacterial 16S check of the first triplicate in PCR1.



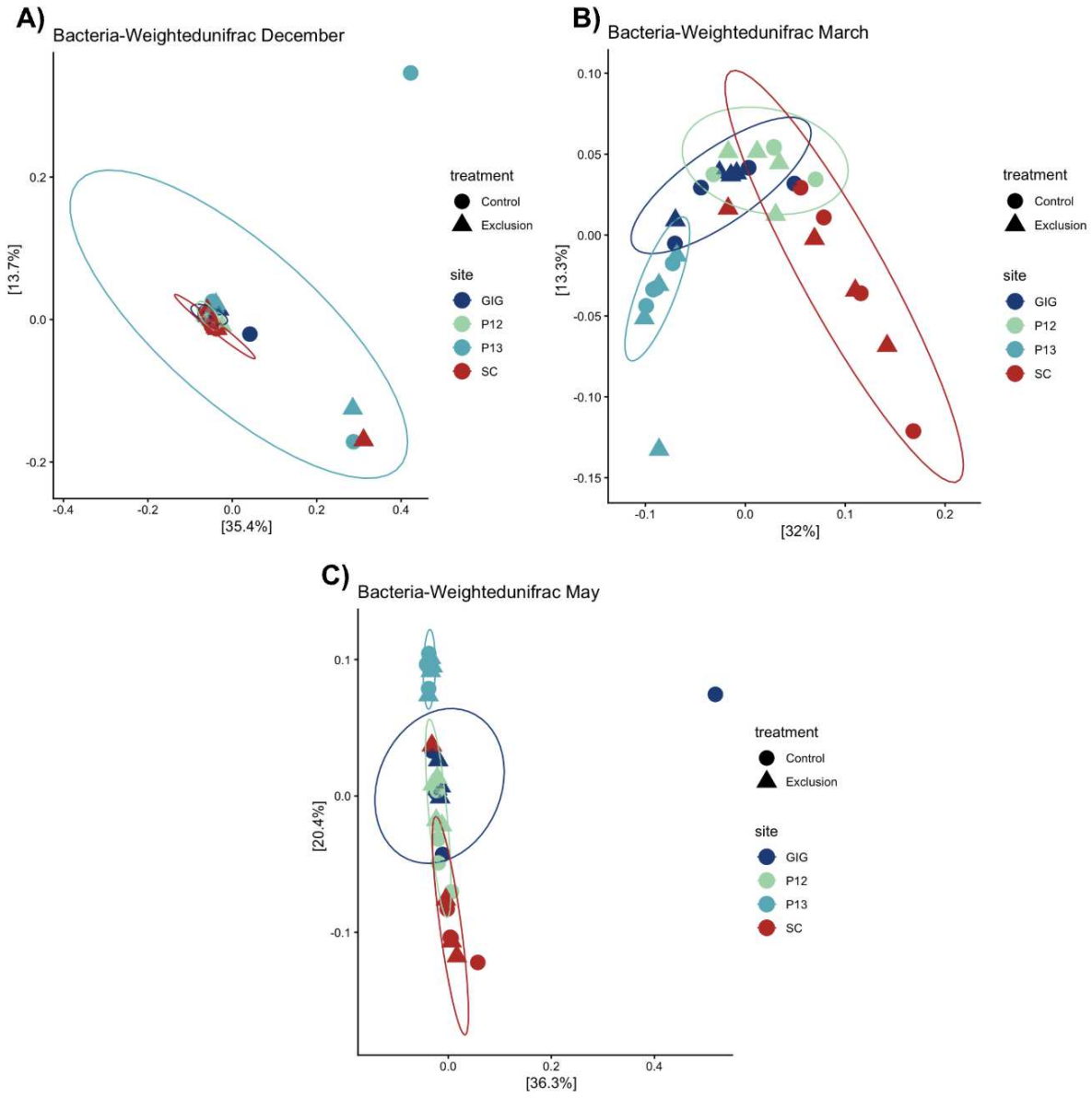
SI Figure 3: ASVs and number of sequences per for the bacterial samples, indicating even read depth across all 96 samples.



SI Figure 4: ASVs and number of sequences per for the fungal samples, indicating even read depth across all 96 samples.



SI Figure 5: Principal coordinate analysis of bacteria using the Unifrac metric. Sampling forest is shown by color, and treatment is indicated by shape, ellipses indicating grouping at a 95% confidence level.



SI Figure 6: Principal coordinate analysis of bacteria using the Weighted Unifrac metric. Sampling forest is shown by color, and treatment is indicated by shape, ellipses indicating grouping at a 95% confidence level.