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Doctorado en Ciencias en Recursos Naturales y Medio Ambiente en Zonas Áridas

SECUESTRO DE CARBONO EN AGROECOSISTEMAS DE AMBIENTES TROPICALES Y ÁRIDOS CON DIFERENTES MÉTODOS DE MANEJO

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SECUESTRO DE CARBONO EN AGROECOSISTEMAS DE AMBIENTES TROPICALES Y ÁRIDOS CON DIFERENTES MÉTODOS DE MANEJO

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ABREVIATURAS USADAS

Abreviatura	Palabra
B-gal	Beta-galactosidase
B-glu	Beta-glucosidase
BS	Bacterial Sequences
BS	Bacterial Sequences
C	Carbon
C/N	Carbon nitrigen ratio
CAM	Conventional Agronomic Management
CAS	Conventional Agronomic System
CCA	Canonical Correspondence Analysis
CD	Chimeras Discarded
CFU	Colony-Forming Units
CO2	Dióxido de carbono
CONACYT	Consejo Nacional de Ciencia y Tecnologia
Conv	Convencional / Conventional
DNA	Ácido desoxirribonucleico
EEG	Easily Extractable Glomalin
F1	Very labile fraction
F2	Labile fraction
F3	Less labile fraction
F4	Recalcitrant fraction
FA	Fulvic Acids
H_2SO_4	Ácido sulfúrico
HA	Humic Acids

HS	Humins
INIFAP	Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias
K	Potassium
LAC	Lacasse
MBC	Microbial Biomass Carbon
MOS	Materia orgánica del suelo
MOS	Microorganisms
N	Nitrogen
OAM	Organic Agronomic Management
OAS	Organic Agronomic System
Org	Orgánico / Organic
OTUs	Operational Taxonomic Units
P	Phosphorous
PDA	Potato Dextrose Agar
PER	Peroxidase
PPO	Polyphenol Oxidase
QIIME	Quantitative Insights Into Microbial Ecology.
QS	Quality Sequences
rRNA	Ácido ribonucleico ribosomal
SADER	Secretaría de Agricultura y Desarrollo Rural
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TOC	Total Organic Carbon analyzer
USD	United States Dollars

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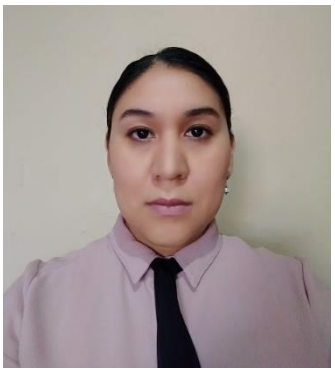
DATOS BIOGRÁFICOS



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RESUMEN GENERAL

Secuestro de carbono en agroecosistemas de ambientes tropicales y áridos con diferentes métodos de manejo

Los microorganismos del suelo son fundamentales en los procesos de descomposición de la materia orgánica y el ciclo de nutrientes, y sus capacidades funcionales son fuertemente influenciadas por el manejo agronómico. Se determinaron variables químicas y biológicas del suelo capaces de responder a manejos agronómicos orgánico (Org) y convencional (Conv), en cultivos de nogal de agroecosistemas áridos y cultivos de café de agroecosistemas tropicales, en México. Además, se estudió el microbioma del suelo a lo largo de la secuenciación masiva en ambos agroecosistemas de estudio. En el cultivo de nogal bajo manejo Org fueron significativamente mayor aquellas variables que están relacionadas con compuestos de carbono recalcitrantes, así como comunidades bacterianas relacionadas con la dinámica del ciclo del carbono. Así mismo, en el agroecosistema tropical bajo manejo Org, se presentó la estructura de la comunidad bacteriana que se correlaciona positivamente con la materia orgánica y al mismo tiempo participa significativamente en el secuestro de carbono. De esta manera, se encontró que el manejo agronómico en los diversos agroecosistemas estudiados influye en la concentración de nutrientes, diversas variables químicas y biológicas del suelo, así como en la estructura de las comunidades bacterianas. Más aún, la agricultura Org en ambos agroecosistemas parece contribuir a la captura de carbono recalcitrante y a la proliferación de ciertas bacterias fundamentales en el ciclo del carbono.

Palabras clave: manejo agronómico, agroecosistemas, secuenciación masiva, estructura de la comunidad bacteriana, carbono orgánico del suelo.

GENERAL ABSTRACT

Carbon sequestration in tropical and arid environment agroecosystems with different management methods

Soil microorganisms are fundamental in the processes of organic matter decomposition and nutrient cycling, and their functional capacities are strongly influenced by agronomic management. There were determined chemical and biological variables of soil capable of responding to organic (Org) and conventional (Conv) agronomic management, in pecan crops of arid agroecosystems and coffee crops of tropical agroecosystems, in Mexico. In addition, soil microbiome was studied along the massive sequencing in both study agroecosystems. In pecan cultivation under Org management, variables related to recalcitrant carbon compounds and bacterial communities related to carbon cycle dynamics were significantly higher. Likewise, in the tropical agroecosystem under Org management, it was presented the structure of the bacterial community that is positively correlated with the organic matter and at the same time it participates significantly in the carbon sequestration. In this way, it was found that the agronomic management in the diverse studied agroecosystems influences the concentration of nutrients, diverse chemical and biological variables of the soil, as well as the structure of the bacterial communities. Moreover, Org agriculture in both agroecosystems seems to contribute to the capture of recalcitrant carbon and to the proliferation of certain fundamental bacteria in the carbon cycle.

Key words: agronomic management, agroecosystems, massive sequencing, bacterial community structure, soil organic carbon

CAPITULO I

1. INTRODUCCIÓN GENERAL

El suelo es uno de los ecosistemas terrestres más complejos debido a su diversidad biológica, la cual abarca alrededor del 25% de la biodiversidad mundial de especies (Decaëns, 2010; Coleman & Wall, 2015). Los microorganismos que habitan en el suelo se encuentran entre los organismos más abundantes y diversos de la tierra (Delgado-Baquerizo et al., 2016); un gramo de suelo puede contener hasta 1 billón de bacterias y varios metros de hifas fúngicas, así como una cantidad considerable de protistas y nematodos (Gans, Wolinsky & Dunbar, 2005; Trevors, 2010). Estos microorganismos comprenden una amplia gama de formas de vida y funciones en procesos ecológicos, proporcionando servicios ecosistémicos vitales, incluida la descomposición de la materia orgánica y el ciclo de nutrientes, así como la regulación del clima (Lavelle et al., 2006; Liu et al., 2020).

En este sentido, las bacterias son claves en los procesos de transformación del carbono orgánico del suelo (COS), ya que, gracias a los aportes de materia orgánica en forma de exudados de raíz y residuos vegetales, son capaces de consumirlo y transformarlo en biomasa o mineralizarlo en forma de dióxido de carbono (CO_2) (Simpson et al., 2007; Lee & Schmidt, 2014; Soares & Rousk, 2019). Así mismo, en el proceso de descomposición de la materia orgánica del suelo (MOS), producen metabolitos y enzimas extracelulares que protegen al COS de una mayor descomposición, contribuyendo a mantener la fertilidad del suelo y estabilizar las fracciones de carbono más recalcitrantes (Goodfellow & Williams, 1983; Aragón, Sandans & Peñuelas, 2014; Singh et al., 2018). Además, se han identificado diversas bacterias quimioautótrofas y fotoautótrofas que habitan el suelo capaces de asimilar CO_2 atmosférico y almacenarlo en forma de carbono (Tolli & King, 2005; Selesi et al., 2007). Este mecanismo se da a través del ciclo de Calvin en donde la enzima ribulosa-1,5 bifosfato

carboxilasa/oxigenasa cataliza la carboxilación de la molécula aceptora de CO_2 (Raines, 2003; Kumar et al., 2018). Dicha asimilación es un proceso importante en el secuestro de carbono en los ecosistemas terrestres, ya que se puede reducir la concentración de CO_2 atmosférico y, por lo tanto podría mitigar impactos ambientales (Chen et al., 2014; Lynn et al., 2017). Sin embargo, los cambios climáticos y la composición de vegetación son factores claves en la variabilidad de los microorganismos presentes en diversos agroecosistemas (Singh & Gupta, 2018).

Al respecto, en agroecosistemas áridos y semiáridos, prevalecen bacterias y hongos, capaces de fijar COS, nitrógeno y mantener la fertilidad, a pesar de la temperatura y radiación solar extrema, la sequía y tener relativamente poco tiempo húmedo para la actividad metabólica (Belnap, 2003; Van Der Heijden, Bardgett & Van Straalen, 2008). Así mismo, en los agroecosistemas tropicales, se desarrollan principalmente hongos micorrízicos los cuales captan diversos nutrientes proporcionando resistencia ante condiciones de suelos degradados (Van Der Heijden et al., 2008). A pesar de ello, las alteraciones del suelo debido al incremento del uso de fertilizantes sintéticos y la conversión de las tierras de cultivos, han venido causando cambios en la estructura de las comunidades microbianas (Lupatini et al., 2017). En este sentido, el manejo agronómico convencional tiene como objetivo aumentar la productividad dependiendo del uso excesivo de fertilizantes sintéticos, fungicidas y plaguicidas, en superficies extensas mecanizadas (Kremen & Miles, 2012; Brambila-Paz et al., 2015). Contrariamente, en la agricultura orgánica, la funcionalidad del agroecosistema se basa en la disponibilidad natural de nutrientes vegetales, control biológico de plagas, siembra con labranza reducida o sin ella, y la aplicación de nutrientes naturales capaces de aumentar la diversidad biológica y el COS (Van Bueren, Struik & Jacobsen, 2002; Ghimire et al., 2017).

Es por ello que, el uso de biofertilizantes en una de las prácticas de agricultura orgánica que ha ido incrementando debido a que promueve la sustentabilidad y

productividad de los agroecosistemas (Ghany et al., 2014). Consiste en el empleo de microorganismos benéficos capaces tomar sustancias para ser asimiladas por las plantas, movilizar nutrientes e inducir resistencia de las plantas antes situaciones de estrés biótico y abiótico (García et al., 2015). Dichas cualidades son posibles mediante diversos mecanismos como la fijación biológica del nitrógeno atmosférico (Tairo & Ndakidemi, 2013), promoción del crecimiento de las plantas (Babalola, 2010), solubilización de fosfatos (Babalola, 2010; Akram et al., 2020), producción de fitohormonas (Glick, 2012), entre otras, lo cual en conjunto promueve la fertilidad del suelo (Kumar et al., 2018). Dado que aumentan la calidad del suelo a largo plazo sin tener efectos residuales y nocivos, además de que se reducen los costos de fertilización sintética, son mucho más rentables (Akram et al., 2020).

En el contexto tanto del costo como del impacto ambiental de los fertilizantes químicos, la dependencia excesiva de los fertilizantes químicos no es una estrategia viable a largo plazo debido al costo, tanto en recursos nacionales como en divisas, que supone el establecimiento de plantas de fertilizantes y el mantenimiento de la producción. Los biofertilizantes son productos que probablemente serán comercialmente prometedores a largo plazo una vez que los productores y agricultores dispongan de información adecuada (Kumar et al., 2018). Se han llevado a cabo investigaciones en distintos agroecosistemas sobre el impacto en cultivos de diversos sistemas de manejo agrícola en la estructura y dinámica de las comunidades bacterianas del suelo y de cómo ello afecta las reservas de COS (Van Diepeningen et al., 2006; Berthrong, Buckley & Drinkwater, 2013; Lee & Schmidt, 2014; Pan et al., 2014;Delgado-Baquerizo et al., 2016; Vasconcellos et al., 2016; Xu et al., 2017). Además, se ha sugerido realizar estudios que empleen tecnologías de secuenciación masiva de siguiente generación (NGS) (Liu et al., 2020), ya que a través de ella se puede realizar la amplificación del gen 16S rRNA (Pan et al., 2014), proveyendo información que puede ayudar a inferir el potencial funcional de las comunidades bacterianas en el suelo (Cadena et al., 2016; Liu et al., 2020). Sin embargo, en México los

estudios sobre la diversidad de microorganismos en suelos con manejo orgánico y convencional y su impacto en el secuestro de carbono, son escasos. Por lo tanto, resulta interesante realizar el estudio de las características del suelo relacionadas con la dinámica general del carbono, así como de la estructura y función de las comunidades bacterianas en suelos de cultivos de interés comercial en agroecosistemas áridos y tropicales de México bajo distintos manejos agronómicos.

2. OBJETIVOS

2.1 Objetivo general

Evaluar efecto de métodos de manejo orgánico y convencional en agroecosistemas de regiones áridas y tropicales, con respecto a la acumulación y secuestro de carbono orgánico y la diversidad de la comunidad bacteriana del suelo.

2.2 Objetivos específicos

- Identificar variables químicas y biológicas del suelo que son afectadas por el manejo orgánico y convencional en cultivos de nogal y café y su relación con las reservas de carbono orgánico.
- Determinar la estructura y composición de las comunidades bacterianas del suelo en cultivos de nogal (en zona árida) y café (en zona tropical) bajo manejo orgánico y convencional, a través de la secuenciación masiva de una región del gen 16S rARN.

3. HIPÓTESIS

El manejo orgánico en cultivos de regiones áridas y tropicales genera una mayor diversidad bacteriana del suelo y mayor eficiencia en el secuestro de carbono que el manejo convencional.

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CAPITULO II

EFFECT OF ORGANIC AND CONVENTIONAL SYSTEMS USED TO GROW PECAN TREES ON DIVERSITY OF SOIL MICROBIOTA

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ABSTRACT

Agronomic management modifies the soil bacterial communities and may alter the carbon fractions. Here, we identify differences in several chemical and biological soil variables, as well as bacterial composition between organic (Org) and conventional (Conv) agronomic management in pecan (*Carya illinoinensis*) orchards located in Coahuila, Mexico. The analyzed variables were pH, N, P, K, soil organic matter, organic matter quality, soil organic carbon, C/N ratio, carbon fractions, microbial biomass carbon, easily extractable Glomalin, colony-forming units, CO_2 emissions, and the enzyme activity. The DNA of soil bacteria was extracted, amplified (V3-V4 16S rRNA), and sequenced using Illumina. To compare variables between agronomic managements, t tests were used. Sequences were analyzed in QIIME (Quantitative Insights Into Microbial Ecology). A canonical correspondence analysis (CCA) was used to observe associations between the ten most abundant phyla and soil variables in both types of agronomic managements. In Org management, variables related to the capture of recalcitrant carbon compounds were significant, and there was a greater diversity of bacterial communities capable of promoting organic carbon sequestration. In Conv management, variables related to the increase in carbon mineralization, as well as the enzymatic activity related to the metabolism of labile compounds, were significant. The CCA suggested a separation between phyla associated with some variables. Agronomic management impacted soil chemical and biological parameters related to carbon dynamics, including bacterial communities associated with carbon sequestration. Further research is still necessary to understand the plasticity of some bacterial communities, as well as the soil–plant dynamics.

Keywords: organic agriculture; soil organic carbon; 16S rRNA; sequencing; structure of the soil bacterial community.

INTRODUCTION

Microorganisms that live in the soil are among the most abundant and diverse organisms on earth [1]. The structure and metabolism of the soil bacterial communities are influenced by elements of the ecosystem such as climate, type of soil, and plant composition; however, one factor that greatly affects their composition and functioning is agronomic management [2–5]. Thus, conventional agronomic management (CAM) alters the distribution of organic material and affects the rate of mineralization of micro and macro elements in the soil [3], negatively impacting the long-range productivity of the soil due to the loss of organic matter and erosion [6]. In CAM, inorganic supplies, such as synthetic fertilizers and pesticides, are used [7]. These supplies affect the availability of nutrients in the soil, contaminating the surface and underground water, thus affecting the native biotic community [8]. Conversely, in organic management, the traditional cultivation methods (conservationist) are combined with modern techniques, excluding conventional supplies [9]. In these systems, crop rotation is practiced and residues from animals and organic vegetables are used to increase soil fertility and productivity [7–10]. Likewise, it has been reported that these practices also affect the long-term structure of the microbial community through the accumulation and chemistry changes of soil organic matter (SOM) [2].

Regarding the above, greater attention should be given to organic agricultural systems in perennial woody crops with commercial interest, such as pecan trees (*Carya illinoinensis*). These systems have the potential to improve soil fertility through microbial activity and carbon accumulation in the soil [10,11,12,13]. For pecan tree orchards, the essential practices to promote soil fertility involve using leguminous plants or wild herbs as ground cover, as well as using organic fertilizers [14]. The pecan tree is a perennial woody crop which produces the pecan nut, which is a highly nutritious food [15]. The world's leading producers of pecan nuts are China, United States, Iran, Turkey, and Mexico [16]. Furthermore, in Mexico, the states with a greater volume of pecan production are Chihuahua,

Sonora, and Coahuila. These pecan nuts are exported mainly to the United States, China, and Vietnam, at an annual amount in 2018 worth USD 751 million [17]. Considering the cultivation of organic pecan trees in Mexico, in 2011 there were 1000 hectares certified as organic [14].

Given the importance of preserving and increasing soil fertility in pecan tree cultivation, several research studies have been developed to determine soil organic carbon (SOC), SOM, carbon–nitrogen relation, the mineralization of carbon and nitrogen, microbial biomass carbon, enzyme activities in diverse management systems, age of the crops, and association with leguminous plants and grasses [11,12,18]. Other research has focused on the qualities of the essential oils in nuts and on the nutritional deficiencies of the foliage, as well as on pests and diseases [15,19,20]. However, although the role of microbial communities in carbon sequestration is relevant, there are not enough research studies related to the structure and functioning of bacterial communities in the soil wherein pecan trees are grown under different agronomic management. Therefore, the objectives of this study were to identify the soil variables that respond to agronomic management related to carbon dynamics, and to determine the structure and composition of the bacterial communities in the soil wherein pecan trees are grown under organic and conventional management. We hypothesize that the organic management of pecan tree cultivation will generate greater soil bacterial diversity, capable of promoting greater efficiency in carbon sequestration than conventional management.

MATERIALS AND METHODS

Study area

Soil samples were collected from pecan orchards (*Carya illinoinensis* (Wangenh.) K. Koch) containing Cheyenne, Wichita, and Western varieties under organic (Org) and conventional (Conv) managements, located in the municipality of Allende, Coahuila, Mexico (Org: N 28°20′05.72″ W −100°49′24.73″; Conv: N 28°21′57.30″ and W −100°46′06.02″) (Figure 1). Both orchards are located in the

same geographical region, where the type of soil corresponds to haplic xerosol and the texture is clay [21]. The prevalent climate is dry and semi-warm, where annual mean temperature ranges between 20 and 24 °C, and the annual mean rainfall is about 461 mm [22]. The orchard under organic management (100 ha) is 20 years old, and the commonly applied practices to the soil are the use of plant covers and the placing of organic matter (pecan tree residues). On the other hand, the orchard under conventional management (35 ha) is 30 years old, and chemical fertilizers and pesticides are applied in accordance with the technical guide for pecan tree management proposed by INIFAP (Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias) [23].

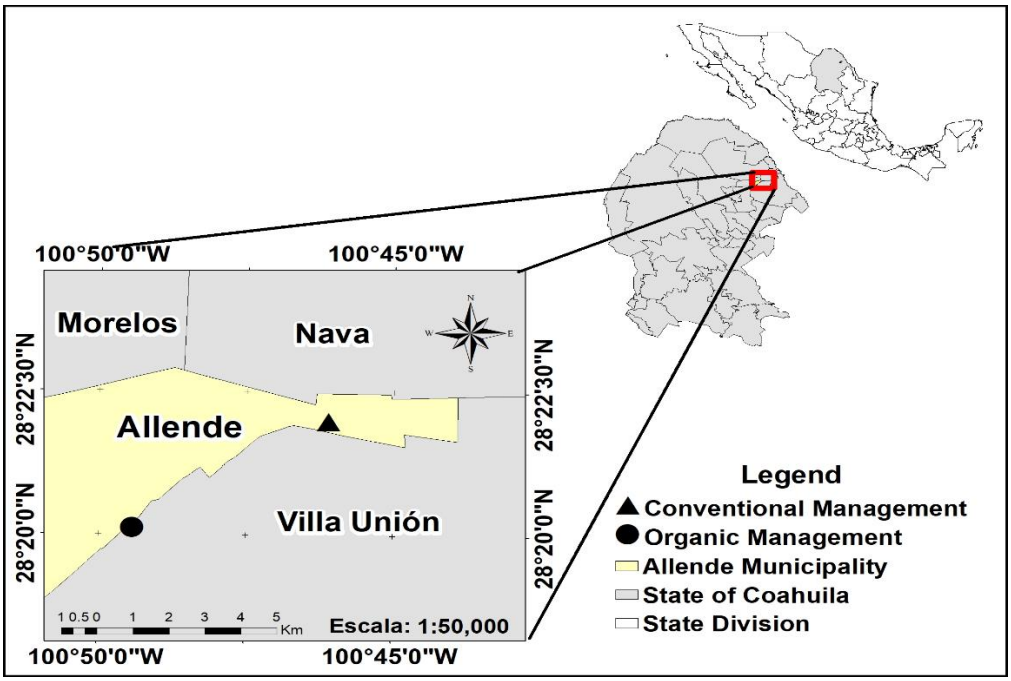

Figure 1.- Geographical location of pecan trees (*Carya illinoinensis*) orchards in Coahuila, Mexico.

Soil sampling and analysis of chemical and biological variables

Four pecan trees were systematically selected from each orchard. From each tree, four sub-samples were taken (one from each cardinal point) at a depth of 20 cm. Respective sub-samples were mixed obtaining composite samples (approximately 1 kg each). The samples were air-dried and sieved using a 2 mm mesh before analytical determinations. The pH was determined with a soil/water

ratio of 1:2 [24]. The essential nutrients, nitrogen (N) [25], phosphorous (P) [26] and potassium (K) [27], were quantified. Likewise, the SOM [28] and the organic matter quality (humic acids (HA), fulvic acids (FA), and humins (HS) [29,30]) were obtained. The SOC was obtained using a total organic carbon analyzer (TOC), and later the C/N ratio was estimated. Furthermore, the carbon fractions very labile (F1), labile (F2), less labile (F3) and recalcitrant (F4) were analyzed by digestion with H2SO4 at concentrations of 12, 18, and 24 N [31]. The microbial biomass carbon (MBC) was analyzed using the extraction fumigation method [32]. Furthermore, the easily extractable Glomalin (EEG) was determined [33], as well as the colony-forming units (CFU) by plate count (trypticase soy agar (total aerobic bacteria) and potato dextrose agar (PDA) (filamentous fungi and yeasts). Additionally, the CO_2 emissions from the soil were measured over 42 days under controlled conditions of humidity and temperature [34,35]. Furthermore, the enzyme activity of the soil was evaluated for lacasse (LAC), peroxidase (PER), polyphenol oxidase (PPO) [36,37], B-glucosidase (B-glu), and B-galactosidase (B-gal) [38].

DNA extraction, amplification and sequencing

Three trees from each pecan orchard were randomly selected to collect 0.25 g of soil from the rhizosphere zone, at a depth of 10 cm. Each sample was placed in a BashingBead™ (Zymo Research Corp., Irvine, CA, USA) cell lysis tube, containing 750 µl of lysing/stabilizing solution. Each tube was processed in a cellular disruptor (TerraLyzer™) for 30 s; samples were kept at ambient temperature. DNA was extracted using a Zymo BIOMICS™ (Zymo Research Corp., Irvine, CA, USA) kit. The amount of DNA obtained was measured in a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). The V3-V4 region of the 16S rRNA gene was amplified using the primers suggested by Klindworth et al. [39]: S-D-Bact-0341-b-S-17, 5´-CCTACGGGNGGCWGCAG-3´ and S-D-Bact-0785-a-A-21, 5´ GACTACHVGGGTATCTAATCC-3´, (~460 pb amplicon), using the Illumina protocol [40]. The amplicons were purified with Agentcourt® AMPure® XP 0.8% beads (Beckman Coulter Inc., Brea, CA, USA). The Nextera XT Index KitTM was used to create the library, following the Illumina protocol [41]. The library

quantification, normalization (equimolarity) and next-generation massive sequencing ((MiSeq; Illumina, San Diego, CA, USA) 2 × 250 paired final readings) were developed following the 16S metagenomic protocol [40]. Sequence data were submitted to The National Center for Biotechnology Information (GenBank), accession numbers: Org samples (SAMN15365245, SAMN15365246, SAMN15365247; Conv samples (SAMN15365249, SAMN15365250, SAMN15365252).

Statistical Analysis

After verifying normality and homogeneity of variance, Student's t test or Welch's t test ($p < 0.05$) were used to compare the chemical and biological variables between pecan orchards. The DNA sequences were analyzed using Quantitative Insights Into Microbial Ecology (QIIME) [42] as suggested by García-De la Peña et al. [43]. The absolute abundance of OTUs at genus level was used to visualize the number of sequences vs. the number of OTUs to observe depth cover (asymptote curves); this graph was made in PAST ver 3.15 (44). A simple random rarefaction process was made to standardize all samples. Using the standardized file, relative abundances for the phylum level were obtained and represented as bar charts using Excel. Taxa at the genera level with a relative abundance greater than 1% were listed. Finally, a canonical correspondence analysis (CCA) was used to observe associations between the ten most abundant phyla and soil variables in both types of agronomic managements. The CCA was made in PAST [44].

RESULTS

Chemical and biological variables of the soil

Some chemical and biological variables of the soil showed significant difference between both orchards. The significantly higher variables in soil under Org management were: N, P, SOC, MBC, and HS. For Conv management, the soil variables that showed significantly higher values were: F2, CO_2 emission, EEG, HA, FA, LAC, B-glu, and B-gal (Table 1).

Table 1.- Chemical and biological characteristics of soil in pecan tree (*Carya illinoinensis*) orchards under organic and conventional management in Coahuila, Mexico. Mean values and standard deviation (±) are shown. An asterisk indicates when a Welch's t test was used; df = degrees of freedom; bold numbers indicate significant differences between managements ($p < 0.05$).

Variable	Organic	Conventional	t	df	P
pH	8.1 ± 0.09	8.1 ± 0.05	1	3	0.391
Nitrogen %	0.050 ± 0.004	0.043 ± 0.003	3.13	6	**0.020**
Phosphorous mg kg^{-1}	16.3 ± 5.80	5.6 ± 1.00	4.901	6	**0.003**
Potassium mg L^{-1}	0.52 ± 0.31	0.31 ±0.19	2.06	3.14	0.127*
Organic Matter %	2.97 ± 0.31	3.11 ± 0.11	-812	6	0.448
Organic Carbon %	1.91 ± 0.04	1.72 ± 0.02	7.071	6	**0.000**
Relation C/N %	38.6 ± 3.10	40.5 ± 2.00	-1.116	6	0.307
Very labile fraction C g kg^{-1}	12.30 ± 1.35	12.80 ± 1.59	-0.522	6	0.620
Labile fraction C g kg^{-1}	7.57 ± .0.70	10.65 ± 0.45	-6.392	6	**0.001**
Less labile fraction C g kg^{-1}	18.67 ± 3.17	18.50 ± 0.91	-0.068	6	0.948
Recalcitrant fraction C g kg^{-1}	4.94 ± 3.03	2.43 ± 0.49	1.822	3.58	0.151*
Mineralization of C mg CO_2 g^{-1}	176.0 ± 29.30	278.5 ± 64.80	-2.781	6	**0.032**
Easily Extractable Glomalin mg g^{-1}	0.5 ± 0.00	0.8 ± 0.00	-9.076	6	**0.000**
Microbial biomass carbon µg C g^{-1}	751.1 ± 73.70	77.0 ± 0.00	45.195	3	**0.000**
Colony forming units g^{-1}	516,000 ± 323,777	1, 200 777 ± 701,683	-1.516	6	0.180
Humic acids mg C kg^{-1}	1,657.4 ± 91.90	2,618 ± 181.80	-10.464	6	**0.000**
Fulvic acids mg C kg^{-1}	8,671.4 ± 144.20	9,632.2 ± 144.10	-8.497	6	**0.000**
Humins mg C kg^{-1}	14,316.3 ± 221.80	7,350 ± 166.40	50.229	6	**0.000**
Peroxidase µmol g^{-1} h^{-1}	4.34 ± 0.71	4.50 ± 0.16	-0.472	6	0.653
Polyphenol oxidase µmol g^{-1} h^{-1}	6.41 ± 0.53	7.23 ± 0.57	-2.021	6	0.090
Lacasse µmol g^{-1} h^{-1}	0.02 ± 0.01	0.18 ± 0.00	-13	6	**0.000**
B-glucosidase mg PNP g $^{-1}$	145.1 ± 7.20	510.6 ± 19.5	-42.287	6	**0.000**
B-galactosidase mg PNP g $^{-1}$	24.2 ± 9.50	54.4 ± 1.00	-4.608	3.03	**0.019***

Abundance of bacterial taxa

The average number of sequences assembled for Org was 230,680, and for Conv was 267,539. After taxonomic designation an average of 28,062 bacterial sequences for Org, and 48,775 for Conv were obtained. The average number of OTUs was 5,995 for Org, and 7,937 for Conv (Table 2). Simple random rarefaction was made at 20,000 sequences, since at this point the number of OTUs reached asymptotes (Figure 2).

Table 2.- Soil bacterial sequences of pecan tree (*Carya illinoinensis*) orchards under organic (Org) and conventional (Conv) management in Coahuila, Mexico. CD = chimeras discarded, QS = quality sequences after chimeras were discarded, BS = bacterial sequences, OTUs = operational taxonomic units.

Sample	Total	Assembled	Discarded	CD	QS	BS	OTUs
Org 1	278,980	68,366	210,608	568	67,798	27,154	6,014
Org 2	225,583	52,984	172,599	486	52,498	22,087	5,594
Org 3	187,477	57,859	129,607	610	57,249	34,945	6,377
Mean	230,680	59,736	170,938	555	59,182	28,062	5,995
Conv 1	212,217	51,586	160,618	523	51,063	33,292	6,022
Conv 2	272,201	83,983	188,207	870	83,113	53,264	8,653
Conv 3	318,199	94,434	223,744	842	93,592	59,769	9,135
Mean	267,539	76,668	190,856	745	75,923	48,775	7,937

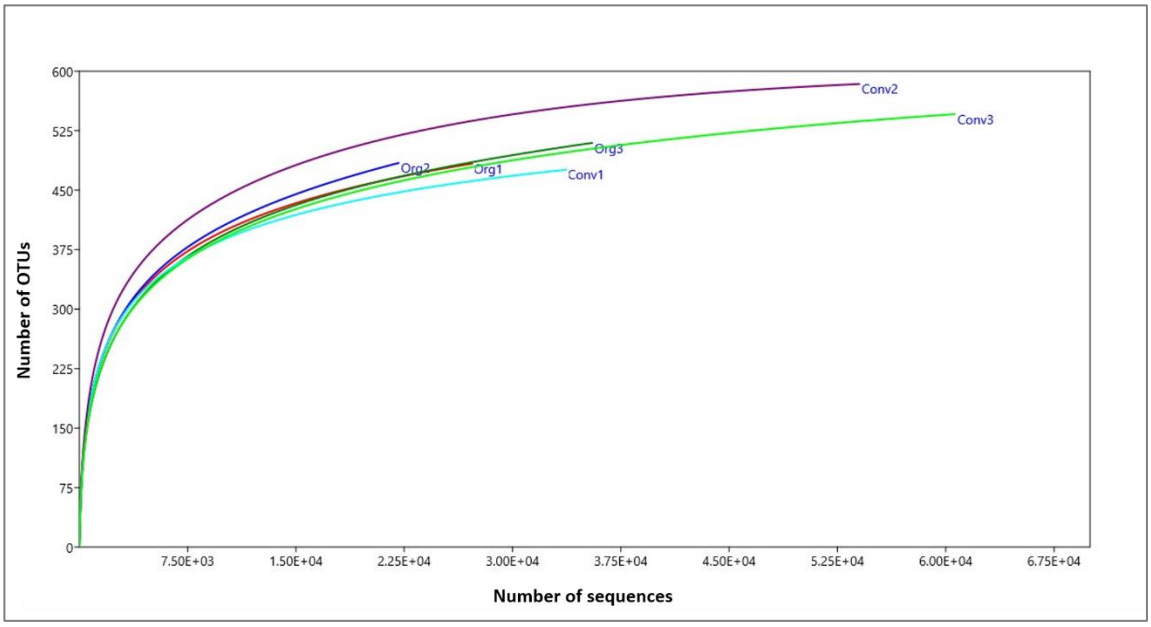

Figure 2.- Rarefaction curves for soil bacteria OTUs identified from pecan tree (*Carya illinoinensis*) orchards under organic (Org) and Conventional (Conv)

management in Coahuila, Mexico.

For Org management, the most abundant phyla were Proteobacteria ($\bar{x}$ = 36 %), Actinobacteria ($\bar{x}$ = 24 %), Planctomycetes ($\bar{x}$ = 18 %) and Chloroflexi ($\bar{x}$ = 13 %), (Figure 3a). A similar phyla composition was observed for the Conv management: Proteobacteria ($\bar{x}$ = 32 %), Actinobacteria ($\bar{x}$ = 26 %), Planctomycetes ($\bar{x}$ = 19 %) and Chloroflexi ($\bar{x}$ = 15 %), (Figure 3b). The remaining phyla were Acidobacteria, Gemmatimonadetes, Verrumicrobia, Cyanobacteria, Parcubacteria, and Saccharibacteria.

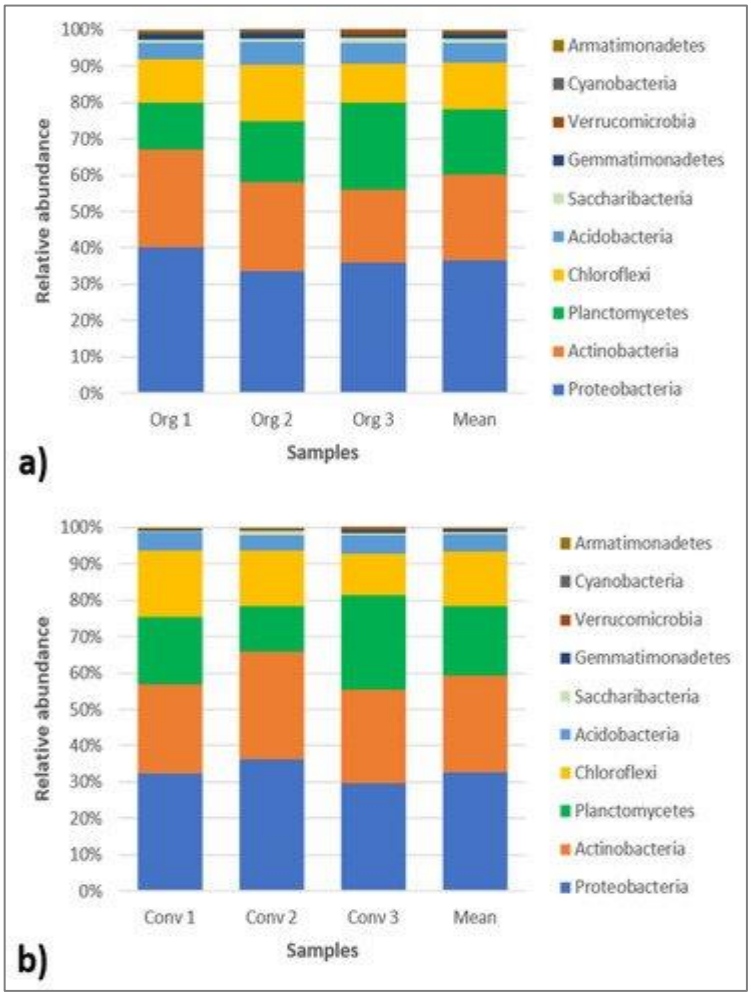

Figure 3.- Relative abundance (%) per sample and means of the main bacterial phyla in soil samples of pecan tree (*Carya illinoinensis*) orchards under organic (a) and conventional (b) management in Coahuila, Mexico.

A total of 776 bacterial genera was obtained, of which 29 had a relative

abundance greater than 1% (20 had a taxonomical name, and 9 had a taxonomical key). The three more abundant cultivated genera were *Tepidisphaera* ($\bar{x}$ = 7.1%), *Sphingomonas* ($\bar{x}$ = 3.2%), and *Gemmata* ($\bar{x}$ = 4.0%). The first two genera were more representative in the Conv management, while the third one was more representative in the Org management. The remaining genera were *Dongia*, *Microvirga*, *Sphingosinicella*, *Streptomyces*, *Rhizomicrobium*, *Stenotrophobacter*, *Pseudolabrys*, *Zavarzinella* and *Catelliglobosispora*; all of these were present in both managements (Table 3).

Table 3.- Relative abundance of the bacterial genera found in the soil of pecan tree (*Carya illinoinensis*) orchards under organic and conventional management in Coahuila, Mexico. Only those genera whose relative abundance was ≥ 1 % are shown. Asterisks indicate greater abundance according to the type of management.

Genera	Relative abundance %	
	Organic	Conventional
Tepidisphaera	3.8	7.1*
GQ396871	3.9	3.7
Sphingomonas	2.6	3.2*
Gemmata	4.0*	3.1
Dongia	3.1*	2.1
FJ478799	4.2	2.0
Microvirga	0.9	1.9*
GQ263023	1.4	1.8
Sphingosinicella	0.5	1.7*
Streptomyces	1.5	1.6*
Rhizomicrobium	1.1	1.3*
EU335288	0.9	1.2
AF370880	1.6	1.2
FJ479444	0.7	1.2
Stenotrophobacter	0.9	1.2*
EF125410	0.3	1.2
EU669599	0.3	1.1
Pseudolabrys	1.2*	1.1
Zavarzinella	1.7*	1.1
EU335161	1.1	1.0
Catelliglobosispora	1.2*	1.0

The CCA suggested that in both managements there is a separation between the phyla, which is associated with some chemical and biological characteristics of

the soil. However, this separation was visible only in the second axis (Figure 4). Regarding axis 1, the phyla located left of the “y” axis belong to Gemmatimonadetes, Cyanobacteria, Parcubacteria, Chloroflexi, Actinobacteria, Proteobacteria, and Saccharibacteria the same that are favored by PER (−0.64) and F3 (−0.62). In contrast, on the right side of the “y” axis, the Verrumicrobia, Planctomycetes and Acidobacteria phyla were located in association with pH (0.66) and C/N (0.94). Above the “x” axis, the Org management samples were found, wherein the phyla belonging to Gemmatimonadetes, Parcubacteria, Proteobacteria, Cyanobacteria, Acidobacteria, and Verrumicrobia were favored by the presence of HS (0.96). On the other hand, the phyla located below the “x” axis were Chloroflexi, Actinobacteria, Planctomycetes and Saccharibacteria, which were associated with EEG (−0.98), FA (−0.97) and B-glu (−0.97).

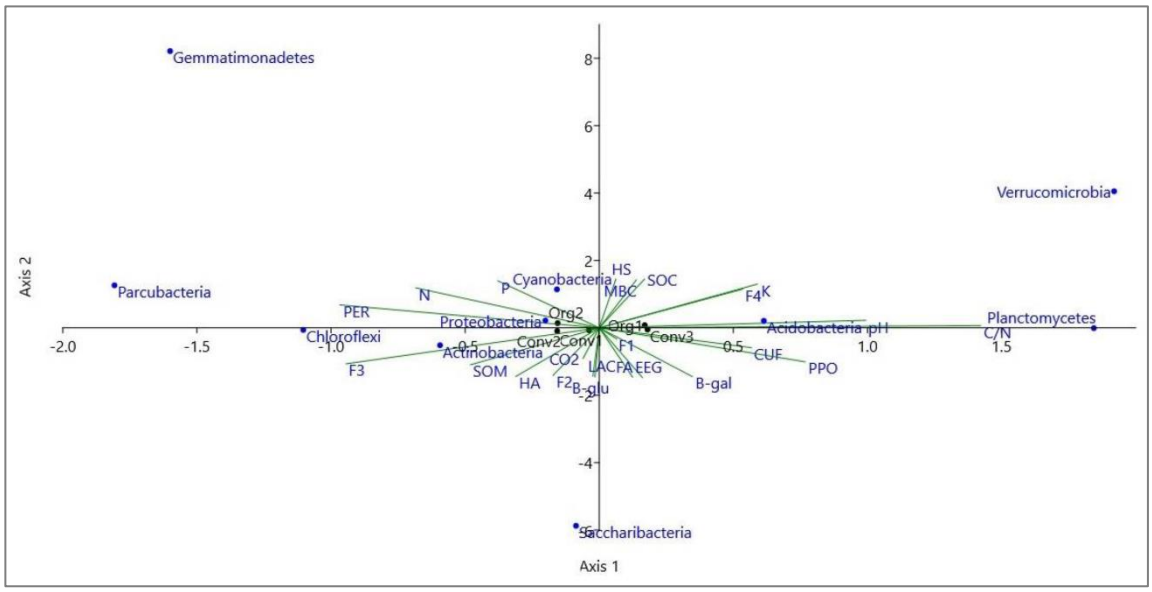

Figure 4.- Canonical Correspondence Analysis of soil from pecan tree (*Carya illinoinensis*) orchards under organic and conventional management in Coahuila, Mexico, identifying associations among the main phyla and the evaluated soil variables.

DISCUSSION

Chemical and biological variables of the soil

The management of the soil in agricultural systems affects its physical, chemical and biological characteristics [5]. It has been demonstrated that the concentration of N is higher in soils under Org management, due to the abundance of microorganisms capable of mineralizing N more efficiently [2,45]. Likewise, the addition of organic amendments generates a greater availability of nutrients such as P, which is mainly found in humic substances or in the microbial biomass of the soil [46,47]. In this study, the SOC content was higher than reported by other authors in pecan tree orchards [11,18]. In addition, HS are considered to be the most recalcitrant fraction of the organic soil and, therefore, are able to stay in the soil for longer [48]. It is likely that most of the SOC under Org management will be stabilized in less labile and recalcitrant forms. With respect to MBC, it has been shown that its content increases with the long-term establishment of plant cover, as in this study [49,50], while nitrogen fertilization tends to decrease it [51]. Likewise, it is the main agent of SOM decomposition, transforming nutrients and making them available [52], which may explain the high content of MBC and the low percentages of SOM in pecan orchards under Org management.

In contrast, it has been shown that the F2 fraction has a high rate of decomposition and a shorter residence time in the soil [53], thus responding to Conv management by releasing carbon into the atmosphere in the form of CO_2, causing losses of nutrients and soil fertility [54] contrary to what happens in soil Org management [55,56]. On the other hand, organic materials with a low degree of humification, i.e., labile, increase the HA and FA fractions [57,58]. According to Vásquez et al. [59], there is a positive correlation between CO_2 emissions and carbon labile fractions. Carbon loss in the form of CO_2 is caused by the decomposition of SOM by heterotrophic microorganisms, and occurs mainly when there is an increase in the availability of SOM and when it lacks a biochemical composition enriched with recalcitrant organic compounds, which makes it more difficult for microorganisms to disintegrate [60]. The results herein reported suggest that in the pecan tree orchard under Conv management, in spite of the fact that the content of SOM is greater than under Org management, the SOC is

less stable and could easily be lost in the form of CO_2, because the SOM lacks recalcitrant organic compounds [60,61,62].

Agronomic management is influential in the increase of concentrations of glomalin [63], which agrees with the current study since the amount of glomalin was greater in Conv management. In this regard, the high concentration of CO_2 produces effects in the increase of the glomalin reserves [64], which suggests that glomalin is related to the high respiration rate in soils under Conv management. Furthermore, the oxidative enzyme LAC (EC 1.10.3.2) participates in the degradation and biosynthesis of lignin, and its activity may be increased by substrates that degenerate rapidly, such as cellobiose and glucose, and with an increase in fungi growth [65,66]. The above may suggest that the increase in oxidative enzymatic activity in the soil under Conv management may be due to the presence of substrates that may easily be degraded by fungi, which could also explain the high content of glomalin under this same management. As for β-glu (EC 3.2.1.21), it participates in the hydrolysis of cellobiose to glucose [67], degrading plant cell walls and contributing to the first phases of plant cell tissue decomposition [68]. The increased activity of β-gal (EC 3.2.1.23) may suggest that soil microorganisms are metabolically more active with rapid proliferation, thus increasing the efficiency of enzyme production [69]. Furthermore, as these enzymes degrade labile carbon compounds, their activity shows how the microorganisms present in the soil under Conv management mineralize this carbon fraction to obtain nutrients, but not to promote carbon sequestration [70].

Abundance of bacterial taxa

Proteobacteria phyla abound when there is a high availability of nutrients due to an increase in SOM, and also, they are able to consume labile organic carbon [71,72,73], which may suggest that the disposition of SOC in soil under Org management is subject to microbial decomposition of the SOM, and the velocity of this decomposition depends on, among other variables, the availability of nutrients [74]. The Actinobacteria phylum has diverse physiological properties, such as the production of extracellular enzymes for the decomposition of organic

matter [75]. It has also been shown that Actinobacteria abundance is associated with the respiration rates of the soil [76,77], which agrees with the current study, since CO_2 emission was greater under Conv management. Regarding bacterial genera that were more abundant in soil under Org management, *Gemmata* is capable of using glucose and galactose as a source of carbon in order to thrive, and therefore it is an important producer in the carbon and N cycles [78,79]. Likewise, it has been shown that *Dongia* and *Pseudolabrys* genera were significantly more abundant in the soils under non-tillage and addition of organic residues [80]. Regarding *Zavarzinella*, in spite of its importance, nowadays its isolation and behavior have been demonstrated in macroalgae [81], while there has been little or no research done in soils. Lastly, the *Catelliglobosispora* genera have also been positively correlated with sucrose [82] and have a high potential for the deconstruction of cellulose and chitin [83]. It seems probable that the establishment of vegetation covers and deposits of organic matter in Org management will promote sources of carbon, which increase the abundance of *Gemmata*, *Dongia*, and *Pseudolabrys*. Regarding the more representative genera under Conv management, *Tepidisphaera* hydrolyzes a broad range of carbohydrates, among which are glucose and galactose, essential components in SOM [84]. On the other hand, in agricultural soils, some species of *Sphingomonas* have shown the capacity for degrading chemical compounds of herbicides into CO_2 that is liberated into the atmosphere [85], which may suggest that the abundance of both genera may be related to the SOM content and the mineralization process of carbon under this management.

The *Streptomyces* genera have been described at length for their adaptation to soils, where they are capable of forming hyphae that branch out to attach to and penetrate into insoluble organic residues from plants and other organisms, as well as recalcitrant insoluble inorganic polymers such as chitin and cellulose [86,87]. In this regard, studies show that the production of oxidative enzymes, such as LAC, associated with the population growth of *Streptomyces*, intervene in the degradation of lignocellulosic compounds [88,89]. Regarding *Rhizomicrobium*, a significant abundance has been reported in soils contaminated with fluoride and

chloride [90] as well as in soils where organic residues are applied [80]. Finally, it has been shown that the *Stenotrophobacter* genera participate in the carbon and N cycle and respond to agronomic management [91,92]. These results suggest that the application of organic and inorganic compounds to the soil used in the conventional cultivation of pecan trees affects the bacterial abundance and functional diversity, which impacts the functional properties of the soil, particularly those related to carbon forms [6,93,94]. The results obtained from the CCA confirm that the bacterial communities in the soil were influenced by the type of management. Under the Org management, the Acidobacteria phylum was benefited by HS content. As was mentioned before, HS are the most recalcitrant fraction of organic soil due to the stabilization of SOC [31,48,95]. Rawat et al. [96] showed that Acidobacteria communities are essential to the cycle of carbon in the soil, and that its activity and dominion depend on them. Likewise, being classified as oligotrophic organisms, they are related to C sequestration, since it has been demonstrated that they are autotrophic and have the capacity to fix atmospheric CO_2 in the soils of arid and semi-arid ecosystems [97,98], and thus contribute to the generation of organic carbon reservoirs [99,100]. Conversely, under Conv management, the bacterial communities of the Actinobacteria Phylum were influenced by the EEG, FA, and B-glu variables. Thereon, the change in the microbial community of fungi and Actinobacteria is due to the amounts of organic residues that result from the availability of resources [6,72,101]. It has been shown that both microbial communities are fundamental in the degradation process of complex compounds such as cellulose, lignin, and chitin [102], where Actinobacteria, in particular, present diverse physiological properties, such as the production of extracellular and metabolic enzymes related to the decomposition of SOM [75].

CONCLUSIONS

The use of organic practices in the cultivation of pecan trees seems to influence the concentration of nutrients and various chemical and biological variables in the

soil, mainly in the capture of recalcitrant C compounds. Furthermore, this type of management could favor bacterial communities capable of promoting greater efficiency in organic carbon sequestration. On the other hand, conventional management practices influence the increase in carbon mineralization, as well as the enzymatic activity of the soil, particularly that of the enzymes related to the metabolism of labile compounds. The use of genomic technologies has allowed the discovery of the soil microbiome in recent years, however it is still necessary to understand the adaptation and plasticity of some bacterial communities and other soil microorganisms, as well as their functional biodiversity and soil–plant dynamics, which are essential in order to preserve the optimal state of the soil.

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CAPITULO III

A METAGENOMIC APPROACH IN THE EVALUATION OF THE SOIL MICROBIOME IN COFFEE PLANTATIONS UNDER ORGANIC AND CONVENTIONAL PRODUCTION IN TROPICAL AGROECOSYSTEMS

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ABSTRACT

The aim of this study was to determine the soil microbiome throughout mass sequencing in coffee plantations managed with either an organic (OAM; i.e., bio-fertilizers *Azospirillum brasilense* and *Glomus intraradices*) or a conventional (CAM; i.e., traditional NPK-fertilization) agronomic systems. Soil microbiome samples were collected in tropical eastern Mexico (Veracruz, 19°28' N & 96° 52' W), with annual average temperature and rainfall of 24.8° C, and 882.6 mm, respectively. Upon DNA soil-microbiome extraction, the V3-V4 16S rRNA region was amplified, and sequenced (Illumina). Results were analyzed with QIIME based on the EzBioCloud reference. Diverse phyla (n=16), classes (n=40), orders (n=90), families (n=135) and genera (n=333) were identified. The diversity index values were similar in both treatments, with Shannon's being 9.7 and Simpson's 0.99. While the phylum Proteobacteria was more abundant in CAM-soils and classified as copiotrophic, the phylum Acidobacteria was more abundant in OAM-soils and classified as oligotrophic. This classification may be related to the application of microorganisms and their effect on the soil´s state of organic matter and carbon fractions. Our research outcomes indicate that the application of bio-fertilizers promoted an increased presence of Acidobacteria, a phylum positively correlated with organic matter while significantly involved in carbon sequestration. Undisputable, metagenomics emerges as an interesting up-to-date genomic technology for unveiling the hidden content of the soil microbiome black box.

Key words: **16S rRNA gene, microorganisms, copiotrophic, oligotrophic, bio-fertilizer.**

INTRODUCTION

Microorganisms (MOS) are the living component of the soil; their abundance and metabolic activity depend mainly on the soil type and management, plant growth, as well as root exudates. Certainly, MOS activity influences the availability of essential elements for the plants in that they participate in the decomposition of organic matter (Schinner, 2012; Singh et al., 2011). In this regard, a lack of nutrients in the soil, triggered by different conventional agronomic practices, modifies the composition, structure, and activity of microbial communities (Hernández et al., 2013). This modification avoids the soil from acting as a substrate in that organic fractions are altered, the last being directly proportional to the amount of organic carbon in soil (Bastida et al., 2015).

Diverse management practices in agricultural production systems can modify the soil microbiome properties, as well as increase the microbial biomass content (Diacono and Montemurro, 2011). One of these practices is bio-fertilization, in which probiotics are used because of their ability, through biological processes, to mobilize nutrients in the soil and make them available to plants (Ritika and Utpal, 2014). These probiotics also promote plant growth and yield, which in turn has a positive effect on humification of organic matter and conservation of microbial structures (Cotler et al., 2016; Ortega et al., 2016). Therefore, it is important to consider this type of organic practice in crops of economic interest, since in these crops conventional practices are usually employed, and they can affect the microbial structure of soil and, consequently, its fertility.

One of the most economically and culturally important crops worldwide is coffee, with a harvesting area of 10.6 million ha and a production of 10.3 million tons. In Mexico, coffee (*Coffea arabica* L.) is also an important crop, harvested in more than 630,000 ha, a production of 158,323 tons while 250 million USD economic value. Nonetheless, from the total planted coffee area in Mexico, only 3.24% is classified as organic production; Chiapas, Veracruz and Puebla are the main producer's states of both conventional and organic coffee, the latter standing out

because of its high demand by North American and European markets. Coffee cultivation constitutes 0.66% of the national agricultural gross domestic product, with an annual per capita consumption of 0.6 kg (Flores, 2015; SAGARPA, 2017 and 2018).

Because of such economic importance, diverse agricultural management practices have been proposed to maximize coffee production through the implementation of organic-based production systems to promote the recovery of the soil's optimal status (SAGARPA, 2017). For these reasons, is important to identify and analyze the presence of microorganism communities in soils under organic production systems where organic practices are being implemented to conserve and enhance biotic resources available in coffee plantations (Hernández et al., 2018). Besides, it is fundamental to know the main processes that affect the soil fertility, such as the presence and dynamics of soil microorganisms (Barea et al., 2005; Carbonetto et al., 2014). Certainly, a better understanding of the dominant bacterial taxa in the soil will improve our understanding regarding the main MOS functional capabilities (Delgado et al., 2018).

Nowadays, metagenomics is a tool for the study of bacterial communities used to determine, explore, and analyze microbial communities from diverse environments, through the sequencing of their genetic material (Cadena et al., 2016; Ospino et al., 2018).

Previous studies have been carried out on coffee plantations to determine how the chemical and biological properties of soil are modified in response to organic or conventional management systems. Some of the most evaluated properties include pH, electrical conductivity, nitrogen, phosphorus, microbial biomass carbon, enzymatic activity, microbial respiration, and bacterial colony forming units (Chemura, 2014; Lammel et al., 2015; Velmourougane, 2016). Similarly, some studies have focused on the isolation and identification of bacteria in plant and rhizosphere organs, using various methodologies. These studies have

confirmed the presence of endophytic bacteria in seeds, leaves, stems and roots, some of which have been isolated to evaluate the inhibition of disease-promoting bacteria (Vega et al., 2005; Shiomi et al., 2006). Other studies have been addressed on identifying the bacteria in coffee cherries through the polymerase chain reaction of the 16S rRNA gene (Silva et al., 2000; Vilela et al., 2010; Oliveira et al., 2013). In addition, the role of various rhizobacteria as nitrogen fixers (Jimenez-Salgado et al., 1997), their potential to synthesize acetic acid and to degrade ethanol precursors (Muleta et al., 2009), as well as their importance as phosphate solubilizers (Muleta et al., 2013) have also been studied.

Nonetheless, little is currently known about the response of soil bacterial communities to the type of management in coffee plantations, using the metagenomics approach in order to get a more accurate picture of the soil-microbiome structure and composition. Based on previous findings, we hypothesized that organic management, through the application of bio-fertilizers, could enhance the presence of bacteria involved in carbon sequestration (i.e. acidobacteria), and then, plant growth. Therefore, we determined the soil microbiome in coffee plantations managed under organic or conventional production systems, through massive 16S rRNA sequencing. Secondary objectives included: 1) to determine the bacterial structure and composition in soils, and 2) to establish the main changes in bacterial structure in soils under organic and conventional management.

MATERIALS AND METHODS

Location and environmental conditions of the study area. Soil-microbiome samples were collected in the State of Veracruz with an annual average temperature and rainfall of 24.8° C, and 882.6 mm, respectively (Díaz et al., 2006). The samples were collected from coffee plantations under organic (OAM) or conventional (CAM) agricultural management located in the municipality of Emiliano Zapata, Veracruz, Mexico (19°28’0.98’’ North, and 96°52’38.20” West;

Figures 1 and 2).

Treatments groups, soil microbiome sampling and samples management. The organic agronomic system (OAS treatment) corresponds to coffee plantations whose soil was treated with bio-fertilizer application (i.e. *Azospirillum brasilense* and *Glomus intraradices*; Biofábrica Siglo XXI®) during a five year period. In the OAS-treatment, two annual doses of bio-fertilizer were added, one at the beginning of the rainy season and the other three weeks later, at a concentration of 5x1011 Colony Forming Units of *A. brasilense* and 9x104 spores of *G. intraradices*.

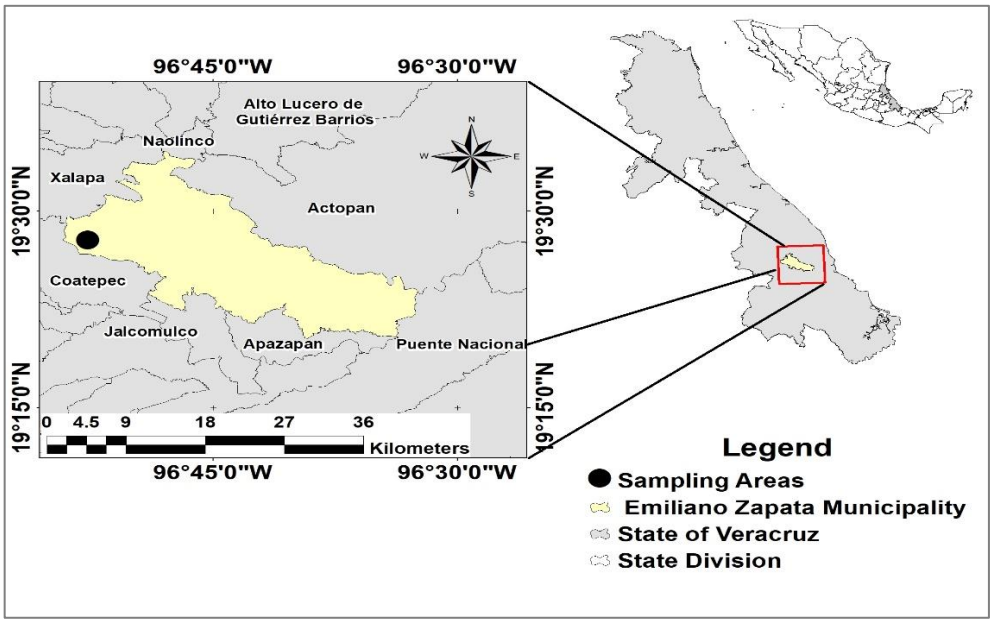

Figure 1.- Geographical location of the soil microbiome samples from coffee plantations either under organic (OAM) or conventional (CAM) agronomic systems in eastern tropical Mexico (Veracruz; 19° N, 96° W).

Figure 2.- (A) Plants of *Coffea arabica* located in the study area. (B) Soil microbiome sampling in coffee plantations either under organic (OAM) or conventional (CAM) agronomic systems in eastern tropical Mexico (Veracruz; 19° N, 96° W).

The control group corresponds to the conventional agronomic system (CAS treatment) where the soil´s coffee plantations were treated with two to three chemical applications of conventional fertilizers (16:16:16 and 17:7:14 NPK) per year were applied aligned to the rainy season. In both treatments groups (i.e., OAS and CAS), 0.25 g of soil was taken in the rhizosphere area at 10 cm deep. Each soil sample was placed in a Zymo Research™ BashingBead™ tube for cell lysis, then 740 µl of lysis/stabilization solution were added, and finally each tube was processed in a TerraLyzer™ cell disruptor for 30 s; samples were kept at room temperature.

DNA extraction from the soil´s microbiome and bioinformatics analyses. Soil microbiome DNA was extracted using the Zymo Research™ Xpedition™ Zymo BIOMICS™ kit in a laminar UV flow hood under sterile conditions and following the manufacturer's instructions. Briefly, the soil microbiome DNA was extracted on a 1.2% agarose gel at 80V for 45 min in a BIORAD electrophoresis

chamber (Bio-Rad Laboratories, Inc.) in order to visualize the presence of high molecular weight DNA. The visualization was carried out in a GelMax™ photo documenter (UVP LLC). The amount of DNA obtained was measured in a Qubit™ fluorometer (Invitrogen). Amplification of the V3-V4 region of the 16S rRNA gene was carried out according to Klindworth et al. (2013): S-D-Bact-0341-b-S-17, 5´-CCTACGGGNGGCWGCAG-3´ and S-D-Bact-0785-a-A-21, 5´ GACTACHVGGGTATC TA ATCC-3´, which produce an amplicon of ~460 bp. The sequences were synthesized with the "overhang" adapters of the Illumina protocol (2017a), being as follows: 5´-CGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3´ and 5´-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCT AAT CC-3´ (amplicon of ~550 bp).

The Illumina PCR protocol (2017a) was used, the amplicons were purified with 0.8% Agencourt® AMPure® XP beads, and then labeled using the Nextera XT Index KitTM for library preparation, following the Illumina protocol (2017b). Finally, quantification, normalization, library clustering and next-generation mass sequencing were performed following the 16S metagenomic protocol (Illumina, 2017a). The sequences were analyzed in the Oracle VM VirtualBox 5.1.14 on the MGLinux platform using Quantitative Insights Into Microbial Ecology bioinformatics software (QIIME) v.1.9.0 (Caporaso et al., 2010), as previously suggested (García-De la Peña et al., 2019). The number of sequences was plotted by the number of taxa at the genus level to observe the coverage depth (PAST version 3.15, Hammer et al., 2001). Using the KRONA program (Ondov et al., 2011), graphs were generated by treatment to visualize the taxonomic levels from phylum to genus. Alpha diversity was determined using the Shannon and Simpson indices.

RESULTS AND DISCUSSION

The arithmetic mean of the total number of sequences obtained for both treatments before assembly was 34,991, whereas the mean of assembled and discarded sequences was 13,791 and 21,200, respectively. On average, 91 chimeras were removed, leaving a mean of 13,700 quality sequences. After taxonomic assignment, a mean of 12,606 bacterial sequences was obtained; however, once the singletons were removed, the final sequences were 5,559. The rarefaction curves for the operational taxonomic units (OTUs) recorded an adequate coverage depth where the samples reaching the asymptote at about 6,000 sequences (Figure 3). The diversity index values were similar in both treatments, with Shannon's being 9.7 and Simpson's 0.99. In both soil samples, 16 phyla were recorded, of which Proteobacteria (54%), Acidobacteria (22%) and Planctomycetes (7%) were the most abundant in the OAM group (Figure 4); corresponding values for the CAM treatment were 56%, 16% and 7% (Figure 5). A total of 40 classes were determined, among which Alphaproteobacteria, Solibacter and Acidobacteria were the most abundant in both treatments; OAM recorded 53%, 11% and 7% (Figure 4), while CAM recorded 56%, 5% and 3%, respectively (Figure 5).

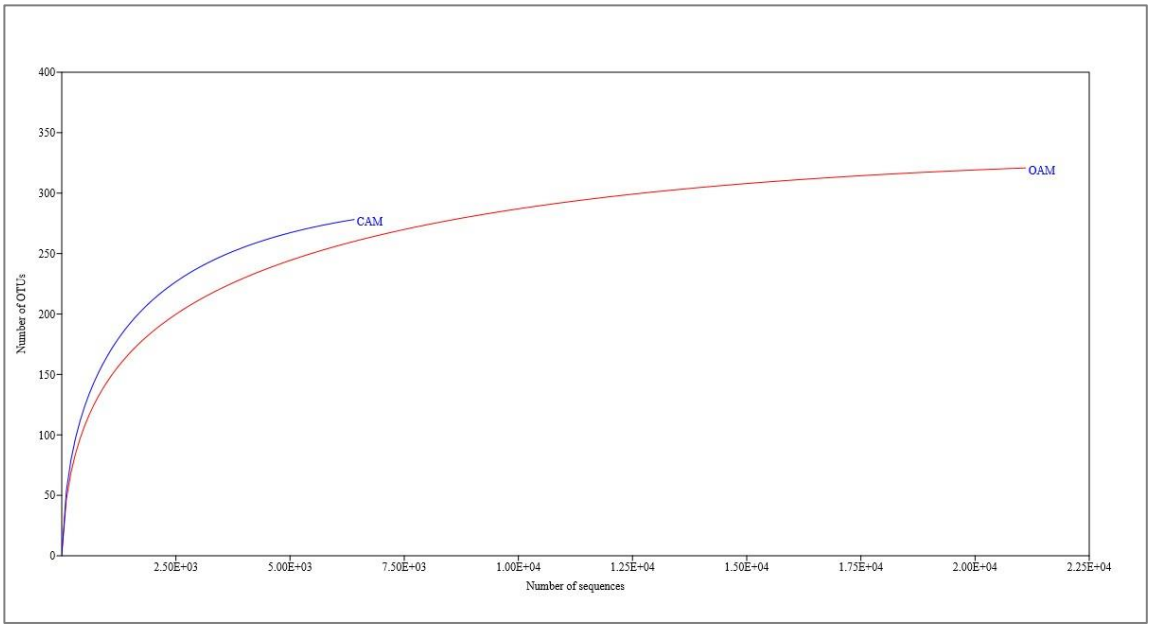

Figure 3.- Number of sequences by the number of operational taxonomic units

(OTUs) in soil microbiome samples from coffee plantations either under organic (OAM) or conventional (CAM) agronomic systems in eastern tropical Mexico (Veracruz; 19° N, 96° W).

From the 90 orders obtained, Rhizobiales, Rhodospirales and Sphingomonadales showed the highest percentages; corresponding values for the OAM were 23%, 20% and 7%, respectively (Figure 4), with corresponding values of 28%, 16% and 10%, for the CAM group. (Figure 5). From the 135 recorded families, Rhodospirillaceae (19%), Bradyrhizobiaceae (16%) and Sphingomonadaceae (7%) predominated in the OAM group (Figure 4), whereas the CAM treatment obtained corresponding values of 15%, 14% and 9%, respectively (Figure 5).

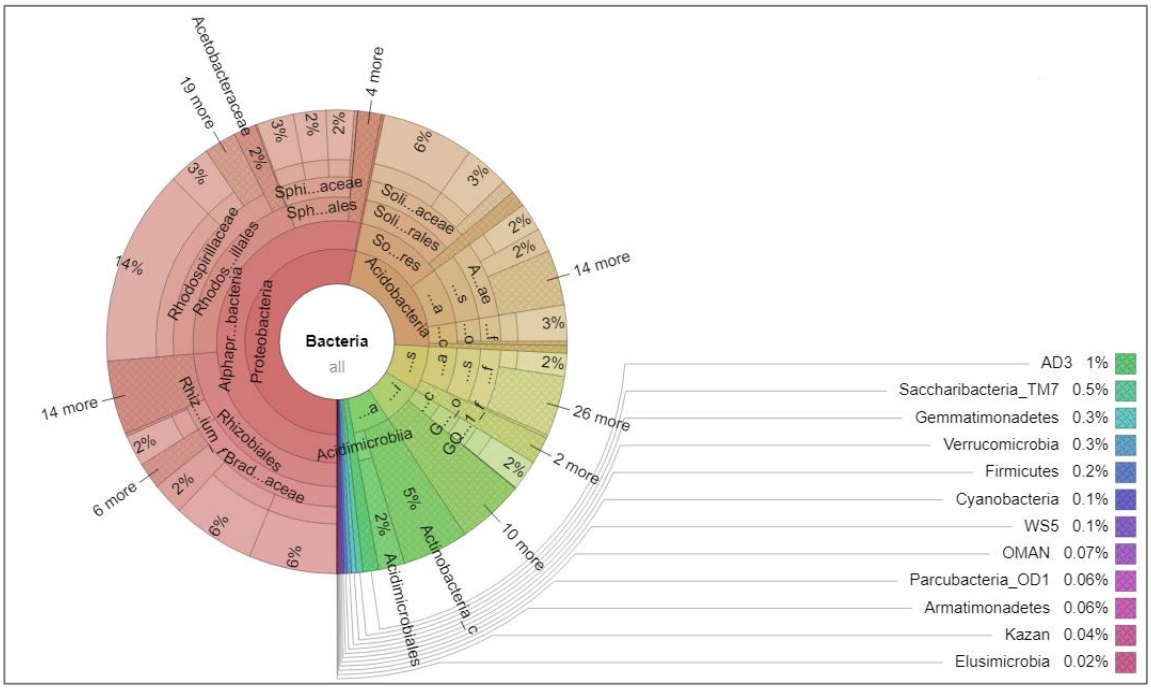

Figure 4.- Relative abundance of bacterial taxa found in soil microbiome samples from organic coffee plantations in eastern tropical Mexico (Veracruz; 19° N, 96° W).

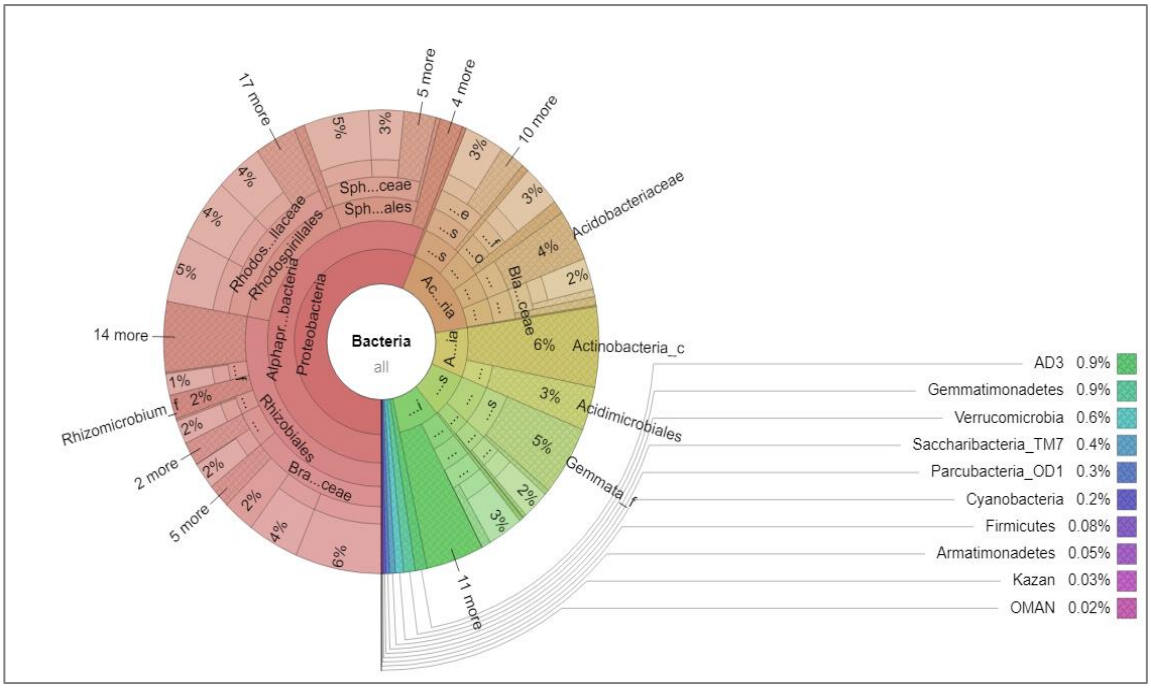

Figure 5.- Relative abundance of bacterial taxa found in soil microbiome samples from conventional coffee plantations in eastern tropical Mexico (Veracruz; 19° N, 96° W).

In both treatments, a total of 333 genera were reported. The most abundant were *Pseudolabrys* (6%), *Rhodoplanes* (5%) and *Solibacter* (6%) in the OAM (Figure 4), with corresponding values of 6%, 3%, and 2%, in the CAM group (Figure 5). Proteobacteria was 2% more abundant in CAM than in OAM, while Acidobacteria was 6% more abundant in OAM, with no difference in Planctomycetes abundance.

The working hypothesis stated that in the production of organic coffee, the application of bio-fertilizers would promote an increased presence of acidobacteria, a phylum positively correlated with organic matter while significantly involved in carbon sequestration. The obtained outcomes of the study support such a hypothesis. The observed soil-bio-fertilizer-biome a scenario in our study suggest increases in the stable fractions of organic matter, making more resistant to decomposition while increasing carbon sequestration in soil, a situation which in at same time, improves plant growth by stimulating root

development, as well as nutrient and water uptake (Dębska et al., 2016; Fatunbi and Ncube, 2009).

Acidobacteria has been reported as one of the most abundant phyla in tropical forests and ecosystems similar to those in the study area (Carbonetto et al., 2014; Delgado et al., 2018). In the same way, the phylum Acidobacteria and Proteobacteria have been grouped as oligotrophic and copiotrophic organisms, respectively (Zhalnina et al., 2014). Most oligotrophic organisms are located in soils with low nutrient availability and a high amount of recalcitrant organic matter. On the other hand, copiotrophic organisms are abundant in conditions with high available nutritional yields and are capable of consuming labile organic carbon (Carbonetto et al., 2014; Koch, 2001; Vigdis and Øvreås, 2008). Moreover, the enrichment of copiotrophic bacteria has been related to the increase of CO2 emission (Sheng and Zhu, 2018). On this regard, Fierer et al. (2007) showed that a greater abundance of Acidobacteria occurs in the soils with low mineralization rates; in contrast, the abundance of Proteobacteria was higher in the soils with high carbon availability. Acidobacteria dominates the soils with high organic matter content and is involved in the microbial degradation of lignocellulose plant biomass. They are also capable of using carbon as an energy source and their distribution is related to the availability of carbon in the soil (Eichorst et al., 2011; Rawat et al., 2012).

Also, members of this phylum, to which the classes Solibacter and Acidobacteria belong, and the genus Solibacter, are essential drivers in ecosystem processes (Kielak et al., 2010; Zhang et al., 2014). In a study by Wang et al. (2020), phylum Acidobacteria was more abundant in soils without agronomic management (23%) than in soils with conventional management (20%). On the other hand, Pan et al. (2014) showed that in grassland soils after applying nitrogen fertilization, there was a positive correlation between the phylum Proteobacteria and different forms of nitrogen (ammonium, nitrate, and nitrite). Otherwise, the most abundant class of pool was Alphaproteobacteria, which is one of the most representative and

abundant in arid and tropical ecosystem soils, such as in the study area (Delgado et al., 2018). Besides, Rhizobiales is considered one of the most important orders due to its ability to establish symbiosis with plant roots through rhizobia, which stabilize soil bacterial communities (Martínez et al., 2015).

The genus *Azospirillum* was not the most abundant in both production systems, however, this genus belongs to the order Rhodospirales and to the family Bradyrhizobiaceae, which were more abundant in the OAM group. On this regard, Aguirre et al. (2011) indicated that in coffee crops, the application of *A. brasilense* induced a greater root development with which the plants developed a better anchorage and greater efficiency in the use of both nutrients and water.

Applying bio-fertilizers increases the stable fractions of organic matter, which makes it more resistant to decomposition, and in turn increases carbon sequestration in soil (Dębska et al., 2016; Fatunbi and Ncube, 2009). Such an increase, as well as the augment in the carbon content of microbial biomass, was demonstrated after three years of biofertilizer application based on *Pseudomonas* spp, *Penicilium* and *Actinomycetes* spp (Piotrowska et al., 2012). Likewise, biofertilization with *A. brasilense* modifies the microbial communities of rhizosphere under field conditions (García de Salamone, 2012). Furthermore, soil bacterial communities in wheat crops responded to different nitrogen fertilization treatments and bio-fertilization with *A. brasilense* (Di Salvo et al., 2018).

Results of this study suggests that the organic use of bio-fertilizers significantly contributed to the increase in recalcitrant forms of carbon, which in turn, improves plant growth by stimulating root development, as well as nutrient and water uptake. On the other hand, conventional management with NPK-fertilization promotes an increase in available forms of minerals for microorganisms, but without accumulation of organic matter and particularly of, recalcitrant carbon. This would explain the relatively greater abundance of Acidobacteria in OAM and the greater abundance of Proteobacteria in CAM. It is likely that long-term continuous management (i.e. bio-fertilization) favors an even greater abundance

of Acidobacteria in the OAM group. Knowing how soil microbial diversity is involved with the function of any edaphic ecosystem is essential to understand the ecosystem compensatory responses to counteract the threat of a changing environment.

CONCLUSIONS

The metagenomics analyses of the soil microbiome in coffee plantations under organic and conventional production systems allowed the classification of the more abundant taxonomic groups into oligotrophic and copiotrophic organisms, respectively. Even though diversity index values were similar in soils under different OAM and CAM production systems, Proteobacteria was more abundant in CAM, while Acidobacteria was more plentiful in OAM, suggesting that agronomic management influences the structure and function of bacterial communities. Moreover, OAM seems to stimulate the proliferation of certain bacteria that are related to soil carbon sequestration and plant growth. Unquestionable, metagenomics emerges as a remarkable up-to-date genomic technology for the unveiling of the taxonomic content in the soil microbiome. Such an approach would help to determine, analyze, and classify the microorganisms of different agroecosystems in order not only to conserve but to enhance the soil´s biotic resources.

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CAPITULO IV

CONCLUSIONES GENERALES

La acumulación y secuestro de carbono en el suelo, así como la diversidad de la comunidad bacteriana fuero afectados de manera similar por el método de manejo (orgánico y convencional) en agroecosistemas de regiones áridas (nogal) y tropicales (café).

En cultivos de nogal de agroecosistema áridos, las prácticas de agricultura Org influyeron en la concentración de nitrógeno y fosforo, y variaron significativamente respecto al manejo Conv en diversas variables del suelo principalmente en las relacionadas con la captura de compuestos de C recalcitrantes: carbono orgánico, carbono de la biomasa microbiana y huminas. Así mismo, el análisis de la comunidad bacteriana mostró la presencia de microorganismos fundamentales en el ciclo del C, fijación de CO_2 atmosférico y la degradación de la biomasa: phylum Acidobacteria y géneros *Gemmata* y *Dongia*. Contrariamente en el manejo agronómico Conv, las variables que variaron significativamente fueron las relacionadas con la mineralización como son las fracciones lábiles y mineralización de C, glomalina fácilmente extraíble, ácidos húmicos y fúlvicos; así como el incremento de enzimas relacionadas con el metabolismo de compuestos lábiles: lacasa, B-glucosidasa y B-galactosidasa. Además, la comunidad bacteriana estuvo compuesta por microorganismos asociados a la descomposición de la materia orgánica, así como al incremento de la tasa de mineralización de C, como lo son los géneros *Tepidisphaera* y *Sphingomonas*.

En tanto, en los cultivos de café de agroecosistemas tropicales, el análisis de la estructura de la comunidad bacteriana en sistemas de producción Org con aplicación de biofertilizantes, permitieron clasificar las bacterias en organismos oligotróficos relacionados con el secuestro de C (Acidobacterias). En cambio, en

el sistema de producción Conv, la comunidad bacteriana estuvo compuesta por microorganismos copiotróficos (Proteobacteria) que promueven el aumento de las formas disponibles de C. Lo anterior sugiere que a pesar de las diferencias ambientales que se presentan en los agroecosistemas estudiados como lo es la temperatura y precipitación, las practicas agronómicas orgánicas (cubiertas vegetales y aplicación de biofertilizantes), influyen significativamente en el aumento de formas recalcitrantes de C, a través de la modificación de parámetros químicos y biológicos del suelo. El conocimiento de los recursos del suelo es esencial para conservar, comprender y mejorar los elementos bióticos en diversos agroecosistemas.