Chapter

Tree Species and Precipitation Effect on the Soil Microbial Community Structure and Enzyme Activities in a Tropical Dry Forest Reserve

Michelle Jovanne Rivera-Rivera, Veronica Acosta-Martínéz and Elvira Cuevas

Abstract

We examined the soil microbial community structure and soil enzyme potential within three dominant tree species at a tropical dry forest during five months. Changes within microbial community in response to sampling periods and tree species were evaluated using fatty acid methyl-ester and enzymes potential. We found that both tree species and precipitation determined microbial community structure and enzyme potential. This is the first study that provides insight into the soil microbial community at Guánica Dry Forest, a valuable contribution that will help elucidate strategies for better management and protection of the soil biota of the area.

Keywords: dry soil, soil enzyme activity, microbial diversity, EL-FAME, phosphatases

1. Introduction

Forest ecosystems are a fundamental part of the biosphere as they govern global primary production and biogeochemical cycling [1]. The dynamics of decomposition and nutrient cycling is driven by soil microbial communities [2] and their enzymatic potential [3]. Assessments of soil microbial community composition are often coupled with the assessment of soil processes (C storage and nutrient cycling) through different enzymatic activities to better address the ecosystem function [4]. Some of the principal enzyme activities assayed in soils are involved in carbon cycling (β-glucosidase, α-galactosidase) with potential importance for C sequestration [5]. Other enzymes such as β-glucosaminidase are important in the decomposition of more complex compounds such as chitin [6, 7] Phosphorus cycling enzymes (acid and alkaline phosphatases and phosphodiesterase) are important since most soils are P deficient [8] and microbial enzyme activity (EA) plays a vital role in the availability of P in soils.

Both microbial communities and enzyme activities are sensitive to biotic and abiotic disturbances. For example, low soil moisture may result in low enzyme
activity [9] and rainfall pulses may result in pulses of microbial growth which may lead to pulsed secretion of soil enzymes promoting a temporary increase of soil enzyme activity [10].

Understanding the dynamics of forest soil ecosystem depends on elucidating the contribution of individual plant species to the soil biota and the process that they regulate [11]. Determining and quantifying plant species effects under natural conditions can be difficult due to environmental noise and the interaction among species present in an area [12]. Little is known about the effects of dominant vegetation on the soil biogeochemical processes such as enzyme activities [13]. Even less is known about the plant-soil-microbial interactions that take place in extreme environments such as tropical dry forest.

Nearly 42% of tropical forests around the world are seasonally dry plant communities, where around half of the Central American and Caribbean land area is characterized by a tropical or subtropical dry forest climate. In the Coastal Plateau of the Guánica Dry Forest (GDF), an UNESCO/MAB Biosphere Reserve, dwarfed trees grow isolated from one another in the cracks of the calcareous platform, forming individual islands of fertility [14]. A lack of interspecific competition is observed, as tolerance to environmental stress and scarcity of space for establishment make it difficult for aboveground and belowground competition, also contributing to the evenness of tree species found in this area [14]. The substrate is derived from limestone made from marine deposits that vary throughout the forest from deep alluvial fans to exposed fractured limestone with shallow soil pockets [15]. This naturally occurring plant community provides the ideal conditions to determine how specific tree species affect the soil microbial community composition and enzymatic potential in a dry forest.

In order to understand how trees impact soil microbial communities, a five-month study was conducted at the GDF. We selected three dwarfed, isolated tree species (a pantropical species and two native species) that are highly distributed among the forest, hypothesizing that these trees may harbour different microbial community structure and activities. The tree species selected complied with the following requirements: (1) that trees were growing in cracks isolated from other trees by exposed rock and (2) that their litter and belowground substrate originated from their own residue decomposition [16]. Additionally, this forest experiences bimodal and pulsed precipitation patterns [17, 18] that may contribute in the alteration of the microbial dynamics and nutrient turnover of the forest. Our objectives were: (1) to determine if tree species traits had an effect on the soil microbial community structure and activities and (2) to determine the effects of sampling period on the soil microbial community structure and activities.

2. Methods

2.1 Study site

The study was located in the coastal association of the Guánica Dry Forest latitude 66°53’30”W longitude 17°58’0”N [16–18]. In this area of the forest, dwarf individual adult trees from different species are separated by exposed rocks which prevent overlap among trees forming “monospecific tree islands” (Figure 1A and B). These “monospecific tree islands” have little or no mineral soil (Figure 1A), with a highly organic substrate composed of shallow monospecific litter and humus (Figure 1C and D), which varies according to variations in the ground relief and season from 2 to 8 cm depth [16]. These characteristics make this forest an ideal system to study tree species effects as there are no confounding effects of overlapping roots from other species. We chose three trees from three species, previously tagged and studied, that grow from 100 m to approximately 300 m from the coast.
The three tree species selected are: *Tabebuia heterophylla* (DC.) Briton, Ann, a facultative deciduous species; *Pisonia albida* (Heimerl) Briton ex Scandal, an obligate deciduous species and *Ficus citrifolia* Mill., Gard., a facultative deciduous species. The trees are distributed more or less randomly across this area and are dominant species of the coastal region [17], no spatial distribution has been detected. During this study, the highest precipitation was reported during the month of August 2011 (212 mm) and the lowest precipitation was reported for the month of October 2011 (85.09 mm). The total accumulated rainfall was 738 mm.

### 2.2 Selected soil substrate analyses

Total organic matter, soil texture, pH and moisture analyses were completed. Soil total organic matter was determined by the ignition method. Soil texture was determined by the pipette method [19]. Soil pH was determined in a 1:5 (soil:water) mixture using an Orion 420A pH Meter [20]. Soil moisture was determined by weighing and drying 1 g of soil at 105°C during 24 h [20].

### 2.3 EL-FAME analysis

Microbial community structure was evaluated by the ester-linked (EL)-FAME method as described by [21]. A total of 3 g of field moist soil was used for each sample, and four steps were completed: (a) saponification and methylation of ester-linked fatty acids were performed by incubating the 3 g of soil in 15 ml of 0.2 M KOH in methanol at 37°C during 1 h—samples were vortexed every 10 min during the
incubation period and, after the incubation period was completed, 3 ml of 1.0 acetic acid was added to neutralize the mixture's pH; (b) partitioning of the FAMEs into an organic phase was achieved by adding 10 ml of hexane and centrifuging the preparations at 480×g for 10 min; (c) the hexane layer was transferred to a clean glass tube and evaporated under a N\textsubscript{2} stream and (d) FAMEs were suspended in 0.5 ml of 1:1 hexane:methyl-tert butyl ether and transferred to GC vials for analysis. Extractions were analysed as described by [4]. A 6890 GC Series II (Hewlett Packard, Wilmington, DE, USA) equipped with a flame ionization detector and fused silica capillary column (25 m × 0.2 mm) with ultra-high purity H\textsubscript{2} as the gas carrier was used to analyse the extractions. The temperature program was ramped from 170 to 250°C at 5°C min\textsuperscript{−1} as described. The fatty acids were identified and quantified by comparing the retention times and peak areas to MIDI standards. The MIDI software provides FAME relative peak areas (percentage) based on the total FAMEs in a sample (based on the Aerobe method of the MIDI system). FAME concentrations (nmol g\textsuperscript{−1} soil) were calculated by comparing peak areas to an analytical standard (19:0, Sigma Chemical Co., St. Louis, MO) calibration curve. The FAMEs are described by the number of C atoms, followed by a colon, the number of double bonds and then by the position of the first double bond from the methyl (ω) end of the molecule. Cis isomers are indicated by c and branched fatty acids are indicated by the prefixes i and a for iso and anteiso, respectively. Other notations are Me for methyl, OH for hydroxy and cy for cyclopropane.

2.4 Soil enzyme activity

Study of enzyme activities was performed as described in [4, 6]. The activities of enzymes relevant in C cycling (β-glucosidase), C and N cycling (β-glucosaminidase), P cycling (alkaline phosphatase, acid phosphatase, phosphodiesterase) and in the S cycle (arylsulphatase) 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 [4]. 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\textsuperscript{−1} soil h\textsuperscript{−1}.

2.5 Statistical analysis

All data analysed with JMP software were checked for normality and transformed to log10. To determine differences in soil FAMEs, enzyme activity and selected soil properties due to tree species and sampling periods, canonical discriminate analysis (CDA) was conducted with the JMP program. The first and second canonical discriminate functions were utilized to determine the distribution of enzyme activity and FAMEs as influenced by each tree species and by sampling period. SigmaPlot 10 software was used to conduct two-way analysis of variance (2-way ANOVA). Two-way analysis of variance was used to establish the effect of plant species and sampling period on soil community structure, enzyme activities and selected soil properties.

3. Results

3.1 Selected soil and plant properties

Soil samples under all tree species were predominantly organic (65–85%). The mineral part of the soil is silty loam to silty clay loam (20–36% clay, 0.4–0.7% sand,
63–80% silt) depending on the tree location in the study area (data not shown). Soil pH was neutral to alkaline for all tree species (Table 1). Two-way ANOVA did not demonstrate an interaction between tree species and sampling months (Table 2). Available N varied significantly with regard to monthly sampling and not to tree species (Table 2). Available Ca and P varied significantly with regard to tree species (Table 2). Total N and C:N ratio varied significantly with regard to tree species.

3.2 Soil enzyme activities

Six enzyme activities (EAs) were assessed representing C (β-glucosidase), N (β-glucosaminidase), P (phosphodiesterase, alkaline and acid phosphatase) and S (arylsulphatase) cycling. There was a clear and significant separation in the soil enzyme activity between the tree species in this forest, while there was no distinct trend due to the sampling time (Figure 2). Most of the separation on the metabolic capacity of the soil according to these six EAs was observed along axis 1, which explained 87.22% of the variability. *Tabebuia heterophylla* aligned along CA 1 separating this species from *Pisonia albida* and *Ficus citrifolia* which aligned along CA 2 (eigenvalue 12.3). The activities of β-glucosidase, alkaline and acid phosphatase were more closely associated with *Tabebuia heterophylla* than with the other tree species while β-glucosaminidase activity was more closely associated with *Ficus citrifolia* trees.

Two-way analysis of variance was used to establish the effect of plant species and sampling period on soil enzyme activities. The only enzymes that were significantly affected by plant species were alkaline phosphatase (F = 8.18; P < 0.001), β-glucosidase (F = 8.86; P < 0.001) and β-glucosaminidase (F = 5.97; P = 0.007) (Table 2). Alkaline phosphatase was the only enzyme affected by monthly variations (F = 4.375; P = 0.007). Two-way ANOVA does not demonstrate significant interactions between tree species and monthly variations.

3.3 Microbial community structure (EL-FAME)

A total of 56 FAMEs were identified in the samples analysed and 13 FAMEs were used as indicators of different microbial groups as affected by tree species traits and sampling time (Table 2). According to CDA, significant differences (P < 0.0001) were detected in the FAME profiles of the soil microbial community structure as affected by tree species traits (Figure 3A). Canonical axis 1 explained 83% of the variation. The soil microbial communities under *Tabebuia heterophylla* were distinct from the microbial communities under *Ficus citrifolia* and *Pisonia albida*. FAMEs that contributed to the separation observed under *Tabebuia heterophylla* are the

### Table 1.
Mean values of selected soil properties under three different tree species.

| Selected Soil Properties | F. citrifolia | P. albida | T. heterophylla | p-value (α 0.05) |
|--------------------------|--------------|-----------|-----------------|------------------|
| *α*id Phosphatase        | 867.79 (221.68) | 1472.65 (1229.14) | 1237.61 (245) | 0.03             |
| *β*glucosaminidase       | 169.70 (42.28)  | 296.90 (251.60)  | 116.78 (46)   | 0.00             |
| Aryl sulphatase          | 434.49 (231.98) | 420.93 (216.88)  | 623.96 (498)  | 0.22             |
| *α*line phosphatase      | 1530.92 (689.80) | 1148.74 (576.70) | 1950.45 (488) | 0.02             |
| *β*phosphodiesterase     | 751.32 (326.18) | 404.28 (197.62)  | 774.60 (238)  | 0.00             |
| *β*glucosidase           | 288.49 (60.40)  | 255.95 (92.73)   | 560.45 (352)  | 0.00             |
| Soil humidity (%)        | 72 (29.98)     | 112.57 (69.16)   | 88.18 (29.2)  | 0.16             |
| Biomass                  | 2606.84 (410.54) | 2306.95 (678.03) | 2746.88 (1061) | 0.28             |
| pH                       | 8.02 (0.23)    | 7.69 (0.71)      | 7.79 (0.51)   | 0.20             |

Mean values of selected soil properties under three different tree species.
Gram-positive (G+) markers $a15:0$ and $a17:0$, actinomycete $10Me18:0$, protozoan marker $20:4\omega_6c$ and the fungal marker $18:3\omega_6c$.

*Ficus citrifolia* and *Pisonia albida* grouped closer together but were still significantly different from each other as species, 95% confidence ellipse are clearly separated. FAMEs that contribute to differences in *Ficus citrifolia* vs. *Pisonia albida* are $cy19:0$ (Gram-negative, G−), $10Me16:0$ or $10Me17:0$ (actinomycete) and $18:2\omega_6c$ (fungal marker). Differences in *Pisonia albida* were due to $i7:0$ (G+), $cy17:0$ (G−), and $16:1\omega5c$ or $18:1\omega9c$ (fungal markers).
Figure 2.
Canonical discriminate analysis of the soil enzyme activity at the Guánica Dry Forest. (A) canonical discriminate analysis of the soil enzyme activity as affected by tree species (Ficus citrifolia, Pisonia albida and Tabebuia heterophylla). Letters (A, B, C, D and E) represent sampling periods (July 2011, August 2011, September 2011, October 2011 and November 2011), respectively. The colours of the letters (pink, green and blue) represent tree species (Ficus citrifolia, Pisonia albida and Tabebuia heterophylla), respectively. The multivariate mean for each tree species is a coloured and labelled circle. The size of the circles corresponds to a 95% confidence limit for the mean. (B) canonical discriminate analysis of the soil enzyme activity as affected by sampling period during July to November 2011 at the Guánica Dry Forest of Puerto Rico. Letters (A, B, C, D and E) represent sampling periods (July 2011, August 2011, September 2011, October 2011 and November 2011), respectively. The colours of the letters (pink, green and blue) represent tree species (Ficus citrifolia, Pisonia albida and Tabebuia heterophylla), respectively. The multivariate mean for each month sampled is a coloured and labelled circle. The size of the circles corresponds to a 95% confidence limit for the mean.
In addition to the effects of tree species on FAME profiles of the microbial community structure, there were also significant monthly variations (p = 0.001) according to CDA along CA1, which explained 68.81% of the variation (Figure 3B). The CDA revealed that July, September and August clustered together along axis 1. August was separated from other months due to the predominance of 17:0 (G+) and cy17:0 (G- marker). This cluster of these 3 months was characterized by the
presence of the fungal marker 18:1ω9c, the G— marker cy19:0 and the actinomycete indicator 10Me16:0. The second cluster formed was composed of October and November due to the predominance of two G+ markers (a15:0 and i17:0), two actinomycete markers (10Me17:0 and 10Me18:0), a protozoan marker (20:4ω6c) and all the fungal markers (16:1ω5c, 18:3ω6c and 18:2ω6c).

Two-way analysis of variance was used to establish the effect of plant species and sampling period on soil community structure. The Gram-positive EL-FAMEs that were significantly affected due to tree species were iso15:0, iso17:0, antesio17:0, 10Me16:0 and 10Me17:0. Gram-negative markers affected by tree species were 17:0cy and 19:0cy and fungal markers were 18:1ω9c, 16:1ω5c, 18:2ω6c and 18:3ω6c. The Gram-positive EL-FAMEs that were significantly affected due to monthly variations were iso15:0, antesio15:0 and 10Me16:0. None of the Gram-negative markers were affected by monthly variations. Fungal markers 16:1ω5c, 18:2ω6c and 18:3ω6c were significantly affected by monthly variations. Two-way ANOVA did not demonstrate significant interactions between tree species and monthly variations (Table 2).

4. Discussion

4.1 Soil microbial communities in a dry forest as affected by tree species

The evaluated tree species had the ability of modifying soil microbial communities. We found that the relative abundance of 8 out of 13 markers was higher under *Ficus citrifolia* than the rest of the tree species. *Ficus citrifolia* is a facultative deciduous tree, which means that it mostly exchanges leaves and has massive litter fall during very dry periods. Higher numbers of FAME markers under this species may be indicative of idiosyncratic effects that may be stabilizing the microbial community. Species traits such as leaf nutrients, leaf toughness and specific leaf area (SLA, cm²/g) are known to contribute to the rate of decomposition of organic matter, which may drive the microbial community under each tree species [22–24]. Previous studies have determined that *Tabebuia heterophylla* leaves are tougher (350 N) and have lower specific leaf area (SLA) (90 cm²/g) than those of *F. citrifolia* (175 N; 110 cm²/g) and *Pisonia albida* (80 N; 100 cm²/g) [16] making them more difficult to undergo decomposition. Soils under *Tabebuia heterophylla* presented a higher relative abundance of saprophytic fungal marker 18:3ω6c which may be indicative of lower rates of litter fragmentation and decomposition.

Differences in the relative abundance of fungal markers at the Guánica Dry Forest could also be due to the microclimate of each tree species. The amplitude of temperature fluctuations encountered in this forest varies among plant species, a factor that has been documented to affect arthropods in this system [16]. The canopies of both *Pisonia albida* and *Ficus citrifolia* generally are taller than the tree canopy of *Tabebuia heterophylla*. This difference in height contributes to differences in temperature, water throughfall and soil moisture. Idiosyncratic tree species characteristics have the ability of modifying the amplitude of daily temperature at the Guánica Dry Forest [16] and these modifications will also affect the fungal community structure that is present under each tree species.

4.2 Soil microbial communities in a dry forest as affected by monthly variations

As found in our study, microbial community structure was influenced by monthly wet/dry events (Figure 3B). Previous studies have described significant responses of the soil microbial communities to wet/dry events [25]. Our results point towards differential responses between sporadic and continuous rainfall.
events. The fungal markers showed lower abundance during July and higher abundance during October and November. Saprophytic fungal marker (18:3 ω6c, 18:2 ω6c) and the mycorrhiza marker 16:1 ω5c were more susceptible to monthly rainfall variations (p < 0.0001) than any other microbial group. As a consequence of rainfall pulses, adventitious roots were observed in the soil substrate, which serves as a surface area for the establishment of arbuscular mycorrhizal fungi. Although arbuscular mycorrhizal fungi enhance P absorption in root systems, we did not find any correlation between available P and mycorrhizal marker 16:1 ω5c.

Although fungal markers showed greater monthly fluctuations, the G+ bacterial marker i15:0 also responded significantly to monthly variations, specifically to water input. Dijkman [26] postulated that FAME marker (i15:0) is found in sulphate-reducing bacteria, which could explain our result. For example, the accumulation of water due to high rain pulses could make the soil habitat anaerobic, contributing to the proliferation of anaerobic and sulphate-reducing bacteria. Sulphate-reducing bacteria are widely spread in anaerobic habitats and play crucial roles in S and C mineralization [27]. For instance, during November, samples were collected after 2 days of continuous rain and the abundance of FAME marker i15:0 increased responding to rainfall pulses, which could support the possibility that certain sulphate-reducing bacteria were represented by this FAME marker and this would be in agreement with the C and S cycling enzyme activity trends during the rainfall pulse.

4.3 Soil enzymatic activity

Our results show that enzyme activity is highly dependent on the soil microbial community structure of each tree species. Tree species was a strong modulator of soil enzyme activity when compared to monthly climatic variations. Although all enzymes tested were active under each tree species, activity of certain enzymes was higher under specific tree species. For instance, acid phosphatase, alkaline phosphatase and β-glucosidase activity was higher under *Tabebuia heterophylla*, and β-glucosaminidase activity was the highest reported for *Pisonia albida*.

Tree species idiosyncratic traits affect not only the structure of microbial communities but also their enzyme activities. Alkaline phosphatase under *Pisonia albida* correlated with many microbial groups (Gram-positive, Gram-negative, actinobacteria, protozoa and saprophytic and arbuscular fungal markers). Alkaline phosphatase under *Ficus citrifolia* only correlated with Gram-positive, saprophytic and arbuscular fungi and finally alkaline phosphatase under *T. heterophylla* correlated with Gram-negative, actinobacteria and saprophytic fungi. Each enzyme activity correlated with specific microbial FAMEs, which varied depending on the tree species. Our results agree with [28] where they found that phosphatase enzymes correlated with higher numbers of fatty acids [28].

Microbial phosphatase activity is crucial for the supply of inorganic phosphate in this system. Soils at the Guánica Dry Forest are P limited due to the high amount of carbonates from the underlying calcareous substrate. In this study, phosphatases were the most active enzymes, microbial communities are allocating resources to balance the P deficiency of the Guánica soils. When P deficiency is present in a system, increased phosphatase activity occurs as a response to P starvation [29]. A mechanism that compensates soil P deficiency is the inoculation of the arbuscular mycorrhizae, which are known to enhance plant P availability via the production of phosphatase enzymes [30]. We identified similar concentrations of the arbuscular mycorrhizae marker 16:1 ω5c for *Tabebuia heterophylla* and *Ficus citrifolia*. Idiosyncratic effect was observed for each tree species; for example, *Ficus citrifolia* enhanced the activity of phosphodiesterase and *Tabebuia heterophylla* enhanced the
activity of both acid and alkaline phosphatase. Wu et al. [31] found that *Poncirus trifoliata* seedlings that were inoculated with arbuscular mycorrhizae exhibited higher total and acid phosphatase activity and higher plant P content when compared to uninoculated seedlings. It is important to mention that other than arbuscular mycorrhizae, bacteria also produce phosphatase enzymes. We propose that microbial phosphatase activity is crucial for the availability of P at the Guánica Dry Forest Reserve.

5. Conclusions

Idiosyncratic effects of tree species coupled with extreme changes in water input contribute in shaping the soil microbial community structure and enzymatic activity at the Guánica Dry Forest. In this system, saprophytic fungi, arbuscular mycorrhizae and anaerobic Gram-positive sulphur-reducing bacteria seem to be more sensitive to rainfall pulses when compared to Gram-positive (including actinobacteria), Gram-negative and protozoan communities. Even though monthly variations play a significant role in microbial community structure, soil enzyme activities did not vary during the months sampled. Our findings demonstrate that, although mesoclimate is a determinant driver of ecosystems, tree species is a stronger modulator of the soil microbial dynamics at the Guánica Dry Forest. To our understanding, this is the first study that provides insight into the soil microbial community of the Guánica Dry Forest, a valuable contribution that will help elucidate strategies for better management and protection of the soil biota of the area.

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Conflict of interest

No conflict of interest.
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