El Colegio de la Frontera Sur

Bacterias asociadas a árboles tropicales en zonas de recuperación de un disturbio antrópico

TESIS
presentada como requisito parcial para optar al grado de
Maestro en Ciencias en Recursos Naturales y Desarrollo Rural
Con orientación en ciencias de la sustentabilidad

Por

Angel Antonio Becerra Lucio

2019

Anexo 9

ECOSUR

PORTADILLA DE TESIS DE MAESTRÍA

El Colegio de la Frontera Sur

10 de junio de 2019.

Las personas abajo firmantes, miembros del jurado examinador de:

Angel Antonio Becerra Lucio

hacemos constar que hemos revisado y aprobado la tesis titulada

Bacterias asociadas a árboles tropicales en zonas de recuperación de un disturbio antrópico

para obtener el grado de **Maestro (a) en Ciencias en Recursos Naturales y Desarrollo Rural**

Nombre	Firma
Director Dr. Yuri Jorge Jesús Peña Ramírez	
Co-Directora Dra. Ana Tztzqui Chávez Bárcenas	
Asesora Dra. Salima Christine Machkour M´Rabet	
Sinodal adicional Dra. Susana Ochoa Gaona	
Sinodal adicional Dra. Ingrid Aileen O´Connor Sánchez	
Sinodal adicional M.C. Natalia Ysabel Labrín Sotomayor	
Sinodal suplente M.C. Víctor Manuel Ku Quej	

Esta investigación va dedicada…

A mis padres y abuelos
que son el pilar fundamental de mi desarrollo y formación como persona,
porque mis conocimientos, mi superación y todo lo que soy se los debo a ellos…

Agradecimientos…

Papá y mamá, gracias por que en cada etapa de mi vida han buscado la manera de darme lo mejor, desde una pequeña sonrisa, hasta ser mi ejemplo de constancia y dedicación, por su trabajo duro, sus enseñanzas y regaños, porque soy quien soy gracias a ustedes.

Ale y Ana, mis compañeras de aventura, les agradezco infinitamente por todo el tiempo brindado, por sus sonrisas, por sus consejos, por ser parte irremplazable en mi vida. Gracias hermanas porque sin ustedes esto no hubiera sido posible.

Abuelos, Angel (†) (sé que estas feliz de ver en lo que nos hemos convertido) y Lidia, gracias por ser los pilares que mantienen una gran familia unida, por estar presentes y apoyándome en cada paso que doy, hoy son parte fundamental de mi vida. De igual manera a mis abuelos Ana (†) y Javier (†) que me cuidan desde lejos. También quisiera agradecer a toda mi familia Becerra-Lucio (tíos y primos) por estar siempre al pendiente de las necesidades que pudieran surgirme.

Flor, mi mejor amiga y el amor de mi vida, no me alcanzan las palabras para expresar lo importante que eres, gracias por apoyarme en cada momento, por las palabras de aliento y por ser parte fundamental de en este trabajo.

Gracias a ustedes amigos, Monse, Jess, Gabo, Gera, Will, Esteban, Arantxa, Luis, Khia, Marina, Edwin, Ilse, Dani, por el apoyo, las risas, las fiestas y convertirse en mi nueva familia. En especial quisiera agradecer a Hugo por ser la primera persona en tenderme la mano cuando llegue a Campeche, además de convertiste en un gran amigo y parte de mi familia.

Gracias Yuri por el tiempo, paciencia, conocimientos, consejos, apoyo, la guía y sobre todo por formar un gran equipo de trabajo. Dra. Tztzqui gracias por seguir apoyándome en mi formación profesional (primero en la Universidad y ahora en la Maestría). Dra. Salima gracias por el apoyo brindado a lo largo de este trabajo. Gracias a mis sinodales por su aporte en el trabajo.

Gracias a todos los integrantes del Laboratorio de flora “Jerzy Rzedowski” (Natalia, Dra. Susana y el Dr. Ben), por su tiempo y apoyo, gracias a ECOSUR que se convirtió en mi segundo hogar.

ÍNDICE

Capítulo 1. Introducción

Las perturbaciones son un efecto permanente o transitorio de un sistema ecológico ante el disturbio, que puede ser por causas naturales o antrópicas (Rykiel, 1985). Los efectos de las perturbaciones antrópicas en el componente vegetal generan un proceso de degradación, ya que existe una pérdida de cobertura, cambio en la composición vegetal y la reducción de la diversidad biológica (Finegan, 1996; Chapin et al. 2000; Gil et al. 2001; Linares et al. 2011; Garcia-Licona et al. 2014).

El componente vegetal interactúa con diversas comunidades microbianas que también son afectadas por las perturbaciones antrópicas como la deforestación, incendios forestales y el cambio de uso de suelo (Matson et al. 1987; Müller et al. 2002; Hartmann et al. 2014). Estas interacciones se encuentran presentes en el suelo y raíces (rizosfera), hasta la parte aérea de las plantas (filosfera); incluso en los tejidos internos de éstas (endosfera) (Ulrich et al. 2008; Cassán et al. 2009; Fierer et al. 2010).

La importancia de las interacciones microbianas con el componente vegetal deriva de los papeles clave que desempeñan para el desarrollo vegetal, por ejemplo, son parte fundamental en los procesos biogeoquímicos, entre los que destacan: 1) el ciclo del carbono: teniendo un impacto directo en la productividad de la planta y descomposición de materia orgánica, 2) el ciclo del nitrógeno: adquisición de nitrógeno vegetal, fijación de nitrógeno y desnitrificación, y 3) el ciclo de fósforo: en la adquisición de fósforo vegetal y mineralización (Falkowski et al. 2008; Heijden et al. 2008; Madsen 2011). Los microorganismos también están implicados en el proceso de resistencia a patógenos (liberación de sustancias antimicrobianas y antifúngicas que favorecen el control biológico) y en el proceso de biorremediación (Kloepper et al. 1999; Matiru y Dakora, 2004; Ryan et al. 2008; Ulrich et al. 2008; Berg, 2009; Compant et al. 2010; Wang et al. 2016).

Los impactos que las perturbaciones antrópicas tienen en el componente microbiano se ha estudiado ampliamente utilizando técnicas tradicionales de cultivo de microorganismos. Con estas herramientas se ha demostrado que los

incendios forestales en bosques de pino reducen la biomasa microbiana hasta en un 50% en la zona superficial del suelo (5 a 10 cm) (Prieto-Fernández et al. 1998), y el cambio de uso de suelo provoca una reducción de la biomasa microbiana hasta 16 veces en comparación con un suelo no cultivado (Yao et al. 2000).

Otros autores utilizaron técnicas de biología molecular y cultivo tradicional de microorganismos para analizar el efecto de los incendios forestales y el cambio de uso de suelo en bosques templados. En suelos de pastoreo y bosques maduros afectados por los incendios, se observó que en cada zona las poblaciones microbianas son únicas, y que las especies bacterianas desconocidas se encuentran mayormente a zonas boscosas (Borneman y Triplett 1997); mientras que en bosques primarios, secundarios y zonas de cultivo, se observó que las prácticas de manejo afectan la estructura de la comunidad microbiana ya que las muestras de bosque tienen una mayor abundancia y diversidad de microorganismos (Bossio et al. 2005).

Sin embargo, utilizar el enfoque de cultivo tradicional de microorganismos presenta un sesgo para entender cómo los disturbios ecológicos afectan a las comunidades microbianas, ya que solo se puede cultivar *in vitro* el 1% de los microorganismos existentes. La biotecnología hace uso de técnicas modernas de secuenciación para el estudio de microorganismos directamente en su ambiente natural, tratando de eliminar el sesgo que se tenía por el enfoque de cultivo de microorganismos (Herrera-Estrella y Castellanos 2007; Hartmann et al. 2012).

La metagenómica (es la aplicación de técnicas genómicas modernas como la extracción masiva y secuenciación de ADN para estudiar las comunidades de microrganismos directamente en su ambiente natural, sin la necesidad de aislar y cultivar cada una de las especies que componen una comunidad) y el análisis bioinformático han permitido conocer la diversidad, abundancia relativa y la posible función de las especies microbianas cultivables y no cultivables en los ecosistemas (Herrera-Estrella y Castellanos, 2007; Hartmann et al. 2014; Llacsa-Sánchez, 2016).

Con la implementación de estas técnicas se ha podido analizar muestras de suelo de bosques y pastizales comprobando el impacto de la deforestación en la comunidad de bacterias fijadoras de nitrógeno (diazotróficas) (Mirza et al. 2014). En bosques templados se encontró diferencia en la abundancia de grupos bacterianos asociados a la rizosfera y suelo de zonas perturbadas y no perturbadas, al igual que en los valores de diversidad de Shannon (H'= 1.2 y H' = 2.5, respectivamente) (Cobo-Díaz et al. 2015).

Otro estudio revela que existen diferencias entre comunidades de bacterias asociadas a pastizales y bosques, encontrando que los suelos de los pastizales son significativamente más diversos que los de bosque (H '= 10.12 y H' = 9.48, respectivamente) (Kaiser et al. 2016). Utilizando este tipo de herramientas también han caracterizado grupos de bacterias que pueden utilizarse como indicadores de sanidad de entornos vegetales semi perturbados, encontrando que especies de la clase Gammaproteobacteria pueden ser indicadoras de sanidad por ser capaces de combatir enfermedades fúngicas (Köberl et al. 2017).

Los estudios realizados del componente microbiano en ambientes tropicales se han enfocado principalmente a la caracterización de la comunidad bacteria asociada a las plantas, reportando la presencia de grupos de bacterias muy similares a los encontrados en zonas templadas, entre los que destacan los fila Proteobacteria y Actinobacteria (Oh et al. 2012; Haruna et al. 2017; Ritter et al 2018).

Las técnicas de biotecnología (metagenómica y la bioinformática) representan una buena herramienta que puede ayudar a entender el comportamiento y función del microbioma, así como evaluar su capacidad de recuperación y observar efectos adversos en el funcionamiento del ecosistema (Berga et al. 2012; Blaalid et al. 2012; Hartmann et al. 2014; Copeland et al. 2015).

En México la mayor área protegida de bosques tropicales se encuentra en La Reserva de la Biosfera de Calakmul (RBC) ubicada al sureste del estado de Campeche y en el centro de la península de Yucatán (Martinez y Galindo-Leal 2002; Porter Bolland et al. 2006). La RBC representa una de las tres mayores

extensiones forestales de Mesoamérica; albergando aproximadamente 1,600 especies vegetales (Martinez y Galindo-Leal 2002).

La RBC está influenciada por factores antropogénicos como la deforestación, agricultura y ganadería (Porter Bolland et al. 2006; García-Licona et al. 2014), lo que genera un proceso de degradación del bosque tropical. Los estudios en la reserva se han enfocado principalmente en el componente vegetal, por ejemplo, Gil et al. (2001) y Garcia-Licona et al. (2014) reportaron que la agricultura y ganaderia son las actividades principales que genera cambios en la compocisión y funcion del ecosistema; por su parte Aryal et al. (2014, 2015) evaluaron los flujos de carbono y nutrientes en hojarasca y árboles durante una cronosecuencia de recuperación de la selvas, demostrando el efecto negativo que genera el cambio de uso de suelo en el ciclo del carbono en ecosistemas tropicales.

Comparado con los ambientes templados, el microbioma asociado en los ambientes forestales tropicales sigue siendo poco estudiado. El estudio de la ecología microbiana en estos ambientes puede poner en manifiesto la enorme diversidad microbiológica presente en las muestras ambientales y los roles funcionales que son fundamentales para el desarrollo de los ambientes forestales; esto representa un gran avance en nuestro entendimiento de cómo funciona la naturaleza y la interacción planta-microorganismo, siendo un punto importante en la gestión de los ecosistemas.

Objetivo

El objetivo del presente estudio fue identificar la composición de las comunidades bacterianas asociadas a las hojas, raíces y suelo en zonas con diferente grado de perturbación antrópica previamente caracterizadas en la RBC, utilizando técnicas de metagenómica y análisis bioinformático en microambientes forestales.

Objetivos particulares

- Identificar los componentes de las comunidades bacterianas utilizando técnicas de metagenómica con el gen 16S ADN.

- Determinar los valores de diversidad de las comunidades bacterianas utilizando los índices de Chao 1 y Shannon-Weaver para cada muestra de los diferentes ambientes.
- Establecer si existe una relación entre las comunidades bacterianas de acuerdo a cada uno de los ambientes muestreados.
- Describir el posible papel funcional de los individuos que forman la comunidad bacteriana asociada a los ambientes forestales tropicales.

Capítulo 2. Articulo

Angel A. Becerra-Lucio[1] , Max M. Apolinar-Hernández[2] , Patricia A. Becerra-Lucio[1] , Flor I. TrujilloElisea[1] , Salima Machkour-M'Rabet[3] , Ana T. Chávez-Bárcenas[4] , Aileen O'Connor-Sánchez[5 2] , and Yuri J. Peña-Ramírez[1∗ 3 4]

Title: Changes in the microbiome associated with Handroanthus chrysanthus (Bignoniaceae) at different recovery stages in the Maya tropical forest

Abstract

Anthropogenic disturbances give rise to a process of regeneration and recovery of the plant component of ecosystems. Throughout this process, plant species interact with various bacterial communities that play a key role in their development. Unlike in temperate environments where the bacterial component and its relationship with the plant component have been widely studied, in tropical areas, it remains poorly understood. In the present study, we studied the bacterial communities associated with soil, roots and leaves in one of the forest species of greatest ecological importance, representing different disturbance phases of a tropical forest in the Calakmul Biosphere Reserve, Mexico. Through massive 16S ribosomal sequencing of environmental samples, the bacterial component was identified in four disturbance phases of forest. The bacterial communities found maintained a similar composition in the different stages of recovery. It was found that the Proteobacteria, Cyanobacteria and Actinobacteria strains are the most abundant; although their diversity values differ in each stage of recovery, the bacterial communities in soil and roots are more diverse than those associated with the leaves. In addition, the greatest diversity found corresponds to samples from pristine jungle and traditional Mayan backyards. The results obtained in this study suggest that bacterial communities maintain a certain degree of resilience to anthropogenic disturbances linked to land-use change.

Introduction

The changes caused by anthropogenic disturbances, such as deforestation or land-use change, give rise to a process of recolonization and regeneration, generating a process of vegetation recovery [1,2]. In this process, plant species interact with various microbial communities, which are present from the soil and roots (rhizosphere) to the aerial part of the plant [3-5]. These communities participate in a positive, neutral or negative way in a large number of processes, such as in the acquisition of nutrients and biogeochemical processes, among which the carbon cycle, the nitrogen cycle and the phosphorous cycle stand out, which has a direct consequence on the development of plants [6-8].

Microbial communities are affected by different anthropogenic activities that alter plant cover and soil (e.g., agriculture, livestock and deforestation), altering their function, biomass and diversity [9-11]. The impact of these activities has been evaluated mainly using traditional tools to cultivate microorganisms, demonstrating that forest fires and changes in land use can diminish the biomass, abundance, and diversity of communities

[12-14]. However, there are strong limitations when using microorganism cultures, as there is a 35 bias towards cultivable microorganisms (1%) [15,16].

In the last decade, the use of new sequencing tools has allowed us to know the diversity, relative abundance, and infer the role of the nonculturable microbial species, extracting their DNA directly from environmental samples [11]. The implementation of these techniques has shown, for example, that deforestation in soils of temperate forests has an impact on nitrogen-fixing bacterial communities [17]; in the rhizosphere of oak forests, a decrease in the abundance and diversity of bacterial communities due to land-use changes has been observed [18], and groups of bacteria have even been identified as indicators of health in partially disturbed plant communities [19].

The Calakmul Biosphere Reserve (CBR) is the largest protected area of tropical forests in Mexico and Central America. It is located southeast of the state of Campeche in the center of the Yucatan Peninsula [20,21]. The CBR is severely affected by different anthropogenic activities, such as deforestation, agriculture and livestock [22]. To date, studies of the effects of anthropogenic disturbances have focused mainly on the impact on the plant component. Previous studies [22,23] reported that agriculture and livestock are the main activities that transform the ecosystem in the CBR, generating changes in the composition and function of the plant component and altering carbon and nutrient flows in a chronosequence of tropical forest recovery, demonstrating the negative effect of land-use change on the carbon cycle and landscape transformation [24, 25].

In these studies, the investigation of the microbial component is pending, without being able to determine to date the impact of these communities on vegetation cover and soil functionality. This is why the objective of the present study was to identify bacterial communities and describe their possible functional role by considering three tropical forest environments the soil, roots, and leaves in zones with distinct phases of antropic disturbance in the CBR in the sites previously analyzed [24, 25]. The contribution to knowledge of this work lies in the analysis of the behavior of the microbiome associated with a model forest species in the face of profound changes in vegetation cover and to compare its composition in light of the recovery of the plant component. This could aid holistic management of tropical ecosystems in the future.

Materials and methods

The present study was conducted in an area where the plant component, nutrient flow and behavior of the carbon cycle before the change in land use were previously characterized [24, 25]. This zone is located in the municipality of Calakmul, Campeche, Mexico, in the communal lands (ejidos) of El Carmen II (18° 09′ 36” N and 89° 24′ 48” W) and Cristóbal Colón (18° 13′ 18” N and 89° 27′ 13” W), where it is possible to find forest 64 environments in various stages of recovery.

Sampling zones

Sampling zones were selected for their degree of disturbance and recovery time from an anthropogenic disturbance associated with changes in land use (agriculture), as described below: 1) traditional Mayan backyard – permanent

disturbance, it is inhabited and constantly modified by the families that inhabit it; its vegetation cover has remnants of isolated arboreal vegetation mainly used as shading or ornamental decoration; 2) pasture – it has a low disturbance, since it has been abandoned for 10 years , during which the native vegetation begins to repopulate the property as a result of which agriculture was abandoned, and the land is destined for animal grazing; this land has remnants of arboreal vegetation dominated by species of shrubs and grasses;; 3) secondary forest – This environment has a recovery time of approximately 25 years during which approximately 47 species of trees and shrubs are established with a height of less than 7 m and a mean diameter of 6 cm; this land is not used for agricultural or silvicultural purposes; and 4) primary forest – this environment has an estimated recovery time of 120 years. Area covered with mature jungle was used as the reference zone, and we found a maximum of 43 species of trees whose individuals had a mean height of 12 m and a mean diameter of 30 cm.

Sampling

The environments to be sampled within the selected areas were chosen based on the presence of the species *Handroanthus chrysanthus*, a perennial forest species reported in the four different sampling areas selected for this study. At each sampling site, three typical, healthy and representative trees were selected, all with a height greater than 3 m, with each individual separated by approximately 100 m. At each site, one sample per tree was taken, collecting 1 kg of the first 30 cm of soil near the roots, 20 g of young roots and 100 g of mature leaves. All samples were transported at 4 °C and stored at -70 °C until processing.

Soil analysis

Soil samples were sent to the Laboratory of Soil, Water and Plant Analysis (Laboratorio de Análisis de Suelos, Agua y Planta, LASPA) of the College of Graduate Studies, Tabasco campus (Cárdenas, Tabasco, Mexico), for physical and chemical analysis (pH, electrical conductivity, organic matter content, nitrogen, phosphorus and potassium content, and soil texture) for the standardized procedures according to the Official Mexican Standard NOM-021-RECNAT-2000 [26], which establishes the specifications of soil fertility, salinity and classification.

DNA extraction and sequencing

Each of the samples of soil, roots (young roots plus rhizospheric soil) and leaves were pulverized with a previously sterilized mortar and pestle until obtaining a homogeneous mixture, from which the extraction of genomic DNA was performed with 0.25 g of each of the samples collected using the commercial PowerSoil® DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) following the methodology proposed by the manufacturer. The quantification and purity of the extracted DNA was determined by spectrophotometry with a Multiskan GO model FI-01620 (Vantaa, Finland) with a µDrop plate and SkanIt software version 4.1 (Thermo Scientific, Waltham, MA. USA). After determining the concentration and purity of each extracted genomic DNA, 12 composite samples were made (one for each evaluated condition: 1) backyard soil, 2) pasture soil, 3) secondary forest soil, 4) primary forest soil, 5) backyard roots, 6) pasture roots, 7) secondary forest roots, 8) primary forest roots, 9) backyard leaves, 10) pasture leaves, 11) secondary forest leaves, and 12) primary forest leaves), each having a volume of 20 µL and a concentration of 20 ng μL^{-1} . Each individual sample contributed 133.33 ng of DNA to the composite sample. The

Samples were sequenced using the MiSeq Illumina 2000 platform in RTLGenomics (www.rtlgenomic.com, Lubbock, TX, USA). The oligonucleotides used for sequencing were Fw 5'-CAGAGTTTGATCCTGGCTCAG-3' (forward) and Rv 5'- 107 AAGGAGGTGATCCAGCC-3' (reverse) [27].

Sample analysis

The products derived from Illumina sequencing were processed in the automated analysis platform Quantitative Insights Into Microbial Ecology (QIIME) [28] (http://qiime.org), the noise reduction and detection and chimera detection were performed with the program UCHIME [29] and were eliminated with an error rate lower than 112 0.1% in de novo mode. The sequences were grouped into operational taxonomic units (OTU) with 80% genetic identity at the phylum level, 85% at the class level, 87% at the order level, 90% at the family level, 93% at the genus level and 97% at the species level using the UPARSE algorithm [30]. All sequences were aligned with the global algorithm UREARCH [31] and compared with the National Center for Biotechnology Information (NCBI) database. Rarefaction curves, diversity and richness indices (Chao 1, Shannon-Weaver H' and Evenness), and Jaccard similarity indices were obtained using RDP tools (RDP-Release 10), sequence analysis tools of the bioinformatics software USEARCH (https://drive5.com/usearc) and the statistical package IBM SPSS Statistics (version 22.0).

Results

Soil analysis

Soils in the four environments sampled (backyard, pasture, secondary forest and primary forest) were classified according to texture as sandy clay soils, with a slightly basic pH (7.23 to 7.43). The organic matter of the samples presented a percentage greater than 15% in all cases, while the levels of nutrients (nitrogen and phosphorous) showed slight variations between each sampling site (Table 1).

Place Table 1 here

Composition and relative abundance of bacterial communities

In total, 202,190 sequences of good quality were obtained with a mean reading length of 280 nucleotides. Taxonomic identification was assigned to 17 phyla, 51 classes, 118 orders, 218 families, 355 genera and 447 species. For the different phases of disturbance in the rainforest, the most abundant phyla were Proteobacteria, Cyanobacteria, Actinobacteria, Acidobacteria, Firmicutes and Bacteroidetes (Figure 1). The most abundant classes belonged to the phyla Proteobacteria and Actinobacteria (α-Proteobacteria, β-Proteobacteria, γ Proteobacteria, δ-Proteobacteria and Actinobacteria). The most abundant known family was Rhizobiaceae, which belongs to the class α-Proteobacteria (Figures S1 and S2). In all the samples that were analyzed, unknown or unclassified bacterial groups were found, increasing their relative abundance according to the level of taxonomic depth. At the phyla level, the organisms classified in this way had a relative abundance in soil of 3% to 20%, in roots from 6% to 12% and in leaves less than 1%. At the class level, the relative abundance of this group varied from 20% to 90%; at the family level, the

abundance ranged from 45% to 95% (Figures S1 and S2); and at the species level, the abundance ranged from 45% to 99% (Figure S4).

Place figure 1 here

In soil samples, the phyla Proteobacteria, Actinobacteria, and Acidobacteria were the most abundant, representing more than 50% of the bacterial community. There was a difference in the relative abundance of the phyla detected in each of the sampled environments. For example, the phylum Proteobacteria was the most abundant in the three zones with highest degree of disturbance (33%, 45.9% and 39.7%), while in primary forests (zone with the longest recovery time), the phylum Actinobacteria was the most abundant (41.1%); phyla with relative abundances less than 1% were Deinococcus-thermus, Nitrospirae, and Planctomycetes (grouped as others) (Figure 1). The most abundant classes in the soil of all environments were α-Proteobacteria (20.7% to 35.4%) and Actinobacteria (3.8% to 29.7%). The most abundant families were Corynebacteriaceae (3.3%) in backyards, Hyphomicrobiaceae (4.3%) in pasture, Bradyrhizobiaceae (2.5%) in secondary forest and Rhizobiaceae (12.6%) in primary forest. The Rhizobiaceae family was the only family found in the four environments analyzed, with a relative abundance greater than 1% (Figures S1 and S2). In backyards and primary forest, Agrobacterium tumefaciens (1.6% and 12.6%, respectively) was the most abundant species, while in pasture and secondary forest, Bradyrhizobium sp (2.6% and 1.6%, respectively) was the most abundant (Figure S4).

Unlike the bacterial community of the soil, in the root samples, the phyla Proteobacteria, Cyanobacteria and Actinobacteria were the most abundant, representing more than 50% of the community. In the backyard, secondary forest and primary forest samples, the phylum Proteobacteria was the most abundant (44.5%, 37.2% and 55.4%, respectively), unlike pasture, where the most abundant phylum was Cyanobacteria (41.3%). On the other hand, the phyla with the lowest abundance were Deinococcus-thermus, Fibrobacteres, Gemmatimonadetes, Planctomycete, Tenericutes and Thermotogae (grouped as others), with relative abundances lower than 1% (Figure 1). Regarding relative abundance at the class level, α-Proteobacteria (12% to 40.2%) and Actinobacteria (4% to 12.8%) were the most abundant in all cases. The most abundant families were Sphingomonadaceae (6%) in backyards, Streptomycetaceae (10.5%) in pasture, and Bradyrhizobiaceae in secondary forest and primary forest (5.2% and 6.3%, respectively) (Figures S1 and S2). Lastly, at the species level, Steroidobacter sp (2.6%) was the most abundant in pasture, while in backyards, secondary forest and primary forest, Bradyrhizobium sp (3.2%, 4.27% and 4.80%, respectively) was the most abundant (Figure S4).

In the leaf samples, the phylum Cyanobacteria was the most abundant in the four recovery times sampled, representing more than 80% of the bacterial community (Figure 1); however, in the areas with the longest recovery time (secondary forest and primary forest), there was a substantial increase in the abundance of the phyla Proteobacteria (4.8% and 12.3%) and Acidobacteria (1% in both cases). These differences occurred at different taxonomic levels, including at the species level, where Beijerinckia sp was the most abundant in backyards and

194 secondary and primary forests (0.1%, 7.2% and 1.2%, respectively), while Sphingomonas sp. was the most abundant
195 in pasture (0.3%) (Figure S4).

196 Diversity and richness of the bacterial community

197 The Shannon diversity index (H') showed that the soil and root bacterial communities were more diverse than were
198 the leaf communities (Table 2). The bacterial community found in backyard, pasture and secondary forest soil
199 samples (phylum H' = 4.01, 3.8 and 3.6; species H' = 7.2, 6.6 and 7.1, respectively) were more diverse than were the
200 bacterial communities found in primary forest samples (phylum H' = 3.2, species H' = 4.7). In the root samples, the
201 areas with an intermediate recovery time (pasture and secondary forest, with phylum H' = 2.4 and 3.3, species H' =
202 4.5 and 6.2, respectively) had the lowest values of diversity. Areas with longer recovery times (secondary forest and
203 primary forest, with phylum H' = 0.27 and 0.17, species H' = 0.26 and 0.16, respectively) presented the highest
204 values of diversity.

205

206 *Place Table 2 here*

207

208 The Chao 1 richness estimator and the Evenness index (Table 2), as well as the rarefaction curves (Figure 2), showed
209 that the bacterial communities of the soil and root samples had greater richness and presented greater homogeneity
210 than did the bacterial community found in the leaf samples. The backyard soil samples and primary forest roots had
211 the highest number of OTUs observed at the phylum level (164 and 220, respectively), while at the species level,
212 secondary forest soil samples and primary forest roots had the highest number of OTUs observed (3.778 and 5.873,
213 respectively). The samples with the lowest number of OTUs observed were those of leaves from backyards and
214 pasture (phylum = 6 and 2; species = 92 and 34, respectively) (Table 2).

215

216 *Place figure 2 here*

217

218 In the rarefaction curves at the phylum level (20% dissimilarity), an asymptote can be observed for all samples,
219 which shows that most of the bacterial community was captured using the experimental strategy employed (Figure
220 2). At the species level (3% dissimilarity), no asymptote was observed for any of the curves (Figure S5). This
221 indicates that the sampling effort was not sufficient to detect part of the bacterial community at this taxonomic level.
222 However, if we compare the number of OTUs with the values estimated by Chao 1 (Table 2), it is possible to
223 include that more than 50% of the richness of the bacterial communities were sampled at the species level.

224 To determine the similarity between each of the samples, the Jaccard index was used. Figure 3 shows that, derived
225 from the analysis of samples at the genus level, three groups were formed in which some groups shared a similarity
226 in their relative abundances. In addition, the soil and root samples were grouped, leaving the group containing the
227 leaf samples separate, with the latter being the most similar to each other. Importantly, the genera Agrobacterium sp.,

Taricella sp., and Propionibacterium sp. and to a lesser degree Beijerinckia sp. and Halospirulina sp. were the most dissimilar taxonomic groups in the samples. These groups, together with the group comprising the less representative taxa (others), provided the greatest heterogeneity to the samples.

Place figure 3 here

Discussion

Soil analysis

The soils that prevail in the region of study are classified as Rendzic Leptosols mixed with Vertisols [25]. The physical and chemical properties of the soil are determining factors for the composition of the microbial community, and pH is considered the most influential factor for the interacting bacterial communities in the soil; although many bacteria develop better at a more neutral pH, some bacterial phyla such as Proteobacteria, Actinobacteria, Acidobacteria and Bacteroidetes can be highly associated with a high pH [32- 36]. However, the pH values in the sampling areas were very similar (Table 1); therefore, the composition of the bacterial community interacted under similar acidity conditions. After pH, texture and soil type are factors that have strong influences on the bacterial community [32, 37, 38]. Based on the samples analyzed, the values found did not a priori represent the expected variability. The values obtained in the different soil physicochemical parameters were indicative of the typicity of the samples, which supported their use for the metagenomic analysis of the bacterial communities.

Composition and relative abundance of bacterial communities

The bacterial groups found in the different samples are consistent with other analyses of this type performed in the same area. Specifically, the phyla detected in soil samples are also reported in another region of the Yucatan Peninsula [39]. In addition, the results obtained in this study at the phylum level coincide with taxa reported in other samples from tropical forest [40], temperate rainforest and grasslands [15, 18, 41-43], with the last two altered by changes in land use. In root samples where rhizosphere soil was included, a phyla bacterial composition similar to that described in rhizospheric soils of temperate forests and agricultural areas with different management intensities was observed [15, 18, 43, 44], and in root samples from tropical forests [45, 46], the phyla Proteobacteria and Actinobacteria were the most abundant. In our samples, however, the relative abundances were different from those previously found [39], which were dominated by the phylum Acidobacteria, which could be due to a lower level of anthropogenic influence or seasonal differences.

Although there were differences in the relative abundances among the bacterial communities (Figure 1), their taxonomic composition was very similar between each group of samples (soil, root, and leaves), resulting in greater similarity between them than with samples of other stages of forest recovery. The similarity in the composition of bacterial communities has been reported in other samples from tropical forests, where it was found that the taxonomic composition of the bacterial community was similar between each of the locations belonging to the same

habitat [40]. Unknown or unclassified bacterial groups that exist at significant levels in these samples can have great relevance, and their observed relative abundance was higher than that reported in other environments, such as temperate forests, tropical forests, cultivated areas and pastures [15, 17, 40, 47, 48].

Possible function of bacterial communities

The phyla Proteobacteria and Actinobacteria were the most abundant in the soil and root samples, which is consistent with previous reports carried out in different soil types, ecosystems and latitudes [15, 18, 40, 43, 47-51], suggesting that in our samples, these bacterial groups dominate, which has proven to be of great ecological importance. The phylum Proteobacteria, which is one of the best represented in this study, groups bacteria that perform different functions in ecosystems, highlighting bacterial species that participate directly in the nitrogen cycle as fixers [39, 52], nitrifying agents [15, 53, 54] and denitrifying agents [15, 55]. Its participation in mineral mineralization has also been described, highlighting the replacement and mineralization of phosphorus (P) [15, 258 56-61] and the mobilization of potassium (K) [60]. Within this bacterial group, we can also find ligninolytic and cellulolytic species linked to the decomposition of organic material [61, 62], methanotrophic bacteria [63], and some other species capable of establishing interactions with some fungi for the benefit of plants [64-67] or with the ability to produce antimicrobial and antifungal compounds [68-70]. The presence of bacteria of this phylum in our samples, particularly in soil and root, is consistent with its possible role in biogeochemical cycles, highlighting its higher abundance in root samples in samples from primary forest, where significant replacement of organic matter has been reported [24, 29].

Similar to the phylum Proteobacteria, different functions have been reported for bacterial species of the phylum Actinobacteria, which was the second most abundant group in the soil and root samples and in two of the leaf samples. This taxonomic group encompasses bacterial genera that have been functionally characterized as 268 pathogen antagonists and inducers of defense mechanisms [51], as possible sources of secondary metabolites [39], which participate in different bioremediation processes [71] and the solubilization of phosphorus [58, 59]. In addition, this phylogeny is mainly associated with the degradation of polysaccharides in plant biomass and phenolic compounds [61, 72, 73]. In this case, the greatest abundance of this group occurred in the primary forest soil samples, which could suggest that these bacterial groups are linked to degradation processes of plant matter below the mulch layer [24, 25].

Other phyla detected in this work, mainly in the leaf samples, are Cyanobacteria, which groups bacteria mainly associated with nitrogen fixation processes, Acidobacteria, a group comprising species of plant biomass degrading bacteria [45, 72], denitrifying agents [55, 74], and phosphorus solubilizing agents [58, 59], Firmicutes, which contains ligninolytic bacteria [51], denitrifying agents [55], and bacteria capable of mobilizing potassium [60] and inhibiting the growth of plant pathogens [75], and Bacteroidetes, which are potent decomposers of cellulose and other biopolymers [62, 72], in addition to their role as potassium mobilizers [60]. The relative abundances of these groups did not seem to follow a defined pattern at this level of taxonomic depth, which could suggest that their role should be established by functional analysis with those cultivable organisms belonging to these phyla.

Diversity and richness of the bacterial community

As shown in Figure 3 and Table 2, there is a marked difference in the composition, diversity and richness between bacterial communities of soil and root samples and of leaf samples, which may be because the environmental conditions and availability of nutrients are less suitable in the leaves. This finding is consistent with previous studies that suggest that leaves can produce microbiomes subject to higher selective pressure, without many microniches, and therefore are less diverse [76-78]. In soil samples, the three areas with the highest disturbance of the rainforest had the highest values of diversity and number of OTUs (Table 1) (e.g., the bacterial community of pasture had a diversity at the species level of H' = 6.6 and was more diverse than the community from primary forest, H' = 4.77). This can also be observed in temperate forests, for which bacterial communities associated with grasslands (disturbed area) (H'= 10.7) were more diverse than were those associated with undisturbed forests (H' = 9.6) [42]. Another study demonstrated that foraging areas with intensive agricultural management (H'= 4.92) were slightly more diverse than temperate forests (H'= 4.37) [48]. In our root samples, the primary forest presented the highest values of diversity and number of OTUs. In pristine temperate forests, similar trends were observed [18]. In this environment, at the phylum level, forests in conserved areas had higher values of diversity and a higher number of OTUs (H'= 2.5 and 17 OTUs) than did forests recovering from forest fires (H'= 2.2 and 13 OTUs).

The similarities in composition that can be observed between each of the groups of samples (Figure 3) could be explained by two main factors. The first is the perennial forest species used for sampling microhabitats. Several studies have corroborated that plant species can directly or indirectly determine the structure of the bacterial community [48, 79], selecting particular populations for their benefit [47, 80, 81] so that the bacterial community can be distinctive for each type of plant species independent of the place where it is found [79]. The second factor that could explain the similarity of the microbial composition found could be associated with the physicochemical characteristics of the soil, which are known to strongly influence the establishment and development of other microorganisms (fungi, lichens, protists or viruses) [15, 34 -38, 48, 77, 82], which were not considered in this study.

The presence of some bacterial groups that do not vary according to different disturbance phase indicate bacterial resistance to disturbances, which can contribute to minimizing changes in the functioning of the ecosystem [83]; this has been demonstrated with some microbial functional groups such as decomposers and denitrifying and nitrifying agents that are constant and relatively insensitive to changes in vegetation cover [84, 86]. Bacterial resistance may have occurred in this case because there was a significant abundance of some phyla performing indispensable maintenance functions for the ecosystem or that were continuously reinoculated and able to establish themselves in environments with different degrees of recovery. Our results suggest that the communities of Proteobacteria and Actinobacteria associated with tropical forest environments in the Mayan tropical forest of Calakmul could have this capacity of resilience in the face of disturbances and that events such as changes in land use or any other disturbance generating a change in the diversity of the plant component is not synchronous, or at least not to the same extent, with changes in bacterial communities, as has been previously described [86]. In this regard, it would be necessary to analyze bacterial species at a deeper taxonomic level or in less abundant groups that would be sensitive to such changes and could serve as indicator species. Another strategy that could be used as a possible response indicator of change in vegetation cover in microbial communities could be to evaluate the change in the expression of certain genes, which would require a metatranscriptomic analysis of these communities.

Lastly, the analysis of the data from the leaf samples shows that the relative abundances found differ from previous reports in temperate zones, where very little or no phylogeny is reported for Cyanobacteria [47]. On the other hand, in tropical forests, sequences of Cyanobacteria linked to nitrogen fixation processes were reported [88]. However, the high abundance of Cyanobacteria detected in this study could also be due to the amplification of compatible regions of the chloroplast of H. chrysanthus because it has a close phylogenetic relationship with Cyanobacteria [47]. Although the sequences assigned to Cyanobacteria show no identity with the ribosomal region of H. chrysanthus, we cannot rule out this possibility. To eliminate the possible bias by this type of sequence, other primers can be used, such as 799F, which was designed to avoid the amplification of plastid sequences; however, several reports show that its effectiveness is limited [89], so a better alternative could be the scrubbing of sequences not required by software [90].

Conclusions

To the best of our knowledge, this is the first report of the bacterial composition associated with soil, roots and leaves of an area with different disturbance phase of the Mayan rainforest in the CBR. This work presents a first view of the bacterial community in this region and its possible behavior in different stages of recovery from anthropogenic disturbance. From these data, we can conclude that the community of bacteria associated with the leaf, root, and soil of H. chrysanthus shows a good degree of resilience to changes in vegetation cover caused mainly by changes in land use. In addition, we have found that the bacteria belonging to the phyla Proteobacteria and Actinobacteria are the most abundant and that, according to their function characterized in other studies, they could be responsible for carrying out a wide range of metabolic activities of great importance in recovering tropical forests. These results are the basis for future studies in which other factors can be analyzed that can determine if the pattern found is characteristic of the model forest species or if this depends on climatic variations at different times of the year. Other studies could consider expanding the study area, the interaction with different types of vegetation and other microhabitats, in addition to evaluating in greater depth the function of known and unknown bacterial groups that are an enormous yet unexplored reservoir of biodiversity that can have a fundamental role in the recovery in tropical areas and a high biotechnological potential.

Acknowledgements

This work was funded by El Colegio de la Frontera Sur (Project #5103711808) and by Consejo Estatal de Investigación Científica y Desarrollo Tecnológico del Estado de Campeche (COESICYDET). AABL thanks to Consejo Nacional de Ciencia y Tecnología (CONACYT) for its Master's scholarship number 624546.

References

1. Finegan B (1996) Pattern and process in neotropical secondary rain forests: the first 100 years of successio. LC-GC North Am. 11:119–124.
2. Walker J, Thompson CH, Reddell P, Rapport DJ (2001) The importance of landscape age ininfluencing landscape health. Eco. Heal. 7:7–14.
3. Ulrich K, Ulrich A, Ewald D (2008) Diversity of endophytic bacterial communities in poplar grown under field conditions. FEMS Microbiol Ecol. 63(2):169–180. doi:10.1111/j.1574-6941.2007.00419.x.
4. Cassán F, Perrig D, Sgroy V, Masciarelli O, Penna C, Luna V (2009) Azospirillum brasilense Az39 and Bradyrhizobium japonicum E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (Zea mays L.) and soybean (Glycine max L.). Eur J Soil Biol. 45(1):28–35. doi:10.1016/j.ejsobi.2008.08.005.
5. Fierer N, Nemergut D, Knight R, Craine JM (2010) Changes through time: Integrating microorganisms into the study of succession. Res Microbiol. 161(8):1–8. doi:10.1016/j.resmic.2010.06.002.
6. Falkowski PG, Fenchel T, Delong EF (2008) The Microbial Engines That Drive Earth's biogeochemical cycles. Science. 320(5879):1034-1039. doi:10.1126/science.1153213
7. Van Der Heijden MGA, Bardgett RD, Van Straalen NM (2008) The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett. 11:296–310. doi:10.1111/j.1461-0248.2007.01139.x.
8. Madsen EL (2011) Microorganisms and their roles in fundamental biogeochemical cycles. Curr Opin Biotechnol. 22:456–464. doi:10.1016/j.copbio.2011.01.008.
9. Matson PA, Vitousek PM, Ewel JJ, Mazzarino MJ, Robertson GP (1987) Nitrogen Transformations Following Tropical Forest Felling and Burning on a Volcanic Soil. Ecol. 68(3):491–502. doi: 10.2307/1938454
10. Müller AK, Westergaard K, Christensen S, Sorensen SJ (2002) The Diversity and Function of Soil Microbial Communities Exposed to Different Disturbances. Microb Ecol. 44:49–58. doi: 10.1007/s00248-001-0042-8
11. Hartmann M, Niklaus PA, Zimmermann S, Schmutz S, Kremer J, Abarenkov K, Lüscher P, Widmer F, Frey B (2014) Resistance and resilience of the forest soil microbiome to logging-associated compaction. 385 ISME J. 8:226–244. doi:10.1038/ismej.2013.141.
12. Prieto-Fernández A, Acea MJ, Carballas T (1998) Soil microbial and extractable C and N after wildfire. Biol Fertil Soils. 27:132–142. doi:10.1007/s003740050411.
13. Yao H, He Z, Wilson M, Campbell CD (2000) Microbial Biomass and Community Structure in a Sequence of Soils with Increasing Fertility and Changing Land Use. Microb Ecol. 40:223–237.doi:10.1007/s002480000053.
14. Bossio DA, Girvan MS, Verchot L, Bullimore J, Borelli T, Albrecht A, Scow KM, Ball AS, Pretty JN, Osborn AM (2005) Soil microbial community response to land use change in an agricultural landscape of western Kenya. Microb Ecol. 49:50–62. doi:10.1007/s00248-003-0209-6.

15. Uroz S, Buée M, Murat C, Frey-Klett P, Martin F (2010) Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. Environ Microbiol Rep. 2:281–288. 396 doi:10.1111/j.1758-2229.2009.00117.x.
16. Hartmann M, Howes CG, Vaninsberghe D, Yu H, Bachar D, Christen R, Henrik Nilsson R, Hallam SJ, Mohn WW (2012) Significant and persistent impact of timber harvesting on soil microbial communities in Northern coniferous forests. ISME J. 6:2199–2218. doi:10.1038/ismej.2012.84.
17. Mirza BS, Potisap C, Nüsslein K, Bohannan BJM, Rodriguesa JLM (2014) Response of free-living nitrogen-fixing microorganisms to land use change in the amazon rainforest. Appl Environ Microbiol. 80(1):281–288. doi:10.1128/AEM.02362-13.
18. Cobo-Díaz JF, Fernández-González AJ, Villadas PJ, Robles AB, Toro N, Fernández-López M (2015) Metagenomic Assessment of the Potential Microbial Nitrogen Pathways in the Rhizosphere of a Mediterranean Forest After a Wildfire. Microb Ecol. 69:895–904. doi:10.1007/s00248-015-0586-7.
19. Köberl M, Dita M, Martinuz A, Staver C, Berg G (2017) Members of Gammaproteobacteria as indicator species of healthy banana plants on Fusarium wilt-infested fields in Central America. Sci Rep. 7:45318. 408 doi:10.1038/srep45318.
20. Martínez E, Galindo Leal C (2002) La vegetación de Calakmul, Campeche, México: clasificación, descripción y distribución. Boletín la Soc Botánica México. 71:7–32.
21. Porter Bolland L, Drew AP, Vergara-Tenorio C (2006) Analysis of a natural resources management system in the Calakmul Biosphere Reserve. Landsc Urban Plan. 74:223–241. doi:10.1016/j.landurbplan.2004.09.005.
22. Garcia-Licona J, Esparza-Olguin L, Martinez-Romero E (2014) Estructura Y Composición De La Vegetación Leñosa De Selvas En Diferentes Estadios Sucesionales en el Ejido El Carmen II, Calakmul, México. Polibotánica. 38:1–26.
23. Gil GG, Mifsut IM, Santiago MAC (2001) Vegetation change resulting from the change in soil use in the Calakmul Biosphere Reserve, Campeche. Investig Geogr Bol del Inst Geogr UNAM. 46:45–57.
24. Aryal DR, De Jong BHJ, Ochoa-Gaona S, Esparza-Olguin L, Mendoza-Vega J (2014) Carbon stocks and changes in tropical secondary forests of southern Mexico. Agric Ecosyst Environ. 195:220–230. doi:10.1016/j.agee.2014.06.005.
25. Aryal DR, De Jong BHJ, Ochoa-Gaona S, Mendoza-Vega J, Esparza-Olguin L (2015) Successional and seasonal variation in litterfall and associated nutrient transfer in semi-evergreen tropical forests of SE Mexico. Nutr Cycl Agroecosystems. 103:45–60. doi:10.1007/s10705-015-9719-0.
26. Norma Oficial Mexicana NOM-021-RECNAT-2000. Especificaciones de fertilidad, salinidad y clasificación de suelos. Estudios, muestreo y análisis. D. O. de la Fed. México Dec 31 2002. Available at: http://legismex.mty.itesm.mx/normas/rn/rn021-02.pdf
27. Marquez-Santacruz HA, Hernandez-Leon R, Orozco-Mosqueda MC, Velazquez-Sepulveda I, Santoyo G. 2010. Diversity of bacterial endophytes in roots of Mexican husk tomato plants (Physalis ixocarpa) and their detection in the rhizosphere. Genet Mol Res. 9(4):2372–2380. doi:10.4238/vol9-4gmr921.

441 28. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG,
442 Goodrich K, Gordon JI, et al (2010) QIIME allows analysis of high-throughput community sequencing
443 data. Nat Methods. 7:335–336. doi:10.1038/nmeth.f.303.QIIME.
444 29. Quail MA, Smith M, Coupland P, Otto TD, Harris SR, Connor TR, Bertoni A, Swerdlow HP, Gu Y
445 (2012) A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific
446 Biosciences and Illumina MiSeq sequencers. BMC Genomics. 13:1–13. doi:10.1186/1471-2164-13-341.
447 30. Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 26(19):
448 439 2460–2461. doi:10.1093/bioinformatics/btq461
449 31. Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat 441
450 Methods. 10:996–998. doi:10.1038/nmeth.2604
451 32. Nacke H, Thürmer A, Wollherr A, Will C, Hodac L, Herold N, Schöning I, Schrumpf M, Daniel R
452 (2011) Pyrosequencing-based assessment of bacterial community structure along different management
453 types in German forest and grassland soils. PLoS One. 6:1–12. doi:10.1371/journal.pone.0017000.
454 33. Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of soil pH as a
455 predictor of soil bacterial community structure at the continental scale. Appl Environ Microbiol.
456 75:5111–5120. doi:10.1128/AEM.00335-09.
457 34. Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Fierer N (2010) Soil bacterial
458 and fungal communities across a pH gradient in an arable soil. The ISME journal. 4:1340.
459 doi:10.1038/ismej.2010.58
460 35. Jeanbille M, Buee M, Bach C, Cebron A, Frey-Klett P, Turpault, MP, Uroz S (2016) Soil parameters
461 drive the structure, diversity and metabolic potentials of the bacterial communities across temperate
462 beech forest soil sequences. Microb Ecol. 71:482-493. doi:10.1007/s00248-015-0669-5
463 36. Siles JA, Margesin R (2017) Seasonal soil microbial responses are limited to changes in functionality at
464 two Alpine forest sites differing in altitude and vegetation. Sci Rep. 7:1–16. doi:10.1038/s41598- 017-
465 02363-2.
466 37. Sessitsch A, Weilharter A, Gerzabek MH, Kirchmann H, Kandeler E (2001) Microbial Population
467 Structures in Soil Particle Size Fractions of a Long-Term Fertilizer Field Experiment. Appl Environ
468 Microbiol. 67:4215–4224. doi:10.1128/AEM.67.9.4215.
469 38. Girvan MS, Bullimore J, Pretty JN, Osborn AM, Ball AS (2003) Soil Type Is the Primary Determinant
470 of the Composition of the Total and Active Bacterial Communities in Arable Soils. Appl Environ
471 Microbiol. 69:1800–1809. doi:10.1128/AEM.69.3.1800.
472 39. Estrada-Medina H, Canto-Canché BB, De los Santos-Briones C, O'Connor-Sánchez A (2016) Yucatán
473 in black and red: Linking edaphic analysis and pyrosequencing-based assessment of bacterial and fungal
474 community structures in the two main kinds of soil of Yucatán State. Microbiol Res. 188– 466 189:23–
475 33. doi:10.1016/j.micres.2016.04.007.
476 40. Ritter CD, Zizka A, Barnes C, Nilsson HR, Roger F, Antonelli A (2018) Locality or habitat? Exploring
477 predictors of biodiversity in Amazonia. Ecographyc. 42:321-333, doi: 10.1111/ecog.03833

478 41. Prober SM, Leff JW, Bates ST, Borer ET, Firn J, Harpole WS, Lind EM, Seabloom EW, Adler PB,
479 Bakker JD, et al (2014) Plant diversity predicts beta but not alpha diversity of soil microbes across
480 grasslands worldwide. Ecol Lett. 18:85–95. doi:10.1111/ele.12381.
481 42. Kaiser K, Wemheuer B, Korolkow V, Wemheuer F, Nacke H, Schöning I, Schrumpf M, Daniel R
482 (2016) Driving forces of soil bacterial community structure, diversity, and function in temperate
483 grasslands and forests. Sci Rep. 6:1–12. doi:10.1038/srep33696.
484 43. Roesch LF., Fulthorpe RR, Riva A, Casella G, Hadwin AK, Kent AD, Daroub SH, Camargo FA (2007)
485 Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J. 1:283–290.
486 doi:10.1038/ismej.2007.53.
487 44. Fulthorpe RR, Roesch LFW, Riva A, Triplett EW (2008) Distantly sampled soils carry few species in
488 common. ISME J. 2:901–910. doi:10.1038/ismej.2008.55.
489 45. Haruna E, Zin NM, Kerfahi D, Adams JM (2017) Extensive Overlap of Tropical Rainforest Bacterial
490 Endophytes between Soil, Plant Parts, and Plant Species. Environmental Microbiology. doi
491 10.1007/s00248-017-1002-2
492 46. Oh YM, Kim M, Lee-Cruz L, Lai-Hoe A, Go R, Ainuddin N, Rahim RA, Shukor N, Adams JM (2012)
493 Distinctive Bacterial Communities in the Rhizoplane of Four Tropical Tree Species. Plant Mic. Interact.
494 DOI 10.1007/s00248-012-0082-2.
495 47. Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N (2010) The ecology of the phyllosphere:
496 Geographic and phylogenetic variability in the distribution of bacteria on tree leaves. Environ Microbiol.
497 12:2885–2893. doi:10.1111/j.1462-2920.2010.02258.x.
498 48. Nacke H, Thürmer A, Wollherr A, Will C, Hodac L, Herold N, Schöning I, Schrumpf M, Daniel R
499 (2011) Pyrosequencing-based assessment of bacterial community structure along different management
500 types in German forest and grassland soils. PLoS One. 6:1–12. doi:10.1371/journal.pone.0017000.
501 49. Lipson DA (2007) Relationships between temperature responses and bacterial community structure
502 along seasonal and altitudinal gradients. FEMS Microbiol Ecol. 59:418–427. doi:10.1111/j.1574-
503 6941.2006.00240.x.
504 50. Kuffner M, Hai B, Rattei T, Melodelima C, Schloter M, Zechmeister-Boltenstern S, Jandl R,
505 Schindlbacher A, Sessitsch A (2012) Effects of season and experimental warming on the bacterial
506 community in a temperate mountain forest soil assessed by 16S rRNA gene pyrosequencing. FEMS
507 Microbiol Ecol. 82:551–562. doi:10.1111/j.1574-6941.2012.01420.x.
508 51. Lladó S, López-Mondéjar R, Baldrian P (2017) Forest Soil Bacteria: Diversity, Involvement in
509 Ecosystem Processes, and Response to Global Change. Microbiol Mol Biol Rev. 81:1–27.
510 doi:10.1128/MMBR.00063-16.
511 52. Gaby, JC, Buckley, DH (2011) A global census of nitrogenase diversity. Environm Microbiol. 13:1790-
512 1799. doi:10.1111/j.1462-2920.2011.02488.x
513 53. Isobe K, Koba K, Otsuka S, Senoo K (2011) Nitrification and nitrifying microbial communities in forest
514 soils. J For Res. 16:351–362. doi:10.1007/s10310-011-0266-5.

515 54. Szukics U, Hackl E, Zechmeister-Boltenstern S, Sessitsch A (2012) Rapid and dissimilar response of
516 ammonia oxidizing archaea and bacteria to nitrogen and water amendment in two temperate forest soils.
517 Microbiol Res. 167:103–109. doi:10.1016/j.micres.2011.04.002.
518 55. Nelson MB, Martiny AC, Martiny JBH (2016) Global biogeography of microbial nitrogen-cycling traits
519 in soil. Proc Natl Acad Sci. 113:8033–8040. doi:10.1073/pnas.1601070113.
520 56. Uroz S, Calvaruso C, Turpault MP, Frey-Klett P (2009) Mineral weathering by bacteria: ecology, actors
521 and mechanisms. Cell Press. 17:378–387. doi:10.1016/j.tim.2009.05.004.
522 57. Uroz S, Oger P, Lepleux C, Collignon C, Frey-Klett P, Marie-Pierre (2011) Bacterial weathering and its
523 contribution to nutrient cycling in temperate forest. Free Radic Biol Med. 162:820–831.
524 58. Bergkemper F, Schöler A, Engel M, Lang F, Krüger J, Schloter M, Schulz S (2016) Phosphorus
525 depletion in forest soils shapes bacterial communities towards phosphorus recycling systems. Environm
526 Microbiol. 18:1988-2000. doi:10.1111/1462-2920.13188
527 59. Bergkemper F, Kublik S, Lang F, Krüger J, Vestergaard G, Schloter M, Schulz S (2016) Novel
528 oligonucleotide primers reveal a high diversity of microbes which drive phosphorous turnover in soil. J.
529 Microbiol. Methods. 125:91-97. doi:10.1016/j.mimet.2016.04.011
530 60. Hu X, Chen J, Guo J (2006) Two phosphate- and potassium-solubilizing bacteria isolated from Tianmu
531 Mountain, Zhejiang, China. World J Microbiol Biotechnol. 22(9):983–990. doi:10.1007/s11274-006-
532 9144-2.
533 61. Brown ME, Chang MC (2014) Exploring bacterial lignin degradation. Curr Opn Chem Biol. 19:1–7.
534 doi:10.1016/j.cbpa.2013.11.015.
535 62. López-Mondéjar R, Zühlke D, Becher D, Riedel K, Baldrian P (2016) Cellulose and hemicellulose
536 decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems.
537 Sci Rep. 6:1–12. doi:10.1038/srep25279.
538 63. Esson KC, Lin X, Kumaresan D, Chanton JP, Murrell JC, Kostka JE (2016) Alpha-and
539 gammaproteobacterial methanotrophs codominate the active methane-oxidizing communities in an
540 acidic boreal peat bog. Appl Environ Microbiol. 82:2363–2371. doi:10.1128/AEM.03640-15.
541 64. Uroz S, Calvaruso C, Turpault MP, Pierrat JC, Mustin C, Frey-Klett P (2007) Effect of the
542 mycorrhizosphere on the genotypic and metabolic diversity of the bacterial communities involved in
543 mineral weathering in a forest soil. Appl Environ Microbiol. 73(9):3019–3027.
544 doi:10.1128/AEM.00121-07.
545 65. Izumi H, Cairney JWG, Killham K, Moore E, Alexander IJ, Anderson IC (2008) Bacteria associated
546 with ectomycorrhizas of slash pine (Pinus elliottii) in south-eastern Queensland, Australia. FEMS
547 Microbiol Lett. 282(2):196–204. doi:10.1111/j.1574-6968.2008.01122.x.
548 66. Kluber LA, Smith JE, Myrold DD (2011) Distinctive fungal and bacterial communities are associated
549 with mats formed by ectomycorrhizal fungi. Soil Biol Biochem. 43:1042–1050.
550 doi:10.1016/j.soilbio.2011.01.022.

551 67. Yu HX, Wang CY, Tang M (2013) Fungal and bacterial communities in the rhizosphere of pinus
552 tabulaeformis related to the restoration of plantations and natural secondary forests in the loess plateau,
553 Northwest China. Sci World J.:1–12. doi:10.1155/2013/606480.
554 68. Kai M, Effmert U, Berg G, Piechulla B (2007) Volatiles of bacterial antagonists inhibit mycelial growth
555 of the plant pathogen Rhizoctonia solani. Arch Microbiol. 187:351–360. doi:10.1007/s00203-006- 0199-
556 0.
557 69. Kai M, Haustein M, Molina F, Petri A, Scholz B, Piechulla B (2009) Bacterial volatiles and their action
558 potential. Appl Microbiol Biotechnol. 81(6):1001–1012. doi:10.1007/s00253-008-1760-3.
559 70. Mendes LW, Kuramae EE, Navarrete AA, Van Veen JA, Tsai SM (2014) Taxonomical and functional
560 microbial community selection in soybean rhizosphere. ISME J. 8:1577–1587.
561 doi:10.1038/ismej.2014.17.
562 71. Kawasaki A, Watson ER, Kertesz MA (2012) Indirect effects of polycyclic aromatic hydrocarbon
563 contamination on microbial communities in legume and grass rhizospheres. Plant Soil. 358(1–2):169–
564 182. doi:10.1007/s11104-011-1089-z.
565 72. Štursová M, Žifčáková L, Leigh MB, Burgess R, Baldrian P (2012) Cellulose utilization in forest litter
566 and soil: Identification of bacterial and fungal decomposers. FEMS Microbiol Ecol. 80:735–746.
567 doi:10.1111/j.1574-6941.2012.01343.x.
568 73. Větrovský T, Steffen KT, Baldrian P (2014) Potential of cometabolic transformation of polysaccharides
569 and lignin in lignocellulose by soil Actinobacteria. PLoS One. 9(2):e89108.
570 doi:10.1371/journal.pone.0089108.
571 74. Rosch C, Mergel A, Bothe H (2002) Biodiversity of Denitrifying and Dinitrogen-Fixing Bacteria in an
572 Acid Forest Soil. Appl Environ Microbiol. 42:3818–3829. doi:10.1128/AEM.68.8.3818.
573 75. Jamalizadeh M, Etebarian HR, Aminian H, Alizadeh A (2010) Biological control of Botrytis mali on
574 apple fruit by use of Bacillus bacteria, isolated from the rhizosphere of wheat. Arch Phytopathol Plant
575 Protec. 43:1836–1845.doi.org/10.1080/03235400902830960
576 76. Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R (2009) Community
577 proteogenomics reveals insights into the physiology of phyllosphere bacteria. Proc Natl Acad Sci.
578 22:16428–16433. doi:10.1073/pnas.0905240106
579 77. Lauber CL, Hamady M, Knight R, Fierer N (2009)) Pyrosequencing-based assessment of soil pH as a
580 predictor of soil bacterial community structure at the continental scale. Appl Environ Microbiol.
581 75:5111–5120. doi:10.1128/AEM.00335-09.
582 78. Remus-emsermann MNP, Tecon R, Kowalchuk GA, Leveau JHJ (2012) Variation in local carrying
583 capacity and the individual fate of bacterial colonizers in the phyllosphere. ISME J. 6:756–765.
584 doi:10.1038/ismej.2011.209.
585 79. Knelman JE, Legg TM, Neill SPO, Washenberger CL, González A, Cleveland CC, Nemergut DR
586 (2012) Soil Biology & Biochemistry Bacterial community structure and function change in association
587 with colonizer plants during early primary succession in a glacier forefi eld. Soil Biol Biochem. 46:172–
588 180. doi:10.1016/j.soilbio.2011.12.001.

80. Acosta-Martínez V, Dowd S, Sun Y, Allen V (2008) Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. Soil Biol Biochem. 40:2762-2770. doi:10.1016/j.soilbio.2008.07.022

81. Haichar Z, Marol C, Berge O, Rangel-castro JI (2008) Plant host habitat and root exudates shape soil bacterial community structure. ISME J. 2:1221–1230. doi:10.1038/ismej.2008.80.

82. Lepleux C, Turpault MP, Oger P, Frey-Klett P, Uroz S (2012) Correlation of the abundance of betaproteobacteria on mineral surfaces with mineral weathering in forest soils. Appl Environ Microbiol. 78:7114-7119. doi:10.1128/AEM.00996-12.

83. Wertz S, Degrange V, Prosser JI, Poly F, Commeaux C, Guillaumaud N, Roux X Le (2007) Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial funct. Environ Microbiol. 9:2211–2219. doi:10.1111/j.1462-2920.2007.01335.x.

84. Griffiths BS, Ritz K, Wheatley R, Kuan HL, Boag B, Christensen S, Ekelund F (2001) An examination of the biodiversity-ecosystem function relationship in arable soil microbial communities. Soil Biol Biochem. 33:1713–1722. doi:10.1016/S0038-0717(01)00094-3

85. Wertz S, Degrange V, Prosser JI, Poly F, Commeaux C, Freitag T, Guillaumaud N, Roux X Le (2006) Maintenance of soil functioning following erosion of microbial diversity. Environ Microbiol. 8:2162–2169. doi:10.1111/j.1462-2920.2006.01098.x.

86. Bardgett RD, Bowman WD, Kaufmann R, Schmidt SK (2005) A temporal approach to linking aboveground and belowground ecology. TRENDS Ecol Evol. 20(11):634–641. doi:10.1016/j.tree.2005.08.005.

87. Haruna E, Zin NM, Kerfahi D, Adams JM (2018) Extensive overlap of tropical rainforest bacterial endophytes between soil, plant parts, and plant species. Microb Ecol. 75(1):88–103. doi:10.1007/s0024

88. Fürnkranz M, Wanek W, Richter A, Abel G, Rasche F, Sessitsch A (2008) Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. ISME J. 2: 561–570. doi:10.1038/ismej.2008.14

89. Hanshew AS, Mason CJ, Raffa KF, Currie CR (2013) Minimization of chloroplast contamination in 16S rRNA gene pyrosequencing of insect herbivore bacterial communities. J Microbiol Methods. 95:149–155. doi:10.1016/j.mimet.2013.08.007.

90. Bengtsson-Palme J, Hartmann M, Eriksson KM, Pal C, Thorell K, Larsson DGJ, Nilsson RH (2015) METAXA2: improved identification and taxonomic classification of small and large subunit rRNA in metagenomic data. Mol Ecol Resour. 15(6):1403–1414. doi:10.1111/1755-0998.12399.

Figure footnotes and table headings

Figure 1 Relative abundance. Bacterial phyla associated with the soil, roots and leaves in zones with different recovery times since anthropogenic disturbance. The represented taxa are > 1% abundance in at least one sample; the taxa with abundances less than 1% are grouped in the category others. Abbreviations: B: backyard, PL: pasture, SF: secondary forest and PF: primary forest.

Figure 2 Rarefaction curves. Taxon accumulation curves as a function of the number of 16S DNA sequences at the phylum level (20% dissimilarity) of the bacterial community associated with the soil (black), roots (yellow) and leaves (green) in zones with different recovery times since anthropogenic disturbance. Abbreviations B: backyard, PL: pasture, SF: secondary forest, and PF: primary forest.

Figure 3 Correlation of bacterial communities in soil, root and leaf. Heat map based on the Jaccard similarity coefficient and relative abundances at the genus level. Abbreviations B: backyard; PL: pasture; SF: secondary forest; PF: primary forest; S: soil; R: root; and L: leaf.

Figure S1 Relative abundance at class level. Bacterial classes associated with the soil, roots and leaves in zones with different recovery times since anthropogenic disturbance. The represented taxa are > 1% abundance in at least one sample; the taxa with abundances less than 1% are grouped in the category others. Abbreviations: B: backyard, PL: pasture, SF: secondary forest and PF: primary forest.

Figure S2 Relative abundance at family level. Bacterial families associated with the soil, roots and leaves in zones with different recovery times since anthropogenic disturbance. The represented taxa are > 1% abundance in at least one sample; the taxa with abundances less than 1% are grouped in the category others. Abbreviations: B: backyard, PL: pasture, SF: secondary forest and PF: primary forest.

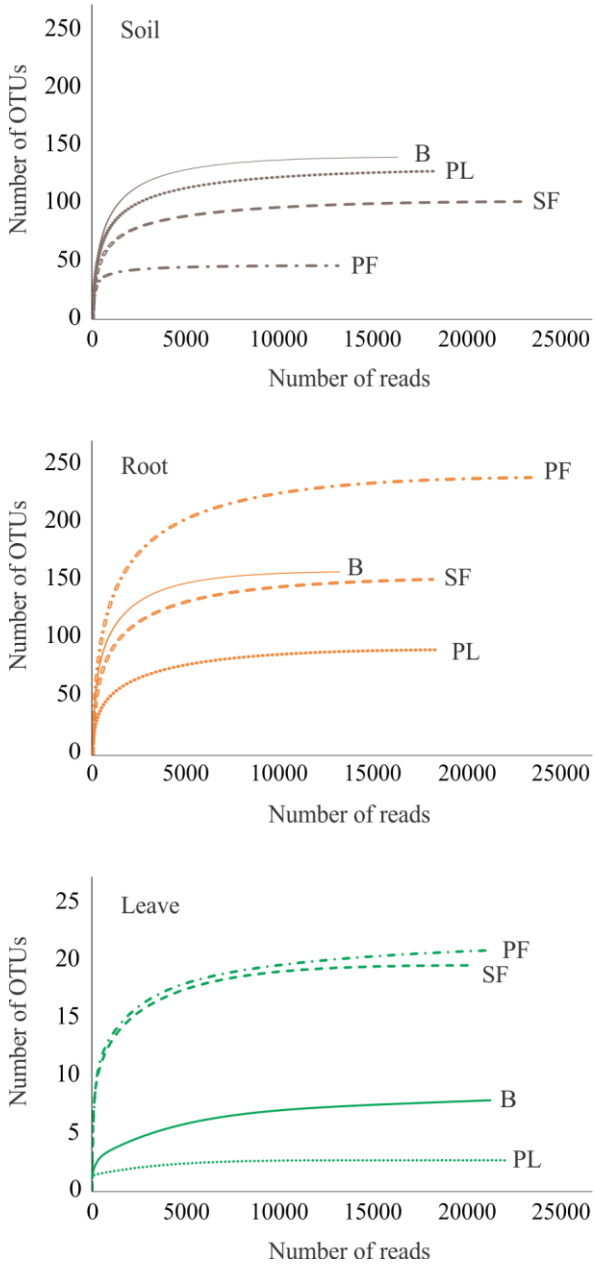
Figure S3 Relative abundance at genus level. Bacterial genuses associated with the soil, roots and leaves in zones with different recovery times since anthropogenic disturbance. The represented taxa are > 1% abundance in at least one sample; the taxa with abundances less than 1% are grouped in the category others. Abbreviations: B: backyard, PL: pasture, SF: secondary forest and PF: primary forest.

Figure S4 Relative abundance at species level. Bacterial species associated with the soil, roots and leaves in zones with different recovery times since anthropogenic disturbance. The represented taxa are > 1% abundance in at least one sample; the taxa with abundances less than 1% are grouped in the category others. Abbreviations: B: backyard, PL: pasture, SF: secondary forest and PF: primary forest.

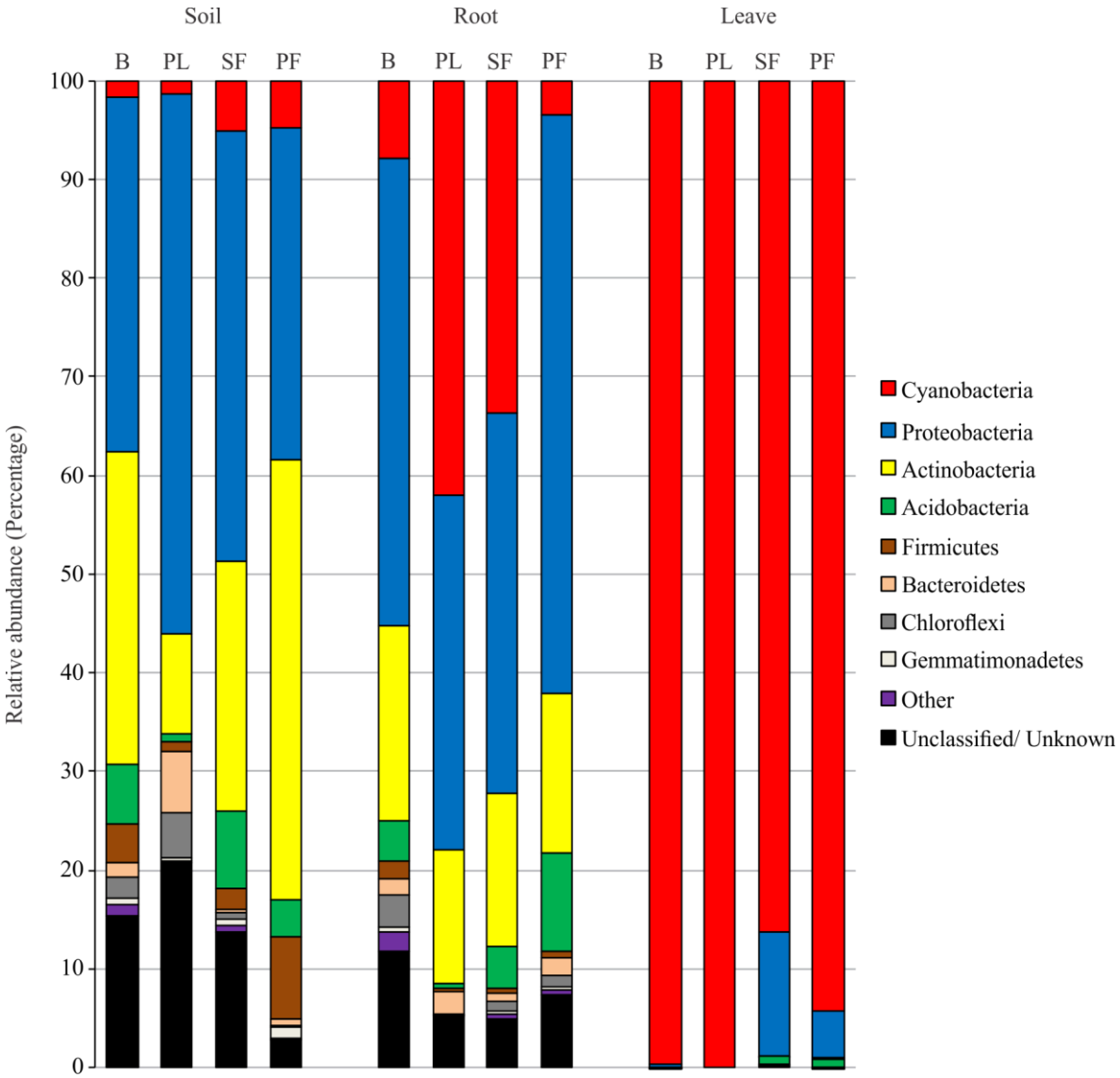
Table 1. General soil analysis. Abbreviations: B: backyard, PL: pasture, SF: secondary forest, PF: primary forest, EC: electrical conductivity, OM: organic matter, N: nitrogen, K: potassium, P: phosphorus. pH correspond to a measure at 1:2 ratio.

Table 2. General analysis of the sequence datasets. The number of OTUs, Shannon H' diversity, Chao 1 and evenness were analyzed at a sequence dissimilarity of 20% (phyla level) and 3% (species level) for each sample. Abbreviations: B: backyard, PL: pasture, SF: secondary forest, PF: primary forest; # seq: number of sequences; MSL: mean sequence length, bp: base pairs.

Figure 1


Soil
Number of OTUs
250
200
150
100
50
0
B
PL
SF
PF
0
5000
10000
15000
20000
25000
Number of reads
Root
Number of OTUs
250
200
150
100
50
0
PF
B
SF
PL
0
5000
10000
15000
20000
25000
Number of reads
Leave
Number of OTUs
25
20
15
10
5
0
PF
SF
B
PL
0
5000
10000
15000
20000
25000
Number of reads

709 Figure 2



710

711

712

713

714

715

716

717

718 Figure 3

719

Figure S1

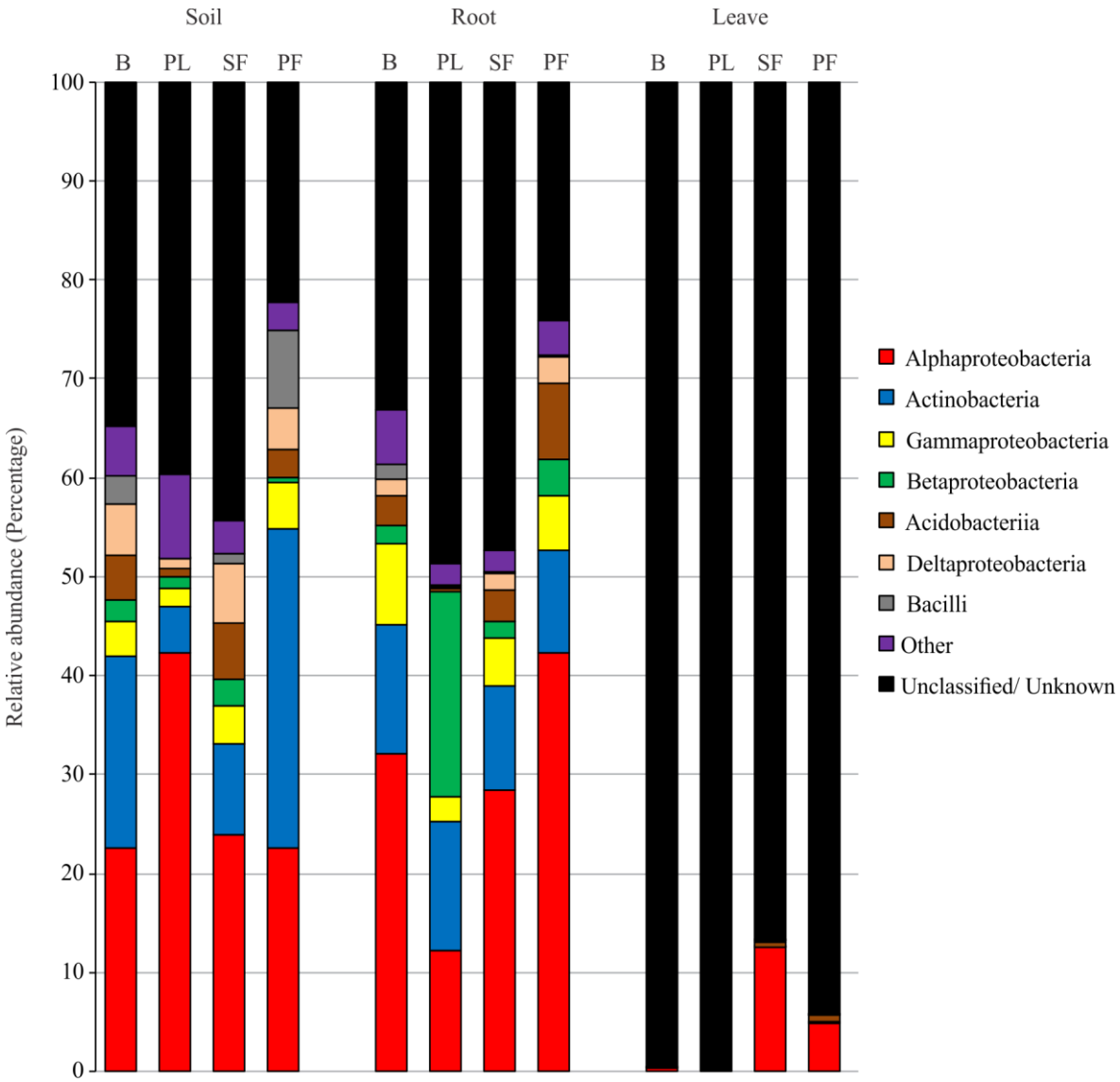

Soil
Root
Leave
B
PL
SF
PF
Relative abundance (Percentage)
100
90
80
70
60
50
40
30
20
10
0
Alphaproteobacteria
Actinobacteria
Gammaproteobacteria
Betaproteobacteria
Acidobacteriia
Deltaproteobacteria
Bacilli
Other
Unclassified/ Unknown

Figure S2

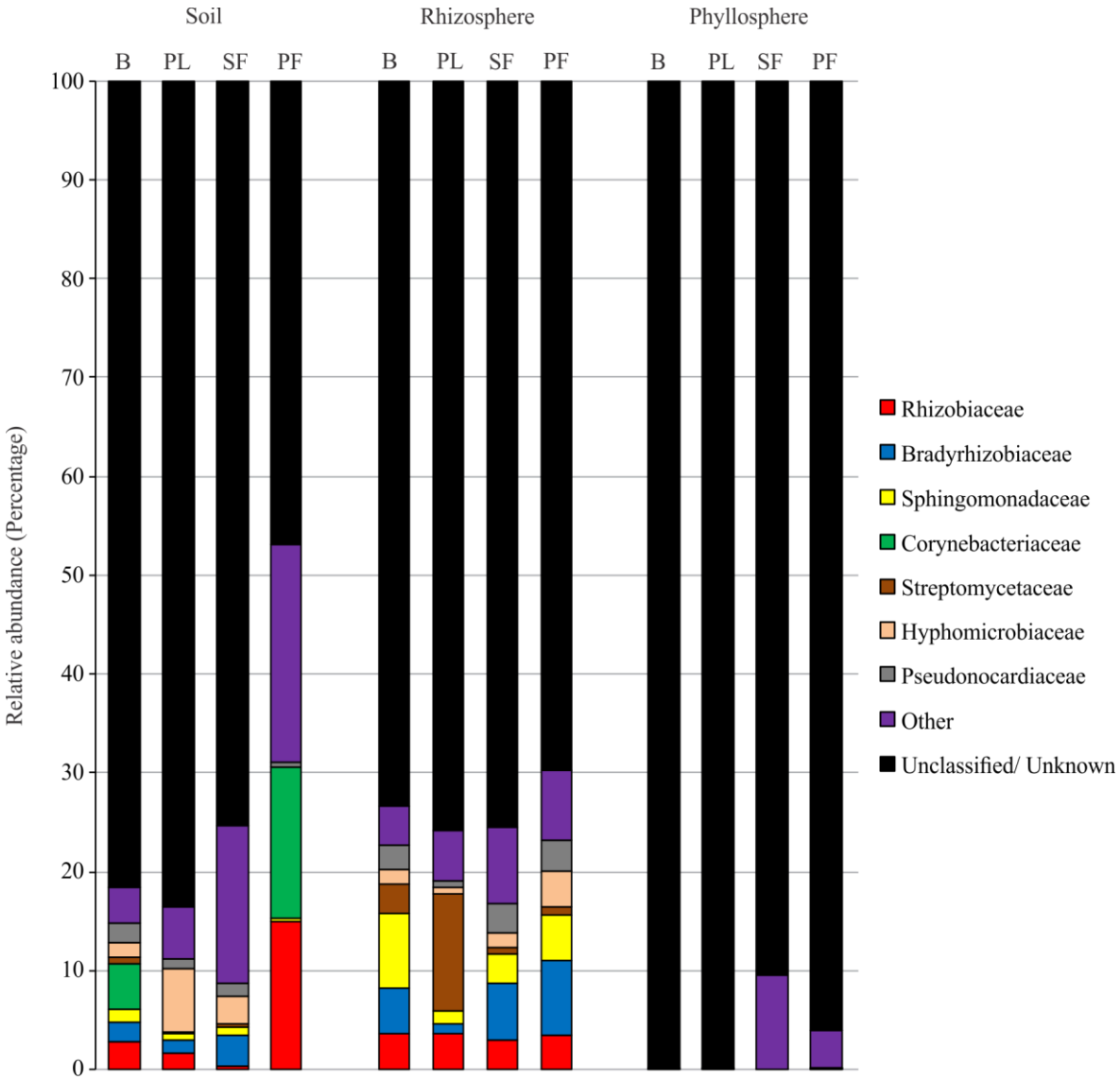

Soil
Rhizosphere
Phyllosphere
B
PL
SF
PF
Relative abundance (Percentage)
100
90
80
70
60
50
40
30
20
10
0
Rhizobiaceae
Bradyrhizobiaceae
Sphingomonadaceae
Corynebacteriaceae
Streptomycetaceae
Hyphomicrobiaceae
Pseudonocardiaceae
Other
Unclassified/ Unknown

Figure S3

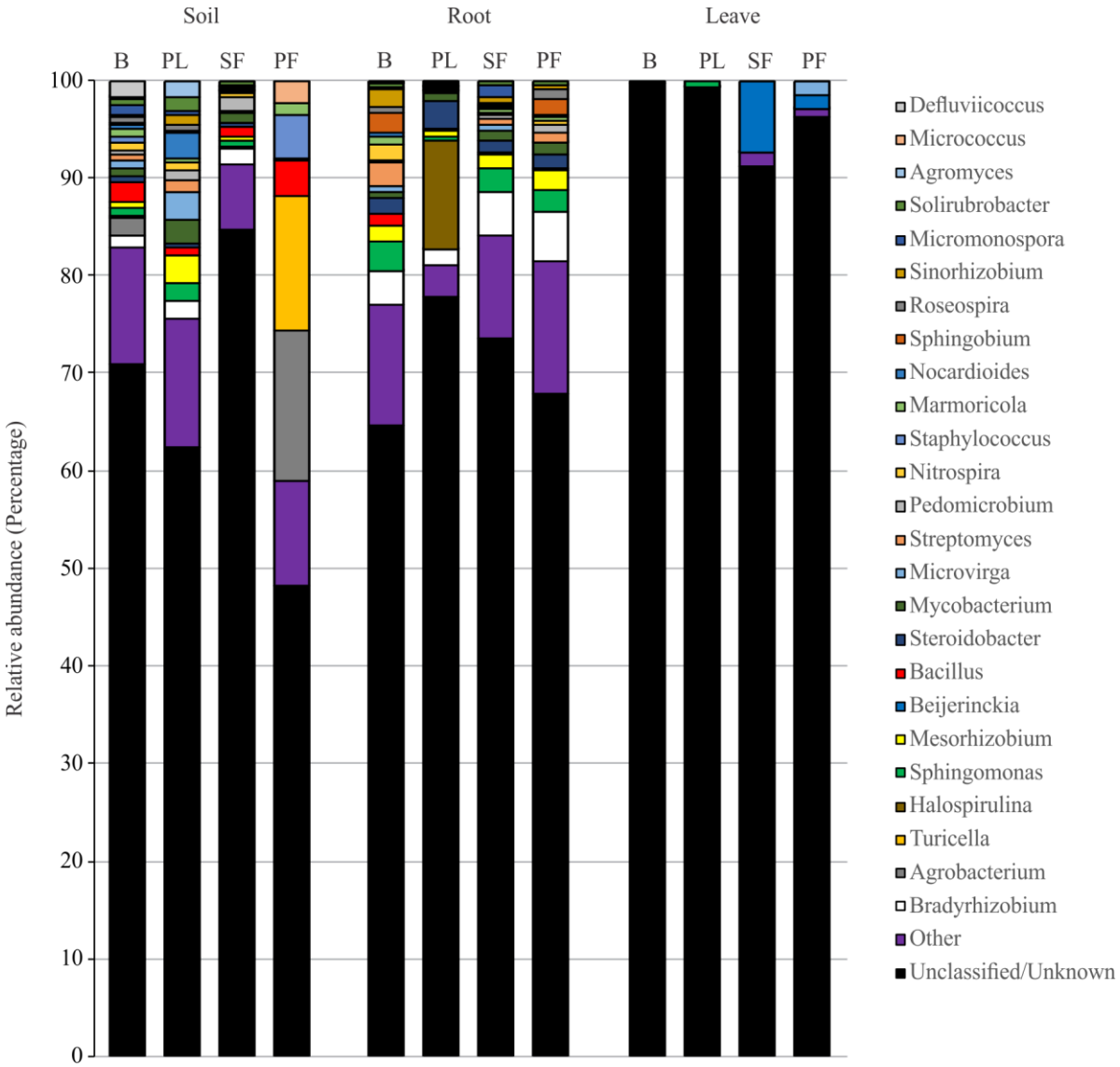
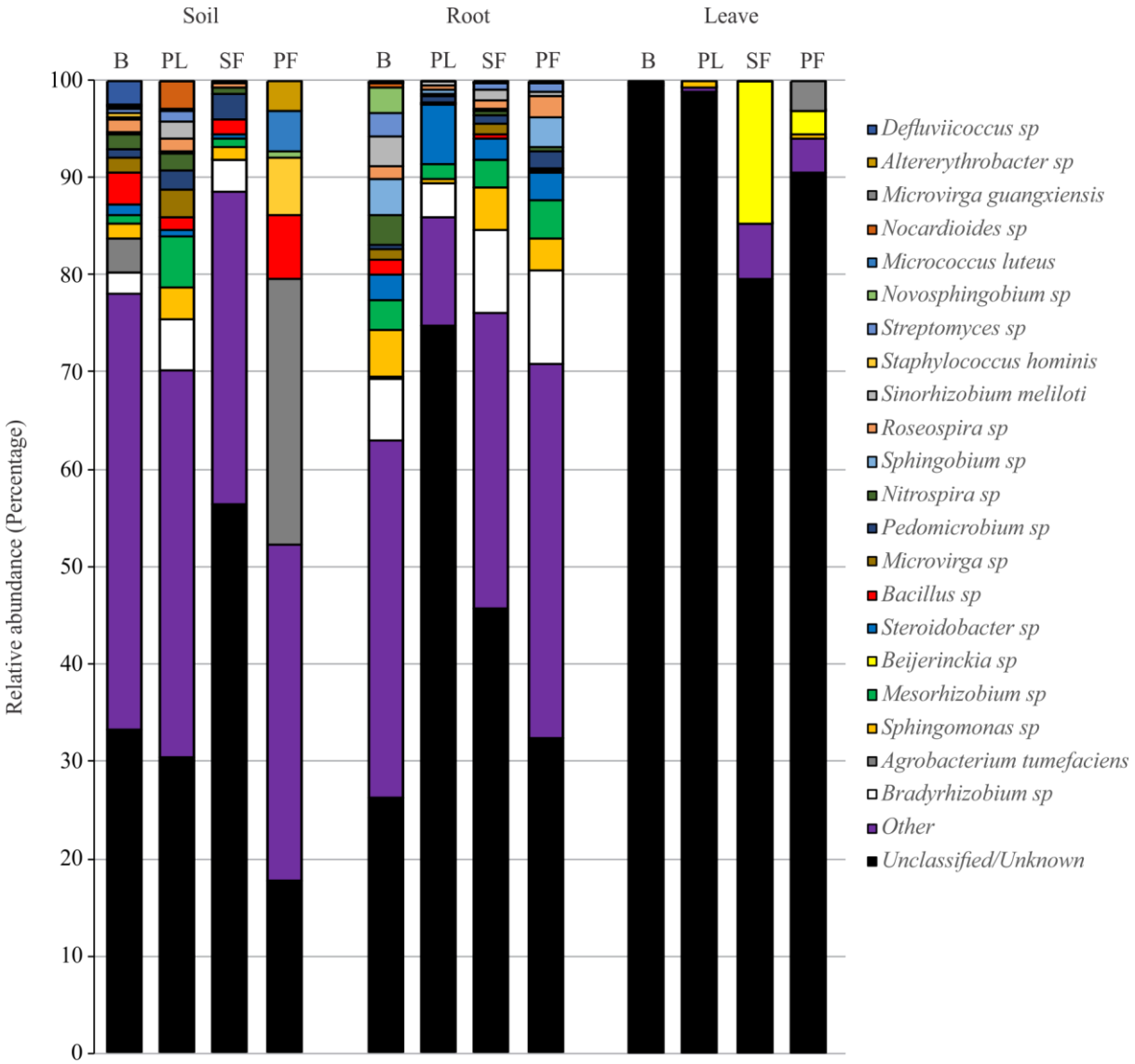
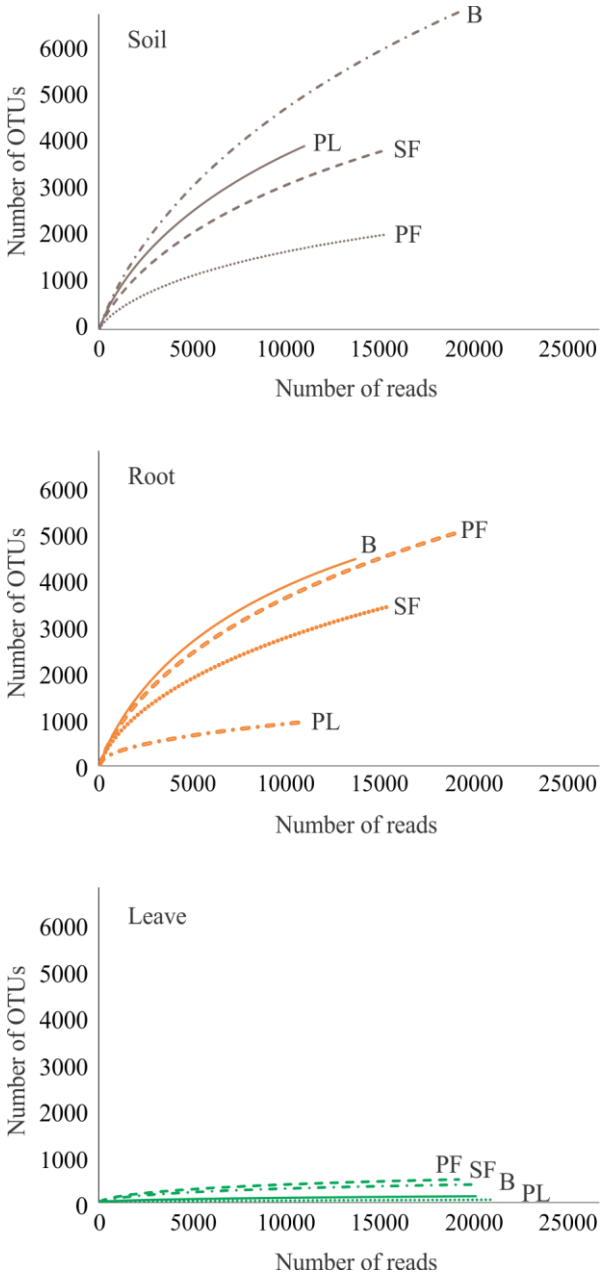

Soil
Root
Leave
B
PL
SF
PF
Relative abundance (Percentage)
100
90
80
70
60
50
40
30
20
10
0
Defluviicoccus
Micrococcus
Agromyces
Solirubrobacter
Micromonospora
Sinorhizobium
Roseospira
Sphingobium
Nocardioides
Marmoricola
Staphylococcus
Nitrospira
Pedomicrobium
Streptomyces
Microvirga
Mycobacterium
Steroidobacter
Bacillus
Beijerinckia
Mesorhizobium
Sphingomonas
Halospirulina
Turicella
Agrobacterium
Bradyrhizobium
Other
Unclassified/Unknown

Figure S4


Soil
Root
Leave
B
PL
SF
PF
Relative abundance (Percentage)
100
90
80
70
60
50
40
30
20
10
0
Defluviicoccus sp
Altererythrobacter sp
Microvirga guangxiensis
Nocardioides sp
Micrococcus luteus
Novosphingobium sp
Streptomyces sp
Staphylococcus hominis
Sinorhizobium meliloti
Roseospira sp
Sphingobium sp
Nitrospira sp
Pedomicrobium sp
Microvirga sp
Bacillus sp
Steroidobacter sp
Beijerinckia sp
Mesorhizobium sp
Sphingomonas sp
Agrobacterium tumefaciens
Bradyrhizobium sp
Other
Unclassified/Unknown

760 Figure S5



761

762

763

764

765

766

767

Table 1

Sample	pH (H_2O)	EC $\mu S\ cm^{-1}$	OM Percentage	N	K $cmol\ Kg^{-1}$	P $mg\ Kg^{-1}$	Clay Percentage	Silt	Sand	Classification texture
B	7.23	1997	15.92	0.67	0.77	16.29	48	8	44	Clay - Sandy
PL	7.34	2059	18.6	0.82	0.77	24.43	45	8	47	Clay - Sandy
SF	7.38	380	21.79	0.77	0.77	18.74	48	5	48	Clay - Sandy
PF	7.43	631	22.04	0.51	0.77	12.37	43	6	52	Clay - Sandy

Table 2

Samples		# seq	MSL (bp)	Phylum level				Species level			
				H'	Chao 1	Evenness	OTUs	*H'*	Chao 1	Evenness	OTUs
Soil	B	13122	281	4.01	164	0.78	164	7.23	4,677	0.89	3,342
	P	14813	293	3.8	151	0.76	150	6.6	4,127	0.84	2,572
	SF	18423	293	3.63	119	0.76	119	7.16	5,931	0.87	3,778
	PF	10547	286	3.2	54	0.8	54	4.71	1,156	0.72	700
Root	B	11947	283	3.9	145	0.78	145	7.18	5,427	0.88	3,364
	P	16640	281	2.47	83	0.56	83	4.5	2,639	0.6	1,730
	SF	16448	281	3.31	140	0.67	139	6.25	5,180	0.77	3,269
	PF	21218	288	4.03	220	0.75	220	7.76	9,746	0.89	5,873
Leaf	B	19802	293	0.04	6	0.02	6	0.2	133	0.04	92
	P	20655	283	0	2	0	2	0.05	196	0.01	34
	SF	18910	293	0.72	15	0.27	15	1.54	501	0.26	372
	PF	19665	293	0.46	16	0.17	16	0.93	383	0.16	288

Capitulo 3. Conclusiones

Hasta donde se tiene conocimiento el presente estudio es el primer reporte en la Reserva de la Biosfera de Calakmul en el que se presenta la secuenciación masiva de ADN para analizar la comunidad bacteriana asociada al suelo, raíces y hojas en un microhabitat modelo, en zonas con distintas fases de perturbación del bosque tropical.

Con los datos obtenidos podemos concluir que en las zonas muestreadas las comunidades bacterianas asociadas suelo y raíces son más diversas que la comunidad bacteriana asociadas a las hojas, en la comunidad bacteriana de suelo y raíces los fila dominantes son Proteobacteria y Actinobacteria, mientras que en las hojas Cianobacteria. Los grupos bacterianos más abundantes (Proteobacteria y Actinobacteria) podrían desempeñar una gran cantidad de funciones importantes para la recuperación de los ecosistemas, sin embargo, en este estudio no se evaluó la funcionalidad bacteriana, por lo que solo se describió, lo cual solo nos permite suponer la gran gama de funciones metabólicas que desempeñan estos grupos. Por ultimo nuestros resultados sugieren que las comunidades bacterianas son resistentes a las perturbaciones antrópicas y que la especie vegetal a la que se asocian, así como las características del suelo son factores determinantes de la composición de la comunidad, ya que podemos encontrar una composición taxonómica similar en cada zona de recuperación.

Cabe señalar que los resultados obtenidos solo nos muestran una visión panorámica de lo que podría estar pasando con la comunidad bacteriana en las zonas tropicales, por lo que es necesario seguir estudiando y poder evaluar algunos aspectos que no se tomaron en cuenta en este trabajo, como la variación en las condiciones climáticas en distintas temporadas del año, la interacción con distintos tipos de vegetación, ampliar el área de estudio y si podemos encontrar el mismo patrón con otros microhábitats, también poder evaluar de manera más precisa las funciones metabólicas de las bacterias conocida y desconocidas, estas últimas encontrándose en mayor proporción que en zonas tropicales, siendo un

gran reservorio de diversidad el cual puede tener un gran potencial biotecnológico y un papel clave en la recuperación de los ecosistemas tropicales.

Para finalizar, es importante recalcar que tomar en cuenta a la diversidad y la gama de funciones que desempeña las comunidades microbianas, puede ser un factor muy importante para el manejo y conservación de los ecosistemas, complementando al componente vegetal y animal, que por lo general son los únicos que se toman en cuenta.

Literatura Citada.

Aryal DR, de Jong BHJ, Ochoa-Gaona S, Esparza-Olguin L, Mendoza-Vega J. 2014. Carbon stocks and changes in tropical secondary forests of southern Mexico. Agric. Ecosyst. Environ. 195:220–230.

Aryal DR, de Jong BHJ, Ochoa-Gaona S, Mendoza-Vega J, Esparza-Olguin L. 2015. Successional and seasonal variation in litterfall and associated nutrient transfer in semi-evergreen tropical forests of SE Mexico. Nutr. Cycl. Agroecosystems 103:45–60.

Berg G. 2009. Plant-microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. Appl. Microbiol. Biotechnol. 84:11–18.

Berga M, Székely AJ, Langenheder S. 2012. Effects of disturbance intensity and frequency on bacterial community composition and function. PLoS One 7.

Blaalid R, Carlsen T, Kumar S, Halvorsen R, Ugland KI, Fontana G, Kauserud H. 2012. Changes in the root-associated fungal communities along a primary succession gradient analysed by 454 pyrosequencing. Mol. Ecol. 21:1897–1908.

Borneman J, Triplett EW. 1997. Molecular Microbial Diversity in Soils from Eastern Amazonia : Evidence for Unusual Microorganisms and Microbial Population Shifts Associated with Deforestation. Appl. Environ. Microbiol. 63:2647–2653.

Bossio DA, Girvan MS, Verchot L, Bullimore J, Borelli T, Albrecht A, K.Scow, Ball AS, Pretty JN, M. O. 2005. Microbial Ecology. Microb. Ecol. 49:50–62.

Cassán F, Perrig D, Sgroy V, Masciarelli O, Penna C, Luna V. 2009. Azospirillum brasilense Az39 and Bradyrhizobium japonicum E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (Zea mays L.) and soybean (Glycine max L.). Eur. J. Soil Biol. 45:28–35.

Chapin SF, Zavaleta SV, Eviners R, Naylor P, Vitousek H, Reynolds D Hooper, Lavorel O, Sala S, Hobbie M, Díaz S. 2000. Consequences of changing biodiversity. Nature 405: 234-242.

Cobo-Díaz JF, Fernández-González AJ, Villadas PJ, Robles AB, Toro N, Fernández-López M. 2015. Metagenomic Assessment of the Potential Microbial Nitrogen Pathways in the Rhizosphere of a Mediterranean Forest After a Wildfire. Microb. Ecol. 69:895–904.

Compant S, Clément C, Sessitsch A. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biol. Biochem. 42:669–678.

Copeland JK, Yuan L, Layeghifard M, Wang PW, Guttman DS. 2015. Seasonal community succession of the phyllosphere microbiome. Mol. Plant-Microbe Interact. 28:274–285.

Falkowski PG, Fenchel T, Delong EF. 2008. The Microbial Engines That Drive Earth ' s Biogeochemical Cycles. Microb. Ecol. Rev. 320:1034–1040.

Fierer N, Nemergut D, Knight R, Craine JM. 2010. Changes through time: Integrating microorganisms into the study of succession. Res. Microbiol. 161:635–642.

Finegan B. 1996. Pattern and process in neotropoical secondary rain forests: the first 100 years of succession. Trends Ecol. Evol. 11:119–124.

García-Licona J, Esparza-Olguín L, Martínez-Romero E. 2014. Estructura y composición de la vegetación leñosa de selvas en diferentes estadios sucesionales en el Ejido el Carmen II, Calakmul, México. Polibotánica 38:1–26.

Guariguata MR, Ostertag R. 2002. Sucesión secundaria. Ecol. bosues Neotrop.:592–616.

Hartmann M, Howes CG, VanInsberghe D, Yu H, Bachar D, Christen R, Henrik Nilsson R, Hallam SJ, Mohn WW. 2012. Significant and persistent impact of timber harvesting on soil microbial communities in Northern coniferous forests. ISME J. 6:2199–2218.

Hartmann M, Niklaus PA, Zimmermann S, Schmutz S, Kremer J, Abarenkov K, Lüscher P, Widmer F, Frey B. 2014. Resistance and resilience of the forest soil microbiome to logging-associated compaction. ISME J. 8:226–44.

Haruna E, Zin NM, Kerfahi D, Adams JM (2017) Extensive Overlap of Tropical Rainforest Bacterial Endophytes between Soil, Plant Parts, and Plant Species. Environmental Microbiology. doi 10.1007/s00248-017-1002-2

Heijden MGA van der, Bardgett RD, Nico M. Van Straalen. 2008. The unseen majority : soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11:296–310.

Herrera-Estrella A, Castellanos F. 2007. Análisis metagenómico de la microbiota edáfica de la reserva le la biósfera de Calakmul. Ideas del CONCYTEG 29:802–823.

Kaiser K, Wemheuer B, Korolkow V, Wemheuer F, Nacke H, Schöning I, Schrumpf M, Daniel R. 2016. Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. Sci. Rep. 6:1–12.

Kloepper JW, Rodriguez-Kabanaa R, Zehnder " GW, Murphya JF, Sikora- E, Fernandez C. 1999. Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases. Australas. Plant Pathol. 28:21–26.

Köberl M, Dita M, Martinuz A, Staver C, Berg G. 2017. Members of Gammaproteobacteria as indicator species of healthy banana plants on Fusarium wilt-infested fields in Central America. Sci. Rep. 7:1–9.

Leiva JA, Mata R, Rocha OJ, Soto MVG. 2009. Cronología de la regeneración del bosque tropical seco en Santa Rosa ,. Rev. Biol. Trop. 57:801–815.

Liebsch D, Marques MCM, Goldenberg R. 2008. How long does the Atlantic Rain Forest take to recover after a disturbance? Changes in species composition and ecological features during secondary succession. Biol. Conserv. 141:1717–1725.

Linares JC, Carreira JA, Ochoa V. 2011. Human impacts drive forest structure and diversity. Insights from Mediterranean mountain forest dominated by Abies pinsapo (Boiss.). European Journal of Forest Research, 130(4), 533-542.,

Llacsa-Sánchez LX. 2016. Identificación molecular de microorganismos asociados a la rizosfera y filosfera de los guayacanes (tabebuia chrysantha y tabebuia billbergii) y evaluación de cepas aisladas en el proceso inicial de desarrollo de plántulas. universidad nacional de tumbes.

Martinez E, Galindo-Leal C. 2002. La vegetación de Calakmul, Campeche, México: clasificación, descripción y distribución. Boletín la Soc. Botánica México 71:7–32.

Matson PA, Vitousek PM, Ewel JJ, Mazzarino MJ, Robertson GP. 1987. Nitrogen transformations following tropical forest felling and burning on a volcanic soil. *Ecology*, *68*: 491-502.

Madsen EL. 2011. Microorganisms and their roles in fundamental biogeochemical cycles. Curr. Opin. Biotechnol. 22:456–464.

Matiru VN, Dakora FD. 2004. Minireview. Potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. African J. Biotechnol. 3:1–7.

Mirza BS, Potisap C, Nüsslein K, Bohannan BJM, Rodrigues LM. 2014. Response of Free-Living Nitrogen-Fixing Microorganisms to Land Use. Appl. Environ. Microbiol. 80:281–288.

Müller AK, Westergaard K, Christensen S, Sørensen SJ. 2002. The diversity and function of soil microbial communities exposed to different disturbances. Microb. Ecol. 44:49–58.

Oh YM, Kim M, Lee-Cruz L, Lai-Hoe A, Go R, Ainuddin N, Rahim RA, Shukor N, Adams JM (2012) Distinctive Bacterial Communities in the Rhizoplane of Four Tropical Tree Species. Plant Microbe Interactions. DOI 10.1007/s00248-012-0082-2.

Porter Bolland L, Drew AP, Vergara-Tenorio C. 2006. Analysis of a natural resources management system in the Calakmul Biosphere Reserve. Landsc. Urban Plan. 74:223–241.

Prieto-Fernández A, Acea MJ, Carballas T. 1998. Soil microbial and extractable C and N after wildfire. *Biology and Fertility of Soils*, *27*: 132-142.

Ritter CD, Zizka A, Barnes C, Nilsson HR, Roger F, Antonelli A (2018) Locality or habitat? Exploring predictors of biodiversity in Amazonia. Ecographyc. 42:321-333, doi: 10.1111/ecog.03833

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. 2008. Bacterial endophytes: Recent developments and applications. FEMS Microbiol. Lett. 278:1–9.

Rykiel JR. 1985. Towards a definition of ecological disturbance. Australian Journal of Ecology, 10(3), 361-365.

Ulrich K, Ulrich A, Ewald D. 2008. Diversity of endophytic bacterial communities in poplar grown under field conditions. FEMS Microbiol. Ecol. 63:169–180.

Wang Y, YANG C de, YAO Y ling, WANG Y qin, ZHANG Z fen, XUE L. 2016. The diversity and potential function of endophytic bacteria isolated from Kobreasia capillifolia at alpine grasslands on the Tibetan Plateau, China. J. Integr. Agric. 15:2153–2162.

Yao H, He Z, Wilson M, Campbell C. 2000. Microbial Biomass and Community Structure in a Sequence of Soils with Increasing Fertility and Changing Land Use. Microb. Ecol. 40:223–237.