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Yucatán in black and red: Linking edaphic analysis and pyrosequencing-based assessment of bacterial and fungal community structures in the two main kinds of soil of Yucatán State

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ABSTRACT

Yucatán State is dominated by two kinds of soil, named "Black Leptosol" and "Red Leptosol", which are interwoven across the State. In this work, we analyzed the relation between the edaphic characteristics and the bacterial and fungal community structures in these two kinds of Leptosol. The results revealed that Black Leptosol (BlaS) had a higher content of calcium carbonates, organic matter, nitrogen, and phosphorus than Red Leptosol (RedS). The most outstanding difference in the bacterial community structure between BlaS and RedS was that while in BlaS Actinobacteria was the most abundant phylum (43.7%), followed by Acidobacteria (26.9%) and Proteobacteria (23.6%), in RedS the bacterial community was strongly dominated by Acidobacteria (83%). Two fungal phyla were identified in both kinds of soil; Ascomycota, with 77% in BlaS and 56% in RedS, and Basidiomycota, with 22% in RedS and only 0.67% in BlaS. The most relevant difference between the two fungal communities was that excepting for *Fusarium* sp., all the species they had were different. Thus, in contrast with bacterial communities, where most of the major OTUs were present in both kinds of soil, fungal communities appeared to be unique to each kind of Leptosol.

1. Introduction

Yucatán State is strongly dominated by two kinds of soil, popularly known as black soil and red soil. In the north part of the State, both of them are very shallow (<25 cm deep) and rich in coarse fragments (Bautista et al., 2005; IUSS, 2014), scientifically they are classified as Leptosols. These two kinds of soil are distributed in a sort of patchwork pattern across the State. The main reason that explains this pattern, as well as the differences between both, is related to the geological history of the region. This part of the Yucatán peninsula belonged to the sea floor in geological times and when, in its evolution, the calcium-rich carbonate rock derived from coral reefs and other benthonic fauna, emerged and was exposed to weathering, it turned into a set of cracked and/or porous permeable limestone layer, which allows water to percolate into the subsoil (Bauer-Gottwein et al., 2011). Calcium carbonates of this limestone are partially soluble in water, what has prompted the formation of a karst topography, characterized by an undulated landscape with slight mounds and plains of varying expanse. The plains correspond to areas where the limestone has been relatively more soluble and permeable; at some points in the plains the rock has even slimmed so much that it has collapsed and fallen into the aquifer, forming sinkholes called "cenotes", the best known characteristic geological feature of the state of Yucatán. But the water that percolates through the most permeable regions not only dissolves the rock, but also leaches out many of the compounds contained in the sediments and the organic matter that is continuously deposited on that rock; thus in the plains the soil is usually poorer in nutrients and soluble compounds than the soil on the mounds, where the underlying limestone is relatively less permeable (Estrada-Medina et al., 2013). Furthermore, Leptosols in the plains normally have aerobic conditions (as a result of rapid water percolation) that promote iron oxidation causing the formation of

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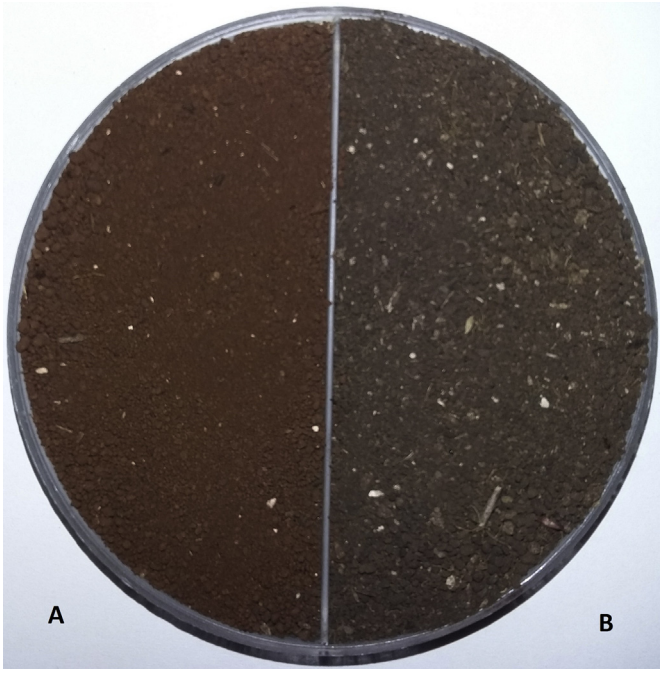

Fig. 1. Physical appearance of BlaS (A) and RedS (B) after drying and sieved to 2 mm.

red-colored hematite. This explains the reddish color of this kind of soil (Fig. 1A) and its denomination of "red soil" (Martín-García et al., 1996; Bautista-Zúñiga et al., 2003). In contrast, Leptosols in the mounds have a higher retention of organic matter imparting a black color (Fig. 1B) (Chesworth, 2008), hence the popular name of "black soil".

There are several previous reports about the edaphic characteristics of black and red Leptosols in Yucatán (*e.g.*, Bautista-Zúñiga et al., 2003; Aguila-Alcantara, 2007; Estrada-Medina et al., 2013); nevertheless, to our knowledge the microorganisms living in these soils have never been identified. As black and red soils are interwoven across the state, they are subjected to the same atmospheric conditions (*e.g.,* rains, atmospheric temperature, seasonality, etc.) in a given area, and they both are subjected to the same animal and human activities, which may carry materials and substances from one type of soil to the other; thus, it is interesting to compare the microorganisms communities living in either soil type, since it is likely that those differences are due to the natural selection imposed by edaphic characteristics. The analysis of these differences might serve to formulate hypotheses to understand the participation of the microorganisms in the biogeochemical cycles of these soils and in the general ecology of the region.

Many ecosystem services such as plant production, safeguarding of drinking water, and C sequestration, are consequences of soil microbial activities (Torsvik and Ovreas, 2002; Schulz et al., 2013). And the other way around, the soil characteristics, such as the quality and amount of the organic matter in the soil, pH, and redox conditions, have a pronounced influence on the dynamics of the structure and function of microbial communities in soils (Lombard et al., 2011). This close interplay between abiotic conditions and the soil biosphere is one of the most attractive issues in earth sciences, and has huge implications on environmental as well as human health (van Elsas et al., 2008; Schulz et al., 2013). Most functional traits, for example the development of food-web structures and closed nutrient cycles, are not a result of a single organism but of complex microbial communities, where microorganisms closely interact with each other (Wargo and Hogan, 2006). It has been demonstrated for example, that bacteria and fungi use to live in association, and that these liaisons may result in negative, neutral, or positive effects for each other (Frey-Klett et al., 2011). Nevertheless, the studies about these relations in the soil are still very scarce.

The recent advent of massively parallel sequencing technologies has revolutionized microbial diversity and ecology studies. The gene encoding the small-subunit of the rRNA (16S) and the nuclear ribosomal internal transcribed spacer (ITS) regions serve as prominent tools for phylogenetic analysis and classification of bacteria and fungi respectively (Vos et al., 2012; Sapkota and Nicolaisen, 2015). Pyrosequencing of these DNA regions has proved to be a cost-effective method for the characterization of bacterial and fungal communities and, although they may be subject to a moderate bias (Kumar et al., 2011), they are widely used to get a cultivation-independent general view about the phylogenetic profile of microorganism communities (*e.g.,* Vancov and Keen, 2009; Archer et al., 2015).

The aim of the present work was to analyze the relation among the edaphic characteristics and the bacterial and fungal community structures in the two main kinds of soil of Yucatán State. To our knowledge, this is the first report on microorganisms in these Leptosols.

2. Materials and methods

2.1. Soil sample collection

Five samples of black Leptosol and five samples of red Leptosol were collected in the town of Xmatkuil in the state of Yucatán, México (Fig. 2), on land belonging to the Universidad Autónoma de Yucatán (UADY). It is a rural locality typical of the Yucatán State, where the natural vegetation has been eliminated by slash-and-burn agriculture, and it was abandoned about ten years ago. The locations where samples were taken were close to a boundary where both types of soil join; each set of soil samples was taken from points about 20 m distant from each other, and samples in each set were approximately 3 m apart from each other. Black-soil samples were collected at: 20°52′06.95″N 89°37′10.68″W, 20°52′06.93″N 89°37′10.76″W, 20°52′06.93″N 89°37′10.60″W, 20°52′06.85″N 89°37′10.67″W, and 20°52′06.81″N 89°37′10.60″W; and red-soil samples were taken at 20°52′06.60″N 89°37′11.12″W, 20°52′06.54″N 89°37′11.18″W, 20°52′06.51″N 89°37′11.11″W, 20°52′06.47″N 89°37′11.04″W, 20°52′06.45″N 89°37′11.18″W. Samples of each kind of soil were collected to a depth of 5–15 cm, in an area free of trees and bushes. Soil samples were placed in sterile plastic bags, sealed, and transported on ice to the laboratory, where they were stored at 4 °C for up to 2 days prior to further processing. Before soil characterization, a fraction of each sample was separated for DNA extraction.

2.2. Soil characterization

Soil samples were submitted to the "Laboratorio de Análisis de Suelos, Plantas y Agua (LASPA) (Mérida, Yucatán, Mexico) for physical and chemical analysis. There, samples were dried to a constant weight in an oven at 105 °C and sieved to 2 mm. Then, to corroborate that none of the samples had any major particularity, samples of each set were visually examined to verify that their differences in color were no larger than two chroma units, that they did not show detectable differences in pH and that their differences in electric conductivity were no larger than 2%. Afterwards, the five samples of black soil were mixed together to get a composite sample, which was called BlaS, and the five samples of red soil were mixed together to get another composite sample, labeled RedS. Subsequently, both composite samples were analyzed following standard procedures for: particle size analysis with a Bouyoucus densimeter

Fig. 2. Geographic location of Xmatkuil in the State of Yucatán, Mexico.

(Gee and Bauder, 1986); REDOX by potentiometry (Patrick et al., 1996); pH by potentiometry (Thomas, 1996); phosphorus by the Olsen method (Kuo, 1996); total nitrogen by the Kjeldahl method (Bremmer, 1996); organic matter by colorimetric determination (Nelson and Sommers, 1996); electric conductivity by potentiometry (Rhoades 1996); carbonates by the acetic acid method (Loeppert and Suarez, 1996).

2.3. Metagenomic DNA extraction

Metagenomic DNA was extracted from each composite sample (combination of each set of five samples) using a PowerMax DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's protocol. Following elution, DNA samples were concentrated by ethanol precipitation, resuspended in 10 mM Tris-HCl (pH 8.0) and quantified with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

2.4. Tag-encoded FLX amplicon pyrosequencing

Purified metagenomic DNA was submitted to the Research and Testing Laboratory (RTL) (Lubbock, TX, USA) for tag-pyrosequencing. Tag-encoded FLX amplicon pyrosequencing (TEFAP) was performed as described previously using 939F (5′-TTGACGGGGGCCCGCACAAG-3′) and 1492R (5′ -TACCTTGTTACGACTT- 3′) primers for Bacteria, and ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′) for Fungi (Dowd et al., 2008; Sen et al., 2009). HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, USA) was used for PCR under the following conditions: 94 °C for 3 min followed by 32 cycles of 94 °C for 30 s; 60 °C for 40 s and 72 °C for 1 min; and a final elongation step at 72 °C for 5 min. Tag-encoded FLX amplicon pyrosequencing analyses used a Roche 454 FLX instrument with Titanium reagents; Titanium procedures were based on RTL protocols (www.researchandtesting.com).

2.5. Data analysis and taxonomic identification

Following sequencing, all failed sequence reads, low quality sequence ends, tags, and primers were removed, and sequence collections were depleted of any non-desired sequences and chimeras using B2C2 software (Gontcharova et al., 2010), as described previously (Ishak et al., 2011). To determine the taxonomic identity of microorganisms in the remaining sequences, these were denoised, assembled into clusters, and queried using a distributed BLASTn.NET algorithm (Dowd et al., 2005) against a high-quality database from NCBI. Database sequences were characterized as high quality based on criteria similar to those used by RDP (Cole et al., 2009). Using a .NET and C# analysis pipeline, the resulting BLASTn outputs were compiled, validated using taxonomic distance methods, and data-reduction analysis was performed as described previously (Andreotti et al., 2011). Based on the above BLASTn-derived sequence identity (percent of total-length query sequence which aligns with a given database sequence) and validation using taxonomic distance methods, the microorganisms were classified at the appropriate taxonomic levels based upon the following criteria: sequences with identity scores (relative to known or well-characterized 16S sequences) greater than 97% identity (<3% divergence) were resolved at the species level, between 95% and 97% at the genus level, between 90% and 95% at the family level, between 85% and 90% at the order level, 80 and 85% at the class level, and 77–80% at the phylum level. After resolution based

Table 1
Physical and chemical characterization of Black and Red Leptosols.

Parameter	Kind of soil	
	BlaS	RedS
Color (Munsell)	7.5YR 2.5/2	5YR 3/3
Sand (%)	64 ± 1.6	53 ± 1.2
Silt (%)	25 ± 3.1	23 ± 1.6
Clay (%)	11 ± 4.4	24 ± 1.4
Carbonates (%)	46.5 ± 2.5	4.3 ± 0.4
EC 1:5 (μS/cm)	414.3 ± 4.2	208.6 ± 3.3
Field Capacity (g/g)	1.16 ± 0.06	0.86 ± 0.02
Nitrogen (%)	1.40 ± 0.02	0.38 ± 0.02
Organic Carbon (%)	12.1 ± 0.2	4.6 ± 0.4
Organic Matter (%)	20.9 ± 0.9	8.0 ± 0.7
pH 1:2	7.8 ± 0.0	7.7 ± 0.0
Phosphorous (mg/kg)	310.5 ± 0.6	15.9 ± 0.4
REDOX potential 1:1 (mv)	102.5 ± 0.6	87.0 ± 2.4
Water content (%)	28.9	28.5

Abbreviations: BlaS, Black Leptosol composite sample; RedS, Red Leptosol composite sample; EC, electrical conductivity; M s.d., mean plus or minus standard deviation.

on these parameters, the percentage of each bacterial and Fungal ID was individually analyzed for each sample providing relative abundance information within individual samples based on relative numbers of reads within each. Evaluations presented at each taxonomic level, including percentage compilations represent all sequences resolved to their primary identification or their closest relative (Ishak et al., 2011).

Sequencing reads were aligned and clustered following the Ribosomal Database Project (RDP-Release 10) Pyrosequencing pipeline (http://pyro.cme.msu.edu/). Shannon, Chao 1, and evenness indices, as well as rarefaction curves, were obtained using the RDP tools.

2.6. Accession numbers

All tag pyrosequence data from this study were deposited and made publicly accessible in the MG-RAST under accession numbers 4641910.3 (BlaS-16S reads), 4641908.3 (RedS-16S reads), 4641911.3 (BlaS-ITS reads), and 4641909.3 (RedS-ITS reads).

3. Results and discussion

3.1. Soil characterization

As it was to be expected, BlaS and RedS had a typical Leptosols texture (Table 1). BlaS had a higher content of sand and silt, but a lower proportion of clay than RedS, possibly a result of clay being easily carried from mounds to plains by winds and water trickling. This is relevant because it is likely that the similar water content of both kinds of soil was given by clay in RedS and by organic matter in BlaS. Black Leptosols have a superior field capacity, but possibly red Leptosols keep humid for longer times, because clay has strong water retention (Gaiser et al., 2000). Furthermore, greater sand contents make the water to filter more easily, thus decreasing water retention (Dagadu and Nimbalkar, 2012). It is likely that the higher redox potential of BlaS is related to its higher water content (Weisbach et al., 2002). Possibly BlaS also has lower aerobic conditions.

The greater content of nitrogen and phosphorus in BlaS is certainly related with its higher content of organic carbon, since organic matter naturally contains these elements and releases them *via* decomposition (Murphy, 2014). Mineralogical composition of Red Leptosols and Black Leptosols are similar, both are mainly composed by, Si, Al, Fe, Ca, and P, however, Red Leptosols have more calcium than Black Leptosols (Bautista-Zúñiga et al., 2003).

The slightly higher pH in BlaS, as well as its larger EC, can be explained by its higher content of carbonates. Perhaps the organic content of this soil reduces the differences in pH between both kinds of soil, because the organic matter decomposition releases humic acids to the soil, thus decreasing its pH (Craswell and Lefroy, 2001).

All the values obtained in this work are in the range previously reported for these kinds of Leptosols (Bautista-Zúñiga et al., 2003; Aguila-Alcantara, 2007; Estrada-Medina et al., 2013), supporting the supposition that the samples we took are typical of these kinds of soil.

3.2. General analysis of the bacterial pyrosequencing-derived dataset

A total of 21,190 rRNA quality sequences were generated through the 454 tag-sequencing, from which 12,830 sequences with an average length of 467 bp belonged to BlaS, and 8360 sequences with an average length of 472 bp belonged to RedS (Table 2).

At phylum level, BlaS showed less than half the number of OTUs than RedS. At species level this difference was even higher, with about eight times less OTUs in BlaS than in RedS. This means that RedS not only had more OTUs at phylum level, but that those OTUs split in more species than the ones contained in BlaS. In agreement with this, the Shannon diversity index values (H') suggest that bacterial diversity at phylum level was only slightly lower in BlackS than in RedS, but at species level RedS was almost twice more diverse than BlackS.

Chao 1 richness estimates suggest that most of the estimated diversity contained within their bacterial communities was captured by our sequencing efforts. At phylum level, Chao 1 for RedS was about two and a half times higher than for BlaS, and at species level RedS showed almost seven times the richness of BlaS, which is consistent with the OTUs and Shannon values. RedS also presented a higher evenness than BlaS, meaning that the relative abundances of their taxa have more homogeneous values. It is not common to find Chao 1 values for soils inferior to 1000 at species level (*e.g.*, Will et al., 2010; Jung et al., 2014; Yun et al., 2014), which might mean that BlaS possibly has not only a lower richness compared with RedS, but that it has a low richness in general. Further studies would be necessary to corroborate this supposition.

Rarefaction curves of both soils (Fig. 3A) showed a leveling off, indicating that the number of analyzed reads was representative of their bacterial communities, both at phylum and species level, although there are still OTUs (mainly in RedS at species level) that were not captured in our work.

3.3. Bacterial community structures

All the generated reads were classified as belonging to the domain Bacteria. The sequences of the two kinds of soil together clustered in a total of 8 phyla (Fig. 4A), out of which 6 had a relative abundance > 1% in at least one of them. These six major phyla were present in both kinds of soil, but the minor phylum Planctomycetes was only observed in BlaS (0.22%), and the minor phylum Chloroflexi was only found in RedS (0.10%). The three most abundant phyla in both kinds of soil were Acidobacteria, Actinobacteria and Proteobacteria. Together, they accounted for about 94% of the total phyla present in each soil. The most outstanding difference in the bacterial community structure between BlaS and RedS was that while in BlaS Actinobacteria was the most abundant phylum (43.7%), followed by Acidobacteria (26.9%) and Proteobacteria (23.6%), in RedS the community was strongly dominated by Acidobacteria, with 83%. A possible explanation for this is that the other phyla cannot thrive in the prevailing conditions of RedS,

Table 2
General analysis of the bacterial and fungal pyrosequencing-derived datasets. The number of OTUs, Shannon diversity, Chao 1, and evenness were analyzed at 20% (phylum level) and 3% (species level) sequence dissimilarity for each Leptosol.

Sample ID	#seq	Phylum level				Species level			
		#OTUs	Chao 1	Shannon (H′)	Evenness	#OTUs	Chao 1	Shannon (H′)	Evenness
BlaS	12,830	472	18	18.5	1.69	0.58	166	256.0	3.23
RedS	8360	467	46	46.1	1.79	0.46	1409	1738.7	6.32
BlaS-ITS	5221	366	405	4.0	0.67	1015	1892	4.8	0.69
RedS-ITS	4437	168	187	3.8	0.74	662	1328	4.8	0.74

Abbreviations: BlaS, Black Leptosol composite sample analyzed for bacteria; RedS, Red Leptosol composite sample analyzed for bacteria; BlaS-ITS, Black Leptosol composite sample analyzed for fungy; RedS-ITS, RedS, Red Leptosol composite sample analyzed for fungy.

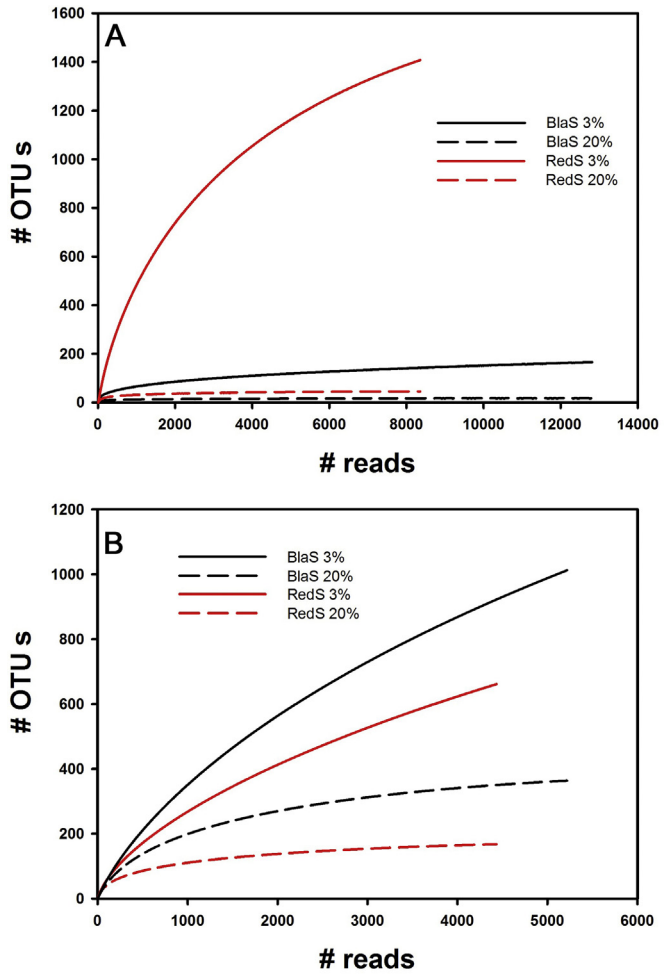

Fig. 3. Rarefaction curves at 20% and 3% dissimilarity. Taxa accumulation curves as a function of the number of sequences using resampling of bacterial 16S rRNA (A) and fungal ITS (B) gene sequences. Curves were calculated using RDP tools. Black Leptosol composed sample (BlaS); Red Leptosol composed sample (RedS). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

thus their relative abundances are lower. Acidobacteria is a very common phylum in soils, nevertheless to our knowledge it is not usual to find it reaching such a high abundance. Usually, it ranges from < 1% to 55% (*e.g.*, Chu et al., 2010; Siles et al., 2014; Kim et al., 2015), and the phyla that dominates most kinds of soils are either Proteobacteria or Actinobacteria (*e.g.*, Hu et al., 2015; Liao et al., 2015; Yasir et al., 2015).

At species level, a total of 103 OTUs were identified in the two kinds of soils together. From these, 21 OTUs were shared by both samples, 7 OTUs were exclusively found in BlaS and 75 OTUs were exclusively found in RedS. Nevertheless, from the total 103 OTUs, only 17 were major OTUs (abundance >1% in at least one kind of soil) (Fig. 4B). Of these major OTUs, 14 were detected in both kinds of soil, *Jiangella* sp. (15.8%) and *Sinorhizobium* sp. (4.3%) were only found in BlaS and *Thiobacter subterraneus* (1.6%) was only detected in RedS. These results reinforce the idea that almost all the major OTUs are present in both kinds of soil but most of them do not flourish in the prevailing conditions of RedS, which are probably given by the vast proportion of calcium phosphates present in this type of soil.

The most remarkable difference in the bacterial community structures between RedS and BlaS was that RedS was strongly dominated by *Acidobacterium* sp. (79.8%), and only *Holophaga* sp. (3.2%), *Nitrospira* sp. (2.3%), *T. subterraneus* (1.6%), *Conexibacter* sp. (1.5%), and *Streptacidiphilus* sp. (1.1%) reached abundances > 1% (Fig. 4B). These results together with the general analysis suggest that RedS (in contrast to BlaS) has one dominant species, five major species (abundance > 1%), and many minor species (abundance < 1%). Meanwhile BlaS seems to have several major species but a much inferior number of minor species. Possibly *Acidobacterium* sp. is fit to thrive in calcium phosphate (which is much more abundant in RedS), while the other major species thrive in the organic matter that both kinds of soil share (though in much lower proportion in RedS), and there are numerous species deriving profit from in some scant materials unique to RedS. We can picture two possible explanations to understand the presence of these rare materials. One is that as the rain leaches out the water-soluble compounds in RedS, the water-insoluble compounds increase their proportion; thus, the relative abundance of bacteria occupying those niches also increases, reaching a level we can detect with our sequencing effort. This would imply that BlaS has most of the species detected only in RedS, but in a level that escaped our perception. The other possible explanation is that there were some contaminants in RedS, such as traces of some fruits or of animal products, which were not detectable by the methods we used to evaluate the edaphic characteristics of the samples, because they were present only in a tiny proportion. Further work would be necessary to find out why RedS had such a high amount of minor OTUs concentrated in only a small fraction of the total bacterial community and whether this is a general characteristic of Red Leptosols in this region.

It is worth mentioning that in neither of the two soil types was found any member of the bacterial family Enterobacteriaceae, which includes many of the most familiar pathogens, such as *Escherichia coli*, *Salmonella*, and *Shigella*. This means either that these kinds of soil do not allow Enterobacteriaceae to flourish (or at least not in detectable amounts) or that the place where the samples were taken did not have an important human or animal impact. Nevertheless, there are some wild mammals (*e.g.*, bats, small marsupials, and rodents) in these lands, and they must have an impact on the soil; thus, we believe that the first supposition is better.

About the phylum Acidobacteria, which was abundant in both kinds of soil, it can be said that its members have been observed in many different habitats (*e.g.*, Elliott et al., 2015; López-Fernández

et al., 2015; Yasir et al., 2015). Based on 16S rRNA gene sequence divergence, Acidobacteria is predicted to be as diverse as the much better studied phylum Proteobacteria (Stamps et al., 2014). Their phylogenetic diversity, ubiquity and abundance suggest that they have an important ecological role and an extensive metabolic versatility (Quaiser et al., 2003; Ward et al., 2009). However, the genetic and physiological information regarding Acidobacteria is very scarce, as the majority of its members have not been cultivated and they have only been identified by their 16S rDNA sequences. Currently, only 17 genome sequences from this phylum are publicly available (Stamps et al., 2014). Thus it is not possible as yet to formulate a well-based hypothesis to explain its high abundance in the two kinds of soils, particularly in RedS.

After *Acidobacterium*, the most abundant genus in BlaS was *Jiangella* (15.8%) (phylum Actinobacteria, class Actinobacteria, order Jiangellales), which was not detected at all in RedS. The four *Jiangella* species that have been described so far are aerobic, Gram-positive, filamentous bacteria (Tang et al., 2014). *Jiangella gansuensis* was isolated from a desert soil in China (Song et al., 2005), *Jiangella alkaliphila* from the soil of a natural cave in Korea (Lee, 2008), *Jiangella alba* from an endophytic environment in China (Qin et al., 2009) and *Jiangella muralis* from wall material of an indoor environment in Germany (Kämpfer et al., 2011). Possibly *Jiangella* is not a common genus, because it is not easy to find reports about it, even in works where 16S pyrosequencing approaches were used.

The third most abundant genus in BlaS was *Streptacidiphilus* (12.5%) (phylum Actinobacteria, class Actinobacteria, order Actinomycetales). At present, this genus contains 10 reported species. On the basis of 16S rRNA gene sequence data, these species are closely related to members of the genera *Kitasatospora* and *Streptomyces*, which are famous as promising sources for secondary metabolites (Komaki et al., 2015). Members of the genus *Streptacidiphilus* have a major role in the turnover of organic matter in acidic habitats (Goodfellow and Williams, 1983), consistent with the relatively high organic carbon in BlaS, and are also reported to be potential producers of antifungal compounds (Williams and Khan, 1974) and acid-stable enzymes (Williams and Flowers, 1978). The genus *Streptacidiphilus* was proposed by Kim et al. (2003) for Actinobacteria that grow between pH 3.5 and 6.0, and BlaS had a pH of 7.8, thus, possibly the *Streptacidiphilus* sp. detected in BlaS has different properties. It might be worth to isolate and study its secondary metabolites, because they may also have also different properties of potential ecological and biotechnological interest.

The only other species with an abundance > 10% was *Stenotrophomonas maltophilia* (phylum Proteobacteria, class Gammaproteobacteria, order Xantomonadales). In BlaS it was the fourth most abundant species (12.4%), but in RedS it only reached trace levels (0.01%). *S. maltophilia* has recently been reported as an environmentally global emerging multiple-drug-resistant bacterium. It is a Gram-negative obligate aerobe species, rod shaped and motile, commonly associated with respiratory infections in humans (Brooke, 2012). As revised by Brooke, *S. maltophilia* has been recovered from diverse environments, such as soils, plant roots, animals, sinkholes, water systems, faucets, and bottled water. A noteworthy feature of *S. maltophilia* is its ability to adhere to surfaces and form bacterial films. Another significant characteristic of *S. maltophilia* is that it can acquire and transfer genes to other bacterial species (Alonso and Martínez, 2000; Berg, 2009). It may be important to investigate whether the *S. maltophilia* in BlaS is a human or animal pathogen, because if so, BlaS might be an important reservoir of this infectious agent.

In RedS, the three most abundant genera after *Acidobacterium* were: (i) *Holophaga* (3.2%), which belongs also to the phylum Acidobacteria (class Holophagae, order Holophagales), and is of interest for its ability to anaerobically degrade aromatic compounds and for its production of volatile sulfur compounds through a unique pathway; until now in this genus only one species has been described and sequenced, named *Holophaga foetida* (Anderson et al., 2012). (ii) *Nitrospira* (2.3%) (phylum Nitrospirae, class Nitrospira, order Nitrospirales), which includes barely studied and mostly uncultured nitrite-oxidizing bacteria that are, according to molecular data, among the most diverse and widespread nitrifiers in natural ecosystems (Anderson et al., 2012). (iii) *T. subterraneus* (1.6%) (phylum Proteobacteria, class β-Proteobacteria, order Burkholderiales) which has been reported as a thermophilic, obligately chemolithoautotrophic, sulfur/thiosulfate-oxidizing bacterium; it was isolated from the subsurface geothermal water in a gold mine in Japan, and its growth was observed at temperatures between 35 °C and 62 °C and pH between 5.2 and 7.7 (Hirayama et al., 2005). Possibly the species of these genera growing in RedS exhibit some differences with respect to their reported relatives; nevertheless, it seems that these genera are adapted to grow in harsh environments, where most bacterial taxa cannot thrive, in agreement with the characteristics of RedS.

Some other interesting genera that were identified, mainly in BlaS, were *Sinorhizobium* (phylum Proteobacteria, class Alphaproteobacteria, order Rhizobiales) and *Herbaspirillum* (phylum Proteobacteria, class Betaproteobacteria, order Burkholderiales) which include nitrogen-fixing bacteria (Barnett and Long, 2015; Seiki-Kadowaki et al., 2012); *Verrucosispora* (phylum Actinobacteria, class Actinobacteria, order Actinomycetales) which is the focus of considerable interest as a source of new bioactive compounds (Goodfellow et al., 2013); and *Gemmatimonas* (phylum Gemmatimonadetes class Gemmatimonadetes, order Gemmatimonadales), which is the only cultured representative of the phylum Gemmatimonadetes and has been suggested as a container of many unique enzymes and metabolic pathways (Zhang et al., 2003).

3.4. General analysis of the fungal pyrosequencing-derived dataset

A total of 9658 fungal sequences were obtained, from which 4437 belonged to RedS, and 5221 to BlaS (Table 2).

At phylum level, BlaS showed about twice the number of OTUs, compared to RedS. Chao 1 richness estimates suggest that both at phylum and at species level, most of the richness contained in the fungal communities of both kinds of soil was captured by our sequencing effort, except for RedS at species level, where about one half was left out. Chao 1 also suggests that phyla in RedS split in more species than phyla in BlaS.

Shannon diversity index values (H') suggest that fungal diversity at phylum level was slightly higher in BlaS than in RedS, but at species level, BlaS seemed to be as diverse as RedS.

BlaS presented a lower evenness values than RedS both at phylum and at species level, indicating that the relative abundances of its taxa had less homogeneous values.

Rarefaction curves of both soils (Fig. 3B) showed a leveling off, indicating that the number of analyzed reads was representative of their fungal communities both at phylum and species level, except for RedS at species level, where, supporting the results of Chao 1 index, an extra effort would be necessary to capture the majority of OTUs.

3.5. Fungal community structures

77.8% of the generated reads belonged to the kingdom fungi and 22.2% had no hit. Two fungal phyla were identified in both kinds of soil; Ascomycota which was dominant, with 77% in BlaS and 56% in RedS, and Basidiomycota, with only 0.67% in BlaS and 22% in RedS (Fig. 5A). This structure, with Ascomycota being the dominant fungal phylum followed by Basidiomycota, is common

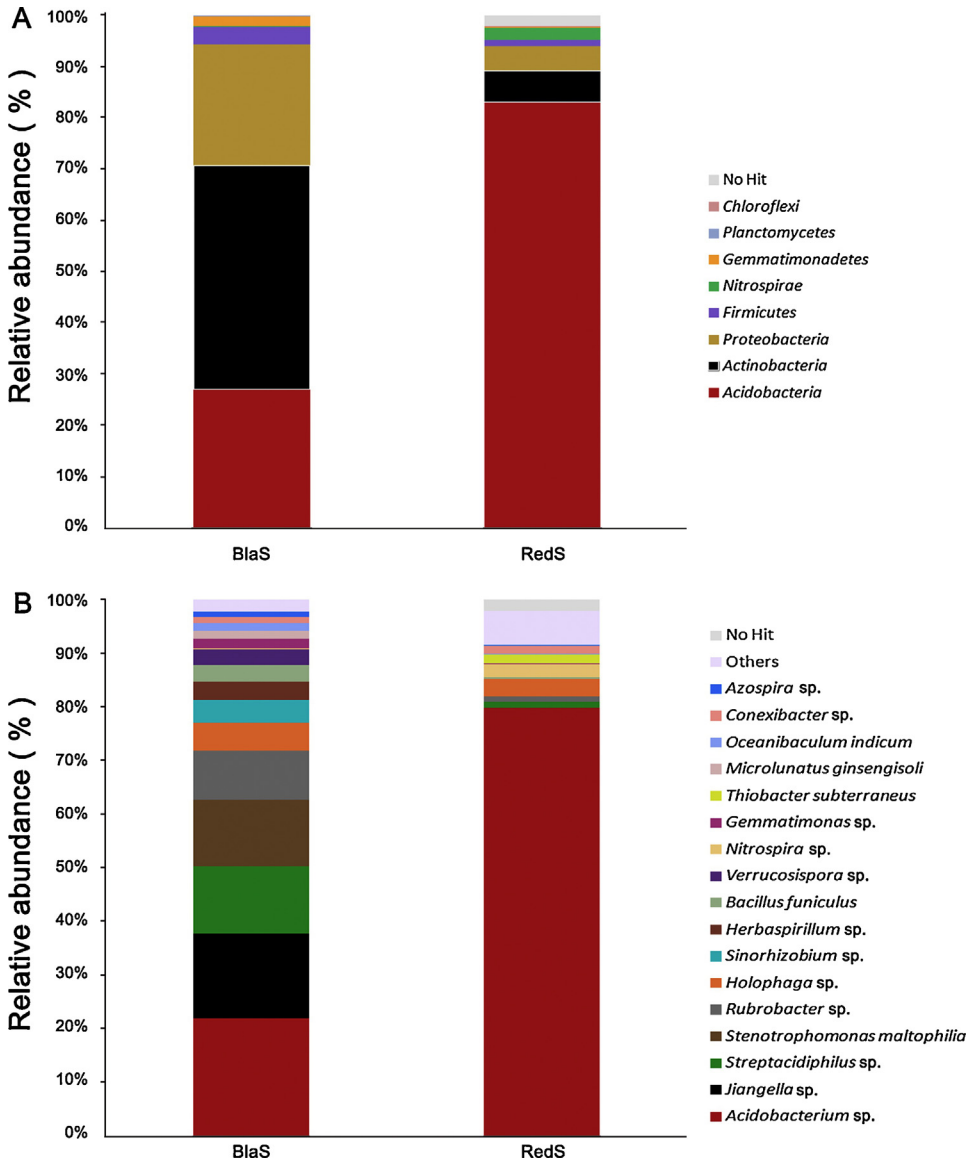

Fig. 4. Bacterial relative abundances at 20% (A) and 3% (B) dissimilarity. Bacterial community composition in Black Leptosol composed sample (BlaS) and Red Leptosol composed sample (RedS). The abundance is presented in terms of the percentage of the total bacterial 16S rRNA sequences in each sample. Only taxa > 1% in at least one sample were individually named; the rest were grouped as "others". (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in many kinds of soil used either for agriculture (*e.g.*, Orgiazzi et al., 2012; Kuramae et al., 2013; Xu et al., 2015) or forestry (*e.g.*, Buée et al., 2009; Liu et al., 2015). Basidiomycota is dominant only in plant litter and organic matter soil horizons, where lignocellulosic biomass accumulates (Baldrian et al., 2012; Tian et al., 2014).

At species level, a total of 52 OTUs were identified in the two kinds of soil together. The most outstanding difference between the two fungal communities was that from these 52 OTUs, only *Fusarium* sp. was shared by both kinds of soil (12.3% in BlaS and 15.7% in RedS). 49 OTUs were exclusively found in BlaS and two OTUs were exclusively found in RedS.

Ten out of the 52 OTUs had abundance > 1% (Fig. 5B). From these major OTUs, seven were only present in BlaS (*Chaetomella raphigera* 31.5%, *Humicola* sp. 8.3%, *Cochliobolus* sp. 3.4%, *Zopfiella* sp. 3.0%, *Stagonosporopsis cucurbitacearum* 1.7%, *Periconia macrospinosa* 1.4%, and *Verticillium* sp. 1.0%), and two were only detected in RedS (*Madurella* sp. 40.8%) and *Malassezia* sp. 22.5%. Thus, in contrast to bacterial communities, where most of the major OTUs were present in both kinds of soil, fungal communities appeared to be unique to each kind of soil. Another important difference was that while the RedS bacterial community had a large number of species contained in a small proportion of the community, in the RedS fungal community this was not the case. A possible explanation for this difference is that the number of sequences obtained for the RedS fungal community was not sufficient to detect the least represented fungal species. This supposition supported by its Chao 1 and its rarefaction curve. Thus we are looking only to its abundant OTUs.

C. raphigera, which was the most abundant species in BlaS (31.5%), has been reported as an endophytic fungi producer of taxol, a drug used to treat breast, lung and ovarian cancers (Gangadevi and Muthumary, 2009). Some strains of *C. raphigera* produce secondary metabolites with antibacterial and antifungal activity (US patent 4375462 A), and additionally, strain M4854 (*C. raphigera* ATCC® 44497™) produces compounds M4854-I and M4854-II, which are potent inhibitors of β-lactamase (Yaginuma et al., 1980), thus decreasing the possibilities of other microorganism to resist its attack. This may explain why this species is so abundant in BlaS.

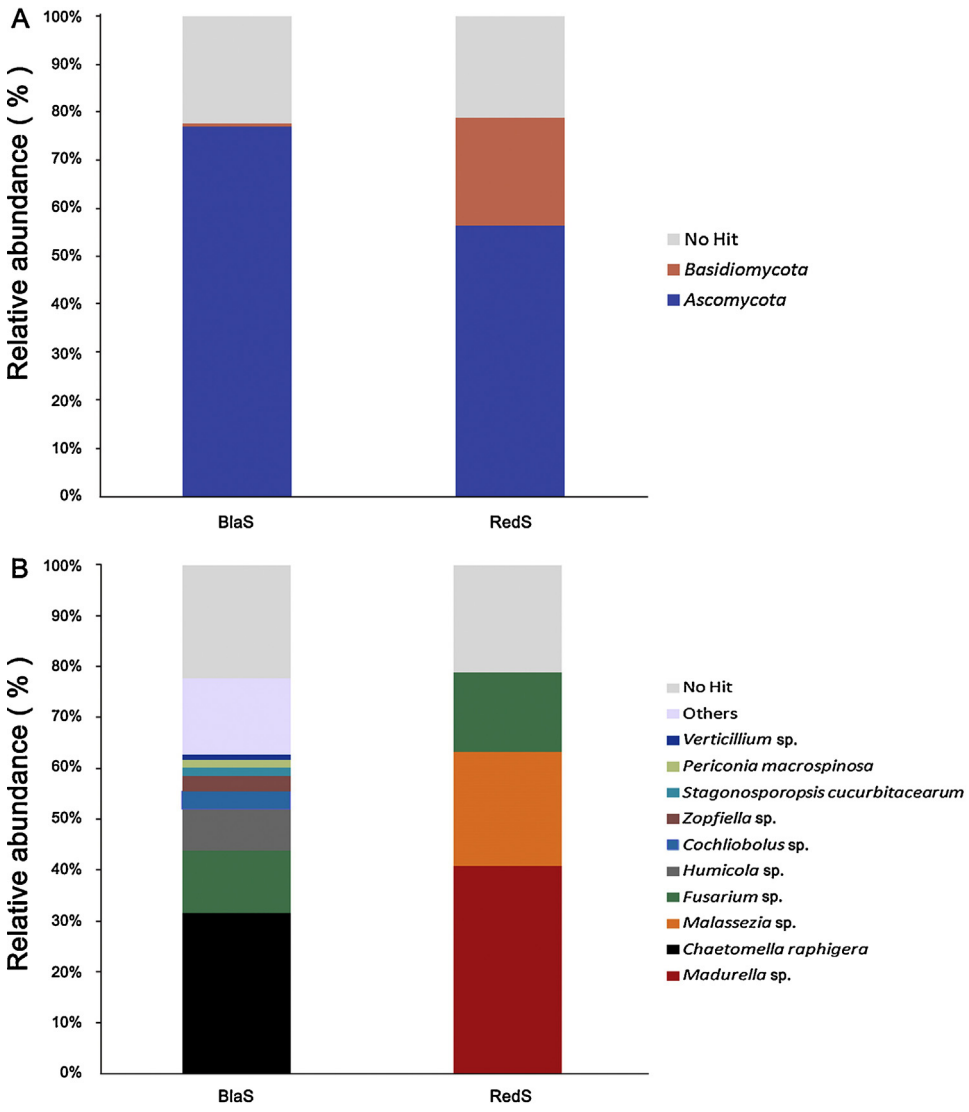

Fig. 5. Fungal relative abundances at 20% (A) and 3% (B) dissimilarity. Fungal community composition in Black Leptosol composed sample (BlaS) and Red Leptosol composed sample (RedS). The abundance is presented in terms of the percentage of the total fungal ITS sequences in each sample. Only taxa > 1% in at least one sample were individually named; the rest were grouped as "others". (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Recently *C. raphigera* was recognized as a pathogen of rosebushes (Zhang et al., 2014), suggesting that this species may have ecological niches as an endophytic fungus in some plants and as a plant pathogen in others. It would be interesting to investigate whether plants in Yucatán are hosts of this fungus, as well as the properties of the secondary metabolites isolated from strains collected in the State.

The second most abundant fungal genus in BlaS, and the third most abundant in RedS, was *Fusarium* (phylum Ascomycota, class Sordariomycetes, order Hypocreales). It was the only genus shared by BlaS and RedS. *Fusarium* is a large cosmopolitan genus; a number of its species are important plant pathogens and produce several toxins of agricultural relevance (*e.g.*, fumonisins, gibberellins, and fusarins, among others). The genomes of *Fusarium* species usually contain a relatively high number of genes codifying for nonribosomal peptide synthetases (NRPS) and polyketide synthases (PKS) (Ma et al., 2013), meaning that they might produce a wide variety of secondary metabolites. Most *Fusarium* species are soil-borne fungi, and can remain alive for long periods before infecting their hosts (Moretti, 2009). Usually this fungus shows broad resistance to antifungal drugs. Thus, as *Fusarium* usually is a major genus in soils (Mandel, 2006; Smith, 2007; Yergeau et al., 2010) it is not surprising to find it as one of the major identified OTUs in both kinds of the analyzed soils.

The third most abundant fungal genus in BlaS was *Humicola*. It is also a cosmopolitan genus, usually isolated from soil and plant litter material (*e.g.*, Wen-Hsiung et al., 2011). *Humicola* species are strongly cellulolytic and usually produce several extracellular enzymes such as amylases, glucoamylases, trehalases and xylanases (Schülein, 1997; Singh et al., 2005; Sharma et al., 2008). Campos and Felix (1995) reported an uncommon β-glucosidase from *H. grisea*, which is insensitive to glucose inhibition. *Humicola* is an important cellulose-decomposing genus; thus, it is very commonly found in soils rich in organic matter (*e.g.*, Singh et al., 2005; Wen-Hsiung et al., 2011). This is consistent with its relatively high abundance in BlaS, where *Humicola* likely has a role in the decomposition of organic matter.

In RedS, the most abundant genus was *Madurella* (phylum Ascomycota, class Sordariomycetes, order Sordariales), which comprised 40.7% of its fungal community. The best known species of this genus are *M. mycetomatis* and *M. grisea*. Both are human pathogens, which have been isolated from soil, and have been reported as

the major causative agents of eumycetoma, a severely debilitating disease in India (Thirumalachar and Padhye, 1968) and Sudan (Ahmed et al., 2002). Eumycetoma is also an important disease in agricultural workers in Latin America, particularly in Mexico. The first report of eumycetoma in Mexico was in Guerrero State in 1998 (Chávez et al., 1998). In 2011, the National Commission for Knowledge and Use of Biodiversity reported the presence of *M. mycetomatis* and *M. grisea* in several locations of Puebla State (Medel-Ortiz et al., 2011), and in 2013, some cases of eumycetoma caused by *M. mycetomatis* and *M. grisea* were detected in the states of Guerrero, Jalisco, Michoacán, Morelos, Nuevo León, and Veracruz (López-Martínez et al., 2013). However, to our knowledge, the present work is the first to report *Madurella* as being present in Yucatán, and the first to give an insight of it being abundant specifically in red Leptosol. It might be important to find out whether the species thriving here are human pathogens. López-Martínez et al. (2013) mention that laboratories in Mexico often have limitations to make precise diagnosis of mycosis, thus eumycetoma cannot be excluded in Yucatán.

The second most abundant genus in RedS was *Malassezia* (phylum Basidiomycota, class Exobasidiomycetes, order Malasseziales), which has been reported as a soil-borne dermatophyte; interestingly another animal and human pathogen. This genus comprises seven species. It is an anthropophilic, lipophilic fungus, normal in human skin flora, but it can turn into an opportunistic systemic pathogen. Its involvement in several animal and human skin diseases has been well documented (Ashbee and Evans, 2002; Aizawa et al., 2001). Thus it is worth to highlight that the two most abundant fungal genera founded in RedS, which together had an abundance > 60%, have been reported as animal pathogens, and the third most abundant one (*Fusarium*, 15.7%) is an important plant pathogen. It may be important to make further investigations to find out whether the specific species thriving in RedS are indeed pathogenic.

Some other potentially important major fungal genus identified in BlaS were *Cochliobolus* (phylum Ascomycota, class Ascomycetes, order Pleosporales) and *Verticillium* (phylum Ascomycota, class Incertae sedis), which have been reported as plant pathogens (Condon et al., 2014; Daayf, 2015; Klosterman et al., 2009), and *Chaetomium* (phylum Ascomycota, class Sordariomycetes, order Sordariales), which has been reported as a decomposer (Barbosa et al., 2012; Pornsuriya et al., 2008).

4. Conclusions

The results obtained in this work revealed interesting differences between BlaS and RedS regarding their edaphic characteristics, as well as their bacterial and fungal communities.

Agriculture in Yucatán is very scarce, mainly because soils in this region are very shallow. Nevertheless, for a local scale, there is an important production of citrus, corn, papaya, cucurbits, avocado, and some other vegetables (http://www.inegi.org.mx/). It is popular knowledge that these plants grow better in black soil than in red soil. However, there are no formal studies about this subject. In future work, it would be interesting to measure, for several cultivated and wild plants, their different abilities to grow in black soil in comparison to red soil, and to investigate whether these differences are due only to the differential nutrient content or if they are also determined by microbes that are preferentially associated with each kind of soil.

We found some microorganisms which likely have a role in ecological processes of these soils, microorganisms which are related to promising sources of biotechnologically-useful compounds, others related to human or plant diseases, and bacteria which have very few well-studied members. Thus, results of this first exploration provide a panoramic view about the microbial communities in these soils, which may serve as foundation for further studies about different aspects of microbiological interest.

Specifically, from a practical point of view, results about human and plant pathogens should guide investigations to better understand some local diseases. In Yucatán many people live in rural and poor conditions, in close contact with soil, and they and their plant cultures often suffer from diseases whose pathogenic agents have not been identified. It is possible that several of the putative pathogenic microorganisms found in this work are related to these diseases, thus the results may serve to design strategies to identify and characterize them, to develop diagnosis tools, and eventually to control them.

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