

Divergent responses of soil microorganisms to throughfall exclusion across tropical forest soils driven by soil fertility and climate history

Stephany Soledad Chacon¹, Daniela Cusack², Aizah Khurram³, Markus Bill¹, Lee Dietterich⁴, and Nicholas Bouskill⁵

¹Climate and Ecosystem Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720

²Department of Ecosystem Science and Sustainability

³Climate and Ecosystem Sciences Division

⁴Department of Ecosystem Science and Sustainability, Colorado State University, Fort Collins, CO, 80523

⁵Climate and Ecosystem Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720

November 23, 2022

Abstract

Model projections predict tropical forests will experience longer periods of drought and more intense precipitation cycles under a changing climate. Such transitions have implications for structure-function relationships within microbial communities. We examine how chronic drying might reshape prokaryotic and fungal communities across four lowland forests in Panama with a wide variation in mean annual precipitation and soil fertility. Four sites were established across a 1000 mm span in mean annual precipitation (2335 to 3300 mm). We expected microbial communities at sites with lower MAP to be less sensitive to chronic drying than sites with higher MAP; while fungal communities to be more resistant to disturbance than prokaryotes. At each location, partial throughfall exclusion structures were established over 10 x 10 m plots to reduce direct precipitation input. After the first nine months of throughfall exclusion, prokaryotic communities showed no change in composition. However, 18 months of throughfall exclusion resulted in markedly divergent prokaryotic community responses, reflecting MAP and soil fertility. We observed the emergence of a “drought microbiome” within infertile sites, whereby the community structure of the experimental drying plots at the lower MAP sites diverged from their respective control sites and converged towards overlapping assemblages. Furthermore, taxa increasing in relative abundance under throughfall exclusion at the highest MAP became more similar to taxa characteristic of the control plots at the lowest MAP site, suggesting a shift toward communities with life-history traits selected 1

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Stephany S. Chacon¹ (ORCID# 0000-0001-7599-9152)

Daniela F. Cusack^{2,3} (ORCID#0000-0003-4681-7449)

Aizah Khurram¹ (ORCID# 0000-0002-3796-5522)

Markus Bill¹ (ORCID# 0000-0001-7002-2174)

Lee H. Dietterich² (ORCID # 0000-0003-4465-5845)

Nicholas J. Bouskill¹ (ORCID# 0000-0002-6577-8724)

¹Climate and Ecosystem Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720

²Department of Ecosystem Science and Sustainability, Colorado State University, Fort Collins, CO, 80523

³Smithsonian Tropical Research Institute, Apartado, 0843-03092, Balboa, Ancon, Panama.

Corresponding Authors: Stephany Chacon (sschacon@lbl.gov), Nick Bouskill (njbouskill@lbl.gov).

Keywords: Field, throughfall exclusion, microbial community, soil, nitrogen, tropical rainforest, Panama, phosphorus, base cations, SOM, mean annual precipitation

Abstract

Model projections predict tropical forests will experience longer periods of drought and more intense precipitation cycles under a changing climate. Such transitions have implications for structure-function relationships within microbial communities. We examine how chronic drying might reshape prokaryotic and fungal communities across four lowland forests in Panama with a wide variation in mean annual precipitation and soil fertility. Four sites were established across a 1000 mm span in mean annual precipitation (2335 to 3300 mm). We expected microbial communities at sites with lower MAP to be less sensitive to chronic drying than sites with higher MAP; while fungal communities to be more resistant to disturbance than prokaryotes. At each location, partial throughfall exclusion structures were established over 10 x 10 m plots to reduce direct precipitation input. After the first nine months of throughfall exclusion, prokaryotic communities showed no change in composition. However, 18 months of throughfall exclusion resulted in markedly divergent prokaryotic community responses, reflecting MAP and soil fertility. We observed the emergence of a “drought microbiome” within infertile sites, whereby the community structure of the experimental drying plots at the lower MAP sites diverged from their respective control sites and converged towards overlapping assemblages. Furthermore, taxa increasing in relative abundance under throughfall exclusion at the highest MAP became more similar to taxa characteristic of the control plots at the lowest MAP site, suggesting a shift toward communities with life-history traits selected

under a lower MAP. By contrast, fungal community composition across sites was resilient to drying; however, biomass declined in the throughfall exclusion plots at all sites. Broadly, our results suggest that the sensitivity of microbial communities in tropical forest soils to frequent periods of drying and re-wetting will be dependent on climate history and soil fertility, with infertile sites likely to respond readily to changes in precipitation.

Introduction

Tropical forest soils contain some of the largest carbon stocks on Earth (Crowther et al., 2019; Jackson et al., 2017). Humid and warm conditions promote high primary productivity, which offsets high ecosystem respiration rates (Bonan, 2008; Malhi & Grace, 2000). This balance in productivity and respiration has resulted in significant carbon accumulation in plant biomass and soils within tropical forests. These vast carbon stocks can be destabilized under a changing climate (Mitchard, 2018; Sullivan et al., 2020), and model projections predict tropical and subtropical regions will experience disturbance to the hydrological cycle, with an increased likelihood of more frequent and prolonged droughts interspersed with periods of intense precipitation (Chadwick et al., 2016; Easterling et al., 2000; Meehl et al., 2006). Drought within tropical regions has previously been demonstrated to disrupt soil nutrient cycling (O’Connell et al., 2018) and may decrease tropical forest C storage (Cusack et al., 2011; Doughty et al., 2014; Gatti et al., 2014; Phillips et al., 2009).

The impact of soil drying on microbial communities within tropical forest soils remains poorly understood. The resistance and resilience of a community are shaped largely by historical contingencies (Evans and Wallenstein, 2014; Hawkes and Keitt, 2015). Thus, past and present climate, in particular, mean annual precipitation and dry season lengths, are likely important in determining the sensitivity of soil microbes to drought (Azarbad et al., 2020). Therefore, regions with high precipitation may be more sensitive to seldomly experienced environmental changes, such as soil drying (Bouskill et al., 2013; Hawkes & Keitt, 2015). Indeed, microbial communities without a historical legacy of drought

have exhibited profound shifts in community composition (Bouskill et al., 2013) function (Bouskill, Wood, Baran, Hao, et al., 2016; Bouskill, Wood, Baran, Ye, et al., 2016), and higher mortality (Veatch et al., 2020). These historical contingencies place constraints on microbial responses to changes in the environment, which in turn could shape the trait distribution of the microbial community as a whole.

While the adaptive loss of function, gene transfer, and genome streamlining have diluted trait-linkage to phylogeny in many cases, there remain several functional traits that exhibit taxonomic conservatism (Martiny et al., 2015). Such conservation might further explain why bacteria show phylogenetically conserved responses to different disturbances (Amend et al., 2016; Isobe et al., 2019, 2020) and highlights the importance of characterizing microbial community response to a disturbance at a taxonomic level. However, the taxonomic responses of microorganisms to drying can be quite variable. Gram-positive bacteria are generally considered to be more drought-tolerant than gram-negative bacteria (Manzoni et al., 2012; Uhlířová et al., 2005); however, several Gram-negative bacteria, including Acidobacteria, Verrucomicrobia, and Alphaproteobacteria, have been observed to tolerate periods of droughts, while Actinobacteria, which are Gram-positive, can be sensitive to soil drying (de Vries et al., 2018; Isobe et al., 2020). Similarly, Ascomycota and Glomeromycota have been observed to be more drought tolerant fungal phyla, whereas fungi in the Mortierellaceae family within the Mucoromycota phylum are more drought-sensitive (de Vries et al., 2018). However, sufficient distinction remains in phylogenetic data-sets at high taxonomic levels to predict the responses of members of a community based on their life-history traits (Evan and Wallenstein, 2014).

To improve understanding of how an increased frequency of chronic drying in tropical forests will impact soil microbial communities during a throughfall exclusion experiment. The shelters were placed in four lowland tropical forests in Panama to study separate varying in background rainfall, dry season length, and soil fertility. We quantified the total biomass as well as measured the alpha and beta diversity metrics of the microbial community in tropical forest soils after 9 and 18 months of chronic drying treatment in four tropical forests. We hypothesized that:

1. Historical contingencies render tropical forest soils sites with lower MAP and longer dry seasons more resistant to throughfall exclusion.
2. A generalizable demographic shift occurs across all sites selecting for gram-positive over gram-negative bacteria in response to throughfall exclusion.
3. Fungal communities will be more resistant to disturbance than bacterial communities.

2. Materials and Methods

2.1 Site Information: This study was conducted in four distinct lowland seasonal forests on the Isthmus of Panama (Fig. 1) that range in rainfall from 2335 to 3300 mm mean annual precipitation (MAP). A detailed description and soil USDA taxonomy classification of these sites have been published recently (Cusack et al., 2018, 2019), and further information is provided in Table 1. The dry season spans from approximately mid-December to mid-April, with longer, stronger dry seasons at the lower MAP sites toward the Pacific Coast, with greater rainfall and shorter dry seasons on the Caribbean Coast. The Isthmus includes great variation in the geological substrate (Stewart et al., 1980), which gives rise to contrasting soil fertility that is uncorrelated with changes in rainfall (Pyke et al., 2001; Turner & Engelbrecht, 2011). We selected four sites that span half of the natural rainfall gradient on the Isthmus of Panama. The Sherman Crane (SC) site is located in the North of Panama, close to the Caribbean coast, with MAP ~ 3300 mm of rainfall per year. Two sites (P12 and P13) are located on Buena Vista Peninsula and receive the same MAP of ~ 2600 mm. The final site, Gigante, receives ~ 2335 mm per year. Three of the forests are on infertile, strongly weathered soils (SC, P12, and GIG), while the P13 site is located on fertile soils with higher base cations, phosphate, and ammonium concentrations than the other three sites (Table 1). This site is situated within close proximity to P12 and thus serves to clarify the role nutrient availability plays in the microbial response to hydrological perturbation.

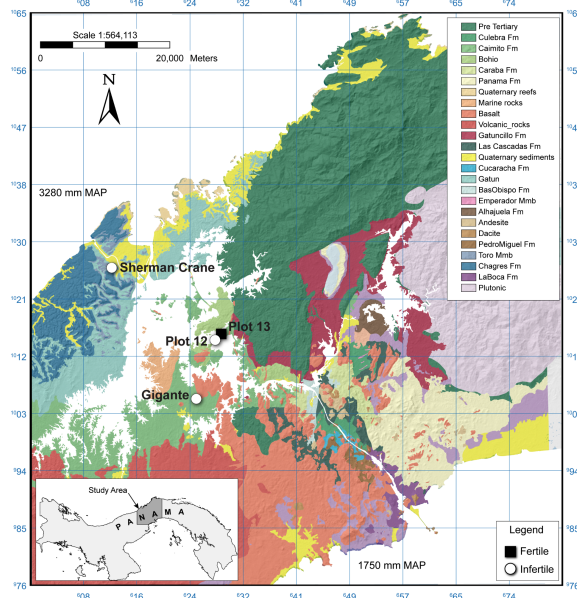


Figure 1: Location of field sites across with varying MAP and fertility in Panama.

Section 2.2 Field Throughfall Exclusion Experiment

Throughfall exclusion structures were erected in each of the four Panamanian forests described above. Briefly, 10m x 10m throughfall exclusion plots were paired with similar 10m x 10m control plots, with four pairs per site assigned according to local topography, spatial proximity, and tree cover. Throughfall exclusion structures were designed to divert ~50% of throughfall away from the plots, reducing the precipitation that reaches the soil. Each plot was trenched, and the trenches lined with heavy plastic and back-filled with soil. This was done to prevent water diffusion and inhibit roots from leaving the throughfall exclusion plots to forage for water. Throughfall exclusion frames were constructed of aluminum support poles and PVC cross-supports, with a peak in the center of the plots. These structures were topped with clear plastic laminates to cover 50% of the plot area. Roof slope from a height of 2.3 m to about 1.1 m over a horizontal distance of about 6 m, producing a slope of about 11.3 degrees. Throughfall exclusion structures were completed between June and December of 2018. Further details on experimental design are provided in Dietterich et al. (submitted).

For this study, we sampled soils in May 2019 (after ~9 months of throughfall exclusion) and again in January 2020 (after ~18 months of throughfall exclusion). The May time point corresponds to the early wet season, while the January time point corresponds to the beginning of the dry season in Panama. For each sampling effort, we collected soil samples from control and throughfall exclusion plots at two depths (0-10 cm and 10-20 cm) using a 2.54 cm diameter soil corer that was cleaned after each collection. Six samples were collected at each depth in each plot and stored in sterile Whirl-Pak bags. Soil samples were shipped overnight to Lawrence Berkeley National Laboratory at ambient temperature. The six replicate soil samples for each depth and plot were pooled into two composite samples to integrate across spatial heterogeneity. The soil composites were separated for biological and chemical analyses and stored at -80°C.

2.3 Biological Analyses: In order to ascertain the impact of throughfall exclusion on the microbial community (i.e., bacteria, archaea, and fungi), we used a combination of biomass quantification and amplicon sequencing to determine alpha and beta diversity metrics.

2.3.1 Microbial biomass: Phospholipid fatty acid analyses (PLFA) were measured from lyophilized soil samples to determine total microbial biomass and the biomass of specific microbial groups according to a previously published approach (Bouskill et al., 2013). Both the prokaryotic and fungal communities sampled in the present study were from the bulk soil and not directly from the rhizosphere or litter layer. PLFAs were measured using gas chromatography-mass spectrometry (Microbial ID, Newark DE). The total biomass, in nmol per gram soil, was further normalized by the concentration of total organic carbon (TOC) in each sample.

2.3.2 DNA extraction and amplicon sequencing: Total genomic DNA was extracted from 0.25g of each soil sample in duplicate using the DNeasy PowerSoil kit (QIAGEN) following the manufacturer’s instructions. The duplicate DNA extractions were combined before PCR amplification and normalized to 10ng/μl. Quantification of the purified PCR products was done using the Qubit hs-DS-DNA kit (Invitrogen) and pooled in equimolar concentrations (10ng/μL for 16S and 20ng/μl for ITS) and sequenced on a single lane for 300 bp paired-end Illumina v3 MiSeq sequencing completed at the Vincent J. Coates Genomics Sequencing Laboratory at the University of California, Berkeley.

2.3.3 Microbial community composition analysis: Raw amplicon sequences were demultiplexed, trimmed, filtered by quality, and resolved into amplicon sequence variants (ASV) using the DADA2 package v.1.9.1 (Callahan et al., 2016) package in R studio software v.1.1.463 (Team, 2016)). Taxonomy was assigned using the Silva reference database (v.132) for 16S and the UNITE database for ITS sequences (Nilsson et al., 2019; Quast et al., 2013). A phylogenetic tree was constructed using the inferred ASVs *de novo* by performing multiple alignments using the DECIPHER R package (v. 2.14.0) and constructed with phangorn package v. 2.5.5 (Schliep, 2011; Wright, 2015). The phylogenetic data was imported into the phyloseq (1.30.0) package to store and analyze the ASV table and the phylogenetic tree (McMurdie & Holmes, 2013). The workflow resulted in 83 archaeal, 10,133 bacterial, and 8,525 fungal ASVs. The total number of reads was converted to relative abundance by dividing the counts of a taxon by the sum of taxon counts across the samples.

2.4 Physical and Chemical determination of Soil Properties: Soils for nutrient analyses were slowly thawed before being shaken in a 1M KCl solution at ratios of 1.0 g of soil per 5 mL of solution for an hour. The extract was filtered through no.45 Whatman filters. Nutrient concentrations within the filtrate were measured in microplates using sodium salicylate assay for ammonium and malachite green assay for inorganic phosphorus (Lajtha & Jarrell, 1999; Weatherburn, 1967). Gravimetric soil moisture was calculated by collecting field moist soil samples and weighing them before and after drying in a 105°C oven until weight stabilized. Bulk density measurements used a 1.5” diameter constant volume corer (AMS Inc, American Falls, ID, USA, part 404.39). For pH, 8.0 ± 1.0 g of soil was weighed, mixed with 40 ml of DI water, allowed to settle for approximately 30 min, and the pH of the resulting slurry was measured with a pH meter (SevenCompact pH/Ion meter S220, Mettler Toledo, Columbus, OH, USA). Total organic carbon (TOC) and total nitrogen (TN) were measured using a Costech ECS 4010 elemental analyzer. All soils had a pH below 7.0, and we did not detect any inorganic carbon; thus, TC concentrations are assumed to represent the TOC concentrations. Soil samples for soil moisture were taken at the time of collection for microbial analysis. Samples were collected and air-dried in a 105°C oven, and the final weight was measured to determine gravimetric water content (Dietterich et al., submitted). Bulk density measurements were used to convert soil moisture to volumetric water content (VWC).

2.5 Statistical Analysis: Significant differences in microbial biomass, ammonium, and phosphate concentrations across sites and between treatments were tested using two-way ANOVA and Tukey post hoc analysis. Community richness and evenness were calculated across the four forests and between the control and treatment plots. The beta diversity was visualized through a Double Principal Coordinate Analysis (DPCoA, (Fukuyama et al., 2012; Pavoine et al., 2004; Purdom, 2011) using the square root of the cophenetic/patristic distance between ASVs for Euclidean dissimilarity matrix. To identify and quantify the phylotypes differentiating control and throughfall excluded soil communities, we used the DESeq2 program (v.1.26.0) for differential analysis of count data to model the dispersion abundances using geometric means for each ASV (Love et al., 2014). ASVs that had statistically significant changes in abundance between treatments were identified by calculating the binary logarithm fold change ($\log_2\text{foldchange}$) of median counts of the control versus the treatment variable using the DESeq2 program. For significance testing, we used the Wald test within the DESeq2 program with Benjamini and Hochberg adjusted P-values. Variance partitioning approaches, including permutational multivariate analysis (PERMANOVA) and constrained principal components analysis (CCA), was applied to relate phylogenetic responses to changes in soil moisture or chemistry using DPCoA distance measurements. PERMANOVAs were run on weighted Unifrac distance matrices using the *adonis* function in the vegan package v.2.5-7 (Oksanen & Others, 2011). Tree-based visualizations of relative abundances for taxa identified in the samples were done using Metacoder v.0.3.4 ((Foster et al., 2017)). Heat-trees were generated and used $\log_2\text{foldchange}$ ratios of the median counts for each taxon and used the Wald test within the Metacoder program with Benjamini and Hochberg adjusted P-values. P12, P13, and GIG control plots were compared to SC control plots for tree comparisons across sites. Trees showing comparisons between treatments at one site were also generated.

3. Results

Below, we outline the soil plots' alpha and beta diversity metrics. We initially contrast the control sites across the MAP transect to ascertain how these communities are structured before subsequently moving on to describe how these different communities respond to throughfall exclusion.

3.1: Microbial community structure across sites: The biomass of the microbiota across these soil plots is largely dominated by bacteria and archaea, which compose between 34-47% of the total biomass within the control plots. Across the four sites, there were no significant differences in total, fungal, and non-fungal eukaryotic biomass (Fig. 2a, 2b, 2f). By contrast, the biomass of different bacterial groups increased significantly with decreasing MAP. The biomass of the Actinomycetes, the gram-negative and gram-positive bacteria were ~53%, ~131%, and ~127% higher at the drier GIG sites relative to the SC soils with the highest MAP (Fig. 2c, d, e).

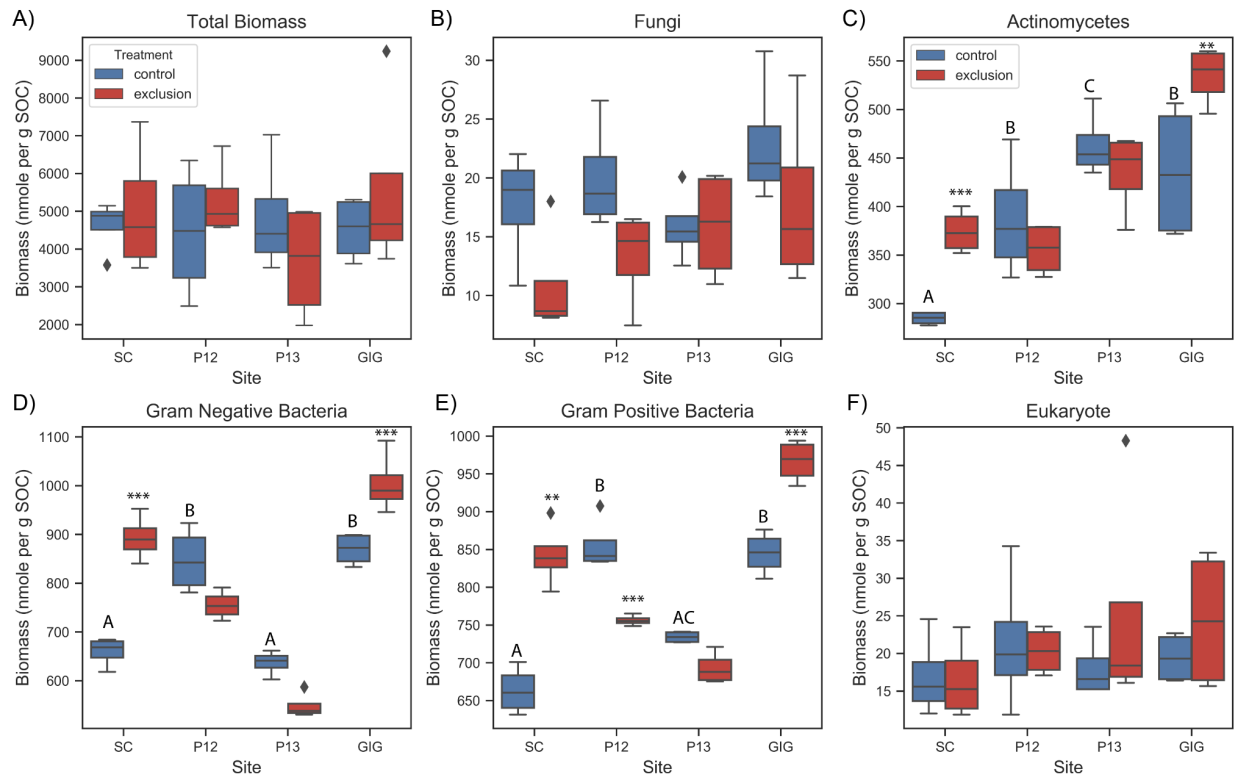


Figure 2: Microbial biomass as determined from phospholipid fatty acid analysis from the top horizon (0-10 cm) from samples taken after 18 months of treatment. Whiskers indicate the minimum and maximum biomass values. Sites are Sherman Crane (SC - 3300 mm), Buena Vista Peninsula Site 12 (P12 - 2600 mm) and 13 (P13 - 2600 mm), and Gigante (GIG - 2350 mm) site. Total biomass

was normalized by total organic carbon (TOC) content. Control plots are indicated in blue, while biomass from exclusion plots is indicated in red. Biomass is separated by microbial type: (b) Fungi, (c) Actinomycetes, (d) Gram-negative and (e) Gram-positive bacteria, and (f) Eukaryotes (non-fungal). Diamonds represent points that are outliers. The biomass results on the topsoil (0-10 cm) since throughfall exclusion had more discernible impacts on this depth range relative to the subsoil (10-20 cm). Significant differences in biomass across control plots are indicated by differing capital letters. Asterisks* above exclusion boxplots indicate significant differences between treatments within a site.

‘Site’ was a significant predictor of community structure (PERMANOVA $p=0.001$). Average taxonomic richness and evenness generally increased for prokaryotes with decreasing MAP (Fig. S5). The major prokaryotic taxa dominating these soils were the Proteobacteria, Acidobacteria, Actinobacteria, and Verrucomicrobia. Phyla present but at smaller relative abundances included the Bacteroidetes, Rokubacteria, Chloroflexi, Nitrospirae, and Entothaeonellaeota. The relative abundance of the Acidobacteria decreased with decreasing MAP, while the Actinobacteria increased with decreasing MAP (Fig S6). Relative abundance of Verrucomicrobia was lowest at GIG, intermediate at SC, and greatest at P12 and P13. The relative abundance of Proteobacteria was greater in P13 than in the other sites. Nanoarchaeaeota increased relative abundance with decreasing MAP except for P12, where it was lower. Figure 3a shows a DPCoA, a phylogenetic distance-based ordination, depicting the relative dissimilarity between the different sites at the nine months and 18 months of throughfall exclusion. The sites with the most dissimilar microbial communities were SC and P13 at both time points. The dissimilarity between sites across the primary axis accounts for 49.9% of the variance at nine months and 52.4% at 18 months (Fig. 3). Compared to the other sites, SC was enriched in Acidobacteria and depauperate in Lactescibacteria, Rokubacteria, Gemmatimonadetes, and Actinomycetes (Fig. S12).

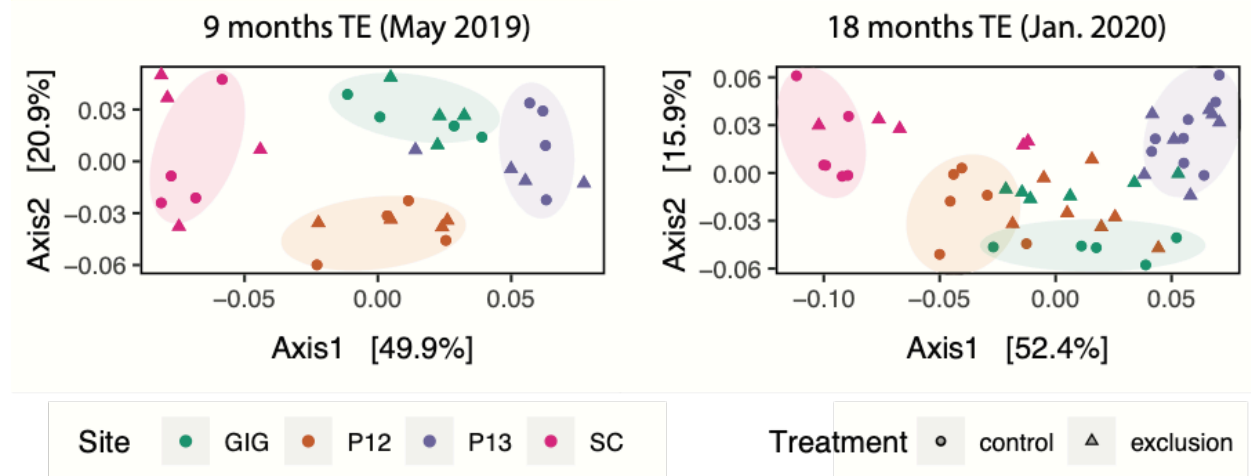


Figure 3: Double Principal Coordinate Analysis (DPCoA) of Bacteria and Archaea after A) 9 months and B) 18 months of partial throughfall exclusion. Control plots are indicated in circles, and exclusion plots are indicated in triangles. Green symbols indicate Gigante (GIG) points. P12 points are indicated in brown, and P13 are purple symbols. The Sherman Crane (SC) samples are indicated in pink. Ellipses highlight the clustering of samples within control plots at a specific site.

The fungal community's beta diversity was indistinguishable from site to site (Fig. S8) and dominated by Ascomycota, Basidiomycota, and Mortierellomycota. Fungal richness and evenness were greatest in P13 and lowest in GIG (Fig. S5). The relative abundance of Ascomycota increased from SC to P13 (Fig. S7) but declined significantly at GIG. The relative abundance of Basidiomycota was lowest at P12, and highest at P13, whereas the Mortierellomycota, decreased in relative abundance along with decreasing MAP. We note that beta diversity measurements in fungal communities did not show clear differentiation by the site ($p=0.114$; Fig S8; Table S3).

A canonical correspondence analysis (CCA) was used to determine which environmental variables best explained the changes in microbial community composition across sites. CCA analysis using the DPCoA distances clustered communities primarily by site (Fig. 4a). For bacteria and archaea, the primary axis explained 46.2% of the variance in the CCA. A number of environmental variables were significant factors in the emergent community structure (PERMANOVA $p=0.001$; Table S4), including soil moisture and Fe, which were the main factors discriminating between communities at SC from the other sites. The dissimilarity of P13 from the other sites was predominantly explained by soil fertility (TN, ammonium, base cations) and pH. Finally, total microbial biomass, inorganic phosphate, and sodium concentrations distinguish prokaryotic communities in GIG and P12. However, the collected environmental variables were unable to explain the emergent fungal community structure as measured by DPCoA distances (Fig 4b; Table S4).

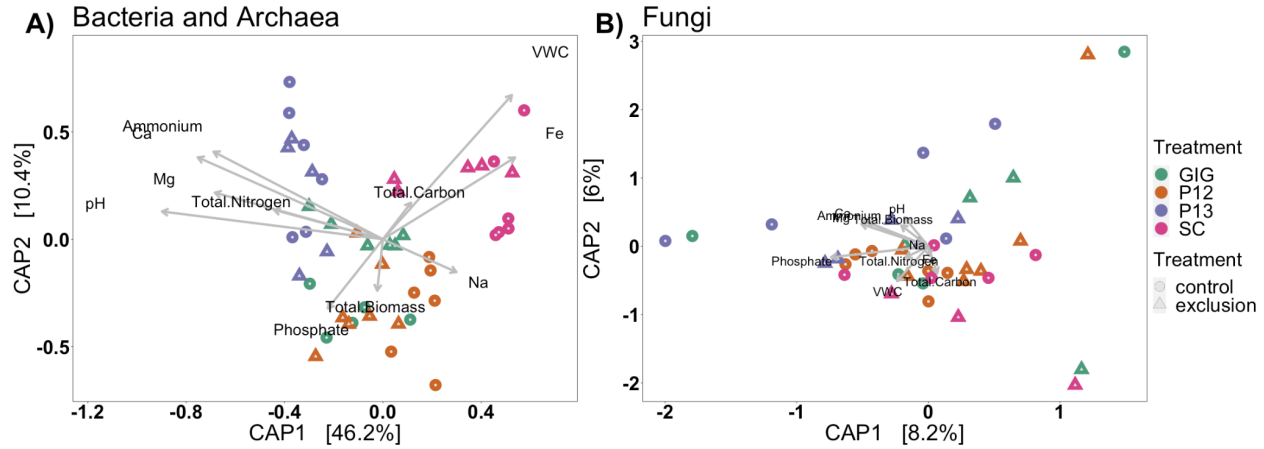


Figure 4: Canonical correspondence analysis (CCA) plots of samples taken in January 2020 after 18 months of partial throughfall exclusion were applied to relate phylogenetic responses to changes in soil moisture or chemistry. Dissimilarity matrices used were distances calculated by Double Principal Component analysis (DPCoA). Variables used for the model are total organic carbon (TOC), total nitrogen (TN), Ammonium concentration, inorganic phosphate (phosphate), total microbial biomass (total biomass), Volumetric water content (VWC), iron (Fe), magnesium (Mg), calcium (Ca), and soil pH. Circle symbols indicated samples from control plots and triangle symbols for exclusion plots. Arrows in CCA are significant factors for bacteria and archaea (PERMANOVA $p=0.001$).

3.2. Impacts of throughfall exclusion on microbial biomass and community composition: Throughfall exclusion imparted no significant effect on total biomass (Fig. 2a) when separated by the domain (i.e., fungi, bacteria, and eukaryote) or cell-wall morphology (i.e., gram-negative, and positive), clear trends emerge (Table S2). However, fungal biomass showed a clear decline under throughfall exclusion at most sites, except at the most fertile site (P13), where no change was observed (Fig. 2b). Gram-negative and gram-positive bacterial biomass both increased with throughfall exclusion at SC and GIG and decreased with throughfall exclusion at P12 and P13 (Fig. 2d, e). Actinomycetes also showed qualitatively similar trends, with throughfall exclusion promoting higher average biomass at the SC and GIG sites but a negligible impact at the P12 and P13 sites. Finally, treatment imparted no impact on the biomass of non-fungal eukaryotic organisms, which did not vary between the control and excluded plots within a site (Fig. 2f). Taken together, these data suggest that, despite not impacting total biomass, throughfall exclusion reshaped community abundance and composition.

Following the initial nine months of treatment, no significant differences were observed between microbial communities' richness, evenness, or composition when comparing control and throughfall exclusion plots at each site (Fig. 3a and S11). However, after 18 months of partial throughfall exclusion, the beta diversity metrics demonstrated an increasing dissimilarity in community composition in treatment relative to controls plots. This effect was confined to the infertile sites (i.e., SC, P12, and GIG) and, when considered with the aforementioned alpha diversity metrics, are indicative of shifts in the relative abundance

of different taxa (Fig. 3b and 4). The dissimilarity between the community composition of control and throughfall excluded plots was strongest at the P12 site, which diverged across the primary ordination axis. However, we also noted similar dissimilarity between control and treatment plots at the GIG site, which separated across the secondary axis, and converged towards a very similar community composition as that emerging under treatment at the P12 plots. The SC prokaryote community in the exclusion plots began to show greater similarity to the community composition at P12 and GIG exclusion plots, albeit with higher plot-to-plot heterogeneity (Fig. 3b). By contrast, the community composition of bacteria and archaea at the nutrient-rich site (P13) showed no divergence from the control site following prolonged (18 months) treatment (Fig. 3b).

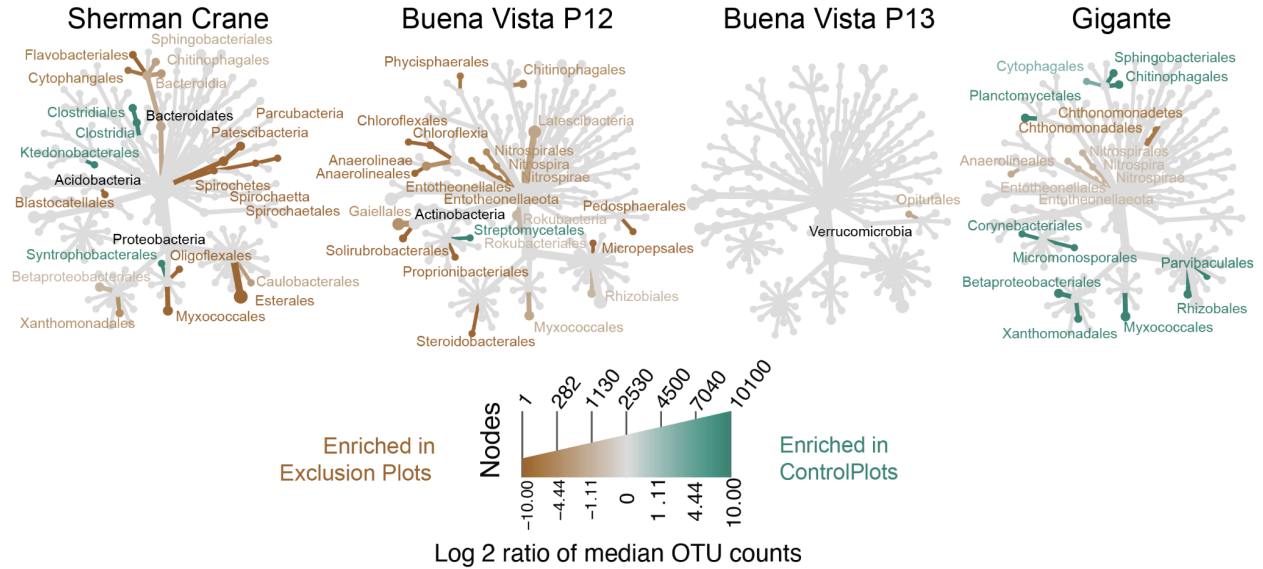


Figure 5: Tree-based visualizations for taxa identified in samples. Colors indicate the \log_2 ratios of median counts between control and exclusion plots. Brown colors indicate taxa enriched in control plots, while green colors indicate taxa enriched in exclusion plots. Trees are separated by the site. Colored branches indicate taxa significantly enriched in plots ($p < 0.05$ Wald test).

The observed shifts in community composition under throughfall exclusion were attributable to the significant enrichment of multiple taxa across the different sites ($p < 0.05$, Fig 5). Within some sites, a phylogenetic signal was discernible amongst the enriched phyla. For example, the Nitrospirae (*Nitrospira*), Chloroflexi (*Anaerolineae*), Proteobacteria (*Deltaproteobacteria*), and Entothaeonella (*Entothaeonellaeota*) were enriched following chronic drying within both the P12 and GIG plots. Similarly, members of the Bacteroidetes (e.g., *Chitinophagales*) and *Deltaproteobacteria* were significantly enriched under throughfall exclusion at both the P12 and SC sites. Actinobacteria, Planctomycetes, and Verrucomicrobia were only significantly enriched following throughfall exclusion in P12

(Fig. 5). Interestingly, members of the Bacteroidetes and Proteobacteria (Xanthomonas and Myxococcales) were enriched both in exclusion plots in SC and control plots in GIG. Distinct archaeal taxa were enriched in exclusion plots at different sites. At P13, Thaumarchaeota relative abundance was much higher in exclusion plots than in control plots. Nanoarchaeaeota were enriched in SC exclusion plots compared to control plots. By contrast, while fungal biomass decreased under throughfall exclusion, we observed no discernable impact on community structure relative to the control plots (Fig. S8) and little discrimination of sites when various edaphic drivers were accounted for (Fig. 4b).

Finally, we used CCA to identify the environmental factors underpinning shifts in community composition within a site as a result of throughfall exclusion in infertile soils (Fig. S4). This analysis revealed a strong relationship between soil moisture (VWC) and ammonium concentrations at GIG exclusion plots ($p=0.007$; Table S5). For communities within the P12 throughfall exclusion plots, separation from the control plots was associated with increasing TC, TN, Mg, Ca, and soil pH. In comparison, communities in SC exclusion plots were associated with changing pH and Ca. Despite the site-to-site divergence in factors influencing the composition of throughfall exclusion plots, it was clear that total carbon and nutrients, including total nitrogen and phosphate, were essential factors structuring microbial community composition in the control plots at SC and GIG, whereas soil moisture was the most important factor associated with community composition within the P12 control plots.

3.3: Physicochemical factors: Within control plots across the four sites on January 2020, we observed soil moisture content (measured as VWC) to decrease from SC to GIG (Fig. S1). TOC and TN in the topsoil did not vary significantly across the four forest soils. TOC was between 4.0-5.8% by weight and 0.32-0.47% for TN (Table 1). Ammonium concentrations were low in the infertile sites (0.46-2.43 mg NH_4^+ kg soil) and significantly higher in P13 sites (~12.0-15.4 mg NH_4^+ kg soil). Similarly, phosphate was also higher in P13 sites and low at the three other sites except for control plots in GIG (Table 1). Yet phosphate concentrations were variable in P13 plots (Fig. S3).

When comparing control soils with throughfall excluded soils, we observed general increases in TOC and TN in P13 and P12 plots at the 18- months but decreases in exclusion plots at SC and GIG. Average ammonium concentration at the P13 exclusion plots did slightly increase compared to average concentrations in the P13 control plots. Bulk measurements of soil moisture (i.e., VWC) showed no significant differences between the control and throughfall exclusion plots at the 18-month time point.

{Insert Table 1. Characteristics of sites sampled. The mean annual temperature across sites is 26°C. Values provided are from the 0-10 cm depth. Total nitrogen, total organic carbon, biomass, ammonium (NH_4^+), inorganic phosphate (PO_4^{-3}), soil pH were measured from samples collected after 18 months of throughfall exclusion.}

4. Discussion

4.1 Bacterial and archaeal responses to throughfall exclusion.

The taxonomic composition of microbial communities shows a profound sensitivity to disturbance (Shade et al., 2012) that may (Louca et al., 2018), or may not (Rocca et al., 2018), reshape the functional diversity of a community and feedback on soil biogeochemistry. As climate changes, the frequency of pulse (e.g., drought) and press (e.g., warming or elevated atmospheric CO_2) disturbances will play an increasingly important role in shaping community composition. The sensitivity of tropical soil communities to the impact of multi-faceted climate change is generally understudied. Tropical microbial communities have previously been shown to be sensitive to warming (Nottingham et al., 2020) and soil drying (Bouskill et al., 2013). However, the factors that regulate a community’s sensitivity relative to its resistance remain poorly understood. Here we show that eighteen months of throughfall exclusion imparted remarkably divergent responses across the study sites. The extent to which bacterial and archaeal communities shifted under treatment was broadly dependent on (a) soil fertility and (b) the length of the dry season and MAP.

4.1.1. High nutrient availability buffers the impact of soil drying: Shifts in bacterial and archaeal community diversity under throughfall exclusion occurred solely within soils that were relatively low in bedrock-derived and atmospherically-deposited nutrients. Throughfall exclusion was associated with strong shifts in community structure in all three infertile sites studied, but no discernible shift in the fertile site. In this case, the availability of soil nutrients could either sustain a metabolic response within the community to resist the ongoing perturbation or facilitate the rapid recovery of the initial community following the onset of perturbation (Bardgett & Caruso, 2020).

As soils dry, shrinking water films can concentrate solutes, which can impart stress on microorganisms (Malik & Bouskill, 2022; Schimel, 2018). In response to matric and osmotic stressors, microorganisms have been shown to increase demand for both nitrogen and phosphorus (Buscardo et al., 2021), alter the composition of phosphorus-rich cell walls (Williams & Rice, 2007) to maintain cellular turgor, and synthesize a range of compatible solutes to maintain macromolecular integrity during this stress (Bremer & Krämer, 2019). These compounds include a range of non-structural carbohydrates and amino acids high in nitrogen and, in some cases, phosphorus. However, such a metabolic response

to stress is energetically expensive (Oren, 1999) and likely only used when substrate availability is sufficient to cover the energetic and macromolecular cost of synthesizing these compounds (Manzoni et al., 2014). This could certainly be the case at the P13 site, which shows higher carbon and nutrient availability relative to the other sites, and provides possible avenues to identify which soil fertility components directly support purported microbial responses to soil drying.

Such nutrient-enabled community resistance to soil drying in tropical forest soils may hold as long as nutrient concentrations do not become severely limited. However, throughfall exclusion and drought in tropical forest soils can bring about a drop in phosphorus availability (Bouskill, Wood, Baran, Ye, et al., 2016; O’Connell et al., 2018), as fluctuating soil redox potential under drying increases phosphorus sorption to soil minerals. Therefore, prolonged soil drying under a changing climate may potentially restrict the metabolic response of the microbial community by reducing nutrient availability.

4.1.2. Impacts of historical contingency on community response to chronic drying: By contrast to the resistance observed at the fertile soil site (P13) the comparatively infertile sites, SC, P12, and GIG, showed shifts in microbial community composition following prolonged throughfall exclusion. The GIG and P12 sites exhibited a ‘treatment microbiome’, whereby an overlapping community composition emerged under throughfall exclusion. Such a strong and complementary response to soil drying at GIG and P12 sites is interesting when contextualized by the lack of measurable differences in soil moisture within control and throughfall exclusion plots. Microbial community composition has previously been shown to be more sensitive to disturbance impacts than bulk soil properties (Ma et al., 2019), and in this case, community composition appeared more sensitive to perturbation than bulk measurements of water content at the plot scale. The emergence of this treatment microbiome might reflect the lower MAP and longer dry seasons at GIG and P12, which could condition it to respond quickly, and to a degree, predictably, to soil drying.

Historical contingencies did factor into determining resistance to disturbance but, as discussed above, are outweighed by fertility. Soil drying at the P12 and GIG sites selected for taxa that might be predicted to respond positively based on their life-history traits. For example, the observed enrichment in gram-positive bacteria, including the Actinobacteria, in throughfall exclusion plots might be expected due to the morphological and physiological traits of this group, including the ability to sporulate under adverse environmental conditions (Jordan et al., 2008; Mayfield et al., 1972). Furthermore, the gram-positive bacteria possess thick peptidoglycan cell walls, which serves as the initial barrier to drying and osmotic stress, allowing gram-positive organisms to maintain activity as water potential declines (Manzoni et al., 2012). Moreover, gram-positive bacteria within the Actinobacteria also possess a large secondary metabolome that plays a role in conferring resistance to environmental stress (Wolf et al., 2013). In addition to producing compatible solutes, some taxa use filamentous

structures to extend their growth during low soil moisture conditions (Wolf et al., 2013). These traits likely explain an overall negative trend with soil moisture of the Actinobacteria (Chanal et al., 2006), and an elevated relative abundance in dry soils (Bachar et al., 2010), and under throughfall manipulation experiments in the tropics and subtropics (Bouskill et al., 2013; Zhou et al., 2019).

While the elevated relative abundance of different gram-positive organisms might be predicted on the basis of their ecology and physiology, we also note an increase in the relative abundance of a number of gram-negative taxa under drying within the infertile plots. For example, we observed the statistically significant enrichment of members of the Acidobacteria phyla under chronic drying across all three infertile sites (GIG, P12, SC). The increased relative abundance of Acidobacteria has been observed in drying manipulations in field and microcosm experiments with tropical and subtropical forest soils (Bu et al., 2018; Supramaniam et al., 2016). A positive response to drying could be facilitated by the metabolic capacity of the phyla to produce cellulose and exopolysaccharides and form biofilms under osmotic stress (Kielak et al., 2017; N. L. Ward et al., 2009). Furthermore, members of the large Proteobacteria phylum also increased in relative abundance in the P12 and GIG treatment plots. This is consistent with previous precipitation manipulation experiments in tropical soils, which have observed the enrichment of Alpha- or Betaproteobacteria (Bouskill et al., 2013; Nemergut et al., 2010). In addition, we also show here an increase in the relative abundance of the Delta- and Gammaproteobacteria in tropical forest soils. However, while not necessarily predicted to increase under soil drying, some taxa within the Proteobacteria (including the Gammaproteobacteria) show the capacity to avoid osmotic and matric stress associated with drought by increasing cellulose and biofilm production (Römling & Galperin, 2015). Biofilm production protects embedded cells from rapid fluctuations in external water potential (Flemming et al., 2016), increasing their relative abundance within the community as mortality reduces the abundance of other non-biofilm forming groups.

The strong response of gram-negative bacteria might also represent metabolic cross-feeding between tolerant and vulnerable organisms under increased cell-to-cell interactions as soils dry (Tecon et al., 2018). Analogs can be drawn with the interactions between microorganisms with and without a significant capacity to produce extracellular enzymes (Allison, 2005; Allison et al., 2014). Organisms that do not invest in the production of exo-enzymes ‘cheat’ by successfully competing with organisms that do. Greater investment in high-affinity transporters that take up monomers faster than other microbes can conserve energy and sustain these populations (Allison, 2005; Allison et al., 2014). Indeed, opportunistic life-history strategies have been observed in soil microbial communities in response to drying-rewetting cycles (Evans & Wallenstein, 2014). In the case of drought-sensitive communities, microorganisms could divert resources to transporters rather than synthesizing secondary metabolites. Thereby accumulating compatible solutes and non-structural carbohydrates from the environment, particularly after cells lyse the following synthesis. These community dynamics may

provide additional strategies for gram-negative bacteria to increase in relative abundance under drying conditions, although further investigation is needed to determine if “cheating” for compatible solutes is a viable and widespread strategy for microbial communities experiencing prolonged drying-wetting cycles.

We observed a less pronounced drying-induced shift in the microbial community at GIG than at the other sites. This might be indicative of a community adapted to drier conditions relative to the other sites with higher MAP, and therefore less sensitive to the imposed disturbance. The sites with higher MAP take longer to shift but the dissimilarity between unperturbed plots is much greater. Seasonal shifts in environmental conditions give rise to dynamic bacterial communities, whereby distinct communities are selected for and recur during specific times of the year (Bouskill et al., 2011; C. S. Ward et al., 2017). Such dynamics might explain why GIG, with prolonged dry seasons and lower MAP, showed smaller, more discrete shifts in beta diversity under throughfall exclusion relative to P12. The implication here is that tropical sites that have prolonged annual dry seasons could harbor a prokaryotic seed bank (Lennon et al., 2021) adapted to an increasing intensity of drought, reducing the impact of this perturbation on microbial assembly and function.

Our finding of similar microbial communities in exclusion plots in SC and control plots in GIG suggests that despite having higher precipitation and shorter dry season length, the SC site still harbors similar organisms to the GIG site. This emphasizes the control soil moisture availability has on community composition and lends a degree of predictability to how tropical microbial communities will change under soil drying. We initially hypothesized that there would be demographically generalizable shifts across all sites in response to throughfall exclusion. There is some evidence for this, however, we also note that across the sites we find no clear morphological signal in the response to disturbance.

4.2 Fungal response to throughfall exclusion

At each site along the precipitation gradient, fungal biomass declined under throughfall exclusion. While there is support for such sensitivity to drying conditions in subtropical forest soils (Zhang et al., 2021; Zhao et al., 2018), fungi have been considered to be broadly resistant to drought (Evans & Wallenstein, 2012; Six, 2012), and to drying and re-wetting cycles (Bapiri et al., 2010; Barnard et al., 2015). However, fungal guilds within tropical and subtropical soils have previously been shown to shift in community composition in response to soil drying. In particular, drying increased the relative abundance of dark septate and phytopathogenic fungi in tropical forest soils (Buscardo et al., 2021; de Oliveira et al., 2020; He et al., 2017) and increased the abundance of Sordariomycetes and Agaricomycetes in tropical grassland soils (He et al., 2017).

Our results show a general decline in fungal biomass under throughfall exclusion, which is contrasted by many observations of fungal resistance to drought

and leads us to reject our final hypothesis inferring that fungi will be resistant to throughfall exclusion. A corollary to this point is that the composition of the fungal community does not change in response to disturbance. Such resistance in demography could be tied to key physiological mechanisms that enable fungal community resistance to water stress and ecological relationships, including hyphal networks and mutualism. For example, filamentous structures have been shown to aid fungi in enduring water stress (Freckman, 1986). This serves to transport water and substrates through the hyphal network and provide mutualistic relationships with microorganisms (Boer et al., 2005). Fungi show additional drought-resistant traits similar to bacteria, including compatible solute synthesis and EPS production (Crowther et al., 2014). Although resistance in fungal community composition to decreased precipitation could be explained by physiological adaptations, it is interesting that fungal biomass in these tropical forest soils decreased under throughfall exclusion. The small fraction of fungi in total biomass may be a result of sampling in bulk soil and not near roots or litter layers. Drying conditions have been observed to decrease fungal activity and biomass in boreal forest soils but also followed changes in community composition (Allison & Treseder, 2008). But in this study, we did not observe significant changes between community compositions as a result of treatment. These clay-rich tropical forest soils may require longer periods of prolonged drying or more intense decreases in soil moisture to see an effect in fungal beta diversity.

5. Conclusion

The present study demonstrates seemingly disparate responses of tropical forest soils to partial throughfall exclusion, which were dependent on site-specific climate history (e.g. MAP, dry season lengths). In general, the historical contingencies that shape community composition across a 1 m MAP gradient in tropical forest soils partially determine resistance to soil drying, but are overshadowed by soil fertility. As such, historical contingencies, and soil properties (e.g., texture and fertility) need to be accounted for when attempting to predict how tropical soil microbial communities may respond to projected disturbances in the hydrological cycle. Further work must connect the observed shifts in community composition to changes in microbial trait distribution and determine whether community responses to the changing climate will alter the carbon cycle within tropical forest soils.

6. Acknowledgments: Funding for this work was provided by the US Department of Energy, Office of Science (BER), Early Career Research Program to N.J. Bouskill (#FP00005182), and Daniela Cusack (#DE-SC0015898). We thank Biancolini Castro, Lily Colburn, Alexandra Hedgpeth, Jason Brawdy, Korina Valencia, and Carley Tsiamas for the help in collecting these samples with us. Special thanks to the Tupper Soil Lab in the Smithsonian Tropical Research Institute for coordination of sample handling and transport.

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@ >p(- 22) * >p(- 22) * >p(- 22) * >p(- 22) * >p(- 22) * >p(- 22) * >p(- 22) * >p(- 22) * >p(- 22) * >p(- 22) * @

Site

&

Soil Taxonomy

&

Lat

&

Long

&

MAP

&

Treatment

&

Total Nitrogen

&

Total Organic Carbon

&

Total Microbial Biomass

&

NH₄⁺

& **PO₄⁻³** & **pH**

& & &

mm

& &

% by weight
 &
 % by weight
 &
 nmole per g of TOC
 &
 mg per kg soil
 &
 ng per kg soil
 &
 Sherman Crane (SC) &
 Typic Kandiudox (Oxisol)
 &
 9° 16' 51.132"
 &
 -79° 58' 28.9194"
 &
 3300
 &
 Control
 &
 0.397
 (± 0.075)
 &
 5.815
 (± 1.493)
 &
 4516.10
 (± 810.4)
 &

1.06
 (± 0.99)
 &
 97.81
 (± 28.25)
 &
 4.82
 (± 0.25)
 & & & &
 Exclusion
 &
 0.316
 (± 0.161)
 &
 4.554
 (± 2.689)
 &
 4986.65
 (± 1934.4)
 &
 0.458
 (± 0.24)
 &
 77.13
 (± 26.86)
 &
 5.02
 (± 0.53)

Buena Vista Península P12 &

Typic Haplohumult (Ultisol)
 &
 9° 10' 45.696"
 &
 -79° 49' 46.5594"
 &
 2595
 &
 Control
 &
 0.362
 (± 0.010)
 &
 4.458
 (± 0.189)
 &
 4769.65
 (± 1807.1)
 &
 2.23
 (± 0.85)
 &
 97.36
 (± 17.96)
 &
 5.63
 (± 0.31)
 & & & &
 Exclusion
 &

0.402
 (± 0.050)
 &
 5.050
 (± 0.695)
 &
 5171.15
 (± 780.1)
 &
 2.43
 (± 1.42)
 &
 152.99
 (± 136.6)
 &
 5.85
 (± 0.43)

Buena Vista Península P13 &
 Mollic Oxyaquic Hapludalf (Alfisol)
 &
 9° 11' 16.3674"
 &
 -79° 49' 15.5994"
 &
 2590
 &
 Control
 &
 0.475
 (± 0.044)

&
5.360
(± 0.435)
&
4860.36
(± 1218.4)
&
11.99
(± 4.03)
&
941.58
(± 458.3)
&
6.19
(± 0.27)

& & & & &
Exclusion
&
0.402
(± 0.066)
&
4.344
(± 0.876)
&
4687.42
(± 1437.5)
&
15.36
(± 3.71)
&

760.68
 (± 760.2)
 &
 6.25
 (± 0.34)
 Gigante (GIG) &
 Typic Hapludox (Oxisol)
 &
 9° 5' 57.084"
 &
 -79° 51' 14.3994"
 &
 2335
 &
 Control
 &
 0.400
 (± 0.07)
 &
 4.36
 (± 0.85)
 &
 4236.15
 (± 863.66)
 &
 1.30
 (± 0.30)
 &
 937.27
 (± 917.9)

&	
	<i>5.65</i>
	<i>(±0.24)</i>
& & & & &	
	Exclusion
&	
	0.392
	<i>(±0.11)</i>
&	
	4.06
	<i>(±1.14)</i>
&	
	5162.27
	<i>(±682.97)</i>
&	
	2.17
	<i>(±0.40)</i>
&	
	40.96
	<i>(±49.2)</i>
&	
	<i>5.59</i>
	<i>(±0.30)</i>

Table 1