Applied Soil Ecology 82 (2014) 1–10

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Identification of culturable microbial functional groups isolated from the rhizosphere of four species of mangroves and their biotechnological potential

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ARTICLE INFO

Article history:
Received 11 November 2013
Received in revised form 7 April 2014
Accepted 8 May 2014

Keywords:
Rhizophora mangle
Avicennia germinans
Laguncularia racemosa
Conocarpus erectus
Microbial population
Terminos Lagoon

ABSTRACT

This study identified microbial functional groups like total culturable bacteria, potential N_2-fixing free living bacteria, N_2-fixing hydrocarbonoclastic bacteria, N-assimilating hydrocarbonoclastic bacteria, total fungi, actinobacteria, P-solubilizers, lipolytic microorganisms, and starch, cellulose, pectin and protein degrading microorganisms, isolated from the rhizosphere of four species of mangroves (Red, Black, White, and Button) from the natural protected area at the Terminos Lagoon, Campeche, México. Overall, microbial populations showed significant differences ($P < 0.05$) among the four mangrove species. The rhizosphere of White mangrove showed better chemical and textural soil properties, and harbored the highest microbial populations when compared to the remaining mangrove species. The principal component analysis indicated that two components accounted the 85.3% of the total variation. The most significant textural and chemical soil properties were the major components, CP1 (organic matter and total organic carbon) and CP2 (sand and clay). Microbial populations correlated ($P < 0.05$, Pearson coefficient) with sand and clay particles, and with some soil chemical properties such as organic matter. The total nitrogen and organic carbon significantly correlated with cellulose degraders, while phosphorus with N_2-fixing bacteria, total fungi, and with pectin and starch degraders.

1. Introduction

There are reported approximately 73 species of mangroves worldwide (Spalding et al., 2010). Many coastal lagoons in the tropics and subtropics support dense mangrove forests. These are some of the most productive areas in the marine and estuarine environments supporting large plants and animal communities, many of which are economically and ecologically important (Jones, 1992; Toledo et al., 1995). Mangroves in Mexico are distributed in shoreline lagoons and estuaries in the Gulf of Mexico and in the Pacific Ocean, covering 655,667 ha. Campeche is the Mexican state with the highest mangrove coverage (196,552 ha), which is mainly found in the natural protected area of the Terminos Lagoon, and in the biosphere reserve Los Petenes (Spalding et al., 2010). Mangrove ecosystems in Campeche consist of four species: *Rhizophora mangle* L. (Red Mangrove), *Avicennia germinans* (L.) Stern (Black Mangrove), *Laguncularia racemosa* (L.) Gaertn. f. (White Mangrove) and *Conocarpus erectus* L. (Button Mangrove) (Day et al., 1987, 1996).

Mangrove ecosystems are characterized by the accumulation of lignocellulose residues that are subjected to mineralization throughout microbial activities (Alongi et al., 1989; Holguin et al., 2001). Plant residue decomposition generates detritus that is the baseline for the food chain in mangrove ecosystems for several organisms such as crustaceans, mollusks, insect larvae, nematodes, and commercial species of shrimps, scallops, oysters and fish (Odum and Heald, 1975a,b; Aburto-Oropeza et al., 2008;

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http://dx.doi.org/10.1016/j.apsoil.2014.05.001
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Duke et al., 2007). Nevertheless, detritus is not only important for species living in the mangrove ecosystems since near to 25% of this material is transported to open seas, mangroves are also considered as nutrient exporting ecosystems (Clough, 1991). Conversely, mangroves are nutrient deficient ecosystems, especially for nitrogen and phosphorus (Holguin et al., 1992; Alongi et al., 1993), but paradoxically, mangroves are highly productive. Thus, microbial activity contributes significantly on nutrient availability in mangroves ecosystems in which biological nitrogen fixation and P-solubilization have ecological relevance (Sengupta and Chaudhuri, 1990; Alongi et al., 1993; Rivera-Monroy and Twilley, 1996; Vázquez et al., 2000; Holguin et al., 2001; Flores-Mireles et al., 2007; Vovides et al., 2011a,b).

Some microbial groups also play significant roles in the carbon cycling by degrading starch, cellulose, lignin, or lipids; and other microorganisms participate in both nitrogen and phosphorus cycles in soil (Bouillon et al., 2008). Therefore, microbial activity considerably contributes on changes of soil chemical properties as well as on plant nutrition by facilitating organic matter mineralization, and increasing nutrient availability and plant uptake (Schloter et al., 2003). Plant growth promoting rhizobacteria (PGPR) are useful biological elements for reforestation and rehabilitation of mangrove ecosystems. In addition, PGPR may serve as important components in bioremediation of contaminated water, sediments, and soil (Alongi, 1994; Bashan and Holguin, 1998, 2002; Holguin et al., 2001; de-Bashan et al., 2012). Mangrove ecosystems are also subjected to anthropogenic pressures such as oil spill contamination in which the presence of hydrocarbonoclastic microorganisms may play a significant role on either detoxifying or decontaminating the impacted water and sediments (Alexander, 1994; Taketani et al., 2009). Thus, the identification of microbial strains able to degrade organic contaminants may be relevant for directing them as part of biotechnological approaches for mangrove rehabilitation (Sivaramakrishnan et al., 2006; Lageiro et al., 2007; Salihu et al., 2012; de-Bashan et al., 2012). We tested the hypothesis that mangrove ecosystem at the natural protected area of the Terminos Lagoon, Campeche (México) harbors functional microbial populations with biotechnological purposes, and that these microbial populations correlate with textural and chemical properties of the rhizosphere soil collected from four predominant mangrove species.

Thus, this study identified culturable microbial functional groups in the rhizosphere of four species of mangroves (Red, Black, White, and Button) that are predominant at the natural protected area of the Terminos Lagoon. The identified microbial groups were total bacteria, potential N_2-fixing free living bacteria, N_2-fixing hydrocarbonoclastic bacteria, N-assimilating hydrocarbonoclastic bacteria, total fungi, actinobacteria, P-solubilizing bacteria, and lipolytic and cellulolytic microorganisms, as well as pectin, starch and protein degraders.

2. Materials and methods

2.1. Site of study and samplings

Rhizosphere soil was collected from the four dominant mangrove species *R. mangle* L. (Red mangrove – RedM-), *A. germinans* (L.) Stern (Black mangrove – BlackM-), *L. racemosa* (L.) Gaertn. f. (White mangrove – WhiteM-) and *C. erectus* L. (Button mangrove – ButtonM-) present at the natural protected area of the Terminos Lagoon, Campeche, México. Soil samples were collected during both rain (September, 2010) and dry season (June, 2011). The sampling site is located along the coastal area between the Botanical Garden of the Universidad Autonoma del Carmen and the Estero Pargo (18° 38′ North and 91° 46′ West) (Fig. 1).

Rhizosphere soil samples were taken at 20 cm of depth from the root environment of each mangrove species, and placed in sterilized glass jars and kept at 4 °C for further microbial or chemical and physical analysis (Paetz and Wilke, 2005). Soil sampling consisted on a randomized collection of two samples of rhizosphere soil (1 kg each, approximately) from three mangrove trees for each mangrove species. Soil samples were used for either analyzing soil texture and chemical properties, or determining target microbial populations.

2.2. Determination of physical and chemical properties of the rhizosphere soil

Soil samples from each type of mangrove were dried and sieved (<2 mm) for determining pH (1:2 H_2O) and electrical conductivity (EC) (1:5 H_2O) (APHA, 1998), organic matter (OM) and total organic carbon (TOC) content (Walkley and Black, 1934). Total content of nitrogen (N), phosphorus (P), and calcium carbonate ($CaCO_3$) were also determined (APHA, 1998; Allison and Moodie, 1965; Olsen and Dean, 1965), as well as soil texture (Bouyoucos, 1962).

2.3. Identification of culturable microbial functional groups

Culturable microorganisms were quantified by the dilution and plate count technique using specific culture media. Total culturable bacteria (TotB), total fungi (TotF), and actinobacteria (ACMY) were determined using nutrient agar, potato dextrose agar, and CZAPEK agar adjusted to pH 8 (Merck®), respectively. Potential P-Solubilizing (PSol) bacteria were quantified by means of Pikovskaya medium amended with tricalcium phosphate (Pikovskaya, 1948). Since potential N_2-fixing free living bacteria (NFB) were determined by using a nitrogen-free medium (Rennie, 1981); this culture medium was modified by applying either crude oil (80 mg L^{-1}) or ammonium nitrate (2 mg L^{-1}) plus crude oil for enumerating potential either N_2-fixing hydrocarbonoclastic bacteria (NFHB) or N-assimilating hydrocarbonoclastic bacteria (NAHB), respectively. Plates were incubated at 28 ± 1 °C for 3–5 days, and bacteria enumeration was performed expressing the results from six replicates, as colony forming units (CFU g^{-1} dry soil). Additionally, populations of lipolytic (LIPOL) and cellulolytic (CELLUL) microorganisms as well as degraders of pectin (PEC), starch (AMYL) and protein (PROT) were also determined (Levine, 1953; Sierra, 1957; Wollum, 1982; Sumaya et al., 1993). Plates were incubated at 28 ± 1 °C for 7 days, and bacteria enumeration was performed expressing the results from six replicates, as colony forming units (CFU g^{-1} dry soil).

2.4. Experimental design and statistical analysis

Average data from six replicates were used for estimating microbial populations (CFU g^{-1} soil), but data from microbial enumeration were transformed to logarithmic units and subjected to an analysis of variance (ANOVA) and to a mean comparison test (Tukey, $\alpha = 0.05$); however, data in graphs are presented using the actual average values. Soil textural and chemical properties were analyzed by means of the principal component analysis (PCA) and main data are showed by means of a dispersion diagram for principal components CP1 and CP2. Also, Pearson correlation coefficients ($P < 0.05$) were performed for microbial populations, and soil textural and chemical properties. Statistical analysis was conducted with the software InfoStat®.

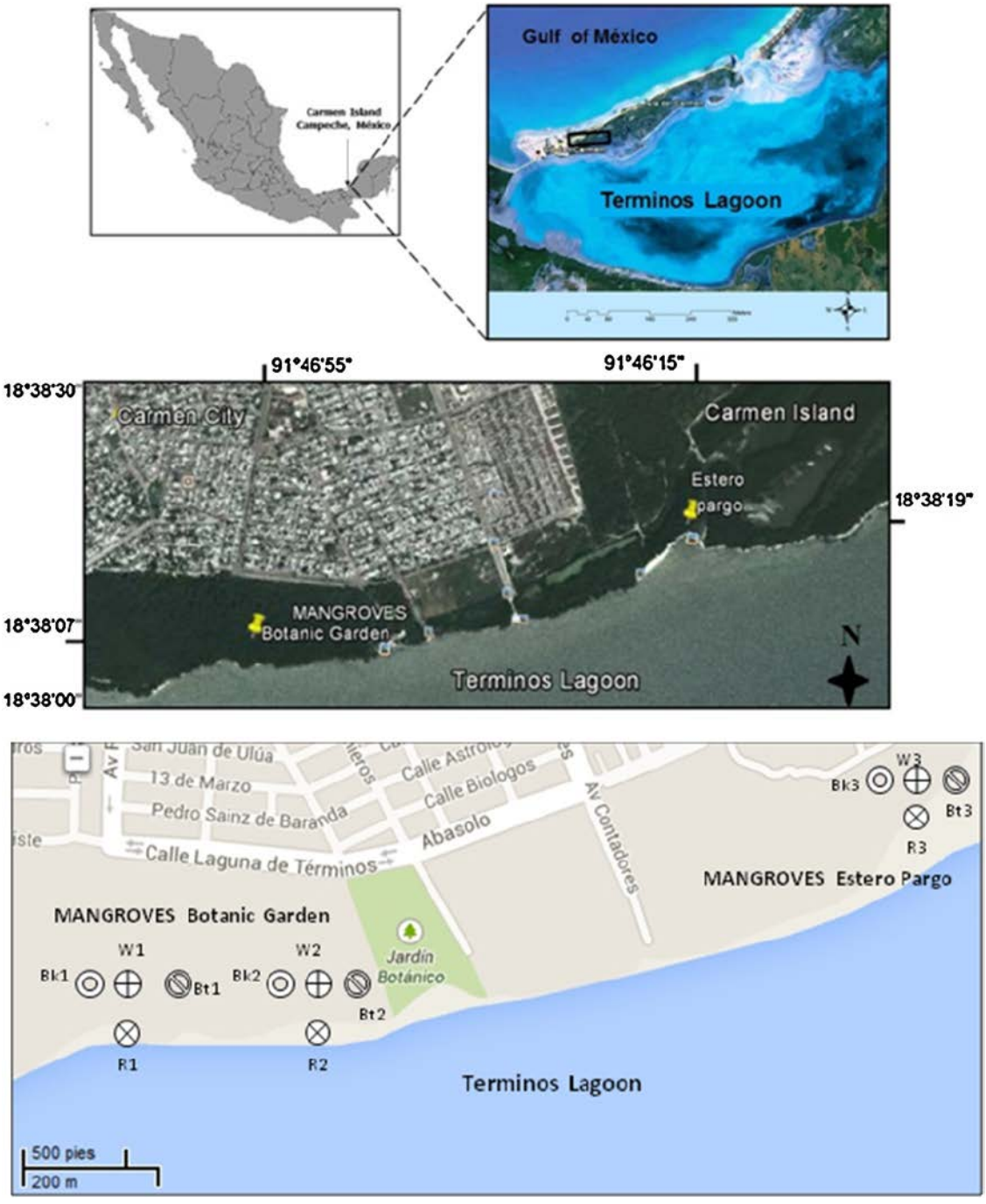

Fig. 1. Location of sampling sites in the mangrove ecosystems at the Terminos Lagoon, Campeche, Mexico.

3. Results

3.1. Physical and chemical properties of the rhizosphere soil

Table 1 shows pH values for the four mangrove species which ranged from 7.4 and 8.9, regardless seasonality. The electrical conductivity (EC) values were higher in samples collected during dry season than rainy season, excepting for the soil sample from the rhizosphere of ButtonM. The highest organic matter (OM) contents were obtained in the rhizosphere of WhiteM, and the lowest values at the rhizosphere from RedM; moreover, the OM contents were high during the rainy season (Table 1). Similar pattern was found for total nitrogen, which was higher in the rhizosphere of WhiteM and BlackM during both rainy and dry season; in contrast, the lowest values were found in the RedM and ButtonM rhizosphere (Table 1). The P content was generally higher during rainy than dry season; at rainy season, the highest values were obtained in rhizosphere of WhiteM, BlackM, and ButtonM; in contrast, during dry season, the highest values corresponded to the rhizosphere of BlackM and ButtonM (Table 1). Regardless mangrove species, the content of $CaCO_3$ during dry season was 7-fold greater than the rainy season (Table 1). The WhiteM rhizosphere showed the highest values of TOC in both seasons; in contrast, the lowest TOC values were achieved at the RedM and ButtonM rhizosphere, respectively (Table 1).

Table 1
Textural and chemical properties of rhizosphere soil samples collected from four mangrove species at the Terminos Lagoon, Campeche, México.

Season	Mangrove species	pH	EC ($dS\,m^{-1}$)	OM (%)	Total N (%)	Total P ($mg\,kg^{-1}$)	$CaCO_3$ (%)	TOC (%)	Soil texture (%)		
									Sand	Silt	Clay
Rain	RedM	8.9 ± 0.02	0.89 ± 0.02	1.2 ± 0.1	0.04 ± 0.01	8.0 ± 0.5	9.6 ± 0.06	0.48 ± 0.05	80 ± 2	9 ± 2	11 ± 2
	WhiteM	7.8 ± 0.03	0.51 ± 0.04	13.9 ± 0.1	0.34 ± 0.06	39.0 ± 2	7.7 ± 0.1	7.15 ± 0.05	80 ± 1	9 ± 1	11 ± 1
	BlackM	8.3 ± 0.2	0.76 ± 0.01	3.0 ± 0.5	0.08 ± 0.01	24.0 ± 1	13.5 ± 0.1	1.26 ± 0.04	84 ± 1	5 ± 1	11 ± 1
	ButtonM	8.6 ± 0.1	0.79 ± 0.01	2.3 ± 0.1	0.07 ± 0.02	15.0 ± 0.5	8.9 ± 0.1	1.88 ± 0.08	82 ± 1	7 ± 1	11 ± 1
Dry	RedM	8.6 ± 0.1	4.27 ± 0.03	0.1 ± 0.05	0.04 ± 0.03	1.0 ± 0.4	88.3 ± 0.75	0.20 ± 0.06	4 ± 2	3 ± 2	93 ± 2
	WhiteM	7.4 ± 0.06	3.20 ± 0.1	11.0 ± 1	0.37 ± 0.02	0.8 ± 0.04	46.4 ± 0.06	6.40 ± 0.1	8 ± 1	9 ± 1	83 ± 1
	BlackM	7.6 ± 0.1	2.67 ± 0.1	6.6 ± 0.1	0.16 ± 0.01	14.8 ± 0.01	56.7 ± 0.03	3.90 ± 0.1	18 ± 1	9 ± 1	73 ± 1
	ButtonM	8.8 ± 0.1	0.21 ± 0.01	0.5 ± 0.05	0.04 ± 0.01	13.4 ± 0.1	84.4 ± 0.4	0.30 ± 0.1	7 ± 1	2 ± 1	91 ± 1

Abbreviations: RedM = red mangrove (*Rhizophora mangle* L.); WhiteM = white mangrove (*Laguncularia racemosa* (L.) Gaertn. f.); BlackM = black mangrove (*Avicennia germinans* (L.) Stern); ButtonM = button mangrove (*Conocarpus erectus* L.).
Means ± standard error.

3.2. Microbial populations

The total culturable bacteria (TotB) population was significantly higher in the rhizosphere of WhiteM than that from RedM and BlackM rhizosphere, during the rainy season; but at dry season, the rhizosphere of WhiteM and ButtonM showed significantly greater population of TotB than BlackM and RedM (Fig. 2A). The Actinobacteria population (ACMY) during the rainy season was significantly high in the rhizosphere of BlackM and ButtonM (19.5 CFU × 10^3 g^{-1} soil in average), but at dry season the ACMY population (15.7 CFU × 10^3 g^{-1} soil in average) was high in the rhizosphere of WhiteM and ButtonM (Fig. 2B). The lowest ACMY population was achieved in the rhizosphere of RedM, regardless seasonality (Fig. 2B).

The highest populations of P-solubilizing (PSol) bacteria were found during dry season, especially in the rhizosphere of ButtonM and WhiteM with 198 and 105 CFU × 10^3 g^{-1} soil, respectively (Fig. 2C); for the remaining mangrove species the PSol population was in average 42.5 CFU × 10^3 g^{-1} soil, regardless seasonality (Fig. 2C). The total fungi population was significantly higher during rainy season, especially in the rhizosphere of WhiteM and BlackM whose fungal populations were 1500 CFU g^{-1} soil and 600 CFU g^{-1} soil, respectively (Fig. 2D).

Population of potential diazotrophic bacteria (NFB) was significantly greater during the rainy season than the dry season; the rhizosphere of WhiteM, ButtonM, and RedM showed higher NFB population than BlackM (Fig. 3A). Potential hydrocarbonoclastic diazotrophic bacteria (NFHB) were significantly higher in samples collected during the rainy season than dry season; moreover, the NFHB populations from the rhizosphere of WhiteM and ButtonM were 129 and 323 CFU × 10^3 g^{-1} soil, respectively (Fig. 3B).

In regards of N-assimilating hydrocarbonoclastic bacteria (NAHB) at the rainy season, its population was significantly higher in samples collected from the rhizosphere of WhiteM and ButtonM (98 and 74 CFU × 10^3 g^{-1} soil, respectively) than that observed for BlackM (Fig. 3C); during dry season the lowest NAHB population was achieved in the rhizosphere of BlackM (27 × 10^3 CFU × 10^3 g^{-1} soil) (Fig. 3C).

There were found significant differences among mangrove species in respect to the population of lypolytic (LIPOL), cellulolytic (CELLUL) microorganisms, as well as for starch- (AMYL), pectin- (PEC), and protein-degrading (PROT) microorganisms (Fig. 4). The rhizosphere of WhiteM showed the highest populations of CELLUL, AMYL, PEC, and PROT in samples collected during the rainy season (Fig. 4B–E); whereas the rhizosphere of ButtonM harbored the greatest LIPOL population, at both seasons (Fig. 4A). In contrast, the lowest microbial population of CELLUL, AMYL, and PEC was detected in the rhizosphere of ButtonM (Fig. 4B–D); whereas the rhizosphere of RedM and BlackM had the lowest populations of LIPOL and PROT microorganisms, respectively (Fig. 4A and E).

Results about grouping soil textural and chemical properties of the rhizosphere soil from the four mangrove species are shown in Fig. 5. Based on the principal component analysis (PCA), it was observed that two principal components represented the 85.5% of the total variation (CP1 with 48.3%, and CP2 with 37%). Thus, soil properties with the most significant influence for CP1 were organic matter and total organic carbon. In opposite way, CP2 was significantly influenced by the textural properties such as sand and clay particles. Highly significant correlations were achieved between sand × clay ($r = -1.0$; $P < 0.0001$), and between sand × $CaCO_3$ ($r = -0.93$; $P < 0.0007$). Also, correlations of parameters associated to either the rainy (sand × phosphorus, $r = 0.62$) or the dry season (clay × $CaCO_3$, $r = 0.95$) were highly significant and positive (Table 2).

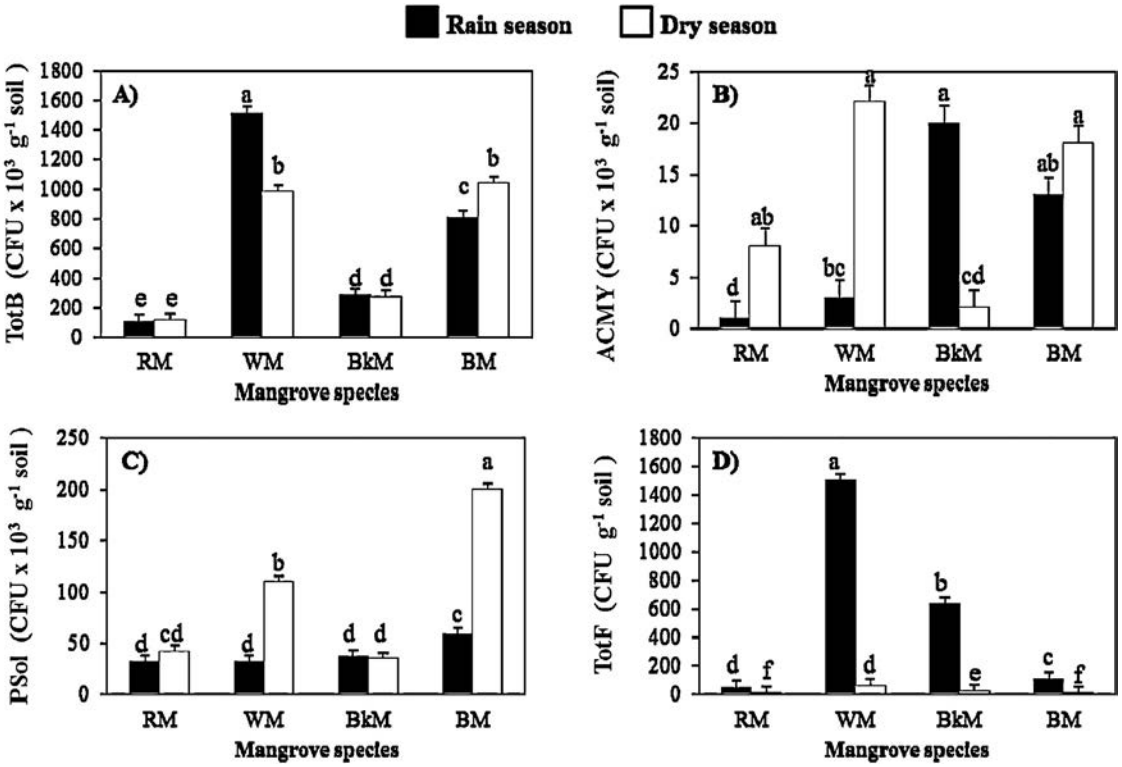

Fig. 2. Populations of total bacteria (TotB), total fungi (TotF), actinobacteria (ACMY), and potential P-solubilizing bacteria (PSol) of rhizosphere soil from four mangrove species collected at the Terminos Lagoon, Campeche, Mexico. Means ± standard error. Identical letters on bars are not statistically different (Tukey, $\alpha = 0.05$), $n = 6$. *Abbreviations*: RM = red mangrove (*Rhizophora mangle* L.); WM = white mangrove (*Laguncularia racemosa* (L.) Gaertn. f.); BkM = black mangrove (*Avicennia germinans* (L.) Stern); BM = button mangrove (*Conocarpus erectus* L.).

Fig. 5 shows that as the two vectors are closer, the *r* values are near to 1.0 as occurred with the estimated correlation coefficient for TOC × OM ($r = 0.99$). On the contrary, when vectors are opposite, the correlation become negative as observed for sand × clay ($r = -1.0$). Based on the dispersion diagram for the principal components CP1 and CP2 (Fig. 6) and on the negative correlation observed for the PCA, the association between sand × clay ($r = -1.0$) was inversely proportional to each other. For instance, the rhizosphere soil from RedM, ButtonM, BlackM, and WhiteM had high values of Sand during the rainy season, but low values of Clay. On the contrary, during the dry season the rhizosphere of the four mangrove species had low values of Sand, but high values of Clay.

3.3. Correlations between microbial populations with physical and chemical soil properties

Table 2 shows the most significant Pearson correlation coefficients ($P < 0.05$) among microbial populations and textural and chemical parameters in the rhizosphere. Parameters with highly significant correlations were NFB, NAHB, total fungi, CELLUL, AMYL, PEC, PROT, pH, OM, N, P, $CaCO_3$, sand, clay and TOC. In contrast, the parameters without significant correlations were NFHB, P-solubilizing bacteria, CE, and silt. Thus, the lowest Pearson correlation coefficient was observed between P × NFB ($r = 0.72$), and the highest significant correlations ($P < 0.0007$) were

Table 2
Overall Pearson correlation coefficients estimated for microbial populations, and textural and chemical soil properties of rhizosphere soil samples collected from four mangrove species at the Terminos Lagoon, Campeche, México.

Parameters	Pearson correlation (*r*)	*P* value (<0.05)	Parameters	Pearson correlation (*r*)	*P* value (<0.05)
N-assimilating hydrocarbonoclasts × N_2 fixing bacteria	0.94	0.0005	Total fungi × N_2 fixing bacteria	0.79	0.0201
Total fungi × N-assimilating hydrocarbonoclasts	0.73	0.0397	Lipolytic × total bacteria	0.75	0.0337
Cellulolytic × total fungi	0.79	0.0206	Pectin × total fungi	0.95	0.0003
Pectin × cellulolytic	0.77	0.0262	Starch degraders × N_2 fixing bacteria	0.84	0.0099
Amylose × total fungi	0.88	0.0042	Starch degraders × cellulolytic	0.83	0.01
Amylose × pectin	0.85	0.0081	Protein × N_2 fixing bacteria	0.92	0.0013
Protein × N assimilating Hydrocarbonoclasts	0.87	0.0055	Protein × cellulolytic	0.74	0.0343
Protein × Starch degraders	0.84	0.0088	Organic matter × cellulolytic	0.88	0.0041
Organic matter × pH	−0.86	0.0058	Nitrogen × cellulolytic	0.84	0.0092
Nitrogen × pH	−0.87	0.005	Nitrogen × organic matter	0.97	0.0001
Phosphorus × N_2 fixing bacteria	0.72	0.0442	Phosphorus × total fungi	0.89	0.0028
Phosphorus × pectin	0.86	0.0056	Phosphorus × starch degraders	0.8	0.0174
Sand × $CaCO_3$	−0.93	0.0007	Clay × $CaCO_3$	0.95	0.0002
Clay × sand	−1	<0.0001	Total organic C × cellulolytic	0.82	0.0125
Total organic C × pH	−0.89	0.0031	Total organic C × organic matter	0.99	<0.0001
Total organic C × N	0.98	<0.0001			

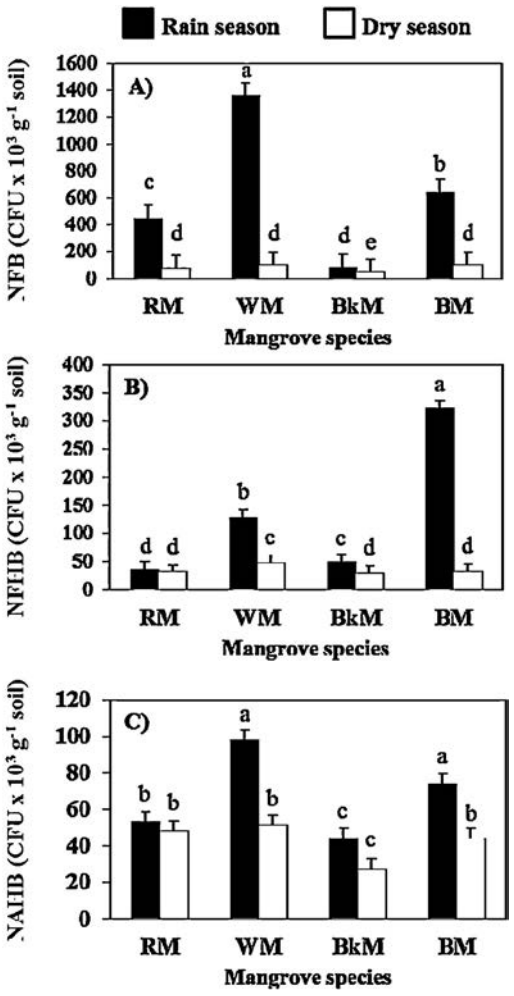

Fig. 3. Populations of potential N_2-fixing free living bacteria, (NFB), N_2-fixing hydrocarbonoclastic bacteria (NF-HB), N-assimilating hydrocarbonoclastic bacteria (NA-HB), of rhizosphere soil from four mangrove species collected at the Terminos Lagoon, Campeche, Mexico. Means ± standard error. Similar letters above bars are not statistically different (Tukey, $\alpha = 0.05$), $n = 6$. *Abbreviations*: RM = red mangrove (*Rhizophora mangle* L.); WM = white mangrove (*Laguncularia racemosa* (L.) Gaertn. f.); BkM = black mangrove (*Avicennia germinans* (L.) Stern); BM = button mangrove (*Conocarpus erectus* L.).

as follows: sand × $CaCO_3$ ($r = -0.93$), NFHB ($r = 0.94$), PECT × total fungi ($r = 0.95$), clay × $CaCO_3$ ($r = 0.95$), N × OM ($r = 0.97$), TOC × N ($r = 0.98$), TOC × OM ($r = 0.99$), and clay × sand ($r = -1.00$).

4. Discussion

Microbial population was significantly influenced due to mangrove species in which the WhiteM rhizosphere harbored the highest microbial population when compared to the remaining three mangrove species. Populations of total bacteria, actinobacteria, P-solubilizers, and potential N_2-fixing free living bacteria were lower than those reported from rhizosphere soil of different mangrove ecosystems (Toledo et al., 1995; Bashan et al., 1998; González-Acosta et al., 2006; Mitra et al., 2008). In contrast, regardless the rhizosphere of the four mangrove species, the greatest fungal population was counted during the rainy season (Fig. 2B). There is not able information about the rhizosphere fungal population in mangroves; however, Kohlmeyer et al. (1995) and Holguin et al. (2001) have reported the vertical distribution of fungi that grew on dead trunks and branches of *R. mangle*, *A. germinans*, *L. racemosa*, and *C. erectus*. Thus, this research provides information about the presence of fungal populations in the rhizosphere of RedM, WhiteM, BlackM, and ButtonM at the Terminos Lagoon.

Furthermore, the determination of specific microbial functional groups such as lipolytic, as well as starch-, pectin-, and protein-degraders has not been previously conducted in mangrove ecosystems. In general, LIPOL, CELLUL, AMYL, PEC, and PROT populations were high in the rhizosphere of WhiteM in both seasons, but the LIPOL population was high in the ButtonM rhizosphere. These two mangrove species are established near to the land or in the inner part of the mangrove forest, in where the OM accumulation contributes as organic reservoirs of N and P. The later concurs with data provided by Bharathkumar et al. (2008) and Dias et al. (2009) that indicated that high OM contents and nutrient availability are determinant for microbial growth and for the proliferation of microorganisms with specific physiological activity such as degraders of cellulose, pectin or starch, among other organic compounds that are present in the litter. In our study, the WhiteM rhizosphere had high chemical and nutritional properties (pH, OM, and N and P content, for instance; Table 1) by which the populations of microorganisms were stimulated. Thus, it seems that the rhizosphere of WhiteM possesses optimal soil chemical properties for harboring microorganisms with more diverse functional physiology as indicated by Dias et al. (2009).

Based on the assessed microbial populations, the present study suggests that mangrove ecosystems harbor diverse functional microbial groups that may potentially be directed to biotechnological approaches for either ecological restoration or agricultural purposes (Toledo et al., 1995; Vázquez et al., 2000; Bashan and Holguin, 2002; Rueda-Puente et al., 2003; Kathiresan and Selvam, 2006; Sundaraman et al., 2007; El-Tarabily and Youssef, 2010; de-Bashan et al., 2012). In this regard, the rhizosphere of WhiteM possessed the highest population of beneficial microorganisms that may play a significant role on the C, N, or P cycles in the mangrove ecosystem, as well as potential degraders of petroleum hydrocarbons that may be deposited in that ecosystem.

The highest microbial populations obtained in the rhizosphere of WhiteM and BlackM may be due to the high amount of rainfall and to flooding conditions during the rainy season, allowing microbial proliferation. In addition to water availability, both pH conditions and nutrient availability may have played a significant role on stimulating microbial populations in the mangrove rhizosphere (Dias et al., 2009). Nevertheless, the microbial populations in either WhiteM or ButtonM were also stimulated during the dry season, and that effect may be due to accumulation of clays and humus (Stotzky, 1993) that may favor microbial adhesion to the soil particles surface, and improve the nutrient accumulation.

Both actinobacteria and fungi are important biological components for organic matter decomposition by means of enzyme releasing that favors degradation of lignin, cellulose, and other plant structural components such as pectin, proteins, and starch (Raghukumar et al., 1994). In this regards, actinobacteria are abundant in alkaline soils, with high organic matter and $CaCO_3$ contents (Waksman, 1950), which agrees with the chemical soil properties obtained from samples collected at the Terminos Lagoon. Similarly, fungi may grow at both high humidity and extremely dry conditions (Waksman, 1950; Abramson et al., 1999), as it was also observed in our data.

P-solubilizing microorganisms play a significant role on P availability for other microorganisms and plants, especially when the P-sources (organic or inorganic) need to be subjected to either mineralization or solubilization processes prior plant uptake (Holguin et al., 2001; Sahoo and Dhal, 2009). Our data show that P-solubilizing microorganisms were significantly high in the rhizosphere of ButtonM at both rainy and dry seasons, suggesting that the P-availability is mediated by this specific microbial group (Vázquez et al., 2000; Holguin et al., 2001). In this regards, the

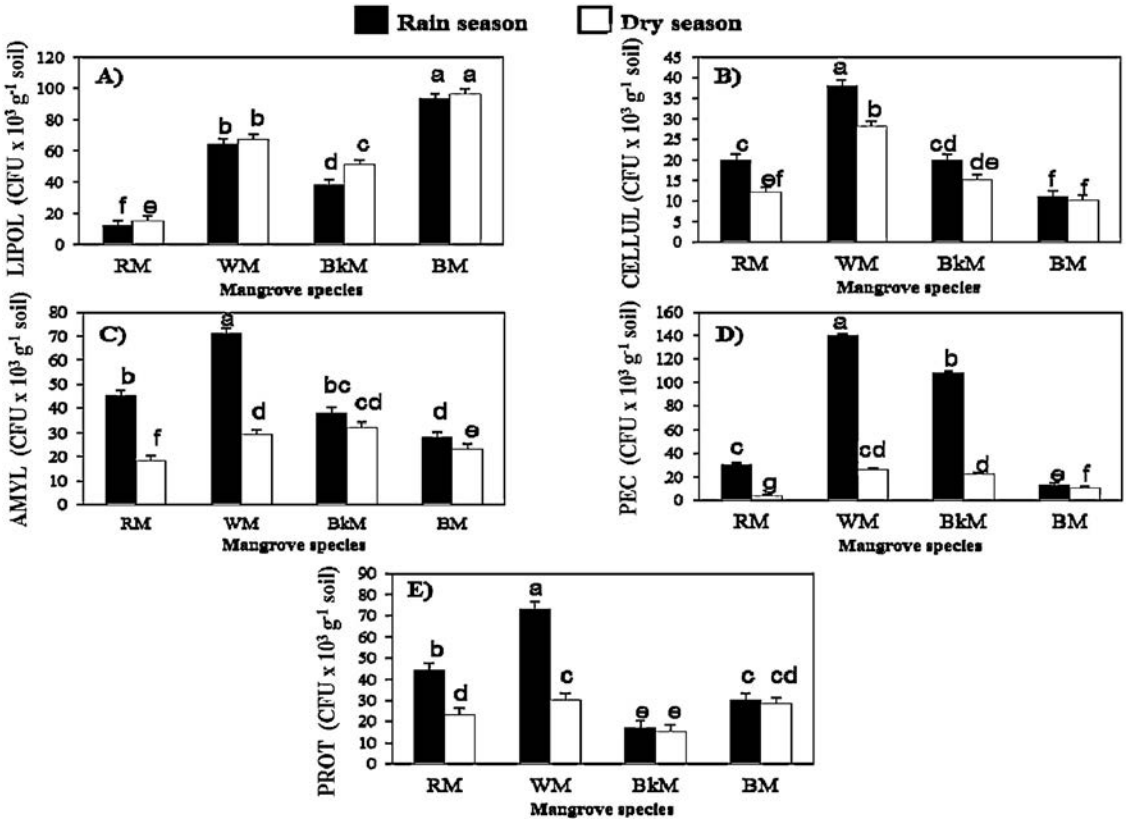

Fig. 4. Populations of microorganisms with specific physiological activity: lipolytic (LIPOL), cellulose degraders (CELLUL), starch degraders (AMYL), pectin degraders (PEC), and proteolytic (PROT) of rhizosphere soil from four mangrove species collected at the Terminos Lagoon, Campeche, Mexico. Means ± standard error. Similar letters above bars are not statistically different (Tukey, $\alpha = 0.05$), $n = 6$. *Abbreviations*: RM = red mangrove (*Rhizophora mangle* L.); WM = white mangrove (*Laguncularia racemosa* (L.) Gaertn. f.); BkM = black mangrove (*Avicennia germinans* (L.) Stern); BM = button mangrove (*Conocarpus erectus* L.).

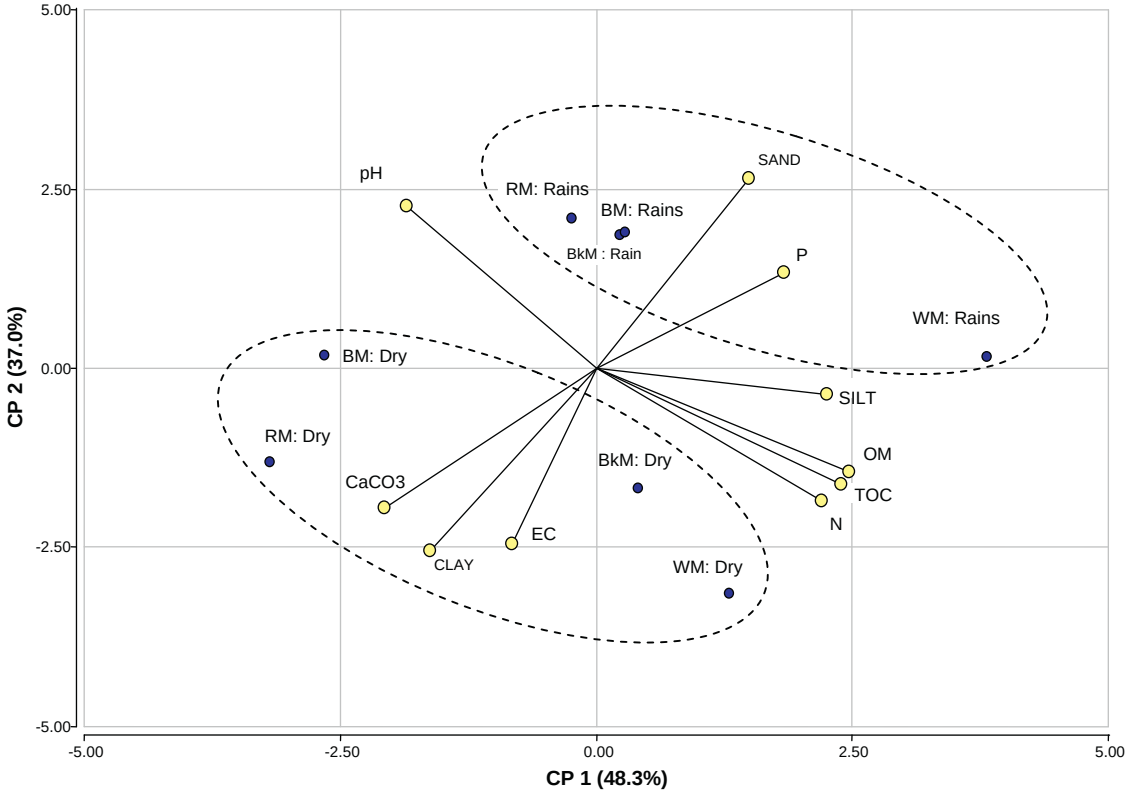

Fig. 5. Principal components plot for physical and chemical soil parameters and seasonality estimated from the four mangrove species at the Terminos Lagoon Campeche, Mexico. Values in parenthesis represent the variation percentage for each component. *Abbreviations*: RM = red mangrove (*Rhizophora mangle* L.); WM = white mangrove (*Laguncularia racemosa* (L.) Gaertn. f.); BkM = black mangrove (*Avicennia germinans* (L.) Stern); BM = button mangrove (*Conocarpus erectus* L.).

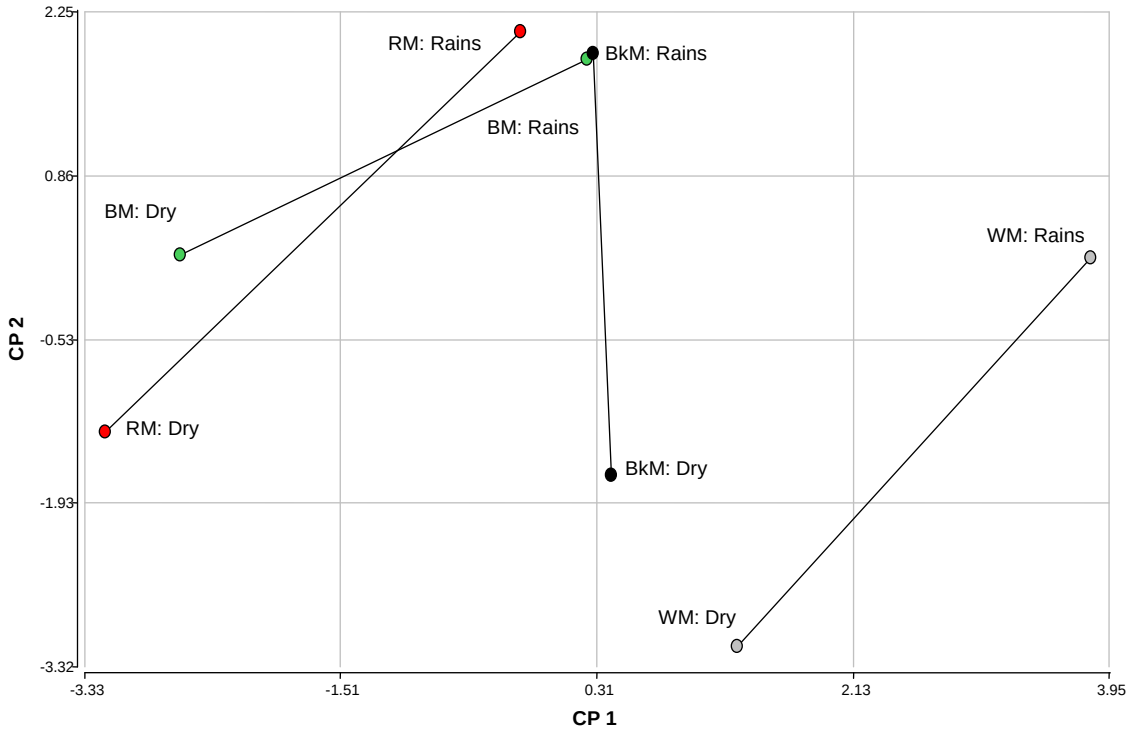

Fig. 6. Spatial distribution of CP1 versus CP2 with respect to mangrove species and seasonality (Rain or Dry) estimated from rhizosphere soil samples collected at the Terminos Lagoon, Campeche, Mexico. CP1 (OM and TOC), and CP2 (SAND and CLAY). *Abbreviations*: RM = red mangrove (*Rhizophora mangle* L.); WM = white mangrove (*Laguncularia racemosa* (L.) Gaertn. f.); BkM = black mangrove (*Avicennia germinans* (L.) Stern); BM = button mangrove (*Conocarpus erectus* L.).

ability of microorganisms to solubilize inorganic phosphates should consider other P-sources for determining this functional microbial groups as suggested by Bashan et al. (2013).

The high proliferation of potential N_2-fixing free living bacteria (NFB), diazotrophic hydrocarbon degraders (NFHB), and N-assimilating hydrocarbon degraders (NAHB) observed at the rhizosphere of WhiteM and ButtonM represents an important source of nitrogen for mangrove ecosystems (Holguin et al., 1992; Toledo et al., 1995). Moreover, these microbial functional groups also indicate that besides reducing N-atmospheric to easily N-assimilable forms also may potentially contribute on degrading organic toxic compounds that can affect the stability of mangrove ecosystems (Rojas et al., 2001; Holguin and Bashan, 1996). The presence of potential hydrocarbonoclastic bacteria has been taken in account in ecological studies conducted from sediments of estuarine shoreline ecosystems (Dias et al., 2009).

In regards to carbon and nitrogen cycles, the populations of CELLUL, AMYL and LIPOL, or PROT and PEC, respectively, are critical for modifying soil chemical properties, and for degrading organic C and N sources (Schloter et al., 2003). Additionally, all the microbial functional groups identified in this research, indicate that the Terminos Lagoon is an important source of microorganisms for being utilized in biotechnological purposes for environmental, agricultural or industrial approaches, as also suggested by Sivaramakrishnan et al. (2006), Lageiro et al. (2007), de-Bashan et al. (2012), and Salihu et al. (2012).

During the rainy season, the rhizosphere from the four mangrove species showed a sandy loam texture (CP1), whereas clay texture (CP2) was predominant during the dry season. This variation in the textural properties may be explained in part by the hydrodynamic processes from the river water flows toward the Terminos Lagoon which is significantly enhanced during the rainy season. The later allows high deposition of sand particles since flooding conditions are maintaining in the sampled locations at the four mangrove species (Rivera-Monroy and Twilley, 1996; Fuentes-Yaco et al., 2001). Based on the correlation coefficients observed among textural, chemical, and microbial parameters, it is possible to accept our hypothesis since microbial populations significantly correlated with textural or chemical soil parameters in the rhizosphere soil collected from the four mangrove species assessed at the Terminos Lagoon.

5. Conclusions

Microbial populations of the four mangrove species (RedM, WhiteM, BlackM, and ButtonM) showed significant differences ($P < 0.05$, ANOVA) during rainy and dry season. The highest microbial populations and the optimal values for textural and chemical soil properties were achieved in the rhizosphere soil from WhiteM (*L. racemosa*), whereas the lowest microbial populations and the highest values of soil properties were obtained in the rhizosphere of RedM (*R. mangle*). The scientific contribution of this research is related to identifying several culturable functional groups of microorganisms that might be directed to further biotechnological approaches.

Acknowledgments

This research was financially supported by the grant SEP-CONACYT 79456. M.A.R.E thanks the financial support provided by the grant PROMEP/103.5/09/4363, the Universidad Autónoma del Carmen (Campeche), CONACYT, and the COMECYT during his PhD-studies. Special thanks for comments and suggestions of two anonymous reviewers that significantly contribute on improving this manuscript.

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