Chapter

# First Insights into the Resilience of the Soil Microbiome of a Tropical Dry Forest in Puerto Rico

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## Abstract

This study evaluated the effect that tree species traits and wet/dry periods display on soil microbial communities in a tropical dry forest in Puerto Rico. Understanding the ecological role of soil microorganisms in tropical dry forests and how they relate to different tree species is necessary to protect these fragile forest ecosystems. Thus, by using 454 pyrosequencing, we explored how microbial diversity was affected by dominant tree species during the wettest and driest periods at the Guánica Dry Forest. We found that 9 out of 17 phyla were more abundant during the dry period demonstrating that soil communities have adapted to historically low rainfall patterns. The most abundant phyla during both periods were Proteobacteria, Actinobacteria, and Bacteroidetes. During the dry period, Actinobacteria increased significantly ($p < 0.0001$), whereas Proteobacteria and Bacteroidetes decreased significantly ($p < 0.0001$; $p < 0.001$). Canonical correspondence analysis (CCA) also demonstrated that soil microbes are shaped by wet and dry periods, thus axis 1 of CCA explained 80% of the variation. This study offers baseline information in order to help elucidate how microbial diversity is affected by climate change in tropical areas and extrapolate this information to agricultural areas in order to develop better management practices.

**Keywords:** historical rainfall patterns, bacterial resilience, soil microbiome, soil microbial ecology, soil enzyme activity, Guánica Dry Forest, Puerto Rico, bacterial diversity, DNA sequencing

## 1. Introduction

Arid and semiarid ecosystems comprise almost 1/3 of the Earth's surface, and it is expected that these ecosystems will increase their total coverage area due to anthropogenic activities and climate change [1]. In tropical dry forests, seasonality and rainfall distribution fluctuate more often than in other ecosystems. Dry periods can extend for many months, and in some cases, they are accompanied by pulsed rainfall that can last from hours to days. These fluctuations control temporal growth patterns, productivity, turnover of organic matter, and other forest soil functional traits [2]. After a dry period, the first pulse of rainfall causes abrupt changes in soil moisture and water potential leading to microbial physiological stress and the reawakening of soil microbial communities. Seasonally tropical dry forests are already towards the extreme of water availability. Climate model predictions for

the Caribbean point towards progressively drier periods, with precipitation loss between −10 and −50% [3]. There is limited information regarding the diversity of soil microbial communities in these ecosystems, and it needs to be assessed in order to establish baseline information that is crucial to help elucidate the degree of the ecosystems resilience to the proposed precipitation changes that are affecting these ecosystems.

The intrinsic effects of vegetation are strong influencers of soil properties. Due to the confounding factor of plant species and plant roots sharing the same area, there is very little information on the effect of specific plant species on microbial diversity and soil enzymatic activities [4, 5]. Seasonally dry ecosystem, such as the Guánica Dry Forest, can serve as a model system to better understand the impact of seasonal variations and tree species effect on microbial community composition and activity. In this forest the trees are growing in the cracks of the calcareous platform, forming individual islands of leaf litter and organic matter under similar climatic conditions [5, 6]. The canopies of the trees are close to ground level limiting the transfer of leaf litter between neighboring trees, thus forming individual islands of fertility. These individual islands of fertility prevent belowground competition for resources [6]. Given that plant species vary in their effects on soil properties [7–9], one of our objectives was to determine how responsive the soil microbial diversity is to plant species effects. Our second objective was to understand to what degree the soil microbial diversity shows resilience to rainfall variability and dry periods. The study was conducted during the rainiest period of 2011 (August) and the driest period of 2012 (January). We selected three tree species (a pantropical species and two native species) that are highly distributed in the forest [10]. We hypothesize that both rainfall and plant species will regulate of modify the soil microbiome.

## 2. Materials and methods

### 2.1 Study site and sampling

The study was carried out at the Guánica Dry Forest which is situated in the semiarid region of Southwestern Puerto Rico (**Figure 1A–C**). Trees in this area are dwarfed, and the vegetation is located between 0 and 150 m from the coastline. The mean annual precipitation of this zone is 750 mm and exhibits a bimodal distribution. Even though monthly rainfall is highly erratic, most studies have documented that almost 50% of the annual precipitation occurs between September and November [11, 12]. Historical data also demonstrates that the forest also exhibits two periods of predominant drought that start in January and June. The data presented here correspond to samples that were taken during July 2011 (wet) and January 2012 (dry) representing the months of higher (wet) and lower (dry) rainfall [13], therefore allowing us to measure maximum and minimum response of the substrates microbiome.

The soils of the area are described as isohyperthermic Calcic Lithic Petrocalcids of the Pitahaya-Limestone outcrop-La Covana Association which consist of shallow, well-drained, very slowly permeable soils that formed or were deposited in material that weathered from limestone bedrock (USDA NRCS 2008). The depth of the substrate varies according to ground relief and among seasons [14]. The low stature woody vegetation grows on a rocky calcareous substrate where plants establish their roots in the holes, cracks, and crevices, of the rocky material accumulating water and very shallow soil substrates. Due to the high variability of the surface area soil

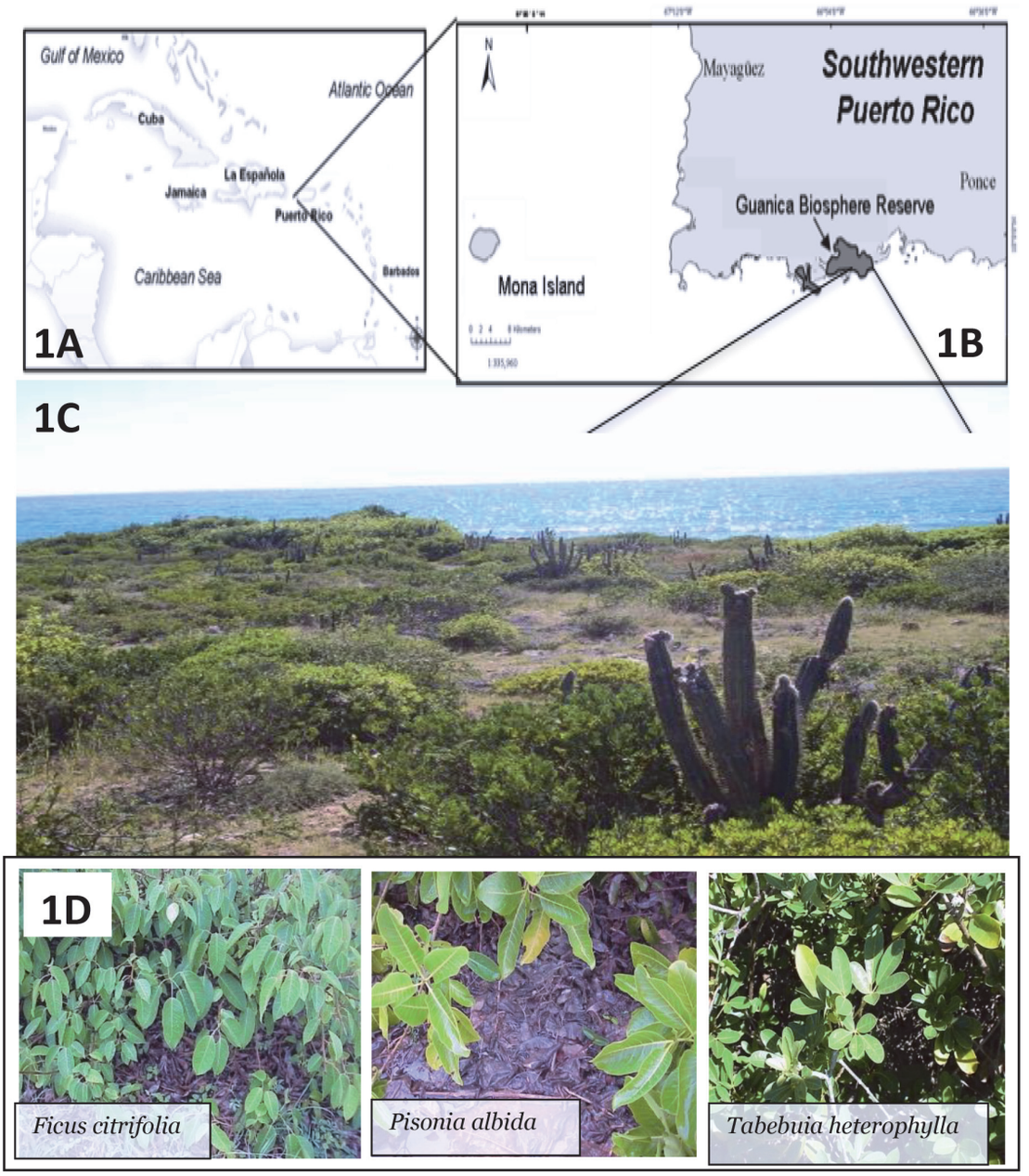


**Figure 1.**
*Location and description of the study site. (A) The Island of San Juan Puerto Rico forms part of the Greater Antilles and is bordered by the Caribbean Sea. (B) The Guánica Dry Forest Biosphere Reserve found in the southwestern area of Puerto Rico. (C) Picture of the landscape of the study site. Here we observe the coastal area of the forest where trees are dwarfed and have established their growth in the cracks and crevices of the rocky substrate. (D) Representation of tree species used in this study.*

sampling depth was not fixed; it ranged from 0 to 8 cm. We selected three (3) trees from three species, previously tagged and studied, that grow from 100 m to approximately 300 m from the coast. The tree species selected complied with the following characteristics: (a) they were interspersed within the study area, (b) each tree formed an island that was isolated from other trees by exposed rock, and (c) that their litter and belowground substrate originated from their own residue decomposition [14]. The three species selected were *Tabebuia heterophylla (DC.)* Briton (facultative deciduous), *Pisonia albida* (Heimerl) Briton ex Standal, (obligate deciduous) and *Ficus citrifolia* Mill., (facultative deciduous) (**Figure 1D**).

Each tree was used as a sampling unit supported by the very high heterogeneity in the vegetation structure of the site and the actual physical separation of the trees.

The minimum distance between any two trees was about 1 m, and the maximum was approximately 30 m. We collected one soil sample of each sampling unit (tree) during the months of the study. Soil samples were sieved in the field with a 2 mm mesh and placed in plastic bags. Samples were then placed on ice, taken to the laboratory, and frozen until they were sent to the Molecular Research Facility at Lubbock Texas. Total soil DNA extraction and 454 pyrosequencing were completed at the Molecular Research Facility. The molecular research facility reported all results as OUT tables.

### 2.2 Soil enzyme activities

Enzyme activities were performed as described in [5, 15]. The activities of enzymes relevant in C cycling (β-glucosidase), C and N cycling (β-glucosaminidase), P cycling (alkaline phosphatase, acid phosphatase, phosphodiesterase), and the S cycle (arylsulfatase) were assayed using 0.5 g of air-dried soil (<2 mm). Duplicate replicates and one control were used for all the soils that were tested; furthermore, the appropriate substrate was used for each assay, and reactions were incubated for 1 h at 37°C at their optimal pH as described in [5]. For the controls, the substrate was added after the 1 h incubation period and subtracted from a sample control value. Enzyme activity is expressed in mg p-nitrophenol (PN) released in $kg^{-1}$ soil $h^{-1}$.

### 2.3 Pyrosequencing data processing and analysis

Amplicon pyrosequencing (bTEFAP) was originally described by Dowd et al. (2008) and has been utilized in describing a wide range of environmental and health-related microbiomes including the intestinal populations of a variety of sample types and environments, including cattle [16–18]. The 16S universal eubacterial primers (F = AGRGTTTGATCMTGGCTCAG, R = GTNTTACNGCGGCKGC TGG) were used for PCR amplification. A single-step 30 cycle PCR using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) was used under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds; 53°C for 40 seconds and 72°C for 1 minute; and after which a final elongation step at 72°C for 5 minutes was performed. Following PCR, all amplicon products from different samples were mixed in equal concentrations and purified using Agencourt AMPure beads (Agencourt Bioscience Corporation, MA, USA). Samples were sequenced utilizing Roche 454 FLX titanium instruments and reagents and following manufacturer's guidelines.

The sequence data derived from the sequencing analysis was processed using a proprietary analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX). Sequences were depleted of barcodes and primers, and all sequences shorter than <200 bp were removed. Sequences with ambiguous base calls were removed, and sequences with homopolymer runs exceeding 6 bp were also removed. All sequences were then denoised and chimeras were removed. Operational taxonomic units were defined after the removal of singleton sequences, clustering at 3% divergence (97% similarity) [16–20]. OTUs were then taxonomically classified using BLASTn against a curated Greengenes database [20] and compiled into each taxonomic level into both "counts" and "percentage" files. Operational taxonomic unit tables (OTU) tables reported by the Molecular Research Facility were used to complete all statistical analysis. Bacterial diversity was estimated by using the Shannon-Wiener (H′) and Equitability (J′) indexes; both were calculated using the

PAST3 statistical program [22]. Nonparametric Kruskal-Wallis was calculated using the JMP10 statistical software to evaluate differences between diversity indexes as affected by tree species and rainfall. In the book "Microbial Source Tracking: Methods applications and case studies," Cao et al. (2011), page 278, discusses that "common multivariate techniques used for the examination of microbial community structure include cluster analysis, principle components analysis (PCA), correspondence analysis (CA), and nonmetric multidimensional scaling (NMDS). All of these techniques belong to a group called indirect gradient analysis, which aims to reveal community similarities among sites or samples through grouping or ordering the sites or samples into either dendrograms or on a two (2D) or three-dimensional (3D) plot." On the other hand, they also mention that "direct gradient analysis such as canonical correspondence analysis (CCA), aims to correlate the overall multivariate community profile with environmental variables." To identify the influence of soil physicochemical characteristics on the bacterial community, canonical correspondence analysis (CCA) was performed using the OTU tables of each community and the soil physicochemical characteristics. Canonical correspondence analysis is a site/species matrix where each site has given values for one or more environmental variables. The ordination axes are linear combinations of the 169 environmental variables. It is a gradient analysis that shows species abundances as a response to an environmental gradient. Environmental variables are plotted as correlations with site scores. I reported two types of scaling. Type 1 emphasizes the relationship between sampling sites and environmental variables, and Type 2 emphasizes relationships between species and environmental variables [21, 22]. Indicator species analysis (ISA) was performed to identify the bacterial species responsible for changes in soil microbial communities between tree species and sampling periods. ISA analysis was completed in R using the IndVal script, where R calculates the indicator value d of species as the product of the relative frequency and relative average abundance [23].

## 3. Results

### 3.1 OTU abundance at the Guánica Dry Forest as affected by sampling period and tree species

Sequencing data revealed 17 predominant bacterial phyla for this forest (**Table 1** and **Figure 2**). The phyla with the highest relative abundance were *Proteobacteria*

| Phyla | Dry | S.D. | Wet | S.D. | p value |
|---|---|---|---|---|---|
| | Bacterial phyla | | | | |
| ***Bacteroidetes*** | **2.80** | **0.94** | **7.60** | **23.26** | **0.01** |
| ***Chloroflexi*** | **2.60** | **0.99** | **0.75** | **0.58** | **0.01** |
| *Cyanobacteria* | 0.08 | 0.15 | 0.00 | 0.00 | 0.47 |
| ***Actinobacteria*** | **37.65** | **6.57** | **16.93** | **6.77** | **<0.0001** |
| ***Verrucomicrobia*** | **0.32** | **0.30** | **0.08** | **0.15** | **0.02** |
| *Spam (candidate division)* | 0.07 | 0.15 | 0.00 | 0.00 | 0.47 |
| ***Planctomycetes*** | **2.26** | **0.68** | **0.68** | **0.40** | **0.01** |
| ***Nitrospirae*** | **0.56** | **0.39** | **0.18** | **0.21** | **0.03** |

| Phyla | Dry | S.D. | Wet | S.D. | p value |
|---|---|---|---|---|---|
| | Bacterial phyla | | | | |
| ***Acidobacteria*** | **2.28** | **1.18** | **0.73** | **0.60** | **0.01** |
| ***Gemmatimonadetes*** | **1.48** | **0.75** | **0.45** | **0.44** | **0.01** |
| ***Firmicutes*** | **5.32** | **2.86** | **2.49** | **1.37** | **0.01** |
| *Tenericutes* | 0.00 | 0.00 | 0.02 | 0.07 | >0.9999 |
| *ws3 (candidate division)* | 0.11 | 0.24 | 0.00 | 0.00 | 0.47 |
| ***Proteobacteria*** | **44.35** | **5.87** | **69.99** | **7.78** | **<0.0001** |
| *op3 (candidate division)* | 0.04 | 0.12 | 0.00 | 0.00 | >0.9999 |
| *tm6 (candidate division)* | 0.02 | 0.07 | 0.01 | 0.02 | >0.9999 |
| *tm7 (candidate division)* | 0.05 | 0.10 | 0.08 | 0.13 | 0.86 |

*Bold numbers represent significant differences ($p < 0.05$).*

**Table 1.**
*Kruskal-Wallis analysis of the relative abundance (%) of bacteria at the Guánica Dry Forest as affected by wet and dry periods ($n = 9$).*

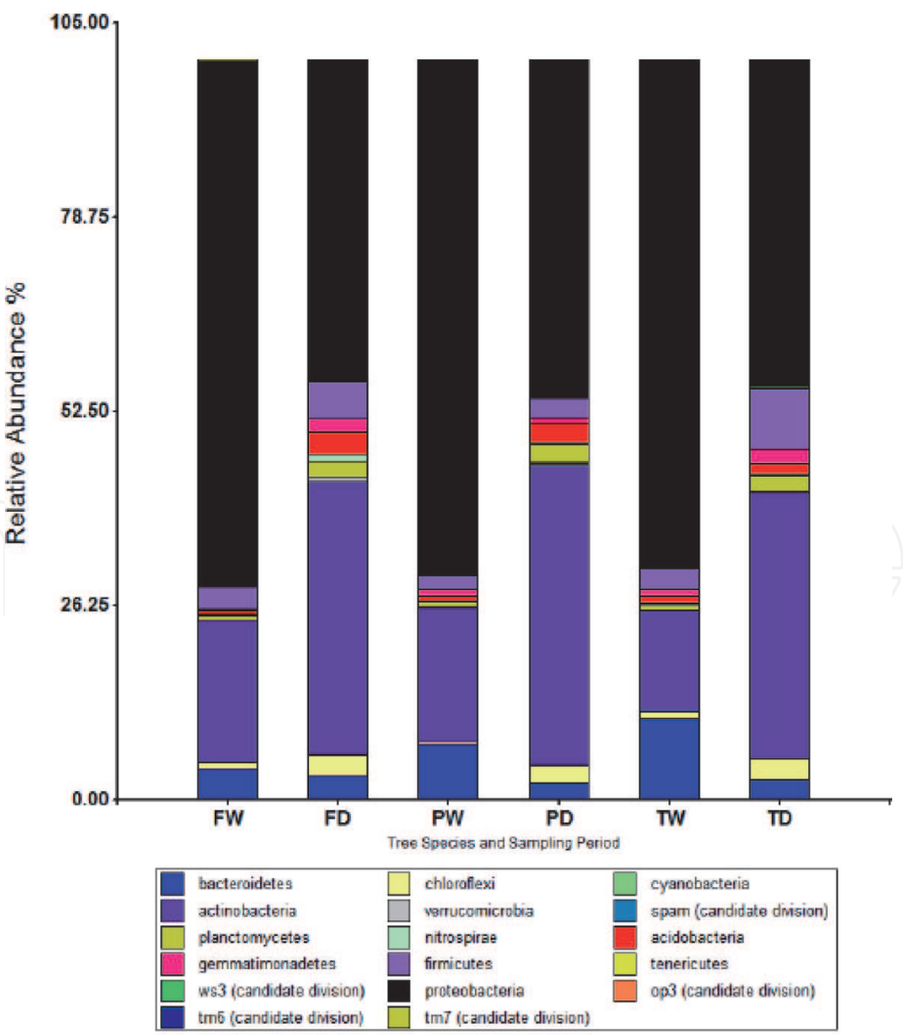


**Figure 2.**
*Relative abundance (%) of bacterial phyla at the Guánica Dry Forest during the wet (W) and dry (D) period under three different tree species (F =* Ficus citrifolia, *P =* Pisonia albida, *T =* Tabebuia heterophylla*).*

| Bacterial phyla | *F. citri.* | S.D. | *P. albida* | S.D. | *T. heter.* | S.D. | p |
|---|---|---|---|---|---|---|---|
| | Tree Species | | | | | | |
| *Bacteroidetes* | 3.71 | 1.27 | 4.97 | 3.45 | 6.92 | 4.89 | 0.69 |
| *Chloroflexi* | 1.91 | 1.39 | 1.29 | 1.24 | 1.84 | 1.19 | 0.70 |
| *Cyanobacteria* | 0.05 | 0.13 | 0.06 | 0.15 | 0.00 | 0.00 | 0.59 |
| *Actinobacteria* | 27.95 | 11.7 | 29.16 | 14.5 | 24.77 | 13.07 | 0.85 |
| *Verrucomicrobia* | 0.30 | 0.39 | 0.25 | 0.17 | 0.07 | 0.14 | 0.18 |
| *Spam (candidate division)* | 0.00 | 0.00 | 0.07 | 0.17 | 0.04 | 0.10 | 0.59 |
| *Planctomycetes* | 1.46 | 1.00 | 1.65 | 1.12 | 1.30 | 0.96 | 0.85 |
| *Nitrospirae* | 0.53 | 0.49 | 0.22 | 0.17 | 0.36 | 0.35 | 0.65 |
| *Acidobacteria* | 1.65 | 1.39 | 1.67 | 1.40 | 1.20 | 0.96 | 0.85 |
| *Gemmatimonadetes* | 1.03 | 0.91 | 0.59 | 0.63 | 1.28 | 0.80 | 0.20 |
| *Firmicutes* | 3.97 | 1.75 | 2.24 | 1.87 | 5.51 | 3.22 | 0.17 |
| *Tenericutes* | 0.04 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.37 |
| *ws3 (candidate division)* | 0.00 | 0.00 | 0.00 | 0.00 | 0.16 | 0.29 | 0.12 |
| *Proteobacteria* | 57.23 | 17 | 57.77 | 15.4 | 56.51 | 14.77 | 0.98 |
| *op3 (candidate division)* | 0.06 | 0.14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.37 |
| *tm6 (candidate division)* | 0.04 | 0.09 | 0.00 | 0.00 | 0.01 | 0.03 | 0.59 |
| *tm7 (candidate division)* | 0.09 | 0.15 | 0.07 | 0.11 | 0.03 | 0.07 | 0.77 |

*Bold numbers represent significant differences ($p < 0.05$).*

**Table 2.**
*Kruskal-Wallis analysis of the relative abundance (%) of bacteria phyla at the Guánica Dry Forest as affected by tree species (Ficus citrifolia, Pisonia albida, and Tabebuia heterophylla).*

| | Tree Species | | | Period | | |
|---|---|---|---|---|---|---|
| | ChiSquare | DF | Prob>ChiSq | ChiSquare | DF | Prob>ChiSq |
| | Bacteria | | | | | |
| S_Taxa | 0.9297 | 2 | 0.6282 | 3.7974 | 1 | **0.0513** |
| Shannon index (H) | 1.1345 | 2 | 0.5671 | 2.3879 | 1 | 0.1223 |
| Equitability (J) | 0.3158 | 2 | 0.8539 | 5.0702 | 1 | **0.0243** |

*Significant differences are found in bold.*

**Table 3.**
*Nonparametric ANOVA for bacterial alpha diversity in soils as affected by tree species and sampling period at the Guánica Dry Forest.*

(44%) and *Actinobacteria* (37%). These dominant bacterial phyla presented the same pattern during the wet and dry period. During the wet period, relative abundance of *Actinobacteria* decreased almost four times when compared to the dry period, and the relative abundance of *Proteobacteria* almost duplicated (**Table 1** and **Figure 2**). Relative abundance of *Planctomycetes*, *Acidobacteria*, *Gemmatimonadetes*, and *Firmicutes* was 1–2 orders of magnitude higher during the dry period when compared to wet period, whereas only *Proteobacteria* and *Bacteroidetes* were 1–2 orders of magnitude higher during the wet period when compared to the dry period. The most predominant bacterial phyla for all tree species during the wet and dry periods were *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* (**Table 2**). Kruskal-Wallis test did not demonstrate significant differences in bacterial relative

| Bacteria Indicator Species | | | | |
|---|---|---|---|---|
| | group | indval | p-value | freq |
| *Acetobacteraceae spp* | Ficus Wet | 0.9036854 | 0.018 | 4 |
| *Brevundimonas wangchunensis* | Ficus Wet | 0.7891088 | 0.019 | 6 |
| *Brevundimonas asdaezzhy* | Ficus Wet | 0.7146215 | 0.011 | 8 |
| *Brevundimonas spp.* | Ficus Wet | 0.6583452 | 0.029 | 12 |
| *Bosea thiooxidans* | Ficus Wet | 0.6415078 | 0.042 | 8 |
| *Devosia spp.* | Ficus Wet | 0.6211101 | 0.009 | 14 |
| *Pseudomonas fluorescens* | Ficus Wet | 0.7762022 | 0.046 | 6 |
| *Pseudomonas stutzeri* | Ficus Wet | 0.6999245 | 0.016 | 8 |
| *Pedobacter ginsengisoli* | Ficus Wet | 0.5858814 | 0.049 | 8 |
| *Delftia lacustris* | Ficus Wet | 0.9448138 | 0.013 | 4 |
| *Streptomyces platensis malvinus* | Pisonia Wet | 1 | 0.014 | 3 |
| *Streptomyces thermolineatus* | Pisonia Wet | 0.7624343 | 0.03 | 4 |
| *Hyphomicrobium zavarzinii* | Pisonia Wet | 0.6518947 | 0.015 | 7 |
| *Aeromicrobium kwangyangensis* | Pisonia Wet | 0.651858 | 0.022 | 4 |
| *Mycobacterium bavariae* | Pisonia Wet | 0.6077151 | 0.004 | 10 |
| *Mycobacterium moriokaense* | Pisonia Wet | 0.5624626 | 0.035 | 10 |
| *Defluviicoccus spp.* | Pisonia Wet | 0.5216104 | 0.031 | 8 |
| *Nitrospira spp.* | Pisonia Wet | 0.4342276 | 0.021 | 14 |
| *Nocardioides spp.* | Pisonia Wet | 0.3847424 | 0.048 | 16 |
| *Rhodospirillaceae spp.* | Pisonia Wet | 0.3789393 | 0.05 | 18 |
| *Streptomyces sakaiensis* | Pisonia Wet | 1 | 0.008 | 3 |
| *Sciscionella spp.* | Pisonia Wet | 1 | 0.01 | 3 |
| *Phenylobacterium spp.* | Pisonia Wet | 0.8661456 | 0.011 | 5 |
| *Bradyrhizobium sp.* | Pisonia Wet | 0.7317982 | 0.011 | 8 |
| *Mycobacterium avium complex* | Pisonia Wet | 0.6628777 | 0.006 | 8 |
| *Frankia spp.* | Pisonia Wet | 0.6492538 | 0.008 | 12 |
| *Bradyrhizobium spp.* | Pisonia Wet | 0.6320272 | 0.045 | 18 |
| *Crossiella spp.* | Pisonia Wet | 0.6190261 | 0.038 | 16 |
| *Candidatus entotheonella* | Pisonia Wet | 0.569092 | 0.025 | 13 |
| *Opitutus spp* | Pisonia Wet | 0.5654218 | 0.035 | 6 |
| *Hyphomonadaceae spp.* | Pisonia Wet | 0.5261401 | 0.004 | 13 |
| *Mycobacterium celatum* | Pisonia Wet | 0.4244481 | 0.006 | 15 |
| *Streptomyces aureus* | Pisonia Wet | 0.966957 | 0.014 | 4 |
| *Nocardioides albus* | Pisonia Wet | 0.8408999 | 0.026 | 5 |
| *Bacillus spp.* | Pisonia Wet | 0.8086407 | 0.011 | 9 |
| *Nitrospinaceae spp.* | Pisonia Wet | 0.601469 | 0.02 | 12 |
| *Sphingomonas spp.* | Pisonia Wet | 0.5841869 | 0.013 | 18 |
| *Microlunatus spp.* | Pisonia Wet | 0.5766045 | 0.006 | 17 |
| *Acidimicrobiales spp.* | Pisonia Wet | 0.5665658 | 0.011 | 18 |
| *Mycobacterium asiaticum* | Pisonia Wet | 0.4652796 | 0.008 | 9 |
| *Paenibacillus spp.* | Pisonia Wet | 0.3844302 | 0.017 | 18 |
| *Bradyrhizobiaceae spp.* | Pisonia Wet | 0.3625066 | 0.035 | 18 |

**Table 4.**
*Bacterial indicator species analysis (ISA) at the Guánica Dry Forest.*

abundance due to tree species (**Table 2**). There were no significant differences in any of the diversity indexes with regard to tree species (**Table 3**). Sampling period exhibited an effect on bacterial species richness (P = 0.05) and on equitability (P = 0.0243). but not on the Shannon index. We found higher bacterial species richness during the wet period and higher equitability during the dry period (**Table 3**).

### 3.2 Evaluation of indicator species analysis (ISA) for this forest ecosystem

The identification of species associated or indicative of groups of samples is a common aspect of ecological research [24]. Indicator species analysis (ISA) identified several bacterial (**Table 4**) species responsible for changes in soil microbial communities. Out of 185 bacterial OTUs, 10 served as indicator species for *Ficus citrifolia* during the wet period (**Table 4**). A total of 31 bacterial OTUs were identified as indicator species for *Pisonia albida* during the wet period. No bacterial indicator species were found for the dry period (**Table 4**).

### 3.3 Relationship between the relative abundance of taxa and enzyme activities

Axis 1 of the canonical correspondence analysis for bacterial community explained 80% of the variation (**Figure 3**). Two groups were segregated with regard

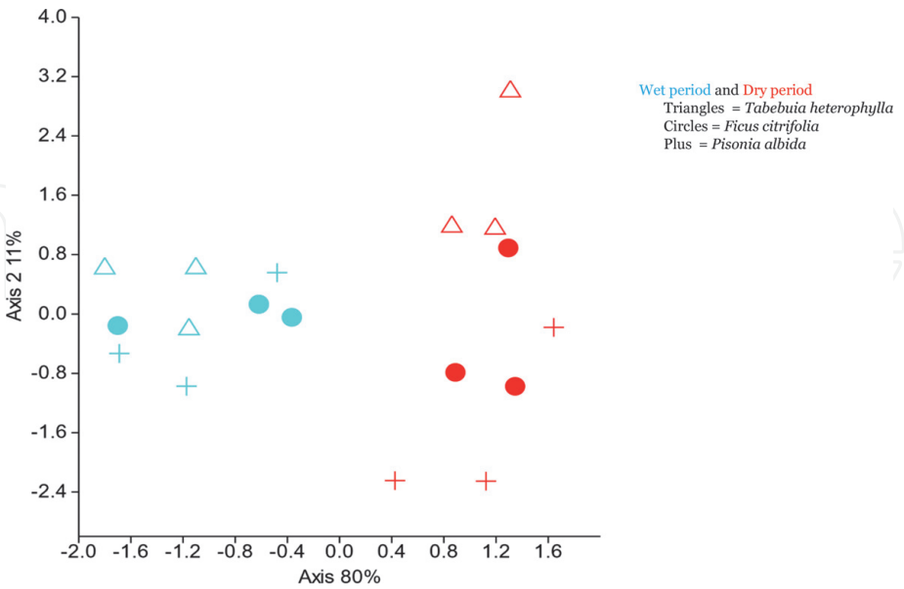


**Figure 3.**
*Canonical correspondence analysis (CCA) of bacterial phyla demonstrating the effect of wet/dry periods at the Guánica Dry Forest. Blue symbols represent wet periods and red symbols represent dry periods. Triangles, circles and plus sign represent tree species (Tabebuia heterophylla, Ficus citrifolia and Pisonia albida), respectively.*

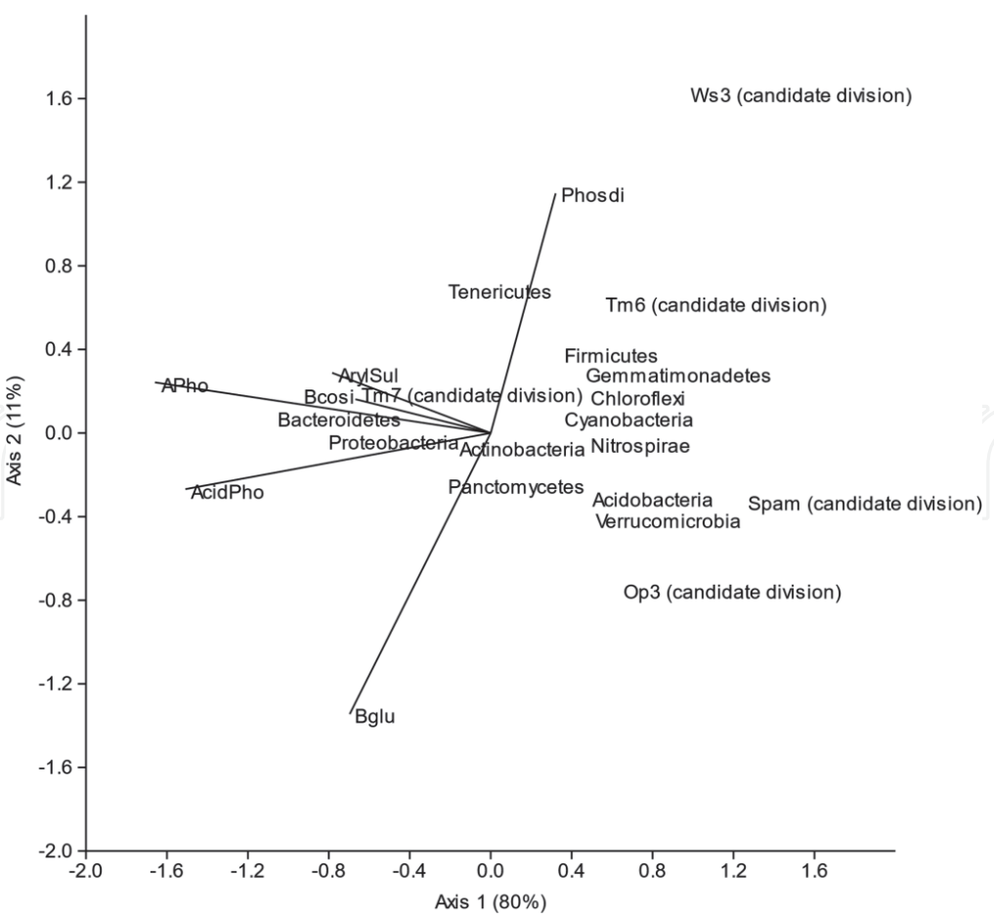


**Figure 4.**
*Canonical correspondence analysis (CCA) of bacterial phyla and soil enzyme activities (Phosdi = phosphodiesterasae, ArylSul = aryl sulphatase, APho = alkaline phosphatase, AcidPho = acid phosphatase, Bcosi = β-glucosaminidase, and Bglu = β-glucosidase). Vectors represent enzyme activities.*

to dry and wet periods (**Figure 3**). Samples that correspond to the wet period were associated with acid phosphatase, alkaline phosphatase, β-glucosaminidase, β-glucosidase, and arylsulfatase (**Figure 4**), whereas samples corresponding to the dry period are associated with phosphodiesterase (**Figure 4**). Microbial taxa associated with wet samples were Bacteroidetes, Proteobacteria, and Tenericutes. Microbial taxa associated with dry sampling points were *Actinobacteria*, *Planctomycetes*, *Acidobacteria*, *Verrucomicrobia*, *Cyanobacteria*, and *Chloroflexi*.

## 4. Discussion

### 4.1 Response of microbial diversity to wet and dry periods at the Guánica Dry Forest

Historical rainfall patterns contribute to the acclimatization and resilience of soil bacterial communities to low and high rainfall events. Bacterial Shannon index (3.9) was similar to values reported by Žifčáková et al. (2016) for a Norway spruce forest (S = 3.5) and lower than the one reported for a hardwood forest (S = 6–6.5) or dry heath in a tundra (S = 7.5) [25]. Our study demonstrates that soil bacterial richness, diversity, and equitability were impacted by rainfall patterns and not by tree species. Both bacterial richness and equitability were higher during the dry period, but bacterial diversity was not impacted by rainfall regime. Our trends imply that a total number of bacterial species do not change during low rainfall events in this forest, but changes occur in the quantity of each species and in their distribution, indicating that soil bacterial communities have adapted to low rainfall at the Guánica Dry Forest. There is indirect evidence that microbial communities do become resistant and function optimally under their historical rainfall regime [26–28]. Cruz-Martínez et al. (2009) [29] found that soil microbial communities were more resilient to long-term changes in rainfall after a 7-year rainfall amendment study. They stated that after 7 years soil microbial communities developed a degree of robustness or acclimatization to the rainfall amendments. Additionally, other studies have reported acclimatization of soil heterotrophic communities to experimental warming and seasonal variation [30]. Compositional changes exhibit historical legacy with regard to moisture regimes [27] suggesting that microbial communities will be shaped in part by the historical climate to which they are exposed [30].

In this study the bacterial communities under all tree species were dominated by *Proteobacteria* (57%), *Actinobacteria* (24–29%), and *Bacteroidetes* (4–7%). All of these bacterial phyla are ubiquitous and have been identified in desert soils [31], agroecosystems, and other types of forest environments [32–35]. *Proteobacteria* are one of the largest bacterial divisions within the prokaryotes and account for most of the known Gram-negative bacteria [36]. Although *Proteobacteria* are one of the top bacterial phyla that are found colonizing many soil types their high predominance at the GDF could be associated with high accumulation of litter and SOM that is reported in these soils. An interesting detail is that Proteobacteria dominated during both periods, with the exception that during the dry period they reduced their relative abundance, suggestion there association with soil moisture as found in other studies [28].

*Actinobacteria*, one of the largest bacterial phyla known, was the second most abundant phylum found in this study. This group is mainly composed of Gram-positive bacteria [32–35] and are known to withstand in harsh environments, due to their metabolic, physiologic, and morphological diversity [36]. Soils of the area

under study have an accumulation of organic matter mainly composed of new and old leaf litter due to the harsh environmental conditions; this bacterial phylum is associated with the degradation of more complex substrates of SOM including lignin, which could explain their predominance in this forest soils [37–39]. Our data suggest that *Actinobacteria* were highly associated with phosphodiesterase at the Guánica forest. Other studies have also found that *Actinobacteria* populations (characterized by TRLFP) correlated with P content in semiarid environments [40]. Other studies have also demonstrated the production of phosphatases by cultivable soil *Actinobacteria* [41–43] serving as a further evidence of the trend observed in this study. I also found that Actinobacteria were the most abundant phyla during the dry period which is consistent with other reports. A similar trend was also found for a semiarid, high desert grassland north of Flagstaff, Arizona [44] and in another study, which evaluated African tropical forest soils and Chinese forest soils [45].

*Bacteroidetes*, the third most abundant phylum in this forest, are widely distributed in different habitats ranging from Antarctic ice, lakes, the gut of animals, and terrestrial environments [46]. Additionally, this phylum has the ability to withstand extreme desiccation conditions including droughts and UV light [47]. They have been found in fine dust traveling thousands of kilometers [48] and inside microaggregates; authors explain that in semiarid agroecosystems this could be a protection strategy employed by these microbes to endure extreme environmental conditions [48]. I found that *Bacteroidetes* were highly associated with the activities of two soil enzymes evaluated (acid phosphatase and alkaline phosphatase). These enzymes mineralize organic P forms into inorganic P forms. Another study provided evidence suggesting that environmental *Bacteroidetes* specialize in the mineralization of high molecular weight organic matter making them a key compartment for carbon fluxes and budgets in ecosystems [49]. *Bacteroidetes* are oligotrophic and are commonly associated with substrates rich in organic matter [50] as is the forest area where I based my study. The high abundance of *Bacteroidetes* could be associated with the rich organic matter present in the sampling area.

### 4.2 Influence of tree species on the bacterial populations in this forest

Even though the most abundant bacterial phyla identified under all tree species were the same (*Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*), some were specific for each tree species. Indicator species analysis (ISA) revealed that specific bacterial species were present under *Ficus citrifolia* and *Pisonia albida* only during the wet period. This trend suggests that these species assemblages may play an important role in the soil ecosystem processes under these specific tree species. One species was *Devosia* spp. whose OUT frequency was 14 (ind val = 0.62; p = 0.009) in soils collected under *Ficus citrifolia. Devosia* spp. forms part of the α-*Proteobacteria*; this genus is a non-rhizobia nodulating, nitrogen fixer [51]. Three different species of *Brevundimonas* spp. also occurred in high frequency under *Ficus citrifolia* during the wet period as indicated by ISA. This genus is actually known to produce phosphodiesterase, a group of enzymes involved in the degradation of organophosphorus [52]. During the wet period, 32 different OTUs were identified exclusively for *Pisonia albida*, and at least 7 of them had a frequency of 18. This high frequency suggests that indicator species may be playing an important role in the soil dynamics of *Pisonia albida*. For instance, three of the most frequent species are nitrogen fixers (*Microlunatus* sp., *Rhodospirillacease* spp., and *Paenibacillus*), and *Pisonia albida* was the tree species with the highest total available nitrogen in this study (data not shown here). Even though we did not cultivate the indicator species nor have information regarding the physiology of the indicator species identified for

*Ficus citrifolia* and *Pisonia albida*, we can infer that they contribute with relevant functions in this soil system. The dwarfed tree species selected for this study influenced the structure and diversity of specific bacterial populations.

### 4.3 Potential disadvantages and bias with 454-pyrosequencing

Amplicon-based pyrosequencing methods have major advantages over the tools that have been used in the past to study microbial community structure. Although the results presented in this chapter have a similar pattern as the results presented Rivera et al. (2018), it important to acknowledge certain biases that have been described for amplicon-based pyrosequencing. Even though 454 pyrosequencing has a higher resolving power than Sanger sequencing or EL-FAME analysis in 454 pyrosequencing, there are some sequencing errors and chimeras that can be retained in the datasets that can inflate the estimated richness of the sample. Bias can also occur with primer selection as the primers used can select for the most predominant DNA present in the sample underestimating the rare DNA in the sample [53]. Using inappropriate primers consequently can lead to questionable biological conclusions. Another concern is that the techniques used for processing amplicon pyrosequencing data can result in the detection of several hundred "false" OTUs, mostly at low abundance, rising the concern that species abundance can be overestimated [54]. More stringent techniques such as shotgun sequencing, Ion Torrent sequencing, and Illumina platforms have been developed that help mitigate some of the concerns with pyrosequencing, but these stringent technologies have biases of their own.

## 5. Conclusions

Soil bacterial communities have adapted to low rainfall at the Guánica Dry Forest; this could be a response to historical rainfall patterns encountered at the Guánica Dry Forest. The fact that 9 out of the 17 bacterial phyla identified were higher during the dry period supports this conclusion. For this forest, bacterial diversity did not change as a response to rainfall; however, equitability and richness changed demonstrating bacterial resilience. We are seeing how the same three bacterial phyla (*Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*) are predominant during both dry and rainfall periods. Even though predominant bacterial phyla were the same during both periods under all tree species, differences were found at a finer scale. For instance, *Pisonia albida* had the soil with the most bacterial indicator species present. It is evident that this tree is shaping the soil microbiome in different ways. The general trend for predominant phyla found for Guánica is similar to the predominant phyla found in other terrestrial ecosystems even though the conditions of Guánica are unique. In the future, it would be nice to compare the sequences obtained in this study with other terrestrial environments but to the species level. This will help elucidate which are the species that could be playing important roles in ecosystem function and resilience.

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## Conflict of interest

No conflict of interest.



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